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Oxidative stabilization of mixed mayonnaises made with linseed oil and saturated medium-chain triglyceride oil

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ABSTRACT

Mayonnaises, made with either saturated medium chain triglyceride (MCT) oil or unsaturated purified linseed oil (LSO), were mixed. Raman confocal microspectrometry demonstrated that lipid droplets in mixed mayonnaise remained intact containing either MCT oil or LSO. Peroxide formation during storage was lower in mixed mayonnaise compared to LSO mayonnaise, while in mixed oil mayonnaise the level of peroxides was constantly low. Mixed oil mayonnaise had a lower rate of oxygen consumption than mixed mayonnaise, LSO mayonnaise having the highest rate. The decay of water-soluble nitroxyl radicals showed radicals are formed in the aqueous phase with the same rate independent of the lipids. This was also reflected in decay of α-tocopherol during storage being similar in MCT and LSO mayonnaises, but being stable in mixed oil mayonnaise and mixed mayonnaise. Results suggest that other effects than simply diluting unsaturated triglycerides with saturated triglycerides is causing the oxidative stabilization observed for mixed mayonnaise and mixed oil mayonnaise.

1. Introduction

Many processed foods contain emulsified fat, and understanding the physical and chemical instability of emulsions has accordingly received a great deal of attention in food science. Oxidation of unsaturated fatty acids, and more recently oxidation of proteins, have been the main focus of chemical instability of emulsions, whereas the change of droplet size and the eventual separation of bulk lipid and water phases has been the focus of understanding the physical instability. Lipid oxidation in emulsified systems has been studied extensively on a macroscopic scale, but there is less knowledge of the physical and chemical processes on the microscopic scale contributing to the overall oxidative reactions.

The effect on oxidative stability of mixing emulsions with different lipid composition is unknown. One proposition is that due to coalescence, the contents of the oil droplets would mix with two or more droplets merging into one, which would cause an increment in the droplet size (McClements, 2005) and lead to physical instability of the emulsion. Another proposition is that the contents of the oil droplets mix, but the sizes of the droplets remain the same; and therefore the emulsion would be physically stable. This could happen due to reversible coalescence, which could include fusion and fission, or permeation of fatty acids from the pores of the interface of one droplet to another in very close proximity (Malassagne-Bulgarelli & McGrath, 2009). On the other hand, fatty acid exchange between two oil droplets with different fatty acid compositions might occur via transportation mechanisms by surfactant micelles. These are able to incorporate fatty acids from one droplet and carry them through the aqueous phase to another droplet, causing mixed contents with characteristics of the two oils and retaining the droplet size (McClements, Dungan, German, & Kinsella, 1992). In addition, a study conducted on oil-in-water emulsion crystallization showed there was intermixing of the contents of oil droplets in the emulsion due to transportation mechanisms supplied by micelles of emulsifier in the aqueous phase (Dickinson, Goller, McClements, & Povey, 1991).

The objective of this study was to investigate the oxidative stability of a food related oil-in-water emulsion containing two different types of oil droplets. This has been carried out by making mayonnaises either with saturated medium-chain triglyceride (MCT) oil or with purified unsaturated linseed oil (LSO). Mixed mayonnaises were made from these two mayonnaises. Also a mayonnaise was made with a mixed MCT and linseed oil. Raman confocal microspectrometry was applied to investigate the composition and spatial distribution of the lipid droplets in the mayonnaises, as well as to determine the physical stability of the emulsions. The oxidative stability has been studied by following the formation of peroxides and the loss of α-tocopherol during storage, as well as oxygen consumption measurements and radical

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trapping with electron spin resonance (ESR) spectroscopy for characterization of oxidation events on a shorter time scale.

2. Materials and methods

2.1. Materials

All chemicals used were of analytical grade. 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and 4[-N,N-dimethyl-*N*-(2-hydroxyethyl)]ammonium-2,2,6,6-tetramethylpiperidine-1-oxyl chloride (TEMPO choline) were obtained from Molecular Probes Inc., Eugene, Oregon, USA. Nonpolar fluorescent stain BODIPY$^{493/503}$ (D-3922) was purchased from Life Technologies Corporation, Oregon, USA. (±)-α-tocopherol was purchased from Fluka Chemie GmbH, Steinheim, Switzerland. MCT (medium-chain triglyceride) oil was from Cognis GmbH, Ludwigshafen, Germany. Linseed oil (LSO) was purchased from a local supermarket and purified before use by alumina column chromatography according to method by Yoshida, Kondo, and Kajimoto (1992) with some small changes: LSO was mixed with hexane 1:1 and purification was done once. The purification process ensured that the oil was cleaned from naturally occurring tocopherols, peroxides and trace metals (Fuster, Lampi, Hopia, & Kamal-Eldin, 1998). After evaporation process the oil was extracted from hexane by rotational evaporator (Büchi Rotavapor R-144, Labortechnik AG, Flawil, Switzerland). Vinegar, Dijon mustard, lemon juice and pasteurized egg yolk were purchased from a local supermarket.

2.2. Mayonnaise

Mayonnaise was produced in small batches and were made of egg yolk (12 wt%), mustard (2 wt%), oil (82 wt%), vinegar (2 wt%) and lemon juice (2 wt%). 83% of the mayonnaise was composed of oil phase and the rest was the aqueous phase. There were two different mixing methods used – either the mayonnaise was made using Ultra-Turrax T25 (IKA Works GmbH & Co. KG, Staufen, Germany) with dispersion element of 8 mm in diameter at a speed of 8000 rpm or mixed manually; and three different mayonnaise systems – either MCT oil or LSO mayonnaise, mixed oils (MO) mayonnaise, which consisted of one part of LSO and four parts of MCT oil mixed before mayonnaise was emulsified, or mixed mayonnaises (MM), which contained one part of LSO mayonnaise and four parts of MCT mayonnaise mixed together. For the ESR experiments mayonnaise samples were made without lemon juice but with 4 wt% vinegar.

2.3. Composition of oils by FAME analysis

MCT oil and LSO were analyzed by gas chromatography for fatty acid methyl esters (FAMEs) according to the method by Jart (1997) with some alterations: 1 ml of 0.025 M sodium methylate was added to the pure oil sample and hexane was added to the clear solution with 4 ml of saturated sodium chloride. Settings for gas chromatography were as follows: GC-chromatograph (5890 A-II; Hewlett–Packard Co., San Fernando, CA) with a 50 m × 0.25 mm × 0.20 μm CP-Sil 88 column (Chrompack, Middelburg, The Netherlands) under the following oven temperature program and conditions: 1 μl was injected to 1:25 split-flow with a constant flow of 1 ml/min, injector temperature was 250 °C and the detector temperature was 300 °C. The oven program was 50 °C for 1 min, and then increased by 15 °C/min to 180 °C. From that point on the rate was changed to 3 °C/min to 240 °C. This temperature was kept for 10 min. The fatty acid composition was calculated from the chromatograms as percentage of total fatty acid content.

2.4. Crystallization and melting temperatures of MCT oil, LSO, MCT mayonnaise, and LSO mayonnaise with differential scanning calorimetry (DSC)

The crystallization and melting temperatures of MCT oil, LSO, MCT mayonnaise, and LSO mayonnaise were investigated with a DSC instrument equipped with STAR^e SW 9.20 system (DSC 1, Mettler Toledo, Columbus, Ohio, USA) according to the following method: Starting temperature 25 °C was kept for 5 min, and then the temperature was increased up to 40 °C and kept for 5 min. After 5 min the temperature was lowered to −30 °C and kept for 5 min. The program finished at 25 °C. The samples were scanned with a scanning rate of 10 °C/min. Samples of 7.26–10.19 mg were weighed into 40 μm aluminium pans (ME-27331 Al-crucibles with pin), sealed hermetically with aluminium covers and measured with an empty aluminium hermetically sealed pan as a reference.

2.5. Confocal laser scanning microscopy (CLSM)

CLSM imaging was done on a typical mayonnaise sample using non-polar fluorescent stain BODIPY$^{493/503}$ (D-3922) at a concentration of 1 μM. Objective HCX PL APO lambda blue 20.0×, NA 0.70 oil immersion, argon laser 488 nm, image resolution 1024 × 1024, pinhole 1 AU, zoom 6.3×, line averaging were used.

2.6. Droplet size distribution

Size distribution of the droplets in the mayonnaises was determined by Laser Diffraction Mastersizer Micro Pro (Malvern Instruments Ltd., Malvern, UK), and calculated using the software based on Mie theory of laser light scattering. It was assumed that the sample was polydisperse. Volume mean diameter D[4,3] and surface mean diameter D[3,2] of the droplets were calculated for each of the mayonnaises.

2.7. The spatial distribution from the variation in chemical composition of the mayonnaises by Raman confocal microspectrometer

Three different emulsion systems were analyzed – MCT oil mayonnaise, LSO mayonnaise and the mixture of MCT oil and LSO mayonnaise (1:1). Raman measurements were performed using a home-built Raman confocal microspectrometer (Pully, Lenferink, & Otto, 2010; Sijtsema, Wouters, de Grauw, Otto, & Greve, 1998; van Manen, Lenferink, & Otto, 2008). A krypton ion laser (Coherent, Innova 90 K, Santa Clara, CA, USA) with excitation wavelength 647.1 nm, and a dry objective Plan Neofluar 40×, NA 0.95 (Carl Zeiss, Thornwood, NY, USA) were used to illuminate and collect the Raman-scattered signal (photons) from the sample. The scattered light was filtered by a razor-edge filter (Semrock Inc., Rochester, New York, USA) to reduce reflected laser and Rayleigh-scattered light, and focused onto a 15 μm diameter pinhole. The Raman images were obtained from three-dimensional hyperspectral (spatial × spatial × spectral) datasets with a spectral resolution of 1.85 cm^{-1}/pixel to 2.85 cm^{-1}/pixel over the wavenumber range from 20 cm^{-1} to 3670 cm^{-1}. The Raman spectra were acquired by raster scanning with a laser output power of 130 mW. The acquired data was converted to real Raman data by using software that removes cosmic rays, subtracts the camera off-set, and converts the wavenumbers axis from pixels to wavenumbers using the bands of toluene as calibration and the emission of a tungsten lamp of known temperature as a pixel-to-pixel transmission correction. The output of the software was organized in a matrix format, where each column represented the position the spectra was

recorded, and each row represented the spectral position. Raman images were created as the integrated intensity of vibrational bands as a function of position.

2.8. Peroxide value (PV)

Samples of different mayonnaises with 50 μM $FeSO_4$ added were stored at 4 °C in a fridge in 50 ml Falcon tubes. The oil phase from the samples taken at different times during storage was used for the determination of peroxide values. The oil phases were isolated by subjecting the mayonnaise samples to freeze–thaw cycles, followed by centrifugation for 3 min at 10,000*g* (Ole Dich Instrumentmakers Aps, Hvidovre, Denmark). The extracted oil was frozen and stored at −20 °C before use. The peroxide values were determined according to the method of ISO 3976:2006 (ISO 3976:2006, 2009). Ten mg of extracted oil was dissolved in 2 ml 1-butanol:methanol solution (1:1 v/v) and 2 ml of 7.7 mM ammonium iron(II) sulphate and 45 mM sulphuric acid in methanol, and 0.13 M ammonium thiocyanate were added. After a fixed reaction time, the absorption of the Fe(III) complex was measured photometrically at 510 nm using UV–visible scanning spectrophotometer (UV 1201, Shimadzu, Japan). The contents of lipid hydroperoxides (LOOH) in the extracted oil were calculated on the basis of an iron standard curve and expressed as mmol of LOOH per kg of oil.

2.9. Decay of α-tocopherol by high-performance liquid chromatography (HPLC)

To the samples of four different mayonnaises – MCT oil mayonnaise, LSO mayonnaise, mixed mayonnaise (MM) and mixed oil mayonnaise (MO) – 0.035 mM of α-tocopherol was added into the lipid phase prior to making mayonnaise, and the samples stored at 5 °C for 5 days. In the case of MM, MCT oil mayonnaise and LSO mayonnaise with added α-tocopherol were mixed. The samples went through a cycle of freeze–thaw and were centrifuged for 3 min at 10,000*g*. The extracted oil was frozen and stored at −20 °C prior to analysis. α-Tocopherol was measured by HPLC (Agilent 1100 Series, Waldbronn, Germany) according to AOCS method (American Oil Chemists' Society (AOCS), 2009) with some modifications. The analysis was run on a silica-based column (Supercosil LC-NH_2, 25 × 4.6 mm, 5 μm; Supelco; Sigma–Aldrich Co.) with the eluting solvent of heptane/ethyl acetate (70:30 v/v) at a flow rate of 1.0 ml/min. The eluent was monitored fluorometrically with an excitation wavelength of 295 nm and detection at the emission wavelength 330 nm, using an Agilent fluorescence detector G 1321A. Fifty mg of oil sample was dissolved in 5 ml heptane and 20 μl of this injected into the HPLC. Linear standard curves were obtained using concentrations between 0.25 mg/l and 10 mg/l of α-tocopherol.

2.10. The rate of oxygen consumption in mayonnaises

Mayonnaises (0.10–3.6 g) and mixtures of them were diluted in air-saturated MilliQ water to make a total volume of 5 ml. The oxygen concentration was determined by a Clark-type oxygen electrode (Radiometer, Copenhagen, Denmark) and recorded by a Unisense picoammeter (Unisense PA2000, Aarhus, Denmark) using a method by Mikkelsen and Skibsted (1992) with some modifications. The electrode was calibrated by a two-point calibration with 100% air-saturated MilliQ water, and 0% air-saturated buffer. The experimental set contained a ~2.7 ml glass reaction vessel, a ground glass stopper with a capillary-size hole through the center, a magnetic stir bar and a water bath set to 40 °C. It has been proven that measuring oxygen consumption with a Clark-type electrode is suitable at 40 °C (Wise & Naylor, 1985). The measurements were recorded after every 30 s for 2 h and plotted as a function of oxygen percentage over time. The 100% recognition of the oxygen concentration by the electrode was not immediate, but showed some time-dependence. The linear part of the oxygen consumption curve was used to ascertain the rate of the process.

2.11. Determining the radical formation by electron spin resonance (ESR) spectroscopy

The rate of radical formation was determined with lipid-soluble spin probe TEMPO as a 5 mM aqueous solution, and with the water-soluble TEMPO choline as 5 mM aqueous solution. Spin probes were added to the mayonnaise samples (at a concentration of 3.0 mM), mixed with a spatula and measured (after approximately 2 min) in 50 μl micropipettes (Blaubrand, Wertheim, Germany) with an ESR spectrometer (Miniscope MS200, Magnettech, Berlin, Germany) (operating in X-band mode). Since the reactions between the spin probe and radicals lead to formation of non-paramagnetic products, the decrease of the ESR signal was measured until the signal disappeared. All the results were expressed as the peak height of the center peak over time. Samples included MCT oil, LSO, MCT oil mayonnaise, LSO mayonnaise, mixture of the both of oils (1:1) and mixture of both mayonnaises in different ratios. The settings in all ESR measurements were as follows: Sweep 68.27 Gauss; sweep time 30 s; modulation 1000 mG; and MW attenuation 10 dB.

2.12. Statistical analysis

ANOVA analysis combined with Sidak multiple comparison test and pair-wise comparisons of means with equal variances combined with Tukey test were used for testing statistical significant differences in oxygen consumption measurements.

3. Results

3.1. Physical composition of mayonnaises

Two mayonnaises were made from either highly saturated medium-chain triglycerides (MCT) oil or unsaturated linseed oil (LSO) with the other ingredients, (egg yolk, mustard, vinegar and lemon juice), being identical. The MCT oil, which contained 97% saturated fatty acids, was selected for this study as a highly oxidation resistant liquid triglyceride oil, whereas LSO with its high level of unsaturated fatty acids (89% in total), was expected to be highly susceptible to oxidation (Supplementary material, Table 1). The pH of all mayonnaises was in the range of 4.11–4.16. DSC thermograms of the two pure oils, as well as the two types of mayonnaises, showed that in the temperature region from −18 °C to +40 °C, no thermal events occurred (data not shown). This implies that both types of oils were liquid when dispersed as droplets in the mayonnaises and they did not undergo phase transitions in the temperature regime used in this study. A separate set of mayonnaises was made without lemon juice, but with a double amount of vinegar. The pH of these mayonnaises was 4.32.

The microscopic spatial structure of mayonnaises was visualized by CLSM by adding a lipid-soluble stain BODIPY$^{493/503}$ (Supplementary material, Fig. 1). The lipid droplets tended to be spherical, but the actual shapes were affected by the close contact between droplets. The particle size distribution by laser diffraction determined that particle sizes of different mayonnaises (MCT oil, and LSO mayonnaises with lemon juice and vinegar or with double amount of vinegar) fell into range from 0.37 (stdev 0.13) μm to 54.65 (19.08) μm, and in most cases gave bimodal distribution of sizes. The D[3,2] value for the mayonnaises was 2.57 (0.11).

3.2. Raman confocal microspectrometry of mixed mayonnaises

Raman spectra were obtained for all the individual components in the mayonnaise (Fig. 1). This allowed identification of spectral features, which were unique and could be used as markers for the following chemical systems: (1) MCT oil had a characteristic peak at 1112–1125 cm^{-1} wavenumbers, which was assigned to the presence of saturated fatty acids; (2) LSO had peaks at 1205–1273 cm^{-1} and 1606–1652 cm^{-1} assigned to the presence of unsaturated fatty acids; and (3) lemon juice, vinegar and egg yolk had a broad peak at 3094–3649 cm^{-1} wavenumbers, which could be assigned to the presence of aqueous domains.

The spectral differences between MCT oil, LSO, and the aqueous phase made it possible to study mayonnaises using Raman confocal microspectrometry, and thereby obtain information about the spatial distribution and physical stability on a microscopic scale of mixtures of MCT oil mayonnaise and LSO mayonnaise. Therefore, the Raman spectra of the mixture of mayonnaises (ratio between MCT oil and LSO mayonnaise was 1:1) were recorded, and separate images based on the characteristic Raman bands were obtained (Fig. 2). The red channel in Fig. 2 illustrates the physical distribution of MCT oil according to the intensity of its characteristic Raman band, and in a similar way, the green channel illustrates the physical distribution of LSO and the blue channel illustrates the physical distribution of the aqueous phase. The presence of clear red colour areas in the combined hyperspectral RGB-image indicates pure MCT oil droplets, while the clear green colour areas indicate pure LSO droplets.

The depth of field with the objective used was around 1.4 μm, which means that all droplets, which are in the focal volume, contribute with their chemical spectra to the overall spatial characterization of the droplets on the integrated image. In other words, areas with many small droplets will have mixed spectra, because more than one lipid droplet will be present in the focal volume. In the integrated image this may be seen as areas with yellowish-light brown color that is the combination of green and red. However, some of the brown regions in the lower part of the combined image coincide with areas that are black in the blue image, which indicate that these areas contain no water. That might be due to small

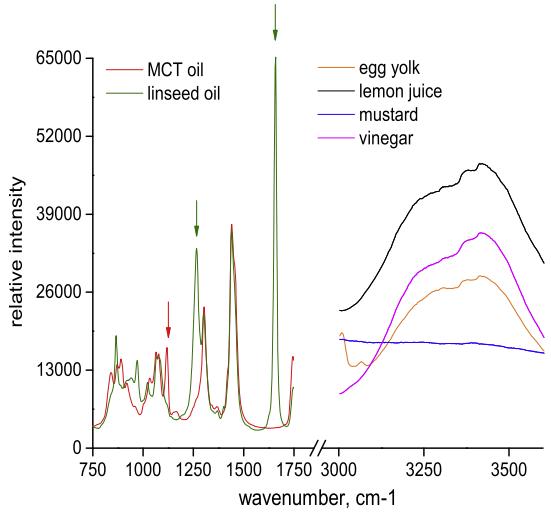

Fig. 1. Selected bands of Raman spectra illustrating variations in the chemical composition of MCT oil, LSO and the aqueous phase. MCT oil is characterized by one narrow peak from wavenumbers 1112 cm^{-1} to 1125 cm^{-1}, LSO is characterized by two narrow peaks from 1205 cm^{-1} to 1273 cm^{-1}, and from 1606 cm^{-1} to 1652 cm^{-1}, and the aqueous phase is characterized by a wider band from 3094 cm^{-1} to 3649 cm^{-1}. The selected bands are chosen to differentiate the two types of oils, and the aqueous phase from one another.

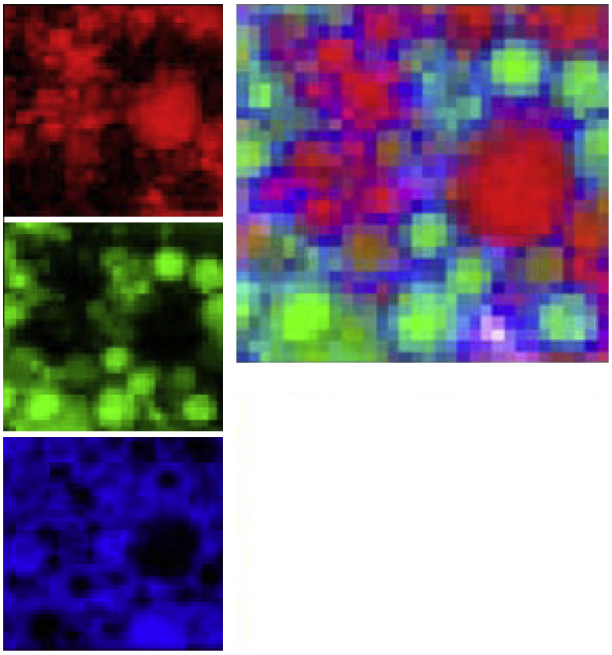

Fig. 2. Raman confocal microspectrometry RGB-image of a mixture of MCT, and LSO mayonnaises (1:1), where the red channel (left top) illustrates characteristic peaks of MCT oil, the green channel (left middle) illustrates characteristic peaks of LSO and the blue channel (left bottom) illustrates characteristic peaks of the aqueous phase. Each RGB-image channel is created from the hyperspectral dataset obtained from the mixed mayonnaise according to the selected bands to characterize important features of MCT oil, LSO and the aqueous phase. Additionally, the fourth image is the integrated image of the three channels and shows that MCT oil droplets and LSO droplets are stable and independent (not merged), and the aqueous phase is surrounding each of the droplets. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

droplets of MCT oil and LSO located close under each other, so that only a limited amount of water is separating these droplets spatially. Due to low resolution, and low signal intensity from the aqueous phase, it is not correct to state that these droplets are a mixture of both oils. In addition, not all the droplets remained intact. There were regions in some samples (data not shown), where large oil droplets showed the spectra of mixed MCT oil and LSO, indicating that the contents of droplets mixed and therefore the mayonnaise was not physically stable. The results obtained showed that mixing of the mayonnaises only induced coalescence in some regions of mayonnaise, and the dominating number of oil droplets still contained the separate pure oils. Images obtained after the same mixed mayonnaise sample had been stored at room temperature for 48 h were similar to the initial images (data now shown), demonstrating the samples were physically stable without coalescence of oil droplets during this period.

3.3. Oxidative changes in mayonnaises

3.3.1. Peroxide values (PV) of mayonnaises

The oxidative stability of mayonnaises was evaluated by measuring the formation of peroxides during storage for 6 days at 4 °C. Mayonnaises were made with purified oil and therefore only contained antioxidants introduced together with the other ingredients. Therefore, all samples were expected to have similar initial levels of antioxidants. The formation of peroxides was accelerated by adding $FeSO_4$ to the mayonnaises immediately before the beginning of storage. The level of peroxides throughout the whole study of the saturated MCT oil mayonnaise was under the detection limit (data not shown). On the other hand, the unsaturated LSO mayonnaise had the highest initial PV, which increased throughout the

whole study (Fig. 3). The mayonnaise made by mixing MCT oil mayonnaise and LSO mayonnaise (MCT:LSO, 4:1) gave initially very low PV, but the peroxide level increased during the storage period and reached a level that was higher than the mixed oil mayonnaise at day 6. During the first two days, the formation of peroxides was in accordance with the content of LSO in the samples, but afterwards the oxidation was accelerated. That indicated MCT oil had some ability to chemically stabilize the LSO mayonnaise during the first two days, but when the rate of oxidation had progressed further, the stabilizing effect of MCT oil vanished, and the oxidation speeded up with rates being similar to LSO mayonnaise. However, the overall formation of peroxides in mixed mayonnaises remained at about half of the amount in the pure LSO mayonnaise.

A mayonnaise was also made with mixed MCT oil and LSO (MCT:LSO, 4:1), and it gave an initial peroxide level similar to the LSO mayonnaise. However, the level remained constant during the whole storage. These results suggest that having mixed MCT oil and LSO droplets decrease the rate of lipid oxidation and thereby the extent of oxidation during the storage, whereas the oxidation of the pure LSO droplets were affected by neighboring MCT oil droplets to some extent in mixed mayonnaise samples.

3.3.2. Oxygen consumption measurements

The rate of lipid oxidation in mayonnaises was further studied by measuring the rate of oxygen consumption, which provides information about the total rate of oxidation in the mayonnaises, whereas the measurement of peroxides only quantifies a single class of oxidation products. The experiments were carried out by diluting mayonnaises in air-saturated MilliQ water in order to vary the total amount of lipids in the measuring chamber and thereby also the amount of LSO present in the experiments. Also, the dilution was necessary to be able to stir the samples during the measurements in order to prevent oxygen gradients. Since the lipid oxidation at ambient temperature is relatively slow (Tian, Dasgupta, & Shermer, 2000; van Dyck, Verleyen, Dooghe, Teunckens, & Adams, 2005), and highly elevated temperatures alter the reaction mechanisms (Tian et al., 2000), a moderate reaction temperature at 40 °C was used. Experiments with LSO mayonnaise gave the highest rates of oxygen consumption, and the rates tended to increase with the increasing amounts of LSO mayonnaise in the diluted sample. On the other hand, the saturated MCT oil mayonnaise had the lowest rate of oxygen consumption of all the tested samples as expected (rate constants of oxidation 14.47 (stdev 5.08)). The mixed oil (MO) mayonnaise samples and the mixed mayonnaise (MM) samples gave lower rates of oxygen consumption as compared to the LSO mayonnaises with the same amount of LSO in the diluted samples ($P \leqslant 0.001$) (Fig. 4). The mixed oil mayonnaises gave in two cases (1.0 g of LSO mayonnaise per 5 ml of diluted sample and 2.0 g of LSO mayonnaise per 5 ml of diluted sample) statistical significant lower rates of oxygen consumption than the comparable mixed mayonnaise samples ($P < 0.05$).

The measurement of peroxide formation during a six-day storage at 4 °C and the measurements of oxygen consumption during a period of few hours at 40 °C both showed that the MO mayonnaise had a much lower tendency for undergoing oxidation than the pure LSO mayonnaise.

In both sets of experiments the pure LSO mayonnaise was oxidizing fastest, whereas the MCT oil mayonnaise only showed negligible signs of oxidation as expected based on its extreme content of saturated triglycerides. The MM had oxidation rates between these two mayonnaises, however it cannot be excluded that in this case some mixing of oils between droplets take place during the continued stirring of the samples.

3.3.3. Radical formation measured by electron spin resonance (ESR) spectroscopy

Autoxidation of unsaturated lipids involves radicals as central intermediates. The two stable spin probes, nitroxyl radicals TEMPO choline and TEMPO, were used to quantify the amount of radicals occurring during the oxidation processes in the mayonnaises. These nitroxyl radicals, which can be detected by ESR spectroscopy, trap radicals with diffusion controlled rates (Beckwith et al., 1986) producing ESR-silent products. The rate of disappearance of the ESR signals can therefore be used for kinetic analysis of the radical formation in the system (Roman, Courtois, Maillard, & Riquet, 2012).

The positively charged TEMPO choline is only soluble in the aqueous phase of the mayonnaise system, and will therefore only trap radicals present in the aqueous phase. The ESR spectrum of TEMPO choline in mayonnaises gave a triplet signal with hyperfine coupling-constant 16.5 G, which is expected for aqueous systems (Tedeschi, D'errico, Busi, Basosi & Barone, 2002). TEMPO partitions between the oil and aqueous phase, with the dominating fraction in the lipid phase (Hubbell & McConnell, 1968). This was confirmed

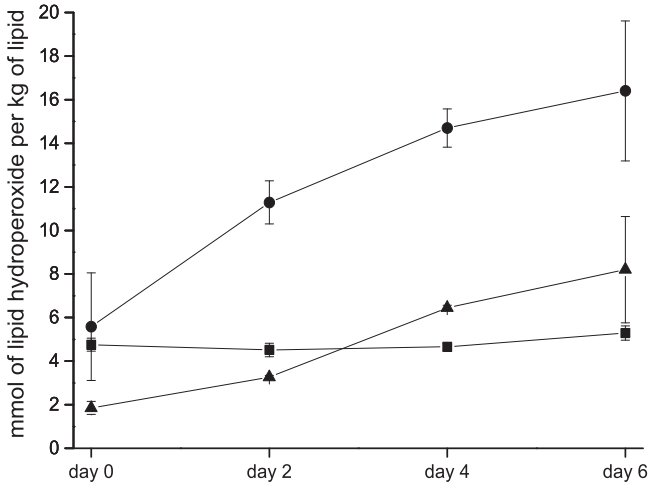

Fig. 3. Development of peroxides during storage of LSO mayonnaise (●), mixed oil mayonnaise made from mixing MCT oil and LSO (4:1) (■), and mixed mayonnaise made from mixing MCT mayonnaise and LSO mayonnaise (4:1) (▲). The mayonnaises were stored at 4 °C and 50 μM $FeSO_4$ was added at the beginning of the storage. Mayonnaise made from MCT oil was also studied, however the peroxide levels were below the detection limits throughout the whole storage period.

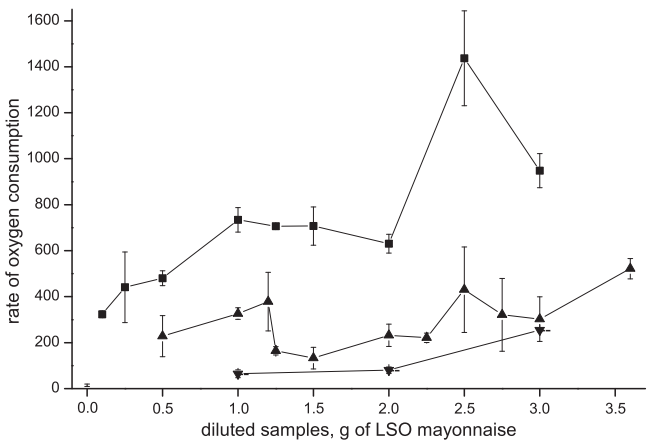

Fig. 4. The rate constants of oxygen consumption of diluted mayonnaises at 40 °C. LSO mayonnaise (■), mixed mayonnaise (MCT:LSO, 4:1) (▲), and mixed oil mayonnaise (MCT:LSO, 4:1) (▼). The x-axis is expressed in g of LSO mayonnaise per 5 ml of diluted sample.

by the ESR spectrum of TEMPO in the mayonnaise, which gave a triplet structure with hyperfine coupling constants 15.3 G (Supplementary material, Fig. 2). This value is in agreement with the value for TEMPO dissolved in oil (Nöel, Allendoerfer, & Osteryoung, 1992). TEMPO choline therefore provides information about the radical levels in the aqueous phase of the mayonnaise, while TEMPO provides information about the radical levels in the lipid droplets.

Experiments with the pure bulk oil samples showed that the TEMPO signal decreased fast in the LSO indicating a high level of radicals, whereas it was considerably more stable in the MCT oil (Fig. 5). A mixture of MCT oil and LSO had even lower rates than the pure MCT oil (data not shown). The rates of decay of TEMPO choline in the MCT oil mayonnaise and LSO mayonnaises were found to be high and very similar, whereas the decay rates of TEMPO were much lower and slowest in the MCT oil mayonnaise. However, conventional mayonnaise is made with lemon juice, which contains high levels of ascorbic acid. Ascorbic acid is able to reduce nitroxyl radicals, including TEMPO, to ESR silent hydroxylamines (Paleos & Dais, 1977). It is also possible that ascorbic acid can have a pro-oxidant effect by keeping traces of iron and copper in reduced states, which promotes radical formation through Fenton chemistry (Frankel, 1998), or by releasing iron from egg yolk, thus accelerating the oxidation (Jacobsen, Adler-Nissen, & Meyer, 1999). Mayonnaises were therefore made without lemon juice in order to avoid the complications due to the presence of ascorbic acid. The rates of TEMPO choline decay were considerable lower in the mayonnaises without lemon juice, but the rates of decay were identical in the two pure mayonnaises as well as in the mixed oil (MO) mayonnaise and the mixed mayonnaises (MM). This suggests a similar rate of radical formation in the aqueous phase, which is expected to be similar in all four mayonnaises. The decay of TEMPO was slower than the decay of TEMPO choline in all four systems showing the primary production of radicals take place in the aqueous phase of the mayonnaise systems (Coupland & McClements, 1996). The rates of TEMPO signal disappearance in the MCT oil mayonnaise and LSO mayonnaise were very similar. However, in the MM and MO mayonnaises, lower rates of TEMPO decay were observed. TEMPO is known to react preferably with alkyl radicals (Sobek, Martschke, & Fischer, 2001), and very weakly with peroxides (Brownlie & Ingold, 1967). Even though TEMPO is considered as a highly reactive probe towards radicals, it only traps alkyl radicals and not peroxyl radicals. Therefore, the ESR results may indicate the concentration of alkyl radicals is lower in the MM than in the MO mayonnaise.

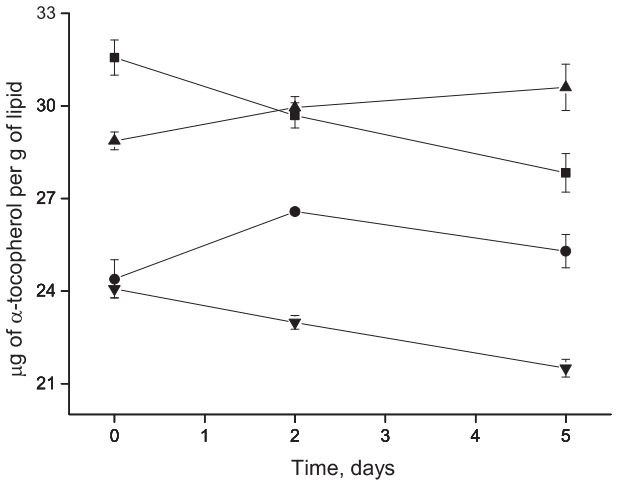

Fig. 6. The level of α-tocopherol during storage of MCT mayonnaise (■), LSO mayonnaise (▼), mixed oil mayonnaise (MCT: LSO, 4:1) (▲), and mixed mayonnaises (MCT: LSO, 4:1) (●) at 5 °C. α-Tocopherol (0.035 μM) was initially added to all mayonnaises.

3.3.4. Decay of α-tocopherol during storage

The development of peroxides in lipid systems is usually associated with uninhibited lipid oxidation, which occurs after an initial induction period with inhibited lipid oxidation, where chain-breaking antioxidants are active and readily oxidized. α-Tocopherol can be considered as an oxidation probe, since due to its chain-breaking antioxidant properties, it will be oxidized before unsaturated fatty acids. The rate of oxidation of α-tocopherol can therefore be used to quantify the extent of reactions leading to the initiation of lipid oxidation. MCT oil mayonnaise, LSO mayonnaise, mixed oil (MO) mayonnaise and the mixed mayonnaise (MM) with added 0.035 mM α-tocopherol were stored for 5 days, and the level of α-tocopherol was monitored during the storage (Fig. 6). The initial levels of α-tocopherol at day 0 varied in the four samples, which were caused by oxidation of α-tocopherol during the preparation of the mayonnaises. In the MO mayonnaise and the MM the levels of α-tocopherol were constant during the storage, while the level of α-tocopherol decreased in the pure MCT oil mayonnaise as well as in the LSO mayonnaise. Tocopherols are expected to be located at the surface of the oil droplets and these results therefore suggest that in the two pure mayonnaise systems, radicals formed in the aqueous phase get in contact with

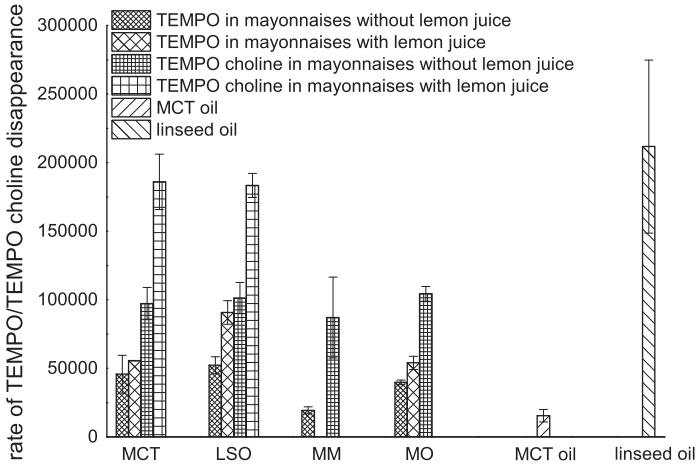

Fig. 5. The rate of TEMPO/TEMPO choline disappearance in bulk MCT oil and LSO, and in different mayonnaises. (MCT) mayonnaise made with MCT oil, (LSO) mayonnaise made with LSO, (MM) mixed mayonnaises made with MCT mayonnaise and LSO mayonnaise, and (MO) mixed MCT oil and LSO mayonnaise. Experiments were conducted at the ambient temperature, the initial concentrations of the spin probes were 3 mM.

oil droplets with the same rate independent of the oil inside the droplet. This is in agreement with the ESR experiment that demonstrated similar rates of radical formation in the aqueous phases of the different mayonnaises. The mixed oil as well as the mixed mayonnaises protected α-tocopherol from oxidation.

4. Discussion

The mixing of mayonnaises with different oil contents was shown by Raman confocal microspectrometry to give a mixed emulsion system where the original oil content of the individual droplets was intact. The droplets in mayonnaise are consequently rather stable since they do not exchange content during the mixing despite the very close contact between droplets due to the high ratio of oil relative to water. The mixing of triglycerides between droplets is entropically favored in mixed emulsions (Dickinson et al., 1991; McClements et al., 1992). Mixing of triglycerides could also occur by coalescence of oil droplets or other physical instability phenomena (Malassagne-Bulgarelli & McGrath, 2009). In the present study, such events were not observed. Raman confocal microspectrometry analysis of two-day old mixture of MCT oil mayonnaise and LSO mayonnaise illustrated that the contents of lipid droplets remained intact, and no mixing of the droplets' contents occurred. Therefore, when the mayonnaise emulsions remain physically stable and retain their droplet size distributions, they also retain the inner triglyceride composition of the individual droplets.

The MCT oil mayonnaise and LSO mayonnaise were, as expected, found to exhibit two extremes of oxidative stability, with the MCT oil mayonnaise containing almost exclusively saturated triglycerides (97%) being resistant to oxidation with respect to formation of peroxides and consumption of oxygen, whereas the LSO mayonnaise (89% unsaturation) gave the highest level of oxidation (Rael et al., 2004). The ESR experiments with TEMPO choline as radical probe demonstrated that in all mayonnaises the formation of the initial radicals mainly took place in the aqueous phase. The oxidation process in the mayonnaise system can therefore be regarded as a collection of separate events, where lipid oxidation is initiated in individual oil droplets by contact with radicals formed in the surrounding aqueous phase. The similar rates of oxidation of α-tocopherol in the MCT oil mayonnaise and the LSO mayonnaises support this model, as α-tocopherol is expected to be located at the surface of the oil droplets where it will be exposed to radicals entering from the aqueous phase.

The mixed mayonnaise (MM) was found to give an intermediate level of oxidation compared to the MCT oil mayonnaise and LSO mayonnaises with respect to oxygen consumption, formation of peroxides and decay of α-tocopherol. In the oxygen consumption study, plotting the data as function of the content of LSO (Fig. 4) clearly demonstrates that a dilution effect cannot explain the lower rates of oxygen consumption observed for the mixed mayonnaise as compared to the pure LSO mayonnaise. Also α-tocopherol was stable in the MM system indicating a protection against oxidation. Although a lower level of peroxide formation was observed in the MM than in the pure LSO mayonnaise, a possible lowering of peroxide formation was only seen in the beginning of the storage, whereas during the later stages of the storage the rates of peroxide formation were identical in the two systems, and thus not indicating any extra protection against oxidation.

The mayonnaise made with mixed MCT oil and LSO gave the lowest rates of peroxide formation and oxygen consumption, as well as stabilization of α-tocopherol towards oxidation. This suggests that the mixing of LSO with MCT oil itself can have a major effect on the extent of lipid oxidation. Coupland et al. (1996) have proposed that the rate of lipid oxidation in emulsion droplets can be affected by the presence of non-oxidazable compounds. At low concentrations, the oxidazable substrate may be able to obtain an optimal orientation at the droplet surface and the initiation of the lipid oxidation will be efficient. At higher concentrations the initiation becomes less efficient due to tighter packing of the oxidazable substrate, but the propagation steps will proceed more efficiently. The surface activity and concentration of the oxidazable substrate are expected to have a major impact on the efficiency of the initiation steps, since these reactions will be located at the droplet surface due to the contact with radicals formed in the aqueous phase. A likely explanation for the inhibiting effect of MCT oil on the level of oxidation in the MO mayonnaise could be the displacement of unsaturated triglycerides from the droplet surface by the medium-chain triglycerides. It has been reported that MCT oil has high polarity, and even a slight solubility in water (Galante & Tenore, 2005). This would give a surface covered with non-oxidazable triglycerides, forming an insulating layer between the radicals in the aqueous phase and the unsaturated lipids inside the oil droplets, and efficiently preventing the initiation of lipid oxidation. The similar rates of oxidation of α-tocopherol in the pure MCT oil mayonnaise and LSO mayonnaise suggest that α-tocopherol is not expelled from the droplet surfaces, since it is more surface active than medium-chain triglycerides as well as long-chain triglycerides, and its surface concentration is therefore not affected by the triglyceride composition. However, the rate of oxidation of α-tocopherol was nevertheless lower in the MO mayonnaise and the MM, suggesting that other factors associated with mixing either the oils or the mayonnaises also affect the oxidation reactions. Further studies are needed in order to identify these effects.

5. Conclusion

Raman confocal microspectrometry of two-day old mayonnaise samples showed the lipid droplets in the mixed mayonnaise remained intact and the contents did not mix. Peroxide value measurements, α-tocopherol studies, the rate of oxygen consumption, and ESR measurements confirmed that LSO mayonnaise was more prone to lipid oxidation than MCT mayonnaise, and mixed oil (MO) mayonnaise and mixed mayonnaise (MM) were less prone to oxidation than LSO mayonnaise. Therefore, it can be concluded that the presence of MCT oil in isolated lipid droplets in the mixed mayonnaises decrease the oxidation of LSO in separate droplets.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2013.11.141.

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