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RESEARCH ARTICLE

Interlaboratory study on lipid oxidation during accelerated storage trials with rapeseed and sunflower oil analyzed by conjugated dienes as primary oxidation products

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Abstract

Accelerated storage tests are frequently used to assess the oxidative stability of foods and related systems due to its reproducibility. Various methods and experimental conditions are used to measure lipid oxidation. Differences between laboratories make it necessary to determine the repeatability and reproducibility of oxidation tests performed under the same conditions. The objective of the present interlaboratory study was to evaluate the outcome of a storage test for two different bulk oils, sunflower oil (SFO) and rapeseed oil (RSO), during a period of 9 weeks at 20°C, 30°C, 40°C, and 60°C. Sixteen laboratories were provided with bottled oils and conducted the storage tests according to a detailed protocol. Lipid oxidation was monitored by the formation of conjugated dienes (CD) and the activation energy (E_a) was determined for comparative purposes and statistically evaluated. An increase in CD formation was observed for both oils when the storage temperature was increased in all laboratories. The $E_{a,1}$ ranged from 47.9 to 73.3 kJ mol^{-1} in RSO and from 27.8 to 62.6 kJ mol^{-1} in SFO, with average values of 58.2 and 46.8 kJ mol^{-1}, respectively. The reproducibility coefficients were 10.9% and 18.2% for RSO and SFO, respectively.

Abbreviations: CD, conjugated dienes; E_a, activation energy; FAMEs, fatty acid methyl esters; RSO, rapeseed oil; SFO, sunflower oil.

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Practical applications: In order to compare results on oxidative stability of foods derived from different studies, the reproducibility of storage tests and methods employed to evaluate the oxidation level should be considered. This study provides fundamental data on the reproducibility of lipid oxidation under accelerated storage conditions and defines important parameters to be considered for the conduction of experiments.

KEYWORDS
activation energy, Schaal Oven test, statistical modeling, temperature, vegetable oil

1 | INTRODUCTION

Lipid oxidation is a major cause of oxidative deterioration of lipid-containing foods and the stabilization of lipids is of great interest for research and industry to improve the food shelf life. Because the oxidation rate is quite slow for most oils at room temperature, accelerated oxidation tests are used to obtain results in a reasonable time scale.[1] Fast methods applying high temperature, such as the Rancimat test, must be considered with prudence,[2,3] because thermodegradation becomes a predominant effect modifying the oxidation reaction mechanisms or even occurrence of side reactions that may be not relevant at room temperature. The most wide-spread methods to accelerate lipid oxidation are based on moderately elevated temperature, usually 40°C to 60°C, that is, the samples are stored at constant temperatures above ambient conditions. This approach is also referred to as Schaal Oven tests. It is based on the fundamental rule for chemical reaction kinetics, which states that the reaction rate increases exponentially with the temperature. In this regard, an increase of 10°C in the storage temperature approximately doubles the reaction rate. The advantage of the Schaal Oven test is that no specific equipment is required and that it can be used for a wide variety of oils, fats, and complex foods systems that are stored at room temperature in praxi.[4] Besides the temperature, several parameters can also modify the oxidation rate and must be therefore controlled. These include oxygen, light, pH value, the addition of prooxidants such as iron and copper ions, and the use of radical initiators. However, some of these parameters can change the reaction mechanisms compared to oxidation at room temperature in the dark. For this reason, storage tests conditions need to be well defined to be reproducible.

A plethora of studies investigating lipid oxidation in various foods during storage have been conducted to compare different samples or evaluate the effect of added antioxidants.[5] However, as different methods and experimental conditions were used to assess lipid oxidation level, the comparison of results between different studies is a challenging task. Interlaboratory trials that investigate the reproducibility and repeatability of the methods used are lacking. Only data for the automated Rancimat test have been reported in the literature.[6] The need to conduct an interlaboratory study on lipid oxidation in vegetable oils stored under accelerated oxidation conditions was identified during a roundtable discussion at the "2nd International Symposium on Lipid Oxidation and Antioxidants."[7]

The present study used a kinetic approach to evaluate the oxidation degree of vegetable oils by the determination of their conjugated

dienes (CD) content during storage at different temperatures. CD was selected as the preferred method because it is a well-standardized, a low error method that has shown to correlate with the hydroperoxide content of samples[8] and can be performed by many laboratories without special equipment. Lipid hydroperoxides, representing primary lipid oxidation products,[9] are of high relevance to determine the oxidative status of fats and oils.[10] As sample material, we chose two different vegetable oils that were obtained in the same industrial production batch from an oil manufacturer. Thus, we were able to ensure that all study participants received a highly comparable sample in which no matrix effects were expected as an influencing factor on lipid oxidation, as might be the case for more complex foods like emulsions.[11] For comparison, the activation energies (E_a) of formation and decomposition of primary oxidation compounds were calculated for each oil and each laboratory and results were statistically evaluated to determine repeatability and reproducibility. The measurement of conjugated dienes is based on the formation of a lipidic hydroperoxide with a conjugated double bond system formed by the rearrangement of bis allylic radicals during the polyunsaturated fatty acids oxidation.

The aim of the present study was to find out whether the outcome of storage experiments conducted in different laboratories under conditions well defined in a standardized protocol (e.g., incubation temperature, sampling, sample volume) is reproducible.

2 | EXPERIMENTAL SECTION

2.1 | Materials

Commercially available fully refined bottled sunflower oil (SFO) and rapeseed oil (RSO), both from the same production batch, were kindly provided by Brökelmann + Co – Oelmühle GmbH + Co. The oil samples were shipped to the study participants under ambient conditions (no temperature control) by mail. Within Europe, the packages reached the study participants within a few days. The international shipment of samples, on the other hand, was associated with some delays at customs, which is why the samples for one laboratory (no. 3) were stored for several weeks under nondefined conditions before they were delivered to the study participant. These samples differed significantly from the other oxidation courses or the initial starting values, which is why the results of this laboratory were excluded from the overall comparison.

Each laboratory received at least one closed PET-bottle (1 L) of SFO and one closed PET-bottle (1 L) of RSO. The fatty acid (FA) compositions and the tocopherol content of the oils are shown in Table 1.

A detailed experimental description (see Supplementary Material) was sent to all participants prior to the study. The instructions and procedural steps were reviewed by all participants in advance and optimized for feasibility in all laboratories. In addition, each laboratory was provided with an Excel template in which raw data and other observations were recorded.

TABLE 1 Compositions of the major fatty acids in the sunflower (SFO) and rapeseed (RSO) oil samples and their tocopherol content ($n = 3$).

fatty acid/tocopherol content	SFO	RSO
C16:0 [%]	6.34 ± 0.03	4.61 ± 0.03
C18:0 [%]	3.21 ± 0.02	1.55 ± 0.02
C18:1 cis (*n*-9) [%]	29.42 ± 0.47	62.74 ± 0.1
C18:2 cis (*n*-6) [%]	59.23 ± 0.58	19.63 ± 0.14
C18:3 (*n*-3) [%]	0.20 ± 0.12	8.46 ± 0.14
∑ SFAs [%]	10.77	7.15
∑ MUFAs [%]	29.77	64.68
∑ PUFAs [%]	59.44	28.16
α-tocopherol [mg kg^{-1}]	589.12 ± 12.38	218.22 ± 3.31
β-tocopherol [mg kg^{-1}]	< 0.50	0.81 ± 0.01
γ-tocopherol [mg kg^{-1}]	1.86 ± 0.17	293.01 ± 8.65
δ-tocopherol [mg kg^{-1}]	0.60 ±0.06	5.73 ±0.14
∑ tocopherol [mg kg^{-1}]	591.58	517.77

2.2 | Characterization of the SFO and RSO (fatty acid composition and tocopherol content)

The fatty acid composition (Table 1) was determined by the laboratory of Kiel University after they received the oil bottles from the oil manufacturer by gas chromatography after sample preparation (modified according to AOAC official method 991.39). The sample preparation is based on saponification of the lipids with methanolic NaOH and methylation by addition of borontrifluoride-methanol.[12] Heneicosanoic acid (Sigma-Aldrich) was used as internal standard. Fatty acid methyl esters (FAMEs) were measured with a HP Agilent 6890 Series gas chromatograph equipped with a J&W DB-23 column (60 m × 0.25 mm × 0.25 µm) as described by Amft et al. (2020).[13] FAMES were identified by comparing their relative retention times with those of authentic standards and their percentages were calculated using Agilent Chem Station software version B.04.03 and "R" for automated read out of the peak areas.

Tocopherols (Table 1) were quantified by HPLC analysis by laboratory A.C.T. FOODS GmbH (Bad Fallingbostel) according to the DGF method F-II 4a with some modifications. In brief, RSO and SFO were dissolved in n-heptane and analyzed by HPLC (Agilent 1100 Instrument; Agilent) at UV 295 nm and FLD 295/332 nm. An isocratic elution was performed with n-heptane/isopropanol (0.12% isopropanol), using a Nucleosil-OH column (125 × 2 mm, 5 µm) and a flow rate of 0.4 mL min^{-1}.

2.3 | Storage and sampling of the oils

The detailed experimental protocol as well as a table with laboratory-specific information can be found in the Supplementary Material. In brief, from both oil bottles, 90 g oil was weighed in triplicate in cleaned

250 mL glass bottles and closed airtight. The glass bottles were provided by each laboratory itself. The cleaning was carried out according to their own laboratory standards and used after their standard cleaning procedures. The oils were stored at 20°C (RT) or 30°C (the ambient temperature depended on the climatic conditions in the participants' country) and at 40°C and 60°C in the dark for 9 weeks. The oils were sampled at 10 time points during storage (days 0, 3, 7, 10, 14, 21, 28, 35, 49, and 63). Before each sampling, the glass bottles were carefully swiveled to ensure a homogeneous sample (air bubbles were avoided). Then, 3 g per bottle were removed, pipetted in a new 15 mL falcon tube, and subjected to analyses. The samples were analyzed immediately after sampling. For the laboratory 2 the samples from day 49 were stored for 3 days until analysis at −20°C. Five Laboratories froze their samples until analysis: laboratory 5 (−20°C), laboratory 6 (−26°C), laboratory 7 (−80°C), laboratory 9 (−20°C), and laboratory 11 (−25°C).

2.4 | Determination of conjugated diene concentration

Each laboratory performed the analysis of CD according to Stöckmann et al. (2000).[8] Therefore, 20 mg oil was dissolved in 5 mL 2-propanol, vortexed, and diluted if necessary. The samples were measured at 234 nm in a quartz cuvette in a spectrophotometer against 2-propanol as blank. A table with laboratory-specific information, for example, type of the used photometer, can be found in the Supplementary Material. The concentration of hydroperoxides was calculated using a molar extinction coefficient for methyl linoleate hydroperoxides of 26 000.[8,14] Data of CD were expressed as change in concentration using unoxidized RSO and SFO from day 0 as controls.[15] Data were collected in an Excel file, exported to CSV and imported in "R" for further evaluation.

2.5 | Lipid oxidation kinetics: Arrhenius plot and reaction rates

The dependence of the rate coefficient (k) with temperature is given by Equation (1), where A is the preexponential factor, E_a is activation energy, R is the gas constant, and T is temperature:

$$k = A \times e^{-E_a/(R \times T)}. \quad (1)$$

The rate k of a reaction (dc/dt = delta c/delta t) is defined by the change of the concentration in time. In this study, lipid hydroperoxides (LOOH) were estimated as conjugated dienes (CD). Therefore, the variation of the concentration of CD ([CD]) with time under storage conditions, can be written as the differential term $\frac{d[CD]}{d(t)}$. The resolution of $\frac{d[CD]}{d(t)}$ is complex as lipid oxidation is an autocatalyzed reaction, which involves unimolecular reactions and bimolecular hydroperoxide decomposition reactions.[3] By linear regression of the concentration of CD, the unimolecular reaction constant is commonly estimated at low concentrations, when the bimolecular reaction rate is still low. The rate coefficients at different temperatures were used in their logarithmic form to calculate the E_a by linear regression from the Arrhenius equation.

A semiempirical method was used based on Equation (2).[16] In Equation (2), the autocatalyzing unimolecular reaction nature of lipid oxidation is considered by the first term $k_{formation}$[CD], whereby the change of concentration of CD is connected to the concentration of itself. The second term represents the bimolecular degradation reaction.

$$\frac{d([CD])}{d(t)} = k_{formation}[CD] - k_{decomposition}[CD]^2. \quad (2)$$

Combining Equations (1) and (2) results in Equation (3), the E_a of the lipid hydroperoxide formation reaction can be solved using differential regression with an estimated initial lipid hydroperoxide value [CD_0] using R Script with the packages ggplot2, dplyr, deSolve, and minpack.

$$\frac{d([CD])}{d(t)} = A_1 \times e^{-E_{a,1}/(R \times T)} \times [CD] - A_2 \times e^{-\frac{E_{a,2}}{R \times T}} \times [CD]^2. \quad (3)$$

Fitting the differential equations, a nonlinear least square regression was conducted using the Levenberg–Marquardt algorithm.[17] In brief, a set of initial parameters (A_1 = 1 247 714 531 d^{-1} or 11 814 878 d^{-1}, A_2 = 4.76431e+15 d^{-2} or 4 772 687 d^{-2}, $E_{a,1}$ = 60 320 or 48 350 J mol^{-1}, $E_{a,2}$ = 110 408 or 53 470 J mol^{-1}) and an estimated CD concentration for the time point 0 (e.g., [CD_0] = 1.39 mmol kg^{-1}) was used to calculate the concentration of CD over time for the given temperatures (20°C or 30°C, 40°C, and 60°C). The least squares were calculated by the difference of the calculated versus the measured CD concentration at a given temperature. The sum of the least squares was then minimized by slightly changing the parameters using the Levenberg–Marquardt algorithm. The limits of 0.1 and 5 mmol kg^{-1} were used for CD_0, 5 d^{-1} and infinity for A_1, 0.0001 d^{-2} and infinity for A_2, and 1 kJ mol^{-1} and infinity for $E_{A,1}$ and $E_{A,2}$.

2.6 | Statistical analyses

All experiments were conducted in triplicate. Repeatability (s_r) and reproducibility (s_R) were used to evaluate the results across all laboratories.[18]

$$s_r = \sqrt{\frac{1}{N-p}\sum_{i=1}^{p}(n_i - 1){s_i}^2}, \quad (4)$$

$$s_R = \sqrt{s_r^2 + s_L^2}. \quad (5)$$

Variation between laboratories (${s_L}^2$) was calculated as follows:

$$s_L^2 = \frac{1}{\bar{n}}\left[\frac{1}{p-1}\sum_{i=1}^{p} n_i(\bar{x}_i - \bar{\bar{x}})^2 - s_r^2\right]. \quad (6)$$

In these equations, p is the number of laboratories, N is the number of measured values, n is the number of values in the laboratory, s_i is the variation in the individual laboratory, and $\bar{x}$ is the mean value in the individual laboratory and $\bar{\bar{x}}$ is the mean value across all laboratories (overall mean).

The statistical software R (2023) was used to evaluate the data. The calculation of the above parameters was based on an appropriate fixed and random effects model.[19,20] The fixed effects model included the factors laboratory, oil and their interaction term. The random effects models were split for the oils and included laboratory as a random effect. The residuals of these models were assumed to be normally distributed and to be homoscedastic within the specific oils. These assumptions are based on a graphical residual analysis. Repeatability (s_r) was obtained as the residual standard errors from the above fixed effects model. It represents the (oil-specific) "average" standard deviation over all laboratories. Variation between laboratories (s_L^2) was obtained as (oil-specific) variance corresponding to the random effect (laboratory) from the above random effects models. Reproducibility (s_R) hence represents the (oil-specific) "total" standard deviation. After these calculations, multiple contrast tests [21,22] were conducted to compare the laboratory means with the (oil-specific) overall mean of all laboratories and to make a comparison of the two oils for each laboratory. The used R-code can be found in the Supplementary Material.

3 | RESULTS AND DISCUSSION

3.1 | Evolution of conjugated dienes concentration during oil storage at the selected temperatures

The time course of CD formation for SFO and RSO at different temperatures for all laboratories is shown in Figures 2 and 3. All laboratories showed the same trends. The formation rate of CD increased with increasing storage temperature and the formation of CD was slower in RSO (Figure 1) than in SFO (Figure 2) at each temperature, which is in line with the study of Wójcicki et al. (2015).[23] The higher formation of oxidation products in SFO can be in part attributed to its higher total content of polyunsaturated fatty acids (PUFA) (59.4%) when compared to RSO (28.2%) (Table 1). The major PUFA in SFO was linoleic acid (C18:2; 59.2%). In addition to C18:2 (19.6%), RSO contained also 8.5% alpha-linolenic acid (C18:3), which with three double bounds is more susceptible to oxidation than C18:2. The fatty acid compositions are in line with data published by Orsavova and colleagues, who analyzed commercial vegetable oils after methylation with boron trifluoride.[24] The tocopherol content (sum of α-, β-, γ-, and δ-tocopherol) was 591.6 mg kg^{-1} in SFO and 517.8 mg kg^{-1} in RSO (Table 1) in accordance to data published for RSO and SFO.[25] The tocopherol composition differed between the oils. α-Tocopherol was the major analogue in SFO, whereas in RSO it was γ-tocopherol. As γ-tocopherol is more effective than α-tocopherol in inhibiting the formation of primary oxidation products in this range of concentrations,[26] the higher oxidative stability of RSO is also attributable to the content and composition of tocopherols.

The shape of the oxidation curve for RSO (Figure 1) and SFO (Figure 2) differed depending on the storage temperature and similar patterns were found across all laboratories. Each frame (1–16) represents the formation of CD in an individual laboratory at different temperatures. It should be noted that all laboratories stored their samples at 40°C and 60°C. For ambient temperature, 20°C or 30°C was used, depending on the climatic conditions in the country of the participants. The results of laboratory 3, in particular the initial start values, differed from the other laboratories due to the international shipping (see Section 2.1). However, the curve progression is very similar.

The curve for RSO was flat at 20°C storage temperature. Similarly, SFO showed a low increase at the same temperature and only in one laboratory (laboratory 12) was observed a strong increase at the end of the storage period, that is, after 60 days.

At a storage temperature of 40°C, the oxidation curve was divided into two parts for RSO, that is, a flat part at the beginning followed by a steep increase after approximately 28 days in most cases. These curves clearly indicated the induction and propagation phase of oxidation in the stored oil. The flat part of the curve in SFO was much shorter and a steep increase was observed after approximately 10 days.

When the oils were stored at 60°C a s-shaped curve was observed for RSO. A very short flat part of 1 or 2 days was followed by a steep increase that finally flattened into a plateau between 28 to 35 days. The curve clearly indicated that formation and degradation reached an equilibrium after ca. 30 days in RSO. For SFO, the curve also reached a plateau in most laboratories after approximately 30 days. However, the oxidation level was higher, that is, approximately 80 mmol CD kg^{-1} oil versus 35 mmol CD kg^{-1} oil in SFO and RSO, respectively.

Crapiste and colleagues reported comparable results in sunflower oil oxidized at 30°C, 47°C, and 67°C. Peroxide values increased progressively, only samples stored at 67°C had a maximum PV followed by a decrease.[16]

At the beginning of the storage period, in a few laboratories, a slight decrease in CD was observed at 20°C compared to the oil before storage. This observation can be related to the degradation of UV-active substances, while at the same time the formation of CD is still very low. Mei and colleagues found similar trends for CD and lipid hydroperoxides in corn oil-in-water emulsions after the start of storage. The authors explained this effect with a possible breakdown of preexisting peroxides in the corn oil.[15]

3.2 | Activation energy

The activation energy (E_a, Equation 1) is the minimum amount of energy required to activate molecules to a condition in which they can rearrange and undergo a chemical reaction. Thereby, E_a is independent of the amount of energy released, and can be linked to a chemical reaction rate coefficient k and the reaction temperature by Equation (1), known as Arrhenius equation.[27]

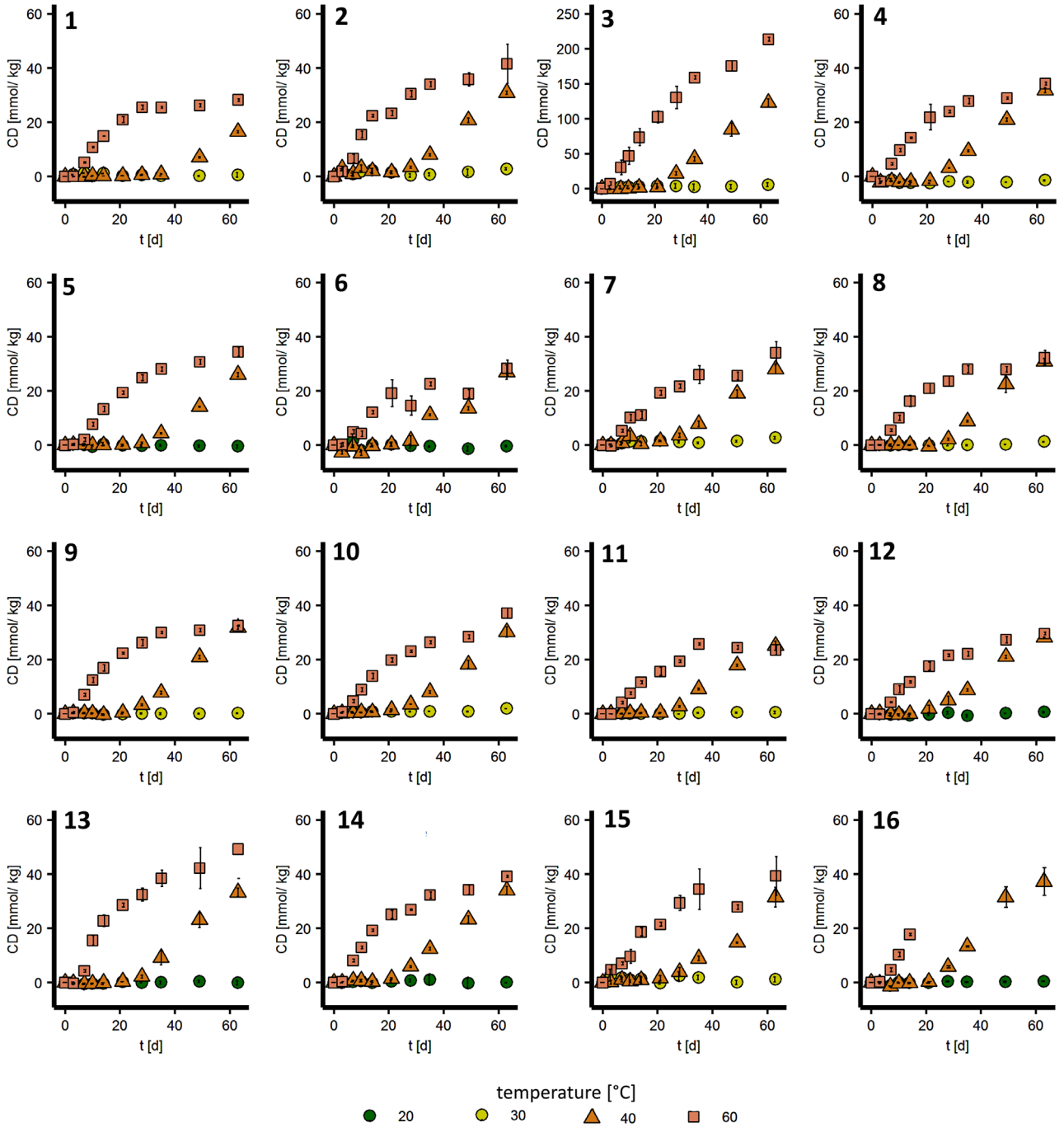

FIGURE 1 Formation of CD during storage in RSO at different temperatures (20°C or 30°C, 40°C, and 60°C) in the 16 different laboratories (anonymized and numbered from 1 to 16). In the laboratories 2, 5, 6, 7, 9, and 11, the samples were frozen until analysis.

To calculate the E_a, we fitted in the first step Equation (2) for all oxidation experiments (SFO and RSO for each laboratory) with a nonlinear least square regression. Equation (2) is based on a kinetic model proposed by Crapiste,[16] which is composed of two terms, a first-order autocatalytic reaction for the formation and a second-order reaction for decomposition of primary oxidation products. Figure 3 shows the fitted curve and the measured values for laboratory 8 as an example. The fit was less accurate for SFO at 40°C compared to RSO and other temperatures. In general, we found in most laboratories a better fit for RSO than for SFO, indicating that Equation (2) is based on a simplified model that does not consider the entire complexity of lipid oxidation in varying oils and temperatures, which is particularly the case at later stages of oxidation. The concentration of conjugated diene groups matches and represents that of compounds only until the degradation begins, at later stages secondary or further oxidation compounds are formed that may not carry a conjugated diene group. Further, we used a molar extinction coefficient of 26 000, which corresponds to conjugated dienes with *Z,E* isomerism, whereas

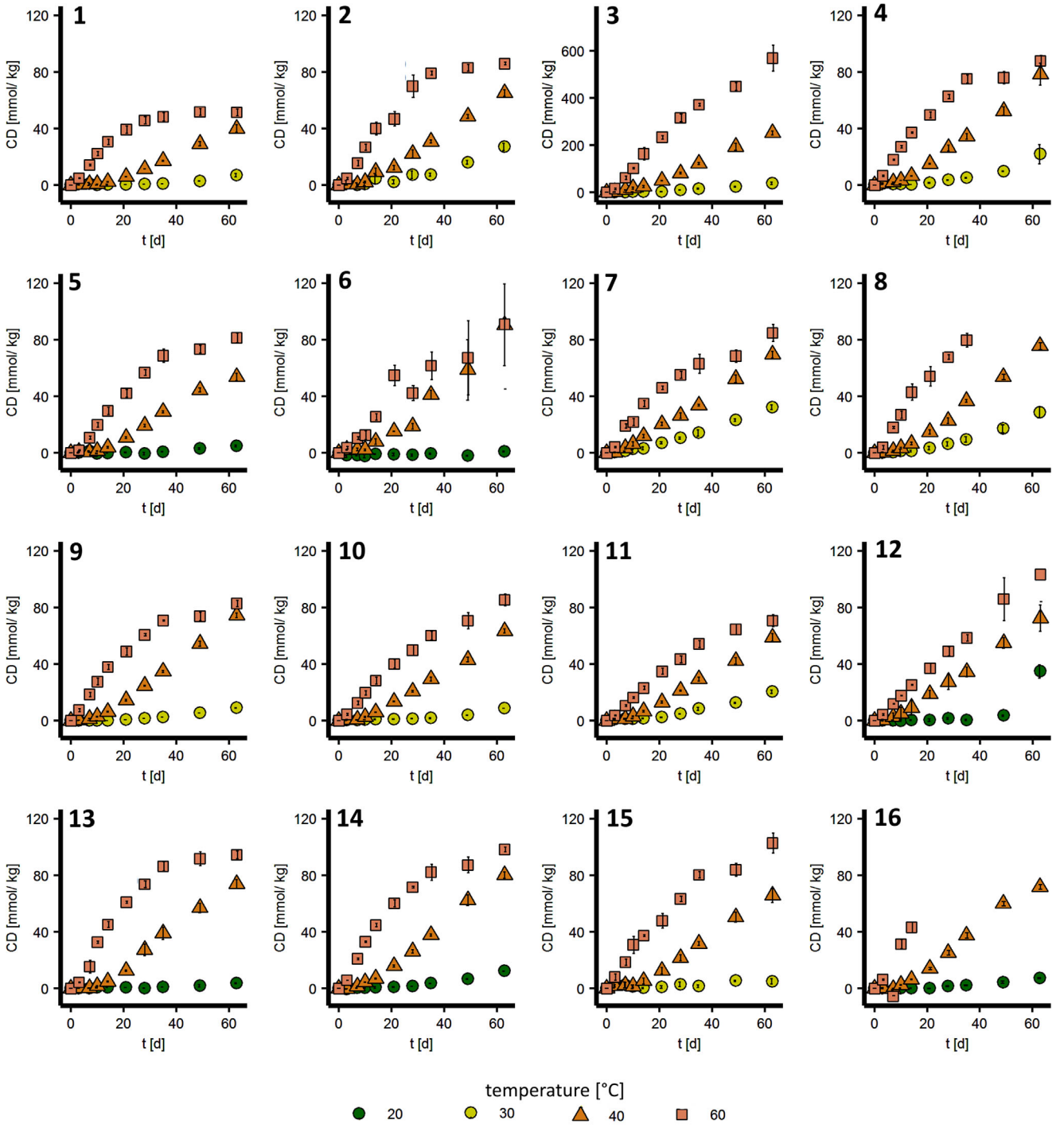

FIGURE 2 Formation of CD during storage in SFO at different temperatures (20°C or 30°C, 40°C, and 60°C) in the 16 different laboratories (anonymized and numbered from 1 to 16). In the laboratories 2, 5, 6, 7, 9, and 11, the samples were frozen until analysis.

E,E isomers, which are also formed, have a higher coefficient of 28 600.[14]

Fitting the curve for the formation of primary oxidation compounds at different temperatures for both oils enables to calculate the activation energy (E_a) for all laboratories. The E_a aggregates all measured CD values for SFO or RSO in only one value. $E_{a,1}$ ranged from 47.9 to 73.3 kJ mol^{-1} in RSO and from 27.8 to 62.6 kJ mol^{-1} in SFO (Figures 4 and 5 and Table 2) for the participating laboratories. Comparing E_a for both oils in the individual laboratories, this was lower in all cases for SFO than for RSO, which is in accordance with the course of oxidation found in the oils (Figures 1 and 2). In addition, variations between the labs seem to be consistent between the 2 oils, that is, labs with lower $E_{a,1}$ for RSO, also have the lowest $E_{a,1}$ for SFO.

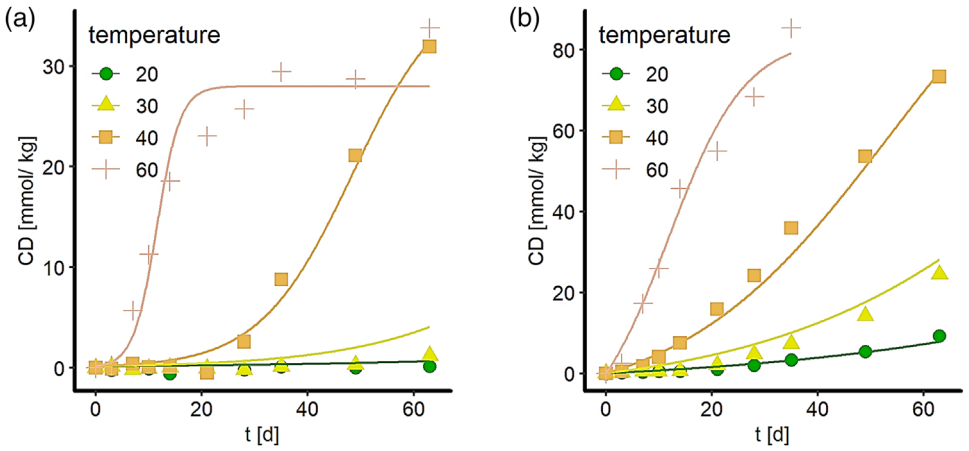

FIGURE 3 Comparison of measured CD values and fitted curve for (a) rapeseed (RSO) and (b) sunflower oil (SFO) for laboratory 8 as an example.

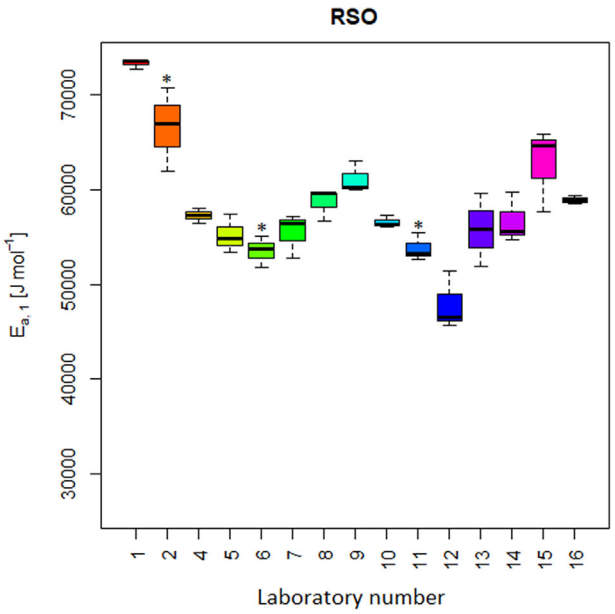

FIGURE 4 Activation energy ($E_{a,1}$; J mol^{-1}) for RSO during storage at different temperatures (20°C, 30°C, 40°C, and 60°C) in 15 out of 16 different laboratories (anonymized and numbered from 1 to 16). Laboratory 3 was not included in the calculations. In the laboratories 2, 5, 6, 7, 9, and 11, the samples were frozen until analysis. Marked with an asterisk are laboratories 2, 6, and 11 that differ significantly ($p \leq 0.05$) from the remaining laboratories that analyzed the samples directly after sampling.

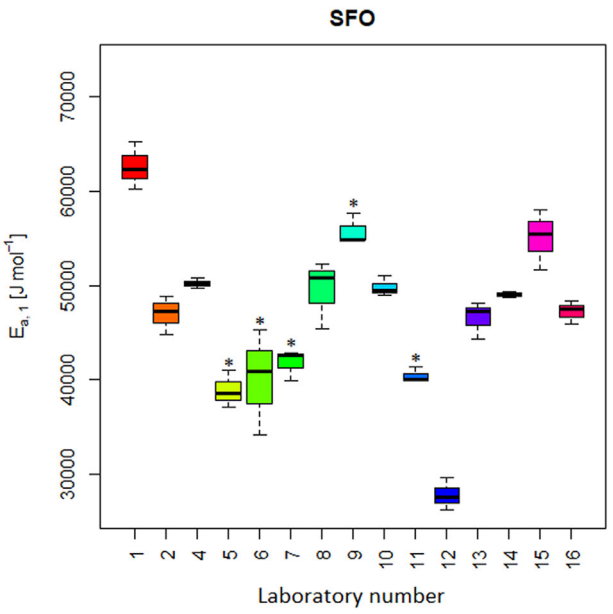

FIGURE 5 Activation energy ($E_{a,1}$; J mol^{-1}) for SFO during storage at different temperatures (20°C, 30°C, 40°C, and 60°C) in 15 out of 16 different laboratories (anonymized and numbered from 1 to 16). Laboratory 3 was not included in the calculations. In the laboratories 2, 5, 6, 7, 9, and 11, the samples were frozen until analysis. Marked with an asterisk are laboratories 5, 6, 7, 9, and 11 that differ significantly ($p \leq 0.05$) from the remaining laboratories that analyzed the samples directly after sampling.

3.3 | Comparison of individual laboratories

The repeatability of the experiments was tested by running the experiments in triplicate. The coefficients of variation for repeatability of the activation energy were 4.2% and 5.1% for RSO and SFO, respectively, for all labs (Table 2). The coefficients of variation for the reproducibility were 10.9% and 18.2% for RSO and SFO, respectively (Table 2). The difference in the coefficients of variation for the reproducibility between the oils can be due to the fact that SFO is a less stable oil than RSO as a consequence of a higher degree of unsaturation mainly. This was also demonstrated by the lower E_a. As a result, the fluctuations between the laboratories were more pronounced for the SFO. However, these values are approximately in the range of coefficients of variation for repeatability and reproducibility reported for lipid analyses in the DGF Standard Methods, for example, for polar compounds content in frying oils (coefficients of variation for reproducibility 3.0–6.5/coefficients of variation for repeatability 0.7–2.9),[28] polymerized triacylglycerols (coefficients of variation for reproducibility

TABLE 2 $E_{a,1}$ and other statistical parameters from oxidation of RSO and SFO (laboratory 3 was not included in the calculations).

	RSO	SFO
Grand mean ($\bar{\bar{x}}$) [kJ mol^{-1}]	58.2	46.8
Range of means ($\bar{x}$) [kJ mol^{-1}]	47.9–73.3	27.8–62.6
Repeatability standard deviation (s_r) [kJ mol^{-1}]	2.47	2.39
Range of standards deviations (s_i) [kJ mol^{-1}]	0.42–4.46	0.34–5.61
Reproducibility standard deviation (s_R) [kJ mol^{-1}]	6.3	8.5
Repeatability coefficient of variation [%]	4.2	5.1
Reproducibility coefficient of variation [%]	10.9	18.2

17.8–29.5/coefficients of variation for repeatability 9.0–16.2)[29,30] or unsaponifiable matter (coefficients of variation for reproducibility 9.6–60.9/coefficients of variation for repeatability 5.0–24.7).[31] However, in these studies, the sample material analyzed was identical, whereas in the present study the samples were obtained from storage experiments with RSO and SFO that were conducted in different laboratories.

We also compared those laboratories that stored the samples in the freezer until analysis with those laboratories that measured the CD directly after sampling. Because the statistical analysis was performed using 90 samples in total small but significant differences were found between the two sets of data. In fact, E_a of SFO samples that were stored under frozen condition was in average 4.65 kJ mol^{-1} lower than those that were immediately analyzed. In contrast, for RSO, the difference of 1.04 kJ mol^{-1} was not significant. We therefore recommend to include the information about sample storage after sampling and prior to analysis. This implies in particular for oil that exhibit medium to low oxidative stability.

There are only very few studies in the scientific literature that report the reproducibility of the determination of oxidative stability of edible oils. Woestenburg and Zaalberg evaluated the reproducibility of the automated Rancimat test in an interlaboratory trial with 11 laboratories.[6] They found coefficients of variation of repeatability and reproducibility of 3.3% and 9.1%, respectively, for rapeseed oil, and 7.9% and 13.5% for palm oil.[6] These results can be well compared to those of the present study as the calculations were conducted based on the same guideline (ISO 5725, release 1985) even it was a newer edition (ISO 5725, release 2000).[18] The coefficients are in the same range as those found in the present interlaboratory trial. Unlike the Rancimat test, the oxidation experiments in the present study were not automated and, therefore, all relevant factors were standardized in the present protocol appropriately to achieve reproducible results. In addition, it has to be considered that results for the E_a compared in this study were calculated based on 90 data points, that is, 90 oil samples were collected from three storage experiments at three different temperatures.

Other studies in the scientific literature did not compare experiments, but individual samples. Moreover, it is not clear whether repeatability or reproducibility was reported. In a study, triglycerides and cholesterol levels in nine serum samples were measured in 12 laboratories over a period of 12 months and the overall coefficients of variation across all laboratories were 6.7% and 3.4%, respectively.[32] However, in the mentioned study it must be considered that all participating laboratories first carried out a standardization procedure by measuring samples before the analyses of the nine serum samples that were finally used for calculation of repeatability and reproducibility.[32] In the present study, on the other hand, the protocol (Supplementary Material) for conducting the storage experiment and performing the analysis of the conjugated dienes was agreed with all the participants in the study, but no practical analytical exercise was performed prior to this interlaboratory trial.

4 | CONCLUSIONS

This work demonstrated for the first time the reproducibility of lipid oxidation analysis during accelerated storage conditions (Schaal Oven test). The outcome of the study is highly representative as 16 different laboratories participated in the trial and only one laboratory has to be excluded because of significantly higher oxidation levels justified by shipping problems with the oil bottles. It has turned out to be important to ensure that samples are shipped quickly worldwide so that the basic conditions are the same. In conclusion, the experimental procedure followed in this study provides sufficient reproducibility for storage tests investigating the stability of bulk oils. All laboratories found an equal temperature dependent effect during the storage experiments based on the analysis of conjugated dienes. Therefore, the protocol (Supplementary Material) can be used as a reliable method for studying bulk oil stability. In addition, information about storage of samples between sampling and analysis should be included. The protocol provides the foundation for the use of this methodology in the study of factors that further impact bulk oil stability such as the presence of antioxidants. Using the activation energy as an aggregated value allows the use of established statistical analyses for interlaboratory studies.

AUTHOR CONTRIBUTIONS

Jonas Amft: conceptualization; data curation; investigation; project administration; resources; visualization; writing—original draft; writing—review & editing. Philipp Matthias Meissner: conceptualization; formal analysis; investigation; resources; visualization; writing—original draft; writing—review & editing. Anja Steffen-Heins: investigation; resources; writing—review & editing. Mario Hasler: formal analysis; resources; visualization; writing—original draft; writing—review & editing. Heiko Stöckmann: investigation; resources; writing—review & editing. Anne Meynier: investigation; resources; writing—review & editing. Lucie Birault: investigation; resources; writing—review & editing. Ann Vermoesen: investigation; resources; writing—review & editing. Ines Perez-Portabella:

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The authors have declared no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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