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## Greener aromatic antioxidants for aviation and beyond

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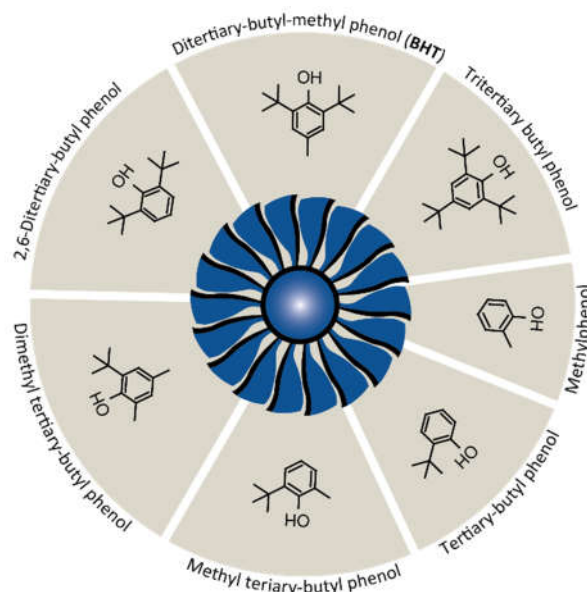
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Antioxidants (AO) inhibit unwanted unspecific reactions of oxygen and are added to most susceptible industrial products, including liquid hydrocarbon based aviation fuels. Regulatory approved jet fuel, whether synthetic or crude oil based, mandates the addition of performance antioxidants to inhibit radical-induced oxidation during storage and transport. While presently used antioxidants are petroleum based, there have been no sustainable, less-toxic, bio-based equivalents reported so far. This study addresses the initial evaluation of natural carotenoid-based antioxidants as a substitution for current alkyl-phenolic antioxidants to kerosene. Inspired from natural products of *Brevibacterium* and *Synechococcus* genus synthesised analogs of candidate aromatic carotenoid cleavage products were evaluated for their effects on combustion characteristics of jet fuel, comparative radical scavenging, and endothelial cell-culture cytotoxicity. These bioinspired antioxidants did show no adverse effect on jet fuel bulk properties and combustion chemistry. The radical scavenging properties of the here proposed phenolic carotenoid cleavage products are superior to non-aromatic  $\beta$ -carotene cleavage products and in range with current alkyl-phenolic additives. Cytological tests showed a similar, low toxicity towards human endothelial cells when compared to butylated hydroxytoluene (BHT), one of the approved alkyl-phenol based additives to jet fuel, food and cosmetics. From these initial performance data, it appears likely that the proposed biobased compounds do not interfere with normal operations of jet engines and human fuel handling. Further developments towards the usage of aromatic carotenoid cleavage products as antioxidants could contribute to reduced non-renewable consumption and possibly being an environmentally more tolerated alternative due to their biosynthetic origin.

### Introduction

Hydrocarbon fuels are predicted to remain the medium termed aviation energy source for bulk transport of time critical goods and passengers<sup>1</sup>. Thermal and chemical stability of these are crucial, as oxidation can lead to the formation of insoluble oxidation products and possible fuel system

failures<sup>234 2-4</sup>



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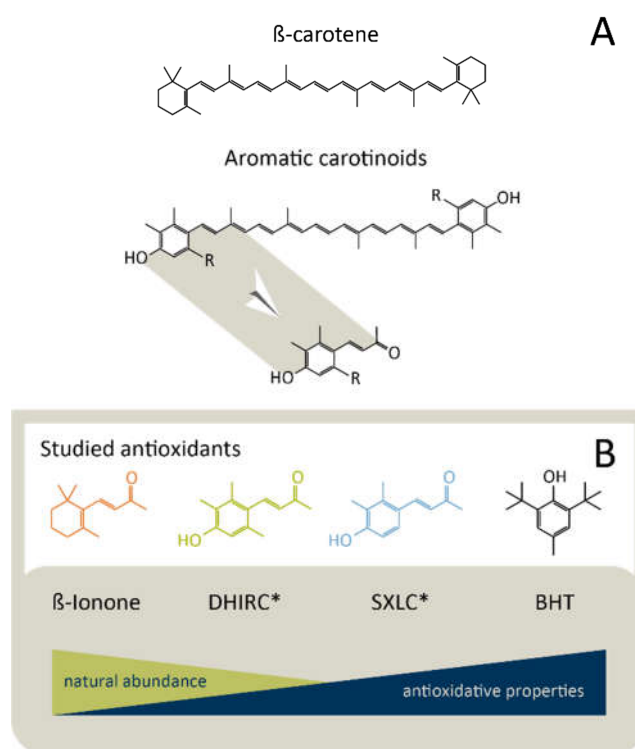
**Fig. 1** Chemical structures of currently certified jet fuel antioxidants. These are added to jet fuel as pure compounds or mixtures at a maximum concentration of  $24 \text{ mg L}^{-1}$  in total. Further regulative information can be found in the ASTM standard specification for aviation turbine fuels<sup>5</sup>.

Consequently, autoxidation reactions in jet fuels have to be prevented, that can be triggered during storage at ambient temperatures as well as upon exposure to elevated temperatures, particularly within the engine fuel supply system<sup>6,7</sup>. Therefore, the addition of antioxidants to aviation fuel formulations is made mandatory by the American Society for Testing and Materials (ASTM). Since operational aspects and certification requires aircraft fuels to be used at temperatures as low as -47 °C, especially starting of the engine has to be ensured at these conditions<sup>8</sup>. Already small amounts of physically active additives may have a significant influence on the viscosity and the surface tension. Temperature dependent properties like (kinematic) viscosity, density, and surface tension are critical parameters for the fuel's spray characteristics, i.e., during atomization at the combustor. Therefore, these properties were investigated in addition to the combustion chemistry.

Among the certified antioxidants for kerosene, only alkyl-phenols are listed as illustrated in Fig. 1. The antioxidative mechanism of alkyl-phenols (ArOH) depends on hydrogen atom transfer (HAT) and single electron transfer (SET) towards oxygen radicals ROO•, quenching further chain reactions<sup>9</sup>. These approved kerosene additives encompass the commodity chemical butylated hydroxytoluene (BHT), exhibiting superior antioxidative properties when compared to similar non-phenolic AOs<sup>9</sup>. Generally, radical scavenging rates of alkyl-phenols are superior to non-alkylated isomers due to positive inductive effects, stabilizing the AO after electron transfer<sup>9</sup>. Consequently, alkyl-phenols are widely used as antioxidants in drugs, cosmetics, food, and fuel<sup>9</sup>.

Although there is a large amount of natural antioxidants, none are used so far in jet fuels<sup>10,11</sup>. Partially, the required physicochemical properties of jet fuel additives exclude many biosynthetic alternatives, besides the extreme financial expense for ASTM approval. When considering biogenic antioxidants, most compound classes are excluded due to either their poor solubility in kerosene, high acidity or limited volatility (e.g. ascorbic acid, glutathione). Research on microalgae as carbon fixation platforms resulted in the detection of different natural antioxidants as documented in the literature<sup>12,13</sup>. Carotenoids are photosynthetically active and/or antioxidant molecules in plants, algae, and bacteria, where they constitute one of the most abundant and accessible antioxidant pigment classes<sup>14,15</sup>. Commercially, racemic carotenoids are generated by conventional chemical synthesis using petrochemical precursors<sup>16</sup>. Recently, sustainable microalgae processes based on either *Dunaliella sp.* or *Haematococcus sp.* as production organisms have been developed into industrial scale for production of enantiomerically pure carotenoids<sup>17,18</sup>. When exposed to osmotic stress, *Dunaliella* produces carotenoids, with reported relative concentrations reaching one to ten percent in dried

biomass<sup>19</sup>. This significant production yield could enable sustainable antioxidants production. When considering carotenoids as jet fuel antioxidants, their cleavage to more volatile products is required to comply with ASTM jet fuel specifications. To the best of our knowledge, volatile cleavage products of carotenoids have never been studied as antioxidants in jet fuel formulations. To that end, antioxidants based on aromatic carotenoid structures (Fig. 2A), resemble those of conventional alkyl-phenols (Fig. 1).



**Fig. 2** [A] Chemical structure of  $\beta$ -carotene, aromatic derivatives and its cleavage products<sup>20,21</sup>. For R = methyl; dihydroxy isorenieratene (DHIR) and the dihydroxy isorenieratene cleavage product (DHIRC\*) for R = hydrogen; synthetic aromatic carotenoid and cleavage products (Synchocoxanthin like cleavage product (SXLC\*) based on structural similarity to the microalgae aromatic carotenoid synchocoxanthin<sup>22</sup>. [B] Chemical structure of all tested jet fuel antioxidants. \*Purified synthetic material was used in all experiments.

Aromatic ring structures in carotenoids are rare and were first identified in a sea sponge by Yamaguchi *et al.* in 1958<sup>23</sup>. Isorenieratene, and its hydroxylated form (DHIR), have first been described and isolated from bacteria of the *Brevibacterium* genus originally isolated from cheese products<sup>24</sup>. Moreover, aromatic carotenoids are not limited to heterotrophic bacteria, but also occur naturally as synchocoxanthin in phototropic cyanobacteria of the *Synechococcus* genus<sup>22</sup>. Enzymatic cleavage of carotenoids with molecular oxygen as the oxidant results in the production of volatile flavor compounds in plants flower and fruit tissues<sup>25</sup>. Enzymatic cleavage of conventional and aromatic carotenoids has been shown *in vitro*, but yields remain marginal due to product inhibition and limited substrate solubility<sup>21,26,27</sup>. Most biotransformation reactions require a

chromatographic separation of the generated products, which in addition diminish its scalability<sup>21</sup>. In contrast, recent oxidative carotenoid cleavage experiments by chemical catalysis lack high regioselectivity and scalability<sup>20,28</sup>. Indeed, long term target of our investigation is the utilization of cleavage products from naturally derived aromatic carotenoids. For the initial tests, chemically synthesized products were used to assess the applicability of derived antioxidants. To comply with volatility specifications of jet fuel additives, this study evaluated possible aromatic carotenoid cleavage products of dihydroxy isorenieratene (DHIR) and synechoxanthin like carotenoids, obtained by a pure synthetic production approach. For the first time, this study presents the carotenoid cleavage inspired products **DHIRC** and **SXLC** as substitutes for alkyl-phenol based jet fuel additives. These compounds (Fig. 1B) were tested for their suitability, with the respective **BHT** and  **$\beta$ -ionone** controls, in combustion processes and for radical scavenging. Furthermore, toxicity in human epithelial cell lines was determined to ensure secure handling properties. The reported new evidence on semisynthetic alternatives to alkyl-phenol based antioxidants opens possible applications of these to jet fuel and beyond.

## Methods

### Chemical synthesis of aromatic carotenoid cleavage products

Pure compounds (E)-4-(4-hydroxy-2,3-dimethylphenyl)but-3-en-2-one (**DHIRC**) and (E)-4-(4-hydroxy-2,3,6-trimethylphenyl)but-3-en-2-one (**SXLC**) were obtained by chemical synthesis starting from commercially available building blocks by a new route improving a protocol of Yeung *et al.*<sup>29</sup>. Details are presented in the Experimental part.

Products were characterized by <sup>1</sup>H-NMR and <sup>13</sup>C-NMR on an Avance III HD 500 MHz NMR spectrometer from Bruker BioSpin equipped with a CryoProbe™ Prodigy broadband probe and by HR-MS on a Thermo Finnigan LTQ FT Ultra Fourier Transform Ion Cyclotron Resonance Mass Spectrometer. Purity of the samples was determined by HPLC on an Agilent Series 1100 HPLC system (Waldbronn, Germany).

### Direct radical scavenging of studied antioxidants

Comparative radical scavenging properties of **DHIRC** and **SXLC** including control **BHT** and  **$\beta$ -ionone** were analyzed using two independent stable radical assays.

**DPPH assay.** Conducted as previously published by Xie and Schaich with some modifications, according to Deng *et al.*<sup>30,31</sup>. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) stable radical reagent was solubilized in ethanol at 100  $\mu$ M. Dilution series were prepared from 10 mM stock solutions. For each triplicate stock

solution of  **$\beta$ -ionone**, **BHT**, **SXLC**, and **DHIRC** triplicate reactions were performed and measured upon addition of the stable radical reagent. 200  $\mu$ L of each stock solution (10 mM – 1 mM) and negative control was added to 24 well microtiter plates on ice. After synchronous addition of 1800  $\mu$ L DPPH reagent at 30 °C, the plates were immediately measured with a plate reader (EnSpire®, PerkinElmer, Inc., Waltham, USA) in 30-sec intervals at 517 nm for 20 min at 30 °C and once after 72 h at RT in a sealed plate.

**ABTS assay.** Conducted as previously published by Re *et al.* with some modifications<sup>32</sup>. For radical formation, an aqueous solution of 2,45 mM potassium persulfate (KPS) and 7 mM 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) was prepared and stirred for 24 h at RT in the dark. The formed ABTS•/ ABTS reagent solution was diluted with ethanol to a concentration of 85  $\mu$ M ABTS• and ABTS with a relative absorbance at 734 nm of 0,7. Dilution series by pipetting were prepared from 1 mM stock solutions. For each stock solution of  **$\beta$ -ionone**, **BHT**, **SXLC**, and **DHIRC** triplicate reactions were performed and measured upon addition of the stable radical reagent. 200  $\mu$ L of each stock solution (1 mM – 100  $\mu$ M) and negative control was added to 24 well microtiter plates on ice. After synchronous addition of 1800  $\mu$ L ABTS reagent, the plates were immediately measured with a plate reader (EnSpire®, PerkinElmer, Inc., Waltham, USA) in 30-sec intervals at 734 nm for 20 min at 30 °C and once after 72 h at RT in a sealed plate.

### Physicochemical properties of additive blends

Approval of new fuels and fuel additives is regulated by ASTM D 4054 which sets all required tests at four times the concentration being sought for approval. For antioxidants, a maximum concentration of 24 mg L<sup>-1</sup> is specified<sup>33</sup>. Back-to-back tests are performed on the additive blend (96 mg L<sup>-1</sup> antioxidant) and a neat control sample without additive, which provides a baseline for comparison<sup>34,35</sup>. A representative jet reference fuel close to the specifications given by ASTM D 4054 was chosen as a surrogate for kerosene-type fuel. The jet reference fuel was formulated by blending the following technical grade solvents in the respective volumes as shown in Table 1.

**Table 1** Jet reference fuel composition as applied in the experimental investigations. Antioxidant additives were added with a concentration of 96 mg L<sup>-1</sup> respectively.

Jet reference fuel (JRF) model formulation		
Type	Component	Volume %
Paraffins	Exxsol D40	38.02
	Exxsol D80	38.02
Aromatics	Aromatic 100	7.69
	Aromatic 150	15.37
Sulfur	tert-Butyl Disulfide	0.75
Mercaptan	Decanethiol	0.01
Fuel System Icing Inhibitor	DiEGME	0.15

Subsequently, kinematic viscosity, density and surface tension were determined in dependency of the temperature to investigate the physiochemical behavior of the additive blends with the synthesized antioxidants **DHIRC** and **SXLC** to the neat jet reference fuel in compliance to ASTM D4054<sup>33</sup> recommendations.

#### Influence of the novel antioxidants on combustion chemistry

Within the approval process of new fuels or additives, extensive engine tests are required to exclude disadvantageous effects on combustion<sup>36</sup>. To minimize the risk of this significant effort, we present a preliminary investigation of the influence of the antioxidative additives on the combustion processes in a model laboratory scale experiment. Measurements were performed at the DLR high temperature flow reactor with coupled molecular beam mass (MBMS) spectrometer. This system allows for a detailed observation of stable and radical combustion intermediates at ideal, pre-vaporized and premixed conditions. It consists of a high-temperature laminar flow reactor and a molecular beam mass spectrometer (MBMS) with time-of-flight detection (TOF) system. Species are measured at the reactor outlet for varying temperatures of the oven housing the reactor tube. A detailed description on this setup has been published previously<sup>36,37,38,39</sup>.

#### Viability assays of melanoma cell line MEL2

The toxicity of all antioxidants studied was evaluated by viability assays of melanoma cell line SK-MEL-28 upon treatment with these. SK-MEL-28 cells were grown in DMEM medium substituted with 10% FBS at 37 °C and 5% CO<sub>2</sub>. For the viability assays, 5x 10<sup>3</sup> cells per well were seeded in technical triplicates in a 96 well plate. The initial growth medium was changed one day after seeding to a growth medium containing Green Cytotoxicity reagent (EssensBioscience). Next, cells were treated with a 9-point dilution series with each corresponding

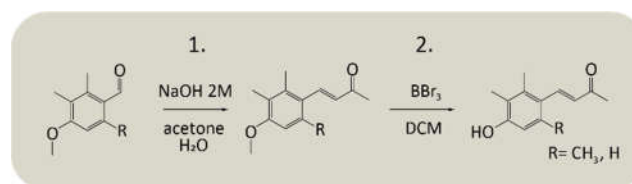
substance (30 μM – 3 nM). Cells were then incubated for four days and viability was estimated by measuring confluence and green fluorescence every 2 h in an IncuCyte® device.

## Results and discussion

### Chemical synthesis of the aromatic carotenoid cleavage products

The two proposed cleavage products of aromatic carotenoids were synthesized from commercially available aldehyde building blocks. As the synthesized compounds are derived from carotenoid compounds we abbreviated them accordingly and the compounds are referred as such for all further results and discussions: (*E*)-4-(4-hydroxy-2,3,6-trimethylphenyl)but-3-en-2-one as **DiHydroxy-IsoRenieratene (DHIR)** Cleavage product (**DHIRC**) and (*E*)-4-(4-hydroxy-2,3-dimethylphenyl)but-3-en-2-one as **SynechoXanthin Like Cleavage product (SXLC)**.

The two-step synthesis (Fig. 3) consists of a Claisen-Schmidt aldol condensation of a methoxy-protected phenolic aldehyde with acetone and subsequent *O*-demethylation with BBr<sub>3</sub>.



**Fig. 3** Chemical synthesis route to **DHIRC** (R = CH<sub>3</sub>) and **SXLC** (R = H).

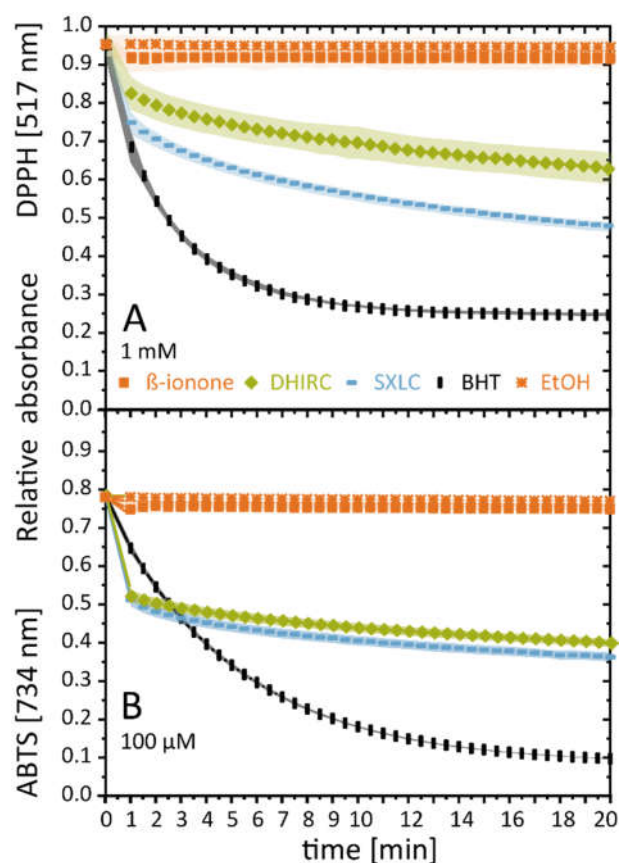
### Anti-radical activity of carotenoid cleavage product based antioxidants

The DPPH assay is widely used for mixed mode radical scavenging experiments, featuring fast single electron and slower hydrogen transfer reactivity. To measure radical scavenging of the novel jet fuel antioxidants, **DHIRC** and **SXLC** were compared to non-phenolic control **β-ionone** and certified alkyl-phenol **BHT**.

**β-ionone**, the cleavage product of the potent natural antioxidant β-carotene, was initially evaluated for its radical scavenging capacity. To that end, no radical scavenging activity even over a timeframe of a couple of hours was observed for **β-ionone**. **DHIRC** demonstrated an initial fast reaction with the stable radicals and slow further scavenging as depicted in Fig. 4. This behavior suggests a bi-mechanistic reaction, possibly a mixture of SET and HAT. The data indicates furthermore that **DHIRC** is a superior antioxidant to the non-phenolic β-carotene cleavage products. Hence, the presented results on radical scavenging indicate significant antioxidative properties of **DHIRC**, suggesting stabilizing effects on jet fuel and other oxidation susceptible products. Nevertheless, the currently certified alkyl-phenol **BHT** remains superior regarding radical

scavenging rates observed in both assays (Fig. 4A and B). Equivalent data for **SXLC** indicates that this compound has similar radical scavenging properties to **DHIRC**, while being slightly more active in the selected assays. One reason for this slightly higher antioxidant activity may be the different methyl substitution pattern of the two compounds under investigation. In that regard **SXLC** is less sterically hindered.

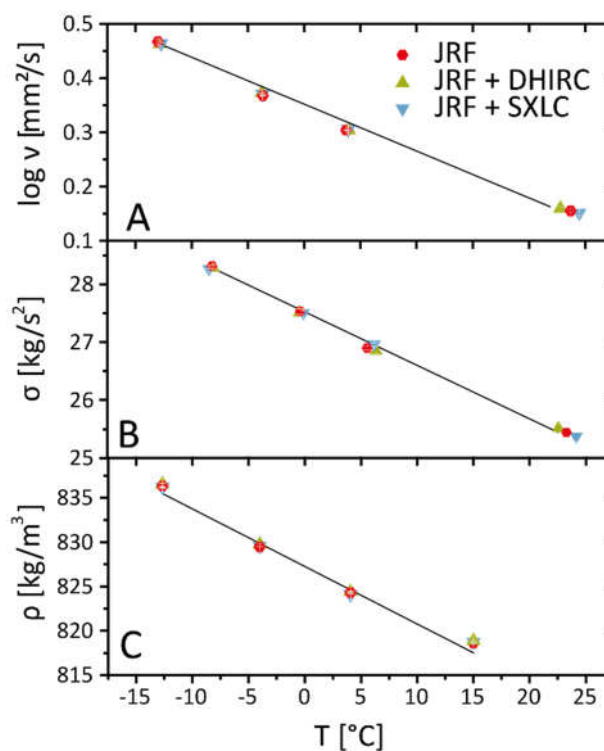
Whether **SXLC** or **DHIRC** will comply with long term storage stability requirements of jet fuel remains to be investigated in a more comprehensive study and during possible certification processes. Interestingly, recent publications on AOs in emulsions suggest a higher product stability when more hydrophilic antioxidants are added to water in oil emulsions<sup>9</sup>. This could also hold true for jet fuels under long term storage conditions, when the more hydrophilic AOs like **DHIRC** are added. Alternatively, there are means to broaden the portfolio of available cleavage products. **SXLC** is one example of a possible semi-synthetic approach, which could include various chemical or biochemical functionalization's to fulfill ASTM D4625 long term stability target specification.



**Fig. 4** Spectrophotometric ABTS and DPPH radical absorption quenching curves with labels according to the analyzed antioxidant candidate. [A] DPPH at compound concentrations of 1 mM. [B] ABTS radical scavenging at 100  $\mu$ M. SD+ are represented by the underlying colored surface. Additional measurements on various concentrations are appended in the Electronic Supplementary Information (see ESI<sup>†</sup>).

### Physicochemical properties of aromatic carotenoid cleavage products additive blends

The range of the kinematic viscosity, density and surface tension for the jet reference fuel (JRF) are in accordance with the approved fuels listed in the world fuel sampling program of the CRC (Coordinating Research Council) aviation committee and the CRC handbook of aviation fuel properties, making the JRF an excellent candidate for back-to-back tests with the additive by providing a baseline for data comparison<sup>34,35</sup>. Typical temperature-dependency was displayed by both additive blends and the neat jet reference fuel for kinematic viscosity (Fig. 5A), surface tension (Fig. 5B) and density (Fig. 5C). For the JRF blend with **SXLC** minor solubility issues arose at low temperatures, which could be met by ultrasonic resuspension. Note that kinematic viscosities are usually denoted in a logarithmic scale.

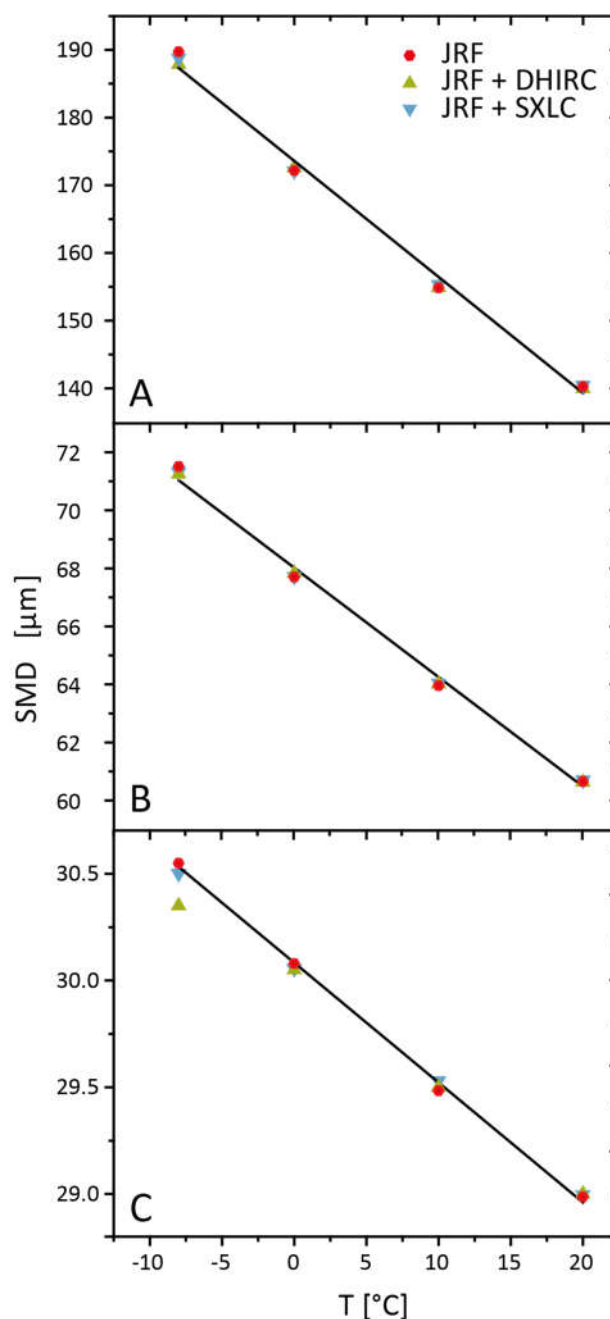


**Fig. 5** Temperature dependent physical properties of the neat jet reference fuel (JRF) and the additive blends with **DHIRC** and **SXLC**: [A] kinematic viscosity (logarithmic scale); [B] surface tension; [C] density.

No significant effects of the antioxidative additives on the jet reference fuel's physical properties could be observed. Minuscule deviations of the measured data points of the different additive blends and the jet reference fuel are within the error margins. This tallying performance, however, should be considered advantageous, since an alteration of physical properties upon addition of an additive could create problems concerning jet engine operation. Any deviation would raise the question of batch reproducibility and in worst case placing blends outside the required fuel specifications.



Spray characteristics and evaporation rates are crucial fuel parameters for their combustion behaviour. To investigate the influence of the regarded antioxidants on the atomization of the jet reference fuel, the Sauter Mean Diameter (SMD) for three typical aero-engine injection principles, namely plain orifice (Fig. 6 A), pressure-swirl (Fig. 6 B) and pre-filming airblast (Fig. 6 C) atomization was determined. The SMD, reflecting the volume to surface ratio of a spray, is an important characteristic diameter for the fuel placement and vaporization<sup>40</sup>. In plain-orifice atomizers, the fuel is introduced into the combustion chamber via a small orifice by means of a pressure differential<sup>41</sup>. In pressure-swirl atomizers, in addition to the pressure differential a swirl is added causing a widening of the spray<sup>40</sup>. The third investigated atomization principle is the so-called pre-filming airblast atomization, where a thin fuel film is spread on a surface<sup>42</sup>. This film is subsequently torn apart by the shear forces resulting from high-velocity air streams above the film surface. For the correlations used to calculate the SMD for the individual atomization principles, the reader is referred to the Electronic Supplementary Information (see ESI<sup>†</sup>). As can be seen from Fig. 6, the effect of the change in physical properties due to the antioxidative additives on atomization is marginal for all three investigated fuel injection principles. The differences are negligible compared to the uncertainties of the empirical correlations and the underlying measurement of the physical properties.

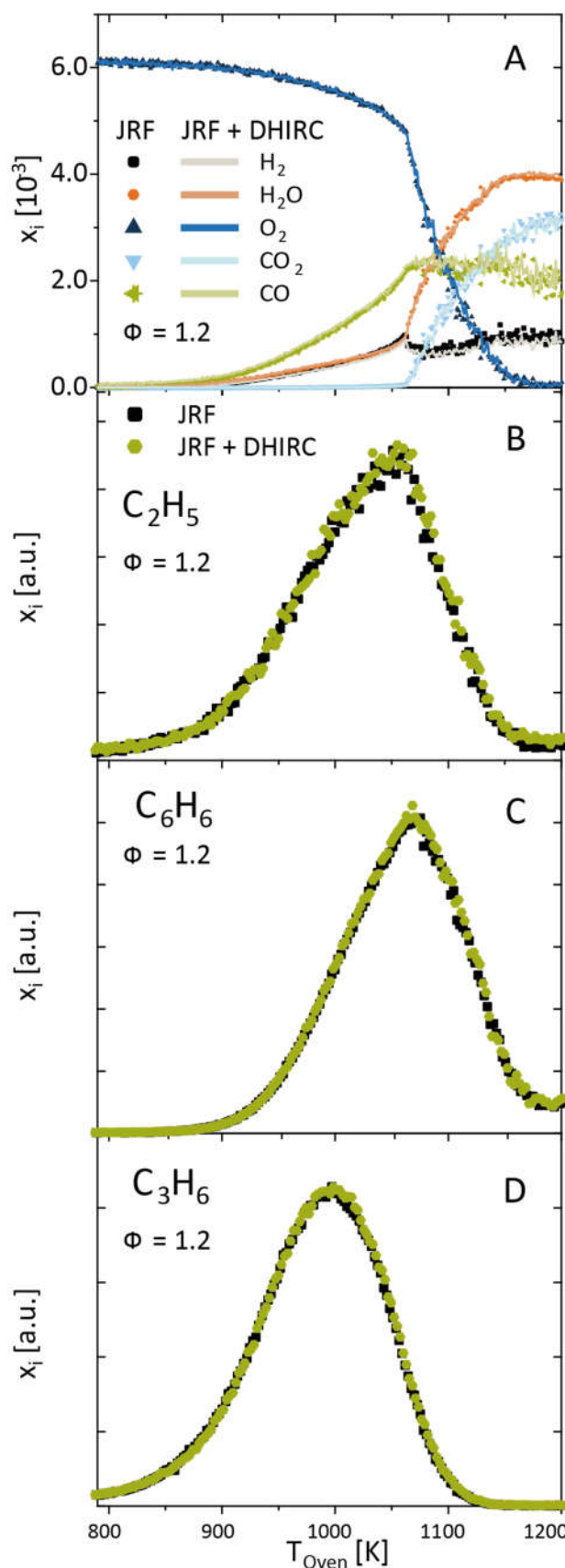


**Fig. 6** Sauter Mean Diameter (SMD) of the neat jet reference fuel (JRF) and the additive blends with **DHIRC** and **SXLC**: [A] plain orifice; [B] pressure swirl; [C] pre-filming airblast

### Jet fuel combustion chemistry of aromatic carotenoid cleavage products additives

Combustion is driven by radical chain reactions. Therefore, the presence of an antioxidant, i.e. a radical scavenger, could have a significant impact on the reaction network. Combustion was carried out at fuel rich conditions ( $\Phi = 1.2$ ) to cover the full reaction regime of the soot precursor chemistry and to determine even slight concentration shifts. Results from the DLR high temperature flow reactor are presented in Fig. 7. For this work the jet reference fuel blend with **DHIRC** (Fig. 7 A

lines) was chosen in comparison to the neat jet reference fuel (Fig. 7 A symbols). The measured mole fraction profiles of the major species in combustion are plotted versus the oven temperature (Fig. 7A), which can be interpreted as the reaction progress and detailed kinetic modelling is feasible<sup>43,44</sup>. At low temperatures, the reactants pass the reactor without changes. With increasing temperature first reactions occur within the residence time of the reactor and first intermediates are formed. At the same time a small amount of oxygen is consumed. Increasing temperature accelerates the reaction network and more intermediates as well as CO and H<sub>2</sub> are formed. Once a certain level of radicals is reached, chain branching reactions exceed the chain termination reactions and fast conversion towards the stable, non-radical products can be observed. In this slightly fuel rich environment these products are H<sub>2</sub> and CO beside H<sub>2</sub>O and CO<sub>2</sub>. The neat jet reference fuel and the jet reference fuel blend with **DHIRC** show no deviation regarding the concentration of the major species, nor on the profile shape, i.e. the temperature where conversion occurs.



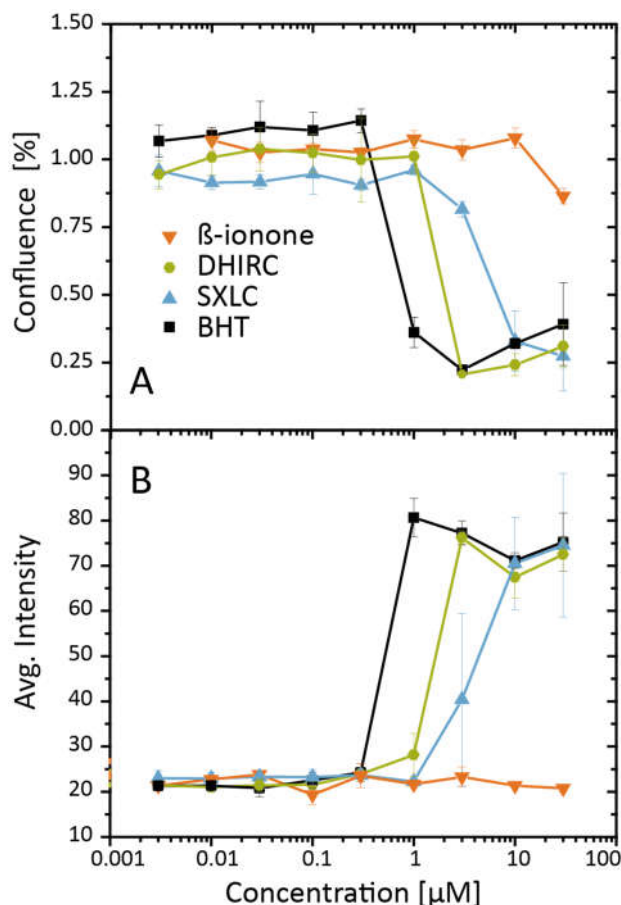
**Fig. 7** Combustion chemistry of neat JRF and JRF + **DHIRC** blend: [A] Mole fraction profiles for major combustion products; [B] Mole fraction profiles for ethyl radical; [C] Mole fraction profiles for typical soot precursor benzene; [D] Mole fraction profile for typical combustion intermediate propene

Since **DHIRC** is investigated for its antioxidative potential, the ethyl radical  $C_2H_5$  formed as combustion intermediate was investigated (Fig. 7B) as an example for a typical radical present at combustion<sup>45</sup>. No significant effect on the mole fraction profile could be observed for the addition of **DHIRC**; however, it has to be considered that the added antioxidants aim at preventing autoxidation within the fuel and the subsequent formation of insoluble oxidation products and non-typical radical species during combustion. Benzene was monitored, as it is a typical aromatic soot precursor formed in hydrocarbon combustion and studied for an estimation of the pollutant formation potential<sup>46</sup>. No deviation between the benzene profiles of the jet reference fuel and the blend could be observed (Fig. 7C). Finally, propene was investigated as a representative stable combustion intermediate. In accordance with the major combustion products, no deviation between the mole profiles could be observed (Fig. 7D).

In summary, no significant deviation of the combustion intermediate species pool is caused by the addition of **DHIRC** to the neat jet reference fuel. Therefore, an impact on the combustion in technical combustors by **DHIRC** can be considered as unlikely. Hence, an unaltered soot tendency and typical flame radical formation during combustion should be considered advantageous, since an alteration of the global combustion chemistry could lead to divergent reaction kinetics and soot formation potential, which would have to be investigated closely prior to regulatory approval procedures for each antioxidant additive. Since **DHIRC** and **SXLC** only differ in a methyl group at the benzene ring in their chemical structure and a single methyl unit most likely should not alter the overall combustion chemistry, only **DHIRC** was studied for combustion influence.

#### Comparative toxicity towards human melanoma cell lines

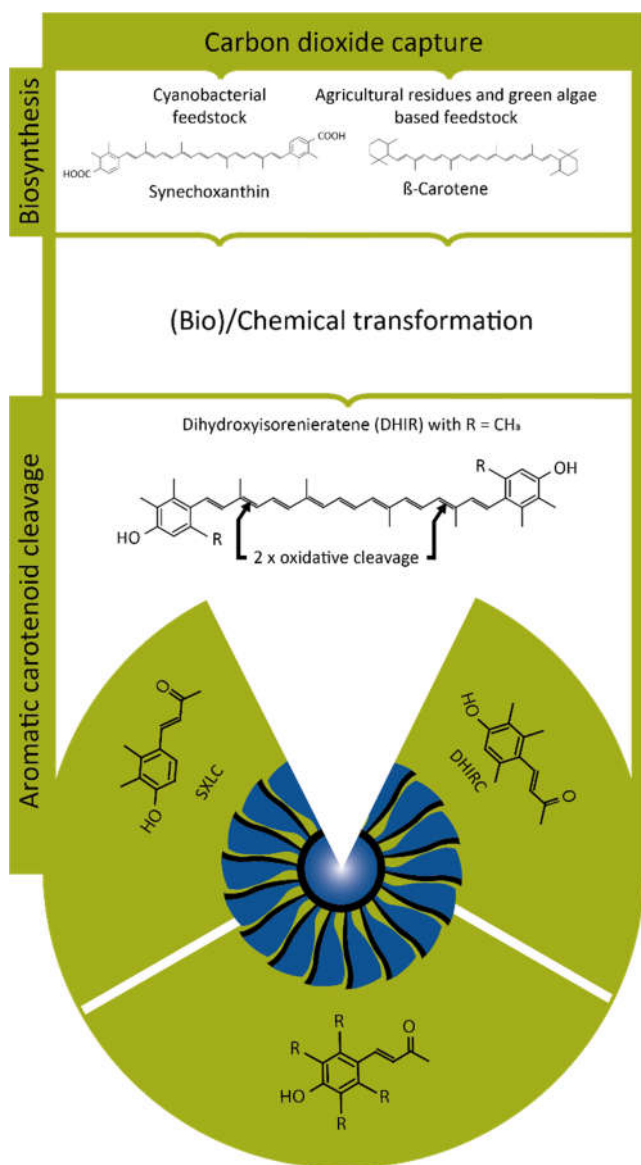
Toxicity of highly concentrated additive blends does impose health risk for professionals and humans in direct contact to jet fuels<sup>47</sup>. Considering environmental safety, the aviation industry is taking all possible actions to reduce the toxicity footprints. One prominent example for these actions is the exclusion of tri-tert-butylphenol (TTBP) from the widely used ETHANOX<sup>®</sup> formulations in favor of environmental protection<sup>48,49</sup>. The carotenoid cleavage products **DHIRC** and **SXLC** did show no increased apoptosis induction (Fig. 8A and B) when compared to the certified product **BHT**, thereby neglecting more toxic alkyl-phenol variants included in Fig. 1. Comparative images of the treated cell cultures can be found in the Electronic Supplementary Information (see ESI<sup>†</sup>).



**Fig. 8** Viability curves after treatment of melanoma cells with the studied antioxidants **DHIRC**, **SXLC**, **BHT** and **β-ionone**. [A] shows reduced cell confluence when exhibited to AO concentrations in the µM scale. Also apoptosis based on staining results in [B] increased when reaching µM concentration ranges for **SXLC**, **DHIRC** and **BHT**. With **β-ionone** showing no response in concentrations up to 30 µM.

#### Natural source and cleavage of aromatic carotenoid-based antioxidants precursors

The introduction of carotenoid cleavage product based antioxidants as additives for jet fuel open an additional field of utilization for algae and other biomass resources. The analysis of β-carotene derived, non-aromatic, cleavage products like β-ionone (Fig. 2) showed limited suitability, as no antioxidative activity was measured in DPPH and ABTS radical scavenging assays, leading to the investigation of structurally similar compounds derived from aromatic carotenoids.



**Fig. 9** Overview of possible natural sources of aromatic carotenoid cleavage products as antioxidants for jet fuel.

These steric defined  $\Phi,\Phi$  and  $\chi,\chi$  carotenoids are exclusive to a limited number of species, in particular bacteria. Wild type bacterial systems synthesize the proposed aromatic substrate molecules autotrophically, by the second most abundant microalgae *Synechococcus elongates*, and heterotrophically, by *Brevibacterium linens*<sup>22,24</sup>. The latter is known for its utilization in cheese production. Carotenoid extracts of *Brevibacterium linens* contain the precursor DHIR, as shown in the aromatic carotenoid cleavage section of Fig. 9 (with R = CH<sub>3</sub>), which can be cleaved in  $\mu\text{g}$ – $\text{mg}$  scale enzymatically by MtCCDs as shown by Scherzinger *et al.* or chemically by unspecific oxidation to **DHIRC**<sup>20,50</sup>. The efficient cleavage of DHIR by chemical or industrial biotechnological processes remains the largest research hurdle before predictions on the scalability of the production can be made. Synechocanthin is an aromatic carotenoid detected in a highly abundant marine and fresh

water research model microalga genus *Synechococcus*<sup>51</sup>. On this basis, further research work can be carried out on the synthesis of aromatic carotenoid molecules with microalgae. Although, requiring a multistep chemical or biochemical transformation to yield the volatile synthetic precursor of Synechocanthin like cleavage product (**SXLC**), the hypothetical aromatic cleavage product was included to show the feasibility of alternative candidate compounds. These expansions to the native natural pathway repertoire are addressable with synthetic biotechnology approaches. The so far studied biochemical pathways towards the precursor dihydroxy isorenieratene (DHIR) all start from  $\beta$ -carotene with a two-step catalysis, requiring the carotene desaturase/methylase CrtU and cytochrome P450 only<sup>52</sup>. Thus, making such processes, as shown in Fig. 9, seems conceivable.

## Conclusion

Data presented in this study suggest that the aromatic carotenoid cleavage products **DHIRC** and **SXLC** have good chances to fulfil the high demands of jet fuel regulations. The conducted measurements indicate that the compounds have a benign influence on operations of jet engines and fuel handling. The application of natural product derived antioxidants could contribute to the reduction of “non-renewable hydrocarbon consumption” and possibly be a more tolerable alternative, due to their biosynthetic origin. The identification of further photosynthetic microorganisms capable of producing aromatic carotenoids, by screening and mutagenesis, could enable simple substrate harvesting processes. Catalysis research will address the selective cleavage of the natural carotenoid substrates by follow-up projects on the petrochemical free production of renewable and biogenic antioxidants like **DHIRC** and **SXLC**.

## Experimental

### Synthesis of aromatic carotenoid cleavage products

Starting material 4-methoxy-2,3-dimethylbenzaldehyde was purchased from TCI Germany ( Eschborn, Germany) and 4-methoxy-2,3,6-trimethylbenzaldehyde from Sigma Aldrich (Merck Group, Darmstadt, Germany). All solvents used were of HPLC grade purity.

**Step 1: Claisen condensation.** Acetone (15 mL) and H<sub>2</sub>O (5 mL) were added to the aromatic aldehyde (5.5 mmol). In an ice bath, 2M NaOH (3.0 mL) was added to the mixture and stirred for 30 min. Then the ice bath was removed and the reaction mixture was stirred overnight at RT. The mixture was acidified to pH 5 with AcOH and the solvent removed *in vacuo*. The residue was dissolved in dichloromethane (DCM) (25 mL), the solution washed with sat. NaHCO<sub>3</sub> solution, dried (MgSO<sub>4</sub>) and the solvent removed *in vacuo*. A silica gel column chromatography using a 0 to 25% ethyl acetate in isohexane

gradient was performed to obtain the desired unsaturated ketones.

**Step 2: Ether cleavage.** Dry DCM (25 mL) was cooled to  $-78\text{ }^{\circ}\text{C}$  and the respective methoxy intermediate (4.12 mmol) was added. Then 12.4 mL of  $\text{BBr}_3$  (1M in DCM) was added dropwise under stirring. The reaction mixture was allowed to reach  $10\text{ }^{\circ}\text{C}$  and stirred over night at  $10\text{ }^{\circ}\text{C}$ . After addition of water (25 mL) and extraction with ethyl acetate (3 x 20 mL), the combined organic layers were dried ( $\text{MgSO}_4$ ) and evaporated to dryness. A silica gel column chromatography, using a 0 to 20% ethyl acetate in isohexane gradient was performed to obtain the phenolic products.

**SXLC** was obtained in 61% yield over both steps and appeared as pale yellow crystals, with no distinct smell and a melting point of  $182\text{ }^{\circ}\text{C}$ .  $^1\text{H-NMR}$  (500 MHz,  $\text{DMSO-d}_6$ ):  $\delta$  (ppm) = 9.80 (s, 1H), 7.83 (d,  $J = 16.0\text{ Hz}$ , 1H), 7.42 (d,  $J = 8.5\text{ Hz}$ , 1H), 6.72 (d,  $J = 8.5\text{ Hz}$ , 1H), 6.52 (d,  $J = 16.1\text{ Hz}$ , 1H), 2.31 (s, 3H), 2.30 (s, 3H), 2.10 (s, 3H);  $^{13}\text{C-NMR}$  (126 MHz,  $\text{DMSO-d}_6$ ):  $\delta$  (ppm) = 198.2, 157.8, 142.0, 138.7, 125.8, 125.6, 124.6, 123.4, 113.4, 27.9, 15.8, 12.4, (see ESI<sup>+</sup>); HR-MS (ESI): calculated for  $\text{C}_{12}\text{H}_{13}\text{O}_2^-$  [M-H]<sup>-</sup>: 189.0921, found: 189.0919; purity (HPLC, 210 and 254 nm): >99%.

**DHIRC** was obtained in 38% yield over both steps and appeared as pale greenish-yellow crystals, with a slight turpentine oil-like smell and a melting point of  $103\text{ }^{\circ}\text{C}$ .  $^1\text{H-NMR}$  (500 MHz,  $\text{DMSO-d}_6$ ):  $\delta$  (ppm) = 9.44 (s, 1H), 7.67 (d,  $J = 16.6\text{ Hz}$ , 1H), 6.57 (s, 1H), 6.16 (d,  $J = 16.5\text{ Hz}$ , 1H), 2.32 (s, 3H), 2.21 (s, 3H), 2.19 (s, 3H), 2.04 (s, 3H);  $^{13}\text{C-NMR}$  (126 MHz,  $\text{DMSO-d}_6$ ):  $\delta$  (ppm) = 197.9, 155.3, 142.5, 136.7, 134.5, 131.5, 124.8, 120.6, 114.5, 27.2, 20.9, 17.2, 11.8, (see ESI<sup>+</sup>); HR-MS (ESI): calculated for  $\text{C}_{13}\text{H}_{15}\text{O}_2^-$  [M-H]<sup>-</sup>: 203.1078, found: 203.1075; purity (HPLC, 210 and 254 nm): >99%.

### Radical scavenging of Antioxidants

Chemicals and reagents were purchased from Sigma Aldrich (Merck Group, Darmstadt, Germany) in analytical grade. All compounds were stored at  $-80\text{ }^{\circ}\text{C}$  under nitrogen atmosphere. Stock solutions were prepared gravimetrically in triplicates at  $25\text{ }^{\circ}\text{C}$  using a cubis<sup>®</sup> analytical balance (Sartorius AG, Göttingen, Germany).

### Chemicals for jet reference fuel

Chemicals were purchased from the following distributors and utilized without further purifications: Exxsol D40 (Exxon Mobil, Irving, USA), Exxsol D80 (Exxon Mobil), Aromatic 100 (Kraft), Aromatic 150 (Kraft), di-tert-butyl disulfide 97% (Sigma Aldrich), 1-decanethiol 99% (Sigma Aldrich), DiEGME 99% (Sigma Aldrich). Volume concentrations have been adjusted for the formulation of the jet reference fuel.

### DLR high temperature flow reactor

The reactor features an alumina ( $\text{Al}_2\text{O}_3$ ) ceramic tube (40 mm inner diameter, total length of 1497 mm), where premixed laminar flowing gases are fed highly diluted ( $\sim 99\%$  Ar) into the reactor. High dilution suppresses heat release and a self-sustaining reaction. Boundary effects are minimized by the reactor design with dimensions including relatively large inner diameter. The vaporizing system is a commercial setup (Bronkhorst, CEM) with a pneumatically driven fuel supply equipped with a Coriolis flow meter (Bronkhorst, Mini Cori-Flow M12). All input streams were metered in high precision (accuracy  $\pm 0.5\%$ ) by Coriolis mass flow meters. Complete evaporation was ensured by the small fuel fraction and the low partial pressure (typically below 100 Pa) needed. The premixed gases were fed into the reactor by a tempered flange equipped with a porous bronze plug to create homogeneous flow conditions. The reaction segment has a total length of 1,000 mm, heated by a customized high temperature oven (Gero, Type HTRH 40–1000) capable of temperatures up to 1900 K. Gases were sampled at the reactor exit, transferred to high vacuum ( $10^{-4}$  Pa) by a two-stage differential pumping system and finally detected by an electron impact (EI) time-of-flight (TOF) mass spectrometer (Kaesdorf, mass resolution  $R = 3,000$ ). To avoid species fragmentation at the ionization process, soft electron energies were applied. A quadrupole mass spectrometer was placed in the ionization chamber and operated at a higher electron energy (70 eV) allowing for tracking of major species simultaneously to the MBMS-TOF measurements.

### Physical properties of additives

Low temperature measurements were performed in an ethanol equipped cryostat connected to an IKA RC2 basic circulation thermostat (IKA<sup>®</sup>-Werke GmbH & Co. KG, Staufen, Germany); temperature range:  $-25\text{ }^{\circ}\text{C}$  to  $35\text{ }^{\circ}\text{C}$ ; temperature stability:  $\pm 0.1\text{ }^{\circ}\text{C}$ . Kinematic viscosities were measured with an Ubbelohde type I viscometer by Lauda (Lauda Dr. R. Wobser GmbH & Co. KG, Lauda-Königshofen, Germany) (accuracy:  $\pm 0.8\%$ ). Densities were determined by underfloor weighing with a high precision scale by Mettler Toledo (linearity:  $\pm 0.1\text{ mg}$ ). Surface tensions were measured with the Nouy ring method on an automatic TD 4 tensiometer by Lauda (surface tension resolution:  $0.01\text{ Nm m}^{-1}$ ).

### Viability assays

SK-MEL-28 cells were grown in DMEM medium substituted with 10% FBS at  $37\text{ }^{\circ}\text{C}$  and 5%  $\text{CO}_2$ . For the viability assays  $5 \times 10^3$  cells were seeded in 100  $\mu\text{L}$  medium per well in a 96 well plate. For each compound a 9-point dilution series in technical triplicates was performed. Following seeding, the medium was changed the next day to 100  $\mu\text{L}$  medium with Green Cytotoxicity reagent (Sartorius AG, Göttingen, Germany, 5  $\mu\text{L}$  of reagent diluted in 14 mL of medium) and cells were treated with a 50  $\mu\text{L}$  compound in medium dilution series (prepared from stock solutions in DMSO; final concentrations in well ranging from 30  $\mu\text{M}$  – 3 nM and 0.1%

DMSO). Confluence and green fluorescence intensity (indicator of apoptosis) were measured every 2 h in the IncuCyte® device (Sartorius AG, Göttingen, Germany) for three days. For the EC<sub>50</sub> estimation, confluence and cytotoxicity were analysed 16 h and 50 h after treatment. After the three-day measurement series, the analysis time was selected based on the best dynamic range of the readout parameters.

### Conflicts of interest

The authors declare no conflict of interests.

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### Notes and references

- 1 A. W. Schäfer, S. R. H. Barrett, K. Doyme, L. M. Dray, A. R. Gnadt, R. Self, A. O'Sullivan, A. P. Synodinos and A. J. Torija, *Nat. Energy*, 2019, **4**, 160–166.
- 2 S. P. Heneghan, D. L. Geiger, S. D. Anderson and W. D. Schulz, *J. Propuls. Power*, 1993, **9**, 1–9.
- 3 W. J. Balster and E. G. Jones, *J. Eng. Gas Turbines Power*, 1998, **120**, 289–293.
- 4 M. Odziemkowska, J. Czarnocka and K. Wawryniuk, in *Improvement Trends for Internal Combustion Engines*, eds. B. A. Ceper and Melih Yıldız, InTech, Rijeka, 1st edn., 2018, pp. 1–17.
- 5 ASTM D1655-16a, *Standard Specification for Aviation Turbine Fuels*, ASTM International, West Conshohocken, PA, 2016.
- 6 R. E. Morris, R. N. Hazlett and C. Linden McLvaine, *Ind. Eng. Chem. Res.*, 1988, **27**, 1524–1528.
- 7 T. Edwards and S. Zabarnick, *Ind. Eng. Chem. Res.*, 1993, **32**, 3117–3122.
- 8 H. A. Conley, D. L. Pumphrey, T. M. Toland and M. David, *Arctic economics in the 21st century, the benefits and costs of cold. A report of the CSIS Europe Program.*, 2013.
- 9 W. A. Yehye, N. A. Rahman, A. Ariffin, S. B. Abd Hamid, A. A. Alhadi, F. A. Kadir and M. Yaeghoobi, *Eur. J. Med. Chem.*, 2015, **101**, 295–312.
- 10 C. J. Chuck and J. Donnelly, *Appl. Energy*, 2014, **118**, 83–91.
- 11 N. Yilmaz and A. Atmanli, *Energy*, 2017, **140**, 1378–1386.
- 12 S. Davinelli, M. E. Nielsen and G. Scapagnini, *Nutrients*, 2018, **10**, 1–12.
- 13 A. C. Guedes, H. M. Amaro and F. X. Malcata, *Mar. Drugs*, 2011, **9**, 625–644.
- 14 W. Stahl and H. Sies, *Mol. Aspects Med.*, 2003, **24**, 345–51.
- 15 Y. M. A. Naguib, *J. Agric. Food Chem.*, 2000, **48**, 1150–1154.
- 16 B. D. Ribeiro, D. W. Barreto and M. A. Z. Coelho, *Food Bioprocess Technol.*, 2011, **4**, 693–701.
- 17 S. Boussiba, *Physiol. Plant.*, 2000, **108**, 111–117.
- 18 Y. Xu and P. J. Harvey, *Antioxidants*, 2019, **8**, 1–14.
- 19 M. A. Borowitzka, L. J. Borowitzka and D. Kessly, *J. Appl. Phycol.*, 1990, **2**, 111–119.
- 20 C. Caris-Veyrat, M. J. Amiot, R. Ramasseul and J. C. Marchon, *New J. Chem.*, 2001, **25**, 203–206.
- 21 C. Nacke, S. Hüttmann, M. M. W. Etschmann and J. Schrader, *J. Ind. Microbiol. Biotechnol.*, 2012, **39**, 1771–1778.
- 22 J. E. Graham, J. T. J. Lecomte and D. A. Bryant, *J. Nat. Prod.*, 2008, **71**, 1647–1650.
- 23 M. Yamaguchi, *Bull. Chem. Soc. Jpn.*, 1958, **31**, 739–742.
- 24 H. D. Martin, S. Kock, R. Scherrers, K. Lutter, T. Wagener, C. Hundsdörfer, S. Frixel, K. Schaper, H. Ernst, W. Schrader, H. Görner and W. Stahl, *Angew. Chem. - Int. Ed.*, 2009, **48**, 400–403.
- 25 P. J. Harrison and T. D. H. Bugg, *Arch. Biochem. Biophys.*, 2014, **544**, 105–111.
- 26 D. Scherzinger, E. Scheffer, C. Bär, H. Ernst and S. Al-Babili, *FEBS J.*, 2010, **277**, 4662–4673.
- 27 M. Schilling, PhD thesis, Technical University of Munich, 2008.
- 28 C. M. de J. Benevides, M. C. da C. Veloso, P. A. de Paula Pereira and J. B. de Andrade, *Food Chem.*, 2011, **126**, 927–934.
- 29 L. Yeung, L. I. Pilkington, M. M. Cadelis, B. R. Copp and D. Barker, *Beilstein J. Org. Chem.*, 2015, **11**, 1991–1996.
- 30 L. L. Deng, M. Taxipalati, F. Que and H. Zhang, *Sci. Rep.*, 2016, **6**, 38–160.
- 31 J. Xie and K. M. Schaich, *J. Agric. Food Chem.*, 2014, **62**, 4251–4260.
- 32 R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans, *Free Radic. Biol. Med.*, 1999, **26**, 1231–1237.
- 33 ASTM D4054-16, *Standard Practice for Qualification and Approval of New Aviation Turbine Fuels and Fuel Additives*, ASTM International, West Conshohocken, PA, 2016.
- 34 J. Hadaller, O.; Johnson, *World fuel sampling program. Coordinating Research Council, Inc., CRC Report 2006, (647)*, .
- 35 *Handbook of Aviation Fuel Properties. Coordinating Research Council, CRC Report 2004, (635)*, .
- 36 S. Jürgens, P. Oßwald, M. Selinsek, P. Piermartini, J. Schwab, P. Pfeifer, U. Bauder, S. Ruoff, B. Rauch and M. Köhler, *Fuel Process. Technol.*, 2019, **193**, 232–243.
- 37 P. Oßwald and M. Köhler, *Rev. Sci. Instrum.*, 2015, **86**, 105109.
- 38 M. Köhler, P. Oßwald, D. Krueger and R. Whitside, *J. Vis. Exp.*, 2018, DOI:10.3791/56965.
- 39 P. Oßwald, R. Whitside, J. Schäffer and M. Köhler, *Fuel*, 2017, **187**, 43–50.
- 40 A. H. Lefebvre, *Atomization and sprays*, Hemisphere Publishing Corporation, Taylor & Francis Inc., 1989.
- 41 M. M. Elkotb, *Prog. Energy Combust. Sci.*, 1982, **8**, 61–91.
- 42 A. H. Lefebvre, *Prog. Energy Combust. Sci.*, 1980, **6**, 233–261.
- 43 T. Kathrotia, C. Naumann, P. Oßwald, M. Köhler and U. Riedel, *Combust. Flame*, 2017, **179**, 172–184.
- 44 T.-C. Chu, Z. J. Buras, P. Oßwald, M. Liu, M. J. Goldman and W. H. Green, *Phys. Chem. Chem. Phys.*, 2019, **21**, 813–832.
- 45 F. L. Dryer, *Proc. Combust. Inst.*, 2015, **35**, 117–144.
- 46 T. Bierkandt, P. Hemberger, P. Oßwald, M. Köhler and T. Kasper, *Proc. Combust. Inst.*, 2017, **36**, 1223–1232.

- 47 C. Li, X. Cui, Y. Chen, C. Liao and L. Q. Ma, *Environ. Res.*, 2019, **169**, 308–314.
- 48 SI Group, SI Group launches new fuel antioxidant blend ETHANOX® 4737R, <https://www.siigroup.com/pressrelease.asp?ArticleID=2262>, (accessed 13 June 2019).
- 49 E. Pérez-Albaladejo, S. Lacorte and C. Porte, *Toxicol. Sci.*, 2019, **167**, 336–346.
- 50 D. Scherzinger, E. Scheffer, C. Bär, H. Ernst and S. Al-Babili, *FEBS J.*, 2010, **277**, 4662–4673.
- 51 J. E. Graham and D. A. Bryant, *J. Bacteriol.*, 2008, **190**, 7966–7974.
- 52 L. Dufossé and M. C. De Echanove, *Food Res. Int.*, 2005, **38**, 967–973.