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Lipid oxidation in glassy and rubbery-state starch extrudates

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Abstract

This work describes the principle of protecting polyunsaturated fatty acids by holding them in the low moisture/solid/glassy-state starch matrix. One strategy already employed commercially is to encapsulate oil droplets within a solid wall that is highly impermeable to oxygen. These microencapsulated powders can then be added to foods. A shorter route would be to add PUFA-rich oils directly into a food formulation during the processing of a low moisture product. This should effectively encapsulate the valuable oils and protect them from oxidation. ω-6 Linoleic acid was incorporated into a waxy maize starch matrix via extrusion cooking. Linoleic acid oxidation occurred when this model food system was held in both the glassy and rubbery states (0.3 and 0.95 Aw, respectively) at 50 °C. The initial oxidation, not surprisingly, occurs near the surface, but interestingly the highest initial rate of lipid oxidation occurred, not in the rubbery samples, but in glassy state starch extrudates with surface micro-cracks.

Keywords: Linoleic acid; Oxidation; Hexanal; Extrusion; Water activity; Glassy state; Rubbery state; Starch

1. Introduction

Foods with a water activity (Aw) below 0.6 can be described as dehydrated or low moisture and are resistant to microbial spoilage. This does not, however, mean that chemical reactions associated with loss in food quality are arrested below this Aw. Lipid oxidation rates decrease on hydrating a system until a minimum is reached at the BET (Brunauer, Emmett and Teller) monolayer region. This is the Aw limit before relatively 'free' water exists in the system (Labuza, Maloney, & Karel, 1966; Labuza, Silver, Cohn, Heidelbaugh, & Karel, 1971; Chou & Labuza, 1974; Labuza & Chou, 1974; Maloney, Labuza, Wallace, & Karel, 1966). The model systems used by these workers were taken to their final Aw either by adsorption of water by a freeze-dried matrix, or by desorption by direct-mix of ingredients to a predetermined Aw. This principle was extended to real food systems such as chicken and pork, and lipid oxidation measured at intermediate moisture contents (Aw of 0.6–0.9) (Labuza, McNally, Gallagher, Hawkes, & Hurtado, 1972). The humectant glycerol was used to limit the Aw at relatively high moisture contents. From such studies the importance of moisture content as well as Aw in affecting lipid oxidation kinetics was highlighted.

As pointed out by Nelson and Labuza (1992), the physical state of a food matrix is also likely to play an important role in determining lipid oxidation kinetics. 'Glassy' and 'rubbery' are terms used to describe distinct amorphous physical states of a material that can be observed by a step change in enthalpy on heating. For example a sugar melt can be rapidly frozen into the glassy state and on heating a glass transition temperature (T_g) is reached where it changes into the rubbery state. T_g is dependant on the material, and on water which can act as a plasticiser, so reducing T_g. One distinguishing feature of a glass is the reduced free volume compared with the rubber – this can lead to reduced diffusion rates and so can result in a reduction in the rate of diffusion limited reactions.

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Maltodextrin, sucrose-maltodextrin-gelatin and lactose glasses containing orange oil, medium chain triacylglycerols and methyl linoleate, respectively (Andersen et al., 2000; Ma, Reineccius and Labuza, cited by Nelson and Labuza, 1992; Shimada et al., 1991), are examples of systems that have been used to study the lipid oxidation kinetics in glassy systems. No work has been reported on lipid oxidation within glassy starch-based matrices formed by extrusion cooking, and, further, few attempts have been made to directly compare lipid oxidation in equivalent systems in the glassy and rubbery states. Our hypothesis was that lipid oxidation will occur in the glassy state of our starch extrudates, but at a much slower rate than in the rubbery state. The results, however, challenged this prediction.

2. Materials and methods

2.1. Sample preparation

Samples composed of waxy maize starch (Variety Amioca, source confidential), 30% (w/w) water and 4% (w/w) free fatty acids (FFA) (Fisher Chemicals, Loughborough, UK, technical grade −60% linoleic acid, 40% other free fatty acids) were prepared by extrusion using the Clextral BC-21 co-rotating, intermeshing twin-screw extruder with a barrel length of 400 mm (Firminy, France). Extrusion parameters used were as follows: barrel thermal profile = Zone 1, 40 °C; Zone 2, 88 °C; Zone 3, 145 °C; Zone 4, 80 °C; screw speed 200 rpm; water feed rate 1.43 l/h; starch feed rate 5 kg/h.

The FFA were added directly to the extruder barrel through an electronic syringe pump, to give a lipid content of 4% of the dry weight of starch. Samples were extruded into flat ribbons with cross-sectional width and height of 2.5 cm and 0.3 cm, respectively. Following extrusion, samples contained approximately 30% moisture (wet weight basis, w.w.b) and were designated as existing in the rubbery state at 50 °C and had an Aw of 0.95 (Bowen, 2005). To generate the glassy state samples, 10 g portions of these extrudates were freeze-dried (Edwards Freeze Drier Super Modulyo, Pirani 1001) at −40 °C and 0.1 mbar for 48 h, to a final moisture content of around 10% moisture (w.w.b.). These samples were glassy at 50 °C and had an Aw of 0.3.

2.2. Accelerated storage trial

Triplicate samples (10 g equivalent prior to drying, approximately 5.5 cm by 2.5 cm by 0.3 cm) for each time point were placed in separate 250 mL glass Schott bottles at 50 °C for 14 days. Replicated samples were removed at each time point and individually vacuum-packed in foil bags and stored at −80 °C prior to analysis.

2.3. Headspace analysis

The samples were analysed for evidence of lipid oxidation by measuring hexanal concentration in the headspace over the sample. A platform quadropole mass spectrometer (Micromass, Altrincham, UK) with a modified source for introduction of gaseous materials (APcI-MS), was used in positive selected ion mode to quantify the hexanal headspace over the samples. The measurement was made on whole extruded preparations, without prior lipid extraction. A transfer line heated to 160 °C and a flow rate of 10 ml per min were used. On the day of analysis, samples were defrosted and allowed to reach room temperature before they were removed from the foil bags. Each sample was added to 100 ml glass Schott bottles, and allowed to equilibrate. For all sample bottles, a 3 mm diameter hole was made in each lid and plugged with PTFE (polytetrafluoroethane) tubing. On removing the plug the bottle was sampled until a peak plateau was recorded. The system was calibrated with 0.4 ppmv (parts per million (by volume)) hexanal. Using the data, it was possible to calculate the concentration of hexanal in the headspace over the sample.

2.4. Hydration of starch extrudates prior to hexanal analysis

Extrudates (10 g equivalent prior to drying) were frozen in liquid nitrogen, then ground for 10 s in a Knifetec mill, with water-cooled basin. The ground sample was placed back in its 100 ml sampling bottle, and HPLC grade water (50 ml) was added. The hydrated samples were allowed to equilibrate for 4 h before hexanal headspace analysis on the APCI-MS (atmospheric pressure chemical ionisation-mass spectrometry).

2.5. Data analysis

MS Data was analysed in the Mass Lynx software, in which the peak heights were measured and the sample headspace concentration calculated in parts per million per volume (ppmv), using the following equation:

$$\text{Headspace concentration (ppmv)} = (\text{SI}/\text{CI}) \times C_{\text{cal}} \times (75/f)$$

where SI is the sample intensity, CI is the calibration intensity, C_{cal} is the concentration of injected calibrant in ppmv, f is the actual sampling flow rate API is set to, 75 is the theoretical flow rate on which C_{cal} is calculated: explained as follows:

The hexanal in cyclohexane calibrant (C_{cal}) delivers volatile to the APCI-MS independent of the sampling flow rate, as it is injected directly. Therefore, the signal intensity (peak height) was adjusted to make the standard (CI) and the sample (SI) comparable. The calibrants were made up to pre-specified concentrations that were calculated from experimental work by previous researchers at the Univer-

sity of Nottingham. These calibrant concentrations were developed assuming a sampling flow rate of 75 ml/min, which was a typical flow rate at one time. So, if for example, the actual sampling flow rate (f) was 25 ml/min, the concentration of the volatile delivered from the sample would be 1/3 of that from the injected calibrant of the same concentration. Therefore, to obtain a real headspace concentration, we multiply by 75/25 to make the sample and standard comparable. The headspace concentration for each ion was calculated, for all of the samples, in the storage trial. These concentrations were plotted against storage time. This allowed the rates of formation of the ions of interest to be monitored, and the differences between these rates for different samples to be observed.

2.6. Statistical treatment of data

Triplicate extrudate samples were analysed at each time point; from this data mean, standard deviation and standard error values were calculated. The apparent accumulation of headspace-hexanal data was obtained by sequential summation of the mean value from triplicate samples at each time point.

3. Results and discussion

3.1. Physical comparison of samples

A mixture of free fatty acids enriched in linoleic acid (60%) was incorporated into starch extrudates or held as 'bulk oil'. Free fatty acids were used in preference to an oil with equivalent fatty acid composition (e.g. sunflower oil) because they hindered mould growth in the rubbery samples and oxidised more rapidly than oil, so allowing sampling over days rather than weeks (Bowen et al., 2006). The extrudates were formed by thermo-mechanical extrusion; the moisture content of one set of samples was subsequently reduced through controlled freeze-drying to form material in the glassy state. The final moisture contents of the samples, their Aw and T_g are shown in Table 1. The surface area of the extrudates was greater than that of the 'bulk oil' (32.3 and 3.14 cm^2, respectively), the latter (an equivalent mass of fatty acids as that contained in extrudates) being held in open-topped glass cups (diameter: 2 cm).

3.2. The rate of hexanal release from rubbery and glassy starch extrudates containing linoleic acid

Starch extrudates were stored in bottles in the dark at 50 °C. Samples were removed at regular intervals over a period of 2 weeks and stored under vacuum in a foil pouch at −80 °C until analysed by APCI-MS. The extruded starch control, 10% moisture w.w.b. (containing no oil), showed no significant production of the lipid oxidation marker hexanal, confirming that the volatiles produced in the samples originated from linoleic acid, and not as artifacts from the starch matrix. Linoleic acid stored as 'bulk oil' showed a much slower onset of oxidation compared with the linoleic acid present in the starch matrix; the peak of hexanal release (258 ppmv) was reached gradually after 200 h compared with 24 h and 48 h for the glassy and rubbery extruded samples, respectively (see Fig. 1). The surface area of the extruded samples is 10 times greater than that of the surface area of the 'bulk oil' and this probably explains much of this difference.

The equilibrium headspace concentration of hexanal initially increased then declined in both extruded systems (see Fig. 1). The rate of these changes was higher for the glassy samples, where a relatively short lag period was observed, however the overall apparent accumulation of hexanal in the headspace over time is greater in the rubbery sample (see Figs. 5 and 6). The results suggest that, as hypothesised, lipid oxidation does indeed occur in the glassy state.

What is not clear from these results is why, after peaking, do hexanal concentrations in the headspace for all samples appear to decline with time? Either the chemistry of hexanal is changing, e.g. oxidation, or hexanal is becoming bound to the matrix during storage, resulting in the build up of a reservoir of retained hexanal in the matrix over time. An increase of hexanoic acid was observed by APCI-MS over storage time (data not shown); this supports the hypothesis that hexanal is further oxidised into hexanoic acid, explaining at least in part, the loss of hexanal from the headspace over time. One limitation of using APCI-MS is that no measure of bound hexanal is provided, and it is possible that bulk or surface changes in the matrix during storage increase the binding of hexanal and so

Table 1
Moisture content, Aw and T_g of starch extrudates

	Moisture content (w.w.b.)	Aw	T_g onset
Glassy	11.2% (±0.6)	0.3	172.2 °C (±5.5)
Rubbery	28.1% (±1.2)	0.95	−10 °C[a]

± Range of values from the mean calculated from triplicate samples.
[a] T_g' of the freeze-concentrated glass.

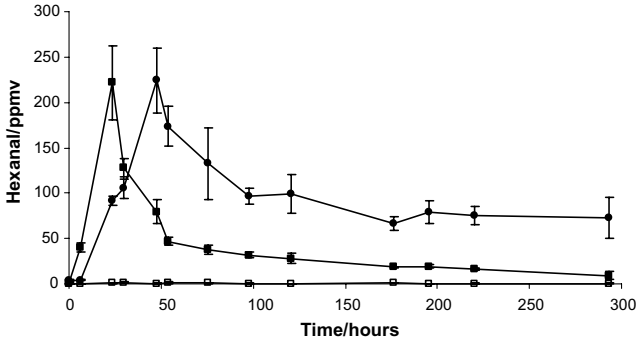

Fig. 1. Effect of physical state on apparent lipid oxidation kinetics in starch extrudates. Glassy (■) and rubbery (●) starch extrudates containing 4% free fatty acids (60% of which is linoleic acid) were stored at 50 °C. Control starch extrudates (□) contained no linoleic acid. Extrudates were harvested from storage bottles at each time point, and stored at −80 °C until analysed. The equilibrium headspace concentration of hexanal was used as a marker of linoleic acid oxidation. Standard error values from triplicate samples are shown.

reduce its partition coefficient. It was therefore necessary to study the effect of hydration on hexanal release from test extrudates.

3.3. Effect of hydration of the starch matrix (just prior to analysis) on hexanal release

At this stage of the work it was unclear whether the differences in hexanal headspace concentrations above the glassy and rubbery samples were effects of the physical state of the matrix on (i) lipid oxidation, or (ii) on volatile release from the matrix, or both. To address this issue, glassy and rubbery samples, derived from a storage trial, were milled in liquid nitrogen then hydrated and their equilibrium headspace hexanal concentration measured (Fig. 2A and B). Although the absolute values of hexanal in the headspace above stored samples declined between 10 and 20 times on sample milling and hydration, presumably due to unavoidable loses to the atmosphere, the profile of hexanal concentration in the headspace derived from the storage trial data did not change significantly as a result of this intervention prior to analysis. It therefore appears, at least in our system, that a bound reservoir of hexanal is not accumulating over storage time, and that APCI-MS analysis of the headspace volatiles can therefore be used to compare hexanal generation in glassy and rubbery state starch-based extrudates.

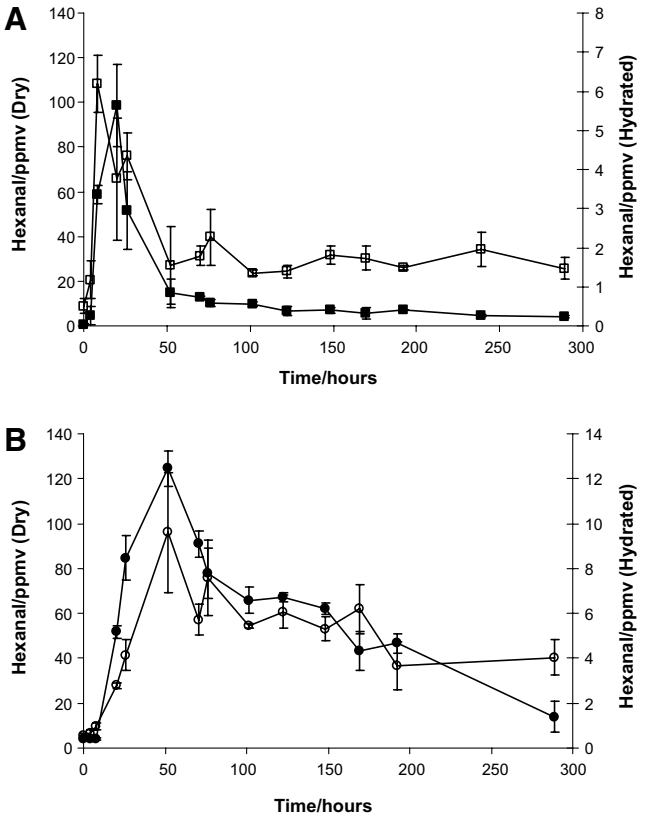

Fig. 2. Effect of sample hydration on the measurement of hexanal in glassy and rubbery starch extrudates. Glassy (squares) and rubbery (circles) starch extrudates containing 4% free fatty acids (60% of which is linoleic acid) were stored at 50 °C. Extrudates were harvested from storage bottles at each time point, and stored at −80 °C until analysed. The equilibrium headspace concentration of hexanal was used as a marker of linoleic acid oxidation. Extrudates were either intact (closed symbols) or milled and hydrated (open symbols) for headspace analysis. Standard error values from triplicate samples are shown.

Post-storage extraction of linoleic acid and measurement by GC revealed negligible loss of linoleic acid in the extruded samples. This suggests that only a small fraction of linoleic acid has been oxidised, and, along with the results from the hydration study, begs the question: is oxidation only occurring at the surface of our samples?

3.4. Surface topography of the starch-extrudates

The surface area of the extruded samples, calculated from basic geometry (32.3 cm^2) is 10 times greater than that of the surface area of the 'bulk oil'. The relatively slow rate of linoleic acid oxidation in the 'bulk oil' sample compared with the extrudates could be explained by this difference. The negligible loss of linoleic acid from the extrudates after storage also points to the importance of surface lipid oxidation in our model system. As part of an examination into the contribution of surface-lipid oxidation in our samples, the surface topography of a range of glassy samples was studied by microscopy (see Fig. 3). Micro-cracking, or more significant cracking, on the surface of extrudates after freeze-drying were frequently evident and the extent of cracking varied from sample to sample.

The impact of surface micro-cracking on the rate of lipid oxidation in glassy extrudates was investigated by selecting freeze-dried samples that had different degrees of cracking. From the results (see Fig. 4) it is clear that glassy samples with micro-cracks are susceptible to oxidation and release hexanal into the headspace; conversely, under the storage conditions used in our study, glassy samples without micro-cracks appear to protect the linoleic acid from oxidation. Limited oxygen permeability and solubility in the glass with no cracks probably explains this observation. Surface lipid appears to be exposed to, and react with, atmospheric oxygen in the cracked regions of the extrudates; this reaction should therefore be minimised if the surface lipid is washed off prior to the storage trail.

3.5. Impact of removal of surface lipid on lipid oxidation

Extrudates formed en-route to the glassy state were hexane-washed, to remove surface lipid, either before or after freeze-drying. Apparent hexanal accumulation was measured with storage time. All glassy state samples used in this experiment displayed a similar extent of surface micro-cracking. The results from this experiment (see Fig. 5) revealed that the timing of hexane-washing does affect the tendency of the samples to oxidise. It appears that micro-cracks on the surface can develop on freeze-drying, exposing a new un-washed face where lipid can react with oxygen. Extruded samples in the rubbery state were

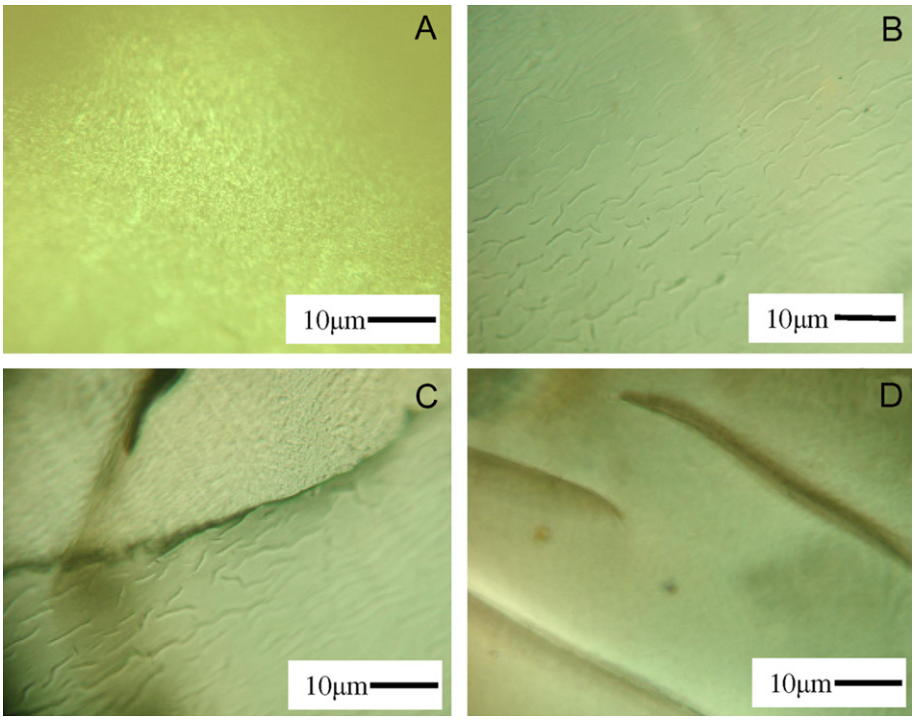

Fig. 3. Micro-cracks on the surface of separate glassy state samples ranging from non-cracked (A), to micro-cracking (B) to much larger cracks in (C) and (D). The scale-bar on each picture represents 10 μm.

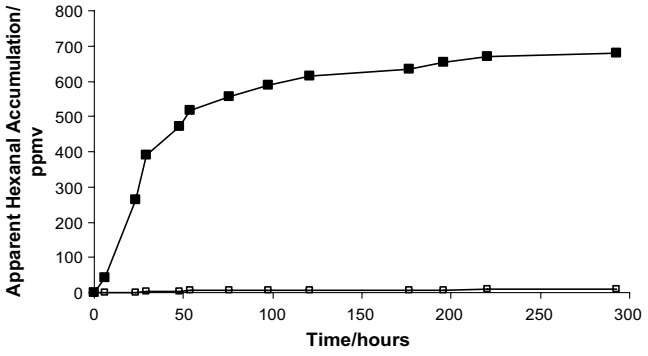

Fig. 4. Effect of surface cracking on apparent hexanal accumulation in the headspace above glassy starch extrudates. Glassy starch extrudates, with (■) or without (□) micro-cracks on the surface, containing 4% free fatty acids (60% of which is linoleic acid) were stored at 50 °C. Extrudates were harvested from storage bottles at each time point, and stored at −80 °C until analysed. The equilibrium headspace concentration of hexanal was used as a marker of linoleic acid oxidation. The apparent accumulation of headspace-hexanal data was obtained by sequential summation of the mean value from triplicate samples at each time point. Error bars are therefore absent, but the CV at C_{max} for the cracked samples was 28%.

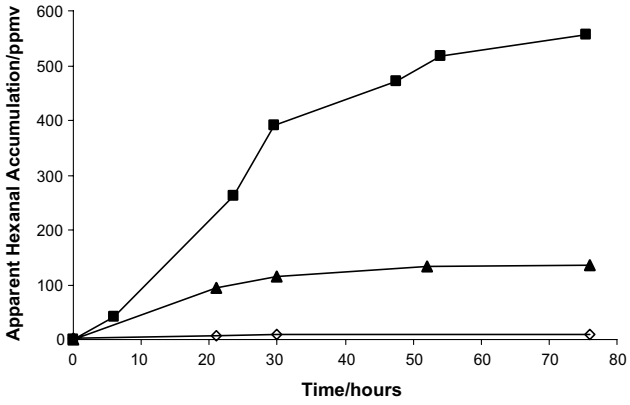

Fig. 5. Effect of surface washing on apparent hexanal accumulation in the headspace above glassy starch extrudates. Glassy starch extrudates, with micro-cracks on the surface, containing 4% free fatty acids (60% of which is linoleic acid) were produced without hexane-washing (■), hexane-washed prior to drying (▲), and hexane-washing just after drying (◇). Extrudates were stored at 50 °C, harvested from storage bottles at each time point, and stored at −80 °C until analysed. The equilibrium headspace concentration of hexanal was used as a marker of linoleic acid oxidation. The apparent accumulation of headspace-hexanal data was obtained by sequential summation of the mean value from triplicate samples at each time point. Error bars are therefore absent, but the CV range at C_{max} in each of the three regimes was 15–20%.

less dramatically affected by hexane washing prior to storage than samples in the glassy state (see Fig. 6).

Many systems have been tested to encapsulate PUFA-rich edible oils to limit quality deterioration by oxidation during storage (Augustin & Sanguansri, 2003; Bustos, Romo, Yáñez, Díaz, & Romo, 2003; Chang, 1997; Galobart, Barroeta, Baucells, Cortinas, & Guardiola, 2001; Kolanowski, Laufenberg, & Kunz, 2004; Partanen,

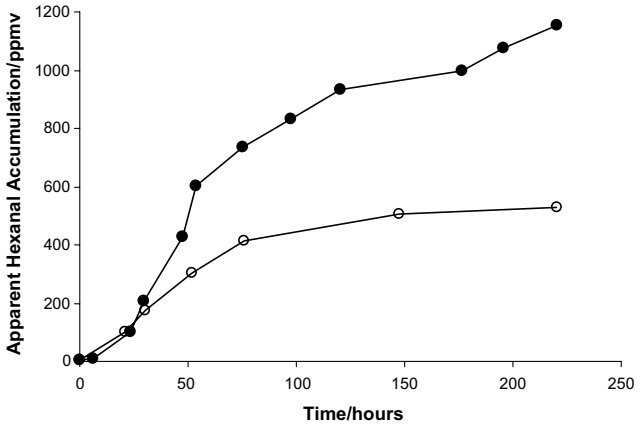

Fig. 6. Effect of surface washing on apparent hexanal accumulation in the headspace above rubbery starch extrudates. Rubbery starch extrudates containing 4% free fatty acids (60% of which is linoleic acid) were produced without hexane-washing (●) and with hexane-washing (○) after extrusion. Extrudates were stored at 50 °C, harvested from storage bottles at each time point, and stored at −80 °C until analysed. The equilibrium headspace concentration of hexanal was used as a marker of linoleic acid oxidation. The apparent accumulation of headspace-hexanal data was obtained by sequential summation of the mean value from triplicate samples at each time point. Error bars are therefore absent, but the CV range at C_{max} in both of these regimes was 10–15%.

Hakala, Sjövall, Kallio, & Forssell, 2005; Yoshii, Furuta, Yasunishi, Linko, & Linko, 1996). Frequently sugars or oligosaccharides are used as encapsulants, and spray- or freeze-drying is used to take the material into the relatively stable glassy state. Freeze-drying is often associated with improved stability because liquid phase reactions are avoided and the vacuum limits the exposure of PUFAs to oxygen; on the other hand, freeze-drying can result in the generation of free radicals and the development of a porous structure and so allow oxygen access to the oil (Kolanowski et al., 2004).

In this present study freeze-drying was used to reduce the water content of starch extrudates from approximately 30% to 6% (w.w. basis). Micro-cracks often appeared as a result of this drying step. Lipid oxidation was negligible over the course of the study in glassy samples without surface micro-cracks, but was rapid (even relative to the rubbery sample in the initial stages) when micro-cracks were present. But why does this newly exposed lipid in the glassy sample oxidise at significantly higher rates than the surface oil in both of the intact, crack-free glass and rubber samples?

Washing samples with hexane to remove surface lipids rendered the glassy sample with micro-cracks stable to oxidation. The accessibility of hexane to surface lipid in the glassy sample with cracks indicates the newly created surfaces have exposed lipid that is denied the protection of the bulk glassy state. Washing the rubbery state extrudates with hexane did reduce the rate of lipid oxidation, but not as dramatically as in the glassy samples. This suggests that, unlike the glassy samples, the rubbery material has a low enough viscosity to allow oxygen diffusion into the matrix within the time limit of the storage trial. This is further supported by the greater increase in the apparent hexanal accumulation value over time relative to the glassy samples (see Figs. 5 and 6). In the glassy samples, the concentration of hexanal generated in the headspace was considerably reduced if surface lipid was removed, suggesting that either the lipid within the matrix is protected from lipid oxidation, or that hexanal generated within the matrix cannot escape, or both. This is likely to be as a result of the smaller amount of free volume within a glassy system reducing the ability of oxygen to diffuse to the lipid and for the hexanal to diffuse out of the matrix, (Kollengode & Hanna, 1997; Parker, Gunning, Lalloue, Noel, & Ring, 2002; Voilley & Le Meste, 1985) and the higher viscosity, limiting molecular mobility and reducing the chance of reactants colliding (Orlien, Andersen, Sinkko, & Skibsted, 2000).

Solubility of oxygen can also be a limiting factor in lipid oxidation reactions within a matrix. In low water sugar glasses the solubility of oxygen is 10^5–10^6 times less than the solubility of oxygen in pure water. The relatively high moisture content of the rubbery samples compared with the glassy samples (30% and 6% on a wet weight basis, respectively) may therefore not only be assisting oxygen permeation by reducing matrix viscosity, but also by increasing oxygen solubility.

The glassy and rubbery samples in this study had Aw values of 0.3 and 0.95, respectively. According to the relationship between Aw and lipid oxidation kinetics (Labuza et al., 1972) lipid oxidation rate should be at its lowest at Aw 0.3, which is consistent with our findings. Labuza and co-workers proposed that the mimimum rate of lipid oxidation at the BET monolayer (Aw 0.2–0.4) was due to:

(i) The hydration of transition metal cations rendering them less active promoters of autoxidation.
(ii) Lipid hydroperoxides move to oil/water boundaries and are taken out of the propagation reactions due to H-bonding with water.
(iii) Free radical termination reactions increase as polar radicals congregate at the oil/water interface.

They also suggested that from Aw 0.4–0.7, lipid oxidation rate increases again due to:

(i) increased mobility of transition metal cations and
(ii) reduced viscosity.

Lipid oxidation rate decreases again above Aw 0.7 probably due to dilution of initiators such as transition metal ions. One could therefore entertain the idea that the relatively high rate of lipid oxidation in our glassy starch extrudates with micro-cracks is also, at least in part, explained by a localised change in Aw. Aw is an equilibrium value, but within the timescale of this work it is possible that micro-regions develop with higher or lower Aw values than the bulk material.

The Aw of our rubbery material was 0.95 and was therefore liable to microbial spoilage. Indeed mould growth was common on rubbery starch extrudates containing 4% sunflower oil (Bowen, 2005). For this reason linoleic acid was tested instead of sunflower oil; it successfully inhibited mould growth, and acted as a good substrate for lipid oxidation in accelerated storage trials with a time restriction. This allowed us to measure lipid oxidation rate in material with a higher Aw than the highest Aw material represented on the Labuza 'map' (Labuza, 1980). Speculative extrapolation of his lipid-oxidation-rate plot beyond Aw 0.8 suggests that oxidation rates at Aw 0.95, despite being on the decline, will be higher than lipid oxidation rates at Aw 0.3. Notwithstanding the unexpected oxidation results with a cracked glassy matrix, this expectation appears to be justified. It could be argued that measuring lipid oxidation at such high Aw values is pointless since microbial spoilage would ruin the material before lipid oxidation had an impact. However, modern approaches to protecting 'wet' foods against microbial spoilage, such as modified atmospheric packaging and irradiation, effectively justify the ongoing need to understand lipid oxidation in food at high Aw values.

4. Conclusions

- The measurement of hexanal in the headspace above stored starch-extrudate samples was a reliable marker for comparing oxidation rates in the glassy and rubbery samples.
- ω-6 Linoleic acid became oxidised in an extruded starch matrix that was stored in both the glassy and rubbery states.
- Initial oxidation rate was most rapid in the glassy state samples. This was significantly reduced when surface linoleic acid and micro-cracks were eliminated. Linoleic acid is effectively protected against oxidation in starch-extrudates held in the glassy state and devoid of micro-cracks.
- It is difficult to measure the relative contribution of Aw, water content and physical state to the rate of lipid oxidation.

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