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Hydroperoxide formation in rapeseed oil encapsulated in a glassy food model as influenced by hydrophilic and lipophilic radicals

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Abstract

A glassy food model based on emulsions of sucrose, maltodextrin 10, gelatine and stripped rapeseed oil was established by freeze-drying. The glassy food model had a glass transition temperature of $61 \pm 2°C$ determined by Differential Scanning Calorimetry, and encapsulated oil as confirmed by Optical and Scanning Electron Microscopy. Radicals were generated thermally with either the hydrophilic radical generator 2,2′-azobis(2-amidinopropane)dihydrochloride, or with the lipophilic radical generator 2,2′-azobis(2,4-dimethyl-pentanenitrile), as confirmed by Electron Spin Resonance spectroscopy. Early events in lipid oxidation were monitored by analyses of conjugated dienes and peroxides during dry storage at 25°C for up to 7 weeks. Formation of lipid peroxides followed 0th order kinetics. The oxidation of the encapsulated oil was enhanced by radicals generated in the oil, and oxygen was able to diffuse through the glassy matrix in a rate determining process. The hydrophilic radicals were found not to enhance oxidation and were concluded to be immobilized in the glassy matrix.

1. Introduction

Dehydrated foods have thermal and physical properties characteristic of amorphous materials and, during dehydration, the amorphous state may encapsulate compounds such as lipids and thereby protect them from oxidation. The structural stability of the glassy matrix (Gejl-Hansen & Flink, 1977; Labrousse, Roos & Karel, 1992; Shimada, Roos & Karel, 1991), the diffusivity of oxygen (Anandaraman & Reineccius, 1986; Imagi, Muraya, Yamashita, Adachi & Matsuno, 1992), the moisture content (Labuza, Maloney & Karel, 1996; Maloney, Labuza, Wallace & Karel, 1966) and the presence of pro- and antioxidants are among the factors to be considered when the oxidative stability of products with encapsulated lipids is evaluated.

Earlier investigations of glass-encapsulated oils have used the almost instantaneous autoxidation of the lipids which occurs when a large surface area is formed and exposed to the atmosphere to detect sugar crystallization or collapse of the glassy matrix (Labrousse et al., 1992; Shimada et al., 1991). The time-dependent phenomena of crystallization or collapse have been induced in such investigations by elevating the temperature or the moisture content. Normally neither a food matrix nor food lipids are pure and, prior to dehydration, contain pro-oxidants capable of forming radicals during subsequent storage. Such pro-oxidants may initiate oxidation of the encapsulated oil, provided that oxygen and the pro-oxidants are available at the lipid–matrix interface or in the lipid. This often requires that either the pro-oxidants or oxygen can diffuse through the food matrix. The kinetics of such processes in amorphous food systems are not well understood and rate-determining steps have not been identified.

An amorphous matrix at a temperature below the glass transition temperature has an extremely high viscosity and changes in macromolecular conformation are extremely slow (Slade & Levine, 1995). This further causes low molecular mobility in the glassy state, which is why collision of reactants in the glassy matrix is limited. The glassy state is therefore expected to inhibit most chemical reactions involving reactants trapped in the glassy matrix. A number of studies have investigated the diffusion of small molecules in high-viscosity carbohydrate systems (Champion, Hervet, Blond, Le Meste & Simatos, 1997; Champion, Hervet, Blond & Simatos, 1995; Goubet, Le Quere & Voilley, 1998; Menting, Hoogstad & Thijssen, 1970; Parker & Ring, 1995; Quast & Karel, 1971; Roozen & Hemminga, 1990; Roozen, Hemminga & Walstra, 1991; Tromp, Parker & Ring,

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1997), whereas others have dealt with the consequences of the physical state on the rate of chemical reactions, such as non-enzymatic browning (Bell, 1996; Buera & Karel, 1993; Karmas, Buera & Karel, 1992; Lievonen, Laaksonen & Roos, 1998), acid-catalysed sucrose hydrolysis (Buera, Chirife & Karel, 1995; Schebor, Buera, Chirife & Karel, 1995) and aspartame degradation (Bell & Hageman, 1994). General conclusions seem to be that the diffusivity of molecules in a carbohydrate system increases dramatically at temperatures close to the glass transition temperature (Roozen & Hemminga, 1990; Roozen et al., 1991; Tromp et al., 1997). A low molecular diameter of the diffusing molecule is also of importance and most pronounced in cases where no interaction between diffusing molecules and the glassy matrix (such as by hydrogen bonding) is expected (Goubet et al., 1998; Menting et al., 1970; Parker & Ring, 1995). Seen in relation to the fact that an increase of the molecular weight of the molecules forming the glassy matrix results in increased diffusion coefficients, most likely due to a less dense packaging of the glassy matrix, this supports a theory of the diffusant travelling in cavities in the glassy matrix (Parker & Ring, 1995; Roozen et al., 1991; Tromp et al., 1997). Theoretical models, such as the WLF model (Williams, Landel & Ferry, 1955), describing viscosity as a function of temperature, can furthermore not be used for describing diffusions of small molecules in high viscosity systems, as concluded by Parker and Ring (1995) and Champion et al. (1997) from experimental work. This is in agreement with the general results obtained from kinetic modelling of reactions in glassy states, from which it seems that molecular mobility and diffusion rate cannot be used as the sole parameters for quantitative descriptions, and that water activity, moisture content and temperature, as well as the size of the reacting molecules, are among other important factors to be taken into consideration.

As for lipid oxidation, the situation is expected to be even more complex, since lipid oxidation proceeds through an initiation phase followed by propagation and subsequent formation of secondary oxidation products. However, improved experimental techniques may now be combined, and we have embarked on studies of lipid oxidation in food with glassy states. In the present study we develop a glassy food model which does not collapse during storage at 25°C and focus on initiation of oxidation of an oil encapsulated in a glassy matrix in comparison to bulk oil, using both hydrophilic and lipophilic radicals.

2. Materials and methods

2.1. The glassy food model

A glassy food model composed of carbohydrates, proteins and polyunsaturated lipids was established. The criteria for establishing the glassy food model were that it should be capable of forming a glassy state at ambient temperature and that the lipid is encapsulated in the glassy matrix. Furthermore, the glassy food model had to maintain its structure at 40°C, during incubation at this temperature. Emulsions made with different concentrations of sucrose (extra pure, Merck, Darmstadt, Germany) and maltodextrins (from maize starch, Fluka, Neu-Ulm, Switzerland), together with 5% gelatine as emulsifier (225 bloom, from calf skin, Aldrich, Milwaukee, USA) and 25% rapeseed oil (Aarhus Oliefabrik A/S, Aarhus, Denmark) were freeze-dried and examined by Differential Scanning Calorimetry (DSC) before choosing the optimal glassy food model (cf. Table 1). The glassy food model chosen for the study consisted of 25% sucrose and 45% maltodextrin 10 (dextrose equivalent of 10) as glass former. The rapeseed oil used was analysed to have approximately 60% oleic acids, 21% linoleic acid and 10% linolenic acid as the main fatty acids using standard GC-methods following transformation to methyl esters (Jart, 1997). The rapeseed oil was stripped according to the method of Lampi, Hopia, Ekholm and Piironen (1992) which yields an oil with less than 10 ppm α-tocopherol, as quantified by the method of Jensen et al. (1997). Carbohydrates were dissolved in hot water (MilliQ, Millipore Q-Plus, Millipore Corporation, Bedford, MA), and gelatine was dissolved in boiling MilliQ water. The solutions were mixed, cooled on ice and divided into three parts. To one portion the hydrophilic radical generator 2,2′-azobis(2-amidinopropane)dihydrochloride (AAPH from Wako chemicals GmbH, Neuss, Germany) was added (0.60 mg/g oil), to the second portion the lipophilic radical generator 2,2′-azobis(2,4-dimethylpentanenitrile) (AMVN from Wako chemicals GmbH, Neuss, Germany) was added in the same concentration, while the last portion served as a blank. The stripped rapeseed oil (25%) was added to each part, and emulsified using a homogenizator (Ultra Turrax, Jankel & Kunkel IKA-Labortechnich, Staufed, Germany) at 9500 rpm for 1 min in an ice bath. The emulsions (435 g, 82% water) were stored for a few minutes in an evacuated desiccator, frozen (−50°C, 15 h) and freeze-dried ($p \sim 0.1$ mbar, 48 h). The dried matrices were stored in an evacuated desiccator over P_2O_5 (relative humidity 0.0%) for further dehydration (room temperature, 36 h). The glassy food models were washed three times with hexane (HPLC Applications, Fisher Scientific, Leicestershire, UK) to remove non-encapsulated surface oil and stored in an evacuated desiccator to dry off hexane. As reference food models, stripped rapeseed oil and stripped rapeseed oil with AMVN (0.60 mg/g oil) without glass-phase were used. 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 in an incubator at 25°C. Karl Fischer titration (Mettler DL18, Schwerzenbach,

Table 1
Glass transition temperatures as determined by DSC and water content as determined by Karl Fischer titration of the glassy food models with 70% carbohydrates, 5% gelatine and 25% rapeseed oil with a varying ratio between maltodextrin 10 and sucrose

Maltodextrin 10 (%)	0	15	25	35	45	55	70
Sucrose (%)	70	55	45	35	25	15	0
T_g (°C)	27	34	49	55	61	n.d.[a]	n.d.
Water content (%)	1.5	1.5	2	2.5	3.7	4.9	3.7

[a] n.d., not detected.

Switzerland) was used to determine the water content in the glassy food models by incubating samples in methanol (< 0.005% H_2O, Merck, Darmstadt, Germany) for water extraction for 24 h. Water activity was measured using an AquaLab CX2 (Decagon Devices, Inc., Washington, USA).

2.2. Differential scanning calorimetry

The glass transition temperature was determined using a DSC 820 from Mettler Toledo (Schwerzenbach, Switzerland). The DSC 820 is based on the heat flux principle and cooled with liquid nitrogen. Calibration of heat flow and temperature was performed with indium as standard (mp = 156.6°C, $\Delta_{fus}H = 28.5$ J/g, Mettler Toledo Calibration Kid, ME 119442). The linearity of the calibration was verified with zinc (mp = 419.5°C, $\Delta_{fus}H = 107.5$ J/g, Mettler Toledo Calibration Kid, ME-119441). 5–15 mg of sample were hermetically sealed into 40 μl aluminium DSC crucibles (ME 27331). As reference, an empty sealed aluminium crucible was used. Heatings at a rate of 5°C/min were used over an appropriate temperature range. Duplicates and rescans verified in each case 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. Microscopy

Optical Microscopy (OM) and Scanning Electron Microscopy (SEM) were used to investigate the structure of the glassy food model: OM for subsurface structure, and SEM for surface morphology. For OM examination, a Zeiss Axiolab microscope with Abbe condenser 0.9/1.25 for bright-field, dark-field, and phase contrast Ph1, Ph2, Ph3, was used (Carl Zeiss, Jena, Germany). An adequate amount of the glassy food model was rehydrated with a drop of water, and the approximate size of the oil droplets was determined. SEM was used to study the fracture planes of the glassy food model. Flakes of about 5×5×2 mm were placed on aluminium stubs, gold–palladium sputtered (Polaron SC 7640 Sputter Coater), and examined in a JEOL JSM T-20 SEM accelerating at 20 kV.

2.4. Electron spin resonance (ESR) spectroscopy

The radicals formed in the glassy food models were detected with ESR. The ESR experiments were carried out using an ECS 106 spectrometer (Bruker Analytische Messtechnik GmbH, Karlsruhe, Germany) equipped with an ER 4103 TM cylindrical mode X-band resonator. Typical instrument parameters used were: Microwave power 20 mW, modulation frequency 100 kHz, modulation amplitude 1.01 Gauss, conversion time 81.92 ms, time constant 81.92 ms, and resulting sweep time 83.89 s. The flakes were contained in cylindrical 710-SQ quartz ESR tubes with an inner diameter of 4 mm (Wilmad Glass Co., Buena, NJ). Separate series (Table 2) of the food models were prepared for ESR measurements, as spin trap were necessary in order to detect the radicals formed. Series 1 was prepared for detecting radicals in the glassy food model with no radical generators added. Glassy food models were prepared containing the spin-trapping agent PBN (*N-tert*-butyl-alpha-phenylnitrone, Molecular Probes Europe B.V., Leiden, The Netherlands) for detecting oil-soluble radicals or the spin-trapping agent POBN (4-pyridyl-1-oxide-*N-tert*-butylnitrone, Sigma, St. Louis, MO) for detecting water-soluble radicals. Series 2 was prepared for observing radicals generated by thermal decomposition of either AMVN or AAPH. When detecting AMVN radicals, the stripped rapeseed oil was replaced by a saturated medium chain triglyceride oil (MCT-oil, Brøste, Lyngby, Denmark), as no radical-consuming reactions are expected to take place in the non-oxidizable MCT oil. When detecting hydrophile AAPH radicals, no oil was added to the glassy food model. The spin-trap agents were added in a concentration of 2.0 mg/g.

A third series was prepared with AMVN and PBN dissolved in MCT oil and AAPH and PBN dissolved in water showing the free mobility spectra of the radical generators.

The partition coefficient of AMVN in the glassy food model was measured by preparing the oil-in-water emulsion with AMVN and reseparating the phases by centrifugation. PBN was added to each phase and the amount of radicals measured by ESR. The water phase was freeze-dried prior to analysis. All spectra were

Table 2
Series prepared for electron spin resonance detection of radicals formed in oil and in the glassy food model

Series	Purpose	System composition
1	To detect radicals formed in the glassy food model with no external radical generators added	Glass + rapeseed oil + PBN Glass + rapeseed oil + POBN
2	To detect the radicals generated from the thermal decomposition of AMVN and AAPH	Glass + MCT oil + PBN + AMVN Glass + PBN + AAPH
3	To show the free mobility spectra of the radical adducts of AMVN-PBN and AAPH-PBN in liquid references	MCT oil + PBN + AMVN Water + PBN + AAPH

recorded following heat incubation. The spin adducts were measured at 50°C.

2.5. Lipid oxidation

Oxidation was monitored by analysis of conjugated dienes and peroxides. Conjugated dienes were measured directly by dissolving 0.10 g powder of the glassy food model in 7.0 ml of ethanol and centrifuging. The absorption spectrum (200 nm $\leq \lambda \leq$ 300 nm) of the supernatant was recorded with a HP 8452 UV–vis diode array spectrophotometer (Hewlett Packard CO., Palo Alto, CA). 10 mg of the bulk oils were dissolved in 3.0 ml of ethanol and the absorption spectrum was 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 until analysis. The peroxide values were obtained using a method based on IDF (1991) standard, entailing reaction of peroxides with iron(II) chloride and ammonium thiocyanate, followed by absorbance measurement at 500 nm after a reaction time of exactly 5 min, recording the red iron(III) thiocyanate complex and using a standard curve based on H_2O_2.

2.6. Statistical analyses

The measurements of the conjugated dienes and peroxide value were subjected to linear model analysis of variance including main effects of storage time and the radical generator content and interaction as fixed factors using SAS version 6.12 software (SAS Institute Inc., Cary, NC). Duncan's Multiple range test was applied to compare means of least significant difference between the main effects. The experiments were performed four times with modifications of storage length, radical generator concentrations and oxidation measurements. The results were found to be consistent regarding the overall results, though not directly comparable regarding the rate and level of oxidation reached, i.e. absolute values for conjugated dienes or peroxide values.

3. Results

3.1. The glassy food model

Emulsions with varying compositions were prepared and freeze-dried according to the scheme of Fig. 1 in order to develop a glassy matrix that would encapsulate the oil and retain a $T_g > 50$°C. A total of 70% (weight of dry matter) of carbohydrates was found appropriate for such a system, and the contents of sucrose and maltodextrin 10 were varied according to Table 1. T_g and water content were determined from the DSC thermograms and from Karl Fischer titration, respectively, and both T_g and water content were found to increase with increasing fractions of maltodextrin 10 up to approximately 50% (Table 1). No glass transition could be detected with maltodextrin 10 contents above 45%, which could be due to a general degradation of the system at higher temperatures. 5% gelatine was added in each case for encapsulating the (up to 25%) rapeseed oil added. No difference in T_g of the glassy matrix with 45% maltodextrin 10 and 25% sucrose was noted when varying the oil content (0, 1, 5, 10 and 25% of the dry matrix). Higher oil contents than 25% were not tested, as such higher concentrations were considered of less relevance from a practical point of view and also would

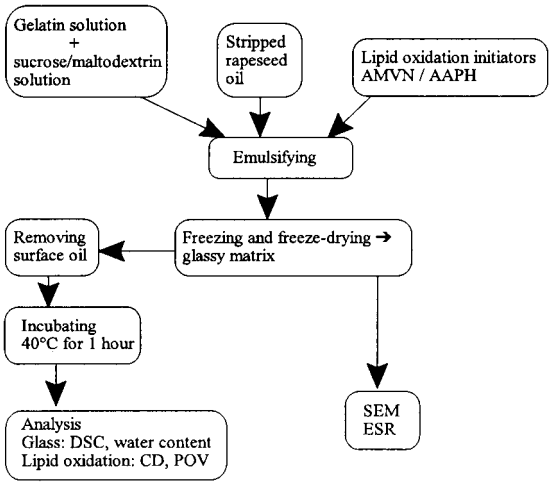

Fig. 1. Scheme for preparation of glassy food model and its analysis.

make diffusion distances too short for kinetic modelling. The freeze-drying of the above mentioned emulsions led to the formation of a glassy matrix, which successfully entrapped a high fraction of the rapeseed oil for periods longer than 7 weeks. The volume of the glassy food model was 20 cm^3 and could displace 5 ml of hexane, giving a free volume of 15 ml. Non-encapsulated oil (10–12%) on the surface of the glassy food model was washed out by hexane immediately after freeze-drying. The developed glassy food model (45% maltodextrin 10, 25% sucrose, 5% gelatine and initially 25% oil) had a water content of $2.0 \pm 0.4\%$ and a water activity of 0.15 as measured at intervals throughout the storage period. The DSC scans of the glassy food models showed a step change in specific heat at $60.7 \pm 1.5°C$ identified as the glass transition temperature. The glass transition temperature was accordingly well above 50°C, and there was no sign of recrystallizations or collapse taking place in the matrix during heat incubation and subsequent storage.

Examination of the glassy food models by OM showed entrapped oil droplets of approximately 20 μm in diameter. Smaller bubbles identified as air were also visible in the rehydrated glassy food model but not within or adhering to the oil droplets. The Scanning Micrograph of the flakes (Fig. 2) showed the glassy food model having a relatively smooth surface and a rather fragile and porous structure. The structure is caused by cavities formed by ice crystals during freezing. The holes (Fig. 2C) may originate from sublimated ice crystals or air bubbles, as for the spherical hole in the upper right corner.

3.2. Radical formation and lipid oxidation

The radicals generated by the thermal cleavage of the azo compounds were found to be trapped by PBN. Spectra of the AMVN-PBN spin adduct and AAPH-PBN spin adduct formed in the glassy food models are seen in Figs 3B and 4B, respectively. Experiments without radical generator but with spin traps (series 1) showed the glassy food model to be ESR-silent (Figs 3A and 4A). Since the oil-soluble spin trap PBN would have trapped radicals formed in the oil, and the POBN spin trap would have trapped water-soluble radicals, it was concluded that radicals were not formed at a detectable level in the food model itself (data for the POBN spin trap not shown). AMVN and PBN in bulk MCT-oil

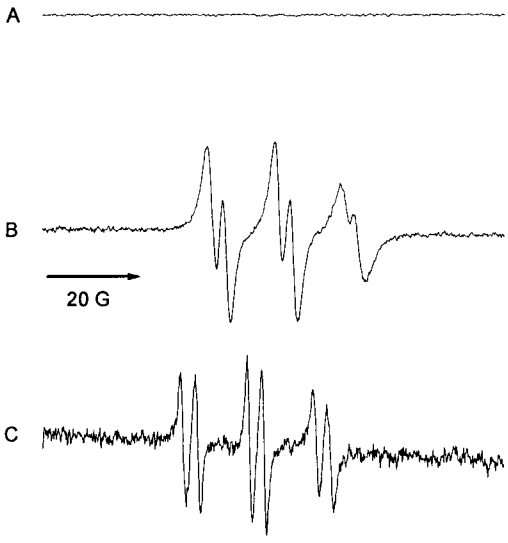

Fig. 3. ESR spectra of PBN spin adducts: (A) the glassy food model with stripped rapeseed oil after 15 min incubation at 50°C without radical generator; (B) the glassy food model encapsulating 25% MCT-oil with AMVN after 30 min at 50°C; (C) AMVN in MCT-oil after 30 min at 50°C. The intensity of the spectra (B) and (C) is not directly comparable.

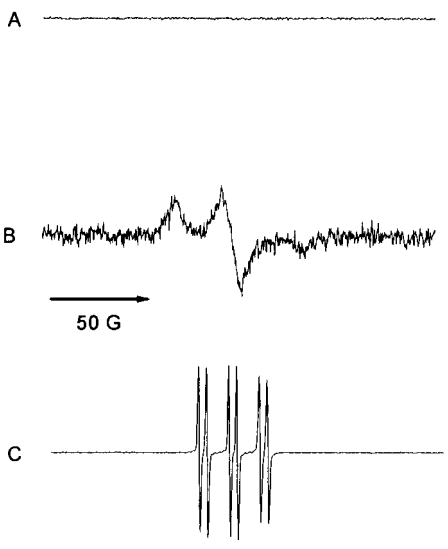

Fig. 4. ESR spectra of PBN spin adducts after 15 min incubation at 50°C: (A) the glassy food model with stripped rapeseed oil; (B) the glassy food model with AAPH; (C) AAPH in water. The intensity of the spectra (B) and (C) is not directly comparable.

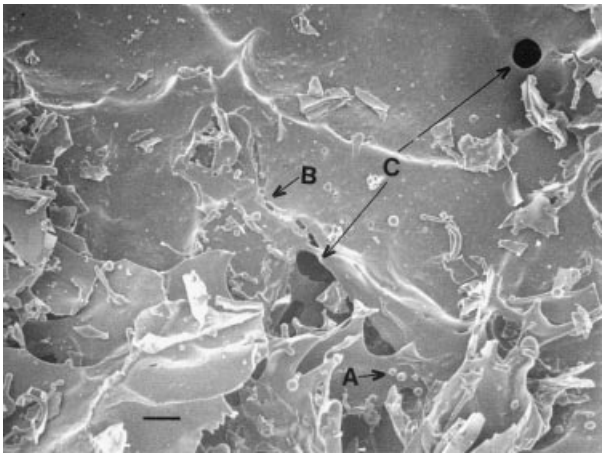

Fig. 2. Scanning electron micrograph of the glassy food model. Bar = 150 μm. A: Protrusions, B: Fracture in the surface, C: Holes.

and AAPH and PBN in bulk water, serving as liquid references to the corresponding glassy food models, show the free mobility of the spin adducts (Figs. 3C and 4C). The AMVN-PBN spin adduct spectrum consisted of a triplet of doublets ($a_N = 14.1$ and $a_H = 3.1$) (Fig. 3C). The AAPH-PBN spin adduct spectrum also consisted of a six-line spectrum ($a_N = 15.4$ and $a_H = 4.0$) (Fig. 4C). The lines in the APPH-PBN adduct in water are narrowed compared to the AMVN-PBN adduct in the oil due to a lower viscosity of water compared to the MCT-oil. On comparison of the spectra of AAPH-PBN spin adduct formed in the glassy matrix and in water (Fig. 4B and C), it may be seen that the line broadening is significant. This line-broadened spectrum is typical for powder spectra and may be explained by anisotropic motion caused by a high viscosity causing a very slow molecular motion of the spin probe on the ESR time scale. The spectrum of the AMVN-PBN adduct is also broadened when comparing the oil sample (Fig. 3C) with the glassy food model (Fig. 3B), but to a lesser degree, and the spectrum of Fig. 3B does not resemble a powder spectrum. Rather this broadening may be explained by oxygen broadening (Swartz & Glockner, 1989) since oxygen is trapped between the glassy food model flakes in the ESR tube but not in the homogeneous oil.

AMVN was found to concentrate fully in the oil and not in the glassy matrix, as found by comparing the ESR spectra of the glass phase to the oil phase of the reseparated glassy food model. Comparing relative intensities of the ESR signals of the bulk oil to the oil phase, initially containing equal amounts of AMVN, furthermore confirmed that all AMVN added to the glassy food model was found in the oil phase.

The formation of primary oxidation products in the glassy food model following heat activation was measured as conjugated dienes (Figs. 5 and 6) and peroxides (Fig. 7). As for the conjugated dienes, absorption maxima at 236 and 244 nm are partly obscured by background absorption, but second derivative spectra provide relative concentrations of *trans*, *trans*-conjugated dienes and of *cis*, *trans*-conjugated dienes, respectively (Corongiu, Poli, Dianzani, Cheeseman, Slater & Slater, 1986). *Cis*, *trans*-conjugated dienes were formed prior to *trans*, *trans*-conjugated dienes in the present system, in agreement with what is also seen in homogeneous oils (Corongiu et al., 1986). The relative amount of conjugated dienes was measured as $d^2A/d\lambda^2|_{254} - d^2A/d\lambda^2|_{244}$ during the initial phase of oxidation and, when the minimum moved to 236 nm, the amount of conjugated dienes was expressed as $d^2A/d\lambda^2|_{254} - d^2A/d\lambda^2|_{236}$ (Figs. 5 and 6). The formation of conjugated dienes confirmed that the lipid in the glassy food models and bulk oil had oxidized. Lipid oxidation was found to be at the same low level for all glassy food models and bulk oil immediately after incubation. After approximately 3 days of sub-

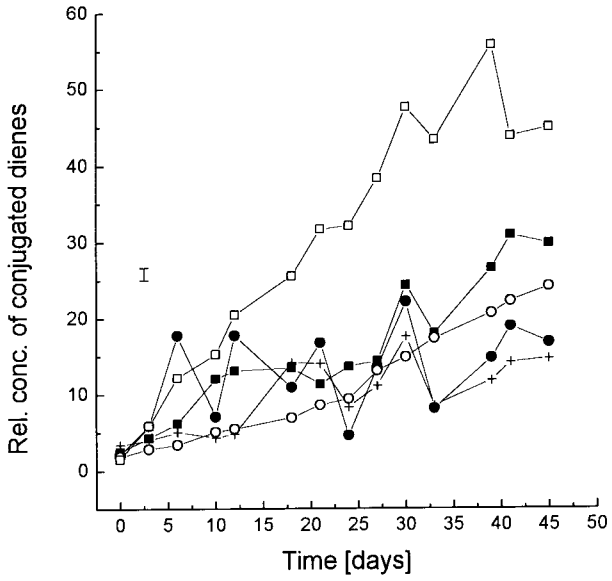

Fig. 5. Formation of conjugated dienes in the glassy food models during 45 days of storage at 25°C following heat incubation at 40°C: ■ = glassy food model with AMVN; + = glassy food model with AAPH; ● = glassy food model without initiator; ○ = bulk oil without initiator and □ = bulk oil with AMVN. Conjugated diene concentrations are on a relative scale and are the sum of *trans*, *trans* and *cis*, *trans* isomers as detected by second derivatives absorption spectroscopy. Bar is the standard deviation.

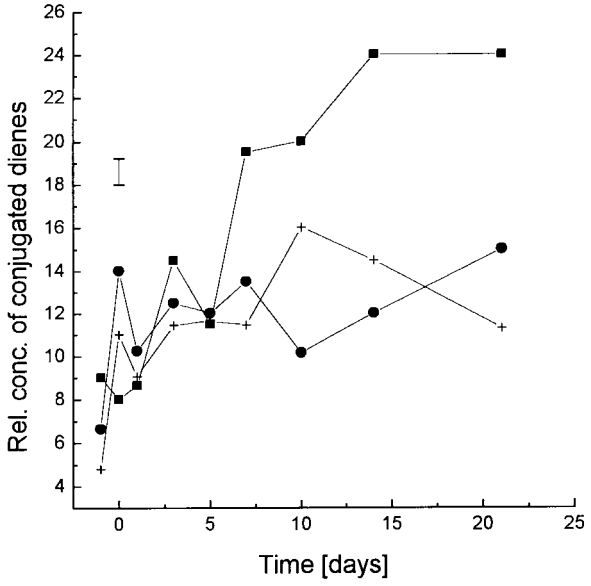

Fig. 6. Formation of conjugated dienes in the glassy food models with encapsulated oil during 21 days of storage at 25°C following heat incubation at 40°C; ■ = glassy food model with AMVN; + = glassy food model with AAPH; ● = glassy food model without initiator. Conjugated diene concentrations are on a relative scale and are the sum of *trans*, *trans* and *cis*, *trans* isomers as detected by second derivatives absorption spectroscopy. Bar is the standard deviation.

sequent storage, the rates of oxidation were clearly different. AMVN is seen to efficiently initiate oxidation of the bulk oil (Fig. 5). Although the glassy food models show a very high day-to-day variation, especially the glassy food model with no initiator, the statistical analysis showed that the glassy food model with AMVN has a significantly higher rate of formation of conjugated dienes than the other glassy food models ($p < 0.0001$), the other glassy food models not being significantly different. In a separate experiment, glassy food models without initiator and glassy food models with the lipophilic initiator AMVN and with the hydrophilic initiator, AAPH, were further compared, and the results (Fig. 6) confirm the initiating effect of the AMVN radical generator on the oxidation of the oil as compared to the AAPH radical generator and no initiator.

The high day-to-day variation found for the conjugated dienes analysis in both series of experiments warranted a more robust analytical method to be used for measurement of the primary lipid oxidation products. Peroxides were accordingly determined (Fig. 7). Similarly to what was found for the conjugated dienes, the level of peroxidation remain the same for all glassy food models during the initial 2 days. After 3 days, the peroxidation in the glassy food model with AMVN proceeds with a significantly higher rate than in the other glassy food models ($p < 0.0001$), the latter two not being significantly different. The peroxide values for the three glassy food models increase linearly with time, indicating pseudo zeroth-order kinetics as confirmed by linear regression, from which the rate constants shown in the legend of Fig. 7 were calculated. In a similar experiment, peroxides were determined in the glassy food model with half the concentrations of the radical generators AMVN and AAPH; regression lines of results are shown in Fig. 7. In spite of generating only half the radical concentration, the rate constants were not significantly different in the two experiments. The saturation solubility of oxygen in vegetable oils is in the range of 4.5–5.6 mmol/kg (Battino, 1981), but the actual level of oxygen in the oil is expected to be lower, due to the evacuation during preparation and freeze-drying. As the level of peroxides reaches levels above 10 mmol/kg, it may be concluded that the observed oxidation cannot be accounted for by lipid-dissolved oxygen alone.

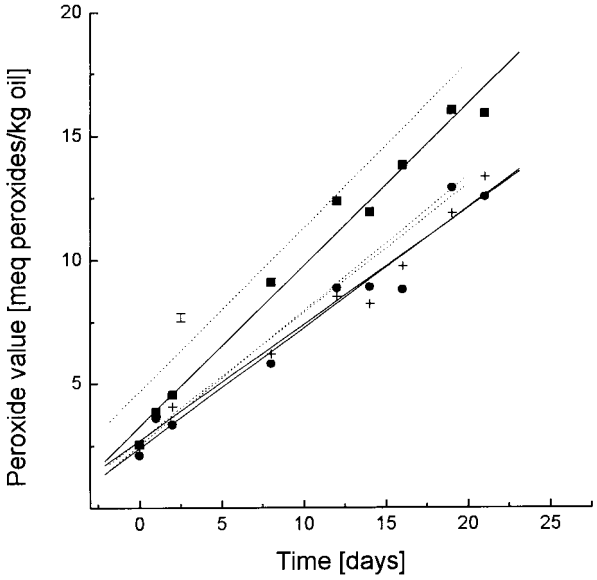

Fig. 7. Formation of peroxides in the glassy food models during 20 days of storage at 25°C following heat incubation at 40°C. ■ = glassy food model with AMVN (0.60 mg AMVN/g oil), k_{AMVN} 0.66 ± 0.03 mmol kg^{-1} day^{-1}; + = glassy food model with AAPH (0.64 mg AAPH/g oil), $k_{AAPH} = 0.47 \pm 0.03$ mmol kg^{-1} day^{-1}; ● = glassy food model without initiator, $k_{NI} = 0.48 \pm 0.04$ mmol kg^{-1} day^{-1}. k is the 0th-order rate constant of peroxide formation determined by linear regression shown with a solid line. Dotted lines show linear regression lines for similar experiment with half the concentration of radical generators, that is 0.29 mg AMVN/g oil, $k_{AMVN} = 0.66 \pm 0.04$ mmol kg^{-1} day^{-1}, 0.35 mg AAPH/g oil, $k_{AAPH} = 0.53 \pm 0.02$ mmol kg^{-1} day^{-1} and $k_{NI} = 0.52 \pm 0.01$ mmol kg^{-1} day^{-1}. Bar is the standard deviation.

4. Discussion

Lipids in dried foods such as milk powder, dried soups and biscuits are often encapsulated in an amorphous food matrix of saccharides and proteins. Lipid oxidation is known to be more significant at interfaces than in bulk and, due to the large surface area of encapsulated oil, it is often found to be rather vulnerable to lipid oxidation. On the other hand, encapsulation in a glassy matrix could be expected to give some protection from oxidation by: (i) preventing or at least limiting oxygen diffusion through the glassy matrix to the lipid phase; and by (ii) protecting the oil from oxidation through immobilizing radicals formed by pro-oxidants in the hydrophilic phase. For milk powder, radicals generated during storage have recently been shown to be immobilized and, on reconstitution of the milk, to initiate formation of secondary lipid oxidation products (Stapelfeldt, Nielsen & Skibsted, 1997). The developed glassy food model is less complex than most real dried food systems like milk powder, and allows controlled generation of radicals in either the lipid phase or in the glassy matrix encapsulating the lipid during incubation without collapse or other structural changes related to the glassy matrix. The glassy food model is believed to be of value also for future investigations, since storage conditions often encountered for milk powders during tropical conditions can be imitated without collapse of structure for temperatures up to at least 55°C.

In the present study we have focused on the formation of primary oxidation products. We found that peroxide value yielded more reproducible results than conjugated

dienes, most likely due to differences in optical spectra of different isomers. The primary oxidation developed in the glassy food model is dependent on the presence of radical generators and the localization of pro-oxidants has a significant effect on the rate of oxidation. The formation of conjugated dienes showed that bulk oil with AMVN, with no limit concerning oxygen accessibility, oxidized at a higher rate than any other food model. In the glassy food model, with AMVN in the same concentration in the oil phase, the oxidation proceeded with a lower rate, indicating that the oxygen accessibility is the limiting factor. The lipophilic radical generator (AMVN) significantly increased the formation of peroxides compared with the other glassy food models (Fig. 7). The two experiments, differing in the concentration of added radical generators, showed the same level of peroxidation and in both experiments oxidation proceeded with a higher rate when the glassy food model contained AMVN, showing that the amount of lipophilic radical generator cannot be the limiting factor. When relating this to the concentration of peroxides exceeding the solubility of oxygen in the oil by a factor of more than 4 during 3 weeks of storage, it may be concluded that oxygen diffuses but not freely through the glassy matrix into the oil. The observation of 0th-order kinetics could indicate that oxidation is limited by the rate of oxygen diffusion. This is supported by the results from Anandaraman and Reineccius (1986), who in a similar system observed 0th-order kinetics of formation of the epoxides during oxidation of encapsulated orange peel oil. Radicals formed by the hydrophilic radical generator (AAPH) have been shown to be efficient initiators of lipid oxidation by Yamamoto, Haga, Niki and Kamiya (1984). The ESR results confirm that AAPH generate radicals in the glassy matrix following heat treatment and they were not seen to react with other constituents in the glassy matrix. The comparable rate of peroxide formation in the glassy food model containing AAPH and in the glass without initiators shows that the glassy state in practice immobilizes the AAPH radicals formed in the hydrophilic phase and in this manner protects the oil from oxidation. The formation of radicals from AAPH is a unimolecular process and as such only to a lesser degree affected by the viscosity of the medium. The powder spectrum (Fig. 4) of the AAPH radicals in the glassy food model shows that the rotation of the trapped radicals certainly is hindered, but it may be noted that some rotational mobility still prevails, as measured in a similar system by Roozen et al. (1991).

Other reports have dealt with the issue of oxidation of encapsulated oils in glassy carbohydrate matrices, with conflicting results regarding the ability of oxygen to diffuse through the glassy matrix (Anandaraman & Reineccius, 1986; Imagi et al., 1992; Labrousse et al., 1992; Shimada et al., 1991). In the work of Shimada et al. (1991) and of Labrousse et al. (1992), methyl linoleate was encapsulated in lactose–gelatine and sucrose–lactose–gelatine matrices, respectively. In these cases no oxidation of oil was recorded during encapsulation, and it was not until the matrices crystallized or collapsed, where the oil was fully or partially released, that a rapid oxidation was recorded. Anandaraman and Reineccius (1986) and Imagi et al. (1992), on the other hand, measured the oxidation of oil while encapsulated in maltodextrin matrices. Anandaraman and Reineccius (1986) observed an increasing protection of orange peel oil with increasing dextrose equivalents of the encapsulating maltodextrin. In our glassy food model, consisting of maltodextrin, sucrose and gelatin, the diffusion of oxygen is not hindered but is the limiting parameter for oxidation of the encapsulated oil. This supports the idea that a matrix consisting of larger and more randomly lengthed molecules may have a larger free volume, facilitating the diffusion of smaller molecules. However, the radical generated from AAPH with a molecular weight of 121 g/mol, which is still small compared to the larger matrix molecules, did not diffuse on a practical time scale. This indicates that a critical molecular size for diffusion in the present system seems to be between that of oxygen and that of the AAPH-derived radical.

5. Conclusion

In this study we have developed a glassy food model which maintains the amorphous structure and encapsulates oil up to 7 weeks. The lipid oxidation results show that the glassy matrix is not able to protect the encapsulated oil from oxidation, as oxygen can diffuse through the free volume of the glassy matrix. The formation of primary oxidation products reveals that the lipid oxidation in the glassy food model follows 0th-order kinetics with the diffusion of oxygen as the limiting factor for oxidation of the encapsulated oil. The hydrophilic radicals (AAPH) generated in the glassy matrix did not initiate oxidation of the encapsulated oil, which is ascribed to an immobilizing of the hydrophilic radicals by the dense glassy matrix. Furthermore, the ESR results showed a very slow molecular motion of the hydrophilic spin probe. This indicates that a critical molecular size for diffusion in a maltodextrin–sucrose glassy matrix is between that of oxygen and that of the AAPH-derived radicals.

Our current research efforts attempt to measure the rate of oxygen diffusion through the glassy food model by use of oximetry.

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