The Removal of Fatty Residues from a Collection of Historic Whale Skeletons in Bergen: An Aqueous Approach to Degreasing

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The Removal of Fatty Residues from a Collection of Historic Whale Skeletons in Bergen: An Aqueous Approach to Degreasing

L'élimination des résidus gras sur une collection ancienne de squelettes de baleines à Bergen : le dégraissage par voie aqueuse

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Abstract: Before any informed conservation intervention can be designed or implemented it is necessary to have a very clear understanding of the problems that need solving – i.e. an in-depth knowledge of the material in terms of chemistry and structure, as well as its deterioration and responses to different treatments. Of course in practice a more pragmatic approach is normally necessary and previous experience is combined with limited test-cleaning experiments when developing a conservation strategy. Before discussing the problems arising from degraded oils and fats on marine mammal skeletons it is appropriate to review the composition and structure of whale bone and whale oils.

Résumé : Avant que toute intervention de conservation documentée puisse être entreprise il est nécessaire d'avoir une compréhension très claire des problèmes à résoudre - c.-à-d. une connaissance détaillée du matériel en termes de chimie et de structure, aussi bien de sa détérioration que de réactions à différents traitements. Naturellement dans la pratique une approche plus pragmatique est normalement nécessaire et une expérience antérieure est combinée avec des expériences limitées d'essai de nettoyage en développant une stratégie de conservation. Avant de discuter les problèmes résultant des huiles et des graisses dégradées sur des squelettes de mammifères marins il est nécessaire de passer en revue la composition et la structure de l'os et des graisses de baleine.

1. Introduction

Before any informed conservation intervention can be designed or implemented it is necessary to have a very clear understanding of the problems that need solving – i.e. an in-depth knowledge of the material in terms of chemistry and structure, as well as its deterioration and responses to different treatments. Of course in practice a more pragmatic approach is normally necessary and previous experience is combined with limited test-cleaning experiments when developing a conservation strategy. Before discussing the problems arising from degraded oils and fats on marine mammal skeletons it is appropriate to review the composition and structure of whale bone and whale oils.

2. Structure and Composition of Bone

Dried, fully mature bovine bone tissue (acellular tissue oven dried for 24 hr @ 105^{o}C) is approximately 24% collagen, 66% HAP (hydroxylapatite) and 10% water by weight. In terms of the volume fraction these amount to approximately 46% collagen, 46% HAP and 8% water (Campos et al. 2011). The Haversian and Volkman canals, together with osteocyte lacunae and canaliculi occupy a considerable volume and consequently bone tissue has a porosity of about 28%. Bone can therefore be considered to have two densities – a bulk density which includes all of the pore spaces (and is therefore correspondingly lighter) of 1.47 gcm^{-3} and a skeletal density of 2.04 gcm^{-3} in which the volume of the pores is excluded and which therefore is a better reflection of the actual composition of bone tissue. Compared to land mammals there is little available data on the exact structure and composition of whale bone and many of its properties must be inferred from other sources.

There is some variation in the composition of bone tissues between different species, depending upon the mechanical demands made upon bones by the behaviour and lifestyle of the animal. For example, wallaby tibia has weight fractions of 25% collagen, 65% HAP and 10% water; while penguin radius is 28%, 60% and 12%; and red deer antler has values of 36%, 44% and 20% (Zioupos et al. 2000). Figure 1 shows the relative proportions of collagen, mineral (HAP) and water expressed as weight percent for a range of vertebrate skeletal tissues. Using data from Zioupos et al. (2000) and Currey (1988) it is possible to estimate the composition of North Atlantic right whale rib bone as 30%, 56% and 14% for collagen, HAP and water respectively. Thus, whale bone appears to be slightly less well mineralised than that of land animals. These conclusions are supported by data from Tont et al. (1977) who published densities of bones in marine mammals. From their data fin whale rib has a higher collagen content and higher water content than human vertebra, and a correspondingly lower mineral content. Conversely, they showed that sperm whale bone had a higher mineral content, and higher overall density, than human bone (Figure 2).

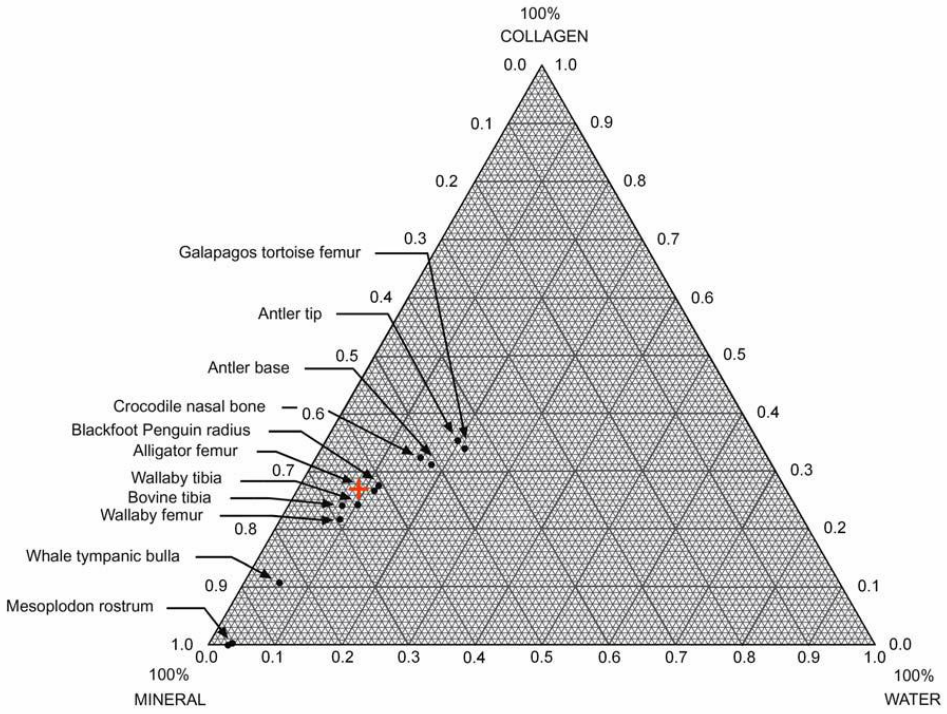

Figure 1: Relative proportions (as wt%) of collagen, HAP and water for a range of species. The red cross represents human femur. Diagram adapted from Zioupos et al. 2000.

The denser bone in sperm whales may reflect the different feeding habits of baleen whales and deep diving sperm whales. Tont et al. used ashing to estimate mineral content which always underestimates the amount of HAP because CO_2 is lost during the ashing process. However, it is possible to estimate the mineral contents of their samples. This gives mineral and collagen contents for sperm whale of ~67% and ~25% and those of fin whale at ~60% and ~29%, respectively. That places bone from fin whale somewhere between bovine and human bone and antler, although there will be considerable variation in the composition of whale bones depending upon skeletal element examined.

The porosity of whale bones is also an issue that influences the bones' mechanical behaviour and their subsequent degradation. Only the mandibles and upper limb bones of whales are beam-like, load-bearing structures and it is only these elements that contain substantial amounts of compact bone. The vertebrae and ribs are composed largely of spongy or trabecular bone with a high porosity. The greater porosity of whale bones means that they have a high storage capacity of oil while the animal is alive, and that they retain a high proportion of that oil even after the bones have been macerated to remove the soft tissues. The role of porosity in the degradation of whale oils will be considered further below.

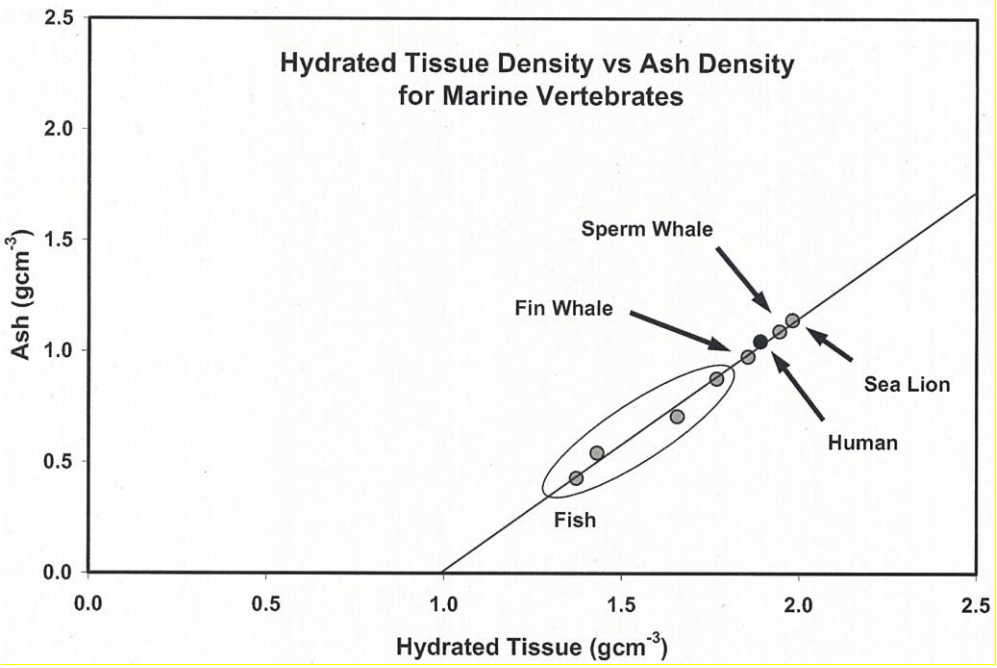

Figure 2: Comparative densities of different marine vertebrates including mammals and fish. Diagram adapted from Tont et al. 1977.

3. The Composition of Whale Oils

The composition of whale oils varies considerably between species but like all oils derived from marine animals it is characterised by a high proportion of triacylglycerols and esters of unsaturated fatty acids – i.e. the fatty acids containing one or more C=C double bonds. Because of the rapid decline in commercial whaling in the second half of the 20th century (leading to a ban in 1986) much of the data on whale oil compositions predate modern analytical methods and are therefore unreliable. The available compositions of whale oils from different species are given in Table 1 below.

In Table 1 the saponification number refers to the number of milligrams of potassium- or sodium hydroxide required to saponify one gram of fat under specified conditions. It is a useful measure of the average molecular weight of all the fatty acids present in the sample. The whale oils extracted from whale blubber differ from those found in whale bones which have a lower percentage of unsaturated fats.

Table 1: Compositions of different whale oils (Data taken from NIIR Board: Modern Technology of Oils, Fats Its Derivatives: Table 32).

Species	% Saturated	% Unsaturated	Saponification Nº	% Unsaponifiable
Blue Whale	13.6-26.3	73.7-86.4	183.0-198.0	0.7-3.5
Humpback Whale	13.0	87.0	183.5-190.1	0.31-0.64
Grey Whale	10.0-14.2	83.8-90.0	191.0-193.0	1.6
Sperm Whale	10.0-19.0	81.9	120.0-150.3	17.5-44.0
Sei Whale	18.5-16.4	73.6-81.5	186.9-193.1	0.56-1.54
Fin Whale	25	75	190.3-196.5	0.32-1.98
Blue Whale (bone)	n.d.#	53*	n.d.	n.d.

* Estimated from data given in Ackman 1966. # n.d. = No data.

Whale bones contain a very high proportion of oil, although the exact values vary from one bone element to another and are dependant on species, sex and maturity of the individual. Table 2 shows a breakdown of lipid content for different bones. Values for the blue whale are averaged over several specimens of different size and sex. The fin whale and blue whale appear to have the greatest oil yields in their bones. Furthermore, data reported in Higgs et al, 2011 indicate that the bones of the skull and mandible, and the lumbar and caudal vertebrae have the highest lipid contents (~40-55%), whilst the thoracic vertebrae have the lowest (~10-25%) in most species analysed. This relative distribution is also representative of the whale skeletons in University Museum of Bergen's Whale Galleries where the tail vertebrae are markedly more disfigured by oil than the thoracic vertebrae.

Table 2: Lipid contents of whale bones (Data taken from Higgs et al., 2011: Table 2).

Skeletal Element	Fin Whale (wt%)	Blue Whale (wt%)
Mandible	n.d.	78.8
Rostrum of Skull	65.4	66.8
Scapula	n.d.	69.3
Rib	32.0	n.d.
Thoracic Vertebra	3.4	17.1
Lumbar Vertebra (anterior)	67.0	37.2
Lumbar Vertebra (posterior)	n.d.	60.6
Caudal Vertebra	52.0	51.6
Humerus	n.d.	64.0
Radius and Ulna	n.d.	66.0
Metacarpals	n.d.	60.0

Not surprisingly, in many bones the overall geometry has affected the distribution of oil and fat. Bones such as ribs with a vertical orientation tend to be more soaked with oil in the lower extremities. A seemingly counterintuitive observation is that the spinous processes of some vertebrae, which stand vertically above the vertebral bodies, and the upper margins of the scapulae are often oily at the uppermost extremities. This presumably reflects the pore size distribution of the spongy bone tissues where oil is drawn into the smallest available pores by capillary forces that can overcome gravity. This wicking of oil into different parts of the bone

architecture can be seen in several skeletal elements (Figures 3-5). Furthermore, oil can be seen to have wicked up into the thick layers of dust lying on the upper surfaces of vertebrae (Figure 6).

Table 3: Fatty acid compositions of whale oils (Data taken from NIIR Board: Modern Technology of Oils, Fats Its Derivatives: Table 33).

Chain Length	Arctic		Antarctic	
	% Saturated	% Unsaturated	% Saturated	% Unsaturated
C_{14}	4.1		7.3	3.1
C_{16}	10.4	18.0	16.9	13.7
C_{18}	3.5	33.0	2.2	37.8
C_{20}		20.0	0.6	11.7
C_{22}		11.0		6.7
Total	18.0	82.0	27.0	73.0

The properties of an oil or fat are dependant upon the fatty acid composition of the triacylglycerol. The fatty acids differ mainly in length but also in the number of unsaturated double bonds. The fatty acids that make up the whale oils range from C_{14} to C_{22} with a higher proportion of unsaturated fatty acids found in Arctic whales compared to Antarctic catches (Table 3).

Figure 3: Oil that has run down the surfaces of two minke whale rib (note also the fungal "foxing").

Figure 4: Oil accumulation in the spongy areas of the distal radius and humerus of the humpback

Figure 5: Oil has collected in the superior margin of this sei whale scapula. Note the oily patch in the lateral surface of the body of the scapula.

Figure 6: Oil wicked into the thick layer of dust on fin whale vertebrae (note white paint spots).

The proportion of unsaturated fatty acids in a lipid determines several of its properties, including whether it is a liquid (oil) or solid (fat) at room temperature. Several of the fatty acids in whale oils are polyunsaturated containing two or more double bonds, with some highly unsaturated fatty acids, including acids of the $C_{18,}$ C_{20} and C_{22} series having 4, 5 and 6 double bonds.

4. The Degradation of Whale Oils

Both saturated and unsaturated lipids are susceptible to oxidation by a number of mechanisms – a process commonly referred to as rancidification when it occurs in edible fats and oils. Hydrolytic rancidity is when water splits the fatty acid from the glycerol backbone of a triacylglycerol (triglyceride). Technically the chemical term for this is ester hydrolysis. The resultant free fatty acid is released into the remaining bulk of the lipid and may alter its properties such as odour, colour and viscosity. Some of the shorter chain fatty acids released my be volatile and escape into the air. Commercial trade in whale oil used a classification of different qualities or grades based on the colour of the oil and proportion of free fatty acids in it (Table 4). Grade 4 was notable for its fishy odour and foul taste. Rancidity can also be caused by bacterial action, in which case the breaking of the ester bond is achieved by bacterial enzymes or lipases.

Table 4: Classification of whale oils according to colour and free fatty acid compositions (Data taken from NIIR Board: Modern Technology of Oils, Fats Its Derivatives: Table 31).

Grade	Colour	Free Fatty Acid Content ($\%_{max}$)
0	Pale yellow	0.5
1	Pale yellow	1
2	Amber yellow	6
3	Pale brown	15
4	Dark brown	30

Generally as the free fatty acid content increases the colour becomes darker, passing from a pale honey colour to dark brown. This picture is also representative of the whale skeletons hanging in University Museum of Bergen with the visible oil drips varying from light brown to dark brown (see Figure 3 above). In principle, the free fatty acids are water soluble but in the whale skeletons, although there may be sufficient absorbed water to initiate hydrolysis of the ester bond in the triacylglycerol, there is no free water and the longer chain fatty acids are likely to accumulate in the oily residues.

In relation to the condition of University Museum of Bergen's whale skeletons there are three major problems that arise from the original oil content of the bones:

1. Fatty acids, aldehydes and other breakdown products
2. Oxidised and cross-linked films
3. Residual un-degraded oil

The first category, although less visibly disfiguring than the others, will be discussed first. Although the fatty acid backbones of lipids (acylglycerols) are reasonably tough they can be cleaved off from the glycerol unit to form diacylglycerols, monoacylglycerols and free fatty acids – either by enzymatic action or by hydrolysis as described in Figure 7. The mono- and triacylglycerols are polar because of the presence of hydroxyl groups. Thus, hydrolysis products in degrading oil in the whalebones are likely to form polar bonds with other moieties within the pores structure, and will also adsorb onto the surfaces of bone apatite (HAP) of the bone tissue

itself. Similarly, free fatty acids may form insoluble salts with calcium if free calcium ions are available (for example from local acid dissolution of bone mineral by micro-organisms). Fatty alcohols and aldehydes are also produced by the catalytic hydrogenation of fatty acids and methyl esters of oils and fats. Commercially this is achieved at elevated temperatures and pressures but can occur at room temperatures by the action of bacterial enzymes.

Chemical analyses of lipid content and degradation products of whale bone extracts and fatty residues taken from the surfaces of the bones are currently underway.

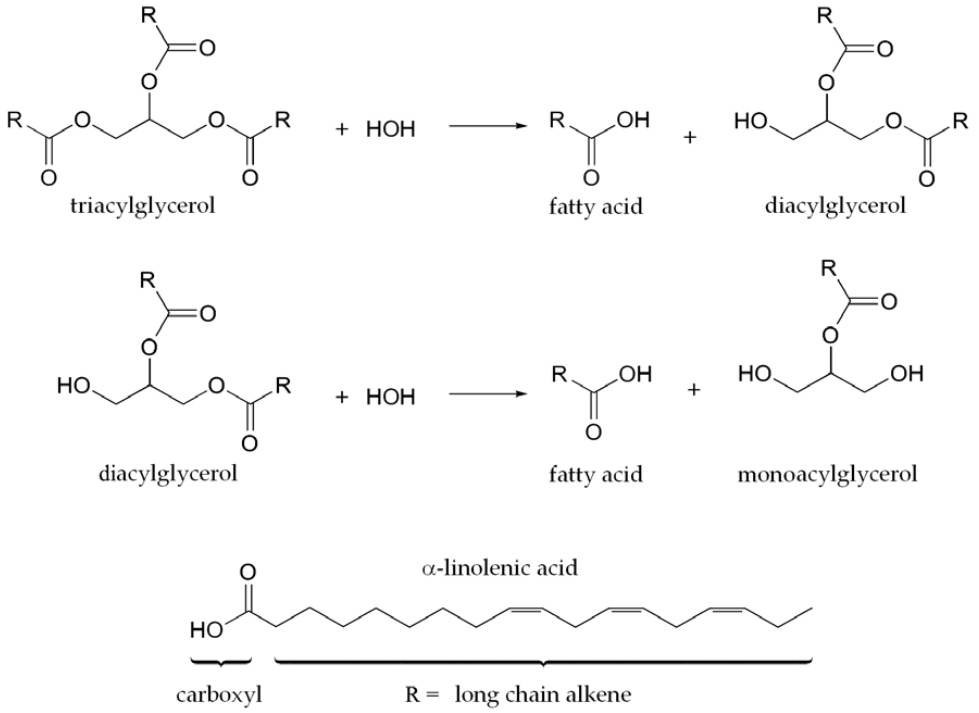

Figure 7: Hydrolysis of a triacylglycerol to form mono- and diacylglycerols. The R-group can be any long chain fatty acid but here the fatty acid is α-linolenic acid.

Polyunsaturated marine oils fall under the classification of drying oils. Drying oils are those that form tough, solid films when exposed to the air. Drying oils have traditionally been used in the manufacture of paints and varnishes where, unlike modern paints and varnishes that dry by the evaporation of solvent, the film forming mechanism is a cross-linking or polymerisation action by atmospheric oxygen. The best known application of a drying oil is in oil paints where linseed oil is used as the binding medium. Other drying oils include walnut oil, poppy seed oil, sunflower oil and – in the Orient – tung oil. Historically, in Norway and Newfoundland marine oils have been used as a binding medium for paints where cod liver oil or seal oil was mixed with red ochre to make a red paint traditionally used on wooden outbuildings.

The mechanism of hardening of the drying oils relies on the presence of unsaturated bonds in the fatty acids – e.g. linseed oil contains a high proportion of α-linolenic acid (~55%) with three double bonds (see Figure 7) and linoleic acid (~15%) with two double bonds. The first stage of the hardening process is the absorption of atmospheric oxygen into the liquid oil. There is a noticeable weight gain in linseed oil films exposed to air, although this declines after the first few days (Figure 8).

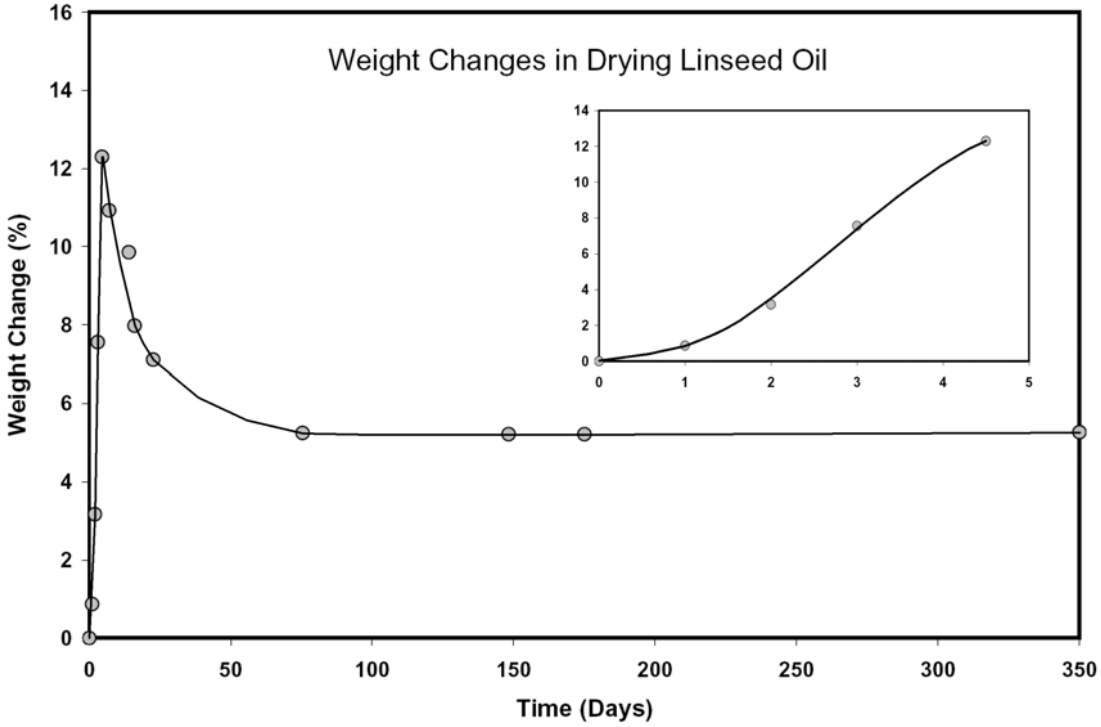

Figure 8: Weight changes in a linseed oil film drying in air. The inset shows the first 5 days of the experiment. (Data reconstructed from Hess & O'Hare, 1952: Figure 6).

If one examines the oxygen content of the drying oil film an interesting pattern emerges. There is a very rapid initial adsorption of oxygen over the first week until a saturation point is reached at around 20% (Figure 9). That level of oxygen is maintained for more than a year despite a considerable loss of weight in the oxidising film. This can only mean that volatile organic compounds are escaping from the polymerising linseed oil film. The horizontal straight lines representing constant weight and constant oxygen concentration further suggests that a dynamic equilibrium is achieved where oxygen is being consumed as quickly as it is being absorbed, although it is very likely that the formation of a hardened film on the surface reduced the diffusion of atmospheric oxygen into the bulk of the oil.

There is little published data on the drying of unsaturated oils from marine mammals but there is a considerable literature on the drying of vegetable oils (Mallego et al., 2001; Stenberg, 2004; Asadauskas et al., 2007; Grehk et al., 2008). The initial stages of drying are thought to be due to free radical attack to the allylic hydrogen of unsaturated fatty acids. Allylic hydrogens are those that are located on carbon atoms adjacent to a double bond and they exhibit higher reactivity than those on carbons forming double bonds with neighbours. Abstraction or removal of one of the allylic hydrogens leads formation of an allylic radical where the bond between the two carbon atoms becomes destabilised. This bond can be stabilised by the donation of electron density (π electrons) from an adjacent double bond (see Figure 10) to form a resonance structure. This allylic radical then undergoes oxidation with atmospheric oxygen that has diffused into the oil to form a peroxide radical. This in turn can decompose via reaction with water molecules dissolved in the oil film (especially in high humidity) or by adjacent fatty acids to form a hydroperoxide, accompanied by the release of another free radical which is then available to take part in further allylic hydrogen abstraction (Figure 10). Hydroperoxides undergo a wide range of decomposition

pathways, including photo-oxidation to form alkoxy radicals with the release of hydroxyl radicals. The reactions described above are commercially important in the oxidative spoilage of foodstuffs containing unsaturated fats and have been widely studied.

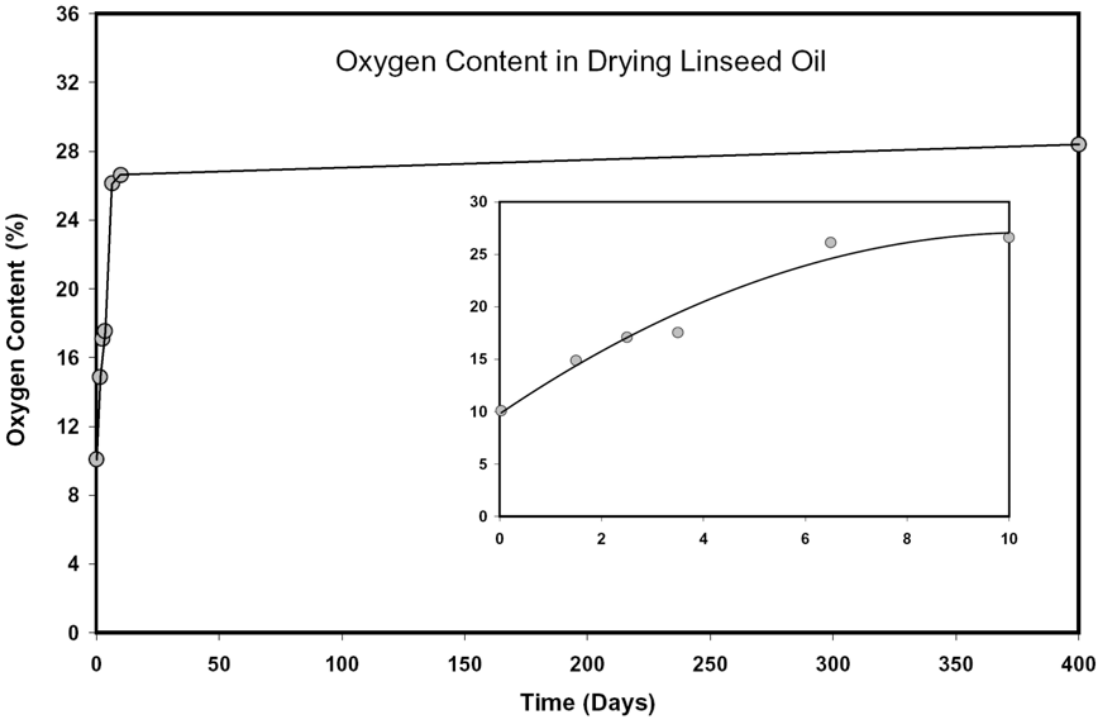

Figure 9: Oxygen uptake in a linseed oil film drying in air. The inset shows the first 10 days of the experiment. (Data reconstructed from Hess & O'Hare, 1952: Figure 7).

The alkoxy- and peroxide radicals formed during hydroperoxide decomposition undergo reactions between themselves and with adjacent molecules by condensation and oxidation reactions (Figure 11). The resulting ether and peroxy bonds lead to a crosslinked network that is characteristic of a dried oil film (Gorkum 2005). The alkoxy radicals also undergo β-scission reactions to form aldehydes and carboxylic acids, which may be saturated or unsaturated (Figure 12). Thus, hydroperoxides decompose to short-chain saturated and unsaturated aldehydes and ketones, as well as short-chain carboxylic acids. These include pentanal, hexanal, hepta-2,4-dienal, decanal, heptan-3-one, nonan-2-one, acetic acid, propanoic acid and butanoic acid (Hsieh et al. 1989). All of these are volatile and may leave the surface of the drying oil film, resulting in the weight losses seen in Figure 8.

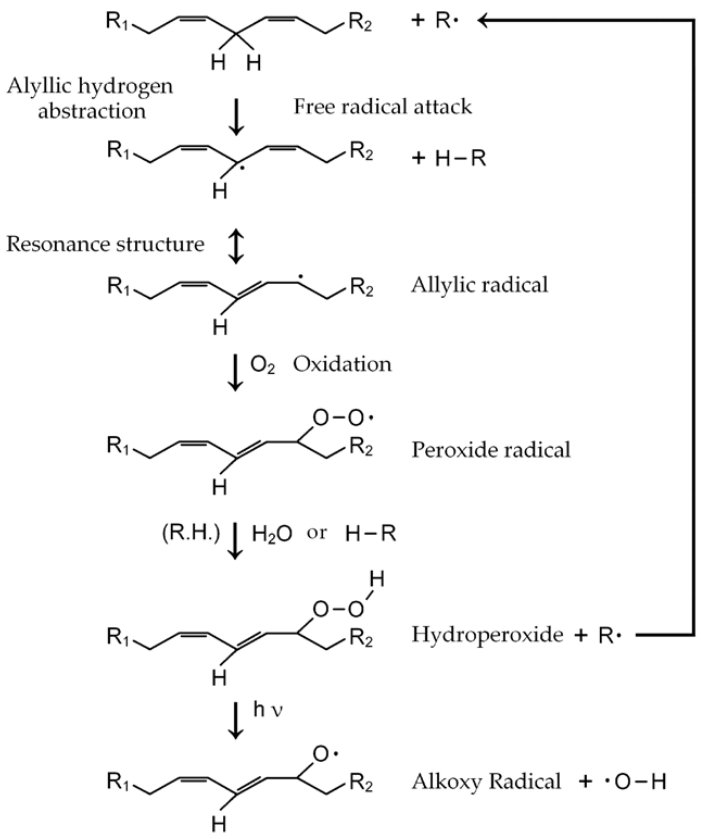

Figure 10: Free radical attack on a polyunsaturated fatty acid to form a hydroperoxide.

These structural changes, and the loss of low molecular weight volatiles, will be accompanied by a decrease in volume. In a drying paint film where there is a limited amount of oil available these effects of these volume changes will be negligible – assuming the shrinkage is not sufficient to disrupt the surface. However, in the case of oil-soaked whalebones one effect of these volume changes will be to allow fresh, un-oxidised oil to wick under capillary forces towards the surface via the bone's natural microporosity. Thus, a thick film of sticky oil can accumulate on the bone's surface until the point where the hardening layer impedes further diffusion of oxygen into the bulk of the bone and reactions slow to a halt. This implies that oil sealed deep inside the porous structure of the bone may remain partly un-oxidised due to the degradation processes being very much slowed, and that there is some kind of gradient from almost un-degraded lipids, through partly- and fully oxidised oils and fatty acids to a dried film on the surface. This has implications for the cleaning of the whalebones – as will be seen later.

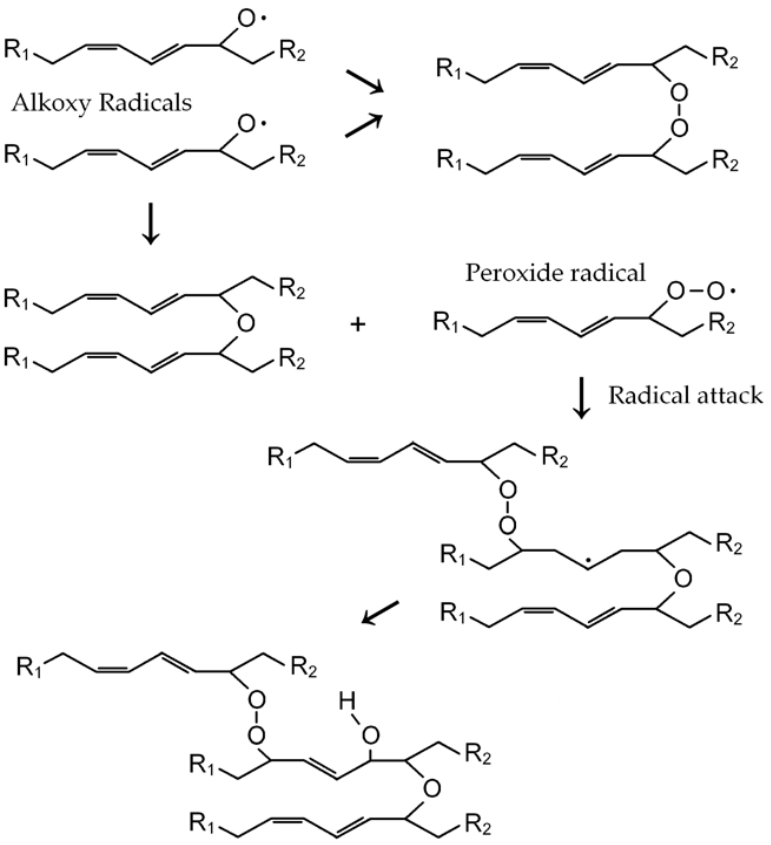

Figure 11: Formation of network in a drying oil film from alkoxy- and peroxide radicals. The resulting ether- and peroxy bonds form cross-links between adjacent chains.

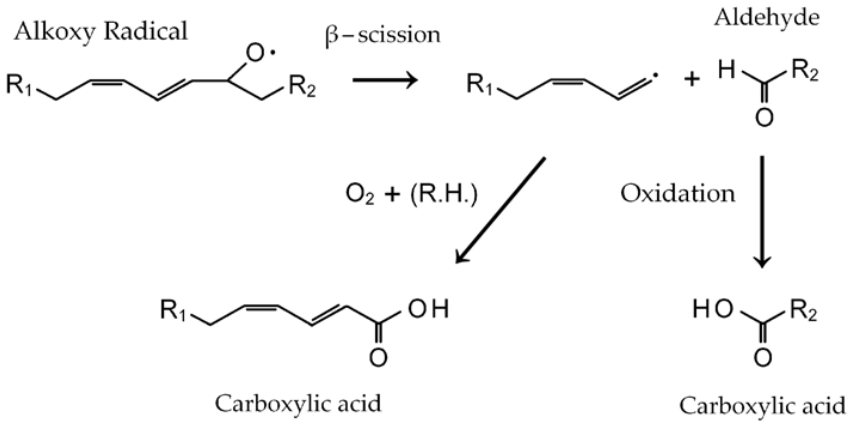

Figure 12: Beta scission of an alkoxy radical leading to production of aldehydes and carboxylic acids.

5. Test-cleaning of Greasy bones

From the beginning the approach to the "cleaning" of the whale skeletons focussed on four major considerations – health and safety to conservation staff and the museum staff and visitors; efficacy (in terms of a good result in a reasonable time); easy of use (skeletons had to be cleaned in situ); and cost. Initial investigation involved test cleanings with a variety of organic solvents, both polar and non-polar:

1. cyclohexane
2. xylene
3. toluene
4. methyl chloride
5. acetone
6. isopropyl alcohol
7. ethanol
8. water

Although one would expect oils and fats to be soluble in non-polar solvents (e.g. aliphatic hydrocarbon solvents such as cyclohexane; aromatics such as toluene and xylene; or chlorinated solvents such as methyl chloride) these solvents proved surprisingly ineffective at cleaning the bone surfaces. In contrast, the polar solvents acetone and the alcohols, had some cleaning effect. This is consistent with the model described above where the surfaces of the bones are covered in a complex mixture of cross-linked and oxidised fatty acids which are themselves polar. The most effective solvent cleaning was achieved using paper poultices wetted with a mixture of acetone:ethanol:water in the ratios 1:1:1 containing a few drops of the surfactant Triton X-100 ($C_{14}H_{22}O(C_2H_4O)_n$). The addition of a few wt% aqueous ammonia increased the cleaning efficiency considerably and tests were undertaken to evaluate using purely aqueous methods (without organic solvents) to solubilise and remove the fatty residues.

6. Degreasing & Cleaning Bones Using Aqueous Ammonia

Aqueous ammonia is used in the saponification of a number of natural resins and oils. It has been used in the production of linseed oil soaps and shellac soap. In general, surfactants produced from the reaction of fatty acids with ammonia have a lower surface tension that those using sodium and potassium (Averko-Antonovich et al., 2004) and this should facilitate their removal from porous substrates.

Aqueous ammonia reacts with fatty acids in the bone to form an ionic soap via a saponification reaction.

$$NH_3 + H_2O \leftrightarrow NH_4^+ + OH^-$$

This is an equilibrium reaction and the ammonia dissolved in the water is only weakly hydrolysed to give hydroxyl ions (OH^-). At room temperature a 25% solution (~ 13 M) has a pH value of around 12.5 but only a small percentage (<1%) of the ammonia is hydrolysed. When spread over an open surface the pH falls quickly to below 11.0 as gaseous ammonia escapes from the liquid. As the pH falls the proportion of ammonia in the form of NH4+ increases in accordance with Le Chatelier's principle.

The hydroxyl ion released by the dissociation of water reacts with fats in a hydrolysis reaction to give free fatty acids which bind to the ammonium ion to give soluble salts.

$$NH_4^+ + OH^- + (R\text{-}OOH) \rightarrow (R\text{-}OO^- \bullet NH_4^+) + H_2O$$

(insoluble) → (soluble)

Where R-OOH is any fatty acid.

The ammonium ion will also bind to some of the cross-linked fatty acids and any that that may be bound to calcium ions in the bone mineral since the ammonium ion forms stronger ligands that Ca^{2+} ions. As the hydroxyl ions are consumed in these reactions more are released by further dissociation of the dissolved ammonia (again in accordance with Le Chatelier's principle). Any excess ammonia will slowly off-gas, leaving no residues and any ammonium salts of low molecular weight carboxylic acids are also likely to sublime and escape from the spongy matrix of the bone.
In practice, cleaning fat-soiled bones with aqueous ammonia involves painting a 25% solution of ammonia directly onto the greasy surface, forming a foam with a soft brush and water, and then removing the foam and any particulate matter from the porous substrate with a wet-vacuum cleaner. Best results are achieved by working in small areas (c. 10cm x 10 cm) and several applications may be necessary. Final drying of the surface is achieved by spraying the surface with absolute ethanol and wiping with a micro-fibre cloth. Ammonia is considerably less soluble in ethanol than water (solubility in water at 25 ºC = 34% w/w; solubility in ethanol at 25 ºC = 10% w/w, Budavari et al. 1996).

It is recommended that the vacuum cleaners are cleaned once a week to remove the dark residues that accumulate in the chamber. These foul smelling deposits contain volatile, short-chain fatty acids such as butyric (butanoic) acid (which gives the smell of rancid butter and vomit) valeric (pentanoic) or caproic (hexanoic) acid (which smells like goats) which are exhausted from the vacuum cleaner during use.

Figure 13: Radius and ulna of humpback whale during aqueous ammonia cleaning.

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