Effect of Relative Humidity on the Oxidative Stability of Microencapsulated Sea Buckthorn Seed Oil

RIITTA PARTANEN, PIJA HAKALA, OLLI SJÖVALL, HEIKKI KALLIO, AND PIRKKO FORSELL

ABSTRACT: The effect of relative humidity (RH) (20 °C; RH 11%, 54%, 55%) on oxidative stability microencapsulated sea buckthorn seed oil was studied using bulk oil as a reference. Microcapsules were prepared by spray-drying using maltodextrin-gum arabic (MD/GA) and corn starch sodium octenyl succinate deivate (HiCap) as the wall materials. The influence of the physical state of the wall material was also evaluated. Under dry conditions, the microencapsulated oils were most stable, but the oxidation of the bulk oil was accelerated. At 20 °C and at RH 11%, the peroxide value of the bulk oil exceeded 20 meq/kg within 1 wk. Microencapsulation prolonged the shelf-life of the oil from 1 wk to 2 mo at 20 °C, when the encapsulating matrix was in glassy state. In conditions in which the HiCap matrix was in a rubbery state (RH 54%, 20 °C), the oxidation proceeded very quickly, reaching a peroxide value of 20 meq/kg just after 1 wk. Caking and collapse of the microcapsule powder were observed in the rubbery state. At accelerated conditions (50 °C; RH 11%, 30%, 45%), the oxidation was noticeably fast, not only in the bulk oil but also in the MD/GA matrix, even in the glassy state. The behavior in the HiCap matrix was more complex as the amount of peroxides started to decrease in time. This was assigned to the structural collapse in HiCap microcapsules. The behavior of the microencapsulated oils under accelerated conditions did not correlate with their behavior at 20 °C.

Keywords: sea buckthorn oil, microencapsulation, oxidation, relative humidity, spray-drying

Introduction

Sea buckthorn (Hippophae rhamnoides) seed and pulp oils have been traditionally used for treating problems of skin (Vlasov 1970; Manku and others 1984; Zhao 1994) and mucous membranes (Nikitin and others 1989; Qiu and Qiao 1997), mainly in China and Russia. Recently, the nutritional value of the berries has also been recognized in the western world because of their special chemical composition (Yang and others 1999; Erkkola and Yang 2003). The oil content is generally high in both seeds and soft parts of the berries. Seed oil contains high levels of 2 essential fatty acids, linoleic (34%) and α-linolenic acids (25%) (Yang and Kallio 2001). The pulp oil has an exceptionally high palmitoleic acid content (12% to 39%), which is not common in the plant kingdom.

The new trend in the application of sea buckthorn oil is to incorporate the oil into daily foodstuffs, such as bread, juice, and yogurts. However, the high content of polyunsaturated fatty acids and other oxygen-sensitive lipid nutrients make the oil susceptible to oxidation, which limits the applications (Yang and Kallio 2002). Sea buckthorn oil within gelatin capsules is stable but is not in a very versatile form for use. Much wider use could be achieved by microencapsulation because it converts the oil into powder form. Microencapsulation may also improve oil stability because of the oxygen and moisture barrier properties of the wall matrix (Gibbs and others 1999).

Microencapsulation has been studied for decades and numerous materials are commercially available to wall matrix. The most widely used encapsulating matrices are starch-based materials and proteins (Goubet and others 1998). When dealing with lipid oxidation, 2 aspects are often considered: water activity affecting the reaction media and glass transition in an amorphous food system (Nelson and Labuza 1992). At both very low and high water activities, lipid oxidation rates are suggested to be considerably higher than at intermediate water activities. On the other hand, the glassy state involving less free volume in a polymer matrix compared with a rubbery state has been suggested to retard the diffusion of small molecules and, possibly, oxidation of lipids. Water is the key factor also in glass transition because it can plasticize amorphous carbohydrates as well as other food macromolecules.

Thus, water aspects should also be considered in relation to stability of microencapsulated oils. So far, there are only a few studies involving varying water content on flavor release (Whorton and Reineccius 1995), on oxidation of volatile compounds (Anker and Reineccius 1988; Beristain and others 2002, Soottitantawat and others 2004), and on oxidation of triglycerides (Hardas and others 2002; Partanen and others 2002) of the microencapsulated lipids. Recently, the effect of relative humidity on storage stability of encapsulated milk fat was studied by Hardas and others (2002). Corn syrup solids and sodium caseinate was used as the shell matrix. The effect of RH on the UV-exposed samples was dramatic between RH 44% and RH 52%, the higher humidity sample suffering the faster oxidation. Interestingly, a much smaller difference between the oxidation rates at RH 44% and RH 14% was observed, the dryer sample being again more stable. In our previous study (Partanen and others 2002) on encapsulated sea buckthorn seed oil, preliminary observations on the importance of humidity control...
were made: maltodextrin and gum arabic microcapsules were more stable when they were stored under controlled conditions (20 °C, RH 50%) compared with ambient conditions (25 °C to 30 °C, RH 50% to 70%). It was suggested that the physical state of the polymer matrix would be important when evaluating their stabilization efficiency for microencapsulation.

Anker and Reineccius (1988) followed the effect of water activity (a_w) on limonene oxide formation in encapsulated orange oil. At the highest a_w (0.54 at 37 °C), the lowest oxidation rate was observed. At the very dry end of the experiment (from a_w 0.11 to a_w 0.01), more limonene oxide was determined from the powder. Gum arabic was used as the sole wall material. Beristain and others (2002) followed limonene oxide formation in encapsulated orange peel oil at different water contents. Consistent with the study of Anker and Reineccius (1988), the authors reported the slowest oxidation rate at high water content (15.83 g water/100 g soluble solids). At this point, structure collapse by water intake had already started, and the optimal water content for the capsule stability was reported at 12.8 g water/100 g soluble solids. The authors concluded that slower oxidation occurred when the matrix was in a rubbery state compared with the glassy state. The conclusion was limited to the water content area, where capsule structure was intact. Recently, Sootitantarawat and others (2004) studied the effect of water activity on oxidative stability of encapsulated D-limonene. Formation of limonene oxide and carvone was studied in maltodextrin/gum arabic and modified starch matrices. Oxidation increased with increasing humidity up to RH 51%, but at higher humidities, lower oxidation rates were again observed. This was assigned to the rehydration of the powder following adhesion of powder particles, which restricted the oxygen intake.

Whorton and Reineccius (1995) studied release of volatile compounds from spray-dried maltodextrin and corn syrup solids microcapsules. Water activity varied from a_w of 0.11 to 0.75, and loss of volatiles was followed by gas chromatographic headspace analysis. The content of volatiles in the powder was also quantified. The results showed that flavor loss increased with increasing a_w until structural collapse took place. After collapse, volatile release decreased. The authors concluded that the high release typically occurred between the glass transition temperature and complete collapse. Full collapse was claimed to effectively re-encapsulate the flavors.

In the studies on stability of microcapsules at varying water contents, a variety of parameters are involved: shell material, encapsulated agent, and the method of determining the stability. The monitored quality can be assumed to be controlled by dissolution and diffusion of small molecules in the matrix: (1) oxygen as well as (2) volatile and oxidized flavor compounds. With the limited number of studies and the controversial results presented, the role of water content and glass transition in stability of microencapsulated powders needs to be further elucidated. The purpose of the present study was to investigate the effect of relative humidity on oxidation of microencapsulated sea buckthorn seed oil during storage. Bulk oil was used as a reference. Oil was encapsulated in maltodextrin with gum arabic or in emulsifying starch by spray-drying. The aim was also to relate the oxidative stability with the matrix rheology.

Materials and Methods

Materials

Sea buckthorn seed oil (SEO) was a supercritical carbon dioxide extract with 500 ppm alpha-tocopherol as added antioxidant. Oil was obtained from Aromtech (Tornio, Finland). The fatty acid compositions of the seed oil analyzed as methyl esters by gas chromatography were as follows: 16:0, 7.4%; 16:1(n-7), 1.5%; 18:0, 2.5%; 18:1(n-9), 18.6%; 18:1(n-7), 2.1%; 18:2(n-6), 36.2%; 18:3(n-3), 31.3%; and 20:0, 0.4%.

Hydrolyzed starch, maltodextrin (MD) with a dextrose equivalent of 18.5, was a product of Cerestar (Neuilly-sur-Seine, France). The modified starch was corn starch sodium octenyl succinate derivative (HiCap) obtained from National Starch & Chemical (Manchester, U.K.). The dextrose equivalent of the modified starch derivative was between 32 and 37. Gum arabic (GA) was purchased from Sigma-Aldrich Finland (Helsinki, Finland). Petroleum ether (boiling point [b.p.] 40 °C to 60 °C) and other chemicals from various sources were analytical grade.

Emulsification of oils

GA was used along with MD to form a stable emulsion of sea buckthorn oil for spray-drying. GA and MD were blended (1:7), and the blend was dissolved in water (40 wt%). The solution was then homogenized with SEO (30% oil of the dry weight of the emulsion) with a Heidolph DIAx 600 (Kelheim, Germany) homogenizer at 2400 rpm 3 times for 1 min. The HiCap emulsion was made, using a similar procedure, from dissolved HiCap (40 wt%) without any additional emulsifier. Droplet-size distribution was checked in fresh emulsion.

Particle size distribution

Oil-droplet-size distribution in emulsion was analyzed by laser diffraction by a Coulter LS230 (Miami, Fla., U.S.A.) instrument as described earlier (Partanen and others 2002). The sample was fed either in emulsion (droplet-size measurement) or as powder (particle-size measurement) suspended in ethanol. For particle-size measurements, ethanol (refractive index 1.359) was circulated in the instrument instead of water to avoid dissolution of the powder during measurement. Oil-droplet size was also measured for microcapsule powder, which was suspended in water (reconstituted emulsion). Duplicate measurements were performed.

Spray drying of emulsions

Emulsions were spray dried by a Niro Mobile Minor (Soeborg, Denmark) laboratory spray dryer with a rotating atomizer. The inlet air temperature was adjusted to 200 °C, and the outlet temperature was kept at 80 ± 2 °C by controlling the flow rate. Rotation speed of the atomizer was 25000 rpm. Samples were collected in the chamber and the cyclone collection vessels.

Surface oil determination

Dry microcapsule powder was extracted for 3 h with petroleum ether (Partanen and others 2002), and the extracted oil was determined gravimetrically. Surface oil was calculated as the ratio of extracted oil and total oil in the sample. Triplicate measurements were performed.

Oil content determination

Oil content was determined by a Maran 23 MHz proton nuclear magnetic resonance (NMR) spectrometer (Resonance Instruments Ltd., Witney, UK). The sample was dried at 105 °C for 30 min to exclude any slowly decaying (liquid) signal from water. Dry sample was weighted into a 10-mm NMR tube. Free induction decay was recorded and signal amplitude (signal/g) at 70 µs was taken after 32 scans. Dwell time was 1.5 µs and 90° pulse length of 3.0 µs was used. The liquid signal (>70 µs) is because of the oil fraction as the signal from the relatively immobile carbohydrate matrix has a rapid decay. In the quantification, signal from pure sea buckthorn seed oil was used as a standard. Triplicate measurements were performed.
Microencapsulated sea buckthorn oil . . .

Table 1—Characteristics of sea buckthorn seed oil (SEO) microencapsulated in sodium octenyl succinate of corn starch (HiCap) and maltodextrin-gum arabic (MD/GA) matrices (mean ± standard deviation)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Oil content (%)</th>
<th>Emulsion oil droplet size</th>
<th>Powder particle size</th>
<th>Reconstituted emulsion oil droplet size</th>
<th>Water content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEO in HiCap</td>
<td>30.5 ± 0.2</td>
<td>0.8 ± 0.0</td>
<td>47.8 ± 0.3</td>
<td>0.5 ± 0.0</td>
<td>2.6 ± 0.5</td>
</tr>
<tr>
<td>SEO in MD/GA</td>
<td>31.0 ± 0.0</td>
<td>4.4 ± 0.0</td>
<td>31.2 ± 0.0</td>
<td>3.9 ± 0.0</td>
<td>2.6 ± 0.1</td>
</tr>
</tbody>
</table>

*Surface oil percentage calculated as the percentage of the easily extractable oil (g) from the total oil (g) in the powder.

Extraction of encapsulated oil for peroxide and p-anisidine value determination

A sample containing about 3 g of oil was weight into a beaker and mixed with 100 mL distilled water. 50 mL of ethanol was added to the dispersion. The dispersion was homogenized (Heidolph DIAX 600) for 2 to 3 min. The homogenization treatment was needed because caking occurred in some samples. High extraction efficiency was needed to ensure that determined peroxide and p-anisidine values represented the values of the total oil and not only those of the surface oil. During homogenization, 100 mL of hexane was added gradually. The main purpose of hexane was to separate the oil from the matrix. If necessary, 100 mL of ethanol was added to improve separation. The phases were separated in a separation funnel and the beaker was rinsed by 50 mL hexane. The hexane solvent with extracted oil was poured into a tared flask and solvent was evaporated. Extraction efficiency was typically 75% to 100% of the initial oil present in the powder. Duplicate determinations were performed.

Peroxide and p-anisidine value determination

The peroxide value (PV) was determined by iodometric titration described earlier (Partanen and others 2002) and the p-anisidine value (AN) was determined by spectrophotometer according to IUPAC method 2.504. The PV analysis was carried out in duplicate.

Water content determination

Water contents of the freshly prepared microcapsules were determined using a Mettler Toledo DL31 Karl Fischer titrator with Hydranal Titrant 2 (Riedel-de Haen, Sigma-Aldrich, Seelze, Germany) as the Karl Fischer reagent. Powder samples (about 100 mg) were weighed into tared vials, and water was extracted with methanol before titration. The titrator was calibrated with water. Analysis was performed in triplicates.

Glass transition temperatures

Glass transition temperatures were determined by differential scanning calorimetry (Partanen and others 2002). The midpoint of the transition was taken as glass transition temperature. Replicates were only performed if complications were observed in the DSC baseline.

Environmental scanning microscope micrographs

An Electroscan model 2020 environmental scanning microscope (ESEM) (Fei UK Ltd, Cambridge, UK) was used to study the surface and inside structures of the microcapsules formed. Before measurements of the inside structure, the microcapsules were split using a 2-sided adhesive tape. Then the microcapsules were attached to ESEM stubs and coated with Pt-Pd using a model SCD 050 Coater (Bal-tec, Balzers, Principality of Liechtenstein).

Storage stability

About 20 g of bulk and encapsulated oil were spread on petri dishes for storage stability tests. The dishes were kept open to allow contact with air and placed in humidity chambers with different salt solutions: LiCl (RH 11% at 20 °C and at 50 °C), MgCl₂ (RH 30% at 50 °C), and Mg(NO₃)₂ (RH 54% at 20 °C and RH 45% at 50 °C). The humidity chambers were balanced before the samples were set in. The samples were not exposed to light during storage. At 20 °C, 1 petri dish was removed for each time point of PV analysis. At 50 °C, 1 additional petri dish was also removed for each time point of the AN analysis. This was performed to ensure that the expected fast deterioration of the oil quality was detected in spite of the peroxides reacting further. Storage time and sampling intervals for powders and oils depended on their stabilities. The sampling intervals were typically ranging from a few days to 1 wk in the beginning of the 20 °C test period to a monthly check in the end of the period. In the accelerated test (50 °C), sampling was performed every 3 or 4 d in the beginning and less frequently in the end.

Color changes of sea buckthorn seed oil during storage at 50 °C

UV-visible spectrophotometer, model UV-1601 (Shimadzu Corp., Tokyo, Japan) was used to measure the absorbance at wavelength 445 nm. About 1.5 g sample was weight into a 25-mL volumetric flask; hexane was added and mixed. Replicates were not made because the standard deviation in preliminary results was very small (<1%).

Results and Discussion

Characteristics of the microencapsulated SEO

Sea buckthorn seed oil (SEO) was microencapsulated by spray-drying using modified starch (HiCap) and MD/GA as the wall materials. The characteristics of microcapsule powders are shown in Table 1. Both microcapsules were homogeneous yellow powders with less than 3% water immediately after preparation. In spite of the similar total oil content, which was about 30%, the surface oil contents (also known as easily extractable lipids) of the powders differed a lot. In the HiCap capsules, surface oil content was below 0.5% of the total oil whereas in MD/GA powder, 30% of the oil was easily extractable (Table 1).

Also, a rather large difference between the wall materials was noticed in their emulsification properties (Table 1): median oil droplet size was below 1 μm in the HiCap emulsion but above 4 μm in the MD/GA emulsion. In both matrices, the oil had essentially the same droplet size after drying and redispersing compared with the original emulsion. The particle size distributions of the 2 powders were rather broad. In the HiCap powder, the particles were somewhat larger with median diameter of 40 μm compared with the 31 μm of the MD/GA microcapsules (Table 1).
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The shape, size, and morphology of the spray-dried microcapsules were studied furthermore by ESEM. Both microcapsules were spherical in shape with smooth surface (Figure 1a and 1c). The inner structure showed that the particles contained large empty vacuoles (Figure 1b and 1d). The formation of central vacuoles in spray-dried particles has been reported and discussed in the literature, but the effect of vacuoles on oxidation kinetics of encapsulated oils has remained unclear (Walton and Mumford 1999a, 1999b; Bylaitë and others 2001; Keogh and others 2001). The present study is not focusing on the formation or consequences of the vacuoles. Interestingly, small spherical holes were observed in the split wall matrix. It was estimated from the ESEM micrographs that in the HiCap capsules, the diameter of the holes was below 1 μm and in the MD/GA matrix below 5 μm. The holes were of a very similar size to the droplets of the reconstituted emulsion (Table 1) suggesting, that the oil droplets were visible as small holes in the micrographs.

The physical state of the encapsulating material is a possible factor affecting the stability of oil (Nelson and Labuza 1992). In the glassy state, the amorphous matrix is considered to be relatively stable. At glass transition temperature, the matrix is transformed into the rubbery state, where caking of powder and collapse of the structure can take place (Roos and Karel 1993). Increased molecular motion in the rubbery state is associated with an increase in free volume in the matrix (Slade and Levine 1993) and increased diffusive mobility of the permeating molecule depending on the molecular structure (Gunning and others 2000). Thus, oxidation of the encapsulated oil is relevant to evaluate in regard to the glassy/rubbery state of the microcapsule wall. Glass transition temperatures (Tg) of HiCap and MD/GA were determined as a function of water content. The well-known plasticization effect of water on food carbohydrates (Zeleznak and Hoseney 1987; Roos and Karel 1991) was observed for both HiCap and MD/GA, but at the respective water contents, Tg of HiCap was observed to be considerably lower than that of MD/GA (Figure 2).

**Effect of relative humidity and temperature on oxidation of bulk SEO**

Oxidation stability of SEO was studied to elucidate the behavior of the bulk oil under conditions, which were applied to the microencapsulated oils. Relative humidities (RH) were 11% (at 20 °C and 50 °C) and 54% (at 20 °C) or 45% (at 50 °C). Peroxide value (PV) was determined during storage (Figure 3). At 20 °C, the oil deteriorated surprisingly fast under dry conditions (RH 11%) compared with the moderate humidity conditions (RH 54%). The PV of SEO exceeded 20 meq/kg (the considered upper limit for acceptable quality oil of sea buckthorn seed oil) within 1 wk at RH 11%. Under moderate humidity, SEO was edible for 1 mo based on PV analysis.

The accelerated storage test was carried out at 50 °C. As expected, oxidation proceeded a lot faster at higher temperature. The first 2 or 3 d were needed before the system reached an equilibrium moisture. After the equilibration, the humidity effect was similar to

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**Figure 1—Environmental scanning microscope (ESEM) micrographs showing the outer topography and inner structure of microcapsules. Shell materials: (a) whole and (b) split sodium octenyl succinate of corn starch (HiCap) microcapsule; (c) whole and (d) split maltodextrin-gum arabic (MD/GA) microcapsule.**

**Figure 2—Glass transition temperatures of the shell materials, maltodextrin-gum arabic (MD/GA), and sodium octenyl succinate of corn starch (HiCap) as a function of water content. The relative humidities of the storage stability test (20 °C) at corresponding water contents are also shown.**

**Figure 3—Peroxide value of sea buckthorn seed oil (SEO) as a function of storage time measured under constant relative humidity and temperature (standard deviation <10%)**
that at 20 °C: in the dry end (RH 11%), oxidation was again faster than at the intermediate humidity (RH 45%). During storage at the elevated temperature, the color of the oil was visibly changing. The color change was followed by measuring the absorbance at wavelength 445 nm (Figure 4). The absorbance decreased during storage, faster at the low RH, consistent with the observed increase in peroxide value. The absorbance of SEO decreased to zero after 1 wk of storage at RH 11%, and the strong yellow-orange color of the original oil had completely disappeared. The slower change in absorbance at RH 45% was also visually detected.

The relative humidity effect on oil oxidation was an early observation reported by many authors both for bulk oils as well as for oils in food matrices (Karel and Labuza 1967; Labuza and Chou 1974; Labuza 1975; Karel 1980; Gopala Krishna and Prabhakar 1992; Gopala Krishna 1992). The rate of lipid oxidation is claimed to be at minimum under moderate RH. A possible explanation for the low rate at intermediate humidity is that water forms a hydration sphere around metal catalysts thus reducing their catalytic activity and slowing down the oxidation rate (Nelson and Labuza 1992).

The huge color change, which was congruent with the peroxide values observed in the present study, may indicate oxidation of natural carotenoids and loss in their antioxidant capacity. However, the tempting approach to use color disappearance as a sign of quality loss in oil could not be applied because the oil quality was completely lost by the oxidation stage at which color changes were visible. More experimental data are needed to understand the effects of all minor constituents of the oil on its oxidation, as SEO is indeed a rather complicated oil to study.

**Effect of the relative humidity and shell matrix on oxidation of microencapsulated SEO at 20 °C**

The stabilization capability of the wall materials HiCap and MD/GA was investigated at 20 °C under low (RH 11%) and intermediate humidity (54%). The changes in PV of the encapsulated oil were determined during storage.

The oxidation rate of encapsulated SEO was dependent both on relative humidity and on the wall material (Figure 5). At RH 54%, MD/GA matrix prolonged the shelf life from 4 wk of the bulk oil to 8 wk. In the HiCap matrix, the oil oxidized within 1 wk at RH 54%. Low environmental humidity (RH 11%) made essentially no difference in the stabilization capability of the MD/GA matrix, but retarded oxidation in HiCap drastically.

At RH 54%, the structure of the HiCap microcapsules was changing with time. At the intermediate humidity, the powder caked rapidly on the surface and caking was complete within a few days. In MD/GA powder there were no significant structural changes observed during the storage period. At RH 11%, both microcapsules remained in powder state without any visible caking. Based on Tg analysis (Figure 2) both wall materials were in a glassy state at 20 °C and RH 11% (corresponding to about 5% water and Tg 50 °C for HiCap and Tg 90 °C for MD/GA). At RH 54%, HiCap contained about 11% water, had a Tg of approximately 10 °C, and consequently was in the rubbery state when stored at 20 °C. Even though MD/GA capsules contained considerably more surface oil, no major difference in the oxidation rate was observed in the glassy matrices. Furthermore, the smaller oil droplets with larger surface area in HiCap could have been more susceptible to oxidation than the coarse droplets in MD/GA. However, as the oxidation rate was essentially the same at RH 11%, the obtained data did not support this behavior. The origin for the high oxidation rate in the HiCap capsules was most probably because of the physical state of the powder. This indicates that the oxidation kinetics is largely determined by oxygen dissolution and diffusion in the matrix.

Several studies regarding the effect of surface oil on oxidation have been conducted (Anker and Reineccius 1988; Buffo and Reineccius 2000; Keogh and others 2001; Hardas and others 2002). Some results have indicated that storage stability was enhanced at low surface oil contents, but several results have also been presented where no connection between surface oil and storage stability could be found. Hardas and others (2002) suggested that surface oil is critical to powder stability because oil droplets on the surface of the particle could be less protected against atmospheric oxygen. On the other hand, Buffo and Reineccius (2000) claimed that surface oil has no relationship to oxidative shelf-life, as the amount of oxidized products on the surface of the powder was not enough to govern the shelf-life. Also Keogh and others (2001) and Anker and Reineccius (1988) have observed that surface oil is not an important determinant of microencapsulated oil shelf-life because powder with no surface oil exhibited a shelf-life very similar to the product with significant quantity of surface oil. Based on the study by Anker and Reineccius (1988), other factors such as matrix porosity, trace mineral level (copper and iron), and presence of antioxidants were believed to be more significant in determining the rate of oxidation of encaps-
Microencapsulated sea buckthorn oil... sulated oils. Thus, the observation in the present study that surface oil has no major role in overall oxidation of microencapsulated oil is in agreement with the most investigations reported earlier.

Studies aiming to understand the relationship between Tg and the stability of encapsulated components have been recently conducted (Gunning and others 1999, 2000; Andersen and others 2000). Diffusion of short-chain alcohols in maltose matrix below and above Tg was investigated by Gunning and others (2000). The temperature difference between the temperature of observation and glass transition temperature (T-Tg) was between –15 °C and 60 °C. The diffusivity decreased with decreasing water content and diffusion was particularly slow in the glassy samples. For the nonglassy samples, decrease in diffusion was reported also as molecular size of the alcohol increased. Diffusion of ethanol was more extensively studied, and it was reported that the changes in diffusion were not directly coupled with changes in the system viscosity. It was, however, concluded that T-Tg was a useful predictor of diffusivity in a maltose-alcohol-water ternary system. Andersen and others (2000) developed a method to determine the rate of oxygen permeation in a glassy food matrix with oil encapsulated. The rate of oxygen permeation was found to increase with temperature. The permeation rate of oxygen through the matrix was described by the Arrhenius equation during the entire range of storage temperatures (from 5 °C to 80 °C, Tg = 65 °C), irrespective of the glass transition. However, the authors pointed out that because of the limited range of temperatures studied above Tg, very solid conclusions of the rubbery state behavior could not be made. The rate of oxygen permeation was believed to be the rate determining step in oxidation, and the results suggested that permeation might be relevant also to glassy state storage stability.

The results of the present study were consistent with those reported by Gunning and others (2000). The transition from the glassy to the rubbery state increased the rate of permeation dramatically, despite the different size of the permeating molecules. Thus under dry conditions, glassy matrices were reasonably stable. At moderate humidity, powders should be handled with special care because wall matrix may even accelerate oxidation. As compared with HiCap, MD/GA offered better protection under ambient conditions because of higher Tg, in spite of the much higher surface oil content and oil droplet size.

**Accelerated oxidation experiment of the microencapsulated SEO**

Because oxidation studies will usually last for several months at room temperature, the stability tests were also carried out at 50 °C. The main question was whether the results from the accelerated conditions could be used to predict the behavior of the encapsulated oil under normal conditions. In addition to PV, oxidation was followed by analyzing p-anisidine value (AN), which is a measure of secondary oxidation products, aldehydes, present in oil.

Both SEO-capsules oxidized very rapidly at 50 °C under moderate humidity (RH 45%). The peroxide value of SEO in MD/GA capsules exceeded the 20 meq/kg limit within a few days under all humidities, RH 45% stimulating the oxidation most (Figure 6). The PV value of the oil encapsulated in HiCap increased with time at first, and then reached a plateau and finally decreased (Figure 7). Formation of secondary oxidation products was relatively slow for 8 d in both matrices, as determined by AN value. After 8 d, a higher AN value with increasing RH was observed in HiCap. In maltodextrin matrix, rate of secondary oxidation product formation did not depend on RH.

The limited primary oxidation in HiCap matrix was observed as a maximum in peroxide value and could be linked to the large structural changes that occurred in the matrix. Similar behavior has been reported earlier by Soottitantawat and others (2004), Whorton and Reineccius (1995), and Labrousse and others (1992). When encapsulated limonene in maltodextrin matrix was hydrated above Tg, the particles began to clump and adhere together (Whorton and Reineccius 1995). Pores in the particle walls disappeared, and the powder entered to a fully collapsed state, which effectively “re-encapsulated” the remaining flavor inhibiting additional evaporation. Soottitantawat and others (2004) studied the effect of RH on retention of encapsulated limonene and on formation of oxidation products in HiCap and maltodextrin gum arabic matrices at 50 °C. A maximum in the amount limonene oxide in the powder was observed for HiCap at RHs 51% and higher and for maltodextrin gum arabic at RH 96%. The effect of collapse on oxidation of encapsulatored flavor was studied by Labrousse and others (1992). Concentration of peroxides in surface oil was determined for lactose-gelatin and sucrose-lactose-gelatin matrices containing mlynolone after storage at 45 °C and 55 °C. Decrease in peroxides during storage was assigned to their decomposition. Lack of oxidation was reported as re-encapsulation because collapse occurred. This was also one explanation for the different behavior of oil in the HiCap and MD/GA carriers in the present study. Possibly HiCap matrix changed to a state, in which oxygen diffusion slowed down. Formation of the primary oxidation products in the oil decreased indicating a lack of oxygen. Based on an assumption that water contents of the samples are a few percentage units lower as temperature shifts from 20 °C to 50 °C, it is found that at least the low humidity (RH

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**Figure 6**—Storage stability of sea buckthorn seed oil (SEO) encapsulated in maltodextrin-gum arabic (MD/GA) under controlled conditions (RH 11%, 30%, 45%; 50 °C) were monitored by measuring (a) peroxide value (PV) (standard deviations <10%) and (b) p-anisidine value (AN). **Figure 7**—Storage stability of sea buckthorn seed oil (SEO) encapsulated in sodium octenyl succinate of corn starch (HiCap) under controlled conditions (RH 11%, 30%, 45%; 50 °C) were monitored by measuring (a) peroxide value (PV) (standard deviations <10%) and (b) p-anisidine value (AN).
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11% and RH 30%) MD/GA samples were deep in the glassy state also at 50 °C, whereas the corresponding HiCap samples were very close to the glass transition range if not in the rubbery state. This observation is not in agreement with the common statement that glassy matrix is more stable because MD/GA powders became unacceptable faster than HiCap powders at RHs 11% and 30%. The results gave further evidence that the different behavior of HiCap and MD/GA capsules is because of their different rheological changes caused by water sorption. The present study did not support the use of accelerated conditions as predictive tools because the behavior differed too much from that at 20 °C.

Conclusions

Relative humidity is of crucial importance when evaluating the protection efficiency of starch–based matrices against oxidation. The choice of the carrier material also greatly affects the behavior. Conditions close to Tg accelerate oxidation. The glassy state was seen to be the most stable. Surface oil did not play any crucial role in overall oxidation, which indicated that the easily extractable “surface” oil was well mixed within the matrix but perhaps not properly emulsified. In spite of the limited results, only the main rate controlling phenomenon was most likely oxygen diffusion, which was much affected by the structural changes occurring at Tg. The accelerated conditions should be applied with specific care.

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