Research Notes

Polymerisation and Oxidation of Docosahexaenoic Acid

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Abstract: Oils rich in polyunsaturated fatty acids, such as linseed oil, can oxidise to form polymers. These can be used as protective coatings and act as an alternative to those derived from petrochemicals. Such renewable source oils usually require the addition of metal ions as catalysts of the polymerisation process and/or the partial pre-oxidation of the oil before use. We hypothesised that increasing the unsaturation of the oil would result in polymerisation occurring without such requirements. Of the oils tested only docosahexaenoic (DHA) acid and boiled linseed oil (BLO) (which contains metal ion catalysts) demonstrated significant increases in viscosity during the incubation period, with both oils thickening at approximately the same rate. The production of the lipid peroxidation product propanal was much greater during DHA polymerization compared to that of BLO. DHA and BLO formed a water proof coating on cotton cloth although DHA formed more brittle coatings than BLO. Our results demonstrate that the greater extent of lipid peroxidation of high purity DHA allows it to polymerise in air to form a water resistant material similar to that obtained using boiled linseed oil but without the addition of toxic metal ion catalysts. Further investigation of this substance as a renewable component of coating materials may be warranted, particularly for applications requiring low toxicity such as those in contact with foodstuffs.

Keywords: Docosahexaenoic Acid, Linseed Oil, Lipid Peroxidation, Propanol

Introduction

Polymer coatings, such as paint, are ubiquitous coverings used to enhance the appearance of and protect a wide variety of materials (Thomas, 1991). The various chemicals used in these coatings are predominantly derived from non-renewable petrochemical sources. The original paints were, however, based on plant derived seed oils which contained high concentrations of unsaturated fatty acids esterified into triglycerides (Mosca, 1997). When such unsaturated oils react with oxygen in the air they form hydroperoxides at carbon-carbon double bonds via a free radical initiated mechanism known as lipid peroxidation (Halliwell and Gutteridge, 2007). These acyl peroxides react further in various ways, including decomposing via multi-step reactions to produce, for example, the aldehyde propanal, or become radicals themselves resulting in further peroxide formation, or by reacting with C-C double bonds on other fatty acid molecules to form a C-O-C crosslink. This latter reaction results in the formation of fatty acid based polymers which underlies the oxidative thickening and hardening of the unsaturated oils used in paints (Van Gorkum and Bouwman, 2005). The use of plant oil derived polymers has a long history including their use in oil paintings, the waterproofing of cloth for such things as canoes, the preservation of wood, and in the production of the floor covering linoleum. The oil traditionally used for this task is a seed oil, specifically linseed oil extracted from the ripened seeds of the flax plant *Linum usitatissimum*, although other oils are also used, such as those derived from cotton (*Gossypium hirsutum or Gossypium herbaceum*), sunflower (*Helianthus annuus*) and tung (*Vernicia fordii*) seeds (Mosca, 1997).

In their natural state these oils, known as drying oils, polymerise too slowly to be useful as either artistic or protective coatings (Mallégol *et al*., 2000). It is for this reason that transition metal ion catalysts e.g., 'Japan Drier', such as acyl salts of cobalt, manganese, chromium, cadmium, iron and, historically, lead, are added to speed up peroxidation by means of the Haber-Weiss reaction (Mallégol *et al*., 2000; Greimel *et al*., 2013). Boiled Linseed Oil (BLO) is an example of this

process in which the oil, which contains the n-3 fatty acid alpha-linolenic acid (18:3; ALA), is heated with fatty acids salts of metal to cause partial polymerisation and thickening before being diluted with a solvent to make it workable as a liquid coating. The addition of metal ions makes the finishes coating unsuitable for applications in which toxicity is of concern such as in food packaging. An alternative to linseed oil, that derived from the seeds of the tung plant, also requires a metal drier in most applications. It may also cause allergic reactions in some persons thereby offering little advantage over linseed oil (Mosca, 1997). Plant oils which require no pre-processing step or the addition of metal ions to be used as practicable coatings are, however, unknown. It was therefore of interest to us to examine other oils for their ability to polymerise in the absence of any additions or pre-treatments. One such possibility are the highly unsaturated fatty acids such as docasahexaenoic (22:6; DHA) and eicosapentaenoic (20:5; EPA), which due to them containing more carbon-carbon double bonds than the ALA in linseed oil, peroxidise at higher rates (Gunstone *et al.*, 2007). Both DHA and EPA can be obtained from both marine sources and from algae (Gunstone *et al.*, 2007). In this study we compare the polymerisation rates of a variety of oils, including DHA and EPA, by measuring the increase in their viscosity over time, as well examining the properties of those that polymerised to 'dryness'.

Materials and Methods

The oils used in the experiments, their source and the typical composition of such oils are described in Table 1.

Polymerisation of Oils

About 15 mL of oil/fatty acid were transferred to 250 mL flasks, the flask loosely covered with aluminum foil and incubated at 21, 60 and 85°C for different times after which the degree of polymerisation was assessed either visually and by touch, or by viscosity measurements.

Viscosity Measurements

The viscosity of the samples were measured through the use of a Visage pocket viscosity comparator (Louis C. Eitzen Co., Glenwood Springs, CO) in units of centistrokes. This device functions by comparing the extent of movement of an air bubble through the oil to that of a calibrated standard oil. Since the device is calibrated at 27°C, oils were first incubated at this temperature in a water bath for 10 min before measurements were taken.

Measurement of Headspace Propanal Concentration

The concentration of the lipid peroxidation marker propanal in the headspace of each flask was measured using a soft-ionisation mass spectrometry technique called Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) as previously described by us in detail (Ross *et al.*, 2013). The SIFT-MS instrument was a Profile 3 model purchased from Instrument Science (Crewe, UK). Briefly headspace gas is sampled by negative pressure at a flow rate of approximately 0.2 mL s^{-1} after the flask had been incubated at 27°C for 10 min.

Table 1. Details of oils used

Oil	Major contents 1	Company, Location
Linseed oil	Alpha linolenic acid (55%) Oleic acid (20%) Linoleic acid (15%)	Metro Inc., Thunder Bay, ON
Evening primrose oil	Linoleic acid (70%) Gamma-linolenic acid (10%) Oleic acid (6%)	Metro Inc., Thunder Bay, ON
Canola oil	Oleic acid (60%) Linoleic Acid (20%) Alpha linolenic acid (10%)	Metro Inc., Thunder Bay, ON
Hempseed oil	Linoleic acid (55%) Alpha-linolenic acid (20%) Gamma linolenic acid (5%)	New Directions Aromatics Inc., Mississauga, ON
Tung oil	Alpha-eleostearic acid (80%) Linoleic acid (10%) Palmitic acid (5%) Oleic acid (5%)	Home Depot Inc., Thunder Bay, ON
Boiled Linseed Oil (BLO)	Linseed oil Petroleum based solvent Metal drier[2]	Home Depot Inc., Thunder Bay, ON
Alpha Linolenic Acid (ALA)	Alpha linolenic acid (95%)	Equatech Ltd., Callanish, UK
Docosahexaenoic Acid (DHA)	Docosahexaenoic acid (95%)	Equatech Ltd., Callanish, UK
Eicosapentaenoic Acid (EPA)	Eicosapentaenoic acid (95%)	Equatech Ltd., Callanish, UK

[1]Contents are approximate and derived from average values (Gunstone *et al.*, 2007).
[2]The relative quantities of the constituents are unknown.

The sampled trace gas molecules are reacted in a flow tube with NO^+ ions in a fast moving stream of helium. The mass/charge ratio of the resulting product ions is dependent on the trace gases present, while their rate of formation can used to calculate the concentration of the analyte of interest. When propanal is reacted with NO^+ the resulting product ion has a mass/charge ratio of 99 which is formed at a known rate from which propanal concentrations can be calculated (Spanel and Smith, 1997; Ross *et al*., 2013). The $NO+$ precursor ion count rate was over 500,000 cps with $NO+$ making up over 99% of all precursors ions present. The flow tube pressure used in these experiments was approximately 1 Torr.

Measurement of Water Resistance

One hundred percent cotton squares (15×15 cm) were liberally coated with an oil, which was then incubated at 21, 60 and 85°C for a period of up to 340 h on a custom built hanging rack. The oil cloths, after the desired polymerisation time period was achieved, were tested for the amount of water column inches that they could withstand before water pentrated the coated cloth. This was accomplished by attaching the cloth to a 0.5 inch PVC pipe using pipe clamps connected to a 40 psi water source in series with a flow control valve and pressure gauge. The water control valve was slowly opened until water penetration was visually detected and the pressure at which this occurred was recorded.

Results

Viscosity Change during Auto-Oxidation of Oils

In preliminary experiments flax, evening primrose, canola, BLO, hempseed oils, or ALA, DHA and EPA, were incubated for 80 h at 21, 60 and 85°C in order to determine which oils exhibited increased viscosity over this time frame. DHA and BLO were the only two oils which exhibited detectable thickening and were deemed suitable for further study. Over time both oils increased in viscosity when incubated at all three temperatures. This was most rapid at 85°C with the maximum measureable viscosity of 400 centistrokes being exceeded during the time course (Fig. 1). Since the DHA used is 95% pure while linseed oil contains 60-70% polyunsaturated fatty acids we diluted the DHA with canola oil to produce a mixture containing approximately 70% polyunsaturated fatty acids. This did thicken but not as quickly as 95% pure DHA and was not used in subsequent experiments (Fig. 2). We also investigated whether the addition of Fe^{2+} ions as a catalyst of oxidation would change the rate at which viscosity would change. As illustrated in Fig. 3 the addition of $Fe(II)Cl_2$ had no effect on BLO polymerization but did increase the rate of that of DHA.

Headspace Propanal Concentration

Propanal concentrations in the headspace above BLO or 95% DHA was measured using SIFT-MS. Under all incubation conditions DHA headspace exhibited higher propanal concentration than BLO (Fig. 4). For both oils incubation at 21°C resulted in the lowest concentration of headspace propanal. When incubated at 60 or 85°C headspace propanal concentration peaked at the 171 s for both oils although propanal concentrations were highest in DHA incubated at 60°C and for BLO incubated at 85°C.
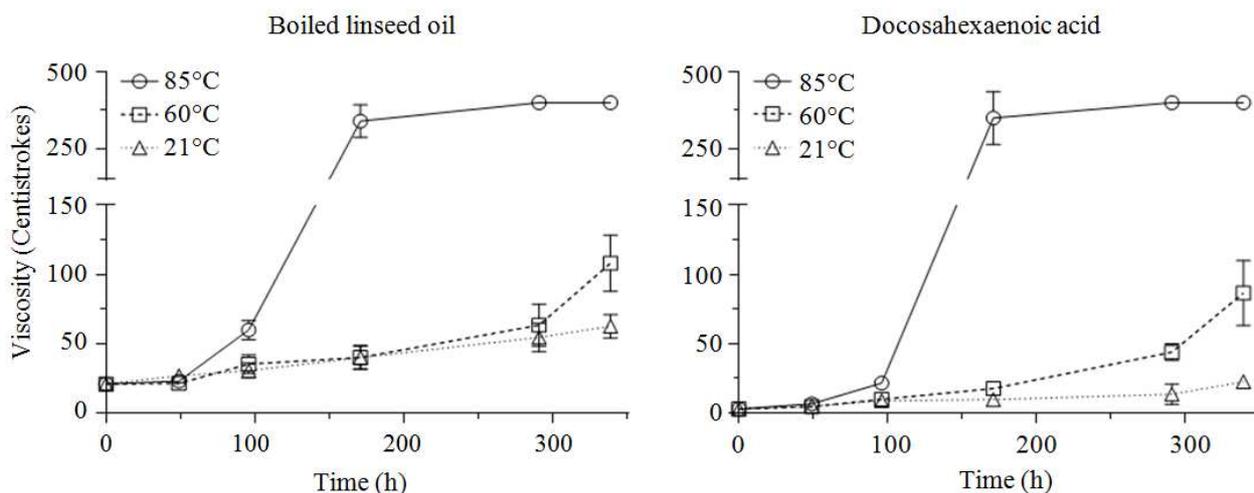

Fig. 1. Effect of incubation time and temperature upon viscosity of boiled linseed oil or 95% purity docasahexaenoic acid. Oils were incubated for various lengths of time at 21, 60 or 85°C as indicated. Data shows the mean viscosity at each time point of 3 experiments; bars indicate the standard deviation
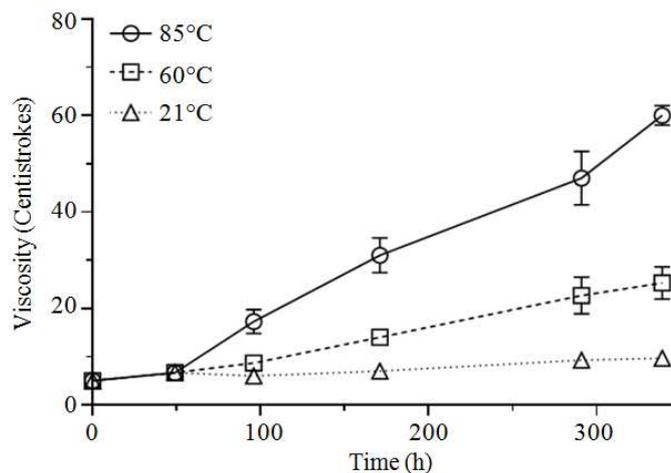
Fig. 2. Effect of incubation time and temperature upon viscosity of docosahexaenoic acid diluted to 70% purity with canola oil. The oil mixture was incubated for various lengths of time at 21, 60 or 85°C as indicated. Data shows the mean viscosity at each time point of 3 experiments; bars indicate the standard deviation
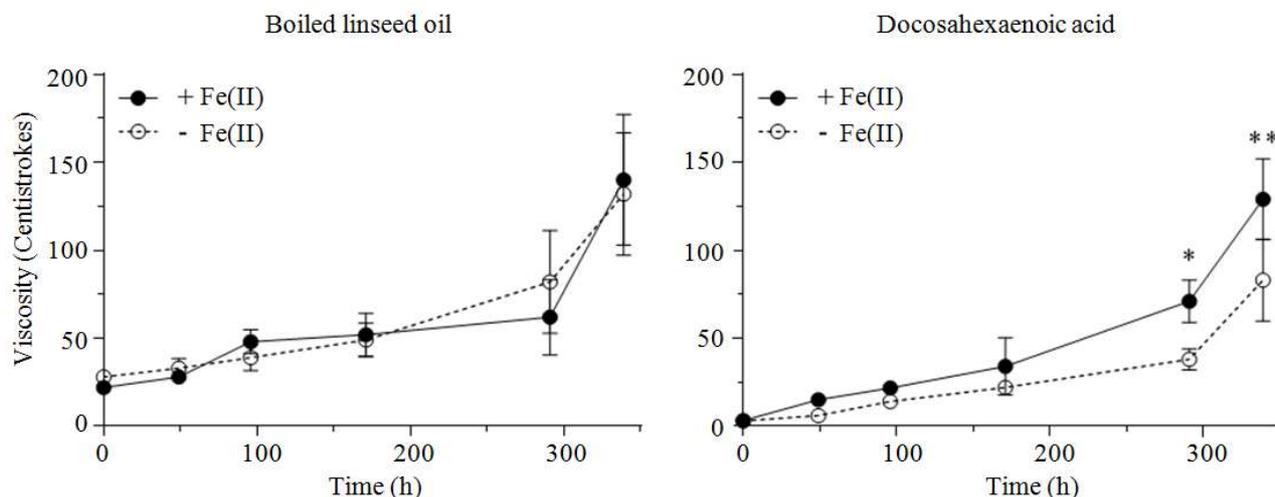

Fig. 3. Effect of the addition of Fe^{2+} ions on the polymerization of boiled linseed oil or 95% purity docosahexaenoic acid. Both oils were incubated at 60°C for various lengths of times with or without the addition of $Fe(II)Cl_2$ in ethanol to a final concentration of 0.5 mM. Data shows the mean viscosity at each time point of 3 experiments; bars indicate the standard deviation. A 2-way ANOVA of the data collected for BLO showed an effect of time ($F_{5,24} = 27.91$; $p<0.0001$) but no effect of the presence of Fe(II) ions ($F_{1,24} = 0.083$; $p>0.05$). For DHA there was both an effect of time ($F_{5,24} = 67.84$; $p<0.0001$) and of the presence of Fe(II) ion ($F_{1,24} = 22.32$; $p>0.0001$) with post-hoc Tukey tests showing a difference at the last two time points: *: $p<0.01$; **: $p<0.001$

Water Resistance of DHA and BLO Coated Cotton

The pressure required to penetrate cloths coated in polymerised BLO and DHA was investigated. When incubated at 85°C the water resistance of BLO cloth reached a maximum after 12 h but had fallen by 24 h after which permeability stayed fairly constant (Fig. 5). This loss of resistance correlated with our subjective impression of decreased pliability of the coated cloth. The resistance of the cloth incubated at 60°C rose more gradually but had reached approximately the same resistance value as the 85°C cloth by the end of the experiment. At 60°C DHA application caused a rapid increase in water resistance after 24 h, but this fell at later time points. In contrast incubation at 85°C resulted in increased resistance at both 6 and 12 h. By 24 h the rigidity of the cloth had increased to such an extent that we could not attach the cloth to the apparatus and hence water resistance could not be measured past 12 h.
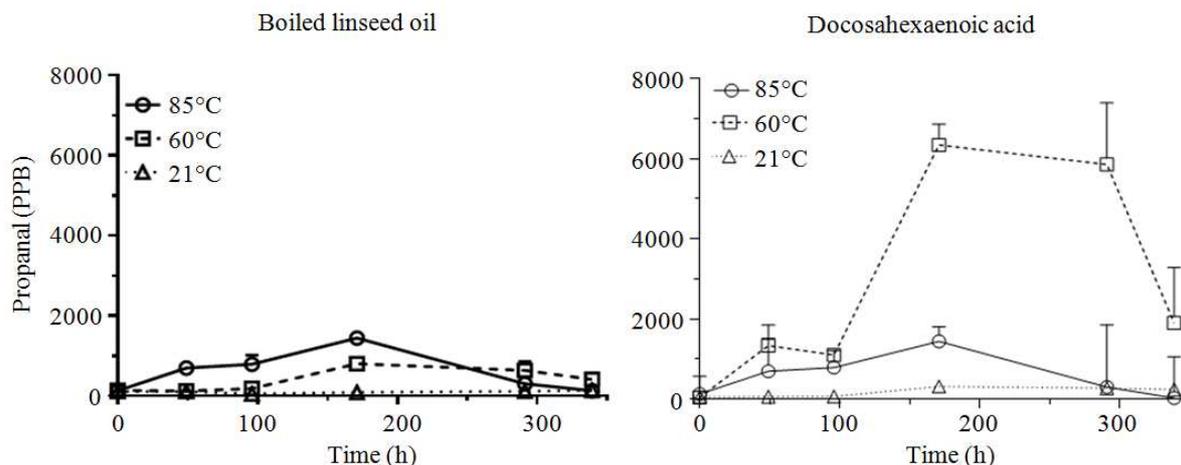
Fig. 4. Effect of incubation time and temperature upon headspace propanal concentration of boiled linseed oil or 95% purity docosahexaenoic acid. Oils were incubated for various lengths of time at 21, 60 or 85°C as indicated. Data shows the mean propanal concentration at each time point of 3 experiments; bars indicate the standard deviation
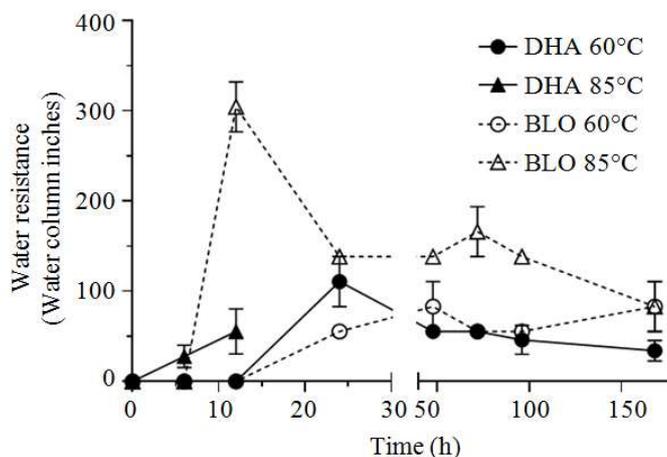

Fig. 5. Effect of incubation time and temperature upon the water resistance of cotton cloth coated with Boiled Linseed Oil (BLO) or 95% purity Docosahexaenoic Acid (DHA). The coated cloth was incubated at 60 or 85°C for various lengths of time as indicated before water resistance was measured. Data shows the mean water resistance at each time point of 3 experiments; bars indicate the standard deviation. Note that no data was collected beyond 12 h for DHA coated cloth incubated at 85°C due to the rigidity of the cloth being too great to allow a measurement to take place

Discussion

Our major finding is that when 95% pure DHA is incubated in the presence of air it thickens and eventually hardens at approximately the same rate as linseed oil which has been preheated and to which metal ions have been added. The addition of Fe(II) ions to BLO has no effect upon the rate of viscosity increased most likely due to metal ion oxidation catalysts already being present. While Fe(II) ions did increase the rate of DHA polymerization (Fig. 3), such additions were not required for DHA polymerisation to occur nor for it to form a coating approximately equivalent to that of BLO. DHA likely does not require the same treatment and additions due to it being a highly unsaturated fatty acid

compared to the less saturated 18 carbon fatty acids (ALA and linoleic acid) which are the major constituents of linseed oil; the higher number of double bonds per molecule may increase oxidation-induced crosslinking which is the basis of the polymerisation process that allows both substances to 'dry'. In addition, the lower relative proportion of unsaturated fatty acids in BLO compared to 95% DHA may also underlie the ability of DHA to polymerise without the additional steps used for BLO. Indeed, when DHA was diluted with canola oil to approximate that proportion of unsaturated fatty acids in linseed oil the rate at which viscosity increased fell markedly. On the other hand 95% pure EPA, a 20 carbon fatty acid containing 4 carbon-carbon double bonds, did not thicken under the same conditions under which the

viscosity of DHA and BLO did increase. This suggests that both the ratio of unsaturated to saturated fatty acids and the degree of unsaturation per molecule play a role in determining the rate of polymerisation. Such a conclusion is supported by our observation that more propanal is released by polymerizing DHA than is by BLO. Propanal concentrations were not correlated with viscosity, rising as they did before viscosity significantly increased and falling towards the end of the time course. Given the similar viscosity changes of the two oils this suggests that increased lipid peroxidation early in the time course allows DHA to crosslink similarly to the pre-processed BLO. Our data also suggests that monitoring the extent of polymerization via measurement of out gassing of volatile compounds is not a feasible approach.

When used as a coating, DHA incubated at 85°C hardened to the point that water resistance could not be measured (Fig. 3). Furthermore when incubated at 60°C while DHA coated cloth initially demonstrated superior water resistance to BLO this decreased with increasing incubation time and was approximately equivalent to BLO by the end of the experiment. Although flexibility was not objectively measured in this study our subjective impression was that water resistance fell in correlation with increasing rigidity of the coated material. As such, BLO appears to be a superior coating material to 95% DHA, however modifying the polymerization process may improve the coating's characteristics. Some possibilities which will be addressed in future work are to further refine the temperature of polymerization, change the thickness of the coating layer, determine the effect of antioxidants such as tocopherol which may be able to modulate the rate of oxidation and the inclusion of additives such as saturated fatty acids or other lipid soluble natural materials. Finally, it should be noted, however, that the water resistance of cotton coated with both oils and incubated at 60°C (approximately 100 inches or 2500 mm of water by the end of the experiment) is at the lower end of water resistance compared to the typical 200-800 inches water demonstrated by synthetic materials (Van Roey, 1992).

Conclusion

In summary we report that 95% purity DHA can polymerise to produce a 'dry' coating with comparable water resistance to that observed using BLO but without the use of potentially toxic metal ion 'dryers'. As such DHA may be suitable for use in applications where toxicity is of importance such as in food packaging. Future work will usefully investigate how coatings containing this renewable substance can be optimized.

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Author's Contributions

Adrian McKee: Assisted in the design of the study, carried out the experimental work and assisted in drafting the manuscript.

Shelly L. Browning: Participated in the conception and design of the study and assisted in the drafting the manuscript.

Brian M. Ross: Participated in the conception and design of the study and was the primary author of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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