European Food Research and Technology (2024) 250:2973–2987
https://doi.org/10.1007/s00217-024-04604-x

ORIGINAL PAPER

Interactions between lipid oxidation and anthocyanins from black carrots in ω-3 fatty acid-rich flaxseed oil-in-water emulsions

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Received: 19 February 2024 / Revised: 8 June 2024 / Accepted: 16 June 2024 / Published online: 30 July 2024
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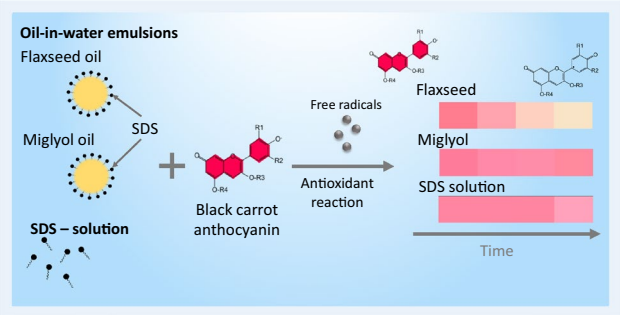
Abstract

The application of anthocyanins as red colorants in lipid-containing foods such as oil-in-water emulsions is challenging due to their ability to act as antioxidants and their instability under various environmental conditions. In this study, we investigated the kinetics of black carrot anthocyanin degradation and the subsequent color loss influenced by lipid oxidation reactions in 1% (*w/w*) flaxseed oil-in-water emulsions stabilized by 0.1% (*w/w*) sodium dodecyl sulfate (SDS) at pH 2 upon storage at 35 °C for ten days under light and in the dark. Oxidatively stable Miglyol oil-in-water emulsions and SDS solutions were used as reference. The results showed simultaneous formation of lipid hydroperoxides and degradation of anthocyanins. The addition of anthocyanins decreased lipid hydroperoxide formation, confirming the antioxidant activity of anthocyanins through radical scavenging mechanism. The kinetic modelling of lipid oxidation and anthocyanin degradation are particularly important for estimating color stability in colored emulsion-based food systems such as dairy or non-dairy milk or yoghurt drinks.

Graphical abstract



Keywords Food colors · Anthocyanins · Oil-in-water emulsions · Lipid oxidation · Antioxidants

Introduction

The use of food colors from plant sources is increasing due to the demands of consumers to replace synthetic colorants with natural ones [1, 2]. For many applications, coloring foods are particularly attractive because of their non-selective, mechanical extraction and often sustainable cultivation from fruits, vegetables and other edible plants [1]. Nevertheless, the application of anthocyanins is pertinent for oil-in-water emulsions as their conjugated ring structure enables delocalization of electrons upon reactions with free radicals,

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making them effective antioxidants but unstable in case of color [3–11]. Oil-in-water emulsions are the basis of many food applications like milk-drinks, yogurts, and beverages. One of the major challenges in emulsion-based food applications is lipid oxidation, which leads to the formation of off-flavors and harmful oxidation products that have a major impact on shelf life [12].

The antioxidant activity of anthocyanins from different sources in lipid oxidation reactions in oil-in-water emulsions has been well documented [5, 9, 10, 13]. In general, lipid alkyl radicals formed during the initiation phase of the lipid oxidation can form peroxyl radicals in the presence of oxygen that can further react to form less reactive lipid hydroperoxides and propagate the formation of more alkyl radicals [14]. This chain reaction can be interrupted or slowed down by antioxidants such as anthocyanins [5, 7, 9, 10, 13]. However, there is limited research on the kinetics of anthocyanin degradation in emulsions. Furthermore, the effect of light on anthocyanins has been described in aqueous solutions, but not in emulsions [15]. Anthocyanin molecules are excited by light irradiation resulting in the formation of anthocyanin radicals that can further react and form peroxyl radicals under oxygen [15].

This study characterizes the degradation of anthocyanins as a function of lipid oxidation in emulsions and includes a kinetic model to investigate color degradation and lipid oxidation in detail. Flaxseed oil was selected as a model oil as it is prone to oxidation due to the high concentrations of polyunsaturated fatty acids such as α-linolenic acid [16]. In order to attribute any color losses to lipid oxidation reactions, a saturated, medium chain Miglyol oil that is stable against oxidation was used. An oil concentration of 1% was chosen as typical low-fat dairy or dairy-like products contain 1% fat. This also falls under the low-fat food definition of no more than 1.5 g of fat per 100 mL for liquids (or 1.8 g fat per 100 mL for semi-skimmed milk) according to Commission Regulation (EU) No 1047/2012 [17]. Anionic sodium dodecyl sulfate (SDS) was chosen as surfactant as it has minor influence on lipid oxidation and it has the ability to efficiently stabilize emulsions [18]. Anthocyanin extract from black carrots (*Daucus carota* L. ssp. *sativus* var. *atrorubens* Alef.) was used as anthocyanin food colorant. Black carrots are commonly used raw materials for red colorants in foods and beverages such as fruit yogurt, fruit gummies, and soft drinks [1]. Furthermore, black carrot anthocyanins are partly acylated, showing a higher stability through intramolecular co-pigmentation than some other anthocyanin types [2, 19]. We hypothesized that the anthocyanins in black carrot extract will reduce lipid oxidation in flaxseed oil-in-water emulsions by acting as an antioxidant, similarly to observations in antioxidant assays, [6, 20] thus causing the color of the anthocyanins to fade over time. We expected that the anthocyanins undergo a radical induced oxidation due to formation of hydroperoxides upon oxidation of the α-linolenic acids [16]. In addition, the lipid oxidation and degradation of anthocyanins was expected to proceed more rapidly under light treatment than in the dark as both polyunsaturated fatty acids and anthocyanins are sensitive to light [10, 11, 15, 21, 22].

Materials and methods

Materials

A commercial black carrot extract (liquid form, 68.7°Brix) was provided by GNT International B.V. (Mierlo, Netherlands). Black carrots (*Daucus carota* L. ssp. *sativus* var. *atrorubens* Alef.) were processed non-selectively by water extraction, solid–liquid separation, enzymatic catalysis, and evaporation. The native flaxseed oil (76% α-linolenic acid, 9% linoleic acid, 4% oleic acid, and 1.5% stearic acid) was purchased from Ölmühle Ditzingen GmbH & Co. KG (Ditzingen, Germany). Miglyol oil 812N comprising mainly saturated medium chain triacylglycerides (C8:0 and C10:0) was obtained from Cremer Oleo GmbH & Co. KG (Hamburg, Germany). Acetonitrile (> 99.9%), ammonium thiocyanate (> 99%), barium chloride (> 99%), 2-butanone (98%), citric acid (> 99.5%), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2-ethylfuran (98%), ferrous sulfate (> 99%), Folin–Ciocalteau reagent, formic acid (> 98%), gallic acid (> 99%), hexanal (98%), hydrochloric acid (32%), methyl pentadecanoate (> 99.5%), phosphoric acid (85%), propanal (97%), sodium azide (> 99%), sodium carbonate (> 99%), sodium citrate dihydrate (> 99%), sodium dodecyl sulfate (SDS) (> 99%) and Supelco 37-compounds FAME-Mix were purchased from Merck (Darmstadt, Germany). 1-Butanol, hexane, isooctane, methanol, and 1-propanol, were obtained from Carl Roth GmbH & Co. KG (Karlsruhe, Germany), and had purities ≥ 99.5%. Cyanidin 3-glucoside standard was purchased from Extrasynthese (Genay, France). Double distilled water was used throughout the study.

Characterization of black carrot extract

The fat content was determined according to AS § 64 LFGB 06.00–6 and the dry matter content according to AS § 64 LFGB 06.00–3 [23]. The ash content was analyzed according to Nielsen [24]. The protein content was analyzed according to Dumas [25] using a nitrogen analyzer (Dumatherm N Pro, Gerhardt GmbH & Co. KG, Königswinter, Germany). A nitrogen-to-protein conversion factor of N × 4.39 was used [26]. The total sugar content was analyzed photometrically before and after enzymatic hydrolysis of sucrose using an enzymatic assay (Enzytec liquid sucrose/ D-glucose/ D-fructose, R-Biopharm AG,

Darmstadt, Germany). In short, sucrose was cleaved into glucose and fructose by β-fructosidase, the monosaccharides were phosphorylated by hexokinase consuming adenosine triphosphate, and then converted into gluconate-6-phosphate by glucose-6-phosphate dehydrogenase, while nicotinamide adenine dinucleotide was oxidized from NAD^+ to $NADH + H^+$. The NADH content was measured at 340 nm and the sugar content was calculated from the difference in absorbance before and after NAD^+ oxidation, which is proportional to the sugar content. To analyze the amount of selected chemical elements (aluminum, calcium, iron, magnesium, manganese, phosphorus, potassium, sodium, sulfur, and zinc), the plant extract was exposed to a microwave pressure digestion in nitric acid (ultraClave, MLS GmbH, Leutkirch, Germany) [27]. The samples were then sequentially extracted, sprayed into argon plasma, and evaporated at 10,000 K to dissociate the elements by using inductively coupled plasma optical emission spectroscopy (5110 SVDV, Agilent Technologies GmbH, Waldbronn, Germany) [27]. The quantification was carried out using respective standard solutions. The total phenolic content was determined photometrically according to the Folin–Ciocalteau method [28]. In short, the black carrot extract was pre-diluted in water (1:100), and 0.1 mL of the sample dilution was mixed with 0.9 mL water and 0.1 mL Folin–Ciocalteau reagent, shortly vortexed, and incubated for 5 min at 25 °C in the dark. Next, 0.8 mL sodium carbonate solution (7.5% *w/w*) was added into the mixture, the samples were further incubated at 25 °C for 40 min, and then the absorbance was measured at 765 nm using a spectrophotometer UV5 (Mettler Toledo, Columbus, OH, USA) at 25 °C. The concentration of total phenolics was calculated using a standard curve made of gallic acid.

The separation of anthocyanins was performed according to a method of Kammerer et al. [2] with some modifications. A 1260 Infinity Prime 2 high-performance liquid chromatograph (HPLC) equipped with a G7104C pump, a G7129C autosampler operated at 8° C, a G7129C column oven, and a G7115A wide range diode array detector with an acquisition range of 280–750 nm (Agilent, Waldbronn, Germany) was used. An ISAspher 100–5 C18 reversed phase column, 250 × 4.0 mm, 5 µm (ISERA GmbH, Düren, Germany) was used, operated at a temperature of 25 °C. The mobile phase consisted of 5% phosphoric acid (*v/v*) for eluent A and 100% acetonitrile for eluent B. The gradient program for anthocyanin analysis was as follows: (i) 5% B isocratic (3 min), (ii) 10% to 12% B (5 min), (iii) 12% to 13% B (2 min), (iv) 13% to 18% B (5 min), (v) 18% to 50% B (4 min), and (vi) 5% B isocratic (2 min) with a constant flow rate of 1 mL/min and a total run time of 21 min. Anthocyanins were quantified using a cyanidin-3-glucoside calibration curve with a concentration of 89 mg/L and varying injection volumes (0.2–12 µL). For sample preparation, 0.35 g of black carrot extract was diluted in 100 mL of 0.1% hydrochloric acid in methanol (*v/v*) and filtered using a cellulose acetate membrane filter with a pore size of 0.45 µm (Merck, Darmstadt, Germany). Absorbance spectra measurements at around 320 nm indicated acylation [29]. Peak assignment was performed using a 1290 Infinity 2 liquid chromatograph (LC) equipped with a G4220 bin pump and an Acquity UPLC HSST3 column, 150 × 2.1 mm, 1.8 µm (Waters, Milford, MA, USA) operated at 40 °C. The mobile phase consisted of 0.2% (*v/v*) formic acid for eluent A and 0.2% (*v/v*) formic acid in acetonitrile for eluent B. The gradient program for anthocyanin analysis was as follows: (i) 10% to 14% B (2.5 min), (ii) 14% to 55% B (9.5 min), (iii) 55% to 95% B (5 min), (iv) 95% B isocratic (1 min), (v) 95% to 10% B (1 min), and (vi) 10% B isocratic (1 min) with a constant flow rate of 0.4 ml/min and a total run time of 20 min. The LC-system was coupled on line to a Q Exactive Plus Hybrid Quadrupol-Orbitrap mass spectrometer (Thermo Scientific, Waltham, MA, USA). Positive ion mass spectra were recorded in the range of mass to charge ratio (*m/z*) from 150 to 1500.

Spectroscopic properties of black carrot extract

To investigate the effect of SDS on the absorbance spectrum of black carrot extract, 0.73 g/L extract was dissolved in 10 mM citrate buffer with and without added SDS (0.1% *w/w*) and the absorbance was measured at 380–780 nm using a Lambda 750 s spectrophotometer equipped with a 60 mm integrating sphere (Perkin Elmer, Waltham, MA, USA).

Antioxidant potential of black carrot extract

The radical scavenging capacity of black carrot extract was estimated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay [30]. The black carrot extract was prediluted (3.33 g/L) to ensure that the reduction in absorbance was within the linear detection range of the spectrophotometer UV5 (Mettler Toledo, Columbus, OH, USA) and the starting absorbance was set to 1.0. An aliquot of 40 µL of the extract solution was mixed with 3 mL of 0.1 mM DPPH in methanol (*w/v*) and the absorbance was measured at 515 nm at 25 °C after 40 min of incubation at 25 °C using methanol as a blank. The DPPH radical scavenging capacity was calculated as percentage of inhibition:

$$Inhibition\,(\%) = \frac{A_0 - A_s}{A_0} \cdot 100 \quad (1)$$

where A_0 is the absorbance of the blank and A_s the absorbance of the sample. The DPPH radical scavenging activity was expressed as EC_{50} value, which is defined as the concentration of the antioxidant which is needed to reduce 50% of the initial DPPH concentration. This expression is used to better compare the activity of different compounds since the

DPPH radical scavenging activity depends on the selected time point of the measurement data, whereas the EC_{50} value does not. Overall, the smaller the EC_{50} value, the higher the antioxidant potential. Four concentrations (0.73–3.33 g/L) of the black carrot extract were measured and EC_{50} was determined using linear regression.

Fatty acid composition of flaxseed oil

The fatty acid composition of flaxseed oil was determined by acidic esterification forming fatty acid methyl esters, which were analyzed by gas chromatography (GC). An aliquot of 100 mg of oil was mixed with 2 mL of 1% sulfuric acid in methanol (*v/v*) and heated at 80 °C for 1 h followed by cooling in an ice bath. Then, 2 mL of hexane, 1 mL of saturated sodium chloride solution, and 2 mL of double distilled water were added to extract the fatty acid methyl esters. After vortexing and subsequent phase separation, 500 µL of the upper organic phase and 2 µL of methyl pentadecanoate as internal standard were transferred to a glass vial (2 mL, 12 × 32 mm, amber) and sealed with a polypropylene screw cap equipped with a polytetrafluoroethylene/silicone septum (Agilent Technologies Germany GmbH & Co. KG, Waldbronn, Germany). Fatty acid composition analysis was performed using an Intuvo 9000 gas chromatograph (GC) coupled with a 5977B single quadrupole mass spectrometer (MS) and a PAL3 autosampler, equipped with an Intuvo MMI guard chip (set to 65 °C) and a 30 m × 0.25 mm internal diameter, 0.25 µm film thickness HP-5MS UI Intuvo capillary column (Agilent Technologies Germany GmbH & Co. KG, Waldbronn, Germany). An aliquot of 1 µL of the liquid sample was injected with an injection flow rate of 100 µL/s, a penetration depth of 45 mm, a penetration speed of 100 mm/s, and an injection signal mode "plunger up". Hexane was filled into a wash vial and the syringe was rinsed before and after each injection with a wash vial depth of 40 mm, a syringe fill of 80%, and a wash aspirate flow rate of 5 µL/s. The inlet temperature was set at 280 °C, the pressure was set at 11.25 psi with a flow rate of 34.2 mL/min, a septum purge flow rate of 3 mL/min, a post run temperature of 250 °C, a post run total flow of 25 mL/min, and a splitless mode was adjusted resulting in a purge flow of 100 mL/min. Helium was used as a carrier gas and the column flow was set to 1.2 mL/min. The oven program was set as follows: (i) 50 °C for 1 min, (ii) heated up to 185 °C at 4.3 °C/min, (iii) heated up to 240 °C at 4 °C/min and (iv) held at 240 °C for 20 min with a post run temperature of 300 °C for 5 min. The MS detector was set to 230 °C with a mass transfer line temperature of 260 °C. The MS was equipped with an EI (electron ionization) source (70°eV) and the mass detection range was from mass to charge ratio (*m/z*) of 40–550, the threshold was set to 100 and the solvent delay was set to 4.5 min. The detection was performed in scan mode with a scan cycle time of 349 ms and a scan speed of 1562 u/s. The quantitation was done by MassHunter Quantitative Analysis software (Agilent Technologies Germany GmbH & Co. KG, Waldbronn, Germany). For compound identification a fatty acid methyl ester standard Supelco 37-compunds FAME-Mix (Merck, Darmstadt, Germany) dissolved in hexane (25 uL/mL) was used.

Preparation of oil-in-water emulsions

Oil-in-water emulsions were prepared by mixing 1% (*w/w*) lipid phase (flaxseed or Miglyol oil) with 99% (*w/w*) aqueous phase containing 0.1% (3.47 mM) SDS (*w/w*) in 10 mM citrate buffer (pH 2). The mixture was homogenized by using a high-shear homogenizer Silent Crusher M (Heidolph Instruments GmbH and Co. KG, Schwabach, Germany) at 15,000 rpm for 2 min followed by a high-pressure homogenization using a LM10 Microfluidizer equipped with a G10Z interaction chamber with a diameter of 87 µm (Microfluidics Corporation, Westwood, MA, USA) at 1500 bar with 3 cycles. After homogenization, black carrot extract (0.73 mg/L) was added to the emulsion samples. The concentration applied was selected at which the absorbance of black carrot extract in 0.1% SDS in 10 mM citrate buffer (*w/w*) was 1.0. Flaxseed and Miglyol oil-in-water emulsions and 0.1% SDS in 10 mM citrate buffer solutions without black carrot extract served as reference samples. Miglyol oil-in-water emulsions were used as control samples since the medium chain triacylglycerides are not prone to lipid oxidation. The emulsion and SDS-solution samples with or without black carrot extract (7.5 mL) were filled into transparent glass vials (10 mL, 18 mm, VWR International, Radnor, PA, USA), sealed with a plastic snap cap, and stored at 35 °C in a climate cabinet in the dark or under light provided by ambient light lamps (TL-D 36W/849, Philips, Amsterdam, Netherlands) and UV lamps (LT36W/009 UV, Narva, Brand-Erbisdorf, Germany). The sample preparation for GC–MS analysis is described in Sect. Secondary oxidation products.

Characterization of oil-in-water emulsions

The particle median diameter ($d_{0.5}$) and particle size distribution were measured using static light scattering device (Horiba LA-950, Retsch Technology GmbH, Haan, Germany). Emulsions were diluted 1:1000 with 10 mM citrate buffer (pH 2) to prevent multiple scattering. The particle size determination is based on the angular dependency of the intensity of a laser beam scattered by the emulsion droplets. The particle size distribution was calculated from the scattering pattern using Mie theory. The refractive indices of 1.48 and 1.45 were used for flaxseed oil and Miglyol oil, respectively.

The hydrodynamic diameter (d_h), polydispersity index (PDI), and ζ-potential were determined using a Zetasizer Nano Series particle size analyzer (Nano-Zs ZEN 3600 with Dispersion Technology Software DTD v 5.1, Malvern Instruments Ltd., Worcestershire, UK). The emulsions were directly filled into disposable folded capillary cells (DTS1070, Malvern Instruments Ltd., Worcestershire, UK) and the size as well as PDI were measured via dynamic light scattering at a wavelength of 633 nm and the backscattered light was detected at a measurement angle of 173°. The ζ-potential was measured to estimate the net electrical charge of the emulsion droplets emulsified with SDS that depends on the velocity and the direction of the moving droplets in the electric field under electrophoresis. Here, the Smoluchowski approximation was applied. During all measurements, the temperature was kept constant at 25 °C.

Lipid oxidation

Primary oxidation products

The formation of lipid hydroperoxides as primary oxidation products was determined according to a method from Shantha and Decker [31]with some modifications. In short, 0.3 mL of emulsion was mixed with 1.5 mL isooctane:2-propanol (3:1, *v/v*) by vortexing (10 s, 3 times), and centrifugated at 1000 g for 3 min. An aliquot of 100 µL from the organic solvent phase was mixed with 2.8 mL of methanol:1-butanol (2:1, *v/v*), followed by addition of 30 µL thiocyanate-ferrous solution, which was prepared by mixing equal volumes of 0.132 M $BaCl_2$ with 0.144 M $FeSO_4$, centrifuging, and then mixing equal volumes of the clear ferrous solution with 3.97 M ammonium thiocyanate. After 20 min of incubation time, the absorbance was measured at a fixed wavelength of 510 nm using a Mettler-Toledo UV VIS–UV7 spectrophotometer (Mettler-Toledo GmbH, Giessen, Germany). The concentration of lipid hydroperoxides (µM) was calculated using a calibration curve made of cumene hydroperoxides (µM).

Secondary oxidation products

The formation of secondary oxidation products was determined by an Intuvo 9000 gas chromatograph (GC) coupled with a 5977B single quadrupole mass spectrometer (MS) and a PAL3 autosampler and equipped with an Intuvo MMI guard chip (set to 65 °C) and a 30 m × 0.25 mm internal diameter, 0.5 µm film thickness DB-HeavyWAX Intuvo capillary column (Agilent Technologies Germany GmbH & Co. KG, Waldbronn, Germany). Headspace glass vials (20 mL, 23 × 75 mm, amber) containing 1.0 mL of emulsion samples were sealed with steel magnetic screw caps with polytetrafluoroethylene/silicone septa (Agilent Technologies Germany GmbH & Co. KG, Waldbronn, Germany), and stored at 35 °C in the dark or under light up to ten days until the measurement time. Vials were heated in an incubator at 55 °C for 15 min prior injection. A headspace volume of 1 mL was injected via headspace syringe heated to 85 °C with an injection flow rate of 10 mL/min, a penetration depth of 15 mm, a penetration speed of 50 mm/s, post-aspiration delay of 0.5 s, an injection signal mode "plunger up", and a post injection time delay of 0.5 s. The inlet temperature was set to 260 °C, the pressure was set to 11.25 psi with a flow rate of 34.2 mL/min, a septum purge flow rate of 3 mL/min, a post run temperature of 250 °C, a post run total flow of 25 mL/min and a split ratio adjusted to 5:1 resulting in a split flow of 6 mL/min. The column flow for carrier gas helium was set to 1.2 ml/min, and the oven temperature program was set to (i) 40 °C for 3 min, (ii) heated up to 80 °C at 5 °C/min, (iii) heated up to 120 °C at 7 °C/min, (iv) heated up to 180 °C at 15 °C/min, (v) heated up to 240 °C at 30 °C/min, and (vi) held at 240 °C for 3 min. The MS detector with an EI source (70°eV) was set to 230 °C and a mass transfer line temperature of 230 °C. The mass detection ranged from mass to charge ratio (*m/z*) of 25 to 250 with a threshold of 100 and a solvent delay of 2.5 min. The detection was performed in scan mode with a scan speed of 781 u/s, a scan cycle time of 313 ms and the evaluation was done by MassHunter Quantitative Analysis software (Agilent Technologies Germany GmbH & Co. KG, Waldbronn, Germany). For compound identification, the *m/z* with 3–4 main qualifiers was used (butanal: *m/z* 27, 29, 41 44 (100), 72; 2-butanone: *m/z* 29, 43 (100), 72; 2-decenal: *m/z* 41, 43 (100), 55, 70; 2-ethylfuran: *m/z* 53, 81 (100), 96; 2,4-heptadienal: *m/z* 41, 81 (100), 110; 2-heptenal: *m/z* 29, 41 (100), 44, 55, 70; hexanal: *m/z* 27, 41, 44 (100), 56, 72; 2-hexenal: *m/z* 39, 41 (100), 55, 69; 2,6-nonadienal: *m/z* 39, 41 (100), 70; nonanal: *m/z* 41, 57 (100), 70, 98; octanal: *m/z* 29, 41 (100), 43, 57; pentanal: *m/z* 29, 44 (100), 58; 2-pentenal: *m/z* 29, 41, 55 (100), 84; 1-penten-3-ol: *m/z* 29, 31, 57 (100); 2-pentylfuran: *m/z* 53, 81 (100), 138 and propanal: *m/z* 27, 29 (100), 57, 58) according to the mass spectra library of National Institute of Standards and Technology (NIST) (NIST tandem mass spectral library, 2020) and the quantification was done by six-point external calibration (hexanal: 0.01–0.16 mM, propanal: 0.02–2.8 mM).

Kinetic modelling of lipid oxidation products

The formation of primary and secondary oxidation products was modelled according to an approach from Farhoosh (2020) [32] (Eq. 2):

$$c = \frac{k_c}{\exp\left[k_c(C - t)\right] + k_d} \quad (2)$$

where c is the concentration of lipid oxidation product (mM), k_c (h^{-1}) is a pseudo-first order rate constant, k_d (mM^{-1}

h^{-1}) is a pseudo-second order rate constant, and C (mM^{-1}) is an integration constant. The data fits can be found in supplementary information in Table S1.

Degradation of anthocyanins

The lipid phase of the flaxseed and Miglyol oil-in-water emulsions responsible for a turbid appearance was removed by solvent extraction to prevent interference upon the absorbance measurements. For this, 6 mL of emulsion sample was mixed with hexane at a ratio of 2:1 (*v/v*), vortexed for 60 s, and centrifugated at 15,000 g for 15 min at 20 °C. The upper clear solvent phase was removed, and the anthocyanin rich water phase was transferred from the centrifuge tubes into polystyrene macro cuvettes (VWR International, Radnor, PA, USA) using a 5 mL syringe equipped with a 0.9 × 70 mm cannula (B. Braun, Melsungen, Germany). The SDS-solutions were measured without solvent extraction. The absorbance from 380 to 780 nm was measured using a Lambda 750 s spectrophotometer equipped with a 60 mm integrating sphere (Perkin Elmer, Waltham, MA, USA). For all measurements, a turbidity baseline correction was performed at 750 nm by subtracting the absorbance at 750 nm from the absorbance at 528 nm. The samples were stored for 48 h until the maximum absorbance (528 nm) of the flaxseed oil-in-water emulsions containing black carrot extract dropped below 0.2, at which no color was perceptible, and the detection limit was reached. The concentration of anthocyanins in SDS-solutions and flaxseed and Miglyol oil-in-water emulsions was calculated according to Lambert–Beer law using a standard curve made of cyanidin-3-glucoside in 10 mM citrate buffer, measuring the absorbance at a maximum wavelength of 508 nm [33].

Kinetic modelling of anthocyanin degradation

The degradation of anthocyanins was modelled via a first order approach (Eq. 3) according to Chen et al. [34] with Excel Solver (Microsoft Inc., Redmond, WA, USA):

$$C_t = C_0 \cdot e^{-kt} \tag{3}$$

where C_t is the anthocyanin concentration (μM) at time t (h), C_0 is the anthocyanin concentration (μM) at $t = 0$ h, k is the reaction rate constant (h^{-1}), and t is the time (h). First order kinetic rate constant (k) was obtained from fitted values and half-life time $t_{1/2}$ (h) (Eq. 4) [34] as follows:

$$t_{1/2} = \frac{ln2}{k} \tag{4}$$

The data fits can be found in supplementary information in Table S2.

CIELAB color spaces

The absorbance spectra obtained from the spectrophotometer (Sect. Degradation of anthocyanins) were used to determine the colorimetric values L* C* h° according to CIELAB 1931 with an observer angle of 2° and illuminant C [35]. Average colors of the samples were extracted by GIMP version 2.10.24 (available at: https://gimp.org/). The color differences (ΔE_{00}) between the sample colors at 0 h and 48 h were calculated according to CIE $\Delta E2000$, which is the latest adjustment of ΔE [35].

Statistical analysis

All experiments were performed in triplicates. Results are presented as calculated means with standard deviations. Statistical analysis was performed using single variance (one-way ANOVA, Tukey or Duncan post hoc test) by SPSS Statistics 27 (IBM Inc., Armonk, NY, USA). Differences at $p < 0.05$ were considered to be significant.

Results and discussion

Characterization of black carrot extract

Chemical composition

The liquid black carrot extract used in this study contained 48 ± 0.1% water (corresponding to a dry matter of 52 ± 0.1%), 27.9 ± 0.1% sugars, 2.8 ± 0.03% protein, and 0% lipids. It contained 3.64 ± 0.09% ash and the following elements: 0.0015% Al, 0.072% Ca, 0.0044% Fe, 1.287% K, 0.044% Mg, 0.001% Mn, 0.274% Na, 0.131% P, 0.0986% S, and 0.0012% Zn.

Phenolic composition

The black carrot extract contained 7.48 g/L total phenolics (as gallic acid equivalents) and 69.73 mg/L anthocyanins (as cyanidin-3-glucoside equivalents). At a water content of 48 ± 0.1% (Sect. Chemical composition), this corresponded to 1.4% total phenolics and 0.0132% anthocyanins on dry matter basis. The total phenolic content was similar to previous literature for different black carrot preparations (1.86–7.70% on dry matter basis) [20]. The anthocyanin concentrations were also consistent with previous research reporting concentrations of 0.00454–1.74% (on dry matter basis) from black carrots [2].

Five major compounds in the black carrot extract were detected, and they were tentatively identified according to an earlier study [2] as sinapic acid derivatives of cyanidin 3-xylosyl galactosides (35.3%; acylated), cyanidin 3-xylosyl

galactosides (33.2%), cyanidin 3-xylosylglucosyl galactosides (12.9%), ferulic acid derivatives of cyanidin 3-xylosyl galactosides (10.4%; acylated), and *p*-coumaric acid derivatives of cyanidin 3-xylosylgalactosides (1.4%; acylated). The absorbance spectra measurements at around 320 nm [29] indicated that around 47.1% of the detected anthocyanins appeared to be acylated anthocyanins. The anthocyanin profile found in black carrot extract agrees with previous data [2, 6].

Antioxidant potential

The DPPH radical scavenging potential of black carrot extract was measured with an EC_{50}-value of 12.73 g/kg dry matter corresponding to a reciprocal EC_{50}—value ($1/E_{50}$) of 0.079. Higher reciprocal EC_{50}- values of 1.83–2.75 have been reported in other commercial black carrot concentrates [36], indicating that they had higher antioxidant potential compared to the extract used in this study.

Variations in both the quantity of polyphenols present in different varieties and the methods used to process them can result in different polyphenol concentrations [1]. Black carrots are known to have a high antioxidant potential, due to the high content of anthocyanins and other phenolic compounds [6, 20]. The protection of anthocyanins against oxidation depends on the structure of the aglycone, specifically on the number and position of free hydroxyl groups around the pyrone ring [3]. The cyanidin aglycone has been shown to have the strongest antioxidant potential in the 3-glucoside range compared to other aglycone types, whereas a further glycosylation decreases the antioxidant potential that can be explained by lower reactivity [9]. On the other hand, acylation of the anthocyanins has been reported to increase their antioxidant potential [3]. Namely, a higher degree of acylation by electron-rich sinapic and ferulic acids found in black carrot extract increases the ability of scavenge radicals [8]. As the determined anthocyanins in black carrot extract were all glycosylated forms of cyanidin with an acylation proportion of 47.1% (Sect. Phenolic composition), they can therefore act as free radical scavengers.

Characterization of oil-in-water emulsions

The 1% (*w/w*) flaxseed and Miglyol oil-in-water emulsions stabilized with 0.1% SDS with or without the black carrot extract were nanosized ($d_h = 110–120$ nm, $d_{0.5} = 126–150$ nm) and monomodally distributed (Table 1, Fig. 1). All the emulsions remained stable for the ten-day test period (Table 1, Fig. 1) due to the highly negative charges ($\zeta = -77$ to -88 mV) of adsorbed SDS (Table 1) that provided electrostatic repulsion between the emulsion droplets. The higher absolute net charge of the emulsions with black carrot extract (Table 1) can be explained by the resulting increase in the concentration of ions in the water phase of the emulsions. The hydrocarbon tails of the SDS are pulled into the oil more strongly, increasing the electrostatic repulsion between the charged heads of the surfactant [37]. Furthermore, the lower net electrical charge observed after a storage time of ten days (ζ-potential from -73 ± 1 mV to -84 ± 3) for Miglyol oil-in-water emulsion without extract suggests the formation of anionic reaction products like free fatty acids which are formed during hydrolysis reactions [13].

Lipid oxidation in oil-in-water emulsions

The purpose of these experiments was to investigate the lipid oxidation reactions in flaxseed oil-in-water emulsions (1% *w/w* oil, 0.1% *w/w* SDS in 10 mM citrate buffer, pH 2) with or without the presence of black carrot extract (0.73 g/L) at 35 °C under ambient light or in the dark during a ten-day storage. A pH of two was chosen to ensure good anthocyanin stability and to avoid hydration reactions with increasing pH that destabilize the molecule [7]. No lipid hydroperoxides or any secondary lipid oxidation products were detected in the Miglyol oil-in-water emulsions (data not shown) that can be attributed to the fact that the saturated medium-chain fatty acids in Miglyol oil do not oxidize.

The flaxseed oil-in-water emulsions rich in α-linolenic acid (76%), linoleic acid (9%), and oleic acid (4%) formed lipid hydroperoxides rapidly after storage at 35 °C (Fig. 2).

Table 1 Hydrodynamic diameter (d_h), median diameter ($d_{0.5}$), polydispersity index (PDI), and ζ-potential of oil-in-water emulsions (1% *w/w* flaxseed or Miglyol oil, 0.1% *w/w* SDS in 10 mM buffer, pH 2) with or without added black carrot extract during a ten-day storage at 35 °C in the dark

$c_{extract}$ (g/L)	d_h (nm)		$d_{0.5}$ (nm)		PDI (-)		ζ (mV)	
	Day 0	Day 10	Day 0	Day 10	Day 0	Day 10	Day 0	Day 10
Flaxseed o/w:								
0	120 ± 2^a	136 ± 2^a	127 ± 0.6^{ab}	120 ± 1.5	0.19 ± 0.00^a	0.18 ± 0.1^b	-77 ± 2^a	-76 ± 2^a
0.73	120 ± 3^a	135 ± 1^a	126 ± 0.5^a	122 ± 2.9	0.17 ± 0.1^a	0.17 ± 0.02^a	-84 ± 4^b	-87 ± 4^b
Miglyol o/w:								
0	121 ± 2^a	140 ± 6^a	150 ± 2.1^c	135 ± 1.1	0.20 ± 0.02^b	0.25 ± 0.07^c	-73 ± 1^a	-84 ± 3^b
0.73	111 ± 4^a	156 ± 8^b	131 ± 3.4^b	136 ± 0.8	0.23 ± 0.02^c	0.29 ± 0.03^d	-88 ± 2^c	-87 ± 11^b

Different small letters within each column denote significant differences according to Tukey post hoc test ($p < 0.05$)

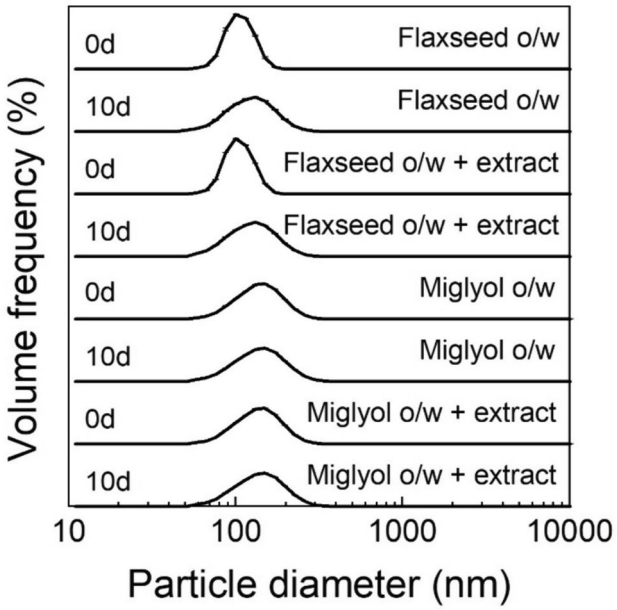

Fig. 1 Volume-based mean particle size distribution (d0.5) of oil-in-water emulsion (1% w/w flaxseed or Miglyol oil, 0.1% w/w SDS in 10 mM citrate buffer, pH 2) with or without added black carrot extract (0.73 g/L) during a ten-day storage. Error bars are within the data curves

This agrees with previous literature that these fatty acids can form a variety of positional lipid hydroperoxide isomers upon oxidation [12, 32]. Some data points showed large variations, particularly during the decomposition of lipid hydroperoxides, that is most likely due to the simultaneous formation and decomposition of lipid hydroperoxides [38]. This effect was especially prominent in the rapidly oxidizing samples, especially those exposed to light. The primary oxidation products were also formed faster when the flaxseed oil-in-water emulsions were stored under light as indicated by shorter lag-phase times ($t_{lag} = 3.45 \pm 1.69$ h) compared to emulsions stored in the dark ($t_{lag} = 20.28 \pm 0.97$ h) (Table 2, Fig. 2).

Light is a well-known initiator of lipid oxidation [14]. Unsaturated lipids absorb light in the ultraviolet wavelength spectra which provides energy for the abstraction of hydrogen from the carbon next to the double bond due to the lower C-H bond energy and accelerates the rate of oxygen consumption by photodecomposition of peroxides into free radicals [21, 39]. Under light storage, the emulsions with added black carrot extract showed significantly ($p < 0.05$) longer lag-times than the emulsions without the extract (Table 2), indicating that the anthocyanins in the extract acted as antioxidants, and decelerated the formation of lipid hydroperoxides. Rate constants k_c and k_d describe the propagation phase of the reaction. Within our kinetic model, k_c can be used as a measure of oxidation susceptibility, indicating the reactivity of the emulsion to peroxidation, and k_d describes the rate at which the lipid hydroperoxides are formed. The k_c and k_d – values showed that the addition of black carrot extract into the flaxseed oil-in-water emulsions slowed down the propagation phase ($p < 0.05$) upon storing under light (Table 2). The propagation phase of flaxseed oil-in-water emulsion with and without black carrot extract under light storage reached their maximum after 24 and 48 h, respectively (Fig. 2A).

Upon storage in the dark, autoxidation of unsaturated fatty acids from flaxseed oil is initiated by triplet oxidation and metal traces [12]. The black carrot extract did not significantly ($p > 0.05$) affect the propagation rate constants (k_c, k_d) (Table 2). Nevertheless, the propagation phase of flaxseed oil-in-water emulsions with and without black carrot extract in the dark storage reached its maximum after 72 and 53 h, respectively (Fig. 2B). In addition, the lower lipid hydroperoxide concentrations (257 ± 83 µM) detected for the emulsions with the added black carrot extracts at the end of the propagation phase compared to the emulsion without the extract (382 ± 25 µM) (Fig. 2B) suggest that the anthocyanins and other phenolic compounds (Sect. Phenolic composition) in the black carrot extract inhibited the reaction to some extent. This agrees with previous studies reporting that anthocyanins from black rice [13], purple sweet potato [10], and black currant [5] inhibited the formation of lipid hydroperoxide in oil-in-water emulsions. To our knowledge, no previous studies on effects of black carrot extract on the formation of lipid hydroperoxides in emulsions are available.

The sudden decrease in the lipid hydroperoxide formation in the flaxseed oil-in-water emulsions with or without black carrot extract stored under light and in the dark at later storage times (Fig. 2) indicated that the primary oxidation products are gradually converted into secondary products due to the instability of lipid hydroperoxides [14]. The most likely pathway of decomposition of unstable hydroperoxides is a homolytic cleavage of oxygen–oxygen bond producing alkoxy and hydroxy radicals [14]. Those radicals undergo homolytic β-cleavage of the carbon–carbon bond forming saturated and unsaturated alkyl radicals and oxo compounds [14]. As a result, volatile secondary oxidation compounds such as aldehydes, ketones, and organic acids can be formed [22].

Indeed, the following secondary oxidation products in the flaxseed oil-in-water emulsions were detected upon storage: Butanal, 2-butanone, 2-decenal, 2-ethylfuran, 2,4-heptadienal, 2-heptenal, hexanal, 2-hexenal, 2,6-nonadienal, nonanal, octanal, pentanal, 2-pentenal, 1-penten-3-ol, 2-pentylfuran, and propanal.

2-Decenal, nonanal, and octanal are oxidation products of oleic acid, produced from 8-hydroperoxide, 9- or 10- hydroperoxides, and 11-hydroperoxides, respectively [16]. Butanal is an oxidation product of oleic acid, most likely formed by

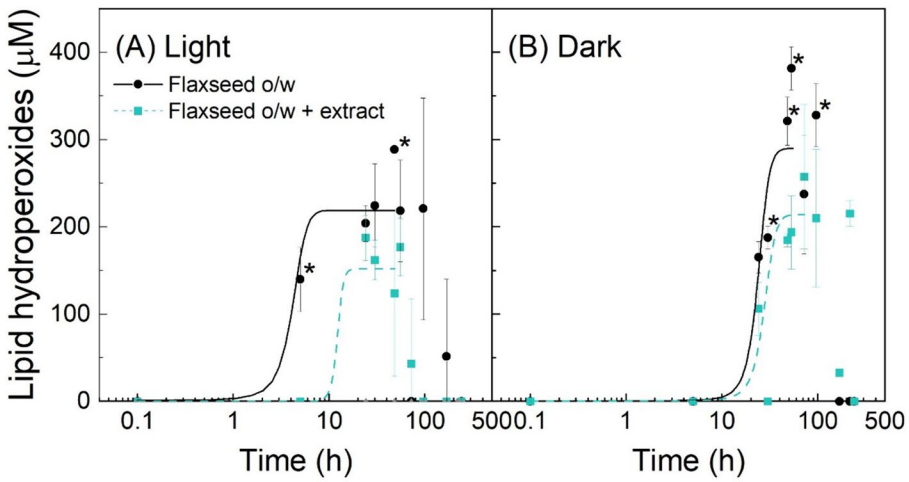

Fig. 2 Formation of lipid hydroperoxides in flaxseed oil-in-water emulsions (1% w/w oil, 0.1% w/w SDS in 10 mM citrate buffer, pH 2) with or without added black carrot extract (0.73 g/L) during a ten-day storage at 35 °C under light (**A**) or in the dark (**B**). The legend applies to both figures. Asterisk * denotes the significant differences ($p < 0.05$) between the emulsion samples with and without black carrot extract within light and dark storage. The lines shown depict sigmoidal data fittings (Supplementary data Table S1)

Table 2 Initial concentration c_0 and kinetic parameters[a] of primary and secondary oxidation products formed in flaxseed oil-in-water emulsions (1% *w/w* oil, 0.1% *w/w* SDS in 10 mM citrate buffer, pH 2) with or without added black carrot extract during storage at 35 °C under light or in the dark

Oxidation product	$c_{extract}$ (g/L)	Light				Dark			
		c_0 (μM)	t_{lag} (h)[b]	$k_c \times 10^2$ (h^{-1})[c]	k_d ($mM^{-1}\ h^{-1}$)[d]	c_0 (μM)	t_{lag} (h)	$k_c \times 10^2$ (h^{-1})	k_d ($mM^{-1}\ h^{-1}$)
Lipid hydroperoxides	0	0 ± 0^a	3.45 ± 1.69^a	1.19 ± 0.25^a	$5.15 \times 10^{-3} \pm 1.62 \times 10^{-3a}$	0 ± 0^a	20.28 ± 0.97^c	$0.29 \times 10^{-2} \pm 0.02 \times 10^{-2a}$	$0.97 \times 10^{-3} \pm 0.21 \times 10^{-3a}$
	0.73	0 ± 0^a	12.31 ± 0.80^b	1.11 ± 0.21^b	$7.30 \times 10^{-3} \pm 1.88 \times 10^{-3b}$	0 ± 0^a	20.54 ± 0.65^c	$0.25 \times 10^{-2} \pm 0.1 \times 10^{-2a}$	$1.15 \times 10^{-3} \pm 0.13 \times 10^{-3a}$
Propanal	0	18 ± 6^b	9.48 ± 2.20^a	11.2 ± 0.30^a	0.06 ± 0.1^{ab}	64 ± 14^a	6.43 ± 3.21^a	10.05 ± 0.07^a	0.05 ± 0.00^a
	0.73	30 ± 3^{ab}	11.43 ± 0.84^a	10.8 ± 0.72^a	0.05 ± 0.01^a	54 ± 24^a	7.95 ± 1.11^a	10.59 ± 0.42^a	0.06 ± 0.00^b
Hexanal	0	0 ± 0^a	na	4.35 ± 0.44^a	0.56 ± 0.09^a	0 ± 0^a	na	5.28 ± 0.40^a	0.60 ± 0.14^a
	0.73	0 ± 0^a	na	4.64 ± 0.33^a	0.59 ± 0.05^a	0 ± 0^a	na	2.15 ± 0.42^b	0.35 ± 0.04^a

Different small letters within each oxidation product denote a significant difference between samples with or without extract stored under light or in the dark according to Tukey post hoc test ($p < 0.05$)

na not applicable: The modelling function could not be calculated due to insufficient data points

[a]The values were calculated from sigmoidal functions fitted on the kinetic curves (Fig. 2, Fig. 3) (for data fits and coefficient of determinations see Supplementary data Table S1)

[b]Lag time t_{lag} was determined by extrapolating the tangent at the inflection point of the propagation phase

[c]Rate constant k_c was calculated according to. Equation 2

[d]Rate constant k_d was calculated according to Eq. 2

the further oxidation of unsaturated aldehydes [12]. Oxidized linoleic acid volatiles including, hexanal and pentanal are formed from 13-hydroperoxides, and 2-heptenal from 12-hydroperoxides [16]. It is assumed that 2,6-nonadienal is an oxidation product of linoleic acid, but the formation is not completely understood [12]. Oxidation of α-linolenic acid leads to the formation of 2,4-heptadienal from 12-hydroperoxides, 2-pentenal from 13-hydroperoxides, and 2-pentylfuran from 10-hydroperoxides [16]. 2-Pentylfuran can be formed from linoleic and linolenic acid [16]. Propanal is an oxidation product of α-linolenic acid, formed through 9-, 12-, 13- and 16-hydroperoxides [14]. 2-Butanone has been shown to form upon oxidation of 2-butanol [40], whereas 2-ethylfuran can form from 2-hexenal under catalysis of amino acids [41]. 1-Penten-3-ol have been identified as a flavor compound in fish oil enriched milk [42].

As propanal and hexanal are major markers for oxidation of ω-3 and ω-6 fatty acids, respectively [12, 16] and subsequently showed the highest peak areas among the detected secondary oxidation products, they were selected for quantification (Fig. 3) and kinetic modelling (Table 2). Overall, the formation of propanal in flaxseed oil-in-water emulsions was substantially higher than that of hexanal (Fig. 3) that is due to the lower activation energy of ω-3 fatty acids compared to ω-6 fatty acids [12].

The kinetic modelling for propanal formation revealed that no significant differences were observed in t_{lag}, k_c, or k_d—values between the flaxseed oil-in-water emulsions with

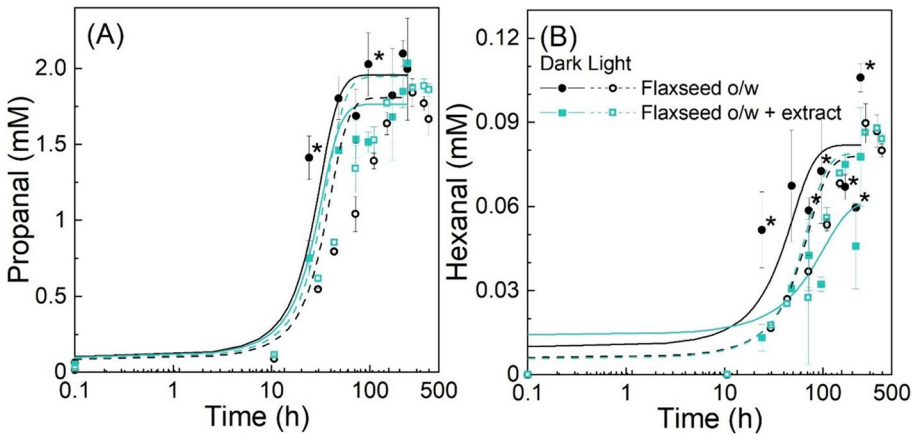

Fig. 3 Formation of propanal (**A**) and hexanal (**B**) in oil-in-water emulsions (1% w/w flaxseed oil or Miglyol oil, 0.1% w/w SDS in 10 mM citrate buffer at pH 2) with or without added black carrot extract (0.73 g/L). The legend applies to both figures. Asterisk * denotes the significant differences ($p < 0.05$) between the emulsion samples with and without black carrot extract within light and dark storage. No significant differences ($p > 0.05$) were found between emulsions with and without the extract within light storage. The lines shown depict sigmoidal data fittings (Supplementary data Table S1)

or without the black carrot extract stored either under light or in the dark, except for k_d – values between emulsions with and without added extract in the dark and between emulsions with extract under light and in the dark ($p < 0.05$) (Table 2). Upon storage in the dark, the propanal concentrations were lower ($p < 0.05$) in the flaxseed oil-in-water emulsions with the added black carrot extract than in the emulsion without the extract during the propagation phase only after 24 h ($c_{0.73\ g/L} = 0.75 \pm 0.11$ mM *vs* $c_{0g/L} = 1.41 \pm 0.14$ mM) and 96 h of storage ($c_{0.73\ g/L} = 1.53 \pm 0.12$ mM *vs* $c_{0g/L} = 2.03 \pm 0.21$ mM), indicating some antioxidant effect of anthocyanins and other polyphenols present in the black carrot extract (Fig. 3A). Under light storage, however, the emulsions with and without the black carrot extract showed no differences in propanal formation ($p > 0.05$) (Fig. 3A).

No lag phase could be calculated for hexanal due to lack of inflection points in the fitted formation curves (Table 2). The formation of hexanal in the flaxseed oil-in-water emulsions with the added black carrot extract was slower in the dark during the propagation phase as depicted by significantly ($p < 0.05$) lower k_c—values (Table 2) and lower hexanal concentrations (Fig. 3B) compared to the one without the extract. Under light storage, however, no differences in hexanal formation were observed between the flaxseed oil-in-water emulsions with or without black carrot extract (Table 2, Fig. 3B).

Anthocyanins from black carrot have been shown to decrease the formation of hexanal in liposomes [43]. Anthocyanins from other sources such as black rice have been shown to slow down the formation of secondary oxidation products (malonaldehydes) in walnut oil-in-water emulsions stabilized by whey proteins (5% oil, 0.5% protein, 10 mM phosphate buffer, pH 7) [13]. The authors described that the antioxidant effect was due to the ability of the anthocyanins to donate hydrogen atoms from their aromatic group, but also due to the physical barrier created by the anthocyanins due to the surface activity that prevents the interactions between the lipid droplets and the pro-oxidants in the continuous water phase [13, 43]. In another study, anthocyanins from black currant were also shown to inhibit the formation of hexanal in Brij-35-stabilized corn oil-in-water emulsions (5% oil, 0.5% emulsifier, 10 mM phosphate buffer, pH 7) [5]. Furthermore, purple sweet potato extract rich in anthocyanins were shown to inhibit the formation of secondary oxidation products (measured as 2-thiobarbituric acid reactive substances; TBARS) in fish oil-soybean oil-in-water emulsions [10].

Overall, the black carrot extract had more impact of slowing down the formation of primary oxidation products than the formation of secondary oxidation products.

Color and anthocyanin stability of oil-in-water emulsions

Next, the color and anthocyanin stability of flaxseed and Miglyol oil-in-water emulsion with added black carrot extract stored for 48 h at 35 °C in the dark or under ambient light was evaluated.

For black carrot extract (0.73 g/L) in 10 mM citrate buffer solution (pH 2) we found a maximum absorbance wavelength of 520 nm with an absorbance of 1.1 (Fig. 4) corresponding to color values of $L^* = 70.9$, $C^* = 59.1$ and $h^\circ = 12.52$. This is in agreement with previous literature on the color values of black carrot extract ($L^* = 68.1$, $C^* = 57.0$, $h^\circ = 10.1$) at acidic pH where anthocyanins preferentially exist in their flavylium cation [2].

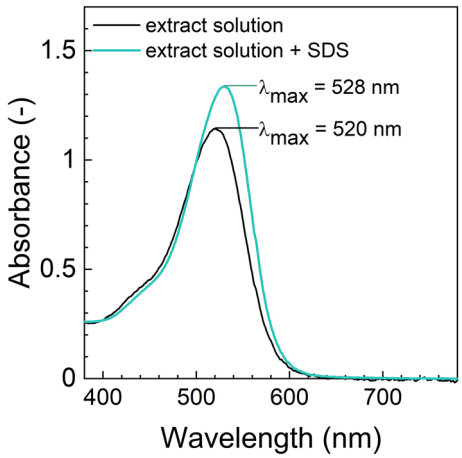

Fig. 4 Absorbance spectra of aqueous black carrot extract solution (0.73 g/L) in 10 mM citrate buffer (pH 2) with and without sodium dodecyl sulfate (SDS, 0.1% w/w) at 35 °C

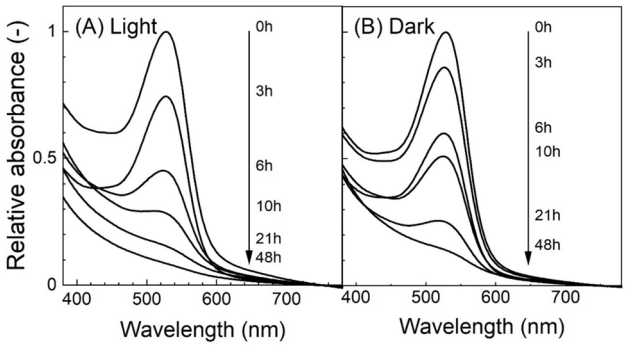

Fig. 5 Relative absorbance of the aqueous black carrot extract phase after hexane extraction from oil-in-water emulsions (1% w/w flaxseed oil, 0.1% w/w SDS in 10 mM buffer, pH 2) with added black carrot extract (0.73 g/L) during a two-day storage at 35 °C under light (**A**) or in the dark (**B**)

To gain a better understanding of the color stability of the emulsions, we first investigated the effect of SDS (0.1%) on the absorbance spectrum of the black carrot extract. This is because the anionic surfactant SDS can form micelles in aqueous solutions at critical micellar concentrations (CMC) of 4.61 mM [44]. Although the applied SDS concentration was below the CMC (0.1%, corresponding to 3.47 mM), the addition even at this concentration into the aqueous black carrot extract led to a hyperchromic and bathochromic shift in the visible spectra (Fig. 4). The bathochromic peak shift was 8 nm from 520 to 528 nm (Fig. 4) that is consistent with the bathochromic shift from 516 to 532 measured for cyanidin, [45] which is the main anthocyanidin in black carrot extract (Sect. Phenolic composition). Addition of SDS above the CMC has been shown to stabilize anthocyanins via distribution of the polar heads of SDS molecules on the micellar surface and the ionic interactions between positively charged flavylium ions and negatively charged SDS [45]. Furthermore, a bathochromic shift was shown to potentially relate to an organized system on the micellar surface [45].

Second, the absorbance spectra of black carrot extract from the flaxseed and Miglyol oil-in-water emulsions and SDS solutions were recorded over time (Fig. 5). Only data for flaxseed oil-in-water emulsions are shown as an example of anthocyanin degradation (Fig. 5). The data from the maximum absorbance values of flaxseed and Miglyol oil-in-water emulsions as well as of SDS solutions containing black carrot extract was converted to anthocyanin concentrations (as cyanidin-3-glucoside equivalents) and plotted as a function of time to show the degradation of the black carrot anthocyanins over time in the different systems with an exponential data fitting (Fig. 6). This exponential data fitting was then used to calculate the kinetic parameters (k and $t_{1/2}$) of anthocyanin degradation (Table 3).

Furthermore, the absorbance data was converted into color units and the color changes (ΔE_{00}) were calculated (Fig. 7). The black carrot extracts solutions containing SDS (0.1% *w/w*) were investigated to determine the potential impact of SDS on the degradation behavior of black carrot extract.

The absorbance of black carrot extract decreased over time when it was added into flaxseed oil-in-water emulsions stored under the light or in the dark (Fig. 5). The maximum absorbance wavelength was at 528 nm, which agrees with the absorbance spectra of the black carrot extract with added

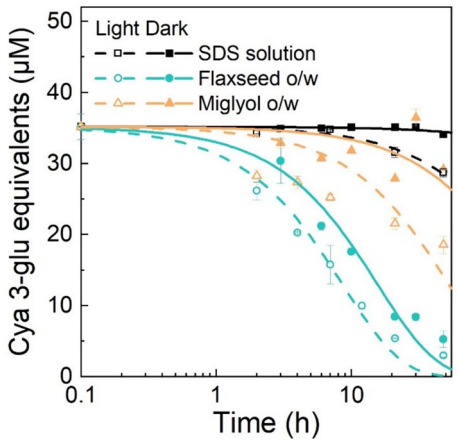

Fig. 6 Degradation of black carrot anthocyanins as cyanidin 3-glucoside (cya 3-glu) equivalents in oil-in-water emulsions (1% w/w flaxseed or Miglyol oil, 0.1% w/w SDS in 10 mM citrate buffer, pH 2) and in aqueous SDS (0.1%, w/w in 10 mM citrate buffer, pH 2) solutions with added black carrot extract (0.73 g/L) during a 48 h-storage at 35 °C in the dark or under light. The lines shown depict exponential data fittings (Supplementary data Table S2)

Table 3 Kinetic parameters[a] of anthocyanin degradation in oil-in-water emulsions (1% *w/w* flaxseed or Miglyol oil, 0.1% *w/w* SDS in 10 mM citrate buffer, pH 2) and in aqueous SDS (0.1% *w/w* in 10 mM citrate buffer, pH 2) solutions with added black carrot extract (0.73 g/L) during storage at 35 °C in the dark or under light

Sample	Dark		Light	
	$k \times 10^{-3}$ (h^{-1})[b]	$t_{1/2}$ (h)[c]	$k \times 10^{-3}$ (h^{-1})	$t_{1/2}$ (h)
Flaxseed o/w	60.23 ± 1.42^{a}	11.51 ± 0.27^{a}	111.33 ± 16.60^{b}	6.32 ± 0.91^{b}
Miglyol o/w	5.31 ± 0.34^{c}	130.90 ± 8.37^{bc}	19.45 ± 2.06^{d}	35.91 ± 3.82^{ab}
SDS solution	0.45 ± 0.04^{c}	1562.96 ± 126.46^{d}	4.24 ± 0.53^{c}	165.17 ± 22.28^{c}

Different small letters within each parameter (for all samples, stored in the dark or light) denote a significant difference according to Duncan post hoc test ($p < 0.05$)

[a]The values were calculated from exponential functions fitted on the kinetic curves (Fig. 6) (for data fits and coefficient of determinations see Supplementary data Table S2)

[b]Rate constant k was calculated according to Eq. 3

[c]Half-life time $t_{1/2}$ was calculated according to Eq. 4

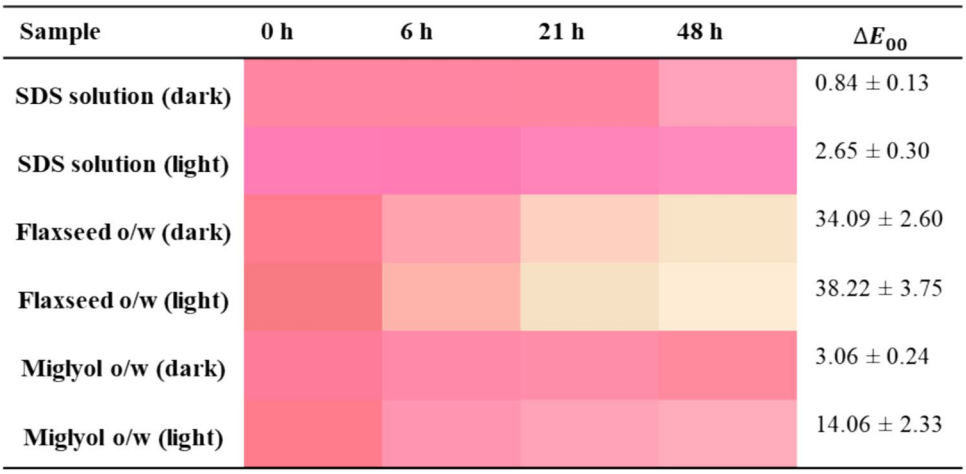

Fig. 7 L*C*h° colors of oil-in-water emulsions (1% w/w flaxseed oil or Miglyol oil, 0.1% w/w SDS in 10 mM citrate buffer, pH 2) and in aqueous SDS (0.1% w/w in 10 mM citrate buffer, pH 2) solutions with added black carrot extract (0.73 g/L) during 2 day storage at 35 °C in the dark or under light. $\Delta E_{00}$was calculated from 0 to 48 h

SDS (Fig. 4). No bathochromic or hypsochromic shifts were observed over time (Fig. 5). The same was observed for the Miglyol oil-in-water emulsions and SDS solutions (data not shown). A irreversible decrease in the absorbance without color shifts can indicate cleavage of the anthocyanin molecule resulting in the formation of colorless compounds [11].

The black carrot extract solutions containing SDS showed no reduction ($p > 0.05$) in anthocyanin concentration upon storing in the dark, whereas storage under light led to slight decrease ($p < 0.05$) in the anthocyanin concentration (Fig. 6). Chen et al. (2020) reported that red raspberry anthocyanins in juice systems degraded by first order reaction with a half-life time ($t_{1/2}$) of 219.8 h [34]. This was similar to the black carrot extract solutions containing SDS stored in the light ($t_{1/2} = 165$ h), but faster than SDS solutions stored in the dark ($t_{1/2} = 1563$ h) (Table 3). The rate constant k, describing the decrease of anthocyanin content over time by first order reaction curve fitting, showed no significant differences between the storage in the dark or under light for the extract solutions containing SDS (Table 3). However, the half-life times ($t_{1/2}$) of the black carrot extracts solutions containing SDS were significantly different ($p < 0.05$) upon storage in the light and in the dark (Table 3). This was also verified by the negligible or minor changes in visual perception of color differences (ΔE_{00}) of black carrot extracts solutions containing SDS in the dark and under light, respectively (Fig. 7). A study by Gérard et al. (2019) described a reduced stability of black carrot anthocyanins in citrate buffer at pH 3.0 under light irradiation in the presence of oxygen due to the excitation of anthocyanins leading to the formation of carbon centered radicals that can generate peroxyl radicals under oxygen [15]. Overall, ΔE-values below 1.0 indicate that the color difference is not noticeable, values between 1 and 2 indicate that only experienced observer can notice the difference, values between 2 and 3.5 indicate that also unexperienced observer notice the difference, values between 3.5 and 5 show a clear difference in color, and values above 5 are perceived as different colors [46].

The anthocyanin concentration in flaxseed oil-in-water emulsions with the added black carrot extract decreased rapidly, and it was faster upon storage in the light than in the dark as depicted by the significantly ($p < 0.05$) different

k-values and $t_{1/2}$-values (Fig. 6, Table 3). This was also illustrated by the rapid decrease of the color in flaxseed oil-in-water emulsions during the 48 h of storage (Fig. 7). The flaxseed oil-in-water emulsions containing the black carrot extract stored under the light began losing their color already after 6 h of storage, and the complete loss of red color occurred after 21 h. The loss of color was slightly slower in the flaxseed oil-in-water emulsions upon storage in the dark; however, the red color was also completely gone after 48 h (Fig. 7).

In Miglyol oil-in-water emulsions, the degradation of anthocyanins in black carrot extract was slower than in flaxseed oil-in-water emulsions in both dark and light storage (Fig. 6). This was also depicted by the significantly ($p < 0.05$) lower k-values (Table 3). However, the $t_{1/2}$-values of anthocyanin degradation showed no differences between the Miglyol oil-in-water emulsions stored in the dark or under light. The color degradation of anthocyanins in the black carrot extract in Miglyol oil-in-water emulsions was closer to the behavior in SDS solutions, showing that the color degraded slowly upon storage in the dark (Fig. 7). Under light, the color in the Miglyol oil-in-water also faded to some extent (Fig. 7). These results indicate that the degree of unsaturation of the lipid droplets influenced the color degradation of anthocyanins. The color degradation of anthocyanins in black carrot extract in Miglyol oil-in-water emulsions and SDS solutions can be explained by the irreversible hydrolysis and autooxidation which is a typically occurring degradation mechanism during storage of anthocyanins [8].

A study by Guldiken et al. (2018) reported that the color from black carrot anthocyanins in liposomes (1, 2, or 4% soy lecithin in acetate buffer, pH 3.5) during a 21 days of storage in the dark at room temperature degraded more the higher the concentration of the unsaturated phospholipids in the liposomes was [43]. This was accompanied by increasing lightness (L^*) and yellowness (b^*) values. This was attributed to degradation of the aglycone by the formation of lipid hydroperoxides [47]. The formation of lipid hydroperoxides typically increases under light storage [12, 14] that can lead to a faster degradation of anthocyanins. Moreover, the general photosensitivity of anthocyanins can accelerate the color loss [15].

Mechanistic insights

The results of the study show a correlation between the color stability of black carrot anthocyanins and lipid oxidation in oil-in-water emulsions. Lipid oxidation was induced by elevated temperature (35 °C) and either with or without light treatment in the presence of catalyzing iron ions from black carrot extract and atmospheric oxygen. These factors are common in inducing lipid oxidation [12, 14, 18, 22]. The high concentration of polyunsaturated fatty acids (89%), especially of ω-3-fatty acids (76%), in flaxseed oil led to rapid oxidation due to the formation of lipid radicals by abstraction of hydrogen atoms from allylic C-H bonds next to the unsaturated double bonds [12, 14, 16]. We demonstrated that the black carrot anthocyanins degraded simultaneously as they acted as radical scavengers (Fig. 6, Sect. Antioxidant potential) to reduce the formation of lipid hydroperoxides and to lesser extent of secondary oxidation products upon oxidation of flaxseed oil-in-water emulsion (Fig. 2, Fig. 3) by interacting with the lipid peroxyl radicals during the initiation and propagation phases [8, 14]. In their native form, the anthocyanins can act as free radical scavengers by donating hydrogen to a lipid peroxide radical and the resulting molecule has a lower energy because of the delocalization in the ring structure [5, 9, 10, 13]. A low energy of the free phenolic radical cannot promote hydrogen elimination from unsaturated fatty acids, thus inhibiting the chain reaction occurring during lipid oxidation [48]. The ability of black carrot anthocyanins to donate hydrogen atoms to DPPH radicals confirmed that they could act as a radical scavenger (Sect. Antioxidant potential).

Consequently, the color loss in the flaxseed oil-in-water emulsions containing black carrot extract (Fig. 7) was linked to that of the proceeding lipid oxidation (Fig. 2, Fig. 3) and degradation of the anthocyanins (Fig. 6). The color degradation is due to a cleavage of the anthocyanin ring structure and subsequent loss of the conjugated double bonds in the aglycone as a result of combined hydrolytic and autoxidative reactions [8]. As expected, the light treatment led to faster lipid oxidation reactions in the flaxseed oil-in-water emulsions and therefore faster degradation of the black carrot anthocyanins as they were reacting earlier with the forming lipid radicals. The degradation of black carrot anthocyanins (and subsequent color) in Miglyol oil-in-water emulsions and in SDS solutions (Fig. 6, Fig. 7) were most due to excitation of anthocyanins with UV radiation (under light treatment) [15] or due to acid hydrolysis [8] (under dark) as no lipid oxidation occurred in these samples.

Conclusion

This study described the effect of anthocyanins from black carrot extract on the oxidation of flaxseed oil-in-water emulsions and their color loss due to their action as radical scavengers. The investigations on oxidatively stable Migyol oil-in-water emulsions and SDS solutions containing no lipids confirmed a direct correlation between lipid oxidation of unsaturated fatty acids and color loss. The study provides a deeper understanding of the use of anthocyanins as colorants in oil-in-water emulsions and the stability issues that arise

due to the antioxidant activity of anthocyanins. To ensure optimal stability in oil containing foods such as flavored milk, a fundamental understanding of the degradation kinetics of anthocyanins is essential.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00217-024-04604-x.

Acknowledgements This research was supported by GNT Europa GmbH.

Author contributions E. Klinger: Conceptualization, Investigation, Formal analysis, Visualization, Writing—original draft. H. Salminen: Conceptualization, Visualization, Writing—original draft, Writing—review and editing, Project management, Supervision. K. Bause: Conceptualization, Writing—review and editing, Supervision. J. Weiss: Conceptualization, Writing—review and editing, Resources, Overall supervision.

Funding Open Access funding enabled and organized by Projekt DEAL.

Data availability All data supporting the findings of this study are available within the paper and supplementary information.

Declarations

Conflict of interest There are no conflicts of interest to declare.

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