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Temperature-dependence of rate of oxidation of rapeseed oil encapsulated in a glassy food matrix

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Abstract

Oxidation of stripped rapeseed oil, encapsulated in a carbohydrate/protein glassy matrix, initiated by a lipophilic free radical initiator (or not initiated) and monitored as peroxide value and conjugated dienes for 45 days, developed differently depending on storage temperature. At low temperature (5 °C), the encapsulated oil and bulk oil, as reference, showed little oxidation which, moreover, could be accounted for by the oxygen dissolved in the oil. At intermediate temperatures (25 and 45 °C), oxidation exceeded the level corresponding to dissolved oxygen and became dependent on oxygen transport through the matrix. At high temperature (60 °C), a rapid, linear increase in peroxide concentration was followed by an autocatalytic phase with a rapid increase in peroxides, subsequently reaching a steady-state concentration. The oxidation of the encapsulated oil was found to have a lower energy of activation (around 60 kJ/mol) than the bulk oil (around 80 kJ/mol), resulting in a protection of the encapsulated oil at higher temperatures. However, the temperature-dependence of the zeroth order rate constants for initial peroxide formation in the encapsulated oil showed a shift from a rate determining reaction at low temperature with a high energy of activation to a reaction at higher temperature with a smaller energy of activation, especially for the encapsulated oil without initiator added. At low temperature, lipid oxidation seems rate-determining while, at higher temperature, oxygen permeation through the matrix with a lower energy of activation becomes rate-determining. The glassy matrix yields only partly protection against lipid oxidation as it allows permeation of oxygen and other small molecules, as further confirmed by the effect of a hydrophilic radical initiator, incorporated in the matrix, on peroxide value of the encapsulated oil.

Keywords: Glassy state; Lipid oxidation; Encapsulated oil; Oxygen permeation; Temperature dependence

1. Introduction

Food in a glassy state is associated with long-term stability due to the extremely high viscosity. High viscosity causes low mobility of molecules entrapped in the glassy matrix and of molecules penetrating the glassy matrix. Consequently, collision of reactants in the glassy matrix will be limited and a glassy state is expected to inhibit chemical reactions involving reactants trapped in the glassy matrix. Foods containing carbohydrates or proteins are capable of forming a glassy state during dehydration. Further, other types of compounds susceptible to degradation, such as lipids, may be encapsulated in the glassy matrix during dehydration, and should thereby be protected against degradation processes such as oxidation.

In an earlier study (Orlien, Andersen, Sinkko, & Skibsted, 2000), in which the oxidative stability of rapeseed oil encapsulated in a carbohydrate based glassy matrix was studied, it was shown that the glassy matrix was not completely able to protect the encapsulated oil from

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oxidation, as oxygen did diffuse through the matrix. The rate of formation of primary oxidation products revealed that lipid oxidation in the glassy food model followed zeroth order kinetics with the diffusion of oxygen as the limiting factor for oxidation. It was further shown that hydrophilic radicals generated in the glassy matrix did not initiate oxidation of the encapsulated oil, an observation which was ascribed to immobilization of the hydrophilic radicals in the dense glassy matrix. Other studies have dealt with oxidation of encapsulated oils in glassy carbohydrate matrices but, regarding the ability of oxygen to diffuse through the matrix and oxidize a given substrate, the results are conflicting. Anandaraman and Reineccius (1986), and Imagi, Muraya, Yamashita, Adachi, and Matsuno (1992) observed oxidation of oil encapsulated in maltodextrin matrices. For an orange peel oil, protection against formation of epoxides in a zeroth order reaction increased for encapsulation in matrices of maltodextrin with increasing dextrose equivalents (Anandaraman & Reineccius, 1986). In contrast, Shimada, Roos, and Karel (1991), and Labrousse, Roos, and Karel (1992) observed no oxidation of methyl linoleate encapsulated in a lactose-gelatin matrix or in sucrose-lactose-gelatin matrix. However, in these studies rapid oxidation was observed when the matrices crystallized or collapsed, and the oil was fully or partially released and thereby exposed to oxygen.

The most important factor regarding oxidation of encapsulated oil seems to be the availability of oxygen in terms of diffusion of oxygen through the glassy matrix. The permeability of oxygen through a sucrose-maltodextrin-gelatine glass was monitored as a function of storage temperature by Andersen, Risbo, Andersen, and Skibsted (2000). It was shown that, when storing the oil-encapsulating glass in 100% oxygen atmosphere, the concentration of oxygen increased in the encapsulated oil. Further, it was found that the rate of diffusion increased with increasing temperature below the glass transition temperature of the system, corresponding to an activation energy of 74 kJ/mol and oxygen diffusion through the matrix was classified as an activated process (Andersen et al., 2000). Accordingly, it may be concluded that the glassy state, as such, only yield partial protection against deteriorative chemical reactions, depending on the nature of the reactants.

In the present study, we report results from a continuation of the studies of Orlien et al. (2000) and Andersen et al. (2000), on oil-encapsulating glassy food models, for which the diffusion of oxygen was found to be the rate-determining step in oxidation of encapsulated oil and for which the rate of diffusion was found to be strongly dependent on temperature. In the present study, we further investigate the protection of encapsulated oil by the glass matrix, including temperature dependence, as we monitor the formation of primary lipid oxidation products of glass-encapsulated oil as a function of time and temperature.

2. Materials and methods

2.1. The glassy food model

A glassy food model, composed of 25% sucrose (extra pure, Merck, Damstadt, Germany), 45% maltodextrin 10 (dextrose equivalent of 10, from maize starch, Fluka, Neu-Ulm, Switzerland), 5% gelatin (225 bloom, from calf skin, Aldrich, Milwaukee, USA) and 25% rapeseed oil (Aarhus Oliefabrik A/S, Aarhus, Denmark), stripped according to the method of Lampi, Hopia, Ekholm, and Piironen (1992), was established. Carbohydrates were dissolved in hot water and gelatin was dissolved in boiling water. The solutions were mixed, cooled on ice and the aqueous solution divided into two parts. Two portions of stripped rapeseed oil were prepared, one portion with the lipophilic radical generator 2,2′-azobis(2,4-dimethylpentanenitrile) (AMVN) (Wako chemicals GmbH, Neuss, Germany) added at concentration 0.60 mg/g oil, while the second portion served as a blank without initiator. The oil portions were added to the aqueous part, and emulsified using a homogeniser (Ultra Turrax, Jankel & Kunkel IKA-Labortechnich, Staufed, Germany) at 9500 rpm for 1 min in an ice bath. The emulsions were frozen (−50 °C, 15 h) in plastic containers giving emulsions sizes of $7 \times 22 \times 2$ cm and freeze-dried ($p \sim 0.1$ mbar, 48 h) using an Edwards laboratory freeze-dryer. The dried glassy food models were stored in an evacuated desiccator over P_2O_5 (relative humidity 0.0%) for further dehydration (1 week at room temperature). The glassy food models were divided into cubes of approximately $2 \times 2 \times 2$ cm and washed three times with hexane (HPLC Applications, Fisher Scientific, Leicestershire, UK) to remove non-encapsulated surface oil and afterwards stored in an evacuated desiccator to dry off hexane. Bulk stripped rapeseed oil and bulk stripped rapeseed oil with AMVN (0.60 mg/g oil), in Petri dishes with a diameter of 9.5 cm, served as reference systems. All samples were incubated at 40 °C for 1 h to generate radicals and initiate lipid oxidation. The food models were then stored over P_2O_5 at 5, 25, 40 and 60 °C (glassy food models and reference systems stored at 60 °C were not incubated at 40 °C). The Karl Fischer titration (Mettler DL18, Schwerzenbach, Switzerland) was used to determine the water contents in the glassy food models by incubating samples in methanol (<0.005% H_2O, Merck, Darmstadt, Germany) for water extraction for 24 h. In an additional experiment, the same glassy food model, encapsulating stripped rapeseed oil, was prepared with the hydrophilic radical generator 4,4′-azobis(4-cyanopentanoic acid) (ABCPA,

Wako chemicals GmbH, Neuss, Germany), added at concentrations of 20, 50, and 100 mg/g oil and peroxide value was determined during storage at room temperature around 25 °C for 35 days, following initiation of oxidation at 48 °C.

2.2. Differential scanning calorimetry

The glass transition temperature was determined using a DSC 820 from Mettler Toledo (Schwerzenbach, Switzerland), which is based on the heat flux principle and cooled with liquid nitrogen. Calibration of heat flow and temperature was done with indium (T_m = 156.6 °C, ΔH_{fus} = 28.5 J/g) and zinc (T_m = 419.5 °C, ΔH_{fus} = 107.5 J/g) as standards. The low-temperature linearity of the calibration was verified with cyclohexane (T_m = 6.47 °C) and decane (T_m = −19.66 °C). Five to 15 mg of sample were hermetically sealed in a 40 μl aluminium DSC crucible. As reference, an empty sealed aluminium crucible was used. Heating, at a rate of 10 °C/min, was used over an appropriate temperature range. Duplicates and rescans in each case, verified the endothermic baseline shift associated with the glass transition temperature. T_g was taken as the onset temperature of the endothermic baseline shift.

2.3. Lipid oxidation

Oxidation was monitored by analysis of conjugated dienes and peroxides. Conjugated dienes were measured directly by dissolving 0.10 g powdered samples of the glassy food model in 7.0 ml of ethanol, followed by centrifugation. The absorption spectrum (200 nm < λ < 300 nm) of the supernatant was recorded with a HP 8452 UV–vis diode array spectrophotometer (Hewlett Packard CO., Palo Alto, CA, USA). Ten milligrams of the bulk oils were dissolved in 3.0 ml of ethanol and the absorption spectrum recorded. The relative amount of conjugated dienes for each sample was expressed in arbitrary units as the second derivative, $d^2A/d\lambda^2$, at 242/236 nm and at 254 nm (Corongiu & Banni, 1994). For peroxide determination, the oil was extracted with hexane and stored at −80 °C prior to analysis. The peroxide values were obtained using a method based on the IDF standard (1991), entailing reaction of peroxides with iron(II) chloride and ammonium thiocyanate, followed by absorbance measurement at 500 nm after a reaction time of 2 min, recording the spectrum of the red iron(III) thiocyanate complex and using a standard curve based on H_2O_2. The peroxide value is reported as mmol peroxide/kg oil.

2.4. Statistical analysis

Results from measurement of lipid hydroperoxide content were subjected to *linear model* analysis of covariance, using storage time as covariate (SAS version 8.2 software; SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. The glassy food model

The glassy food model was designed and found to be stable against crystallisation and collapse. The glass was characterized by optical and scanning electron microscopy, showing a fragile and porous structure (Orlien et al., 2000). The obtained glassy model successfully entrapped a high fraction of the rapeseed oil (79–87%), and the oil drops were found to have sizes in the order of 20 μm and to be distributed throughout the matrix. For the glassy food model, the initial glass transition temperature was measured by DSC to be 55 °C. The glass transition temperatures were measured during storage, and T_gs of 29 ± 3.6 °C for glassy food models stored at 5 °C, 44 ± 4.6 °C for glassy food models at 25 °C, 68 ± 1.8 °C for glassy food models at 40 and 75 °C, for glassy food models at 60 °C, were found. For all glassy food models, the T_gs were above the respective storage temperature throughout storage. The glassy food models had a water content of 1.9 ± 1.6%, as measured at intervals throughout the storage period.

3.2. Lipid oxidation of encapsulated oil versus bulk oil

3.2.1. General oxidation

In order to obtain a specific measure of the protection of the glassy matrix, the conjugated diene (CD) and peroxide values (POV) of the rapeseed oil without antioxidant present were monitored during storage. The development of CD and POV in the bulk oil and in the encapsulated oils, as a function of storage time and temperature, is presented in Figs. 1 and 2, respectively. As may be seen from the figures, conjugated dienes and lipid hydroperoxides are formed, confirming that both the bulk oils and the oil in the glassy matrices oxidized during storage.

The developments of oxidation in bulk oil, and in encapsulated oil, monitored by analysis of conjugated dienes and by lipid hydroperoxides, paralleled one another, as may be seen by a comparison of Figs. 1 and 2. Immediately after incubation, the extents of lipid oxidation are seen to be at the same low levels for bulk oil and the encapsulated oil. Further, it is seen that the time course of formation of conjugated dienes and lipid hydroperoxides depends on the temperature of storage, as the rate of formation of primary oxidation products increases with temperature. After 10 days of storage, the levels of oxidation were clearly different and, notably, the encapsulated oil stored at 60 °C had a considerable higher rate of formation of conjugated dienes and

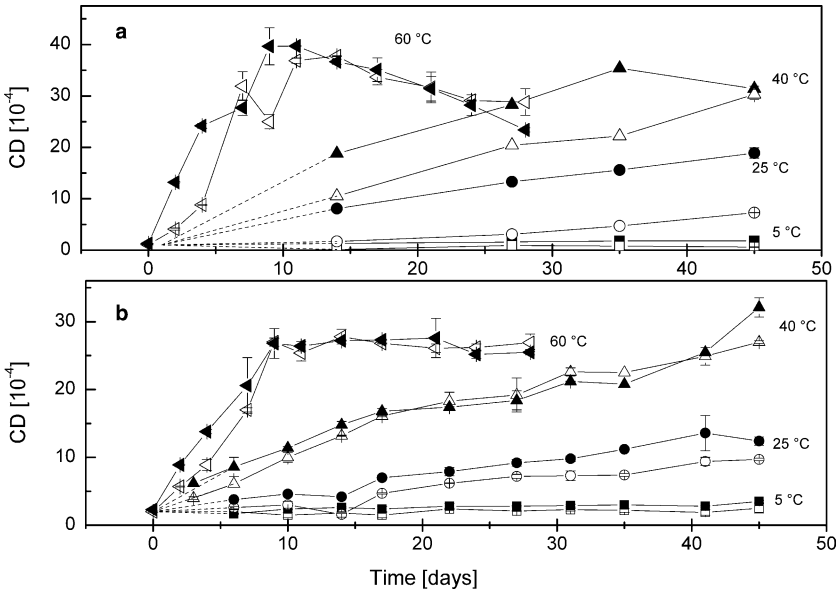

Fig. 1. Formation of conjugated dienes (measurement on a relative scale) in stripped bulk rapeseed oils (a) and stripped rapeseed oils encapsulated in a glassy matrix with a glass transition temperature above the storage temperatures (b) during storage at 5, 25, 40 and 60 °C. Solid symbols refer to oil with AMVN free radical initiator added, while open symbols refer to oil without initiator.

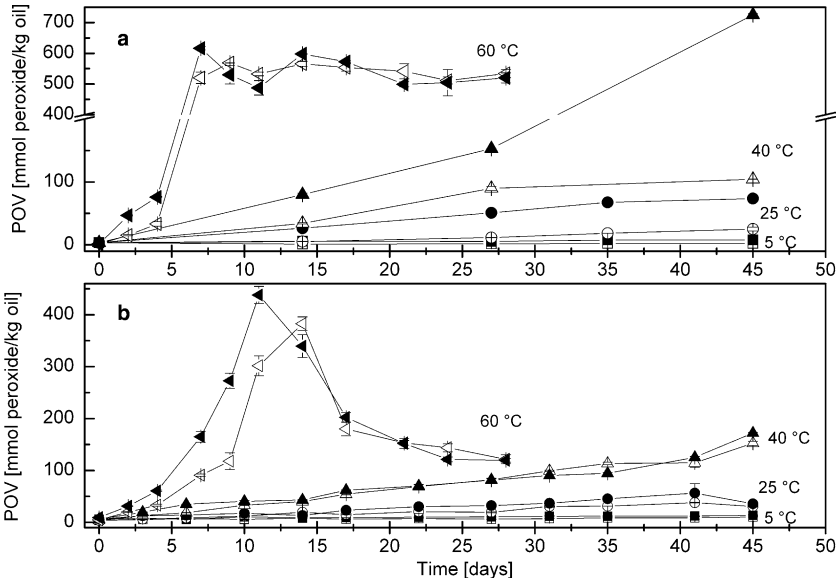

Fig. 2. Formation of lipid hydroperoxides, measured as POV in stripped bulk rapeseed oils (a) and in glass-encapsulated stripped rapeseed oils (b) during storage at 5, 25, 40 and 60 °C. Solid symbols refer to oil with AMVN free radical initiator added, while open symbols refer to oil without initiator.

peroxides than at lower temperatures. As expected, the bulk oil with AMVN and without this initiator showed no lag phase for oxidation. After 5 days, the levels of CD and POV already increased rapidly, with the exception of both bulk oils at 5 °C, and of the oil without initiator at 25 °C.

The developments of CD and POV, as seen in Figs. 1 and 2, show that the lipophilic radical generator, AMVN, is efficient in initiating oxidation of the bulk oil. The statistical analysis on the rate of formation of peroxides at 5, 25 and 40 °C showed that bulk oil with AMVN differed significantly, both from the oil encapsulated by the glass and from bulk oil without initiator. The clear influence of the temperature on the formation of primary oxidation products was also confirmed by statistical analysis, giving significant differences between the groups of oil systems stored at 5, 25, 40 or 60 °C. In order to quantify the effect of temperature on oxidation of the oils encapsulated in the glassy matrices versus the bulk oils, the time courses of the development in POV

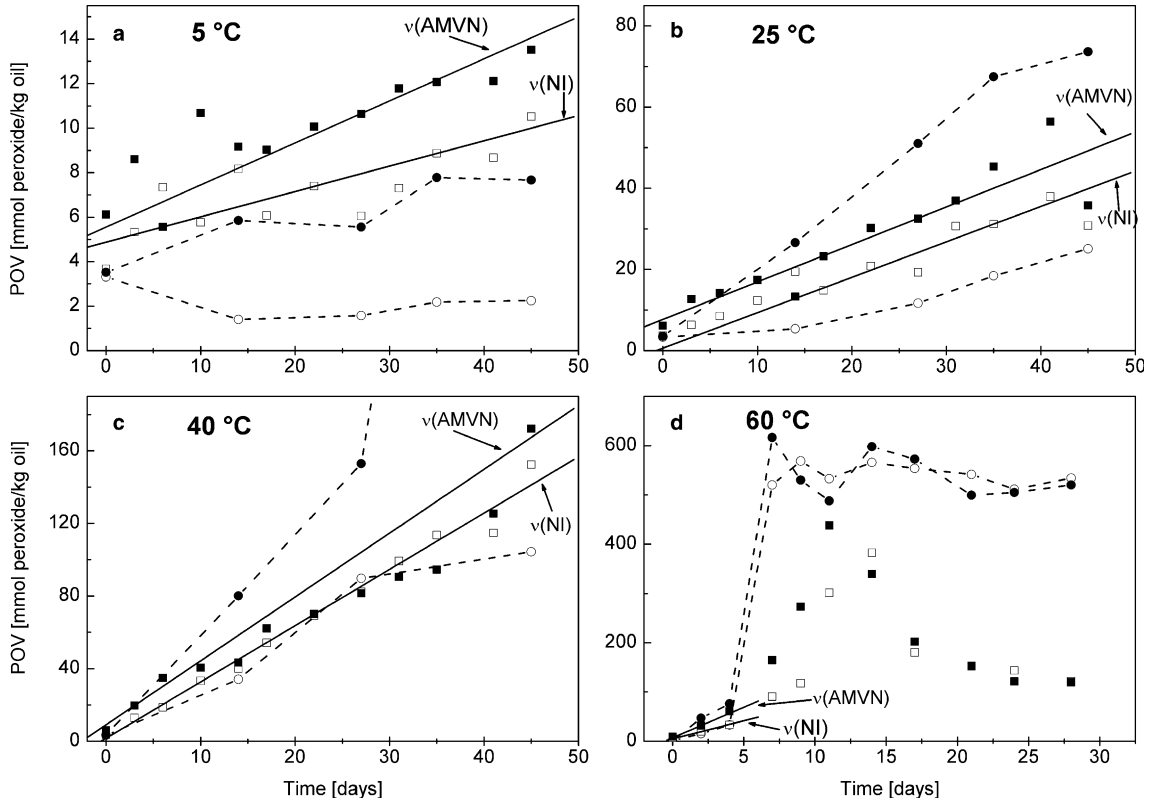

Fig. 3. Development of lipid hydroperoxides in stripped rapeseed oil in the food models divided into groups of storage temperature. (■): glass-encapsulated oil with AMVN free radical initiator, (□): glass-encapsulated oil without initiator, (●): bulk oil with AMVN free radical initiator, and (○): bulk oil without initiator. Solid lines obtained by linear regression are marked *v* (AMVN) and *v* (NI) to indicate that the slopes correspond to the rate for initiated oxidation and non-initiated oxidation, respectively.

are presented separately in Fig. 3. Additionally, the results are presented in Table 1. In the following, a presentation of the results from each storage temperature will be given.

3.2.2. Storage temperature 5 °C

A remarkable pattern appeared for the time course of oxidation for the oils encapsulated in the glassy matrices and in the bulk oils during storage (Fig. 3(a)). Notably, the bulk oils displayed an induction period spanning the entire storage period, despite the removal of the natural content of antioxidants. During the storage period, almost no lipid hydroperoxides were produced and the bulk oils with and especially without initiator maintained the initial low level of peroxides. The low temperature must account for this absent or slow increase in lipid oxidation. In contrast, glass-encapsulated oils did not show an induction period, but exhibited a marked formation of lipid hydroperoxides throughout the storage period, with a significant increase in POV for both glassy food models, with and without initiator, and which could be described as a zero order reaction. The statistical analysis revealed that the rates of oxidation of all four food models were significantly different from each other with the encapsulated oil with initiator oxidizing fastest. As for the storage at 25, 40 and 60 °C (Fig. 3(b)–(d)), the two bulk oils did not exhibit any induction time, an effect which was most prominent in the bulk oil with initiator added.

3.2.3. Storage temperature 25 °C

For storage at 25 °C (Fig. 3(b)), POV increased over the 45-day storage period for the bulk oil with and without initiator, and for the glass-encapsulated oil with and without initiator added. The statistical analysis on the development of primary lipid oxidation products showed that the rates of oxidation of all four food models were significantly different ($p < 0.0001$) from one another.

3.2.4. Storage temperature 40 °C

As may be seen from Fig. 3(c), the bulk oil with AMVN added rapidly oxidized, as expected, and the POV increased remarkably as oxidation accelerated. For the glass-encapsulated oil with AMVN, the glass-encapsulated oil without initiator, and the bulk oil without initiator the increases in POV during the same storage period were similar and not statistically different. The progression in lipid oxidation in bulk oil with AMVN was significantly different from the other food models at 40 °C ($p < 0.0001$).

Table 1
The results of the linear regression and statistical analysis on the formation of lipid hydroperoxide

Food model[a]	Storage temperature (°C)	Rate constant[b] (mmol kg^{-1} day^{-1})	Correlation coefficient	95% confidence limits	Significant grouping[c]
Oil(NI)	5	0.017 ± 0.004	0.516	0.013	a
Oil(AMVN)	5	0.088 ± 0.006	0.948	0.019	b
Glass(NI)	5	0.11 ± 0.006	0.893	0.013	c
Glass(AMVN)	5	0.18 ± 0.009	0.928	0.02	d
Oil(NI)	25	0.58 ± 0.004	0.989	0.013	e
Oil(AMVN)	25	1.79 ± 0.02	0.999	0.073	f
Glass(NI)	25	0.87 ± 0.003	0.995	0.0067	g
Glass(AMVN)	25	1.17 ± 0.005	0.975	0.011	h
Oil(NI)	40	2.25 ± 0.008	1	0.067	j
Oil(AMVN)	40	5.85 ± 0.02	0.951	0.042	i
Glass(NI)	40	3.11 ± 0.03	0.997	0.034	j
Glass(AMVN)	40	3.28 ± 0.02	0.971	0.069	j
Oil(NI)	60	7.29 ± 0.47	0.998	5.95	m
Oil(AMVN)	60	20.1 ± 0.2	0.995	2.62	k
Glass(NI)	60	7.37 ± 0.3	1	3.93	m
Glass(AMVN)	60	12.4 ± 0.5	0.992	6.74	l

[a] Oil(NI): bulk oil without initiator, Oil(AMVN): bulk oil with AMVN, Glass(NI): glass-encapsulated oil without initiator, and Glass(AMVN): glass-encapsulated oil with AMVN.

[b] Rate constants obtained from slopes of weighted linear regression of peroxide formation in Fig. 2. For storage at 60 °C, the slopes were determined from the initial period (first 4 days). SDs are given.

[c] The statistical analysis is performed within each group of storage temperatures; thus only food models at the same storage temperature can be compared. Values with the same letter are not significantly different.

3.2.5. Storage temperature 60 °C

The food models stored at the highest temperature showed different and more complex behaviour in the development of lipid hydroperoxides, Fig. 3(d). After an initial rapid increase in POV during the first 4 days of storage of the bulk oils with and without initiator, an extreme acceleration in lipid oxidation was detected and the amount of lipid hydroperoxide reached a very high concentration, then plateauing to a steady concentration. Almost the same pattern of the time courses of oxidation was found for the oils encapsulated in the glassy matrices. An initial rapid increase in POV was detected, and was followed by acceleration in the development of lipid hydroperoxides. Contrary to the bulk oils, for which POV levelled off, the amount of lipid hydroperoxide in the glassy models decreased after reaching a maximum value. At the initial oxidation stage (first 4 days), there was a significant higher rate of lipid oxidation in the bulk oil with AMVN than in the other food models. For the other food models, the initial rate of oxidation of the glass-encapsulated oil with AMVN was significant higher than the rate of oxidation of the bulk oil without initiator or the glass-encapsulated oil without initiator.

In an additional experiment, lipid oxidation in encapsulated oil in the same type of glassy matrix, with the hydrophilic initiator ABCPA added at varying concentrations, was compared, at room temperature, to oxidation in the glassy food model without the initiator added. Fig. 4 shows the development of oxidation monitored as POV. Although the initial rates of oxidation were not significantly different, oxidation developed more rapidly after 25 days of storage at room temperature in samples with the hydrophilic radical initiator added.

POV for the two bulk oils and the two glassy food models increased linearly with time at the storage temperatures 5, 25 and 40 °C, indicating zeroth order kinetics. The pseudo-zero order rate constant for lipid oxidation at temperatures 5, 25 and 40 °C was obtained

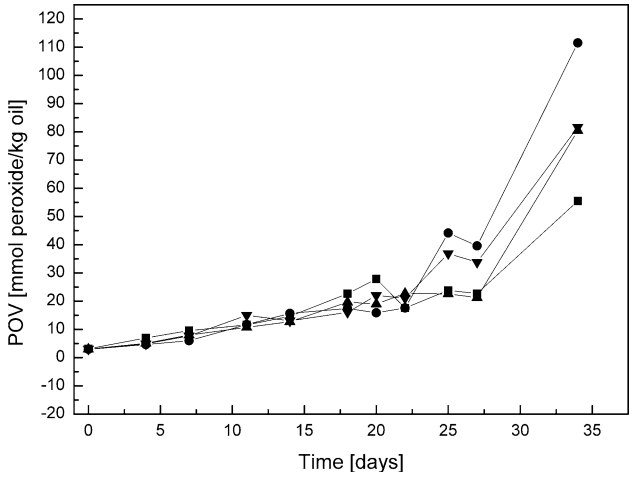

Fig. 4. Formation of lipid hydroperoxides, measured as peroxide value (POV), in oil encapsulated in a glassy matrix with the hydrophilic ABCPA free radical initiator added during storage at room temperature (approximately 25 °C), where (■): no initiator, (●): 20 mg/g oil, (▲): 50 mg/g oil, and (▼): 100 mg/g oil.

by linear regression. For storage at 60 °C, the rate constant was determined from the initial linear region of the plots (first 4 days). The concentration of peroxides was preferred to the CD value for the kinetic analysis, since the POV relates to an actual concentration, where as CD is only measured on a relative scale.

The temperature-dependence of the reaction rate was analysed by the Arrhenius equation. As may be seen from Fig. 5(a), the temperature-dependence of the rate constant may be described by the Arrhenius equation for both the bulk oils and the oils encapsulated in glassy matrices. It should be noted that, for the food models investigated, the rate constant was found to depend more significantly on temperature than on the type of food model. For the glassy food models, the temperature effect on the rate of oxidation was found to be independent of whether the lipid oxidation was initiated with AMVN or not initiated. From the Arrhenius plots, activation energies of 58.9 ± 1.7 and 59.8 ± 5.9 kJ/mol were determined for lipid oxidation in glass-encapsulated oil with AMVN and glass-encapsulated oil without initiator, respectively. For the bulk oils, the activation energy was higher but not very well-defined as the values 75.7 ± 9.4 kJ/mol for oil with AMVN and 84.9 ± 12.7 kJ/mol for oil without initiator added were obtained. In an additional analysis of the temperature-dependence of the rate constant, it was assumed, that the Arrhenius equation did not give an adequate description for the formation of lipid hydroperoxides. In Fig. 5(b), the temperature dependence of $\ln k$ was described by a second order polynomium for the encapsulated oil. Based on comparison of the residuals calculated from the linear model (Fig. 5(b)), with the residuals calculated from the quadratic model (Fig. 5(c)), a better fit is clearly obtained with the polynomium curve. The interpretation of this deviation from linearity will be discussed below.

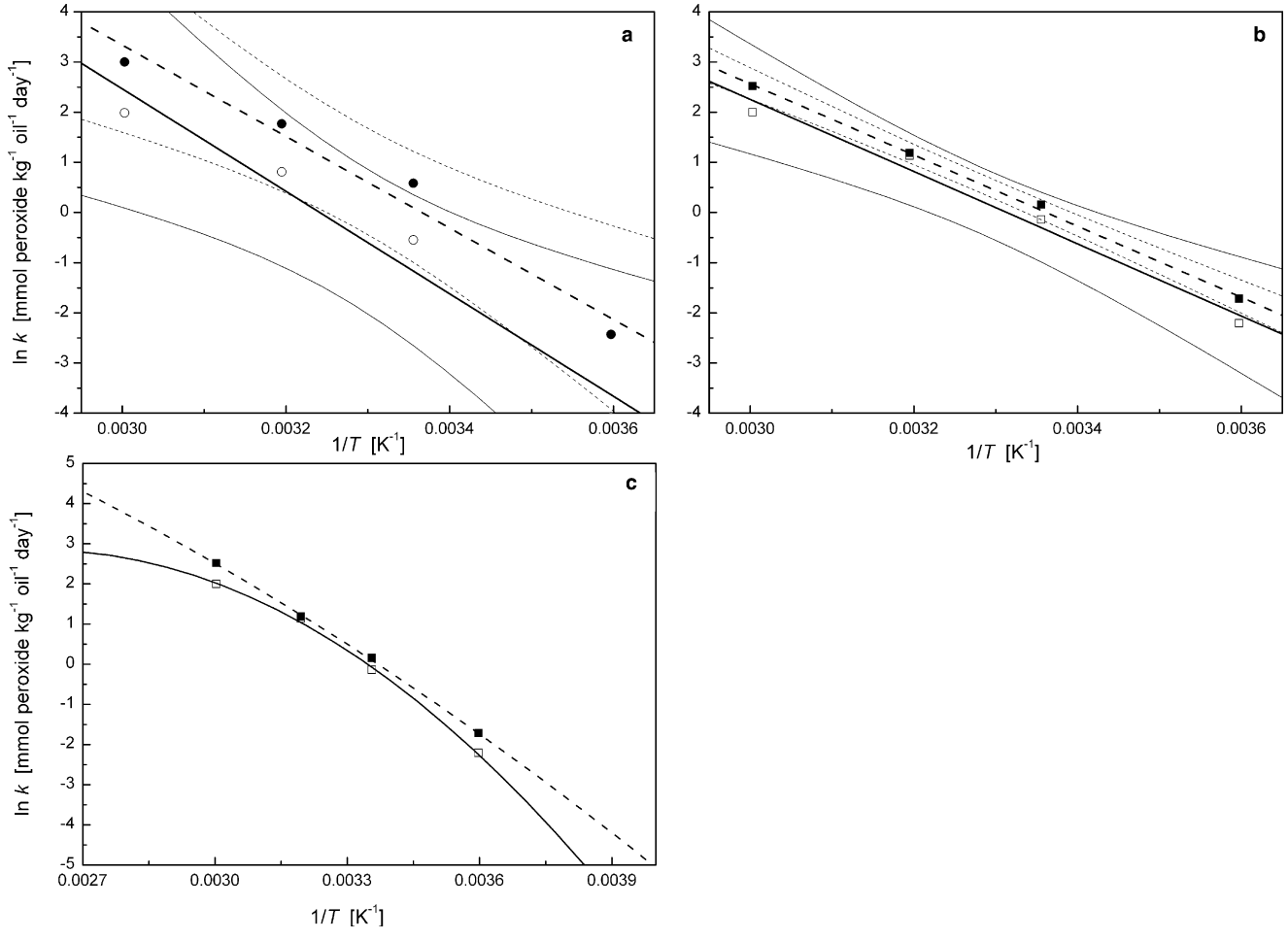

Fig. 5. Arrhenius plots of pseudo-zero order rate constants, k, for formation of primary lipid oxidation products in stripped rapeseed oil and 95% confidence intervals a: bulk oil without initiator (○), regression line (–), confidence intervals (–), and bulk oil with AMVN free radical initiator (●), regression line (– –), confidence intervals (– –). b: encapsulated in a glassy matrix without initiator (□), regression line (–), confidence intervals (–), and with AMVN free radical initiator (■), regression line (– –), confidence intervals (– –). c: The temperature-dependence of the rate constants described by a second order polynomium. (■): glass-encapsulated oil with AMVN, and (□): glass-encapsulated oil without initiator.

4. Discussion

Encapsulation technology is becoming of increasing importance for protection of oxidizable substrates such as fats and oils, for preservation of flavours, and to "convert" liquid and volatile compounds into easy-handling solid ingredients. The rate of deteriorative reactions is a function of several factors, such as porosity of the encapsulating matrix, the water activity, the storage temperature and exposure to light during storage, availability of oxygen, presence of pro-oxidants such as trace metals and presence of antioxidants. The encapsulating materials used to encapsulate liquid or volatile compounds are commonly carbohydrates, natural gums, waxes, gelatins and other proteins, depending on the physical properties of the encapsulated compounds, and efficient encapsulation depends on physical barriers established by the encapsulating matrix.

In the context of the glass theory, the glassy state is hypothesized to enforce complete immobilization of molecules, resulting in prevention of chemical reactions and protection of the encapsulated material during storage below the glass transition temperature.

In the present study, we have investigated the protection of a vegetable oil by glassy encapsulating matrix at varying temperatures below the glass transition temperature. Comparison of the temperature effects on the rate of lipid oxidation in glass-encapsulated oil and bulk oil should make it possible to achieve a mechanistic insight into the protective effect of the glassy matrix. Accordingly, the rate of formation of primary oxidation products in the encapsulated oil was compared with that of bulk oil during storage at different temperatures.

It was found that the oxidation profiles of measurements of conjugated dienes (Fig. 1) and lipid hydroperoxides (Fig. 2) showed similar trends as a function of time and temperature. As expected, the preliminary heat treatment was found to cause a significant increase in lipid oxidation in all food models, except in the case of bulk oils stored at 5 °C, in which no significant oxidation was detected. Unlike the bulk oils, the glassy food models stored at 5 °C exhibited a significant increase in peroxides during the early phase of oxidation. In regard to the glassy theory, this result shows an opposite effect to low temperature storage on the oxidation rate in encapsulated oils and bulk oils, indicating that the oil is not protected by the matrix, but appears more exposed to oxidation, most likely due to an enlarged surface of the oil. Notably, at higher temperature, the bulk oil is oxidized at a higher rate, with 25 °C being intermediate.

Another oxidation pattern was observed in food models stored at 25 °C, at which temperature the progression of oxidation in the bulk oil with AMVN added occurred at a significantly higher rate than with the other food models. Almost the same pattern was observed in the situation of storage at 40 °C. The glass matrix exhibited a retarding effect on the oxidation of both encapsulated oil with AMVN and oil without initiator, as compared to the bulk oil system. The three food models, namely oil without initiator, glass-encapsulated oil with AMVN, and glass-encapsulated oil without initiator, oxidized at approximately the same rate.

Surprisingly, the order of the rate of oxidation of food models stored at 60 °C reversed again and showed a pattern of evolution of oxidation almost similar to the case of storage at 25 °C, although considerably higher peroxide values were found in food models at 60 °C. The bulk oil with AMVN and bulk oil without initiator exhibited similar oxidation profiles at the more advanced stage of oxidation. A "typical" time evolution of the peroxide concentration curve for an oxidating oil starts with an initial low level of peroxides during an induction period, followed by an increase, finally passing through a maximum and decreasing due to decomposition of peroxides. For both bulk oils, the peroxide value increased rapidly and the amount of lipid hydroperoxides accumulated to an extremely high level. For this steady state concentration of peroxides, the rate of formation equals the peroxide decomposition rate. Consequently, the peroxide value levels off at a plateau, irrespective of whether the oil contained an initiator or not. In contrast, after the peroxide content reached a maximum in the oils encapsulated in glassy matrices, the rate of peroxide decomposition actually exceeded the rate of formation, resulting in decreasing POV values. The propagation step in the thermal oxidation process involves hydrogen abstraction from the lipid fatty acid by a radical, yielding an alkyl radical. Once formed, the alkyl radical reacts with oxygen resulting in the lipid peroxyl radical, which is capable of abstracting an H-atom from another lipid molecule, forming the lipid hydroperoxide. It is well known that, during this propagation stage, the formation of hydroperoxides depends on the oxygen concentration. Hence, the decreasing peroxide value could be a consequence of a limited oxygen supply in the oil, due to protection in the glassy matrix.

In summary, consistent results were obtained since bulk oil with AMVN added had a higher rate of formation of lipid hydroperoxides than the other three food models when stored at 25, 40 and 60 °C. Furthermore, it was found that lipid oxidation in the different food models under similar conditions of oxygen accessibility, varied primarily with the temperature and to a lesser degree with the physical environment of the oil, as may be seen from the different extents of lipid oxidation development during storage.

Compared to previous studies, the novel aspect of the present work is the study of the effect of temperature. The oxidation of the lipid was found to develop differ-

ently, depending on the temperature. A simple analysis, according to the Arrhenius equation, showed a higher energy of activation for the non-initiated oxidation in the bulk oil than for the oxidation initiated by the lipophilic radical initiator (Fig. 5(a)). Moreover, the energy of activation was higher for the bulk oils than for the encapsulated oils (Fig. 5(a) and (b)), suggesting that oxidation on the larger surface is favoured by less energy requirement.

As may be seen from Fig. 5(b), the temperature-dependence of the rate may not be adequately described by the Arrhenius equation. In Fig. 5(c), the Arrhenius plot is replaced by fitting to a second-order polynomial. The downward-curved temperature profiles in Fig. 5(c) are characteristic of the temperature-dependence of a sequence of processes when an apparent rate constant, composed of two or more rate constants for the elementary reactions, is analysed within the framework of the Arrhenius equation. One possible reaction sequence, for lipid oxidation in carbohydrate glassy systems, is a sequence of physical and chemical processes. The overall oxidation process may be divided into a physical process, i.e. oxygen permeation,

$$O_2(\text{exterior}) \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} O_2(\text{oil}),$$

followed by a chemical reaction,

$$O_2(\text{oil}) \xrightarrow{k_2} \text{lipid hydroperoxides}$$

Karel and Saguy (1991) reviewed the effects of diffusion on food stability, and proposed a similar approach to explain reaction kinetics in glassy or supercooled food systems.

Assuming reversible oxygen permeation into the oil from the matrix to reach a steady state condition for the concentration of oxygen in oil, followed by an irreversible oxidation, the observed rate constant depends on k_1, k_{-1}, and k_2 (Espenson, 1995):

$$k_{\text{obs}} = \frac{k_1 k_2}{k_{-1} + k_2}.$$

The observed rate constant, k_{obs}, will display a temperature profile that curves downward in an Arrhenius plot. To further explore this temperature profile, the transition state theory can be used to determine the energy of activation for the elementary reactions when more data points become available. The apparent activation energy can be obtained at any temperature by differentiating and it is seen that the activation energy increases with decreasing temperature, as evident from a steeper slope at lower temperatures, inviting further speculation. The reaction with the larger activation energy becomes rate-determining at low temperatures. Above a certain temperature (the isokinetic temperature), the overall reaction rate will change from being dominated by the activation-controlled process into being diffusion-controlled. In summary, the temperature profiles of the observed rate constants of lipid oxidation suggest that the chemical reaction is slow and rate-controlling at low temperatures, whereas the diffusion becomes rate-controlling at high temperatures within the temperature interval under investigation. In Fig. 6 the two processes are illustrated and it is further outlined, by a sketch of an Arrhenius plot, how the different temperature-dependence will change the rate- determining step from chemical reaction at low temperature to oxygen permeation at higher temperature. For the encapsulated oil, the physical process of oxygen permeation becoming rate-determining at higher temperature is still activation-controlled and not a simple diffusion, as was shown by non-invasive oximetry (Andersen et al., 2000). The activation energy for the oxygen permeation was found to be higher than energy of activation for the diffusion-controlled oxidation of the present study. However, the two parameters are not directly comparable since non-oxidising MCT oil was used in the previous study. The apparent activation energies are, however, highest at the lowest temperatures for both initiated and non-initiated oxidation and decrease as temperature

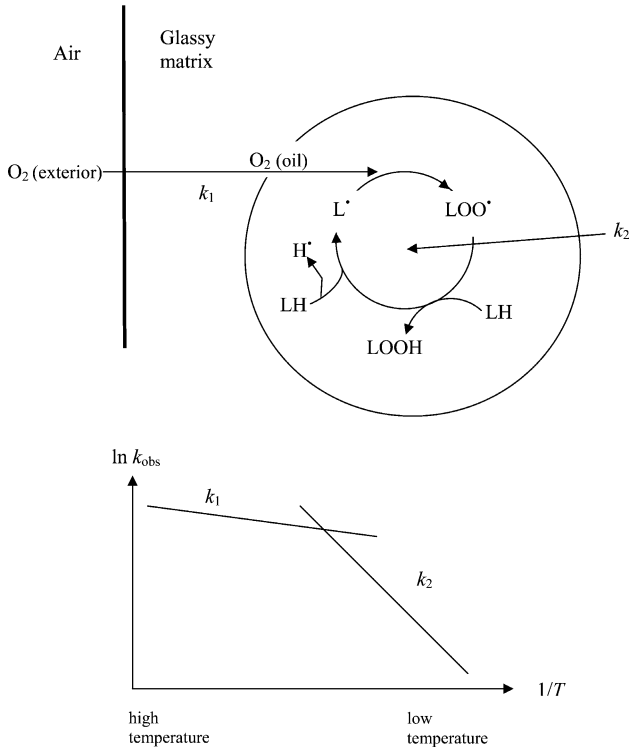

Fig. 6. A schematic representation of the physical and chemical processes involved in lipid oxidation in oil encapsulated in a glassy matrix, together with a sketch of an Arrhenius plot showing that the rate-determining step may change with temperature. k_1 is the rate constant for oxygen permeation, whereas k_2 is an overall rate constant for the propagation phase of thermal lipid oxidation.

increases. The change in the value of $E_{a(app)}$ with temperature, for lipid oxidation of glass-encapsulated oil below the glass transition temperature, is in agreement with a coupled mechanism and shows that the temperature is important for the protective property of the glassy matrix. It should be noted that the shift between the activation-controlled chemical reaction and the physical process is most pronounced for the non-initiated oxidation, Fig. 5(c). By using the elaborated model with two consecutive processes, the apparent activation energies at low temperature becomes different for the non-initiated and the initiated oxidation reaction in the glassy food models, in agreement with the oxidation reaction being rate-controlling, and in agreement with experimental observation that the initiated oil oxidises fastest at 5 °C.

The lipophilic free radical initiator, AMVN, showed a significant effect on lipid oxidation in bulk oil, but only a marginal effect on oxidation of the encapsulated oil, showing that oxygen availability is the limiting factor. Oxygen permeability through the matrix is accordingly the rate-determining step, at least at intermediate and higher temperatures. At lower temperature (5 °C), initiation of lipid oxidation, on the oher hand, becomes slower than oxygen permeation and oxidation is limited to the oxygen dissolved in the oil. Accordingly, formation of lipid oxidation products appears dependent on activation and not on diffusion through the matrix. In a previous study (Orlien et al., 2000), it was shown that a hydrophilic initiator, AAPH, in contrast to oxygen, could not penetrate the glassy matrix. AAPH is rather bulky and in the present study the series of experiments with the initiator ABCPA, with a more linear structure and which is smaller showed that this initiator, is not completely immobilized in the matrix as it, in a concentration-dependent way, promoted oxidation following a lag phase.

In conclusion, the glassy matrix yields only partial protection against lipid oxidation as oxygen permeation is possible through the glassy matrix and this process becomes rate limiting at higher temperatures. The study further confirms the conclusion from a recent ESR spin probe study of molecular mobility in frozen tuna flesh. The mobility of smaller molecules, such as O_2 and the small ABCPA initiator, is decoupled from the glass-forming proteins and carbohydrates (Orlien, Andersen, Jouhtimäki, Risbo, & Skibsted, 2004).

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