Alma Mater Studiorum – Università di Bologna

DOTTORATO DI RICERCA

Science for Conservation

Ciclo XXII

Settore/i scientifico disciplinari di afferenza: CHIM/12

TITOLO TESI

ARCHAEOLOGICAL BALTIC AMBER: DEGRADATION MECHANISMS AND CONSERVATION MEASURES.

Presentata da: **Gianluca Pastorelli**

Coordinatore Dottorato **Relatore**

Prof. Rocco Mazzeo **Prof. Jane Richter**

Esame finale anno 2009

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My work would be incomplete without a mention
of the support given me by my cherished friend,
Clara, to whom this thesis is dedicated.

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Abstract.

The National Museum of Denmark has around 17,000 archaeological objects of Baltic amber in its collections, which are among the largest in Europe.
Approximately 45% of the amber objects has degraded and requires urgent conservation treatment. Degradation has resulted in restricted access to the collection by curators, students, archaeologists and visitors.
The National Museum of Denmark's collection is of interest to archaeologists and curators because amber jewellery reflects the economic, social, religious and other cultural beliefs of the peoples who made and wore it. It is important that the collections are preserved for study and cultural enrichment for future generations.

Baltic amber is sensitive to physico-chemical environmental factors, consequently it is extremely predisposed to degradation.
The aim of this project was to achieve a deeper understanding of the mechanisms by which amber degrades, in order to develop techniques for preventive conservation with the purpose to slow down the rate of degradation of archaeological amber objects.
Early active conservation treatments comprised impregnating crumbling amber with natural and synthetic polymers; with time, these conservation materials have deteriorated and damaged the amber surfaces they were supposed to protect.
For that reason it is more appropriate to create a preventive conservation strategy based on the control of factors, such as temperature, illumination and atmosphere, in the environment where amber objects are placed during storage, transport and display.

A clear understanding about interaction between amber and environmental factors is essential to comprehend degradation processes.
Current state of knowledge presents few degradation features regarding Baltic amber and new experiments were necessary to provide evidence.

In order to study deterioration of Baltic amber, a starting point was to identify and monitor surface and bulk properties which are affected during degradation.
The way to operate consisted of the use of accelerated ageing to initiate degradation of raw Baltic amber samples and, successively, in the use of non/micro-destructive techniques to identify and quantify changes in visual, chemical and structural properties.
For the preliminary experimental phase, a large piece of raw Baltic amber was selected for its visible homogeneity and prepared in two sample sizes: prisms and powder.
Samples were placed inside Pyrex glass flasks and subjected to two different kinds of accelerated ageing: thermal-ageing and photo-ageing.
For both the accelerated ageing procedures, samples were exposed to six different microclimatic conditions, in order to study the role of different environmental parameters (relative humidity, presence of oxygen, pH) on the degradation of amber.
Samples were checked regularly by several analytical techniques, including spectrocolorimetry, infrared and Raman spectroscopies, optical respirometry and gas chromatography – mass spectrometry.

From the interpretation of the obtained results it was possible to identify some relations between amber and environmental factors during the degradation process and to select the conditions to apply for the second ageing experiment.
In the advanced experimental phase, pellet-shaped samples obtained from pressed amber powder (which appeared more appropriate than prisms and free powder in terms of analytical repeatability and homogeneity of results) were exposed to eleven different microclimatic conditions, subjected only to thermal accelerated ageing (since photo-ageing already resulted in considerable degradation) and analysed regularly according to the same techniques and purposes that were employed in the preliminary investigation.

Initial results from the preliminary investigation showed colour change, oxidation of the molecular structure and off-gassing of formic and acetic acids vapours, that is a new important finding for Baltic amber.
Final results from the advanced investigation confirmed some of the previous findings and gave new important outcomes, especially concerning the degrading roles of oxygen, relative humidity and pH.

After the interpretation of the final achieved data, combined with initial information from the preliminary investigation, the main relations between amber and environmental factors during the degradation process became clearer and it was possible to identify the major pathways by which amber degrades, such as hydrolysis of esters into carboxylic acids, thermal-oxidation and photo-oxidation of terpenoid components, depolymerisation and decomposition of the chemical structure.

At the end it was possible to suggest a preventive conservation strategy based on the control of climatic, atmospheric and lighting parameters in the environment where Baltic amber objects are stored and displayed.

List of Abbreviations.

This list contains all the abbreviations used in the thesis.
Units of measurement and chemical formulas were used according to the standards in the scientific field (International System of units –SI–; International Union of Pure and Applied Chemistry –IUPAC–) and they are not included in this list, as well as commercial acronyms related to reagents, consumable experiment supplies and technical equipments.

ANOVA	ANalysis Of VAriance
ATR	Attenuated Total Reflectance
BCE	Before Christian Era
C-H-N	Carbon, Hydrogen, Nitrogen (content)
CIE	Commission Internationale de l'Eclairage
DRIFT	Diffuse-Reflectance Infrared Fourier Transform
DTGS	Deuterated TriGlycine Sulphate
ESR	Electron Spin Resonance
FT	Fourier Transform
FTIR	Fourier Transform InfraRed
GC	Gas Chromatography
ISO	International Organization for Standardization
LED	Light Emitting Diode
MCT	Mercury Cadmium Telluride
MS	Mass Spectrometry
NIR	Near InfraRed
NMR	Nuclear Magnetic Resonance
PC	Personal Computer
PDMS	PolyDiMethylSiloxane
PTFE	PolyTetraFluoroEthylene
Py	Pyrolysis
Ref.	Reference
RH	Relative Humidity
SCI	Specular Component Included
SPME	Solid Phase Micro Extraction
UV	UltraViolet
YAG	Yttrium Aluminium Garnet

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Tables.

Figures.

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1. BACKGROUND TO THE RESEARCH PROJECT.

This chapter offers a brief introduction about the definition and nature of the amber, as well as its chemico-physical properties.
To orient the reader, a statement of the necessity and the importance to preserve museum amber collections, together with a concise description of the current state of archaeological Baltic amber objects in the collections of the National Museum of Denmark, are given.
It is important to remark that the scope of this thesis is not to treat details about geological origin, botanical significance, identification, provenance and restoration of amber, since the literature deals largely with these topics, but to propose solutions for the prevention of its degradation by environmental control.

1.1 Introduction – the amber.

Amber is defined as a solid, discrete organic material found in coals and other sediments as macroscopic and microscopic particles, which are derived from the fossilisation of resins produced by prehistoric trees (figure 1.1; Anderson et al., 1995).
The chemical composition of amber consists of a complex mixture that mainly includes terpenoids and phenols, with minor amounts of alcohols, acids, fats and rarely amino acids (Alekseeva et al., 1966; Urbański et al., 1984; Mills et al., 1984; Mills et al., 1999). This composition is extremely variable and depends on several factors, such as provenance area, geological history, diagenetic alterations, paleoclimate and paleobotanic source (Langenheim, 1969; Savkevich, 1981).
Physical properties of amber also have a high variability depending on the provenance and the typology, while on the other hand, the elemental analysis is rather constant and does not easily allow distinguishing material coming from different deposits (table 1.1 and 1.2; Grimaldi, 1996; Ross, 1998; Clydesdale, 1999; Rice, 1999).

Figure 1.1. Amber nodule in sandstone.

Table 1.1. Main physical properties of amber

Characteristic	Description
Hardness	1.5 – 3 in the Mohs scale
Density	1.023 – 1.125 g/cm^3
Melting point	250 - 300 °C
Solubility	Insoluble in water and variably soluble in most common laboratory organic solvents
Refractive index	n = 1.5388 – 1.5451
Fluorescence	Normally, excitation by a wavelength of 366 nm gives a colouring from white-blue to green-yellow
Structure	Amorphous, with conchoidal fracture
Colour	In not degraded samples it can go from yellow-orange-red to blue-green; translucency depends on the gaseous inclusions content

Table 1.2. Mean elemental composition of amber

Element	% of composition
Carbon	67 – 87
Oxygen	up to 15
Hydrogen	8.5 – 11
Sulfur	0.26 – 0.34
Other elements	up to 0.5

Amber is the result of the fossilisation of the resin that was produced by different groups of trees, some of which are extinct at the present time (table 1.3; Judd et al., 2007).
Resins are organic substances characterized by high viscosity and by a very complex chemical composition, which comprises terpenes, terpenoids, phenols, alcohols and fatty acids (Anderson et al., 1992). The resin is produced by plants in related canals and pockets or blisters (figure 1.2), with several scopes and uses, e.g. defence against fungi and insects, desiccating control, storms protection or simple growing product (Judd et al., 2007).

Table 1.3. Most important tree resin-producing plant families which are considered potential sources of amber

Conifers (gymnosperms)	Flowering plants (angiosperms)
Pinaceae	Leguminosae
Araucariaceae	Burseraceae
Taxodiaceae	Dipterocarpaceae
Cupressaceae	Hamamelidaceae
Podocarpaceae	Anacardiaceae

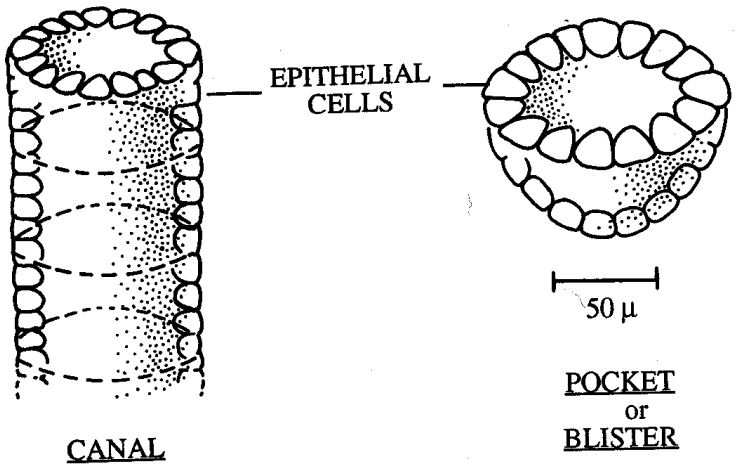


Figure 1.2. Resin secretory structures located in tree resin-producing trunks (modified from Judd et al., 2007).

During the geological ages the climatic and environmental conditions for a high resin production have frequently occurred. The resin, after having dripped to the ground, was buried by sediments and protected by the weathering. When the ideal anaerobic conditions for resin preservation were present, the transformation in amber started. The most volatile compounds (monoterpenes and sesquiterpenes) fled from the material, while, following a free-radical reaction mechanism in the presence of high temperature and pressure, a process of diterpenes polymerisation, accompanied by other processes, such as isomerisation, cyclisation and cross-linking, carried out the formation of high molecular weight chains (Grimaldi, 1996; Ross, 1998; Gold et al., 1999; Lambert et al., 2002). Resin became harder and stiffer, turning into copal. The real amber rose during the last phases of this polymerisation process (figure 1.3). Fossilisation of the resin (the process is known as amberization) has lasted for a variable time, which could be even several millions of years, depending on a number of variables, such as temperature, pressure, water content, oxygen partial pressure and pH (Anderson et al., 1995; King, 2006).

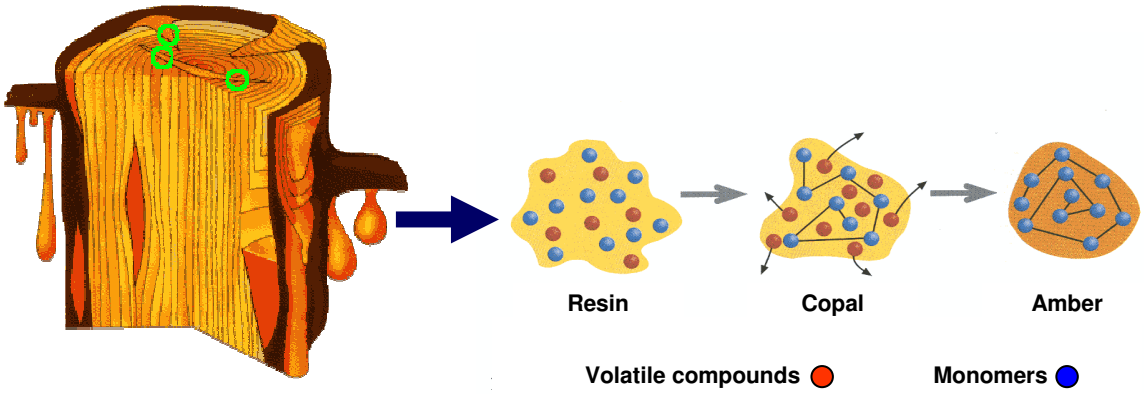


Figure 1.3. Resin fossilisation process that produced amber (modified from Pontin et al., 2000).

Fossilised residues (organic inclusions) of insects, plants and pollens, which remained entrapped in the original resin, can be embedded in the amber (Poinar, 1992; Poinar et al., 1995). The study of these organisms plays a very important role in paleobotany and paleontology fields (Larsson, 1978). It is important to note that the internal parts of the organisms are not preserved in every inclusion trapped in amber. The visible insect or plant is often merely a shell, since the interior is empty and the external structure has been completely desiccated (Clarkson, 1998). Researches regarding microorganisms isolated from amber are also retrievable in literature (Greenblatt et al., 1999).

Amber can also contain inorganic inclusions that likely came from the external environment around the resin during the fossilisation process. For instance, it is frequently possible to detect the presence of pyrite (FeS_2), halite (NaCl) and anhydrite ($CaSO_4$) crystals (Kirchner, 1950).

Figure 1.4. Organic inclusion represented by an insect trapped in amber.

The greatest concentration of amber within the geological time scale starts around 145 millions of years BCE (early Cretaceous), but the oldest amber specimens were found in sediments 300 millions of years BCE dated (Rice, 1999).

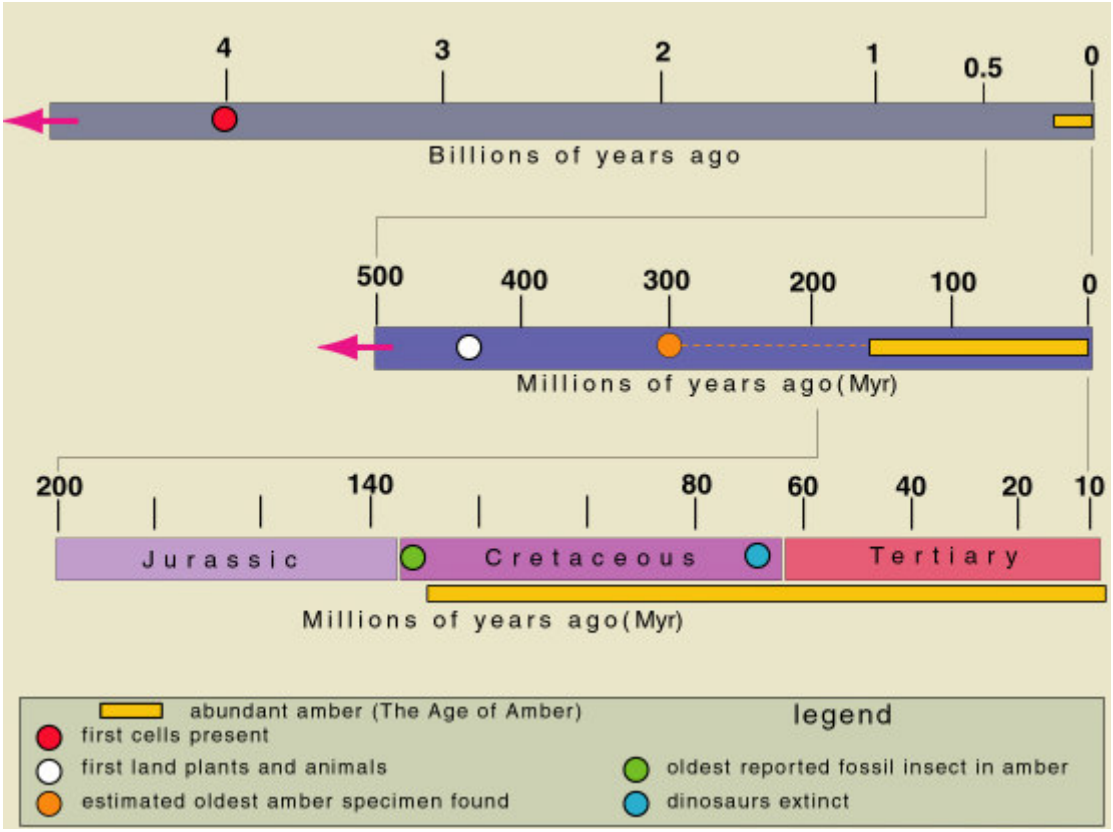


Figure 1.5. The presence of amber in the scale of time (modified from www.3dotstudio.com/amberhome; © 1995-1999 by 3 Dot Studio).

Regarding the classification of different types of amber, there are two kinds of categorisation system: one is founded on the historical-literary tradition, where names come from finding places, historical backgrounds, discoverers (table 1.4; Beck et al., 1986; Grimaldi, 1996), while the second one is based on the structural characterization, where it is possible to distinguish five classes related to the structure of the principal polymer (table 1.5 and figure 1.6; Anderson et al., 1992; Czechowski et al., 1996).

Table 1.4. Historical-literary classification for some European amber deposits

Provenance	Varieties
Baltic area	Succinite, gedanite, gedano-succinite, bekerite, stantienite
Italy	Dolomitic, appenninic, simetite
Romania	Rumaenite, almashite, delatynite, huntenite
Hungary	Ajkaite, kiscellite
Switzerland	Allingite, plaffeiite
Czech Republic	Walchowite, muchite, neudorfite, euosmite

Table 1.5. Structural classification system for fossil resins (modified from Anderson et al., 1992)

Class	Description
Class I	The macromolecular structures of all Class I resinites are derived from polymers of labdanoid diterpenes, including labdatriene carboxylic acids, alcohols and hydrocarbons
Class Ia	Derived from/based on polymers and copolymers of labdanoid diterpenes having the regular configuration, normally including, but not limited to, communic acid and communol, and incorporating significant amounts of succinic acid; also biformene (and related isomers) may be included
Class Ib	Derived from/based on polymers and copolymers of labdanoid diterpenes having the regular configuration, often including, but not limited to, communic acid, communol and biformene (and related isomers); succinic acid is absent
Class Ic	Derived from/based on polymers and copolymers of labdanoid diterpenes having the enantio configuration, often including, but not limited to, ozic acid, ozol and enantio-biformene (and related isomers)
Class II	Derived from/based on polymers of bicyclic sesquiterpenoid hydrocarbons, especially cadinene and related isomers
Class III	Natural (fossil) polystyrene
Class IV	Fundamental structural character is apparently non-polymeric, especially incorporating sesquiterpenoids based on the cedrane carbon skeleton
Class V	Non-polymeric diterpenoid carboxylic acid, especially based on the abietane, pimarane and iso-pimarane carbon skeleton

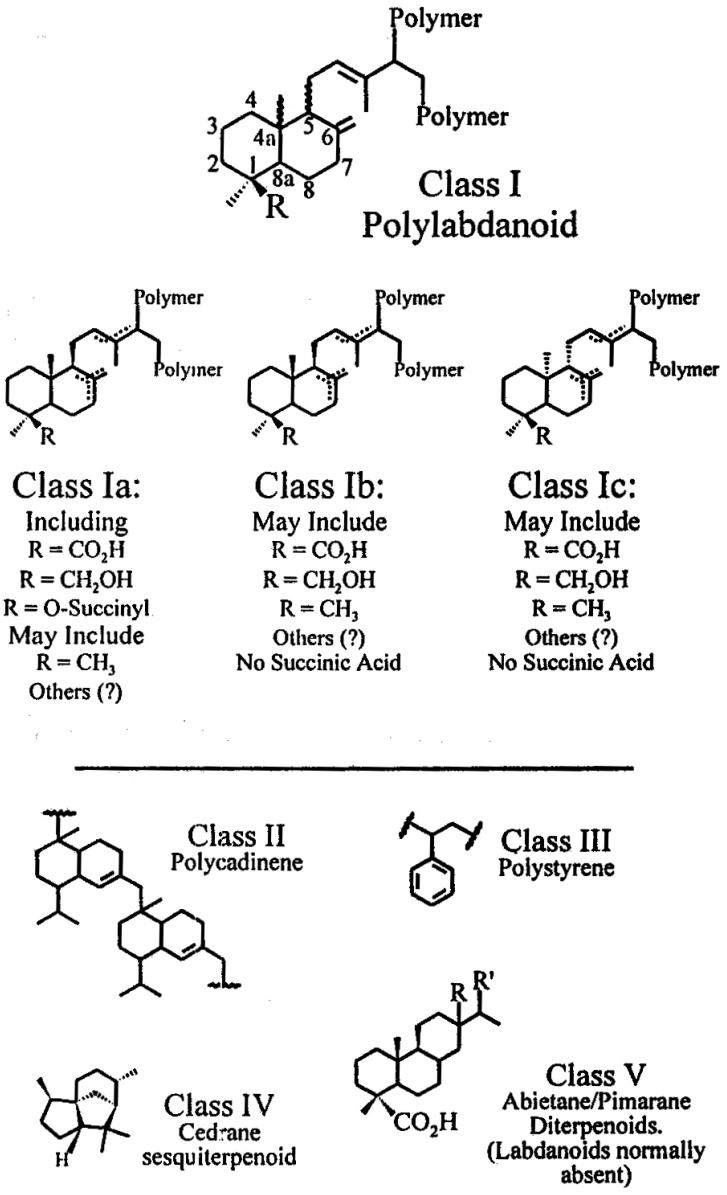


Figure 1.6. Schematic representation of the structural classification system for fossil resins (Anderson et al., 1992).

All over the world there are numerous deposits of amber. The material considered in this work is the amber coming from the Baltic area. Baltic amber is one of the most common and most investigated kind of amber, due to the abundance of its deposits (figure 1.7). Its origin is located in the Northern European area covered by the Baltic Sea and its age is estimated around 35-40 millions of years (Rice, 1999; Pontin et al., 2000).

The succinite, derived from fossilisation of the resin produced by a large number of different conifers (including, but not limited to, *Pinus succinifera*; Langenheim et al., 1965; Langenheim, 1969) and characterized by a content of succinic acid between 3% and 8% (Mills et al., 1984), is the most widespread kind of Baltic amber and it is often, but incorrectly, simply identified as "Baltic amber" in the literature. However, it is necessary to specify that there are several kinds of Baltic amber in addition to succinite, e.g. glessite, stantienite, gedanite, gedano-succinite and many others, which are still less studied and much less common.

Identification of the succinite has been subject of arguments since when in the past its recognition was based on the erroneous hypothesis that the succinic acid was contained exclusively in this type of amber. Substantial quantities of succinic acid are actually found in ambers from Portugal, France, Romania and Italy as well (Beck, 1985; Tonidandel et al., 2009). Nowadays the analysis by infrared spectroscopy allows the identification of succinite in an unmistakable way and its characterization has been abundantly discussed in the literature since the first works of Beck during the 60s (Beck et al., 1965).

Figure 1.7. European distribution of amber deposits. The red line defines the succinite provenance area, where amber was redistributed by glaciers and post-glacial rivers (modified from Koller et al., 1997).

Chemically the succinite is composed of two fractions, which are characterised by different solubilities in ether (Gough et al., 1972; Mosini et al., 1980; Mills et al., 1984; Villanueva-García et al., 2005) and which are described in table 1.6 and figure 1.8.

Table 1.6. Main chemical composition of succinite

Fraction	Class of compounds	Major compounds
Ether-soluble fraction	Aromatic hydrocarbons	Cymenes
	Monoterpenoids	Borneol, camphor, fenchyl alcohol, fenchone
	Sesquiterpenoids	Methyl fenchyl succinate, methyl bornyl succinate
	Diterpenoids	Compounds of the abietane, pimarane and labdane series
	Other compounds	Terpenol succinate and hemisuccinate esters
Ether-insoluble fraction[a]	Labdatrienoid compounds	Communic acid, communol (with its hydroxyl groups partially succinylated by a characteristic content of succinic acid[b])

[a]This fraction is formed by a polylabdanoid (copolymer of labdanoid diterpenes) belonging to the class Ia in the system proposed by Anderson et al. (1992); containing alcohols and acids, this fraction is often indicated as alkyd copolymer.
[b]Succinic acid is suggested to be not an original component of the resin which produced amber, but a degradation product of abietic acid (Rottländer, 1970).

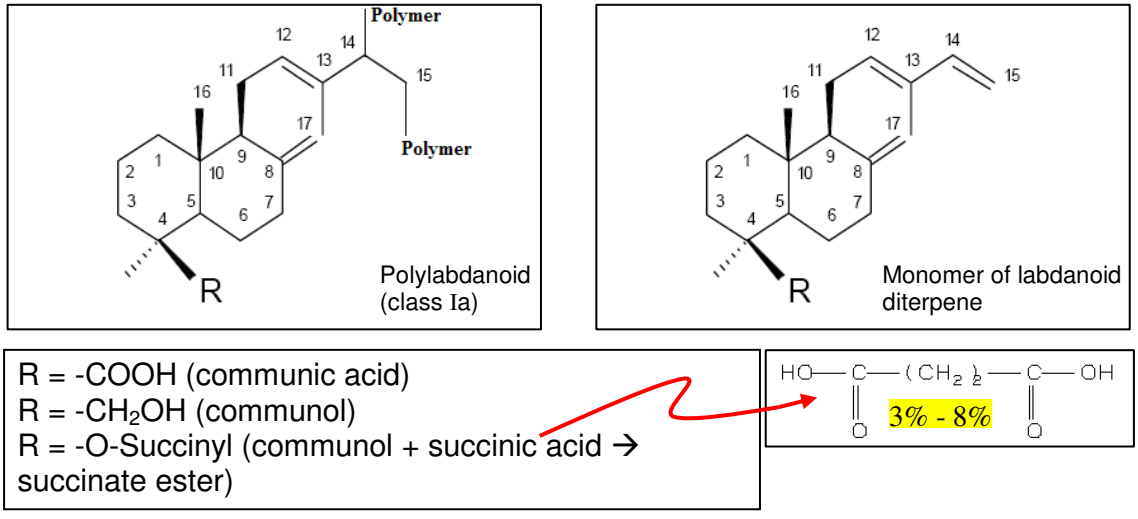


Figure 1.8. Schematic description of the succinite ether-insoluble fraction structure (usual numbering of atoms is employed).

1.2 Amber in museums.

Since the prehistory amber has been considered a precious material. Ancient people used to attribute a great commercial value as well as magic and therapeutic powers to amber; for that reason the mankind has employed amber to produce ornaments, jewellery and decorations (figure 1.9) since the end of the palaeolithic period until the present days (Jensen, 1982; Kristensen, 1986; Botfeldt, 1987; Brost et al., 1996; Koller et al., 1997; figure 1.10).
Museum collections of amber objects are of interest to archaeologists and curators because amber jewellery reflects the economic, social, religious and other cultural beliefs of the peoples who made and wore it.

Once amber objects are registered in museum collections or accessioned, the institution becomes responsible for their long term preservation, until the end of their useful lifetime (Feller, 1994; Shashoua, 2002).
It is important that appearance and meaning of amber objects in museum collections are preserved for study and cultural enrichment for future generations.

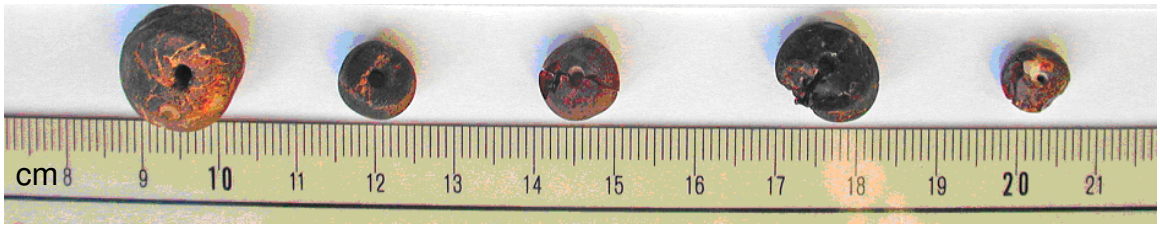
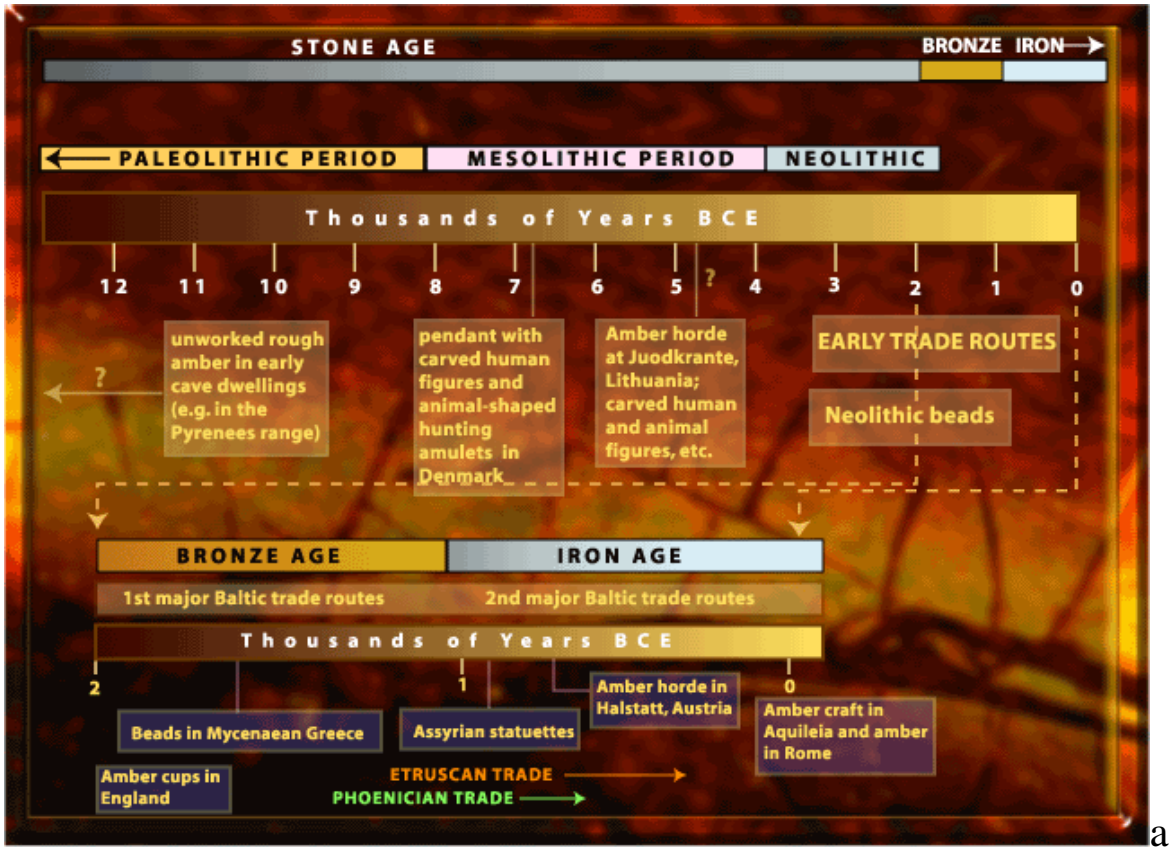


Figure 1.9. Small elements of amber jewellery from the collections of the National Museum of Denmark (Neolithic beads found in Skanderborg -Denmark-, dated 3-2 thousands of years BCE).

1.3 Nature of the problem.

A survey completed in 2000 showed that the National Museum of Denmark has around 17,000 archaeological objects of Baltic amber in its collections (Jensen et al., 2000), which are among the largest in Europe. Approximately 45% of these amber objects has degraded, presenting surface crazing, powdering and discoloration, and it requires urgent conservation treatment (figure 1.11).
Degradation has resulted in restricted access to the collections by curators, students, archaeologists and visitors, and it has limited display and loan of these objects.

Between 1870 and 1980, active conservation treatments, based on the application of polymeric materials used as adhesives and consolidants, aimed to consolidate and stabilize fragmented surfaces of crumbling amber objects (table 1.7; Villemos, 1976; Kunkuliene, 1981; Fraquet, 1987; Cronyn, 1990).
With time, these conservation materials themselves deteriorated, until they damaged the amber surfaces they were supposed to protect, causing changes in colour, fissures and losses. Furthermore, the application of chemicals to amber objects, which may be required to be analysed for identification and provenance studies, can invalidate the analytical results (Clydesdale, 1999).



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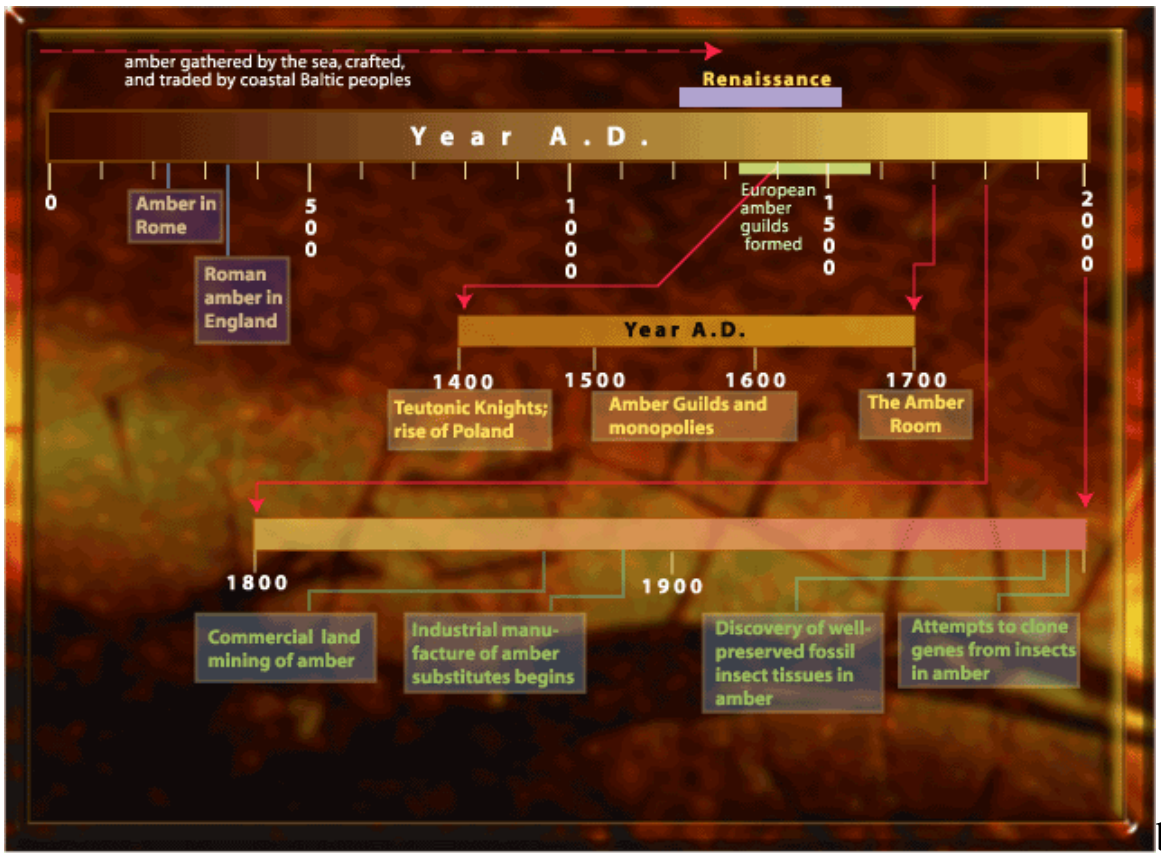


b

Figure 1.10. Timeline of amber cultural history: before the Christian era (a) and during the Christian era (b) (reprinted from www.3dotstudio.com/amberhome; © 1995-1999 by 3 Dot Studio).

a  b 

Figure 1.11. Comparison between a well conserved amber fragment (a) and a typical degraded object observed in Baltic amber collections of the National Museum of Denmatrk (b) (reprinted from Shashoua, 2002).

Table 1.7. Early conservation treatments for deteriorated Baltic amber

Group of materials	Polymeric material[a]
Aqueous solutions of natural products[b]	Gelatin
	Isinglass
	Agar-agar
Pure natural products	Tree resins (e.g. dammar)
	Insect resins (shellac)
	"Oil of amber" and waxes
Solvent based synthetic products	Synacryl 9122X (diluted with xylene)
	Bedacryl 122X (diluted with xylene)
	Paraloid B72 (diluted with xylene or acetone)

[a]Consolidants were often applied under vacuum pressure to aid deeper impregnation.
[b]Physico-chemical properties, e.g. fluidity, hardening and cross-linking, were often improved adding glycerine, phenol or formalin.

2. OVERVIEW OF THE RESEARCH PROJECT.

This chapter explains the need to understand the degradation mechanisms of Baltic amber in order to develop a set of inhibitive conservation techniques. The current state of the art about interactions between amber and environmental factors is also presented, as well as an introduction to the methodology used during previous researches and for this study.

2.1 Research project objectives.

To make possible the preservation of Baltic amber, two different approaches can be considered:

- *active conservation treatments* – practical activities applied as necessary to individual objects to limit further deterioration and repair damages. They include cleaning surfaces, adhering broken sections and filling missing areas to strengthen objects weakened by deterioration;
- *preventive* (or *inhibitive*) *conservation strategy* – group of techniques which are based on the control of parameters, e.g. temperature, illumination and atmosphere, in the environment where objects are placed during storage, transport and display, with the aim of slowing those chemical reactions that cause or accelerate degradation.

Regarding degrading amber, for many years conservation research has focused on active methods, such as testing several polymeric coatings for impregnating and consolidating fragile objects (Thickett et al., 1995; Zivancevic et al., 2006), but these techniques often appeared not suitable, since they carried more surface visual alterations and more structural damages than benefits, like in the National Museum of Denmark's collections case (figure 2.1). In other instances these coatings resulted in not easy reversibility, precluding any chance of further chemical or archaeometric analysis (Clydesdale, 1999).

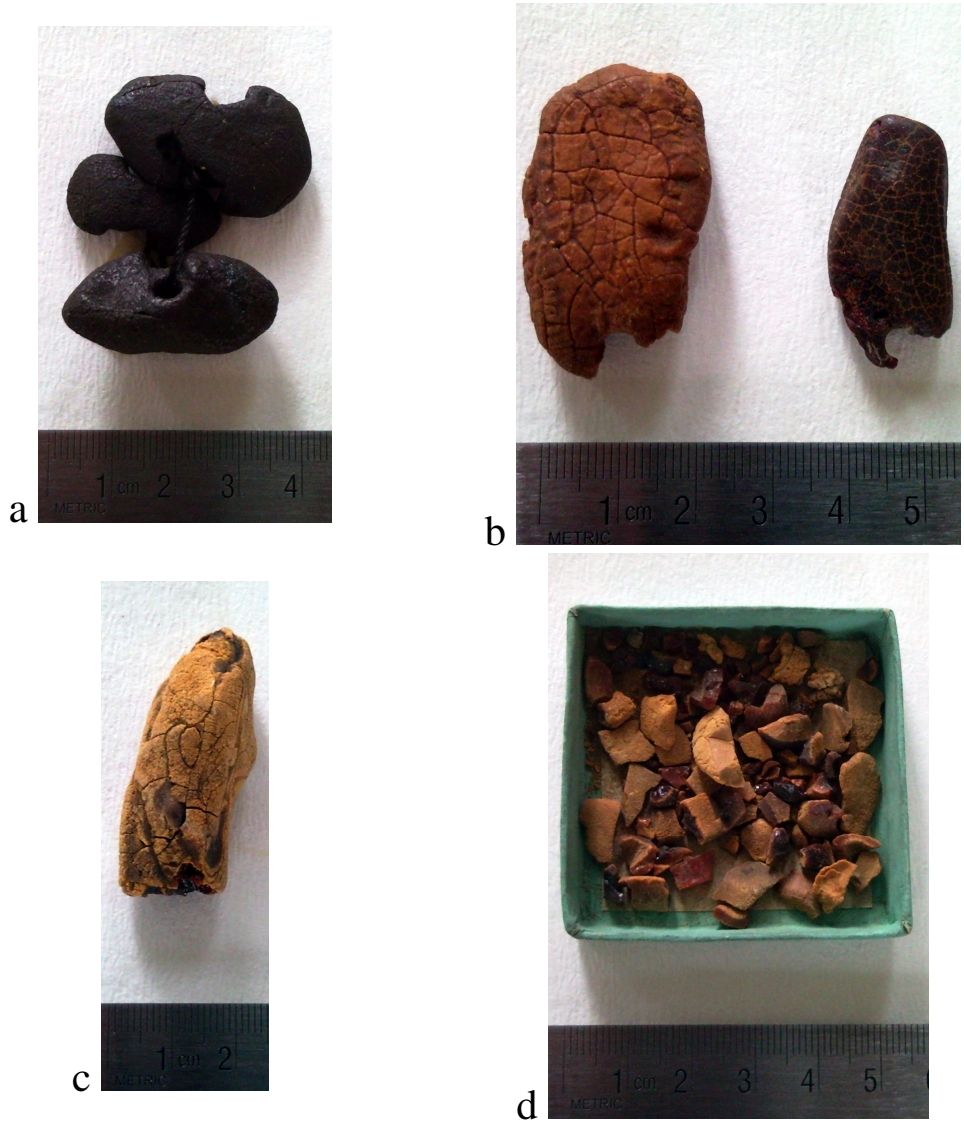


Figure 2.1. Examples of degraded archaeological Baltic amber objects from the National Museum of Denmark's collections, after active conservation treatments: darkened beads after application of gelatin-glycerine solution (a), crazed pieces after application of wax (b - c), completely fragmented objects after various treatments (d).

Consolidants and adhesives on amber should be avoided, unless they are strictly necessary, e.g. for extremely deteriorated and fragile samples (Beck, 1982).
A more effective inhibitive approach, which may eliminate the need of active treatments, could be developed if the causes and pathways of amber degradation were better understood.
The aim of this project was to achieve a deeper understanding of the mechanisms by which Baltic amber degrades, in order to develop techniques for preventive conservation, with the purpose to slow down the rate of degradation of amber objects.
Therefore an extensive literature survey, to clarify what is known today about the degradation of Baltic amber (see references list), and establishment of contact with other researchers in this field, to identify other current studies (see acknowledgements section), were the first steps in the project plan.

2.2 Degradation of Baltic amber – state of the art.

Baltic amber is a sensitive material to physico-chemical environmental factors. It is extremely predisposed to progressive degradation and eventually to complete disintegration by atmospheric oxidation, accelerated by heat and light (Beck, 1982).
A clear understanding about interaction between amber and environmental factors (e.g. heat, light, oxygen, relative humidity and pH) is essential to comprehend degradation processes.
Current state of knowledge presents few degradation features regarding Baltic amber, since research deals predominantly with geological, paleontological and archaeological inquiries, as well as restoration issues.
The role of environmental factors in the degradation process is still subject of studies and during this work new experiments were necessary to provide evidences. For example:

- *energy (heat, light)* – heat and UV radiation can promote rapid chemical degradation (respectively thermal-oxidation and photo-oxidation; Beck, 1982; Williams et al., 1990), while visible light is reported not to affect amber (Williams et al., 1990; Thickett et al., 1995);
- *oxygen* – oxidation of terpenoid components, where unsaturated carbon-carbon bonds are oxidised to acid groups, is thought to be one of the major degradation mechanisms (Beck, 1982; Shashoua et al., 2005); oxidation is a surface phenomenon because of the low permeability to oxygen of the material (Shashoua et al., 2005) and the higher is the surface area to volume ratio, like in small or rough samples, the faster oxidation occurs (Jensen et al., 2000);
- *relative humidity* – amber is sensitive to both high and low RH levels, but particularly to low, since it causes surface cracking, increasing opacity (Williams et al., 1990); the rate of degradation may be slowed by moisture (Thickett et al., 1995; Shashoua et al., 2005);
- *pollutants* – air pollutants, volatile biocides and cleaning agents can cause surface disintegration (Waddington et al., 1989; Williams et al., 1990); fresh surfaces are less affected by pollutants action than surfaces that have been exposed to air for a number of years (Waddington et al., 1989).

2.3 Analytical techniques applied to Baltic amber – state of the art.

Analysis by several techniques, e.g. nuclear magnetic resonance spectroscopy (Beck et al., 1973; Lambert et al., 1982; Lambert et al., 1985; Banerjee et al., 1997; Martínez-Richa et al., 1999), gas chromatography and mass spectrometry, used separately or in combination (Galletti et al., 1993; Heck, 1999; Anderson, 2006; Tonidandel et al., 2009), thermal analysis or pyrolysis combined with mass spectrometry (Wampler, 1995; Ragazzi et al., 2003; Feist et al., 2007) and infrared spectroscopy (Beck et al., 1965; Langenheim, 1969; Lambert et al., 2002; Krishnan et al., 2007), is commonly carried out on amber for different purposes (table 2.1).

Table 2.1. Analytical techniques commonly used for amber studies

Technique	Information available	Applications
Nuclear Magnetic Resonance spectroscopy (^{1}H-NMR and ^{13}C-NMR)	Qualitative and structural analysis	Identification of the paleobotanic source Characterization of geological deposits
Mass Spectrometry (MS)	Isotopic composition (^{13}C, ^{18}O, D)	Characterization of geological deposits Achievement of paleoclimatic information
Pyrolysis - Gas Chromatography - Mass Spectrometry (Py-GC-MS)	Chemical composition	Identification of the paleobotanic source Characterization of geological deposits
Infrared spectroscopy and Raman spectroscopy	Qualitative and structural analysis	Identification of the kind of material Characterization of geological deposits Identification of the paleobotanic source Understanding of degradation mechanisms

The identification of the paleobotanic source is normally used for paleo-environmental and paleo-climatic reconstructions (Langenheim et al., 1965; Langenheim, 1969), while the characterisation of geological deposits (Beck et al., 1965; Stout et al., 2000) can be coupled with the analysis of archaeological objects allowing the recognition of provenance of the raw material and the definition of ancient trade routes (Beck, 1985; Angelini et al., 2005; Ragazzi et al., 2006; Devièse et al., 2007). The identification of the kind of material can be applied on cases of incorrect visual identification (copal, wood-coal and glass can erroneously be identified as amber) and falsification (some synthetic materials, such as nitrocellulose-based plastics, can be used to produce amber imitations; Golloch et al., 1998).

Among the listed techniques, infrared spectroscopy is the most widely applied; the reasons for the wide spread of this methodology are several (Beck et al., 1965; Langenheim, 1969; Lambert et al., 2002):

- *ease of use* and *short analytical time* – the simple and fast analytical procedure allows the analysis of a large amount of samples, making possible the achievement of consistent statistic information;
- *reliability* – the principle on which the analytical process is based (i.e. the absorption of infrared radiation by the material) does not alter the sample, making the method completely trustworthy;
- *micro-destructivity* – requiring samples of the order of 0.2 – 2 mg, sampling does not change the scientific and aesthetic value of the object under analysis, that is a necessary condition in the field of cultural heritage;

- *diagnostic power* – results contain several diagnostic parameters that can be used for diverse scopes, e.g. identification, characterisation, evaluation of chemical changes.

Infrared spectroscopy analysis of amber shows intense absorption bands that are highly characteristic. The region between 3700 and approximately 1350 cm^{-1} (group frequency area) includes bands which are common to all types of amber, while the region between 1350 and 700 cm^{-1} (fingerprints area) presents a series of relatively intense bands, which are different for various types of amber and, consequently, used to identify and characterize specific deposits.

The infrared spectrum of Baltic amber shows a typical pattern characterized by the presence of three peaks at 1150 ± 15, 995 ± 15 and 888 ± 1 cm^{-1}. The main infrared bands characteristic for Baltic amber are listed in table 2.2 and showed in figure 2.2.
The band between 1250 and 1010 cm^{-1}, related to C-O bonds characteristic for succinate ester, is used to discriminate between European ambers of Baltic and non-Baltic origin (Beck, 1986) and normally has a broad horizontal region between 1235 and 1175 cm^{-1}, known as Baltic shoulder. This region becomes more and more sloping going from fresh to deteriorated condition (figure 2.3; Beck et al., 1965).

Infrared spectroscopy analysis of amber can be performed in different geometries: Fourier Transform InfraRed (FTIR; Beck et al., 1965; Williams et al., 1990; Kalsbeek et al., 2007; Pakutinskienea et al., 2007), Diffuse-Reflectance Infrared Fourier Transform (DRIFT; Angelini et al., 2005) and Attenuated Total Reflectance (ATR)-FTIR (Shashoua et al., 2005; Guiliano et al., 2007).
During the study described here it was decided to utilize ATR-FTIR spectroscopy for the extreme ease of use due to the absence of sample preparation for the analysis.

Fourier Transform (FT)-Raman spectroscopy is also successfully applied on the study of amber (Edwards et al., 1995; Moreno et al., 2000; Brody et al., 2001; Kendix et al., 2004; Shashoua et al., 2005; Edwards et al., 2007) and it was used in this work as complementary method of ATR-FTIR spectroscopy, since useful bands at same frequencies in spectra from the two techniques may have different intensities and resolutions (Colthup et al., 1990).
All the details about the mentioned and other techniques which were used during this study are presented and discussed in the materials and methods chapters.

Table 2.2. Main infrared bands characteristic for Baltic amber

Band (cm^{-1})	Group	Type of vibrational change	Change in intensity due to degradation
3700 – 3100	-OH	O-H stretching	Increase due to adsorption of water
3095	$RHC=CH_2$ and $R_1R_2C=CH_2$ (terminal olefins)	C-H stretching	
2930 ± 10 and 2853 ± 10	$>CH_2$	C-H stretching	
2962 and 2861 ± 20	$-CH_3$	C-H stretching	
1735 – 1700	>C=O (of esters and acids)	C=O stretching	Increase due to oxidation
1650 – 1600	>C=C< (non-conjugated)	C=C stretching	Decrease due to oxidation
1450 ± 20	$>CH_2$ and $-CH_3$	C-H bending	
1375 ± 5	$-CH_3$	C-H bending	
1250 – 1010[a]	-CO-O- (of succinate)	C-O stretching	Baltic shoulder negative slope due to oxidation
995 ± 15	$>CH_2$ (of cycloalkanes)	C-H bending	
888 ± 1	$RHC=CH_2$ and $R_1R_2C=CH_2$ (terminal olefins)	C-H out-of-plane bending	Decrease due to oxidation

[a] 1235 – 1175 → Baltic shoulder, 1150 ± 15 → peak.

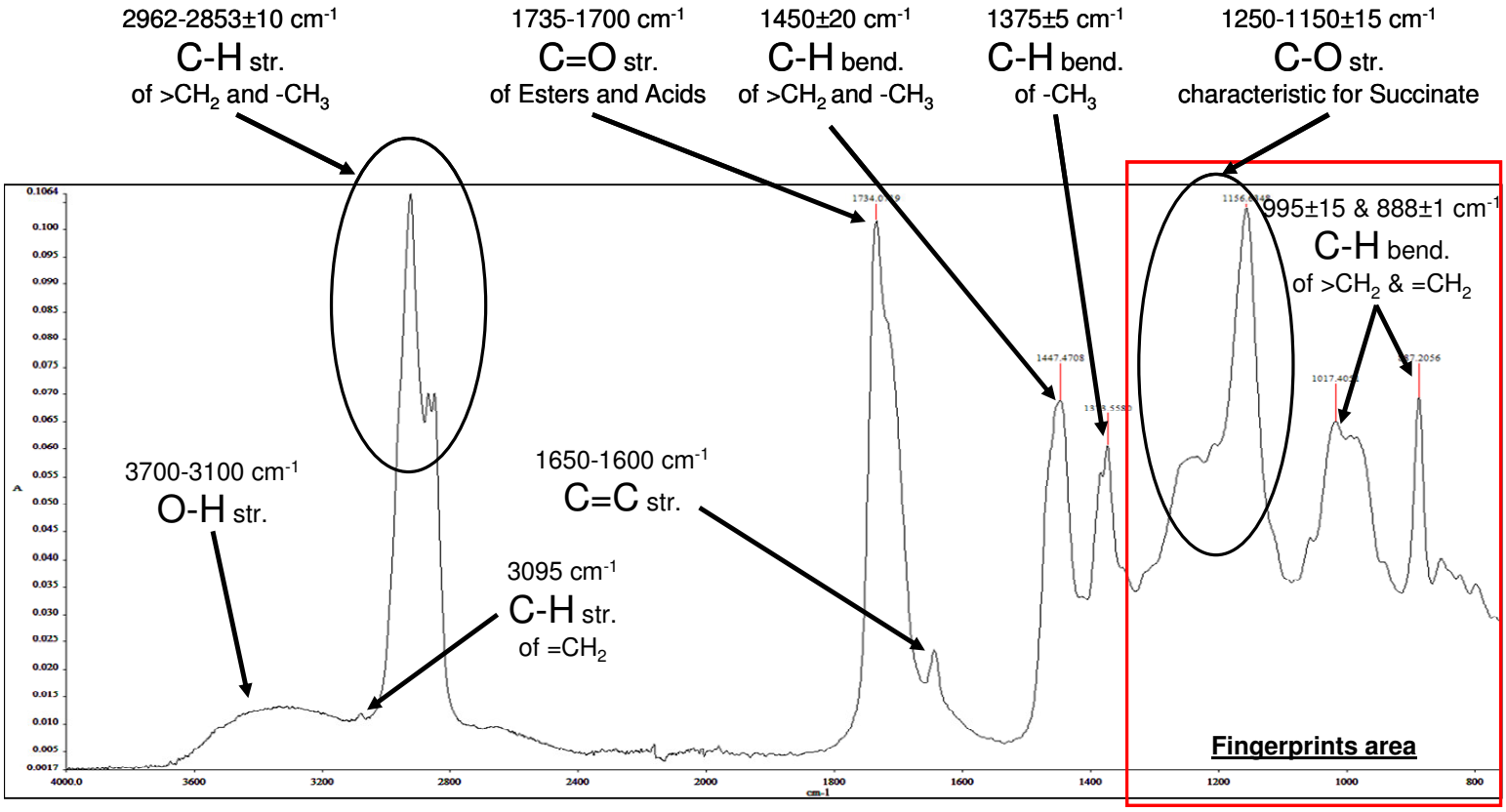


Figure 2.2. Characteristic IR spectrum of Baltic amber.

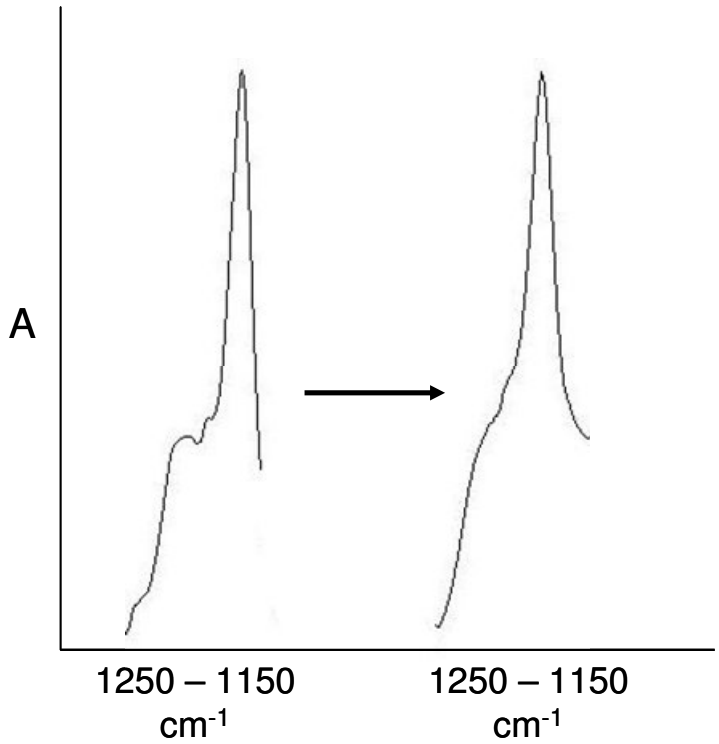


Figure 2.3. Increase in slope of the Baltic shoulder in the region between 1235 and 1175 cm^{-1} during degradation.

2.4 Research project strategy.

In order to study deterioration of Baltic amber, the starting point was to identify and monitor surface and bulk properties which are affected during degradation.
The planned methodology to operate (figure 2.4) consisted of the use of:

- accelerated ageing, by high temperature and light, to speed up degradation processes of raw Baltic amber samples in the laboratory;
- non/micro-destructive analytical techniques, to identify and quantify changes in visual (appearance, colour), chemical (functional groups, elemental composition) and structural (off-gassing from the material, surface roughness) properties, in order to elucidate the chemical reactions (mechanisms) involved in the degradation processes and the related physical consequences.

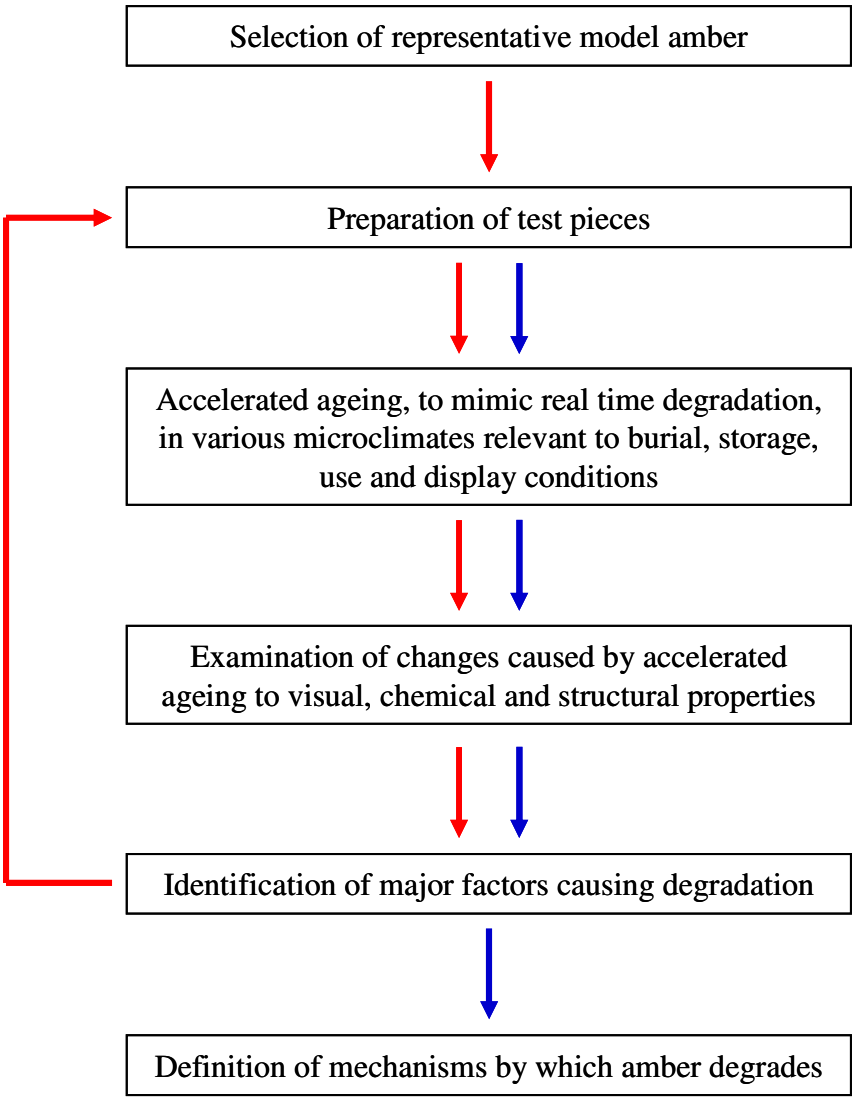


Figure 2.4. Diagram showing the planned experimental methodology (→ preliminary investigation, → advanced investigation).

The ultimate objective of these investigations was the development of inhibitive methods by which the useful lifetime of archaeological Baltic amber objects can be extended.

3. PRELIMINARY INVESTIGATION - MATERIALS AND METHODS.

This chapter describes the experimental design providing details about which materials were employed and how the methodologies were developed and used.
If not specifically noticed, all the tools, devices and analytical instruments used for the preparation, the ageing and the analysis of the samples were available at the School of Conservation of the Royal Danish Academy of Fine Arts, and the operator was the author of this thesis. All the employed instrumental operative settings were considered, after pilot tests, the least required to obtain reliable and repeatable results.

3.1 Selection and characterization of the model material.

For this study no real archaeological object was used (except in few occasions for comparative purposes, as it will be described later on), because of several reasons, e.g. inhomogeneity of samples, unknown storage background and unethical use for research scopes. Consequently, the first step during the experimental phase was to find the most appropriate material to use for the production of the samples. It was necessary that this material was large enough and as homogeneous as possible, in order to produce a big number of samples with similar properties.
It was decided to visit the main office of Ravfehrn ApS, which is one of the biggest producers of amber jewellery in Denmark, placed in Søborg (around 10 km outside Copenhagen).
There, a large piece of raw Baltic amber (approximately 15 x 12 x 6 cm, figure 3.1) was selected for its visible homogeneity. The parameters considered to evaluate the homogeneity were:

- *uniform translucency and colour* – translucency and colour of a given piece of amber depend mainly on the amount of air bubbles contained and on their distribution (Ross, 1998); the selected lump was considered uniform enough in translucency and colour, since only few parts of it contained large concentrations of air bubbles producing a clouded appearance;
- *absence of organic or inorganic inclusions* – the selected lump, by a simple backlight examination, did not show the presence of any biological or mineral inclusion.

The following stage consisted of the characterization of the raw material by the use of ATR-FTIR spectroscopy. The purposes were to verify the actual Baltic provenance (the FTIR spectrum of Baltic amber is absolutely characteristic and diagnostic; Beck et al., 1965) and to characterize the chemical groups present in the unaged material.

To perform the ATR-FTIR analysis, the following procedure was employed:

- *sampling* – three small fragments (around 2 mg each) of the amber chunk were removed from the external surface by the use of a scalpel. The three chips were observed using a reflected light optical microscope, to verify the absence of any sedimentary matrix on the external surface and air-bubbles/inclusions in the interior;

- *analysis* – ATR-FTIR spectra were collected using a Perkin Elmer Spectrum One FTIR spectrometer (details and operative settings are listed in tables 3.1 and 3.2). After the acquisition of an air-background, each fragment was placed on the reflection accessory and consequently analysed.

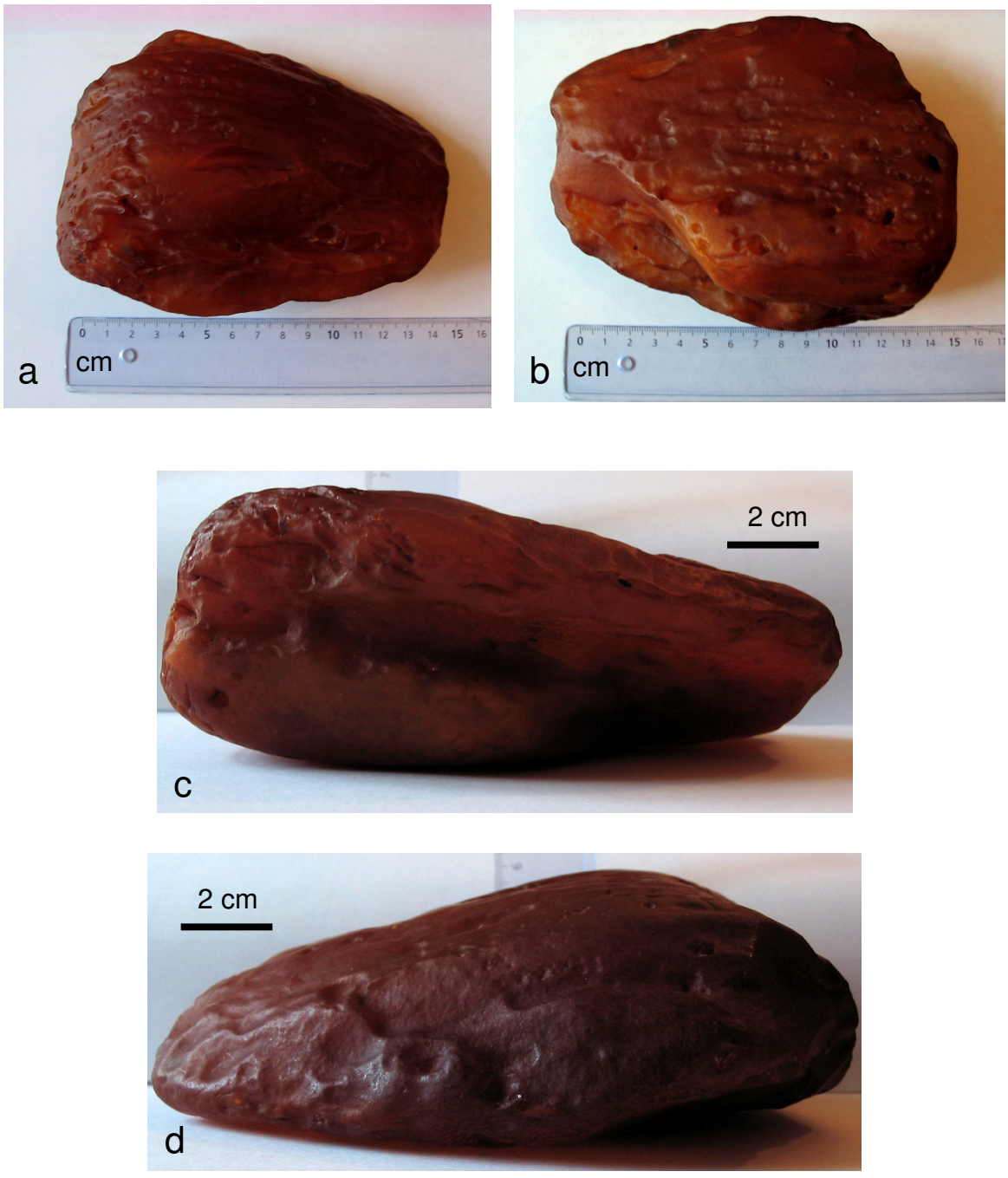


Figure 3.1. Four views (a-b-c-d) of the Baltic amber lump selected for the production of samples.

Table 3.1. Characteristics of the Perkin Elmer Spectrum One FTIR spectrometer used for the analysis of amber fragments

Feature	Description
ATR accessory	ASI DurasamplIR single reflection accessory with an angle of incidence of 45º and fitted with a diamond/ZnSe internal reflection element (active area of 1 mm diameter)
Detector	Pyroelectric: DTGS (deuterated triglycine sulphate)
Applicative software	Perkin Elmer Spectrum version 6.2.0

Table 3.2. Operative settings for ATR-FTIR analysis of amber fragments

Parameter	Value
Range	4000 cm^{-1} – 650 cm^{-1}
Unit	Absorbance
Number of scans	4
Resolution	4 cm^{-1}
Force gauge	70

The observation of collected spectra confirmed that the selected lump of amber belonged to the Baltic type (figure 3.2).

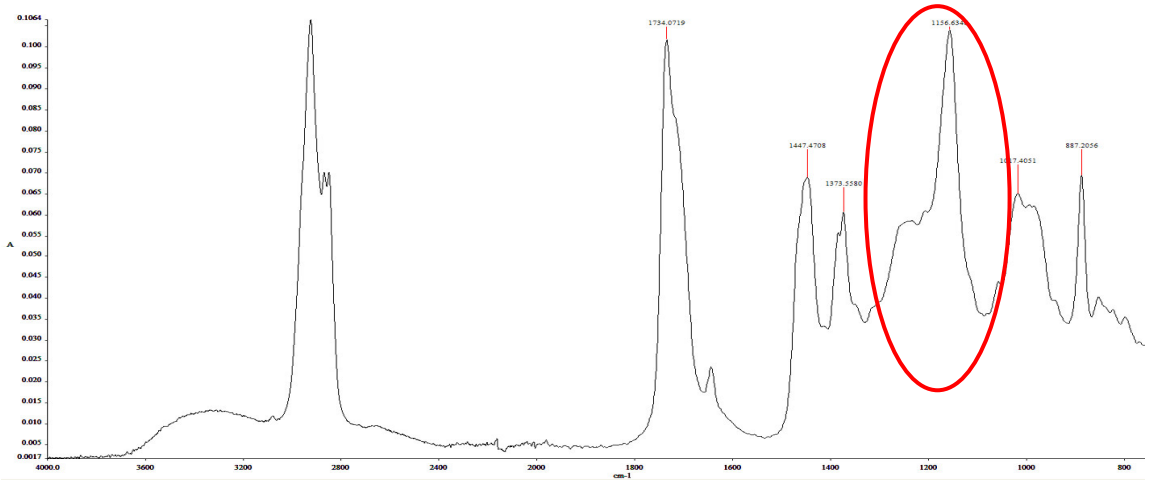


Figure 3.2. One of the spectra (in absorbance) which showed the Baltic nature of the selected lump of amber.

3.2 Preparation of amber samples.

In order to investigate the role of the surface area to volume ratio during degradation, amber samples were prepared in two sample sizes:

- *right rectangular prisms* (simply named prisms later on) of approximately 18 x 9 x 5 mm;
- *powder*, where the surface area to volume ratio is the highest.

The procedures used to produce the samples were planned with the purpose to limit any potential degrading effect, due to the formation of free radicals (Urbański, 1971).

Amber prisms were obtained following this procedure:

- a few unrefined slices of approximately 12 x 6 x 1 cm were cut by a manual saw, with very slow motion to minimize the heat due to the friction as much as possible;
- each slice was cut and refined in prisms of the right size using a Buehler Isomet low speed electrical saw, cooled with a 3% water solution of Struers Additive for Cooling Fluid;
- it was decided not to polish the surfaces of the prisms because they already appeared extremely smooth after cutting.

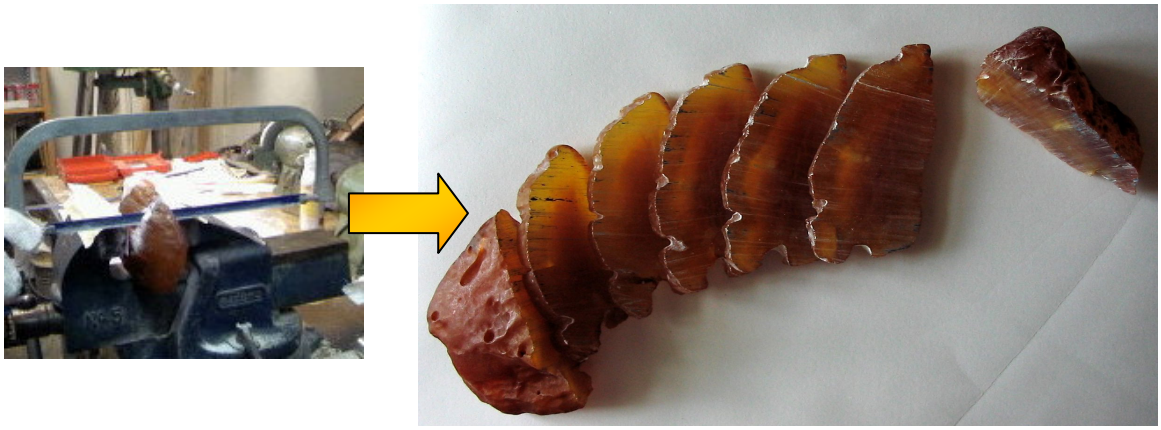


Figure 3.3. Amber slices obtained by manual sawing.

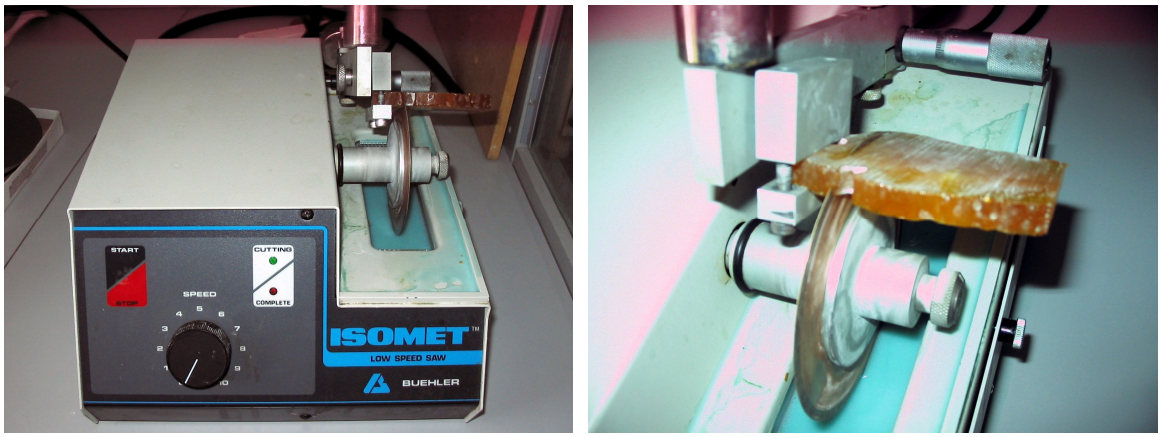


Figure 3.4. Low speed electrical saw used to cut the amber prisms.

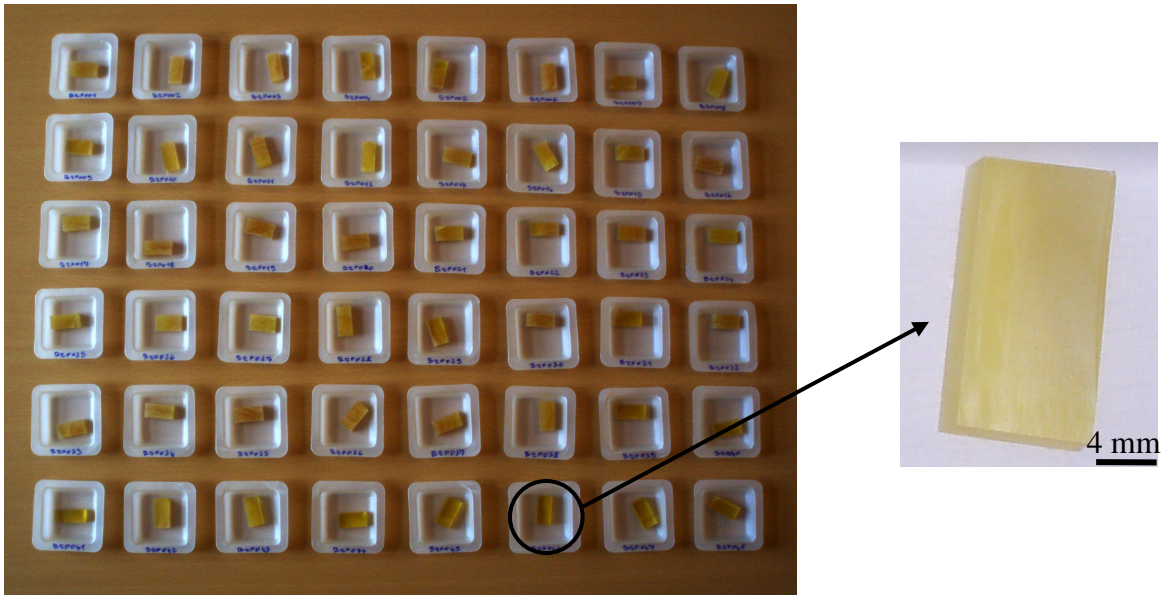


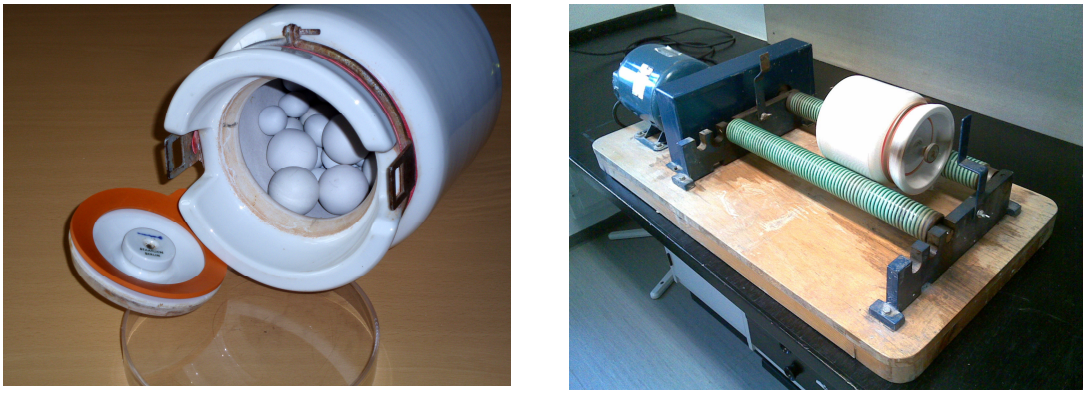
Figure 3.5. Amber prisms.

At the end of the preparation, 80 prisms were obtained. Mean weight was 0.87 ± 0.01 g.

Amber powder was obtained following this procedure:

- a part of the amber lump was smashed in a number of big fragments using a wood hammer (wood absorbs shocks better than metal because of the higher plasticity; Callister, 2002); the impact had to be intense enough to obtain the required number of fragments in one attempt;
- the fragments were placed in a ceramic sphere-mill; once closed, the mill was located and kept in continuous rotation on a Chematex AB rolling machine; sporadically the mill had to be wet with liquid nitrogen in order to keep the temperature down;
- the powder was collected and sieved through a 0.5 mm mesh sieve, to give evenly sized grains.

Figure 3.6. Wood hammer used to smash the amber piece for powder production.



a b

Figure 3.7. Ceramic sphere-mill (a) kept in rotation on the rolling machine (b) used to grind the amber.

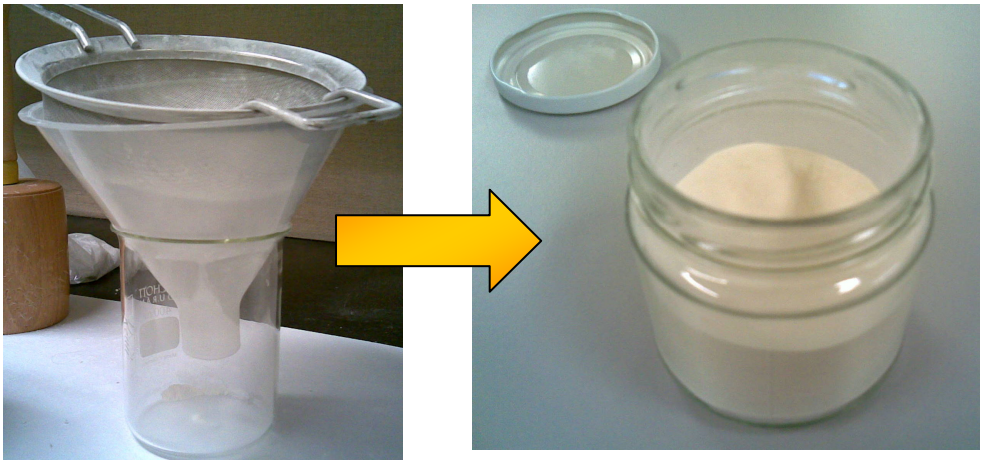


Figure 3.8. Amber powder collected after sieving.

At the end of the preparation, around 100 g of powder was collected.

3.3 Ageing of amber samples.

Samples were placed inside wide neck 100 mL Bibby-Sterilin Pyrex glass flasks with polypropylene cap and silicon gasket (baked out at 100 °C for 4 days before use to remove impurities). Samples were then subjected to two different kinds of accelerated ageing:

- *thermal-ageing* – performed in a Memmert UL 50 oven (see operative conditions in table 3.3);
- *photo-ageing* – performed in an Atlas Ci3000+ light chamber (see operative conditions in table 3.4).

Table 3.3. Operative conditions for thermal-ageing of amber samples

Parameter	Value
Chamber temperature	70 ± 2 °C[a]
Relative humidity	≤ 20%
Light radiation	Absent
Period	35 days[b]

[a]The temperature of 70 °C was considered appropriate since a higher temperature could activate chemical reactions (Feller, 1994) which are different from the natural processes involved in the degradation of amber.
[b]Ageing periods of 35 days were already tested in pilot experiments and resulted in a suitable length of time to make changes, in colour and molecular structure, in the amber samples at 70 °C.

Table 3.4. Operative conditions for photo-ageing of amber samples

Parameter	Value
Chamber temperature	30 ± 2 °C[a]
Relative humidity	Approximately 50%
Radiation source	Xenon lamp
Light radiation type	Daylight behind window glass (325 – 760 nm)[b]
Irradiance	40 W/m^2 [c]
Period	17 days[d]

[a]The ageing environment was kept as cool as possible to separate the degradation effects of heat from light; the temperature of 30 °C was the lowest possible.
[b]To obtain the absorption of UV radiation, a Cira + Sodalime filters combination was applied around the xenon lamp.
[c]The irradiance value was calculated on the region between 300 and 400 nm of the spectrum emitted by the xenon lamp.
[d]It was decided to stop the ageing after 17 days, because changes in colour and molecular structure of the amber samples could be measured.

On the photo-ageing a group of control samples (blind samples later on) was used in order to verify the absence of further ageing factors than light. Three prisms and 1 g of powder were placed inside two open wide neck 100 mL Bibby-Sterilin Pyrex glass flasks, one flask for the prisms and one for the powder. The flasks were wrapped in aluminium foil to block the exposure to light.

a 

b 

Figure 3.9. Ageing of amber samples: oven used for thermal-ageing (a) and light chamber used for photo-ageing (b).

For both the accelerated ageing procedures, samples were exposed to 6 different microclimates (table 3.5), relevant to burial, storage, use and display conditions, in order to study the role of different environmental parameters (relative humidity, presence of oxygen, pH) on the degradation of amber.
Four types of storage were required (see details in figures 3.10 a-d), depending on the kind of ageing (unlike the thermal-ageing, in photo-ageing the samples had to "face" the light to be aged) and on the size of the samples (unlike the prisms, the powder is not a compact object). All materials used for the accelerated ageing procedures were selected for their high physico-chemical stability.

Table 3.5. Microclimatic conditions used for the accelerated ageing of amber samples

Microclimatic condition		How achieved
1	≤ 20% RH, sample exposed to external atmosphere (open air)	Using open containers
2	100% RH, sample exposed to internal atmosphere, pH ≤ 5.5	Placing deionised water inside closed containers
3	≤ 20% RH, sample exposed to internal atmosphere	Placing silica gel inside closed containers
4	≤ 20% RH, sample exposed to internal hypoxic atmosphere	Placing an oxygen absorber (50% iron sulphate + 50% potassium chloride)[a] inside closed containers
5	100% RH, sample exposed to internal atmosphere, pH 4	Placing water solution of acetic acid 33% inside closed containers
6	100% RH, sample exposed to internal atmosphere, pH 10	Placing water solution of ammonium hydroxide 5% inside closed containers

[a]This oxygen absorber was prepared by the author and its composition was suggested by Grattan and Gilberg, 1994.

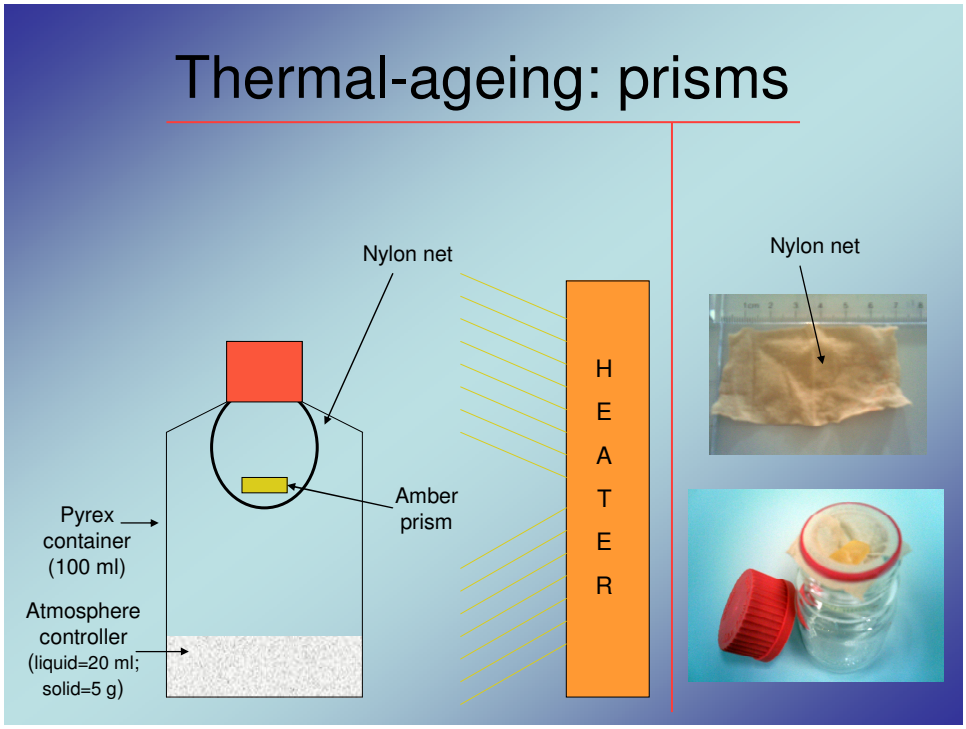


Figure 3.10.a. Experimental storage used for thermal-ageing of amber prisms.

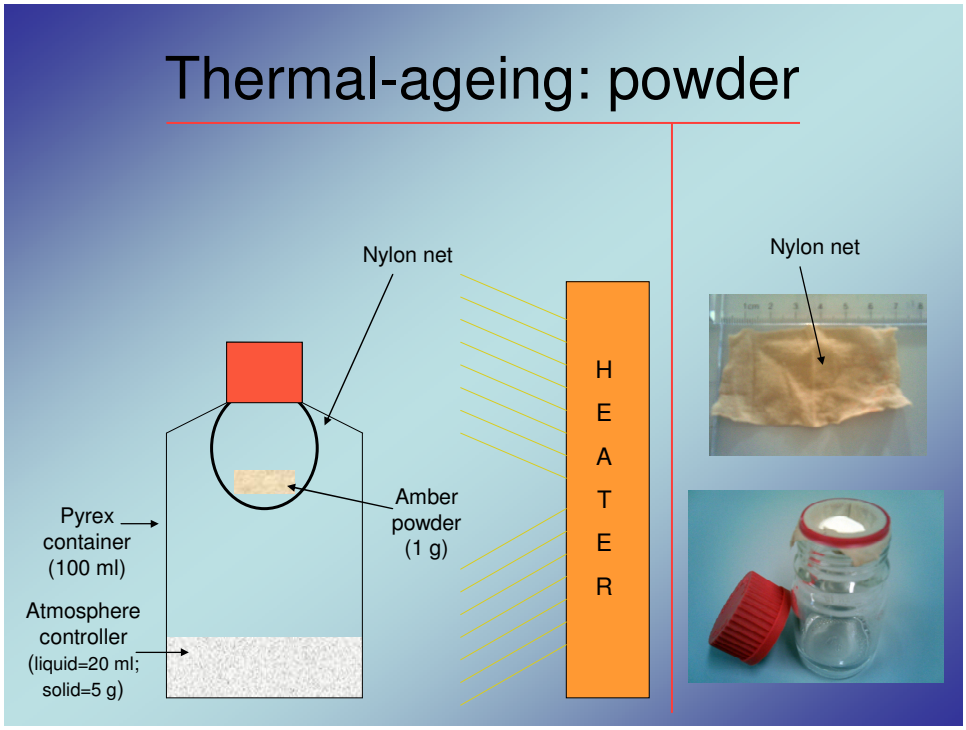


Figure 3.10.b. Experimental storage used for thermal-ageing of amber powder.

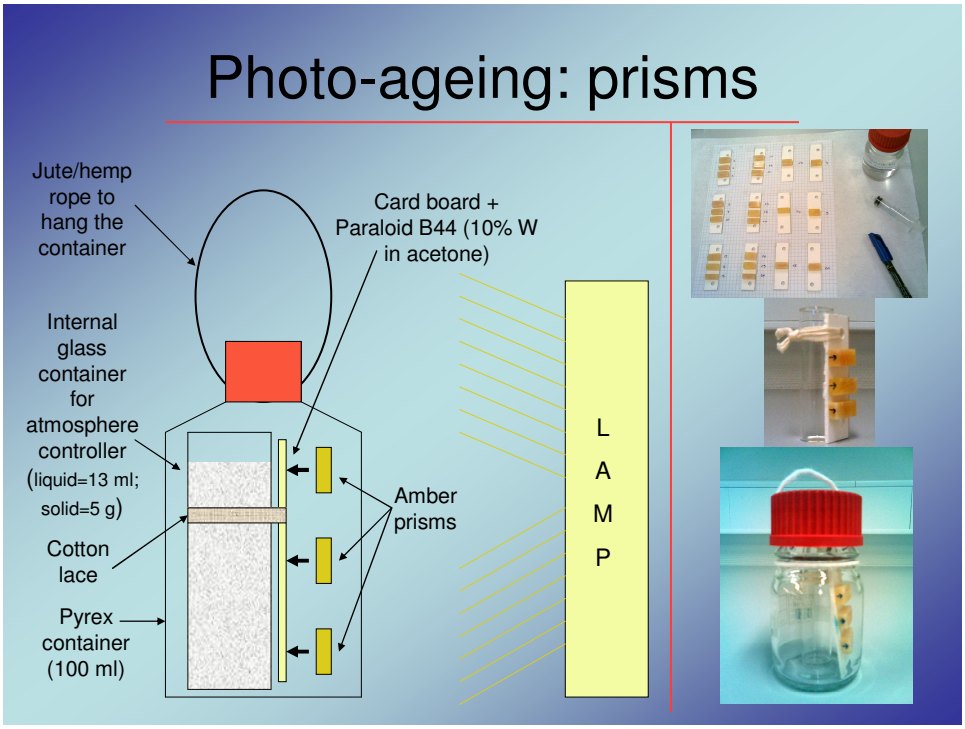


Figure 3.10.c. Experimental storage used for photo-ageing of amber prisms.

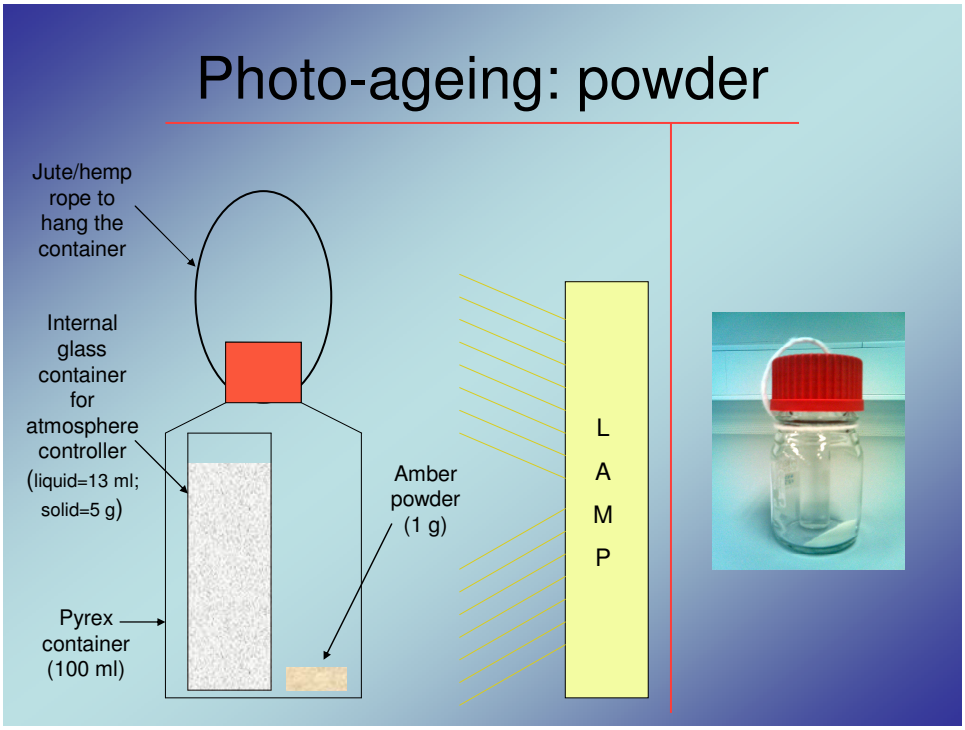


Figure 3.10.d. Experimental storage used for photo-ageing of amber powder.

3.4 Examination of amber samples.

Samples were checked regularly for appearance and colour change, chemical change, rate of oxidation and qualitative analysis of volatiles released by amber, through the analytical techniques listed and grouped in four main categories in table 3.6.

Table 3.6. Analytical techniques used for the examination of amber samples

Group of techniques		Information available
A	Visual examination by naked eye and photography; Colour measurement by CIE L*a*b* spectrocolorimetry	Change in colour; surface appearance
B	Attenuated Total Reflection (ATR)-FTIR spectroscopy; FT-Raman spectroscopy	Chemical groups present at the surface; changes in groups due to the degradation
C	Oxygen measurement by optical respirometry	Determination of the rate of oxidation
D	Solid Phase Micro Extraction (SPME) coupled with Gas Cromatography – Mass Spectrometry (GC-MS) headspace analysis	Identification of volatiles released by amber

To ensure the analysis of the same surfaces of the prisms with all the analytical techniques and every time, one side of each prism was marked by a permanent pen.

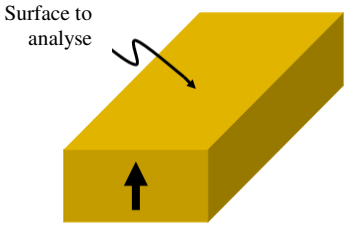


Figure x. Mark on the side of a prism that indicates the surface to analyse.

In most of the cases both prisms and powder were analysed, but to make measurements of amber powder by spectrocolorimetry and FT-Raman spectroscopy it was necessary to create pellet-shaped samples (13 mm in diameter each) obtained from pressed powder before and after ageing, since, to make both the analyses possible, a smooth surface was required to avoid the high radiation-scattering caused by the particles. Pellets were prepared placing 300 mg of amber powder in a 13 mm holder, which was located under a Perkin Elmer hydraulic press with a load of 10 tons for 5 minutes (figure 3.11).
Surfaces of prisms and pellets were washed with deionised water before each analytical stage, in order to remove possible residues that could affect the reliability of the results.

Only amber powder was analysed through SPME-GC-MS analyses, since for this method it was necessary to work with a small amount of material (around 10 mg) not compatible with the size of the prisms.

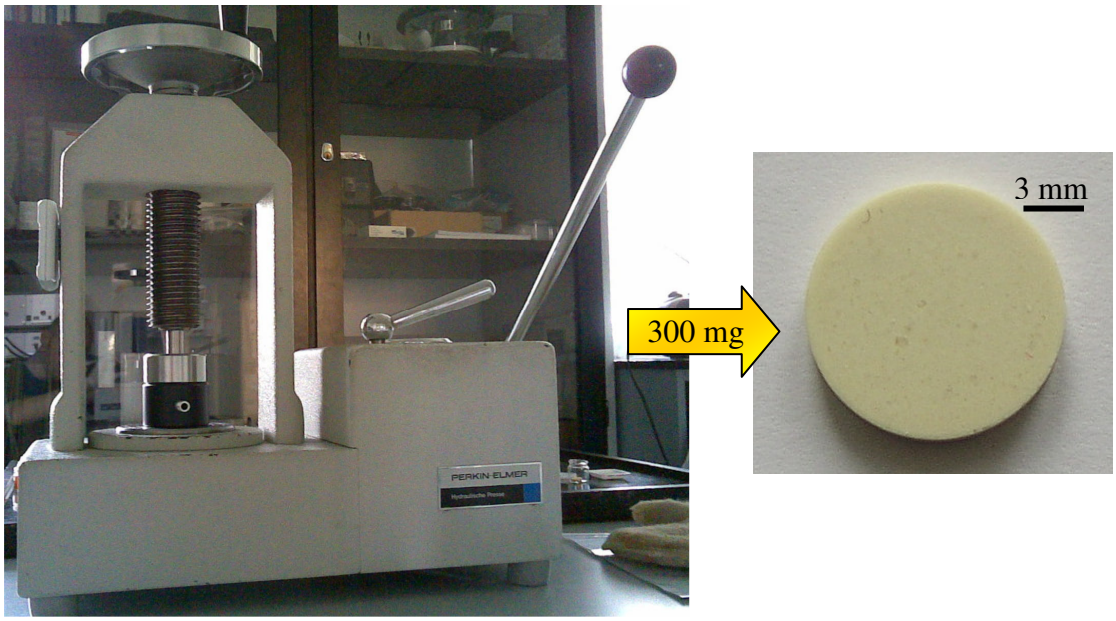


Figure 3.11. Production of amber pellets through the use of a hydraulic press.

More details related to the mentioned analytical techniques have to be illustrated.

3.4.1 Visual examination by naked eye and photography.
Appearance of amber samples was recorded using an Olympus Camedia C-5050 Zoom 5.0 Megapixel digital camera.

Table 3.7. Operative settings for photographic recording of amber samples

Parameter	Value			
ISO parameters	Automatic			
Flash	Excluded			
Picture format	RAW			
Frequency of recordings during ageing		Before	17 days	35 days
	Thermal	X		X
	Photo	X	X	

Regarding the powder samples, it was decided to record pictures of both free powder and pellets used for spectrocolorimetry and FT-Raman spectroscopy measurements.

3.4.2 Colour measurement by CIE L*a*b* spectrocolorimetry.

Colour of samples was measured using a Minolta CM-2600d portable spectrophotometer.

Table 3.8. Operative settings for spectrocolorimetric analysis of amber samples

Parameter	Value			
Geometry	d/8 (diffuse illumination / 8° observation)			
Specular reflection	Included (SCI)			
Measuring field	Ø 3 mm for prisms, Ø 8 mm for powder pellets			
UV content	100% (like daylight)			
Observer	CIE 10°			
Illuminant	D65 (standardized daylight, 6500 K)			
Colour space	CIE L*a*b*			
Colour difference	ΔL*, Δa*, Δb* and ΔE_{ab} [a]			
Frequency of measurement during ageing		Before	17 days	35 days
	Thermal	X	X[b]	X
	Photo	X	X	

[a] $\Delta E_{ab} = \left(|L_1 - L_2|^2 + |a_1 - a_2|^2 + |b_1 - b_2|^2 \right)^{1/2}$

[b] Only prisms.

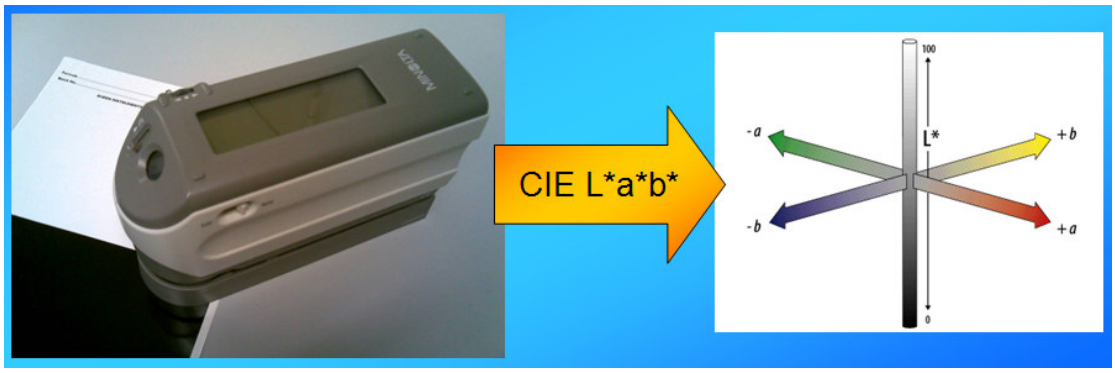


Figure 3.12. Portable spectrophotometer used to measure the colour of samples in the CIE L*a*b* colour space.

Procedure:

- *prisms* – each prism was placed on a Sheen Instruments sheet (Ref. 301-A); because of the slight transparency and heterogeneity of the prism, values of three random points on its surface were measured against the black background, then the procedure was repeated on the white background; the final CIE L*a*b* result was calculated as average of six values;

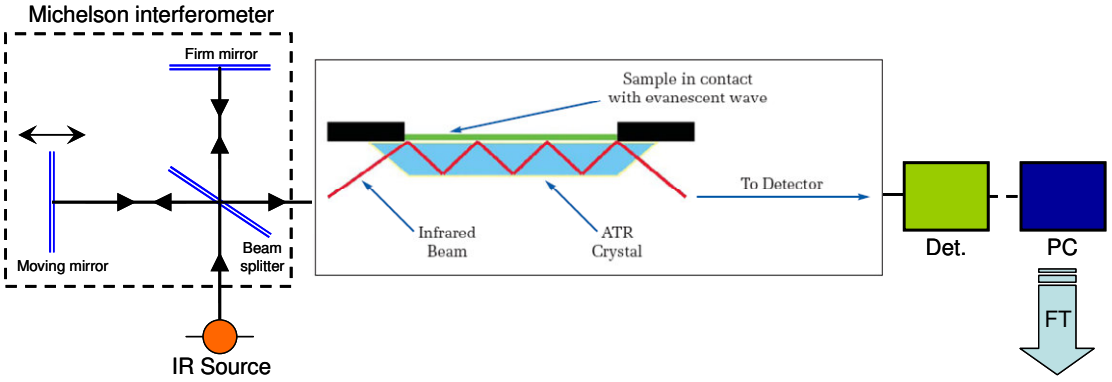
- *powder* – to measure the colour of the powder it was necessary to prepare pellets with the procedure previously described; each pellet was placed on a Sheen Instruments sheet (Ref. 301-A) and, because of its opacity and homogeneity, one single value was measured on the surface against the white background.

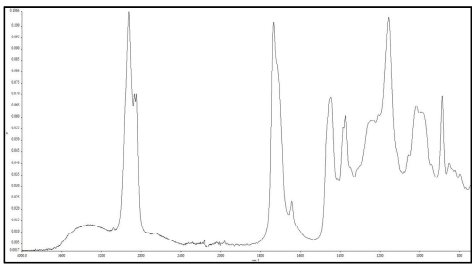
3.4.3 ATR-FTIR spectroscopy.

The characteristics of the Perkin Elmer Spectrum One FTIR spectrometer used for ATR-FTIR spectroscopic analyses have been already described in paragraph 3.1 (a more detailed description of the instrument is showed in figure 3.13).

Table 3.9. Operative settings for ATR-FTIR analysis of amber samples

Parameter	Value			
Range	4000 cm^{-1} – 650 cm^{-1}			
Unit	Absorbance			
Number of scans	4			
Resolution	4 cm^{-1}			
Force gauge	70			
Frequency of measurement during ageing		Before	17 days	35 days
	Thermal	X	X	X
	Photo	X	X	





Amber spectrum

Figure 3.13. Main components of the ATR-FTIR spectrometer used to analyse amber samples (ATR detail is reprinted from www.perkinelmer.com; © 2005 by Perkin Elmer Inc.).

Procedure:

- *background* – background spectra of the empty, clean ATR accessory open to air were run at hourly intervals;
- *prisms* – each prism was placed on the ATR accessory and, because of the slight heterogeneity of the prism, data of three random points on its surface were measured; each absorbance value was calculated as average of three values;
- *powder* – a small amount (around 2 mg) of powder was placed on the ATR accessory and analysed;
- *quantification of degradation* – the method developed to quantify levels of degradation of the amber samples during the accelerated ageing was based on similar methods used in previous works (Shashoua et al., 2005). Beer-Lambert law, which states that spectral absorbance is proportional to the concentration of absorbing species in a material, was applied to the spectra. For this analytical technique the oxidation of the molecular structure was used and it was calculated on the base of relative absorbance values of the infrared band at 1735 – 1700 cm^{-1} (related to C=O groups of esters and acids). Absorbance values at this band were ratioed against a band at 1450 ± 20 cm^{-1} (related to C-H bonds of $>CH_2$ and $-CH_3$ groups) which, during initial trials, did not show relevant changes due to the ageing process. Absorbance of a chemical group may be determined using the area, width or height of its band. Since the two bands of interest were not symmetrical, their maximum heights were determined on raw absorbance spectra, without manipulations or baseline corrections (figure 3.14).

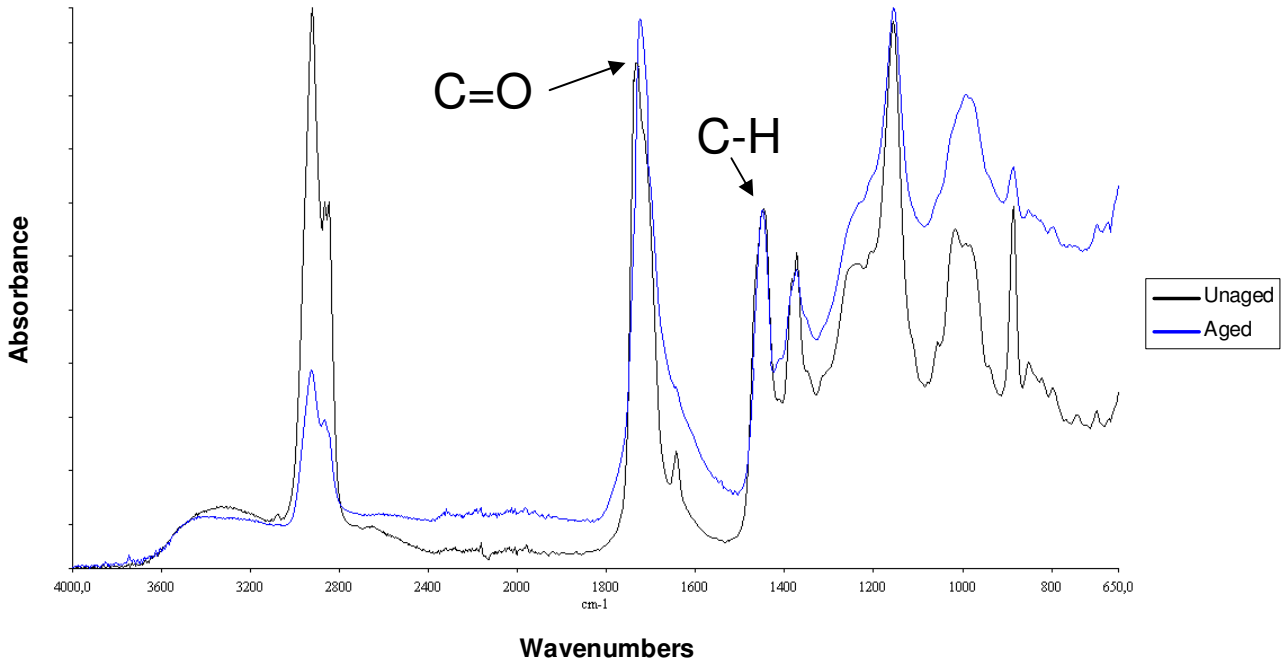


Figure 3.14. Infrared bands observed in ATR-FTIR spectra to quantify levels of degradation of amber samples.

3.4.4 FT-Raman spectroscopy.
FT-Raman analyses were performed with a Bruker RFS 100 spectrometer.

Table 3.10. Characteristics of the Bruker RFS 100 FT-Raman spectrometer used for the analysis of amber samples

Feature	Description
Radiation source	Nd:YAG laser at 1064 nm (NIR)
Detector	Liquid nitrogen cooled Ge-diode
Applicative software	Bruker Optik Opus version 5.5
Location	H.C. Ørsteds Laboratory – Department of Chemistry – University of Copenhagen

Table 3.11. Operative settings for FT-Raman analysis of amber samples

Parameter	Value			
Laser power	350 mW			
Range	3500 cm^{-1} – 10 cm^{-1}			
Number of scans	500			
Resolution	4 cm^{-1}			
Frequency of measurement during ageing		Before	17 days	35 days
	Thermal	X		X
	Photo	X	X	

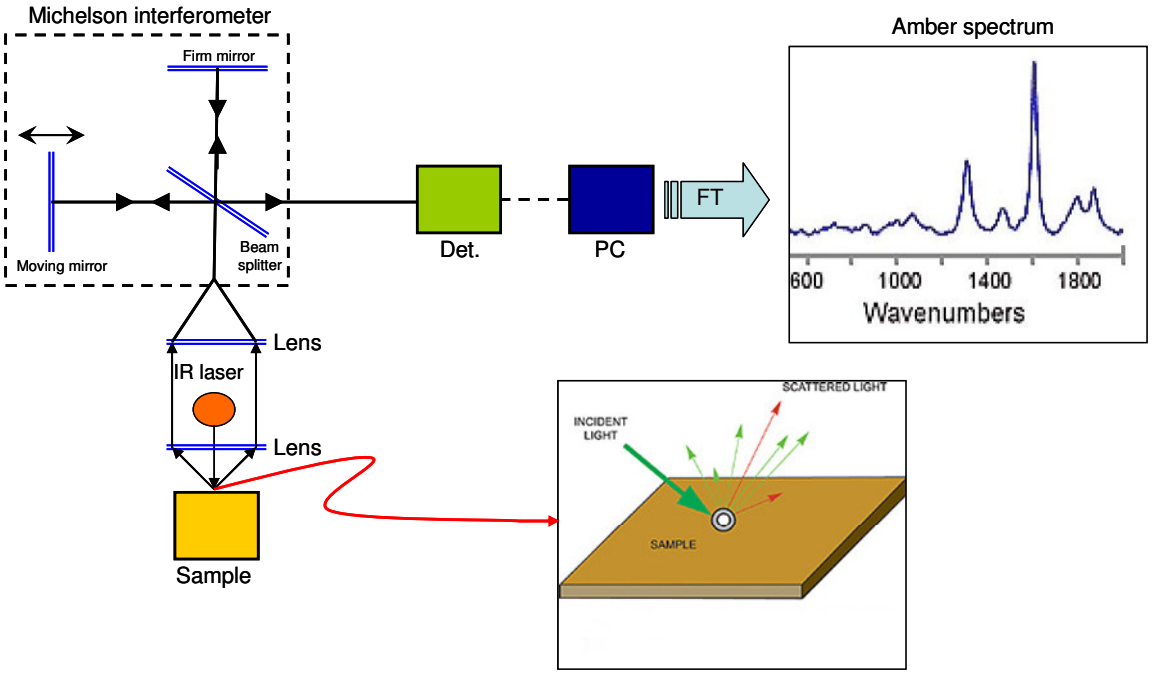


Figure 3.15. Main components of the FT-Raman spectrometer used to analyse amber samples.

Procedure:
Initial trials showed that one analyses on each sample was enough to give repeatable results;

- *prisms* – each prism was placed on the sample holder of the instrument and analysed on one point of the surface;
- *powder* – to collect FT-Raman spectra of the powder it was necessary to prepare pellets with the procedure previously described; each pellet was placed in the sample holder of the instrument and analysed on one point of the surface;
- *quantification of degradation* – the method developed to quantify levels of degradation of the amber samples during the accelerated ageing was based on similar methods used in previous works (Moreno et al., 2000; Shashoua, 2002; Shashoua et al., 2005). For this analytical technique the breakdown/formation of C=C bonds in the molecular structure was used and it was calculated on the base of relative intensity values of the infrared band at 1650 – 1600 cm^{-1} (related to C=C olefin groups). Intensity values at this band were ratioed against the band at 1450 ± 20 cm^{-1} (related to C-H bonds of $>CH_2$ and $-CH_3$ groups) which, during initial trials, did not show relevant changes due to the ageing process. Maximum heights of the two bands of interest were determined on raw Raman spectra without manipulations or baseline corrections (figure 3.16).

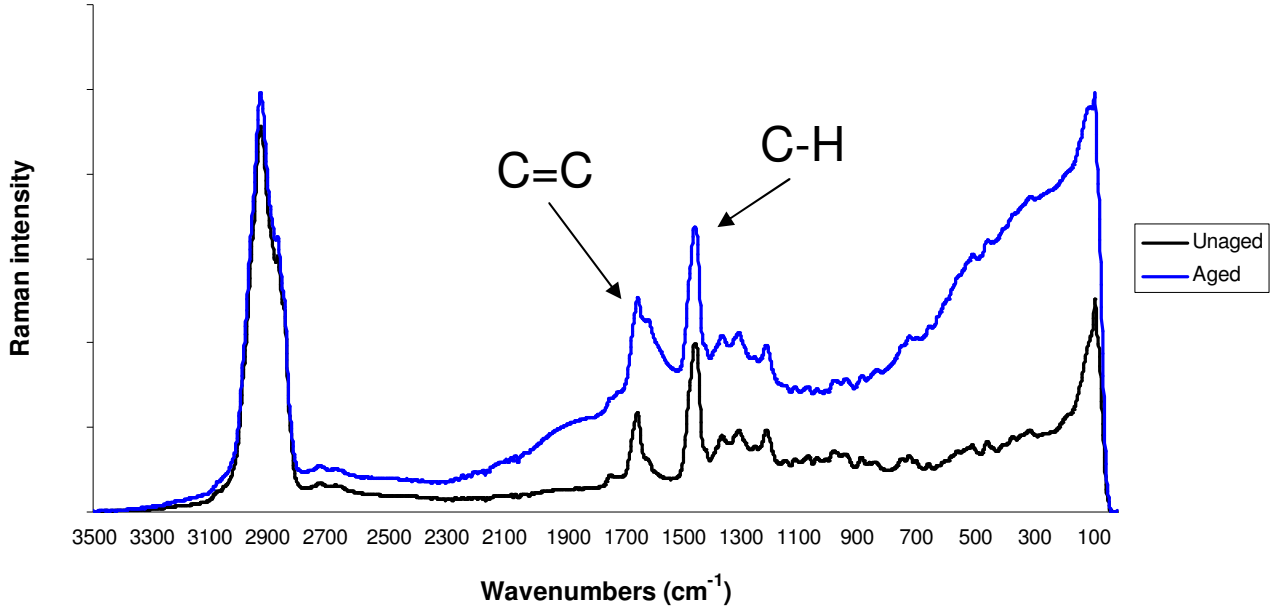


Figure 3.16. Raman bands observed in FT-Raman spectra to quantify levels of degradation of amber samples.

3.4.5 Oxygen measurement by optical respirometry.

For this study, to evaluate the oxygen consumption by the material during the ageing, a new method based on the use of optical oxygen sensor-spots, which allow measurement of the oxygen concentration through the transparent wall of a closed container, was used. By this method, the oxygen concentration is measured optically, using light rather than traditional chemical or electrochemical methods (Matthiesen, 2007).

Oxygen measurements were made out using a PreSens Fibox 3 respirometer coupled with the software OxyView PST3 version 6.02, at the Research, Analysis and Consulting Laboratory of the Department of Conservation – National Museum of Denmark.

Table 3.12. Operative settings for respirometric analysis of amber samples

Parameter	Value			
Excitation wavelength	505 nm			
Operative temperature	Room temperature			
Unit	% air saturation (i.e. oxygen in atmospheric air is assigned 100%)			
Frequency of measurement during ageing		Before	17 days	35 days
	Thermal	X		X
	Photo	X	X	

The working principle is based on the use of optical oxygen sensor-spots. Details about the method are showed in figure 3.17.

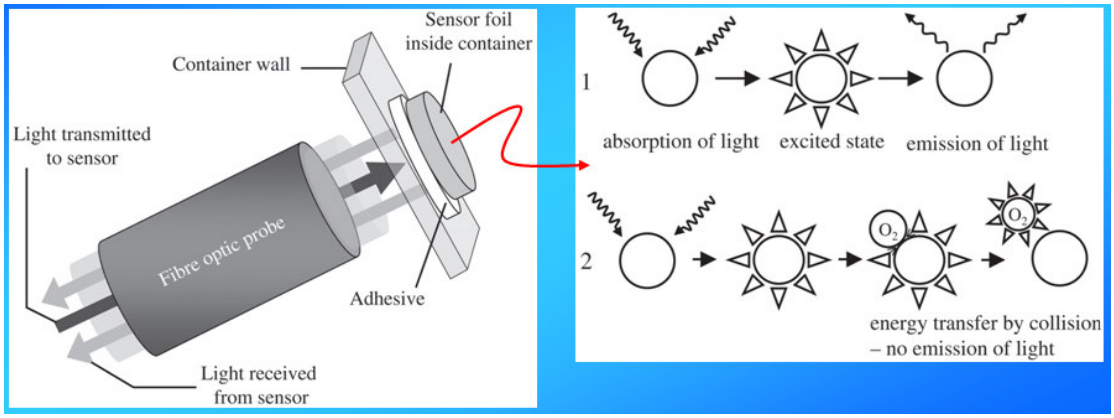


Figure 3.17. Working principle behind optical oxygen sensor-spots. Light is transferred via an optical fibre; the sensor foil with the oxygen-sensitive dye is placed inside the container, and light is transferred and measured through the transparent container wall. The luminescent dye is excited by light at one wavelength and emits light at another wavelength (1). When oxygen is present, the energy of the excited molecules is transferred by collision with oxygen instead of emission of light (2). (Modified from Matthiesen, 2007).

Procedure:

- *preparation* – before the start of the accelerated ageing, a SP-PSt3-NAU-D5-YOP Planar Oxygen-Sensitive Spot from PreSens was adhered to the inside of each bottle with a tiny amount of Paraloid B72, leaving the bottle open for at least 24 hours to allow the complete drying of the adhesive. A stock of experimental storages had to be prepared exclusively for this analytical method, since the containers needed to be sealed for all the duration of the ageing (on the opposite, for the other analyses the containers were opened during the ageing in order to extract the samples for the measurements);
- *analysis* – each sensor-spot related to one sample was excited by the radiation from the optical fibre of the instrument, through the container glass wall; the resultant oxygen concentration value was logged by a PC connected to the instrument.

3.4.6 SPME-GC-MS headspace analysis.

SPME is a patented extractive technique based on the adsorption of analytes directly from the headspace of a sample onto a coated fused-silica fibre. When it is coupled with GC-MS, this sampling technique allows analysing volatile compounds in an innovative, fast and simple way, eliminating the need to heat the sample at high temperature.

Three control samples on thermal-ageing and three on photo-ageing were used in order to verify the absence of external contaminants. Each control sample was prepared placing 10 mg of amber powder on the bottom of a 2 mL Chromacol glass vial with PTFE/silicone closure.

Analyses were carried out by Senior Researcher Jens Glastrup at the Research, Analysis and Consulting Laboratory of the Department of Conservation – National Museum of Denmark, using a Carboxen/PDMS needle from Sigma Aldrich with a Varian Saturn 2000 system (gas-chromatographer coupled with mass spectrometer).

Table 3.13. Characteristics of the SPME-GC-MS equipment used for the analysis of amber samples

Feature	Description
Carboxen/PDMS fibre characteristics	75 µm, bipolar, maximum operative temperature 340 °C
GC carrier	He, total flow 13,7 mL/min
GC column	CP-Wax 58 (FFAP) CB, polar, 25 m x 0.25 mm i.d., 0.3 µm coating, maximum operative temperature 250 °C
MS characteristics	Vacuum pressure $6{,}5 \bullet 10^{-6}$ mmHg, quadrupole mass filter, Silcosteel treated ion trap electrodes
Applicative software	Varian MS workstation version 6.6

Table 3.14. Operative settings for SPME-GC-MS headspace analysis of amber samples

Parameter	Value			
GC injector conditions	Initial temperature 150 °C for 0.10 min, ramp rate 300 °C/min to 250 °C, held for 15 min			
GC column oven	Initial temperature 50 °C for 2 min, ramp rate 10 °C/min to 240 °C, held for 5 min			
MS scanning mode	From m/z 25 to 399, selectively removing the m/z 28 and 32 using the SIS facility			
Frequency of measurement during ageing		Before	17 days	35 days
	Thermal	X		X
	Photo	X	X	

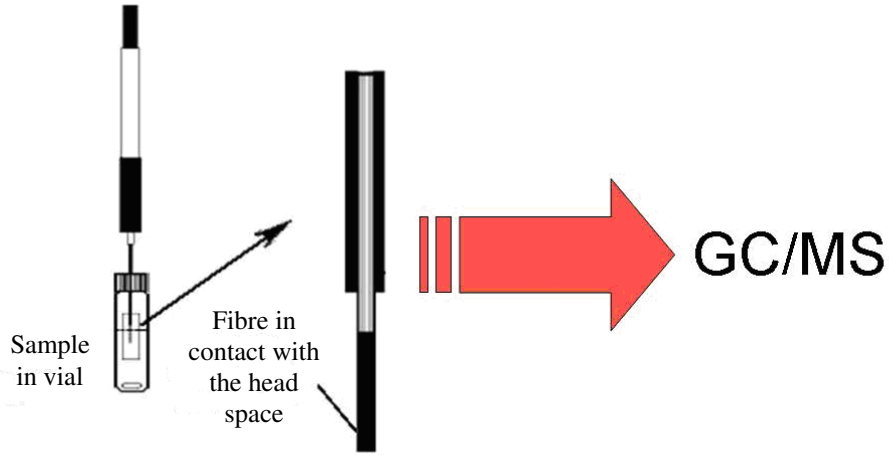


Figure 3.18. Method of the Solid Phase Micro Extraction coupled with GC-MS (modified from www.sigmaaldrich.com; © 1998 by Sigma-Aldrich Co.).

Procedure:

- *preparation* – 10 mg of amber powder from each sample were placed on the bottom of 2 mL Chromacol glass vials with PTFE/silicone closure. The vials were loaded on a Varian 8200 auto-sampler with the SPME option;
- *micro-extraction* – the SPME needle mounted in the auto-sampler was automatically introduced into each vial and the fibre was exposed to the headspace at room temperature for 10 minutes, in order to adsorb the vapours;
- *desorption and analysis* – after the micro-extraction, the SPME needle was automatically transferred to the GC injector and it was desorbed for 10 minutes before the analysis of the vapours.

Blank samples (vials containing just air) were run every three samples to make sure that the SPME needle was cleaned.

3.5 Overview of the experiment design.

To summarize the design of the preliminary investigation, most of the information previously described are illustrated in table 3.15.
It is important to note that in most of the instances three samples for each combination of ageing, sample size and microclimate conditions were used; because of the light chamber smaller size, a minor number of samples was used in some cases during the photo-ageing.

Table 3.15. Ageing experiment design. Unless it is indicated otherwise, each container held one prism or one gram of powder. The different analytical groups are defined according to table 3.6 in paragraph 3.4

Ageing	Size	Microclimatic condition 1	2	3	4	5	6	Analytical group
Thermal-ageing	Prisms	3 prisms						A, B
	Powder							A, B, D
	Prisms							C
	Powder							
Photo-ageing	Prisms	3 prisms	3 prisms	3 prisms	3 prisms	3 prisms	3 prisms	A, B
	Powder							A, B, D
	Prisms							C
	Powder							

Open Pyrex flask.

Closed Pyrex flask.

3.6 Additional analyses.

During the preliminary investigation it was also decided to run a few additional analyses:

- *C-H-N analysis* – a type of elemental analysis to detect possible variations in the elemental composition, precisely in the content of carbon, hydrogen and nitrogen, in the amber material during the ageing;
- *Micro-ATR-FTIR cross sections analysis* – analysis of cross sections obtained from amber prisms, in order to get information about the development of degradation in relation to the distance from surfaces. The purpose was the identification of degradation as surface or bulk phenomenon;
- *Confocal profilometry* – non-contact topographic analysis of the surface of prisms in order to obtain information about any potential changes in the roughness due to the ageing.

In all the cases the experimental design was different from the one described in the previous paragraphs and consisted of the procedures described later on.
Regarding the frequency of the analyses, measurements were performed before the ageing, after 35 days of ageing and after 70 days of ageing, but not all of the samples were analysed every time. This is illustrated in figure 3.19.

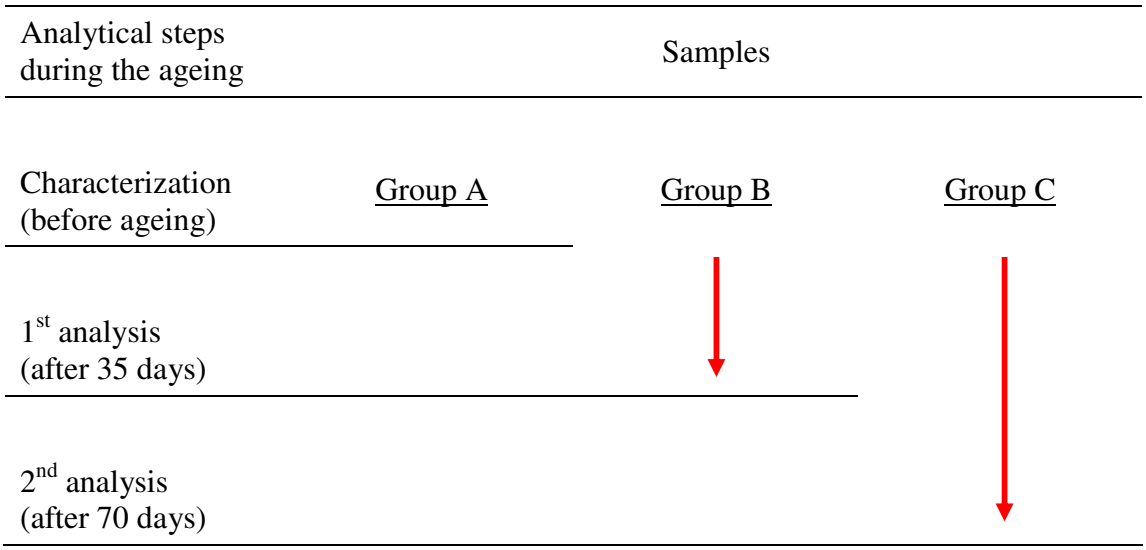


Figure 3.19. Analyses planning during the accelerated ageing of amber samples.

3.6.1 C-H-N analysis.

- *preparation of samples* – 9 small fragments (around 2 mg each) were obtained from one amber prism, which was not used for the main experiment, through the use of a scalpel;
- *ageing of samples* – three fragments (group A) were kept unaged, each one of the remaining six (groups B and C) was placed inside a 2 mL Chromacol open glass vial and subjected to thermal-ageing in a WTB Binder FED 115 oven, at a temperature of 85 °C and a relative humidity ≤ 20%, in absence of light, for 35 + 35 days;

- *examination of samples* – the analyses (that are destructive) were performed following a standard protocol with a CE Instrument Flash 112 series EA, by Associate Professor Jesper Bendix at the Micro Analytical Laboratory - Department of Chemistry - University of Copenhagen;
- *comparison with an archaeological sample* – the same analysis was also repeated on a fragment from the dark brown crust of an archaeological Baltic amber object (Neolithic bead found in Skanderborg -Denmark-, dated 3-2 thousands of years BCE).

3.6.2 Micro-ATR-FTIR cross sections analysis.

- *ageing of samples* – 3 amber prisms, which were not used for the main experiment, were selected; one prism (group A) was kept unaged, each one of the remaining two (groups B and C) was placed in a glass Petri dish and subjected to thermal-ageing, using the same oven and operative settings described in paragraph 3.3, for 35 + 35 days;
- *preparation of cross sections* – before the analysis, each prism was cut along the minor axe in two equal parts (obtaining 6 portions in total), using a Buehler Isomet low speed electrical saw, cooled with a 3% water solution of Struers Additive for Cooling Fluid;
- *examination of cross sections* – the analyses were performed with a Perkin Elmer AutoImage FTIR microscope (details and operative settings are listed in tables 3.16 and 3.17) placing each sample under the microscope objective/ATR crystal and collecting the spectra related to 15 points on the cross section (three random points at five different levels between the external surface and the core); absorbance values of each level were calculated as average of three values. Background spectra of a gold plate were run at hourly intervals;
- *quantification of degradation* – to quantify levels of degradation of the amber samples during the accelerated ageing, the oxidation of the molecular structure was used and it was calculated on the base of relative absorbance values of the infrared band at 1735 - 1700 cm^{-1} (related to C=O groups of esters and acids). Absorbance values at this band were ratioed against a band at 1450 ± 20 cm^{-1} (related to C-H bonds of $>CH_2$ and $-CH_3$ groups) which, during initial trials, did not show relevant changes due to the ageing process. Maximum heights of the two bands of interest were determined on raw absorbance spectra without manipulations or baseline corrections.

Table 3.16. Characteristics of the Perkin Elmer AutoImage FTIR microscope used for the analysis of amber cross sections

Feature	Description
ATR crystal	Diamond/ZnSe
Detector	Photoconductive: liquid nitrogen cooled MCT (mercury cadmium telluride)
Applicative software	Perkin Elmer Spectrum version 6.2.0 + AutoImage

Table 3.17. Operative settings for micro-ATR-FTIR cross sections analysis of amber samples

Parameter	Value
Range	4000 cm^{-1} - 650 cm^{-1}
Unit	Absorbance
Number of scans	50
Resolution	8 cm^{-1}
Measuring field	100 x 100 μm

3.6.3 Confocal profilometry.

- *preparation of samples* – the same samples used for the micro-ATR-FTIR cross sections analysis were also used for this analysis, but in this case the scope of the examination was to investigate the characteristics of the external surface instead of the cross section;
- *examination of samples* – the analyses were performed with a NanoFocus μSurf Explorer (details are listed in table 3.18) placing each sample under the confocal microscope objective and collecting the topographic data related to 3 random points on the surface, with a z-steps width of 0.1 μm; roughness parameters (Ra and Rq defined by standard ISO 4287) and height parameters (Sa and Sq defined by standard ISO 25178) of each sample were calculated as average of three values.

Table 3.18. Characteristics of the NanoFocus μSurf Explorer used for the analysis of amber samples

Feature	Description
Light source	High efficiency LED at 505 nm
Optical module	800 XS
Detector	Fast digital camera 512 x 512 pixel, 10 bit
Applicative software	μSoft control & analysis (standard version)
Location	NanoFocus AG, Oberhausen (Germany)

3.7 Data analysis and documentation.

The results obtained from the above described techniques were analysed through both critical evaluation and statistical study.
Statistical analysis was achieved using professional software such as:

- MS Excel 2007, to create tabulations, summarize data (averages, standard deviations, standard errors), build graphical presentations (histograms, scatter plots) and perform simple tests (t-test for paired data);
- SAS JMP 6, to perform complex tests (ANOVA – analysis of variance).

A further important step during the management of the research results consisted of the creation of a double database which contained all the information related to the analysed amber samples. It was named double database because it was divided in two different electronic environments:

- *informative database* – a data bank created with MS Access 2007, where all the samples data, such as information register, chemico-physical properties, analytical results, comments, statistics, links to pictures and diagrams, were documented and stored;
- *spectrographic database* – a library created with Perkin Elmer Spectrum 6.2.0, where all the ATR-FTIR spectra of the analysed samples were stored. The scope was to create a tool that could be used to evaluate the degradation state in amber samples during following phases of this study.

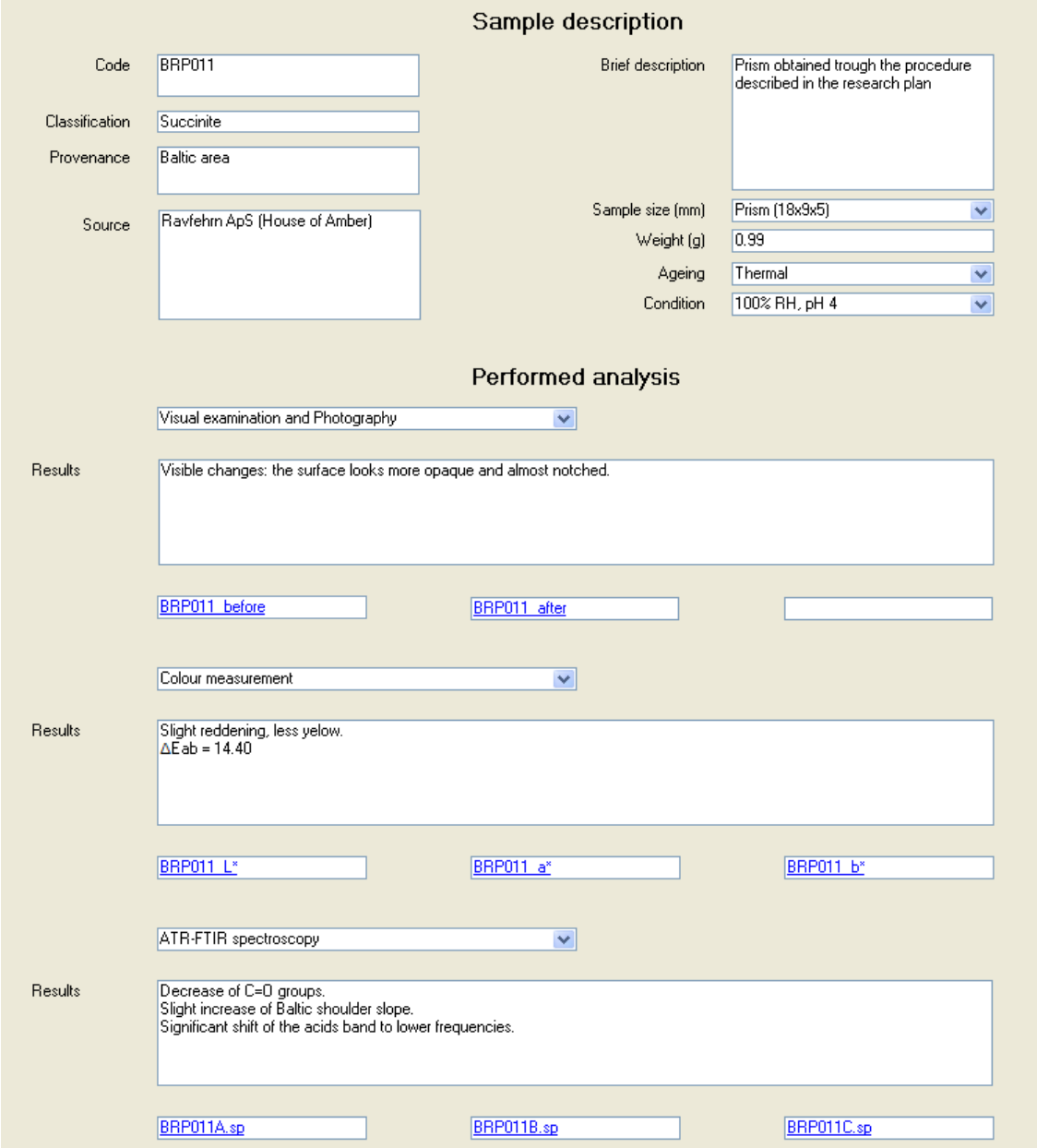


Figure 3.20. Exemplificative detail of a data-form in the informative database created with MS Access 2007.

4. PRELIMINARY INVESTIGATION - RESULTS AND DISCUSSION.

In this chapter, the results related to the different analytical methods which were employed during the preliminary investigation are presented.
During the description of the results, each microclimatic condition is cited by the respective number (according to table 3.5 in paragraph 3.3), with additional comments to remind the reader about the main characteristic.

4.1 Analysis and interpretation of achieved data.

4.1.1 Visual examination by naked eye and photography.
Amber prisms and powder were observed and photographed before and after the artificial accelerated ageing procedures.
Pictures of prisms are showed together with the colour measurement results in appendix A – subsection A.1, to illustrate the correlation between appearance and CIE L*a*b* parameters.

Visible changes in the appearance of the prisms were observable only on samples aged in acidic (condition 5) and alkaline (condition 6) pH, since after both the ageing procedures the surface of these samples looked more opaque and almost notched.
Regarding the powder, it was observed a general darkening and yellowing; on photo-ageing this change in colour was visible only on a thin external layer.
Moreover, a strong cohesion between the powder particles occurred in acidic condition 5, resulting in the formation of a clump of hard material (figure 4.1); it was not possible to determine the hardness of these clumps because of their small size, which did not allow any conventional method to measure penetration or compression resistance. An empirical method based on fingernail scratching suggested that the hardness was lower than 2.5 in the Mohs scale (American Federation of Mineralogical Societies, 2008).
The cause of this cohesion phenomenon is not known and more studies will be necessary.

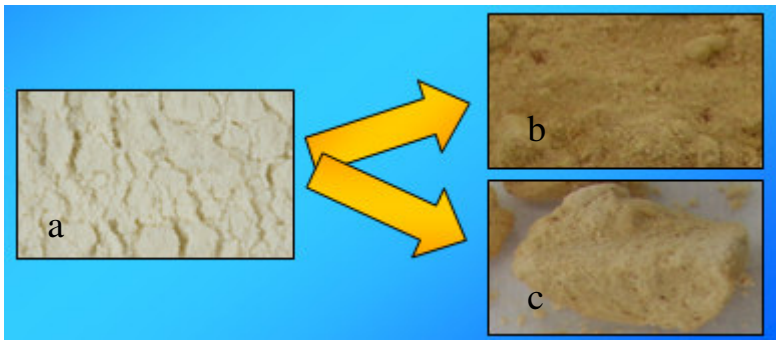


Figure 4.1. Amber powder: comparison between the pre-ageing (a) and the post-ageing appearances (b: general case; c: atypical case of acidic condition 5).

From these observations, it was possible to conclude that acidic and alkaline pH conditions produced the strongest visible changes (deterioration of prisms surfaces and cohesion of powder particles).

4.1.2 Colour measurement by CIE L*a*b* spectrocolorimetry.

Data from colour measurements of amber samples were expressed using the CIE L*a*b* colour system.

In appendix A – subsection A.1, the changes in colour components, the ΔE_{ab} (i.e. index related to the difference between CIE L*a*b* parameters of an aged sample and an unaged one; Johnston-Feller, 2001) and the comparison between pre-ageing and post-ageing appearance of prisms and pellets surfaces, in all the ageing conditions, are illustrated.

These results are summarised in table 4.1.

Table 4.1. Changes in colour of amber samples during the accelerated ageing

Ageing	Size	Description
Thermal-ageing	Prisms	Outcomes were different depending on the microclimatic condition: in conditions 1 and 3, characterized by dry environment, lightening and yellowing of the samples were detected; in conditions 5 and 6, respectively characterized by acidic and alkaline pH values, the samples became less yellow; no relevant changes were detected in conditions 2 and 4, respectively humid and hypoxic
	Powder	Samples showed a general darkening, yellowing and slight reddening
Photo-ageing	Prisms	Samples showed a general lightening and yellowing
	Powder	The general trend consisted of a slight yellowing, but it is important to note that only a thin external layer of each powder sample was actually exposed to the light; even if, during the preparation of each pellet for the colour measurement, it was carefully selected the powder from that external layer, some non-exposed powder was also present in the mixture, affecting the final colour

In most of the instances results showed colour change during ageing: lightening and yellowing were the most frequent effects on prisms, while darkening and yellowing were the most common results about powder. Therefore, it was suggested that the surface area to volume ratio had a role in the change of colour.

Analyzing the distribution of the ΔE_{ab} indexes related to the different experimental conditions, a high concentration of values in a range between 8 and 18 (that signifies a considerable change of colour; Johnston-Feller, 2001) was observed, but a few cases lay out of that interval, as showed in figure 4.2.
The biggest colour changes (highest ΔE_{ab} values) were related to thermal-aged powder samples in low relative humidity conditions; the lowest ones were associated to photo-aged powder samples (because of the reduced exposure) and to the blind samples.

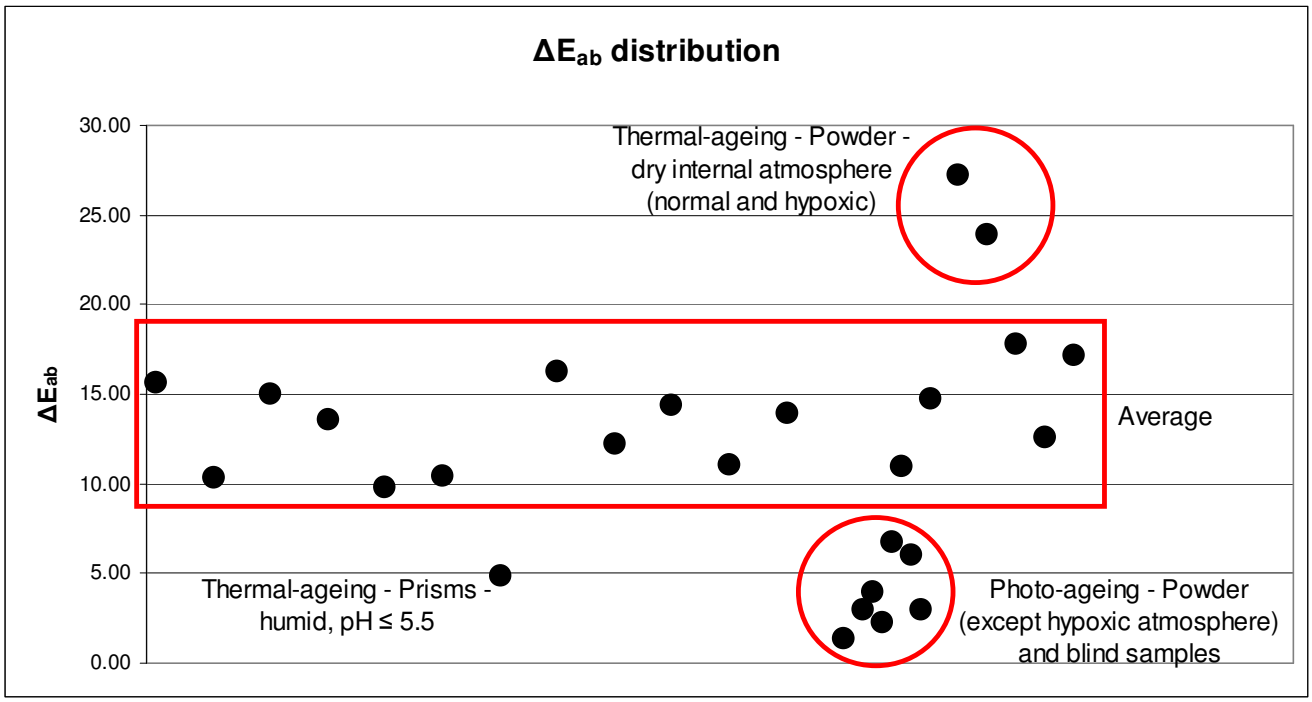


Figure 4.2. Scatter plot representing the distribution of the ΔE_{ab} values related to the different experimental conditions.

It was possible to conclude that regarding the change in colour, factors such as surface area to volume ratio and relative humidity had a more important role than ageing agents such as heat and light, since samples of the same size showed similar changes independently on the kind of ageing. Low relative humidity conditions frequently produced the biggest changes.

4.1.3 ATR-FTIR spectroscopy.
To determine degradation of amber samples during the accelerated ageing, levels of oxidation were evaluated measuring the ratio between absorbance values of C=O (carbonyl group) and C-H infrared bands.
In appendix A – subsection A.2, the carbonyl group absorbance changes in prisms and powder, in all the ageing conditions, are illustrated.
These results are summarised in table 4.2 and showed in figure 4.3.

Table 4.2. Changes in carbonyl group absorbance in amber samples during the accelerated ageing

Ageing	Size	Description
Thermal-ageing	Prisms	Outcomes mainly showed stability or decrease in absorbance of C=O groups; these observations contrast with previously published works (Shashoua et al., 2005), where the results always indicate an increase in the concentration of carbonyl groups
	Powder	Samples showed a general thermal-oxidation, indicated by the increase in absorbance of C=O groups; a decrease in the concentration of the same groups was detected only in condition 6 (alkaline environment)
Photo-ageing	Prisms	Almost all the samples showed a strong increase (slight for blind samples) in absorbance of C=O groups, indicating an intense photo-oxidative activity; this result contrasts with previously published works (Williams et al., 1990), where exposure of amber specimens to visible light is reported to give no appreciable spectroscopic change
	Powder	The general trend consisted of a slight increase (absent in blind samples) in absorbance of C=O groups, but this probably depended on the reduced exposure to light; a decrease in the concentration of the same groups was detected only in condition 6 (alkaline environment)

It was possible to conclude that in most of the occurrences, especially on photo-ageing, the increase in the concentration of carbonyl groups, likely related to the formation of carboxylic acids, was observed. These results showed that the extent of oxidation after ageing was higher to that of unaged amber, confirming that the presence of oxygen is a relevant degradation factor.
Exceptional cases showing different trends (e.g. alkaline condition) were considered for further investigations.
Spectra related to photo-aged prisms also showed extremely strong oxidation in relation to other spectroscopic features, e.g. the intense slope of the Baltic shoulder and the complete loss of the band at 888 ± 1 cm^{-1}.
It is important to note that oxidative processes were also detected in samples exposed to hypoxic atmosphere during ageing. These results suggested the presence of incoming air in flasks which should have been perfectly airtight.

In several cases, during the analysis of the ATR-FTIR spectra, one spectroscopic feature drew the attention: the peak at 1730 cm^{-1} related to C=O groups of esters, present in all the spectra of unaged samples, showed a splitting in two peaks and a following shift to 1715 cm^{-1} during ageing (figure 4.4);
This occurrence suggested the possibility of hydrolytic processes during ageing and it was considered for further investigations.

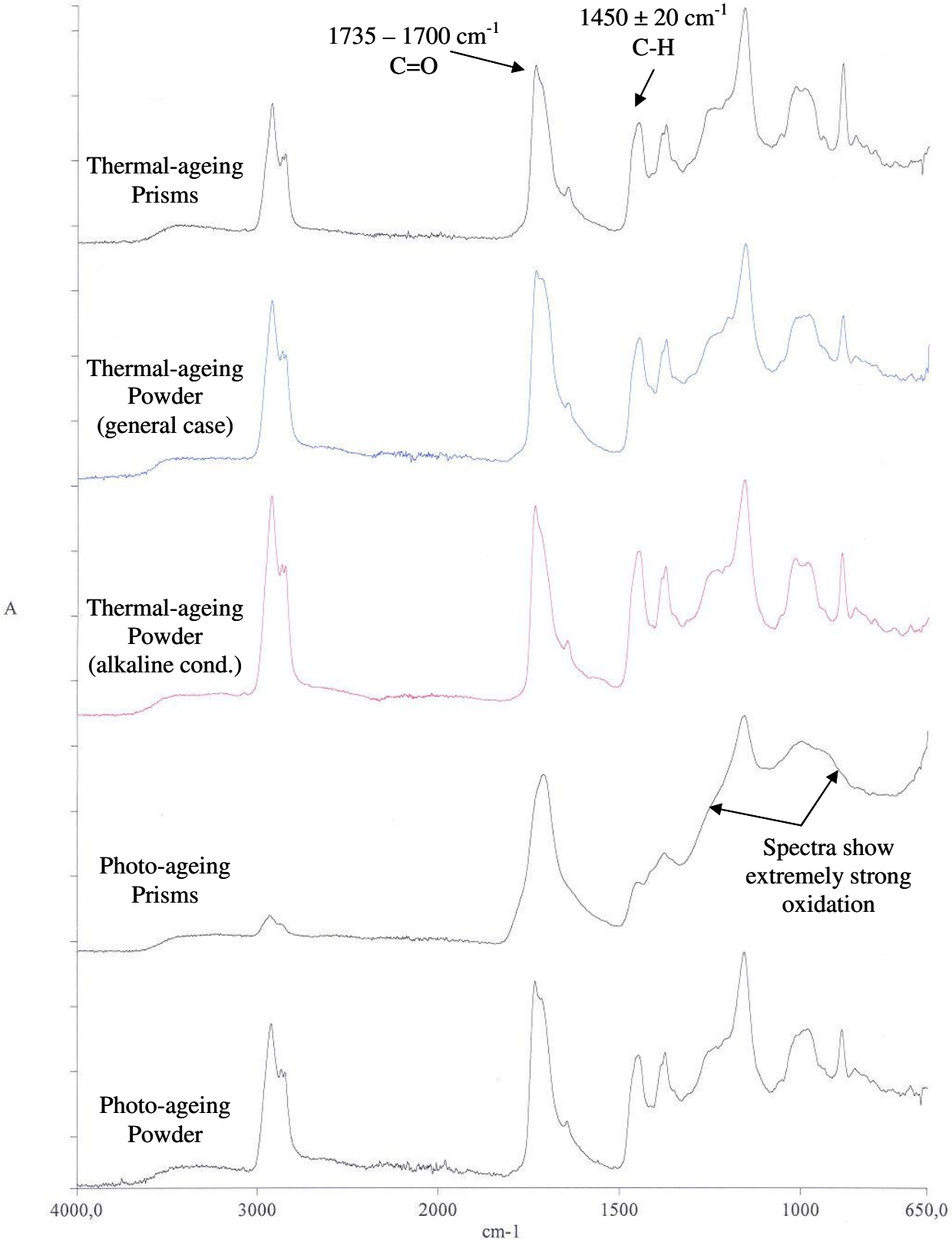


Figure 4.3. Comparison between most representative ATR-FTIR spectra of aged amber samples.

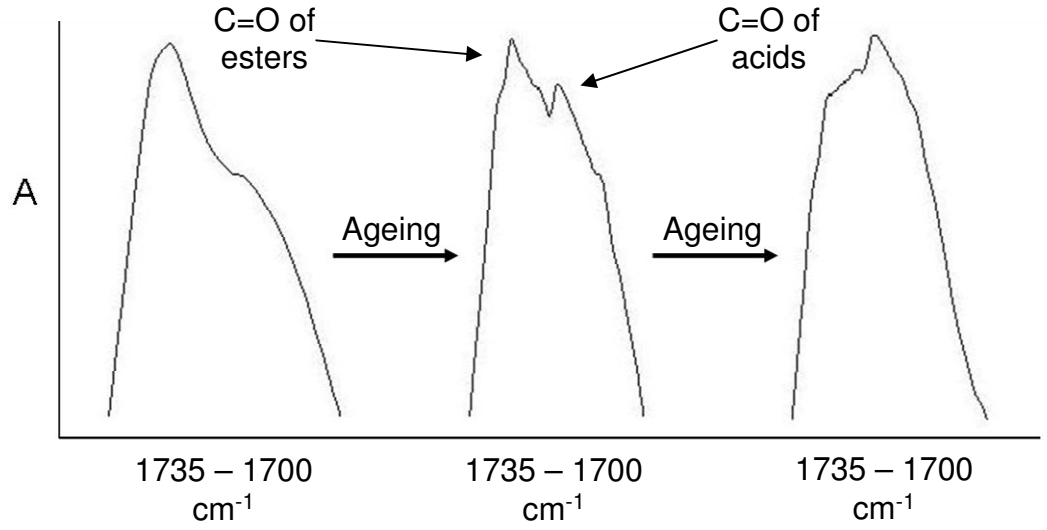


Figure 4.4. Splitting and following shift from 1730 to 1715 cm^{-1} of the peak related to C=O groups of esters and acids during ageing.

4.1.4 FT-Raman spectroscopy.
To determine degradation of amber samples during the accelerated ageing, the breakdown/formation of C=C (olefinic) bonds was evaluated measuring the ratio between intensity values of C=C and C-H infrared bands.
In appendix A – subsection A.3, the olefinic bonds intensity changes in prisms and powder, in all the ageing conditions, are illustrated.
These results are summarised in table 4.3 and showed in figure 4.5.

Table 4.3. Changes in olefinic bonds intensity in amber samples during the accelerated ageing

Ageing	Size	Description
Thermal-ageing	Prisms	Outcomes mainly showed stability in intensity of C=C bonds
	Powder	Samples showed a general formation of C=C bonds, indicated by the increase in intensity of the related band
Photo-ageing	Prisms	Samples (except blind samples) showed a general breakdown of C=C bonds, indicated by the slight decrease in intensity of the related band
	Powder	The general trend was very similar to the one described for photo-aged prisms

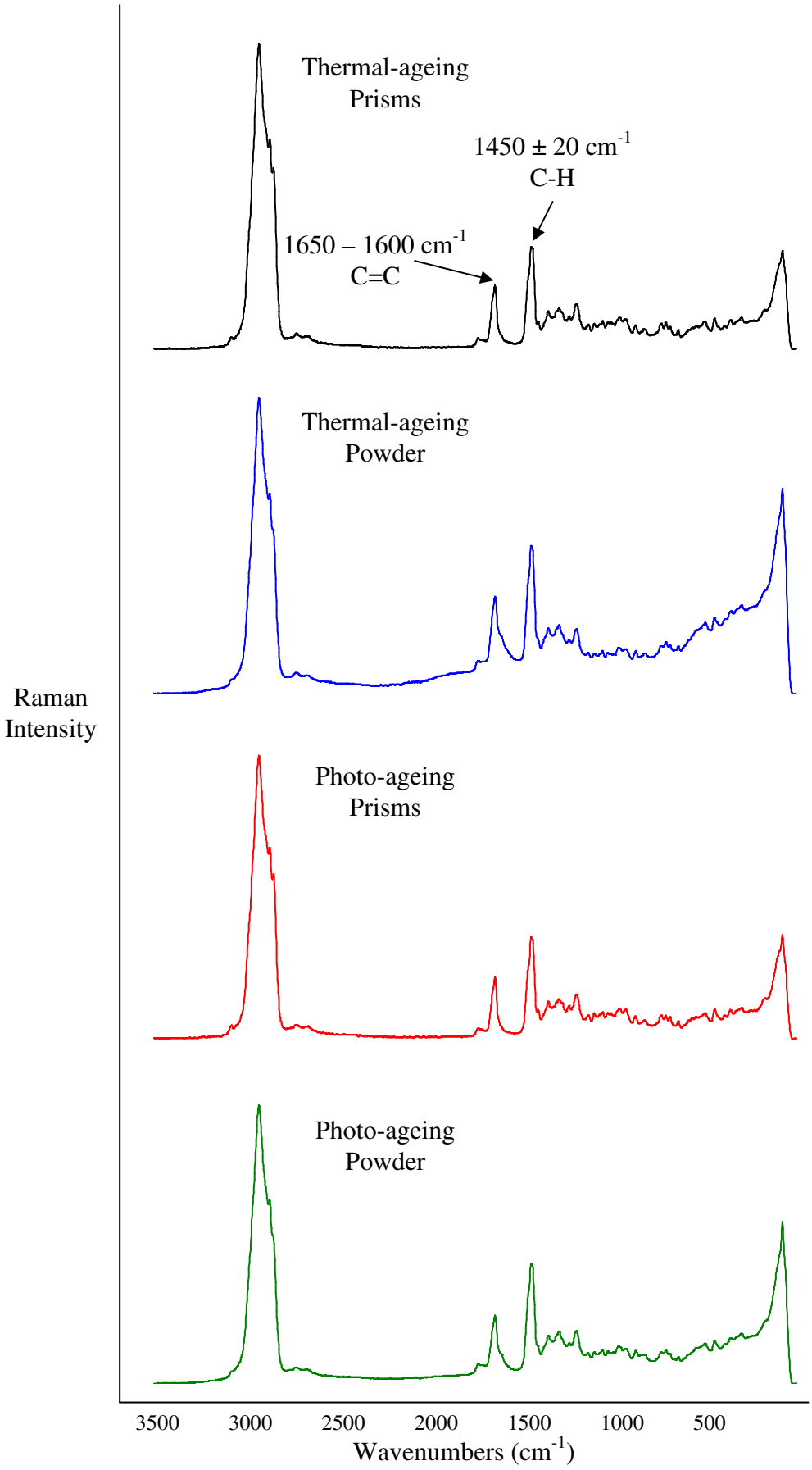


Figure 4.5. Comparison between most representative FT-Raman spectra of aged amber samples.

It was possible to conclude that the results about the concentration of unsaturated carbon-carbon bonds were strictly depending on the kind of ageing: heat seemed to generate the formation of C=C bonds, while light seemed to cause the loss of the same bonds. In similar works (Clifford et al., 1995; Moreno et al., 2000; Shashoua, 2002; Shashoua et al., 2005) the loss in C=C groups appeared the most common effect of maturation or ageing of amber.

Further more, the peak around 1646 cm^{-1}, which was used to evaluate the concentration of olefinic bonds, is actually related to C=C bonds in terminal position (Beck et al., 1965). It is possible that the thermal-ageing caused a depolymerisation of the amber structure with formation of terminal unsaturated carbon-carbon bonds in position 14-15, while the photo-ageing, which already appeared a more intense oxidative process as shown by ATR-FTIR spectroscopy results, drove the mechanism to a further step, represented by the breakdown of the same bonds due to a reaction with oxygen (figure 4.6). Oxygen was likely also involved in the depolymerisation mechanism, since pure breakdown processes occur only in an inert atmosphere (Feller, 1994; Rabek, 1996; Rabek, 2007). Such a condition was theoretically present during this experiment, but in fact it was absent because of the supposed lack of air-tightness of the flasks.

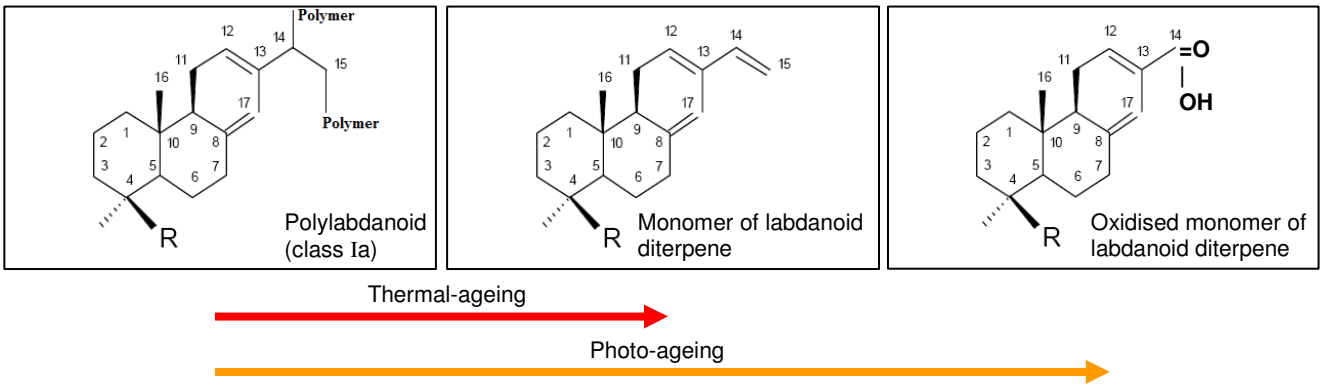


Figure 4.6. Different steps in the degradation process due to thermal-ageing (depolymerisation) and photo-ageing (depolymerisation and oxidation).

Regarding the comparison between FT-Raman and ATR-FTIR spectroscopies results, the detected level of degradation by the two techniques on the same samples appeared different. This may be because FT-Raman spectroscopy analysed samples at a depth of the order of few millimetres, while ATR-FTIR spectroscopy examined surfaces at a depth of the order of few micrometres.
In sum, it was thought that the detection of C=O groups and C=C groups depended on a combination of factors related to the type of ageing and to the applied analytical technique. This hypothesis is illustrated in figure 4.7.

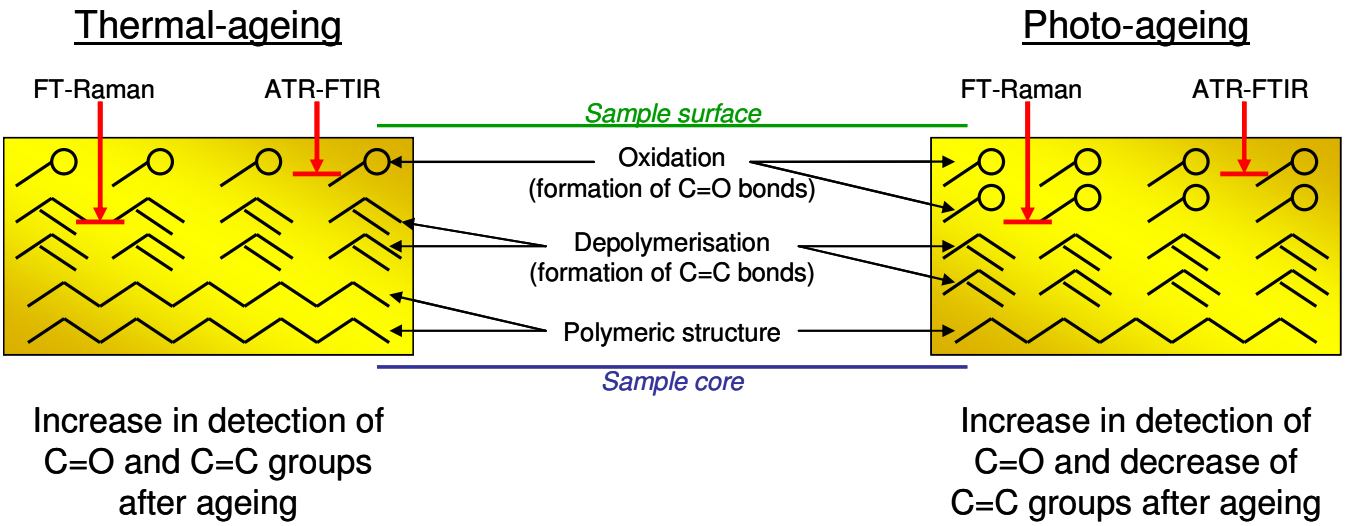


Figure 4.7. Correlation between the analytical results obtained by spectroscopic techniques and the type of accelerated ageing.

It was also thought that other chemical processes involved in the formation of new C=C bonds were the isomerisation and the aromatisation of cyclic regions in the terpenoid components of amber (Anderson, 2001; Matuszewska et al., 2001), but it was not possible to obtain any evidence about the possibility of these phenomena by the analytical techniques which were employed during this experiment.
About a possible correlation between formation of new C=C and colour change, the comparison between data from FT-Raman analyses and colour measurements did not show a clear association, so new studies were necessary.

4.1.5 Oxygen measurement by optical respirometry.
Among the employed analytical techniques, only the oxygen measurements, based on the use of optical oxygen sensor-spots, did not give reliable results, due to two technical problems:

- flasks were not perfectly airtight, so that a continuous exchange of oxygen with the external atmosphere occurred during the artificial ageing. This conclusion was achieved after the observation of the measurements related to samples aged in condition 4 (hypoxic atmosphere), which, in contrast with the expectations, always showed high concentrations of oxygen during ageing. Consequently, it was necessary to find a solution to make the flasks completely airtight during further experiments;
- many optical oxygen sensor-spots were detached during the artificial ageing, making the following analyses not possible. Since Paraloid B72 appeared unsuitable as adhesive for the sensor-spots, especially in acidic and alkaline pH conditions, it was necessary to identify a more appropriate glue to use for further investigations.

4.1.6 SPME-GC-MS headspace analysis.
Volatile compounds in the headspace of amber powder samples were adsorbed and analysed before and after the artificial accelerated ageing procedures.
It was possible to detect a considerable release of formic and acetic acid vapours from all the amber samples (figure 4.8).

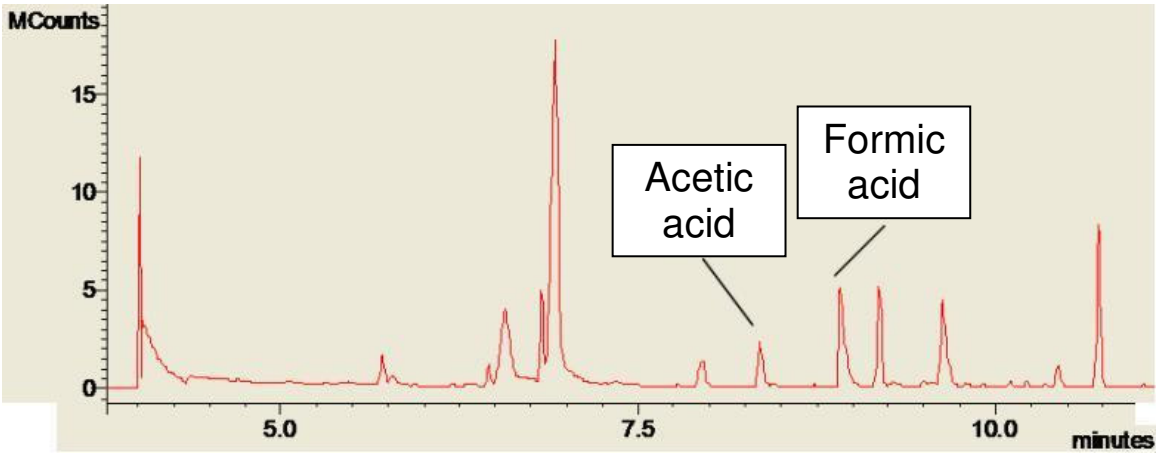


Figure 4.8. Example of chromatogram obtained by GC-MS analysis of volatiles released by Baltic amber. Presence of acetic and formic acids was detected in all the aged samples; the other peaks are related to previously studied volatile compounds (Mosini et al., 1980; Mills et al., 1984), including aromatic hydrocarbons (cymenes) and monoterpenoids (borneol, camphor, fenchyl alcohol and fenchone).

It is important to note that this analysis was only qualitative and not quantitative, since a quantification method requires a long calibration procedure carried out with the use of standards and this procedure was not performed during the present work.
Regarding the unaged powder samples, the mean detected responses (in counts) of formic acid and acetic acid were respectively 23340 and 12300.
About the thermal-aged samples, results are showed in figure 4.9. Concerning the photo-aged samples, results were incomplete due to difficulties collecting the powder aged in humid microclimates, therefore they are not showed.

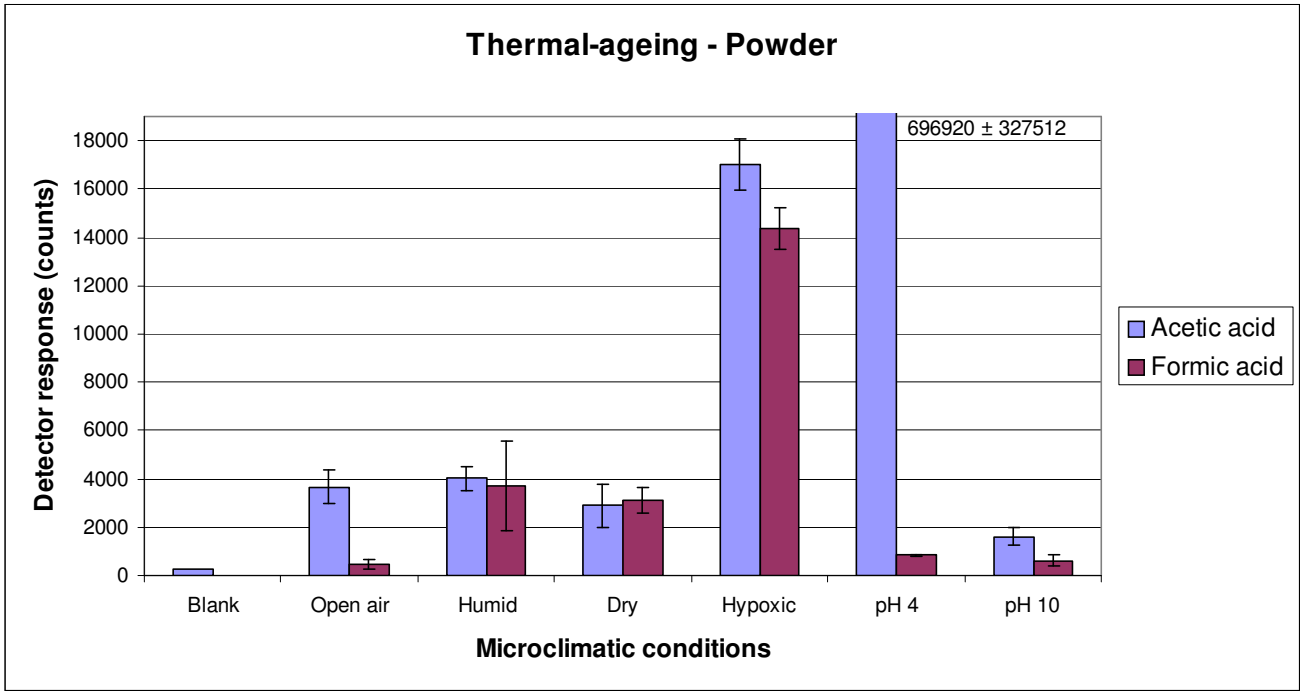


Figure 4.9. Detection of acetic and formic acid vapours in the headspace of thermal-aged powder samples.

A possible correlation between the intensity of off-gassing and the environmental conditions was not clear. Results related to condition 4 (hypoxic atmosphere) were out of the average, but they were not considered reliable because of the confirmed lack of air-tightness of the flasks. The intense detection of acetic acid vapours in samples aged in condition 5 was due to the use of acetic acid to create acidic microclimate.

The detection of acidic vapours was a new important finding for Baltic amber. Additional experiments, based on the Oddy test's principle, were carried out in order to verify the actual release of those acidic gasses.
The Oddy test is habitually used in many museums to evaluate the suitability of materials, which may release corrosive gasses, for use in the display or storage of objects containing certain metals (Oddy, 1973; Oddy, 1975).
During this test, 3 amber prisms and 3 amber powder samples (1 g each) were selected; each sample was placed inside a wide neck 100 mL Bibby-Sterilin Pyrex glass flask with polypropylene cap and silicon gasket (baked out at 100 $^{\circ}$C for 4 days before use to remove impurities), together with a coupon of cleaned lead and a small glass flask containing some water to create high humidity and to accelerate the possible corrosion (amber, lead and glass container were not in contact, as shown in figure 4.10). Samples were then subjected to accelerated thermal-ageing using the same oven and operative settings described in paragraph 3.3. After ageing, the extent of corrosion on the metal coupons was visible as white lead, especially on the ones aged in presence of amber powder (figure 4.11). This was an evidence of acidic vapours off-gassing.
The identification of white lead was performed both by a qualitative spot test (adding solutions of HNO_3 4 M and KI 0.1 M; Odegaard et al., 2000) and by ATR-FTIR, giving basic lead carbonate as result. A reference coupon aged with pure acetic acid produced lead acetate.

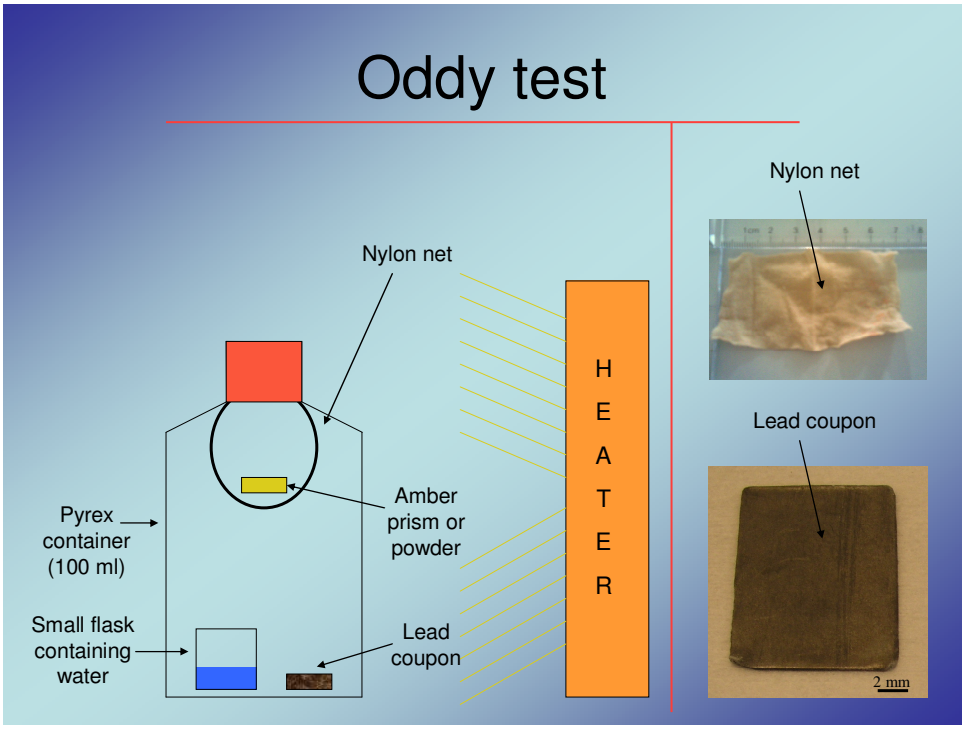


Figure 4.10. Experimental storage used for the Oddy test on lead in presence of Baltic amber.

Figure 4.11. Production of basic lead carbonate on lead coupons after Oddy test in presence of Baltic amber.

The release of formic and acetic acids by amber was likely the result of radical reactions, which involved cleavage of C=C bonds in terminal position, due to the oxidative processes discussed with the results from the spectroscopic analyses (figure 4.12). Such a process, which can be classified as decomposition (Mills, 1966), requires the action of some initiators, which in the composition of Baltic amber are represented by free radicals (Urbański, 1971). This idea was suggested by the improbability to obtain the mentioned acids through other ways, such as hydrolytic processes, since there are not formic and acetic esters in the composition of Baltic amber (Beck et al., 1965; Mills et al., 1984; Anderson et al., 1992). This topic was considered for further investigations.

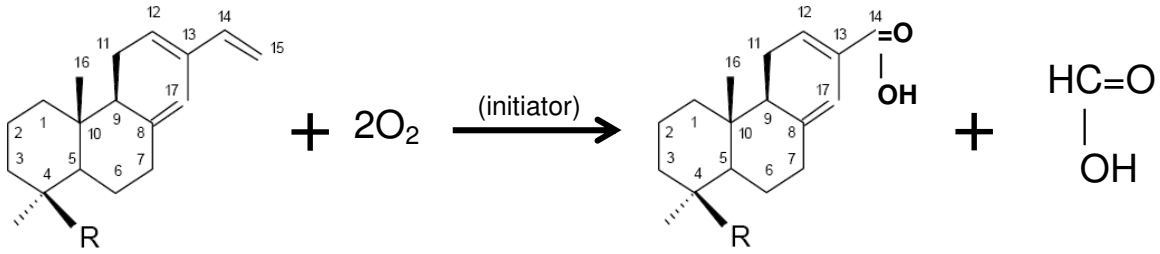


Figure 4.12. Hypothesis of decomposition that produced formic acid through the cleavage of C=C terminal bonds due to oxidation.

4.1.7 C-H-N analysis.

The elemental analysis, to detect the variations of the content of carbon, hydrogen and nitrogen in the amber material during the artificial ageing, was performed on raw and archaeological material. Results are showed in figure 4.13.

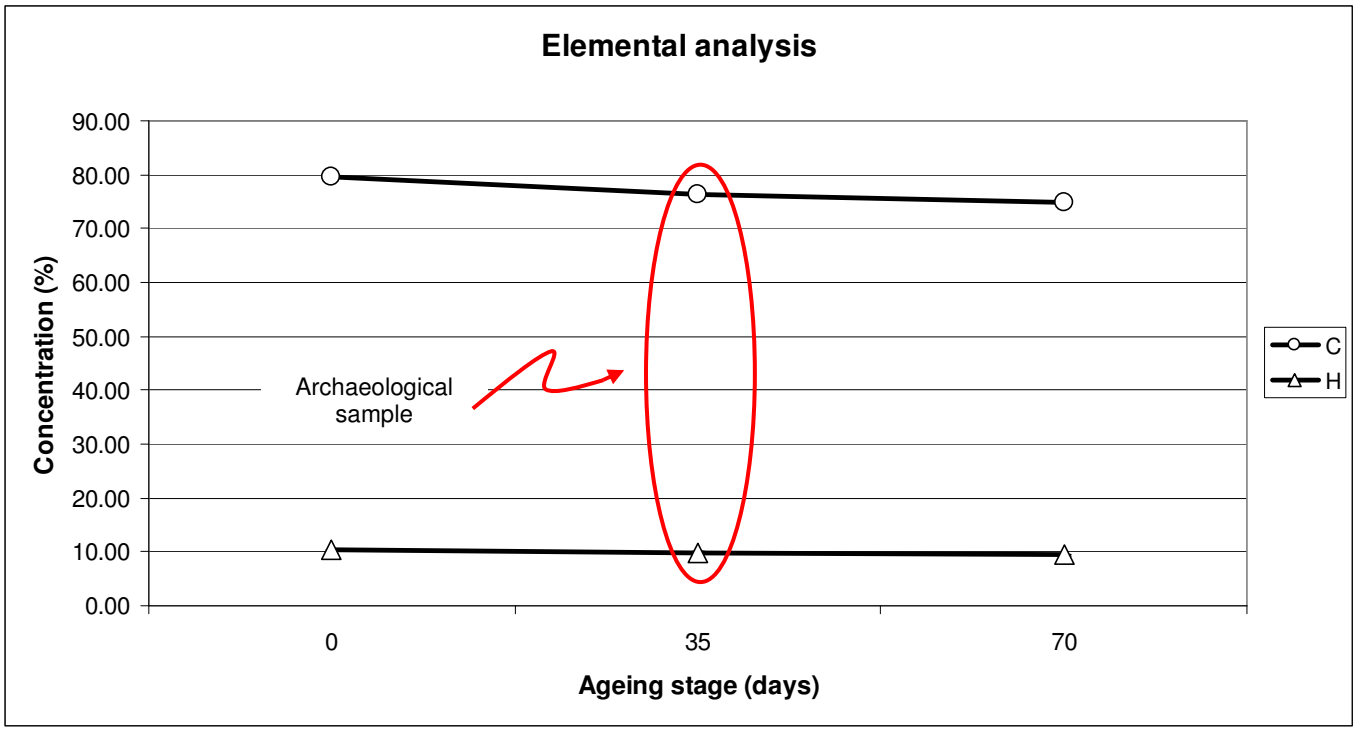


Figure 4.13. Variations of the concentration of carbon and hydrogen (content of nitrogen was irrelevant) in raw Baltic amber samples during ageing. Results related to an archaeological fragment were comparable to the values contained in the red oval.

During ageing of amber samples, a significant decrease of carbon concentration and a substantial stability of hydrogen concentration were observed.
The detected concentrations matched with previously established information (Ross, 1998).
The results related to the analysis of a fragment from a 2-3 thousands of years old archaeological sample showed an elemental composition comparable to the one obtained by a moderately short artificial ageing period (35 days), suggesting a fair physico-chemical stability of the buried condition.

4.1.8 Micro-ATR-FTIR cross sections analysis.
The analysis of cross sections obtained from amber prisms was performed in order to get information about the development of oxidation in relation to the distance from the surface. Results are showed in figure 4.14.

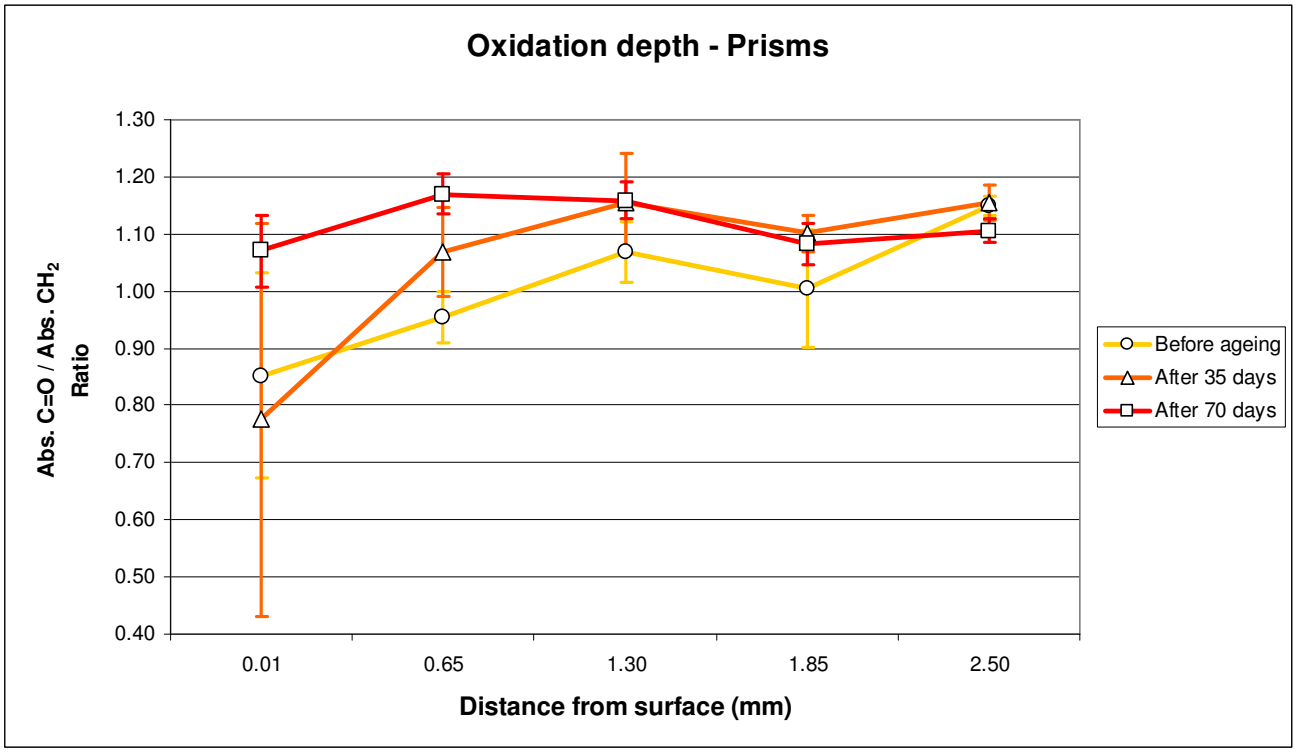


Figure 4.14. Development of the oxidation in Baltic amber prisms cross sections at different levels of ageing.

The detected levels of oxidation showed, especially on the surfaces of the amber samples, too low repeatability (as remarked by the error bars in the graph) to reach consistent conclusions. In any case, this lack of repeatability seemed to reduce with distance from the surface, showing similar oxidation stages in the internal part of the samples independently on the ageing periods.

This might confirm that oxidation of amber occurs initially only at the surface, since oxygen is slow to diffuse through the dense material (Shashoua et al., 2005), but further investigations were necessary to provide evidence.

4.1.9 Confocal profilometry.
Non-contact topographic analyses were performed on the surfaces of amber prisms, in order to obtain information about possible changes in the roughness due to the ageing. Results are showed in figure 4.15.

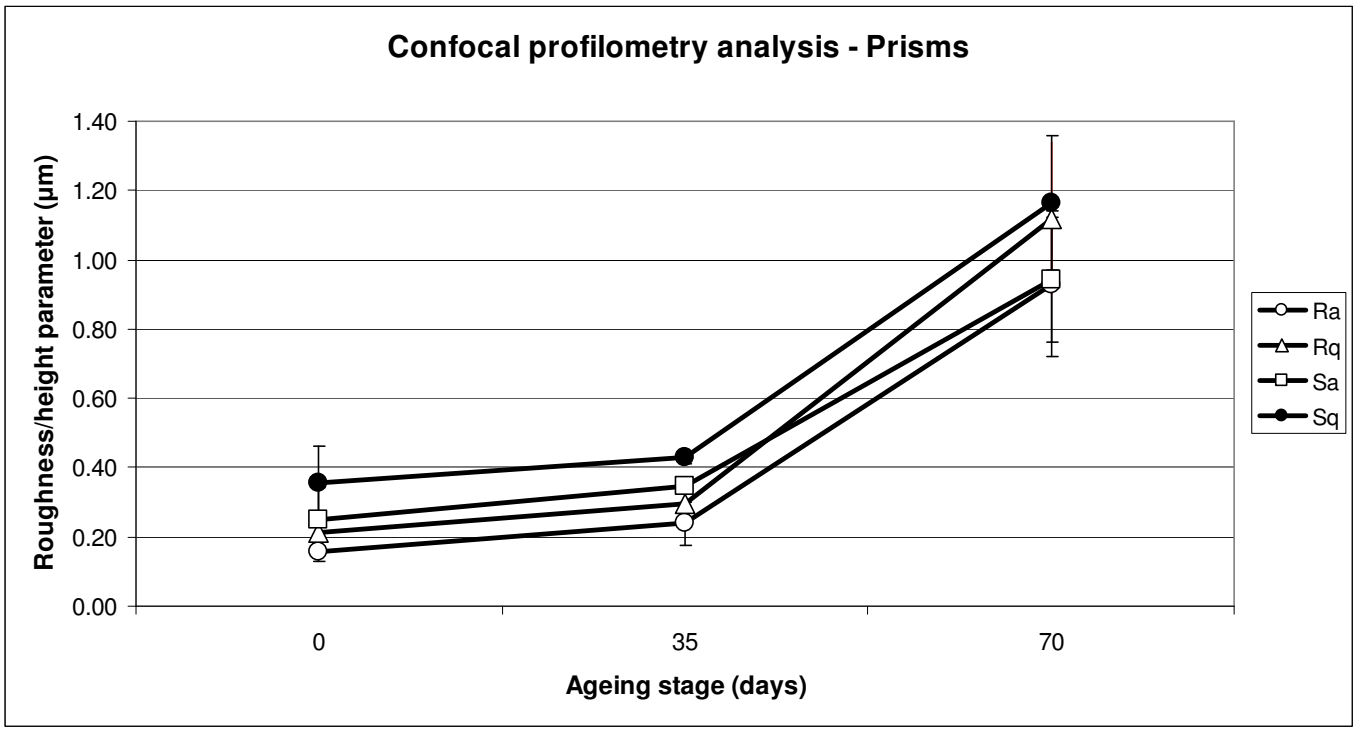


Figure 4.15. Changes in roughness and height parameters on Baltic amber prisms surfaces during ageing.

During ageing of amber samples, a significant increase of all the standard parameters used to evaluate roughness was observed.
It is supposable that the increasing superficial roughness was due to the breaking of structural bonds involved in the depolymerisation of the material, which caused the fragmentation of the surface (surface brittleness, crazing and powdering; Thickett et al., 1995; King, 2006).

4.2 Preliminary assessments.

From the interpretation of the achieved data it was possible to identify a few relations between amber and environmental factors during the degradation process. In sum, a number of assessments were drawn and they are listed in table 4.4.

Table 4.4. Preliminary assessments in relation of different aspects after the initial experimental phase

Aspect	Assessment
Ageing	Photo-ageing resulted in considerably more degradation than thermal-ageing. This photo-degrading activity was pointed out by the intense detection of photo-oxidation effects, i.e. the increase in concentration of C=O groups, the intense slope of the Baltic shoulder and the complete loss of the band at 888 ± 1 cm^{-1}. Consequently light was excluded from further investigations, because it was evidently involved in the degradation of amber
Size	Powder appeared more sensitive to degradation than prisms, as shown by the chemical changes in thermal-aged samples, i.e. increase in concentration of C=C and C=O groups. Thus, it was clearly visible that the surface area to volume ratio had a role in the rate of degradation
Microclimatic condition	Low relative humidity seemed to intensify degradation of amber, as showed by the considerable change in colour of powder samples aged in dry conditions
	Acidic and alkaline pH environments generated the most intense visible changes, such as deterioration of prisms surfaces and cohesion of powder particles. Further more, regarding the formation of C=O groups, alkaline condition results appeared in contrast with the results obtained from other conditions, showing a decrease in concentration of carbonyl groups. This could mean that the alkaline environment caused a different pathway of degradation in the material, therefore more studies were necessary
Degradation pathway	Depolymerisation processes were detected by FT-Raman spectroscopy as formation of C=C terminal bonds, especially in thermal-aged samples. Since depolymerisation was likely based on thermal-oxidative reactions, heat and oxygen were believed to be significant degradation agents
	As oxidative processes were detected by different techniques (ATR-FTIR spectroscopy as formation of C=O groups, FT-Raman spectroscopy as breakdown of C=C groups after photo-ageing, GC-MS as production of volatile organic acids) and in most of the ageing conditions, oxygen appeared to be one of the main degradation agents

Due to the unsettled points risen during this preliminary investigation (possibility of hydrolytic processes, lack of oxygen measurements, doubts about the formation of acidic gasses, oxidation as surface phenomenon), it was decided to select new experimental conditions to apply for a second ageing experiment, with the purpose to identify the mechanisms by which amber degrades.

5. ADVANCED INVESTIGATION - MATERIALS AND METHODS.

5.1 Experiment design.

To design the advanced investigation, a few issues that emerged during the preliminary investigation were considered. In details:

- visual examination, spectrocolorimetry, ATR-FTIR and FT-Raman analyses of amber prisms showed, in general, less repeatability in the results compared to the powder; the reason was due to the differences, even if small, from prism to prism and also from different areas on the same prism;
- free powder appeared unsuitable for some analyses, such as spectrocolorimetry and FT-Raman spectroscopy, because of the high radiation-scattering caused by the particles; to perform the planned analyses, powder samples had to be transformed in pellets during the different ageing steps;
- analytical results related to the two forms of samples gave comparative problems that made the achievement of common conclusions very difficult.

After these considerations, some pilot tests were performed to decide whether it was appropriate to work directly with pellets obtained from pressed amber powder, instead of prisms and free powder, during the setting-up of the second artificial ageing and the following progress of analyses.

The pilot tests were performed following this procedure:

- nine unaged prisms, 9 small quantities (around 2 mg) of unaged powder and 9 unaged pellets were analysed by ATR-FTIR spectroscopy using the same instrument, operative settings and procedure described in paragraph 3.4. The same guidelines used for the prisms were applied also to the pellets;
- all the samples (prisms, pellets and around 1 g of unaged powder) were placed in open containers (glass Petri dishes) and subjected to accelerated thermal-ageing using the same oven and operative settings described in paragraph 3.3;
- at the end of the ageing all the samples were reanalysed by ATR-FTIR spectroscopy;
- for each type of samples, the absorbance values of 5 different bands were collected and averaged; then, the related standard errors were compared to have an idea about the repeatability of the method on the different types of samples, as showed in figure 5.1.

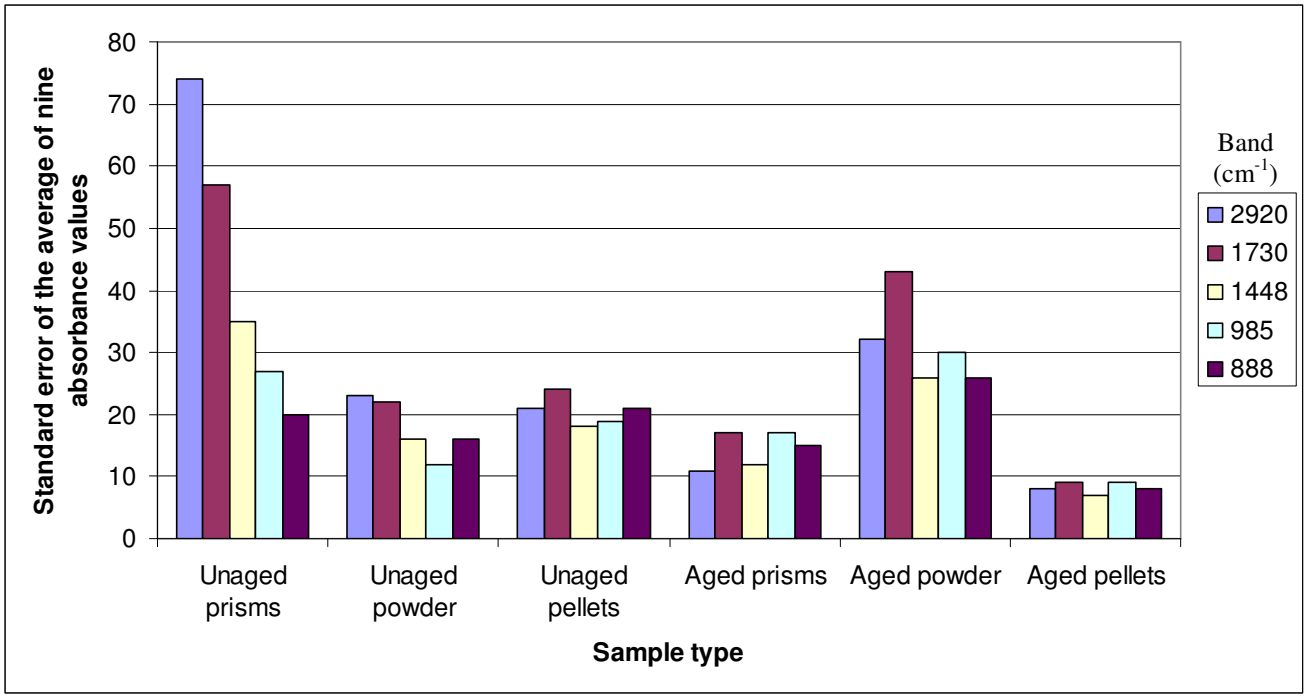


Figure 5.1. Comparison between the standard errors related to repeated ATR-FTIR measurements on different kinds of amber samples.

Analysing the results illustrated in figure 5.1, it is possible to state that among the unaged objects, prisms showed the lowest repeatability (highest standard errors) compared to powder and pellets which gave a similar and fairly high repeatability; among the aged objects, powder showed the lowest repeatability compared to prisms and, especially, pellets which showed a very high repeatability. Consequently, pellets were the only kind of sample that gave high repeatability in both unaged and aged conditions.

To take a final decision, it was necessary to investigate a further aspect: the comparability between the analytical results from the pellets and the results from prisms and powder samples, in order to ensure the continuity between the preliminary and the advanced experimental phases.
To check this factor, for each kind of samples the averaged absorbance value related to the band at 1735 - 1700 cm^{-1} was ratioed against the value related to the band at 1450 ± 20 cm^{-1}, as already made to evaluate the oxidation of the samples during the preliminary investigation.

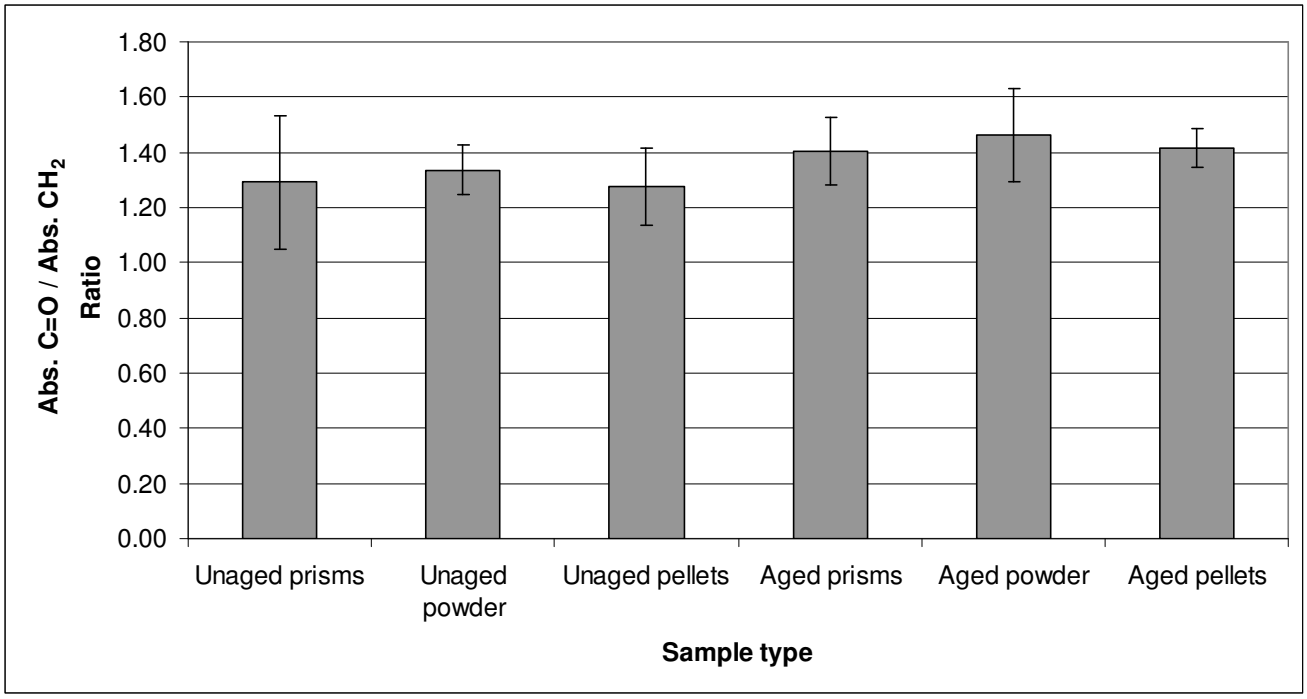


Figure 5.2. Comparison between the oxidation trends related to different kinds of amber samples.

Analysing the outcomes showed in figure 5.2, it is possible to state that the results used to evaluate the oxidation of amber pellets are comparable with the results related to prisms and powder. Consequently, the connection between the preliminary and the advanced experimental phase is confirmed.

After the excellent outcomes in terms of analytical repeatability and comparability with the previous data, amber pellets were finally selected as sample form for the advanced investigation.
It is useful to summarize the numerous advantages related to this new method:

- *greater repeatability on spectrometric analyses* – pellets resulted in much more homogeneous results than prisms (which needed a group of 3 averaged analyses each) because they come from a homogeneous system that is powder, so that one analysis on each sample was enough; moreover, the surface of the pellets is extremely flat, so there was no problem related to imperfections that created poor contact with the ATR accessory affecting the results; in addition, working with only one size gave less comparative problems than with two. All these advantages allowed prolonging the time of each analysis in order to get an optimal signal to noise ratio and, as a result, more resolved spectra;
- *greater suitability* – pellets appeared appropriate for all the employed analytical techniques (except SPME-GC-MS, as it will be discussed later on), no further adaptation of the samples was necessary;
- *complementary information* – pressed pellets are objects with qualities between the ones of prisms and powder. These two sizes were already examined during the preliminary investigation, while the pellet size could give new information, which were anyhow related to the previous results. Therefore the advanced investigation was not a repetition of the previous one, but an extension;

- *faster and less wasteful preparation of samples* – the preparation phase consisted only of powdering and pressing, no cutting. Cutting was actually the most time consuming and wasteful process.

Amber pellets were used for all the employed analytical techniques except SPME-GC-MS, since for this method it was necessary to work with a much smaller amount of material (between 10 and 30 mg).
Tests were carried out to identify the most appropriate form of amber samples to use for this kind of analysis. It was decided to compare the outcomes related to three different shapes:

- powder (10 mg), that was already employed during the preliminary investigation;
- fragments (approximately 10 mg) obtained through the use of a scalpel;
- micro-slices (approximately 9 x 5 x 1 mm) obtained from a few amber prisms using a Buehler Isomet low speed electrical saw, cooled with a 3% water solution of Struers Additive for Cooling Fluid.

Nine samples (three samples belonging to each one of the three categories) were analysed through SPME-GC-MS, using the same instrument, operative settings and method described in paragraph 3.4. The averaged results showed that the most considerable response related to released acidic vapours was detected in the powder samples (figure 5.3).

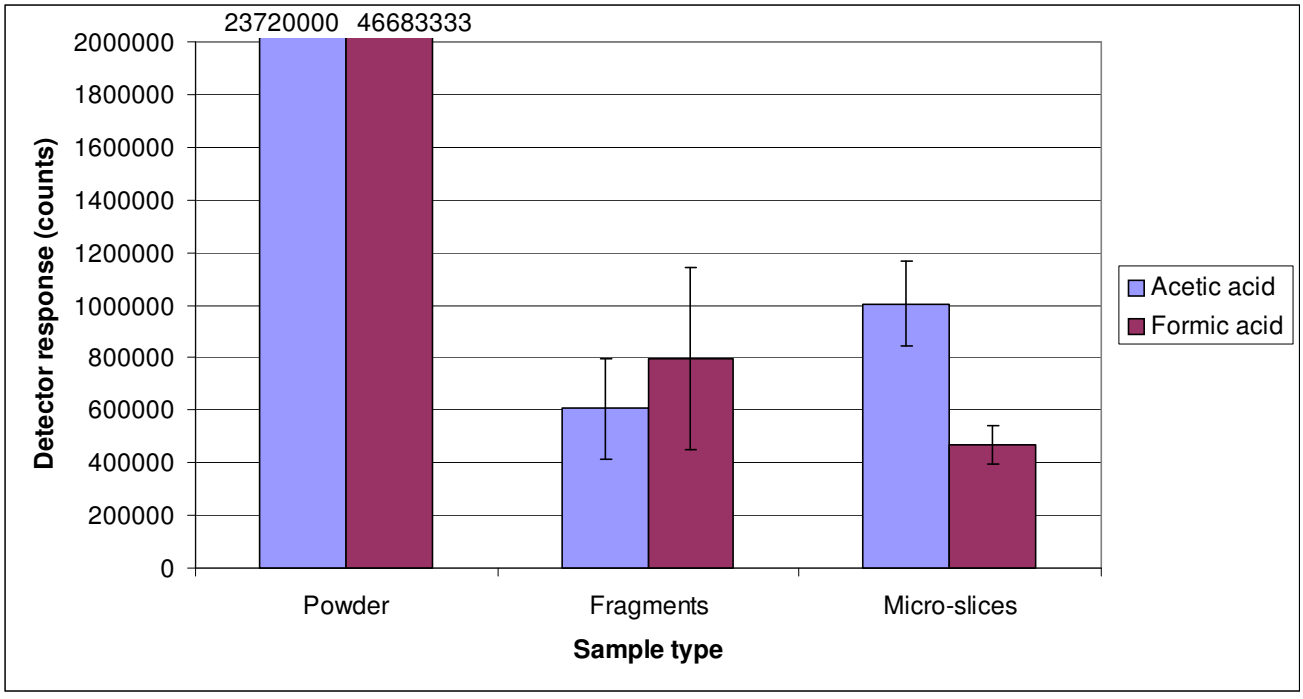


Figure 5.3. Relative responses related to acetic acid and formic acid vapours detected by SPME-GC-MS analysis from different kinds of amber samples.

Despite the result, the powder shape was discarded, since in many of the storage conditions employed during the ageing (the ones where the samples were immersed in a liquid environment, as it will be illustrated in the following paragraph) it would have been complicated to collect the powder for the analyses.
Fragments and micro-slices gave similar results in terms of detection of volatile compounds (not in terms of relative responses, but this was not considered a problem since the analytical purpose was qualitative and not quantitative) and, in the end, it was decided to use the micro-slices, because of the more precise way of production and the higher surface potentially exposed to the degradation agents.

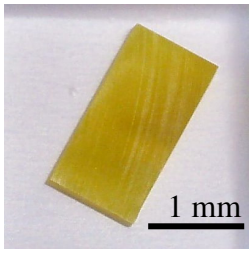


Figure 5.4. Amber micro-slice.

5.2 Preparation, ageing and examination of amber samples.

Seventy amber pellets were prepared using the same hydraulic press and procedure described in paragraph 3.4, and 70 amber micro-slices were prepared using the same electrical saw and procedure described in paragraph 5.1.
Samples were placed inside wide neck 100 mL Bibby-Sterilin Pyrex glass flasks with polypropylene cap and silicon gasket (baked out at 100 °C for 4 days before use to remove impurities). Samples were then subjected to accelerated thermal-ageing using the same oven and operative settings described in paragraph 3.3.
During this advanced investigation, the relative humidity inside the flasks was constantly checked through the use of Indicator Strips 7 Spots from the company Long Life for Art.

For this accelerated ageing procedure, samples were exposed to 11 different microclimates (table 5.1), relevant to burial, storage, use and display conditions, in order to clarify the role of different environmental parameters (relative humidity, presence of oxygen, pH) on the degradation of amber. For each microclimatic condition, three samples were exposed.
Two types of storage were required (see details in figures 5.5 a-b), depending on the placing of the samples: exposed to the atmosphere or immersed in a liquid. All materials used were selected for their high physical-chemical stability.

Table 5.1. Microclimatic conditions used for the accelerated thermal-ageing of amber samples

	Microclimatic condition	How achieved
1	≤ 20% RH, sample exposed to external atmosphere (open air)	Using open containers
2	100% RH, sample exposed to internal atmosphere, pH ≤ 5.5	Placing deionised water inside closed containers
3	100% RH, sample immersed in liquid, pH ≤ 5.5	Placing deionised water inside closed containers
4	≤ 20% RH, sample exposed to internal atmosphere	Placing silica gel inside closed containers
5	≤ 20% RH, sample exposed to internal anoxic atmosphere	Placing silica gel and an oxygen absorber (Ageless Z[a]) inside closed containers
6	100% RH, sample exposed to internal atmosphere, pH 3	Placing buffer pH 3[b] inside closed containers
7	100% RH, sample immersed in liquid, pH 3	Placing buffer pH 3 inside closed containers
8	100% RH, sample exposed to internal atmosphere, pH 5	Placing buffer pH 5[c] inside closed containers
9	100% RH, sample immersed in liquid, pH 5	Placing buffer pH 5 inside closed containers
10	100% RH, sample exposed to internal atmosphere, pH 10	Placing buffer pH 10[d] inside closed containers
11	100% RH, sample immersed in liquid, pH 10	Placing buffer pH 10 inside closed containers

[a]Grattan et al., 1994.
[b]Commercial solution of citric acid, NaOH and HCl from Merck.
[c]Commercial solution of citric acid and NaOH from Merck.
[d]Commercial solution of boric acid, KCl and NaOH from Merck.

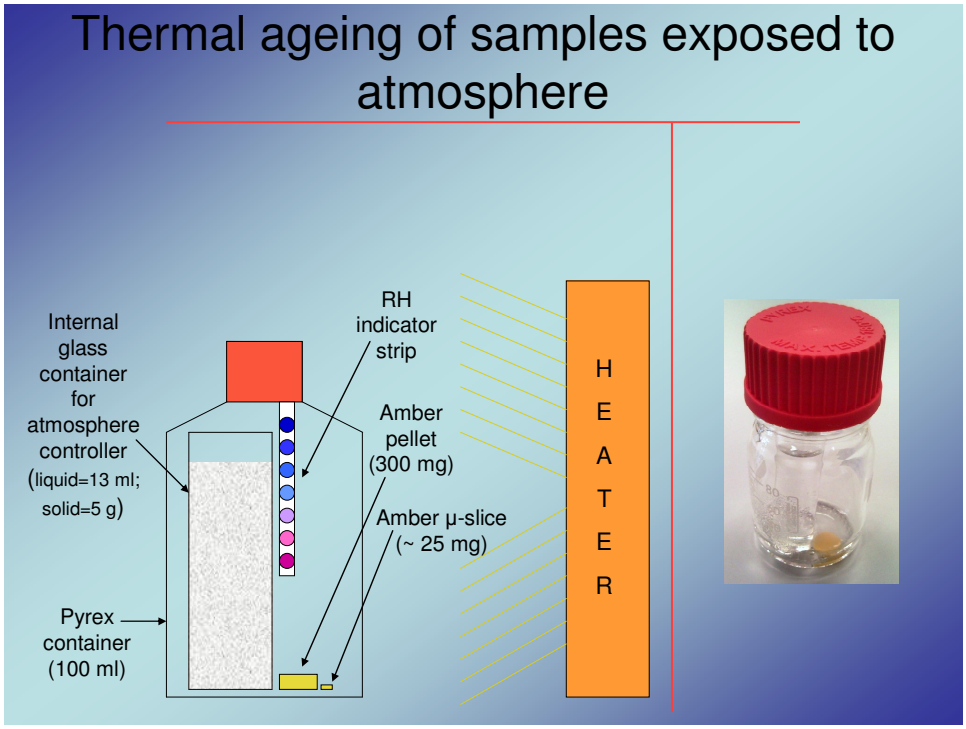


Figure 5.5.a. Experimental storage used for thermal ageing of amber samples exposed to the atmosphere.

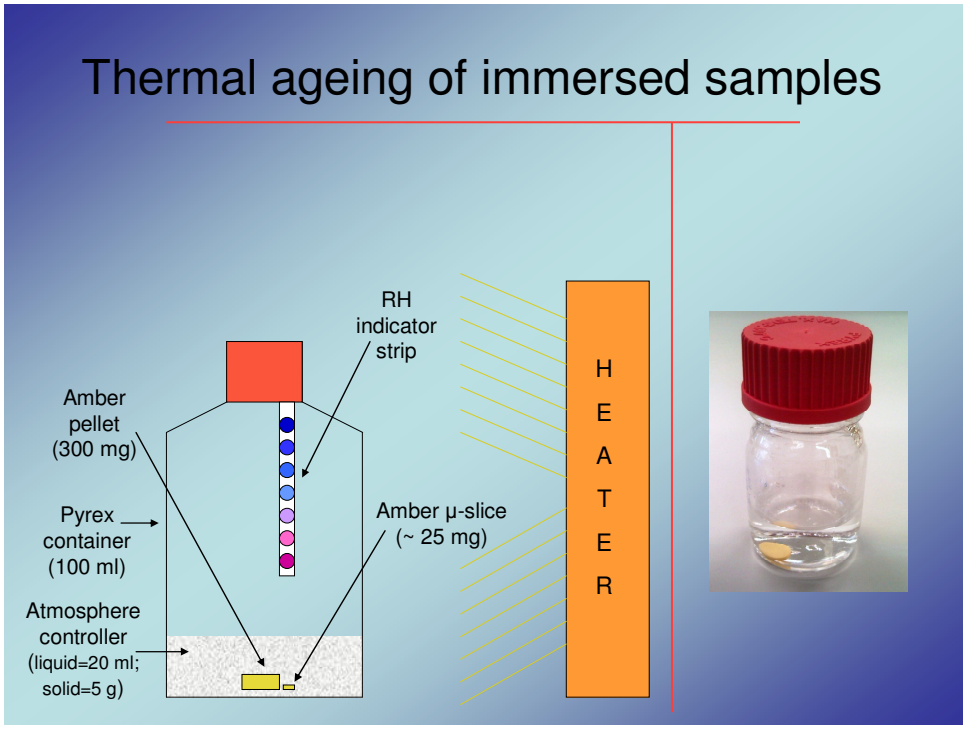


Figure 5.5.b. Experimental storage used for thermal ageing of amber samples immersed in the atmosphere controller.

Samples were analysed regularly according to the same techniques and purposes that were described concerning the preliminary investigation: visual examination, CIE L*a*b* spectrocolorimetry, ATR-FTIR spectroscopy, FT-Raman spectroscopy, optical respirometry and SPME-GC-MS headspace analysis.
To ensure the analysis of the same surfaces of the pellets every time and with all the analytical techniques, the edge of each pellet was marked by a permanent pen in order to indicate the surface to analyse.
Surfaces of pellets were washed with deionised water before each analytical stage, in order to remove possible residues that could affect the reliability of the results.

For all the techniques, the same instruments, operative settings and procedures used for the analyses of amber powder (or amber pellets where it was necessary) during the preliminary investigation, were also used during the advanced investigation, with only two exceptions regarding ATR-FTIR spectroscopy and optical respirometry:

- ATR-FTIR spectroscopy – to improve the quality of the results, two solutions were applied:
  - ✓ the employed number of scans was 64 instead of 4, in order to obtain more resolved spectra;
  - ✓ as additional parameter to quantify levels of degradation of the amber samples during the accelerated ageing, the splitting and the consequent shift from 1730 cm^{-1} to 1715 cm^{-1} of the infrared peak related to the C=O groups was used to evaluate the hydrolysis of esters into acids.
- Optical respirometry – to get reliable results, three solutions were used to fix the problems risen during the preliminary investigation:
  - ✓ Pyrex flasks were made perfectly airtight placing a disc of laminated aluminium foil from Preservation Equipment Ltd, which is normally used for production of airtight bags, between the screw top and the neck of each flask (figure 5.6). Blank tests were performed on three closed flasks filled with atmospheric air (to check any possible consumption of oxygen by the foil) and on three flushed with nitrogen (to check any gases exchange with the external atmosphere); the flasks were subjected to thermal treatment using the same oven and operative settings described in paragraph 3.3, for one week. The results obtained from these tests confirmed the suitability of the method (figure 5.7);
  - ✓ to avoid detachments of optical oxygen sensor-spots, more resistant glue (cellulose nitrate) was employed;
  - ✓ two control samples (one closed Pyrex flask filled with atmospheric air and one flushed with nitrogen) were used during the accelerated ageing, in order to monitor the stability of the system (no consumption of oxygen by the experimental material and no gases exchange with the external atmosphere).

Figure 5.6. Laminated aluminium foil inserted in the screw top of a Pyrex flask.

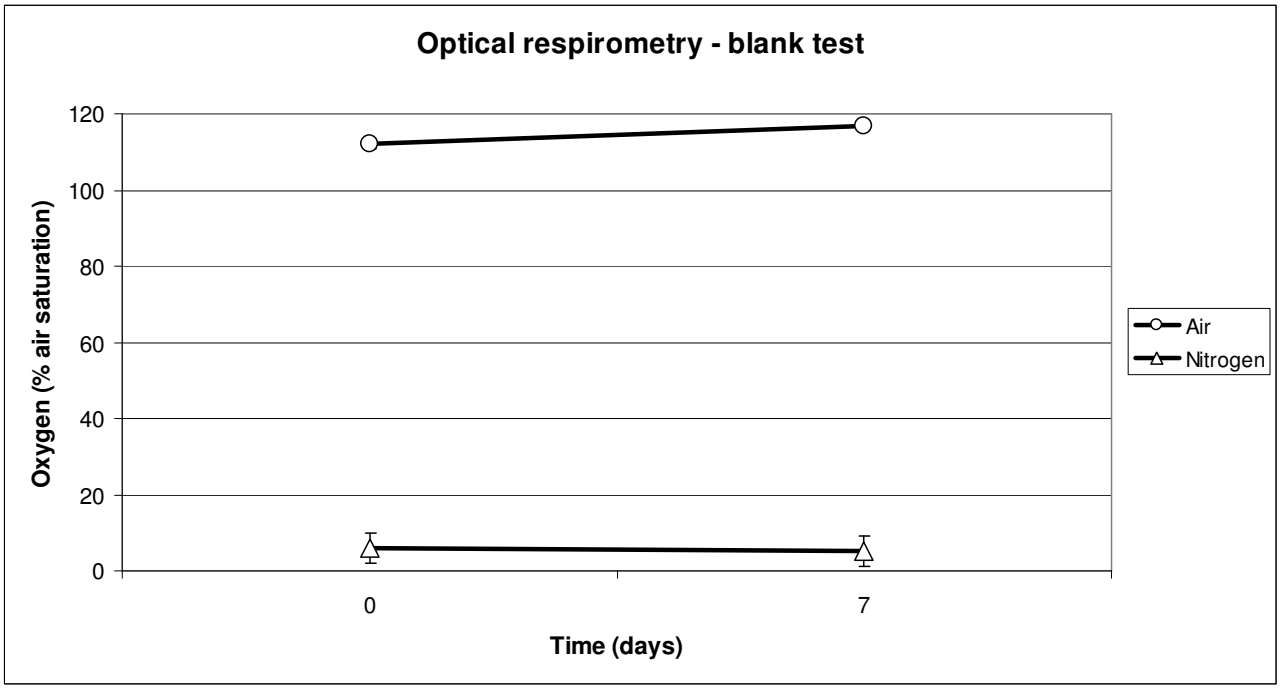


Figure 5.7. Oxygen concentration trend in airtight Pyrex flasks filled with atmospheric air and nitrogen during thermal treatment test. Data related to the flasks filled with atmospheric air showed that there was no consumption of oxygen taking place (the slight increase of the oxygen concentration could be due to either a small difference in temperature or a difference in the air pressure). Data related to the flasks flushed with nitrogen indicated that the system was effectively tight.

Regarding the frequency of the analyses, all the measurements were performed before the ageing, after 11 days of ageing, after 23 days of ageing and after 35 days of ageing, but not all the samples were analysed every time. This is illustrated in figure 5.8.

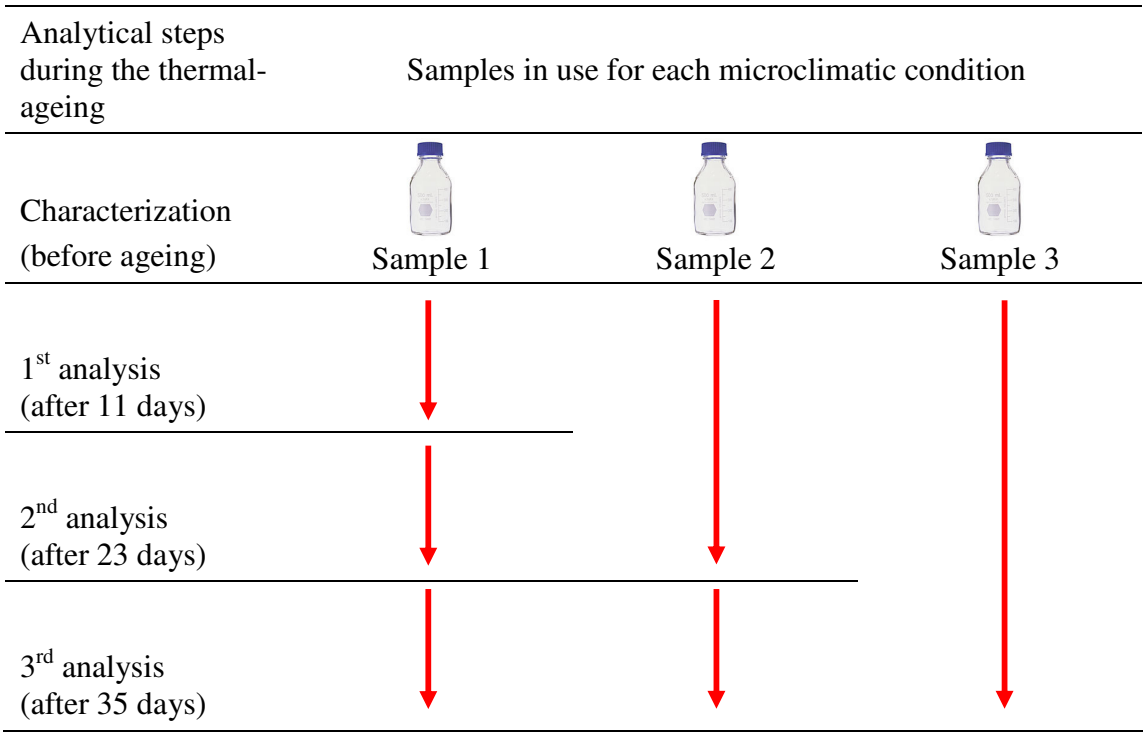


Figure 5.8. Analyses planning during the accelerated thermal-ageing of amber samples.

The purpose to keep some of the samples in the oven during the analytical steps was to check if the interruption of the ageing for one or two days could affect the degradation process.

To summarize the design of the advanced investigation, most of the information previously described are illustrated in table 5.2.

Table 5.2. Ageing experiment design. Unless it is indicated otherwise, each container held one sample. The different analytical groups are defined according to table 3.6 in paragraph 3.4

Ageing	Size	Microclimatic condition 1	2	3	…	10	11	Analytical group
Thermal-ageing	Pellets + micro-slices	3 pellets + 3 micro-slices						A, B, D
	Pellets							C

Open Pyrex flask.

Closed Pyrex flask.

5.3 Additional analyses.

Also during the advanced investigation it was decided to run a few additional analyses:

- *Micro-ATR-FTIR cross sections analysis* – analysis of cross sections obtained from amber pellets, in order to get information about the development of degradation in relation to the distance from the surfaces. The purpose was to confirm degradation as a surface phenomenon;
- *Confocal profilometry* – non-contact topographic analysis of the surface of amber pellets in order to obtain information about any potential changes in the roughness due to the ageing;
- *ATR-FTIR analysis of archaeological material* – analysis performed on a few deaccessioned archaeological Baltic amber objects (Neolithic beads found in Skanderborg -Denmark-, dated 3-2 thousands of years BCE) in order to compare their degradation state with the data obtained from the experiments on the raw material.

In all the cases the experimental design was different from the one described in the previous paragraphs and consisted of the following procedures.

5.3.1 Micro-ATR-FTIR cross sections analysis and Confocal profilometry.

These analyses were performed following exactly the same procedures described in paragraph 3.6, with the only difference that pellets were used instead of prisms.
Regarding the frequency of the analyses, measurements were performed before the ageing, after 35 days of ageing and after 70 days of ageing, following the analyses planning illustrated in figure 3.19 (paragraph 3.6).

5.3.2 ATR-FTIR analysis of archaeological material.

- *sampling* – three small fragments (around 2 mg each) of each amber object were removed by the use of a scalpel from the external surface. The same sampling procedure was repeated on the internal part of each object;
- *analysis* – ATR-FTIR spectra were collected using a Perkin Elmer Spectrum One FTIR spectrometer (details and operative settings have already been described in paragraph 3.1). After the acquisition of an air-background, each fragment was placed on the reflection accessory and consequently analysed;
- *quantification of degradation* – to quantify levels of degradation of the amber samples, the oxidation of the molecular structure was used and it was calculated on the base of relative absorbance values of the infrared band at 1735 - 1700 cm^{-1} (related to C=O groups of esters and acids). Absorbance values at this band were ratioed against a band at 1450 ± 20 cm^{-1} (related to C-H bonds of $>CH_2$ and $-CH_3$ groups), as already made during the experiments on the raw material. Maximum heights of the two bands of interest were determined on raw absorbance spectra without manipulations or baseline corrections.

6. ADVANCED INVESTIGATION - RESULTS AND DISCUSSION.

In this chapter, the results related to the different analytical methods which were employed during the advanced investigation are presented.
During the description of the results, each microclimatic condition is cited with the respective number (according to table 5.1 in paragraph 5.2), with additional comments to remind the reader about the main characteristic.

6.1 Analysis and interpretation of achieved data.

6.1.1 Visual examination by naked eye and photography.
Amber pellets were regularly observed and photographed during the artificial accelerated ageing procedures.
Pictures of pellets are showed together with the colour measurement results in appendix A – subsection A.1, to illustrate the correlation between appearance and CIE L*a*b* parameters.

Visible changes in the appearance of the pellets were observable as colour alterations on all the aged samples. Slight colour changes occurred in condition 5, characterised by the exposure of the samples to anoxic atmosphere, suggesting a deteriorative role of the oxygen.
In condition 11, characterised by the immersion of the samples in alkaline solution, the surfaces of the pellets showed, further than a insignificant change in colour, the presence of cracks, indicating a probable loss of material. Comparing this result to the ones obtained during the preliminary investigation, it was confirmed that acidic and alkaline pH conditions could cause visible structural damages.
No physical damage, such as cracking, was observed on samples aged in dry conditions.

6.1.2 Colour measurement by CIE L*a*b* spectrocolorimetry.
Data from colour measurements of amber samples were expressed using the CIE L*a*b* colour system.
In appendix A – subsection A.1, the changes in colour components, the ΔE_{ab} index and the comparison between pre-ageing and post-ageing appearance of pellets surfaces, in all the ageing conditions, are illustrated.

Results, in all the instances, showed colour change resulting in darkening, reddening and yellowing of the samples, except for samples aged in condition 11 (alkaline environment) which only showed a slight yellowing.
The lowest colour changes, in terms of ΔE_{ab} index, occurred in conditions 5 and 11, respectively characterised by anoxic and alkaline environment (figure 6.1).

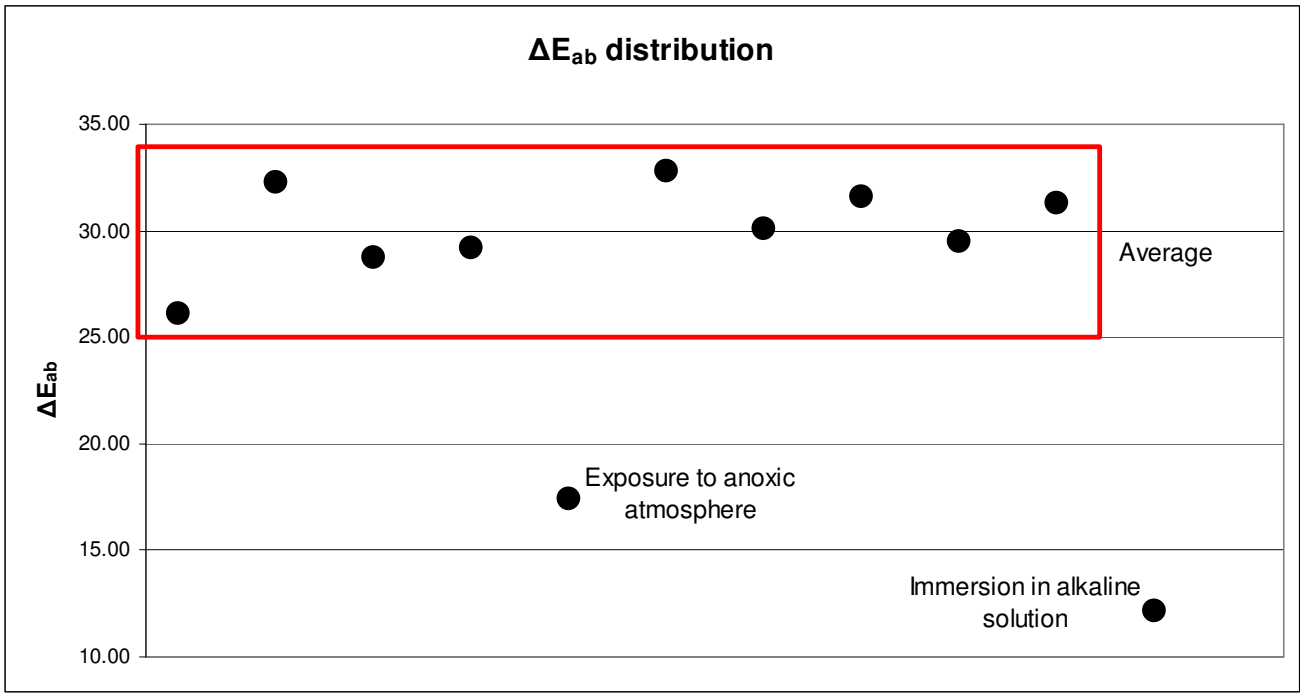


Figure 6.1. Scatter plot representing the distribution of the ΔE_{ab} values related to the different experimental conditions.

Comparing the results to the ones obtained during the preliminary investigation, the changes in colour appeared more related to factors such as presence of oxygen and pH, than relative humidity. It is important to recall the lack of data regarding anoxic conditions from the preliminary investigation.

In conclusion, the absence of oxygen appeared the most important factor to preserve the original colour of amber samples, as indicated by the CIE L*a*b* data; the immersion in alkaline solution also caused slight changes, but it was not considered as advisable preservation method, since, as shown by visual examination, it caused physical damages.

6.1.3 ATR-FTIR spectroscopy.

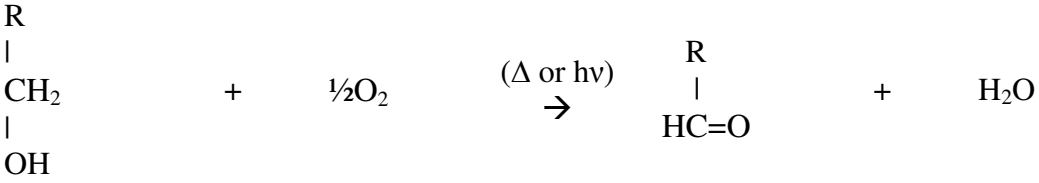
To determine degradation of amber pellets during the accelerated ageing, levels of oxidation were evaluated measuring the ratio between absorbance values of C=O (carbonyl group) and C-H infrared bands; in order to assess the hydrolysis of esters into acids, changes in the shape of the C=O infrared band were also considered.
In appendix A – subsection A.2, the carbonyl group absorbance changes in amber pellets, in all the ageing conditions, are illustrated.

In all the occurrences where the samples were exposed to atmosphere with a normal concentration of oxygen, it was possible to observe the increase in absorbance of C=O groups during ageing; on the other hand, results related to samples that were immersed in liquid environment or exposed to anoxic atmosphere did not show any variation of the same band, or the variation was extremely small.

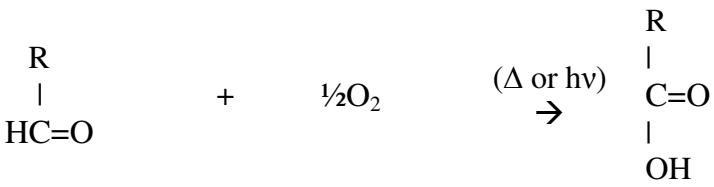
Only in condition 11, characterised by the immersion of the sample in alkaline solution, a strong decrease in absorbance of C=O groups occurred (figure 6.2).

These results, together with the ones from the preliminary investigation, confirmed the presence of oxygen as important degradation factor, since all the samples which were aged in atmosphere with normal concentration of oxygen showed a much higher increase in concentration of C=O groups than the ones aged in hypoxic (immersed in liquid, where the concentration of oxygen is lower than in the atmosphere) or anoxic (in absence of oxygen) conditions.
This outcome was a strong evidence of the progress of oxidative reactions during the ageing. A reaction of this kind could involve the terminal C=C bonds of the labdanoid diterpene monomers, as discussed in paragraph 4.1.4, or it could also cause the production of communic acid from communol; this reaction requires high temperature (thermal-oxidation) or light (photo-oxidation) and proceeds in two phases, giving communic aldehyde as intermediate product (Clayden et al., 2001):

Phase 1

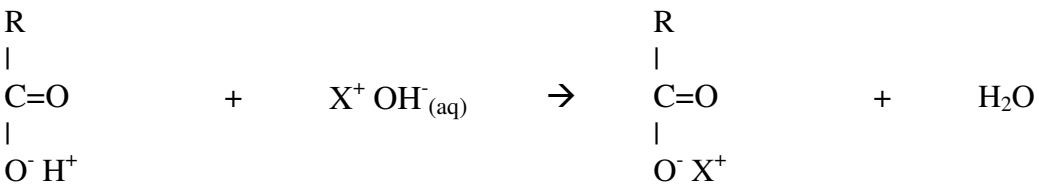


Phase 2



It is possible to note the formation of C=O bonds that was observable by ATR-FTIR analysis.

A decrease in the concentration of C=O bonds was detected only in samples immersed in alkaline solution. This decrease was supposed to be due to a further step after the oxidation process, involving the formation of organic salts rich in C=O groups, which are formed by the reaction of the communic acid with the alkaline buffer solution:

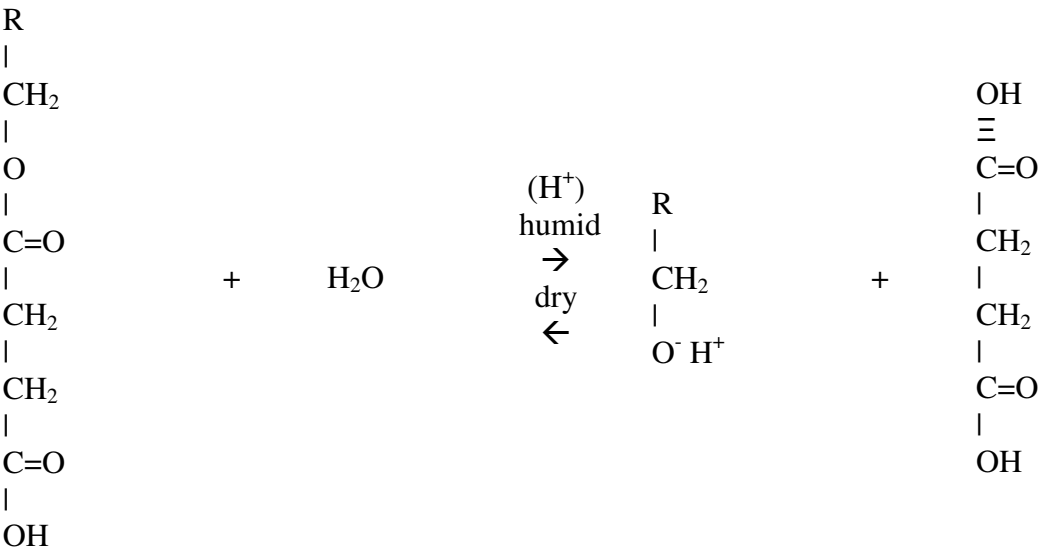


These organic salts, named communates, are soluble in water and, consequently, they migrated from the material to the solution.

The last consideration was verified analysing and identifying the residues contained in the alkaline solution where the samples were immersed, by GC-MS.
To perform the extraction of these residues it was necessary to acidify 10 mL of solution with 0.1 mL of HCl 4 M, then to extract the organic compounds adding 2 mL of diethyl ether and using a separatory funnel. The ether extract was transferred to a glass vial; after evaporation of the ether, 1 mL of diazomethane solution was added to derivatise the acids ($R\text{-}COOH + CH_2N_2 \rightarrow R\text{-}COOCH_3 + N_{2(gas)}$), allowing the analysis of the components by GC-MS.
The analysis permitted the detection of a large amount of diterpenes, while the same test on the solutions belonging to the other microclimatic conditions did not give the same result.

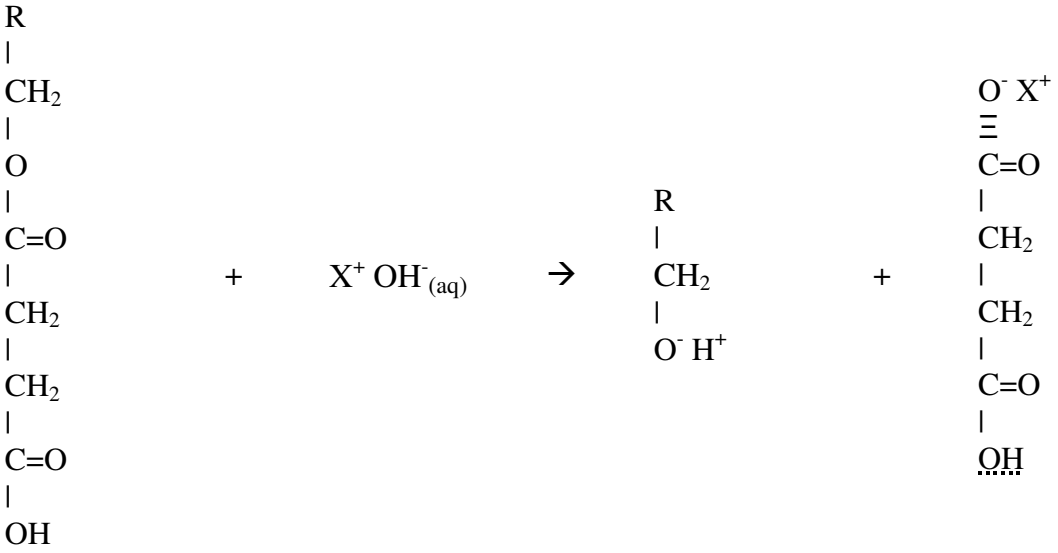
About the hydrolysis of esters into alcohols and carboxylic acids, spectroscopic data (splitting and consequent shift from 1730 cm^{-1} to 1715 cm^{-1} of the peak related to the C=O group) showed that this phenomenon occurred in all the ageing conditions, except in the ones characterized by dry environment, since humidity allows the hydrolysis process, and by alkaline environment, where hydrolysis occurs as saponification, without acids formation. The characteristic ester of Baltic amber is succinate ester and, in acidic and humid conditions (presence of water or dilute acid), it is hydrolysed into communol and succinic acid:

Acidic hydrolysis



In alkaline conditions the succinate ester is hydrolysed into communol and succinate:

Alkaline hydrolysis (saponification)



The hydroxylic group underlined with the dashed line also reacts with the base, forming a second $O^- X^+$ group.
The presence of the cation in the succinate product was detected by ATR-FTIR spectroscopy, since a new infrared band around 1550 cm^{-1} appeared in the spectra related to the samples aged in condition 11 (sample immersed in alkaline solution).

Additionally, it was decided to check the changes in two spectroscopic features used in previous works as oxidation indicator (Beck et al., 1965): the modifications in the slope of the Baltic shoulder at 1235 – 1175 cm^{-1} and the changes in absorbance of the band at 888 ± 1 cm^{-1} related to C-H bonds of terminal olefins. A slight increase of the Baltic shoulder's slope was visible in almost all the ATR-FTIR spectra; the maximum slope was observed in the spectra related to samples aged in condition 1 (sample in open container), indicating a strong oxidation, while the minimum, almost identical to the one of unaged samples, was noticeable in the spectra of samples aged in condition 11 (sample immersed in alkaline solution). This last result was also probably related to the migration of oxidised diterpenes in the alkaline solution.
Regarding the band at 888 ± 1 cm^{-1}, no relevant information was achieved, since no significant correlation between decrease in absorbance of this band and microclimatic conditions was observed.

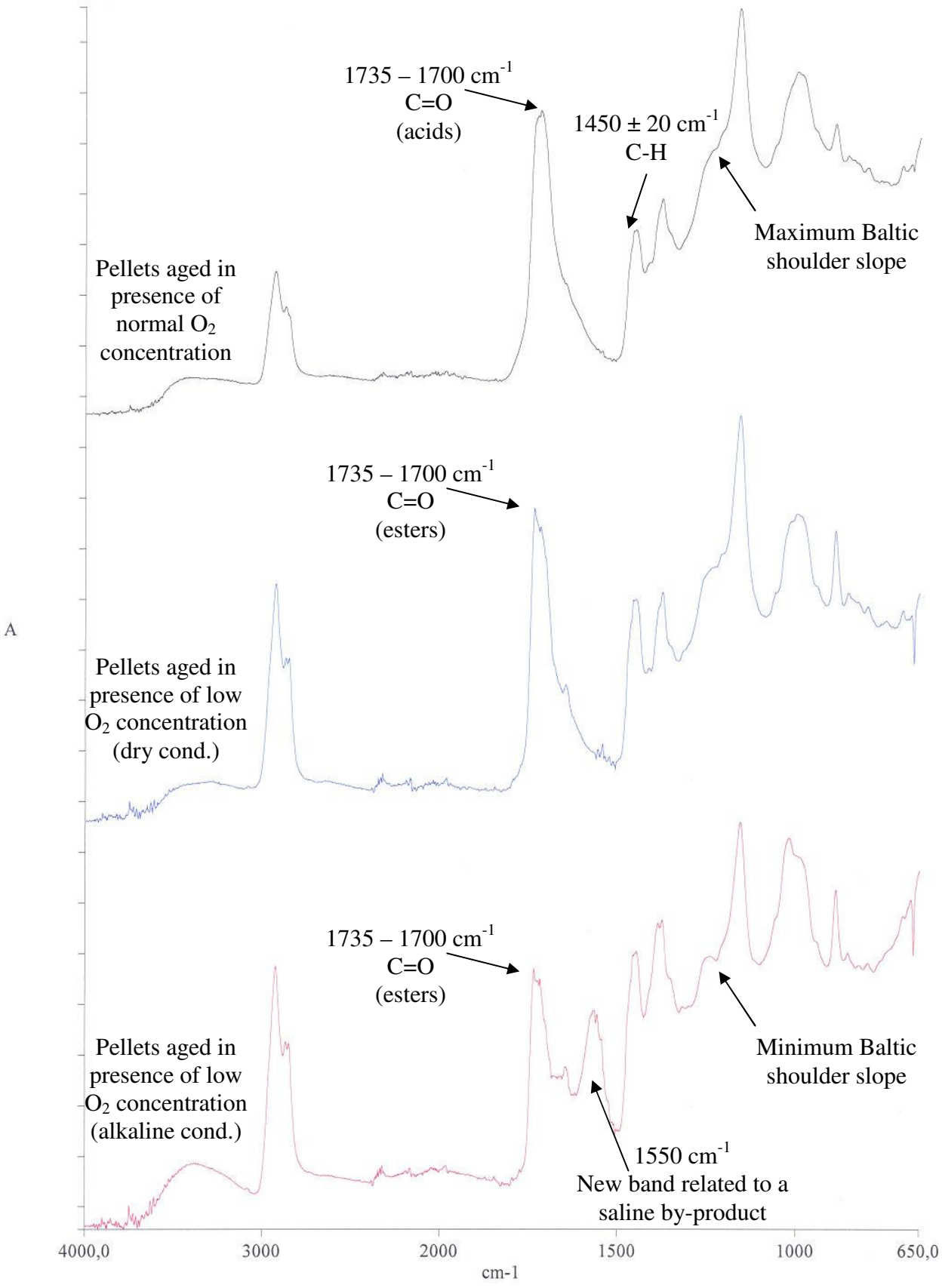


Figure 6.2. Comparison between most representative ATR-FTIR spectra of aged amber pellets.

6.1.4 FT-Raman spectroscopy.

To determine degradation of amber pellets during the accelerated ageing, the breakdown/formation of C=C (olefinic) bonds was evaluated measuring the ratio between intensity values of C=C and C-H infrared bands.

In appendix A – subsection A.3, the olefinic bonds intensity changes in amber pellets, in all the ageing conditions, are illustrated.

In all the cases the increase in intensity of C=C bonds was detected, with the exceptions of condition 11 (sample immersed in alkaline solution), where the variation resulted very small (figure 6.3).

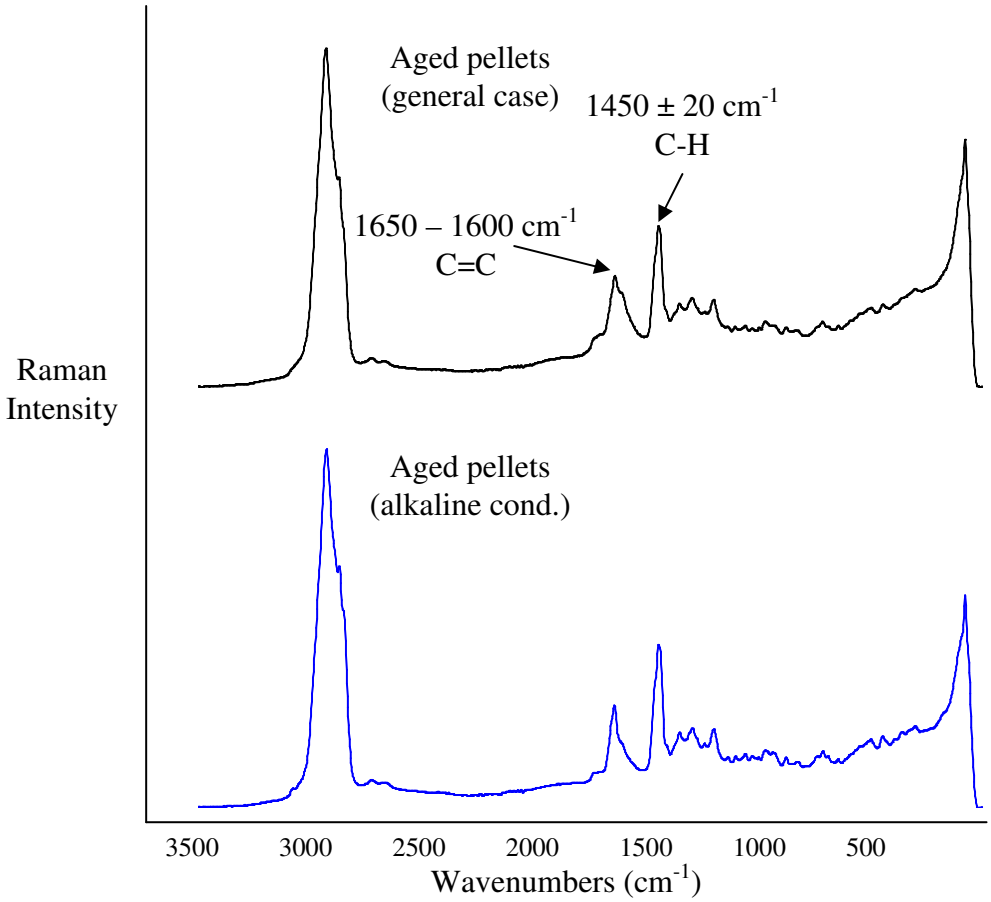


Figure 6.3. Comparison between most representative FT-Raman spectra of aged amber pellets.

From the comparison of these data with the results achieved during the preliminary investigation, the formation of C=C bonds appeared to be the main effect after thermal-ageing at 70 °C, confirming that in this ageing condition the depolymerisation of the amber structure occurred, but the energy was not high enough to cause a further breakdown of the terminal unsaturated carbon-carbon bonds by oxidation. The high level of oxidation detected by ATR-FTIR spectroscopy was related either to other positions in the molecular structure (functional group) or to a lower depth of penetration of the infrared beam.
No significant correlation between the changes in intensity of the C=C band, detected by FT-Raman spectroscopy, and variations in absorbance of the associated C-H band at 888 ± 1 cm^{-1}, detected by ATR-FTIR spectroscopy, was identified, since the repeatability of results related to the latter band was too low to consider the comparison quantitatively reliable.
Analysing the FT-Raman spectra carefully, during ageing it was possible to note a significant change in the shape of the band between 1650 and 1600 cm^{-1}, related to C=C bonds. A shoulder appeared between 1640 and1630 cm^{-1} (at lower frequencies compared to the peak corresponding to C=C terminal bonds) in all the spectra recorded after ageing (figure 6.4).

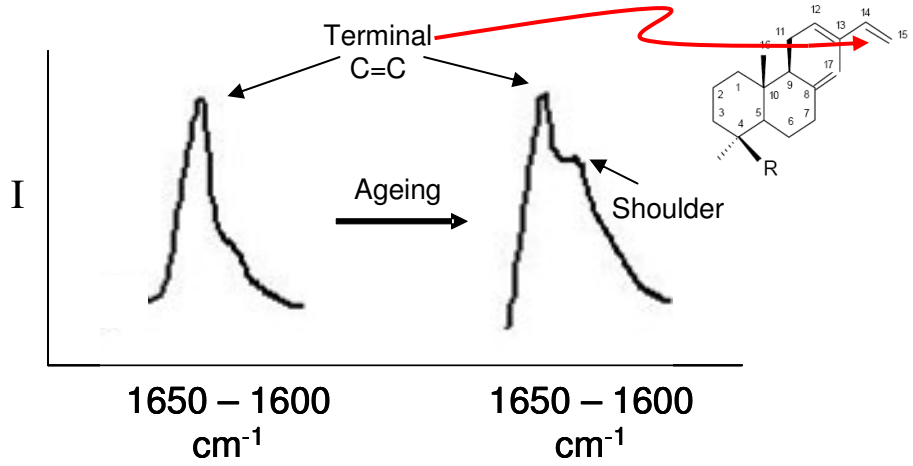


Figure 6.4. Change in the shape of the infrared band related to C=C bonds, detected by FT-Raman spectroscopy.

The infrared region involved in this change is not related to the C=C terminal bonds, consequently the changes were associated to unsaturated carbon-carbon bonds in other positions. It is supposable that this result was due to the aromatisation of cyclic regions in the terpenoid components (figure 6.5; Matuszewska et al., 2001; Pipatmanomai et al., 2001), but it was not possible to achieve any relevant evidence during this work, since other analytical techniques, e.g. NMR spectroscopy, would be necessary.

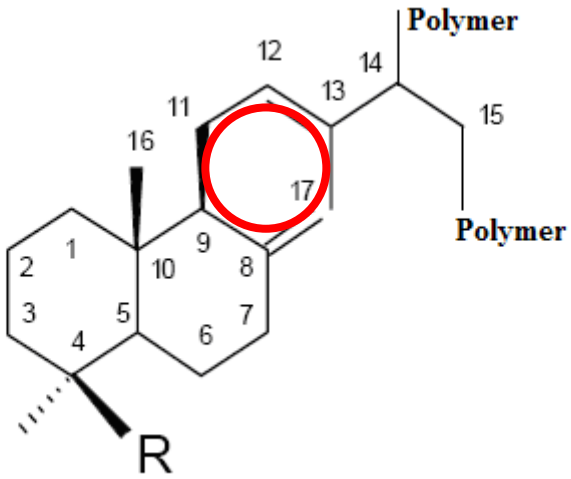


Figure 6.5. Hypothesis of aromatisation of Baltic amber chemical structure. The red circle indicates the position where the aromatic ring could be formed.

Comparing the data from FT-Raman analyses and colour measurements, the correlation between colour change and formation of new C=C double bonds (Zollinger, 2003) appeared clearer.
The only two cases where it was observed both a not significant change of colour and a small increase of C=C groups were represented by condition 5 (sample exposed to dry and anoxic atmosphere) and condition 11 (sample immersed in alkaline solution). In the first case the absence of oxygen could cause a reduction of the depolymerisation process (Feller, 1994; Rabek, 1996; Rabek, 2007), in the second one, as already confirmed by GC-MS analyses, a high concentration of diterpenes containing the C=C bonds were dissolved in the alkaline solution.

6.1.5 Oxygen measurement by optical respirometry.
Oxygen concentration in the atmosphere of each experimental micro-environment was successfully measured by optical method during the artificial accelerated ageing procedure.
In appendix A – subsection A.4, the oxygen consumption trends in amber pellets, in all the ageing conditions, are illustrated. Data related to the control samples confirmed the reliability of the method.

In general, during the ageing it was possible to observe a significant reduction of oxygen percentage inside the flasks where samples were exposed to atmosphere with a normal concentration of oxygen. Results related to samples that were immersed in liquid environment showed a smaller decrease of oxygen concentration.
Obviously, only in condition 5, where samples were exposed to anoxic atmosphere, the concentration of oxygen resulted 0%. Results related to condition 11 (sample immersed in alkaline solution) were incomplete, because of the detachment of two sensor-spots during the last ageing stage, but, despite that, the decreasing trend of the oxygen concentration was clearly deducible.

From the comparison of these data with the results about C=O groups achieved by ATR-FTIR spectroscopy, the direct relationship between oxygen consumption and oxidation development was recognised, since measurements by the two analytical techniques indicated respectively higher oxygen consumption and higher oxidation for samples which were aged in atmosphere with normal concentration of oxygen.

6.1.6 SPME-GC-MS headspace analysis.
Volatile compounds in the headspace of amber powder samples were regularly adsorbed and analysed during the artificial accelerated ageing procedure.
In appendix A – subsection A.5, the acetic and formic acids off-gassing trends in amber micro-slices, in all the ageing conditions, are illustrated.

It was possible to detect a considerable release of formic and acetic acids vapours from all the amber samples; moreover, unlike the preliminary experimental procedure, the collected data were used to develop profiles to define variations in the off-gassing during the ageing.
Generally, irrelevant differences in the response of released gasses were observed during the different phases of the ageing.
Only in conditions 1 and 4, both characterized by the exposure of the samples to dry atmosphere with normal concentration of oxygen, results showed a significant increase of acids (especially formic) off-gassing.
Therefore, dry environment in presence of oxygen seemed to be related to a higher release of acidic vapours, which are likely products of radical reactions. Reactions of this kind occur in other materials, such as oils during drying (Mills, 1966), so it is possible that the same phenomenon happened in Baltic amber when samples were exposed to low relative humidity. The probable reaction that produced formic acid was assumed during the preliminary investigation (paragraph 4.1.6); about the production of acetic acid, more study will be necessary, but a possible way, suggested by Mills' works on drying of oils (1966), might be the one illustrated in figure 6.6.

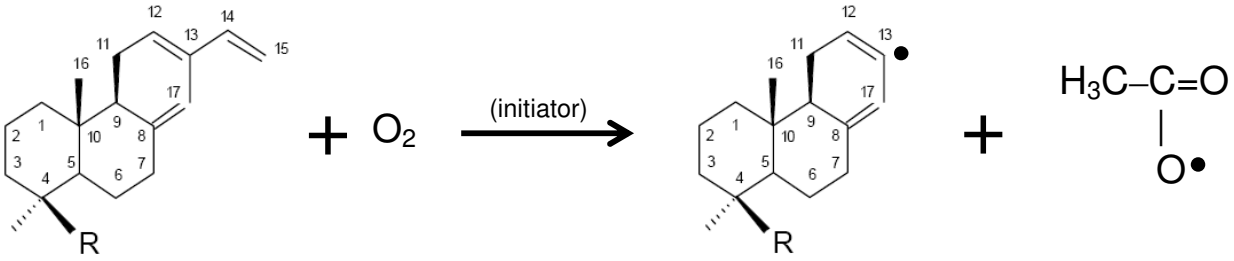


Figure 6.6. Hypothesis of decomposition that produced acetic acid through the cleavage of C-C bonds due to oxidation.

The radicals which are produced by the reaction, might work as new initiators for other reactions of this type.
The described hypothesis requires further and more precise investigations, possibly by NMR and Electron Spin Resonance (ESR) spectroscopies.

6.1.7 Micro-ATR-FTIR cross sections analysis.
The analysis of cross sections obtained from amber pellets was performed in order to get information about the development of oxidation in relation to the distance from the surface. Results are showed in figure 6.7.

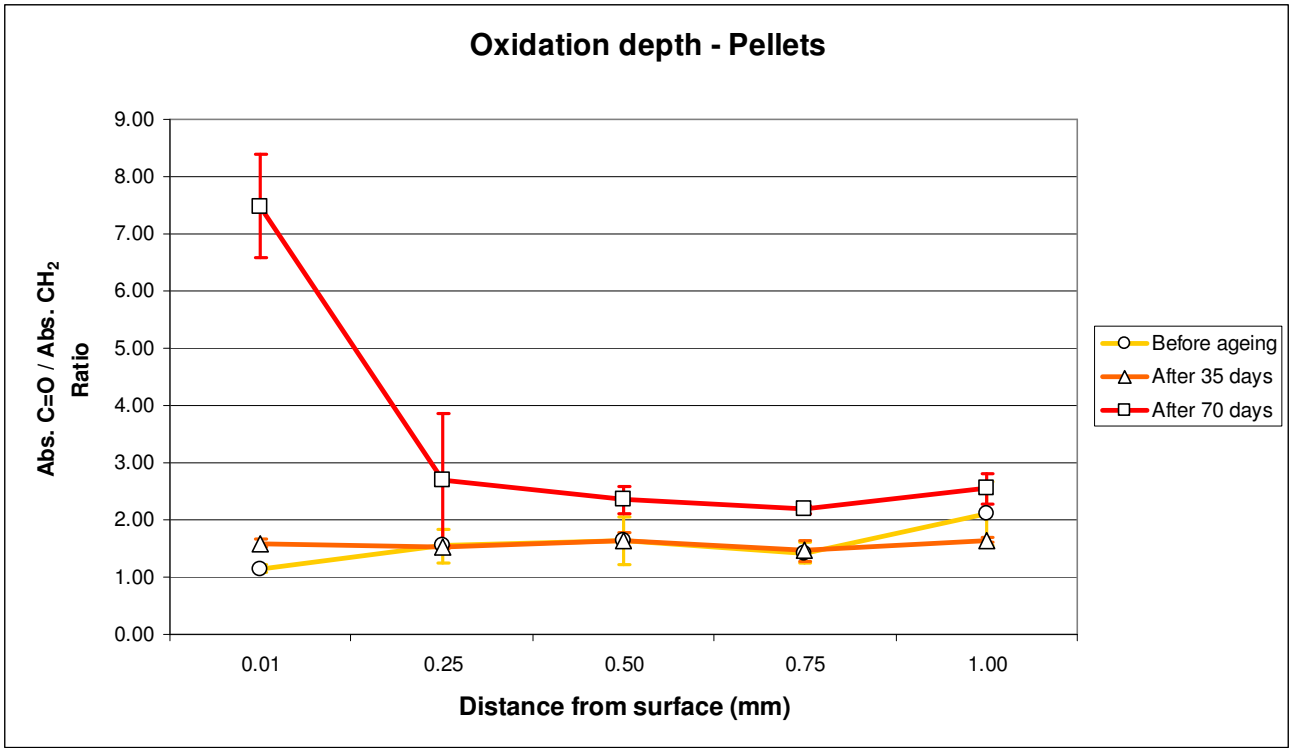


Figure 6.7. Development of the oxidation in Baltic amber pellets cross sections at different levels of ageing.

The detected levels of oxidation did not show a significant difference between unaged and 35 days aged samples. In samples that have been aged for 70 days, it was clearly visible the strong increase of oxidation at the exterior part, confirming that oxidation of amber occurs initially only at the surface.
Due to the rather low resolution of the spectra, it was not possible to obtain reliable data about the development of the esters hydrolysis in the cross sections.

6.1.8 Confocal profilometry.
Non-contact topographic analyses were performed on the surfaces of amber pellets, in order to obtain information about possible changes in the roughness due to the ageing. Results are showed in figure 6.8.

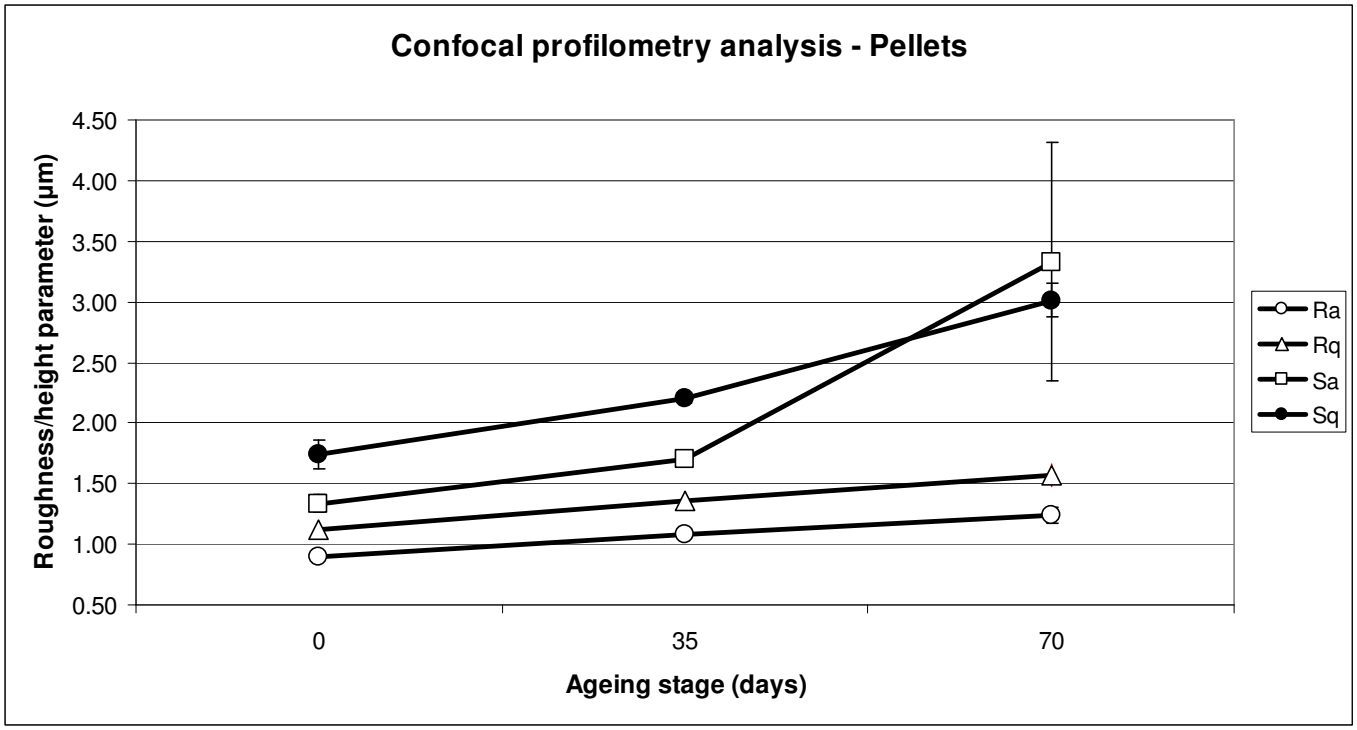


Figure 6.8. Changes in roughness and height parameters on Baltic amber pellets surfaces during ageing.

During ageing of amber samples, a significant increase of all the standard parameters used to evaluate roughness was observed.
This result confirmed the previous information about amber prisms surfaces from the preliminary investigation and it reinforced the hypothesis that the increasing superficial roughness might be due to the breaking of chemical bonds involved in the depolymerisation of the material.

6.1.9 ATR-FTIR analysis of archaeological material.
A few 2-3 thousands of years old archaeological samples were analysed in order to evaluate the oxidation state at different levels and to compare their degradation status with data obtained from the experiments on raw material. Results are showed in figure 6.9.

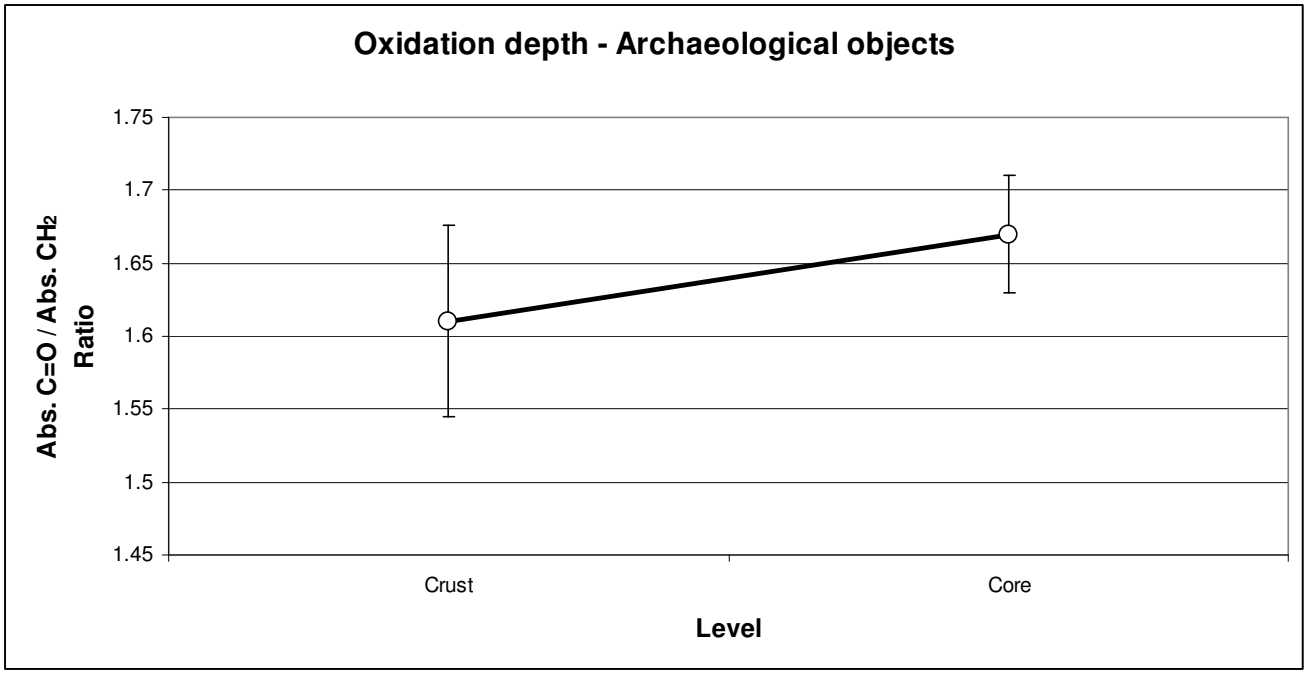


Figure 6.9. State of oxidation in Baltic amber archaeological samples at different levels.

It was possible to notice no relevant difference in the oxidation state between the crust and the internal part of the samples. This result was quantitatively comparable to the one obtained by the micro-ATR-FTIR cross sections analysis of pellets aged for 35 days, which was a moderately short artificial ageing period compared to the age of the objects (2-3 thousands of years). This assessment suggested a fair physico-chemical stability of the buried condition, confirming the conclusions from the elemental analysis on similar archaeological samples (paragraph 4.1.7).
In all the instances, spectroscopic data, related to the peak frequency characteristic for C=O groups, showed the prevalence of acids compared to esters in the chemical composition, confirming the relevant role of hydrolysis as degradation process.

6.2 Final assessments.

After the interpretation of the final achieved data, combined with initial information from the preliminary investigation, it was possible to identify a number of pathways by which Baltic amber degrades (figures 6.10 a-b) and to recognise the main environmental factors which were involved in those degradation processes.
It is important to note that the interruption of the ageing for one or two days, during the analytical sessions on the samples, did not apparently affect the degradation progress.

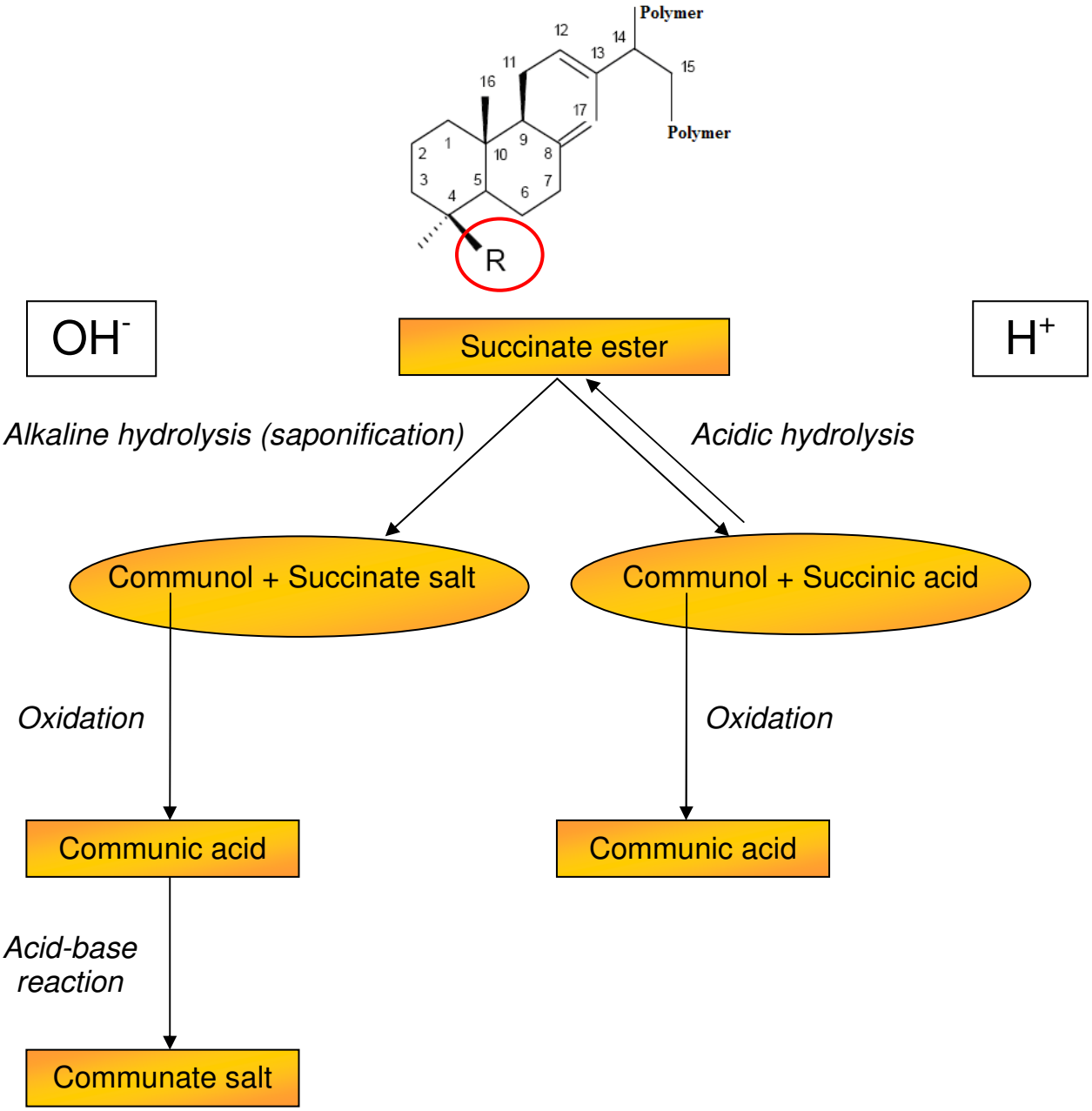


Figure 6.10.a. Degradation pathways related to the functional groups of the labdanoid diterpene molecule.

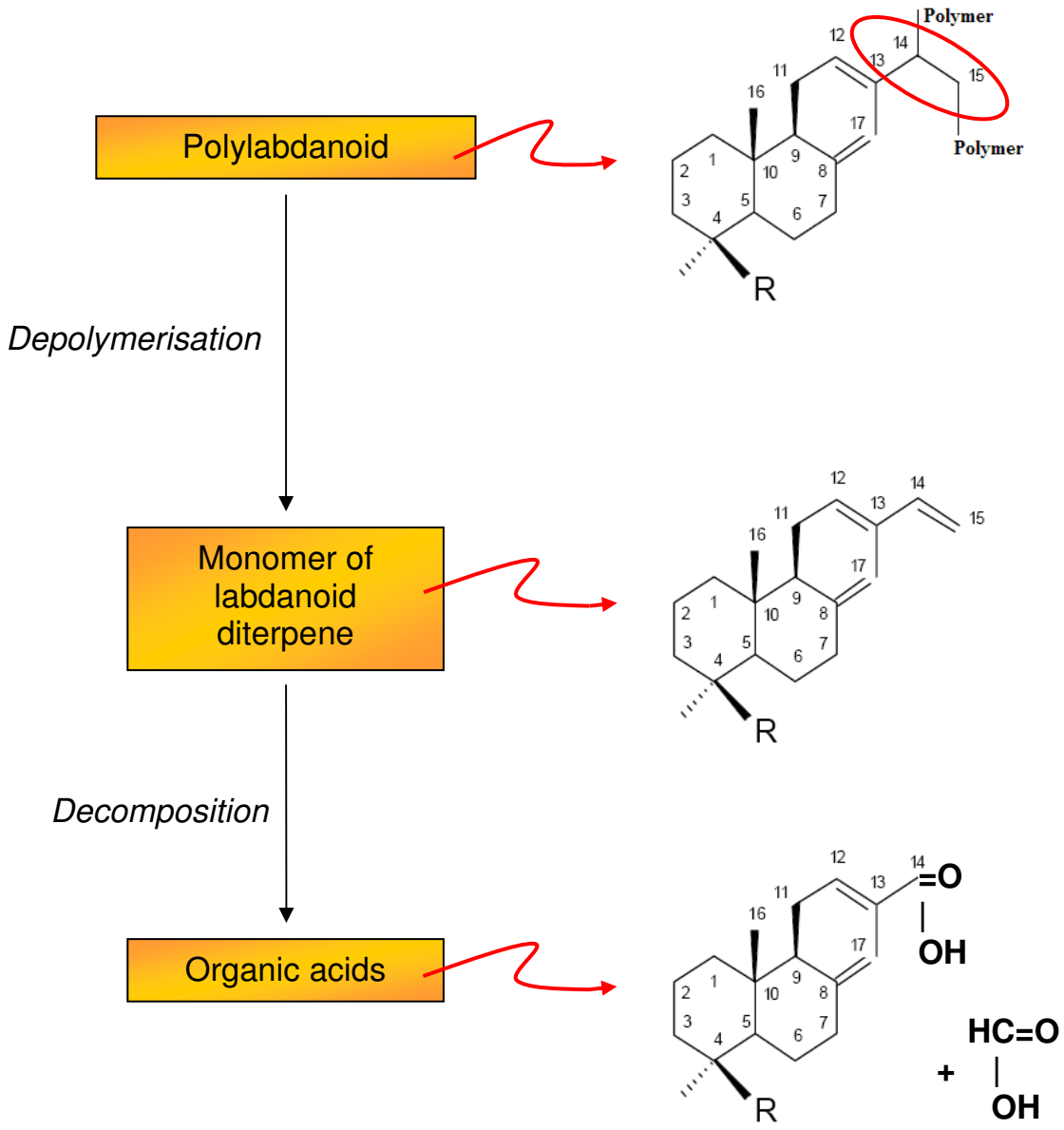


Figure 6.10.b. Degradation pathways related to the terminal bonds of the labdanoid diterpene molecule.

The environmental factors, which worked as degradation agents and which had to be considered for planning a preventive conservation strategy, were:

- *energy (heat, light)* – high temperature and light radiation hastened chemical degradation of Baltic amber, as demonstrated by the use of the accelerated ageing procedures during the experiments;
- *oxygen* – oxidation of terpenoid components was the major cause of degradation of Baltic amber, since oxygen was involved in several of the discussed mechanisms;
- *relative humidity* – humid environments promoted hydrolytic processes, but, on the other hand, dry conditions seemed to be related to a higher rate of acidic vapours off-gassing;
- *pH* – acidic and alkaline conditions were connected to an intense hydrolytic activity.

Having proved that small samples degrade more rapidly than larger pieces, as showed by more significant chemical changes on powder samples, the surface to volume ratio should also be recognised as a degradation agent, but such a factor is not controllable, so it cannot be considered for preventive conservation purposes.

The illustrated degradation pathways and their connection with the listed environmental factors were supported by evidences obtained by all the employed analytical techniques, as showed in table 6.1.

Table 6.1. Environmental factors and related degradation effects on Baltic amber examined by the different analytical techniques

Environmental factor / Degradation process	Energy (heat, light)	Oxygen	Relative humidity	pH
Hydrolysis			ATR-FTIR spectroscopy Visual examination GC-MS	
Oxidation	ATR-FTIR spectroscopy Oxygen measurement Cross sections analysis			
Depolymerisation	FT-Raman spectroscopy Colour measurement Confocal profilometry			
Decomposition		Headspace analysis Oddy test		

The previous table needs to be elucidated with a few comments:
The hydrolysis of succinate ester was proved by changes in the shape of the infrared band at 1735 – 1700 cm^{-1}, clearly visible in ATR-FTIR spectra; humid environments coupled with acidic or alkaline pH generated, further than chemical changes, visible structural alterations, such as deterioration of amber prisms surfaces and cohesion of powder particles; among acidic and alkaline conditions, the second one appeared to cause worse effects (presence of cracks on pellets surfaces), due to the production and release of soluble organic compounds by the material, as verified by GC-MS analysis.
Thermal- and photo-oxidative reactions were detected both by ATR-FTIR spectroscopy, with the increase in the concentration of C=O groups, and by optical respirometry, through the measurement of oxygen consumption; moreover, the infrared analysis of cross sections confirmed that oxidation starts from the surface.
The depolymerisation phenomenon, based on oxidative reactions, was described by the formation of C=C bonds, detected by FT-Raman spectroscopy, and supported by studies on changes in colour and in roughness of the samples surfaces.
Organic volatile decomposition products were identified by GC-MS headspace analysis and their off-gassing was also confirmed by Oddy test through the corrosion of lead coupons; this off-gassing seemed to be more intense in dry conditions.

Consequently, the ideal conditions to obtain a good preservation of Baltic amber objects could be defined:

- *room temperature* – an ambient temperature not higher than 22 °C should avoid any speed-up of degradation of amber objects; during this work, no study concerning effects due to very low temperature was carried out, though previously established information recommend 17 °C as appropriate lower extreme (Thickett et al., 1995). It is also important that the temperature keeps stable, since fluctuating temperatures cause structural damages (as the material expands and contracts; Callister, 2002) and indirectly affect relative humidity;
- *limited exposure to light* – amber objects should not be exposed to intense and direct light, either artificial or natural behind window, which was the kind of radiation used for this work;
- *low concentration of oxygen* – amber objects should be kept in an anoxic or at least hypoxic micro-atmosphere;
- *middle relative humidity* – neither a very low nor a very high relative humidity conditions appeared ideal for Baltic amber preservation, because they both caused degrading effects (respectively, acidic vapours off-gassing and hydrolysis), consequently a middle value around 50% should be appropriate; it is also important that the relative humidity keeps stable (Williams et al., 1990), to avoid structural damages due to shrinking and swelling oscillations;
- *neutral pH* – both acidic and alkaline pH conditions in humid environment caused degrading effects (hydrolysis), even though alkaline pH appeared to cause worse visible effects due to the saponification phenomenon; considering, as proved with this work, that Baltic amber material naturally releases organic acidic vapours, creating acidic conditions in presence of moisture, these chemical compounds should be removed from the environment in order to keep the pH status as neutral as possible.

Elemental and ATR-FTIR spectroscopy analyses on archaeological material gave indications about the stability of buried conditions, since the chemical characterization of these samples was comparable to the one related to moderated artificial ageing conditions.
Buried environments are in general characterised by absence of light and low concentration of oxygen (Cremaschi, 2003). In the instance of the site where the samples used for this work were found (Skanderborg, Denmark), the soil was also characterised by a stable temperature around 8 °C, high relative humidity and acidic pH. The mentioned features partially matched with the ideal conditions necessary to avoid a deep degradation of Baltic amber.

Conclusions.

The aim of this project was to achieve a deeper understanding of the mechanisms by which Baltic amber degrades, in order to develop effective techniques for preventive conservation, with the purpose to slow down the rate of degradation of amber objects.

By the use of accelerated ageing to initiate degradation of raw Baltic amber samples and, successively, by the use of non/micro-destructive techniques to identify and quantify changes in visual, chemical and structural properties, it was possible to recognize the major pathways by which amber degrades:

- acidic hydrolysis of succinate ester into communol and succinic acid;
- saponification of succinate ester in alkaline conditions;
- thermal-oxidation and photo-oxidation of terpenoid components;
- depolymerisation of the chemical structure;
- decomposition of terpenoid components with production of volatile organic acids.

The achieved outcomes advanced the knowledge base within the study of amber degradation, confirming some of the previous information and giving new data:

- not only heat and UV radiation, but also visible light can cause rapid degradation of amber;
- oxidation of terpenoid components, that starts from the surface, is the major cause of degradation, since oxygen is involved in several processes, such as depolymerisation, breakdown of terminal unsaturated carbon-carbon bonds and formation of communic acid from communol;
- amber is sensitive to both high and low relative humidity;
- acidic and alkaline pH conditions can cause chemical changes and surface deterioration in amber objects.

These results can be used to develop a preventive conservation strategy based on the control of climatic, lighting and atmospheric parameters in the environment where Baltic amber objects are placed during storage, transport and display, including the following measures:

- *climate control* – to control temperature and relative humidity of the environment where Baltic amber objects are stored or exhibited, the use of active methods (air conditioning systems or air controlled ventilation systems) or passive methods (temperature and RH buffering, through suitable building materials and natural ventilation; Ryhl-Svendsen et al., 2003; Padfield et al., 2007) is necessary. Systems of these kinds should also be able to keep temperature and relative humidity constant, in order to avoid damaging effects due to fluctuations of the two parameters (Padfield, 2008).
  Descriptions of these systems do not need to be detailed in this work. Each museum should choose the most appropriate system according to its requirements, in terms of energy consumption, maintenance and visitors' comfort level, also making use of psychometric calculations (American Society of Heating, Refrigerating and Air-Conditioning Engineers, 2003);

- *illumination* – moderated exposure to light, either natural or artificial, is relevant to Baltic amber objects in use before they are collected by museums and to objects on display, but not to objects in storage or during transport which are usually covered and consequently not exposed to any kind of illumination.
  During exhibitions light must be accepted, but its intensity and incidence should be regulated to a compromise between the sensitivity of the material and what is required for the proper display of the objects (Ryhl-Svendsen et al., 2003). Continuous artificial illumination could also be avoided, for instance applying timed relays. The use of filtering films is applicable only partially in this context, since Baltic amber is sensitive not only to UV radiation but also to visible light, as demonstrated with this research. When showrooms are closed to the public, showcases should be covered or else the environment should be kept in the dark, closing window shutters and turning off artificial illumination;
- *storage, transport and display* – Baltic amber objects should be enclosed in appropriate storage, transport and display cases for protection against dust, gaseous pollutants, thieves and sudden mechanical shocks, as well as for microclimatic control. About display cases, such a system is produced, for instance, by the Italian company TecnoScianna and they are named Climabox. These showcases are made in fully transparent acrylic material, perfectly sealed and with the possibility to place atmosphere controllers inside, which are hidden in the base of the case and replaceable without rearranging the display.
  Concerning Baltic amber objects, suitable atmosphere controllers should be:
  - ✓ *oxygen absorbers* – oxygen free cases could have many benefits during storage, transport and display of Baltic amber objects, thanks to the exclusion of the main cause of degradation that is oxygen. Ageless Z, supplied by Conservation by Design, appeared to be a very efficient product to recreate oxygen free microclimates during the experiments for this work, therefore it would be an optimal solution in museum contexts;
  - ✓ *humidity stabilisers* – conventional silica gels are useful to stabilise relative humidity at different ranges of values. In the intermediate region around 50%, ideal for Baltic amber objects, specific products such as Pro Sorb and Art Sorb of Long Life for Art are also suitable;
  - ✓ *pollution scavengers* – some case materials, like wood, but also the Baltic amber objects themselves, release air pollutants such as acetic and formic acid, which recreate highly acidic conditions. The more a case is well sealed, the better these compounds are able to concentrate and accumulate, making easier their confinement. Specific pollution scavengers, like activated charcoal supplied by Long Life for Art, coupled with non-emissive materials for storage/display construction, help to neutralize this chemical status.

On the base of the previous statements, in order to preserve archaeological Baltic amber objects in their original state as long as possible, a number of guidelines for storage, transport and display can be listed (table I).

Table I. Guidelines for storage, transport and display of Baltic amber objects

Factor		Guidelines
Climate control		The environment where amber objects are stored or displayed has to be climatically controlled by the most appropriate control system for the related building. Temperature and relative humidity have to be stabilised at values respectively in the range between 17–22 °C and around 50%
Illumination	Storage and transport	Amber objects in storage and during transport have to be kept in the dark
	Display	During exhibitions, the most appropriate illumination system in the room where amber is displayed has to be chosen considering both the visibility and the necessity of limited exposure of the objects. For example, showcases must not be placed close to windows, while artificial illumination must be indirect (not directly pointing toward the objects) and, possibly, timed; the use of camera flashes should be forbidden and the UV component of the light should be reduced as much as possible by the application of filtering-films on the display cases
		During closing times/periods, showcases have to be covered or rooms have to be obscured
Case	Structure	Cases where amber objects are enclosed, during storage, transport or display, must be made in non-emissive materials (e.g. metal, glass, acrylic materials) or they must be coated on the interior surfaces with protective films (e.g. polyester); they must be perfectly sealed and allow the possibility to replace atmosphere controllers inside; amber objects should be displayed on soft shock-absorbent support systems
	Atmosphere control	Oxygen absorbers, humidity stabilisers (at 50% RH) and pollution scavengers must be placed and regularly replaced inside the cases

New methodologies and technologies were applied and developed during this study:

- production of amber powder samples to investigate the role of the surface to volume ratio in the degradation process;
- use of optical respirometry to examine consumption of oxygen by amber;
- use of SPME-GC-MS headspace analysis and Oddy test to detect volatile compounds released by amber;
- spectrometric analysis of amber cross sections to observe the development of degradation in relation to the distance from the surface;
- use of confocal profilometry to obtain information about changes in amber surface roughness due to degradation.

The conclusions of this study can be applied to the preservation of the National Museum of Denmark's collections, which are of interest to curators, students, archaeologists and visitors for the economic, social, religious and cultural value of amber jewellery.

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www.perkinelmer.com

PreSens -
www.presens.com

Preservation Equipment Ltd -
www.preservationequipment.com

The School of Conservation of the Royal Danish Academy of Fine Arts -
www.kons.dk

Sigma-Aldrich Co. -
www.sigmaaldrich.com

TecnoScianna -
www.tecnoscianna.it

Alma Mater Studiorum – Università di Bologna

DOTTORATO DI RICERCA

Science for Conservation

Ciclo XXII

Settore/i scientifico disciplinari di afferenza: CHIM/12

TITOLO TESI

ARCHAEOLOGICAL BALTIC AMBER: DEGRADATION MECHANISMS AND CONSERVATION MEASURES.

Appendixes: A. Analytical results
B. Activities account

Presentata da: **Gianluca Pastorelli**

Coordinatore Dottorato **Relatore**

Prof. Rocco Mazzeo **Prof. Jane Richter**

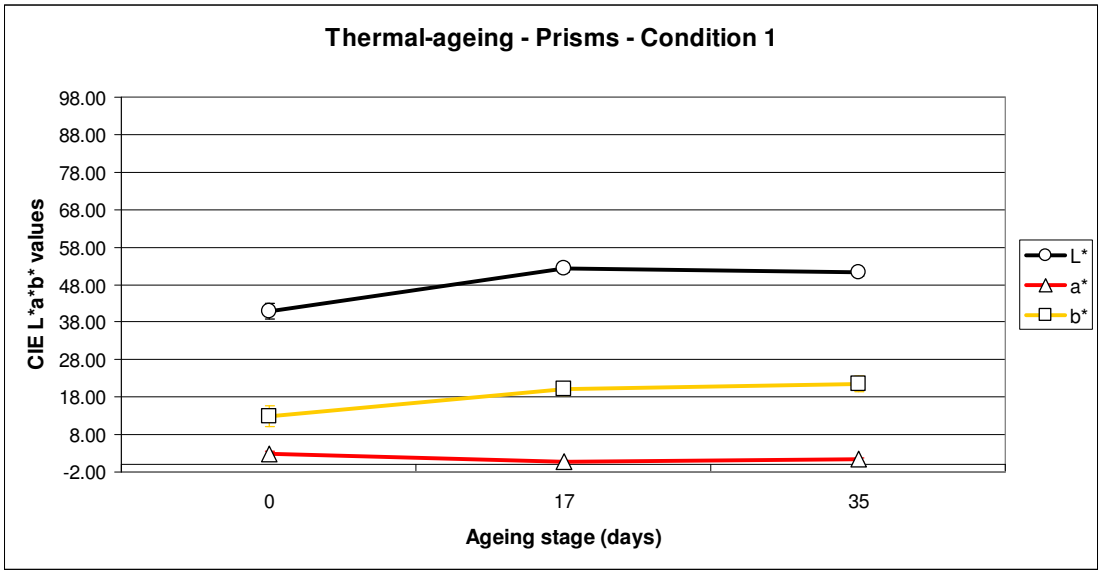
Esame finale anno 2009

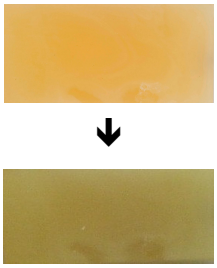
Index.

A. ANALYTICAL RESULTS.

A.1 Visual examination and colour measurement.

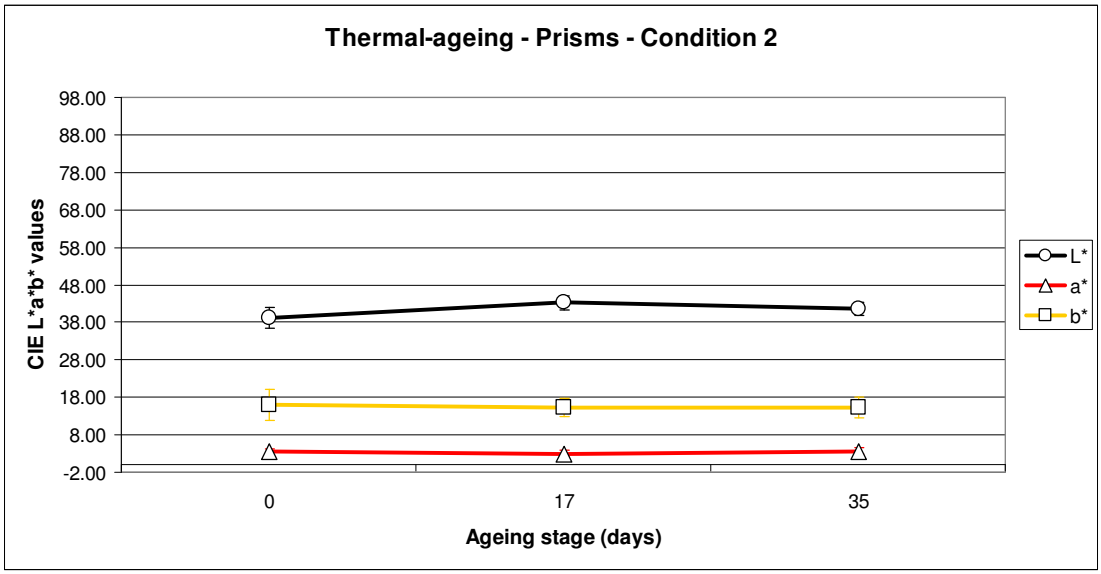
Preliminary investigation:

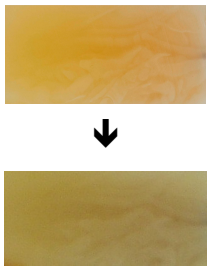




- Lightening, yellowing
- $\Delta E_{ab} = 13.96$

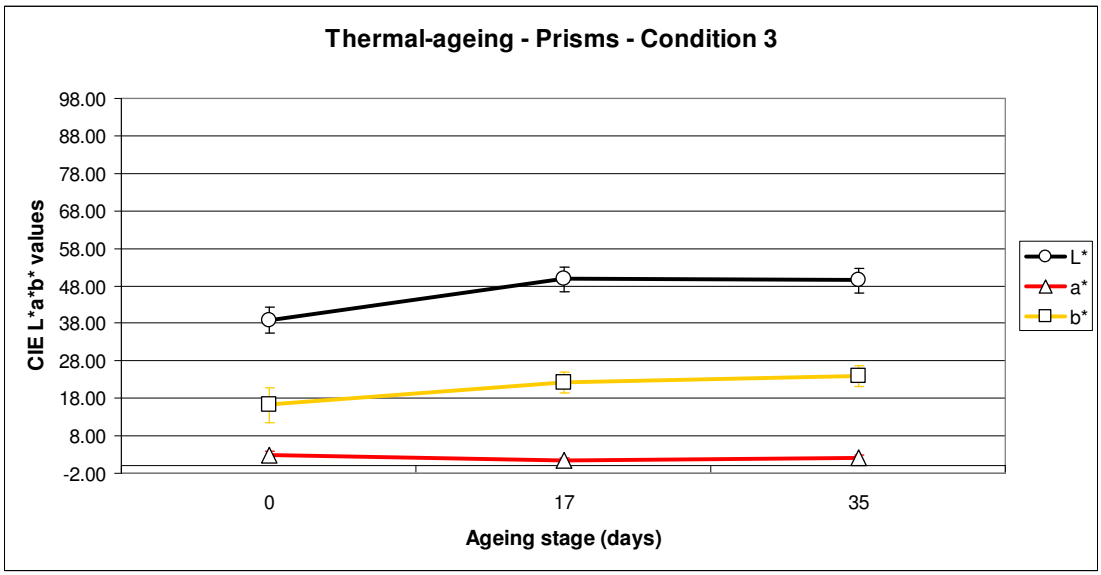
Figure 1. Visual and colour changes in thermal-aged prisms in condition 1 (≤ 20% RH, sample exposed to external atmosphere).

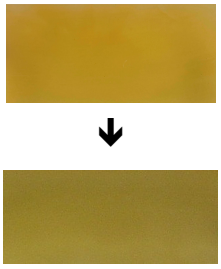




- No significant change
- $\Delta E_{ab} = 4.81$

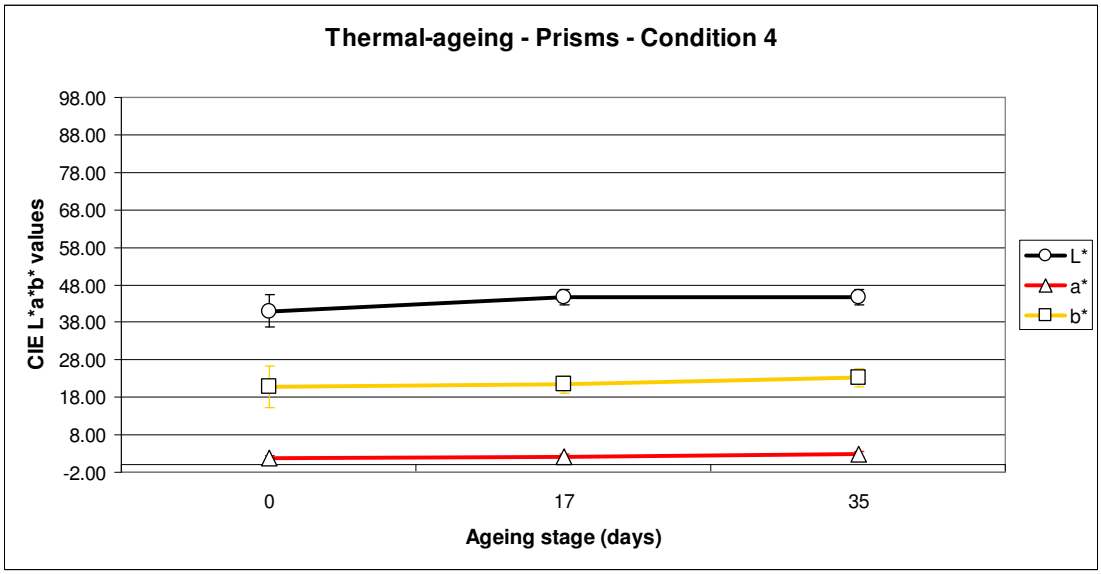
Figure 2. Visual and colour changes in thermal-aged prisms in condition 2 (100% RH, sample exposed to internal atmosphere, pH ≤ 5.5).

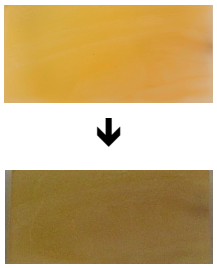




- Lightening, yellowing
- $\Delta E_{ab} = 16.29$

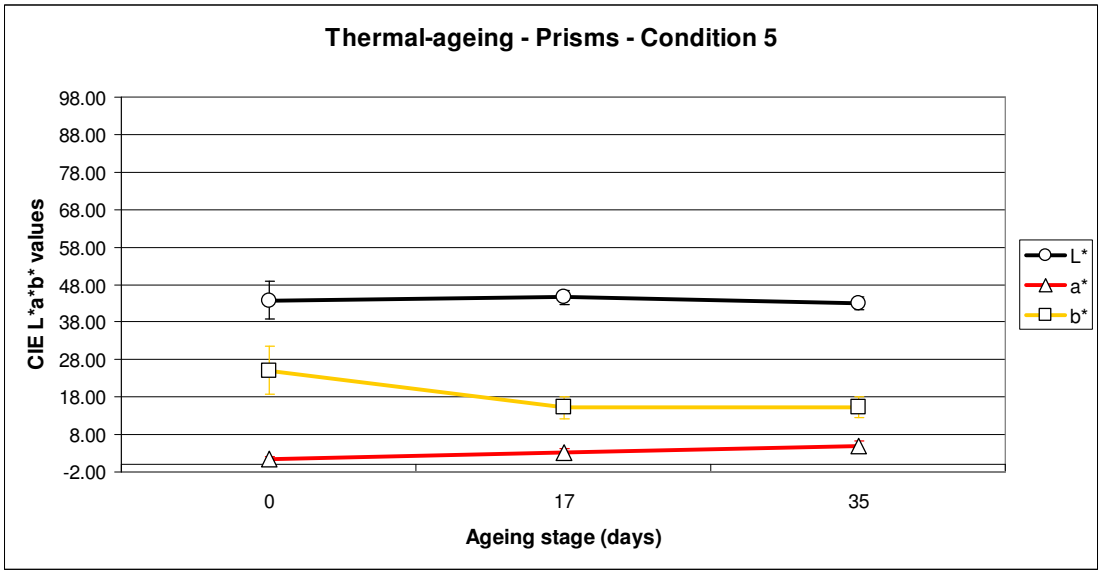
Figure 3. Visual and colour changes in thermal-aged prisms in condition 3 (≤ 20% RH, sample exposed to internal atmosphere).

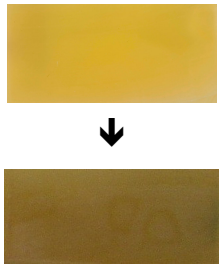




- No significant change
- ΔE_{ab} = 12.18

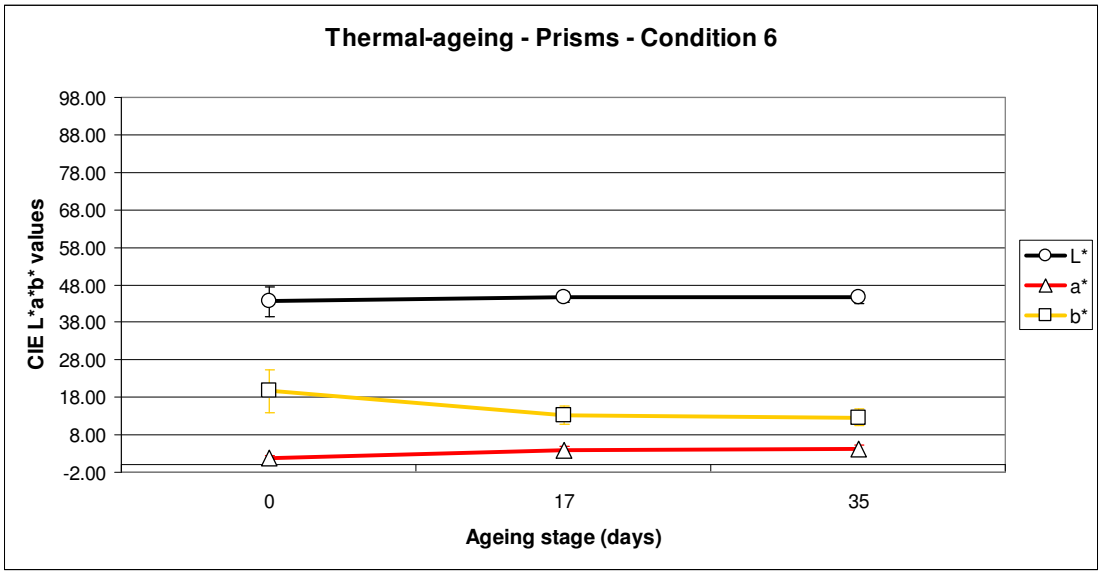
Figure 4. Visual and colour changes in thermal-aged prisms in condition 4 (≤ 20% RH, sample exposed to internal hypoxic atmosphere).

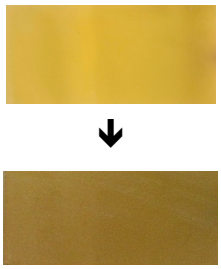




- Slight reddening, less yellow; surface more opaque, almost notched
- ΔE_{ab} = 14.40

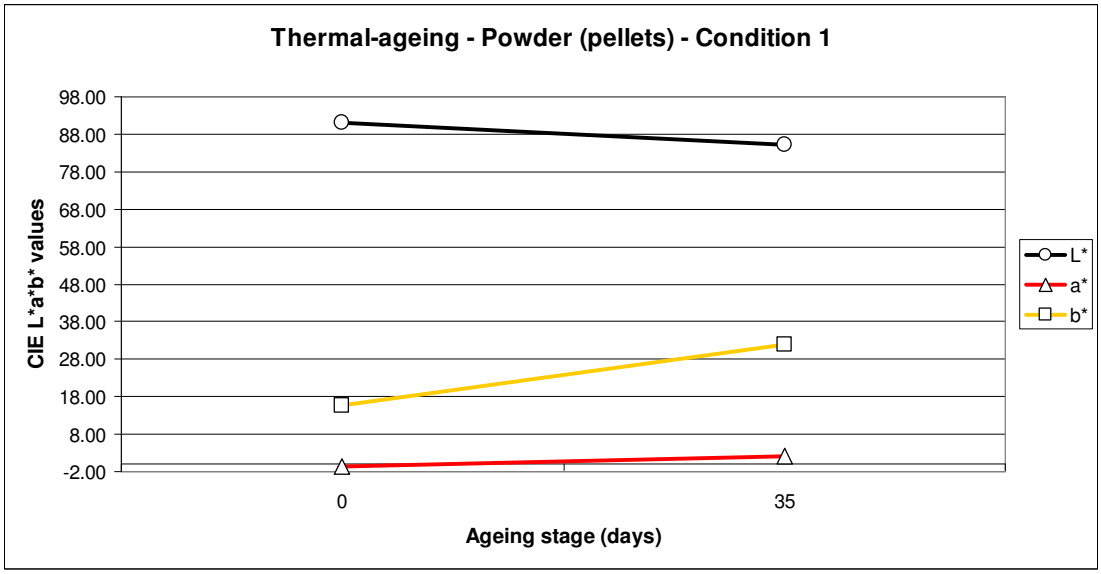
Figure 5. Visual and colour changes in thermal-aged prisms in condition 5 (100% RH, sample exposed to internal atmosphere, pH 4).

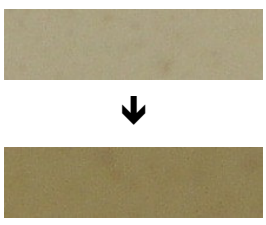




- Less yellow; surface more opaque, almost notched
- $\Delta E_{ab} = 11.05$

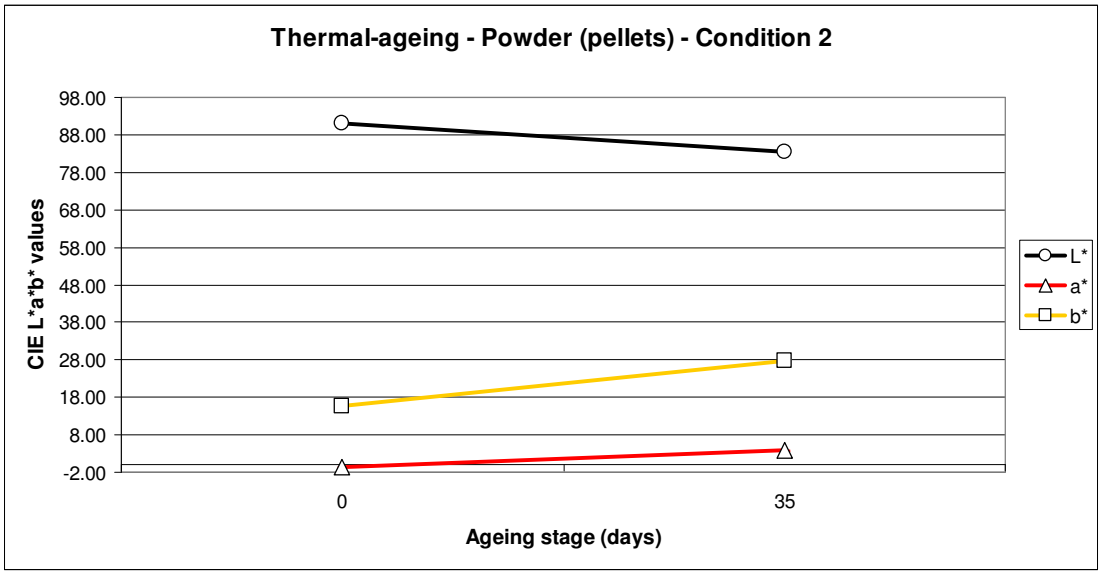
Figure 6. Visual and colour changes in thermal-aged prisms in condition 6 (100% RH, sample exposed to internal atmosphere, pH 10).

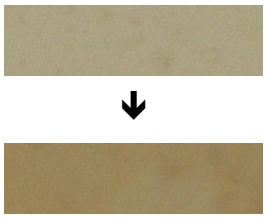




- Darkening, yellowing
- $\Delta E_{ab} = 17.18$

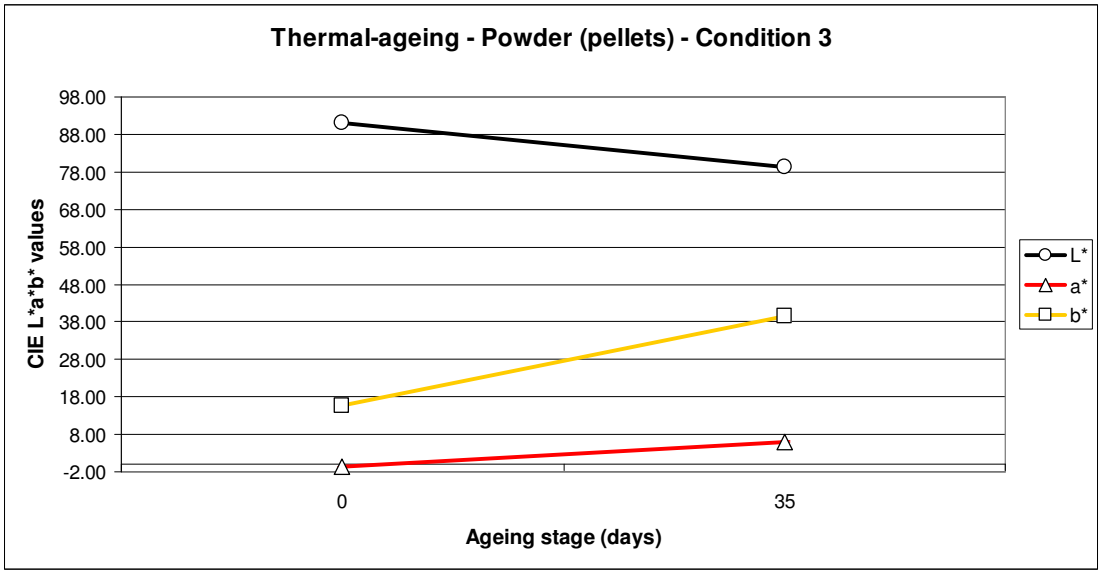
Figure 7. Visual and colour changes in thermal-aged powder in condition 1 (≤ 20% RH, sample exposed to external atmosphere).

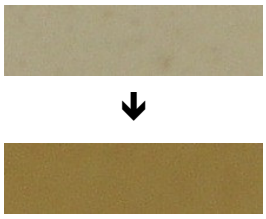




- Darkening, slight reddening, yellowing
- $\Delta E_{ab} = 14.69$

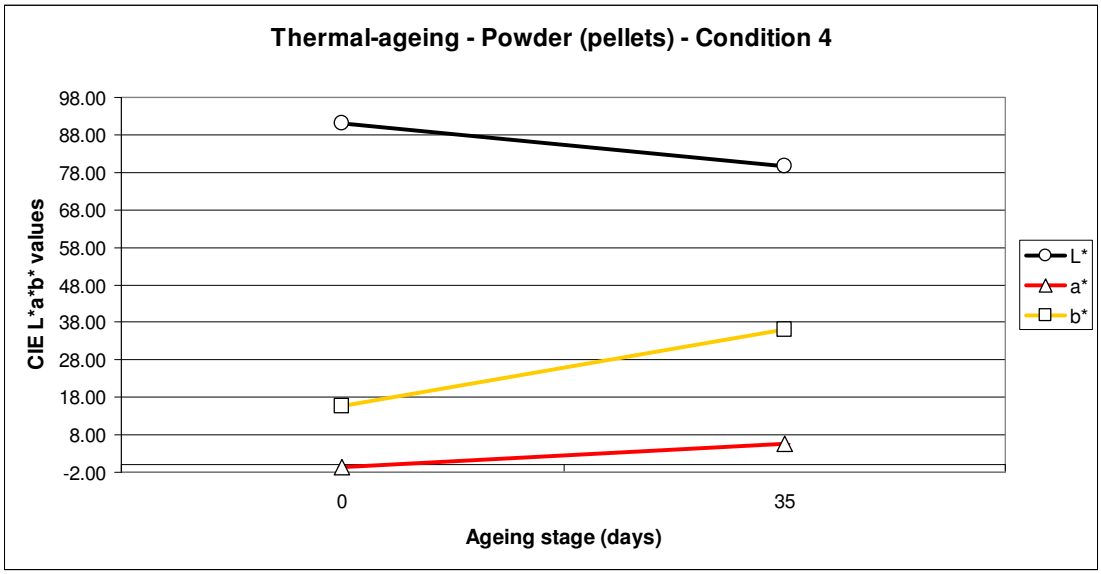
Figure 8. Visual and colour changes in thermal-aged powder in condition 2 (100% RH, sample exposed to internal atmosphere, pH ≤ 5.5).

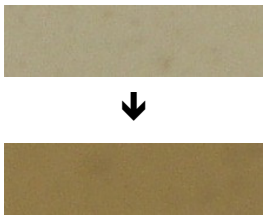




- Darkening, slight reddening, strong yellowing
- $\Delta E_{ab} = 27.20$

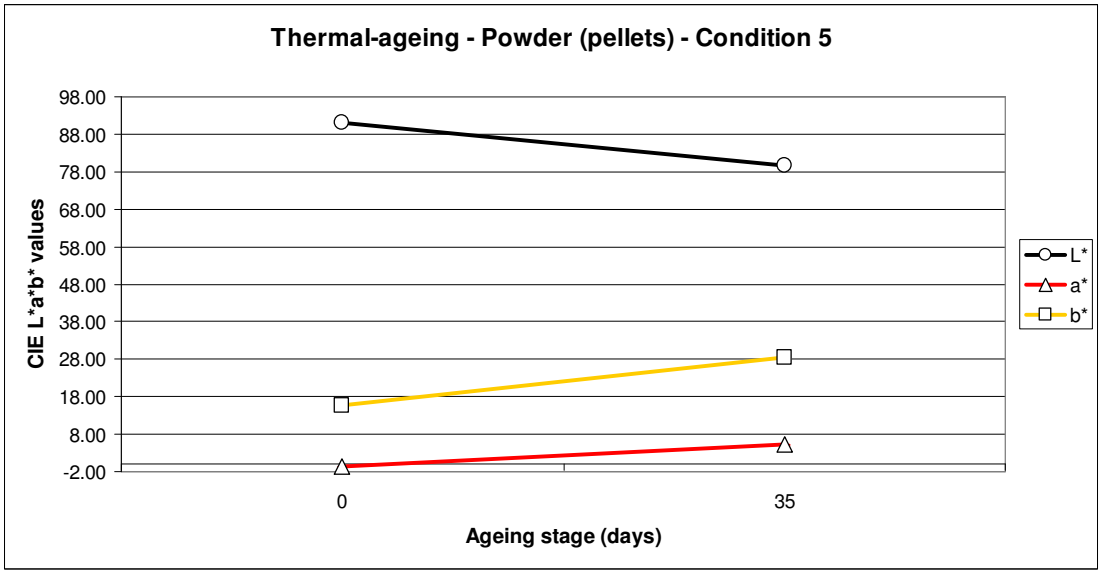
Figure 9. Visual and colour changes in thermal-aged powder in condition 3 (≤ 20% RH, sample exposed to internal atmosphere).

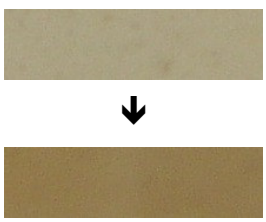




- Darkening, slight reddening, strong yellowing
- ΔE_{ab} = 23.93

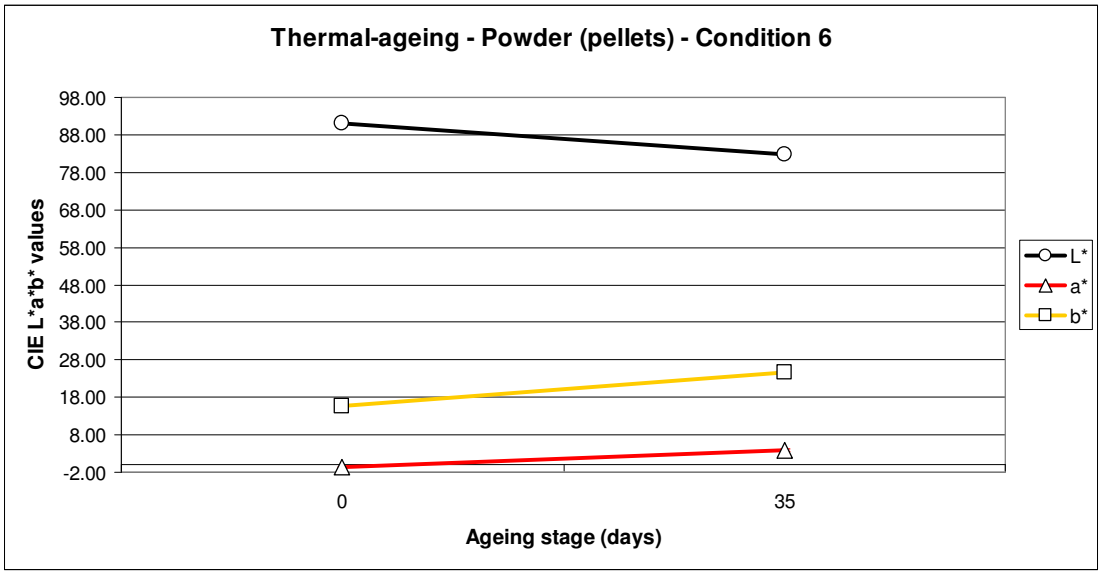
Figure 10. Visual and colour changes in thermal-aged powder in condition 4 (≤ 20% RH, sample exposed to internal hypoxic atmosphere).





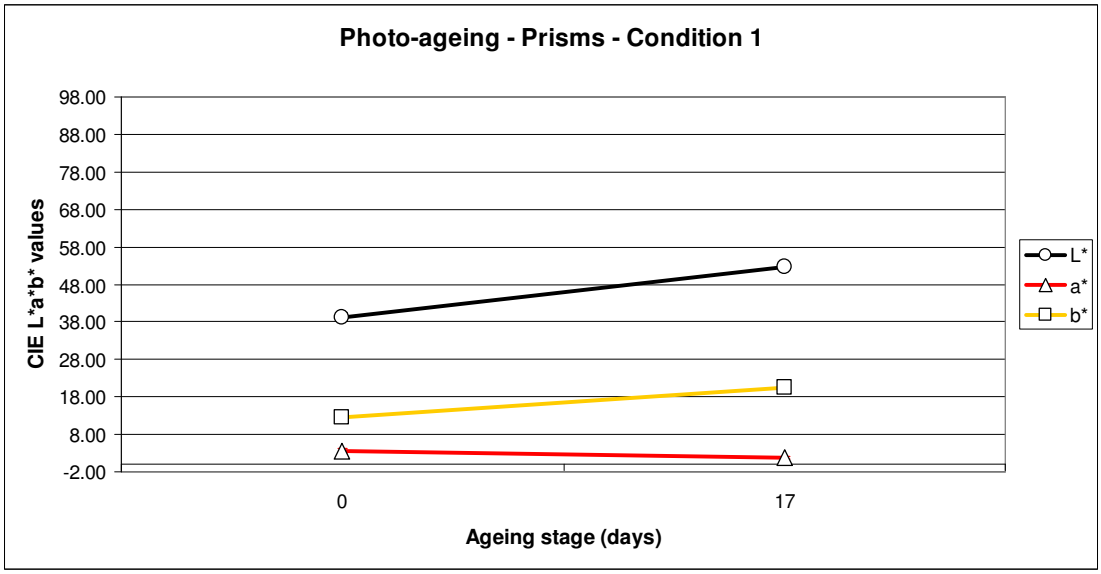
- Darkening, slight reddening, yellowing
- ΔE_{ab} = 17.80

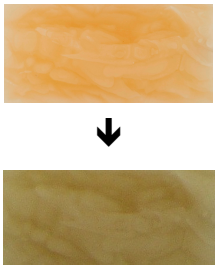
Figure 11. Visual and colour changes in thermal-aged powder in condition 5 (100% RH, sample exposed to internal atmosphere, pH 4).



- Darkening, slight reddening, yellowing
- ΔE_{ab} = 12.61

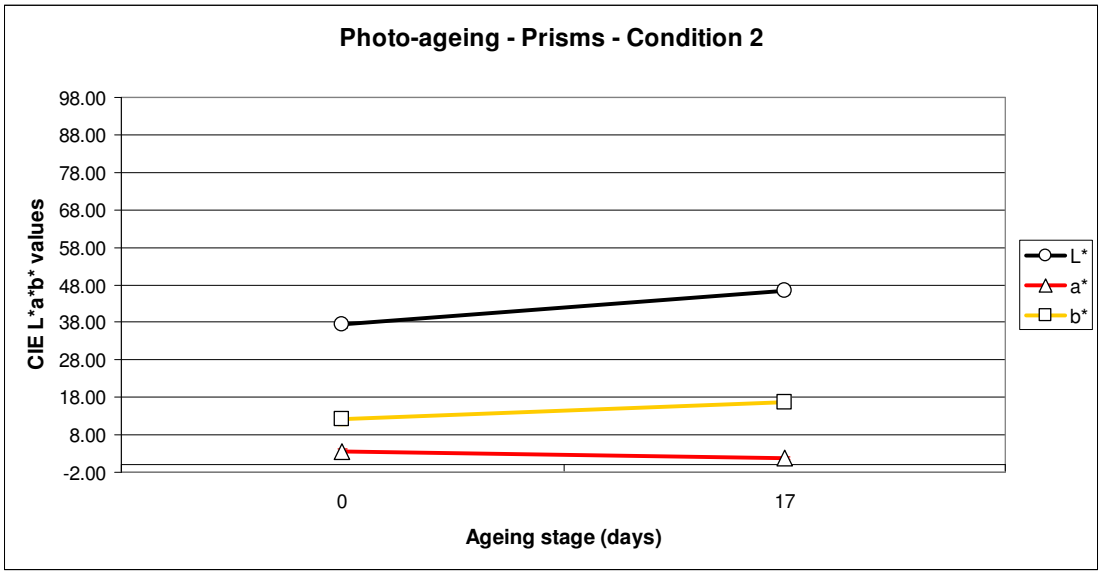
Figure 12. Visual and colour changes in thermal-aged powder in condition 6 (100% RH, sample exposed to internal atmosphere, pH 10).





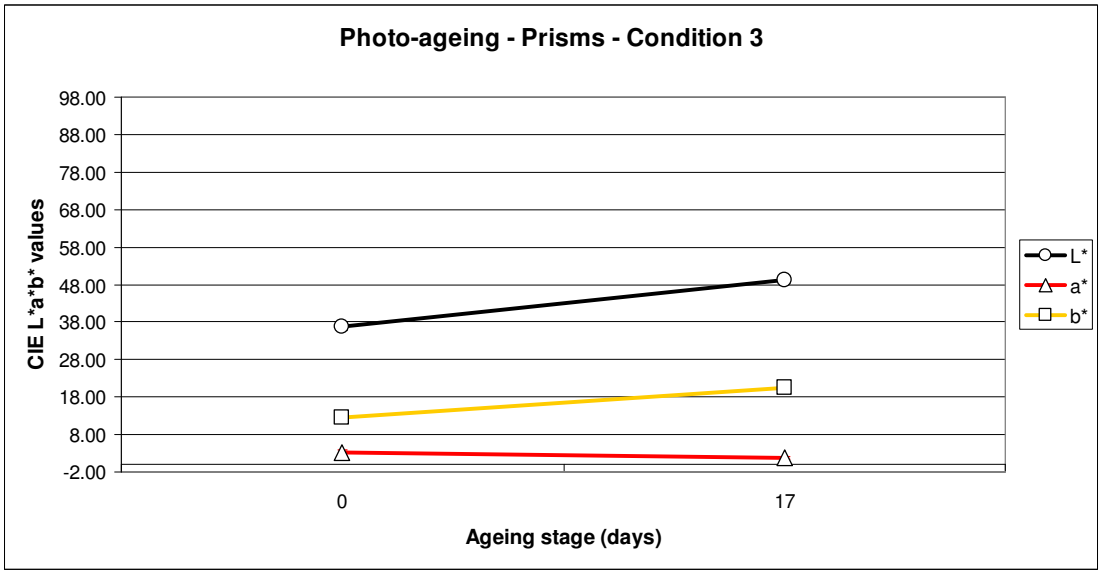
- Lightening, yellowing
- ΔE_{ab} = 15.67

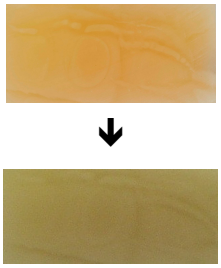
Figure 13. Visual and colour changes in photo-aged prisms in condition 1 (≤ 20% RH, sample exposed to external atmosphere).



- Lightening, yellowing
- $\Delta E_{ab} = 10.35$

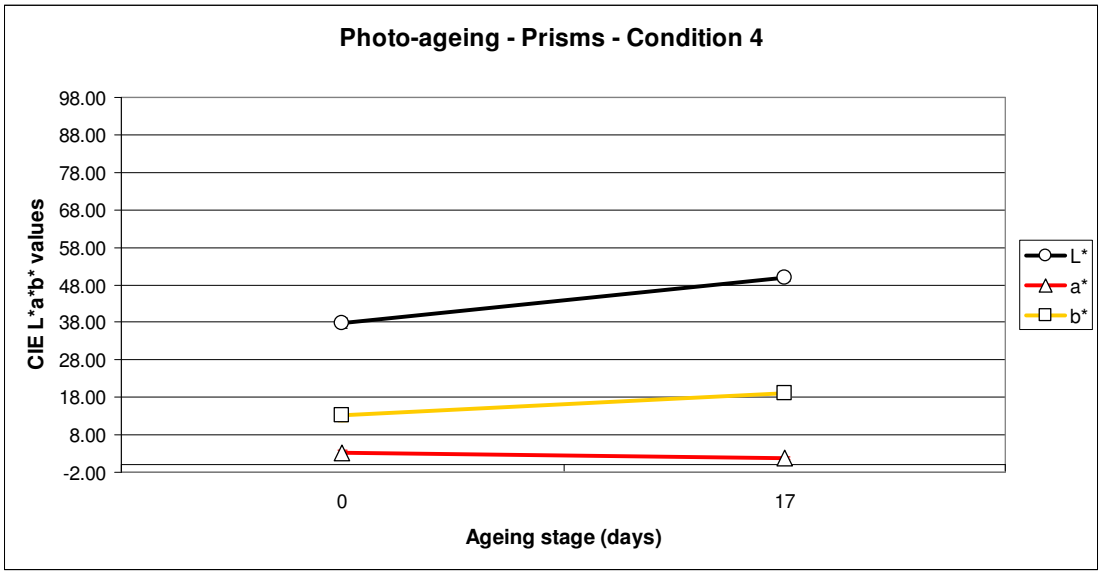
Figure 14. Visual and colour changes in photo-aged prisms in condition 2 (100% RH, sample exposed to internal atmosphere, pH ≤ 5.5).

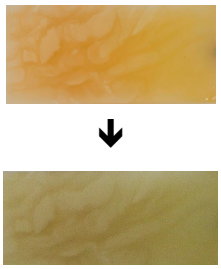




- Lightening, yellowing
- $\Delta E_{ab} = 14.99$

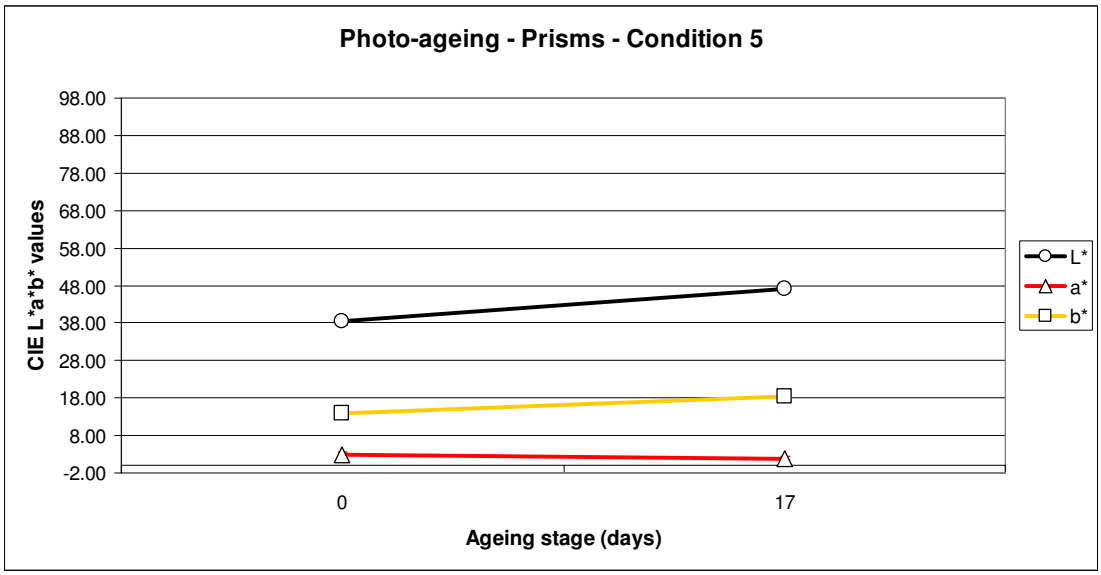
Figure 15. Visual and colour changes in photo-aged prisms in condition 3 (≤ 20% RH, sample exposed to internal atmosphere).

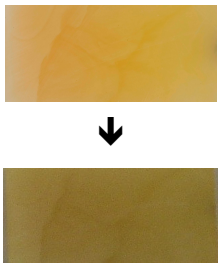




- Lightening, yellowing
- $\Delta E_{ab} = 13.54$

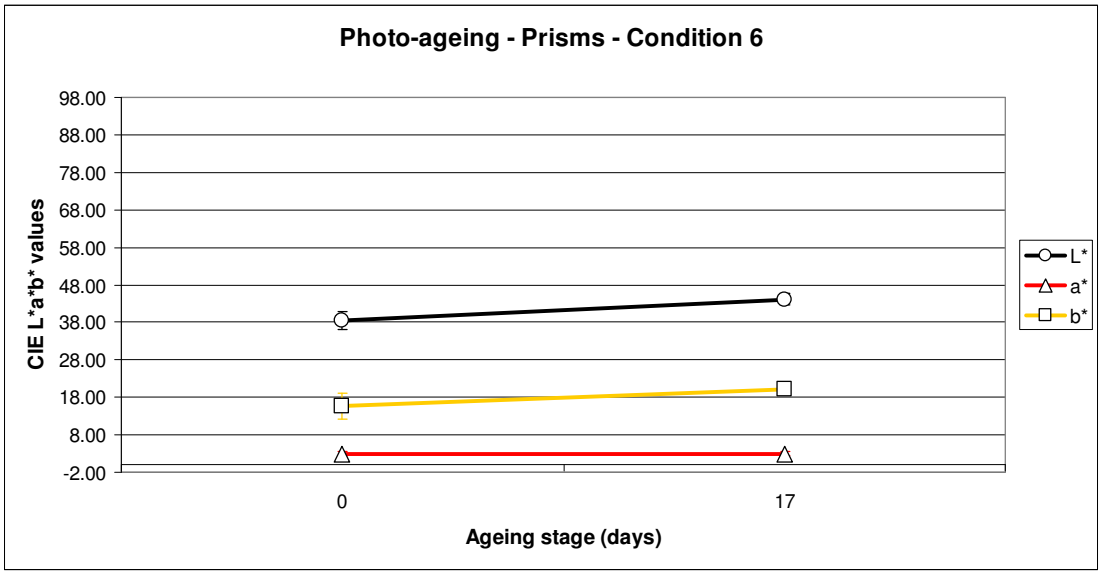
Figure 16. Visual and colour changes in photo-aged prisms in condition 4 (≤ 20% RH, sample exposed to internal hypoxic atmosphere).

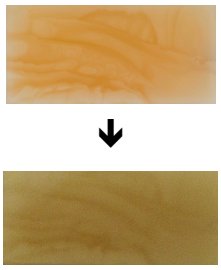




- Lightening, slight yellowing; surface more opaque, almost notched
- $\Delta E_{ab} = 9.77$

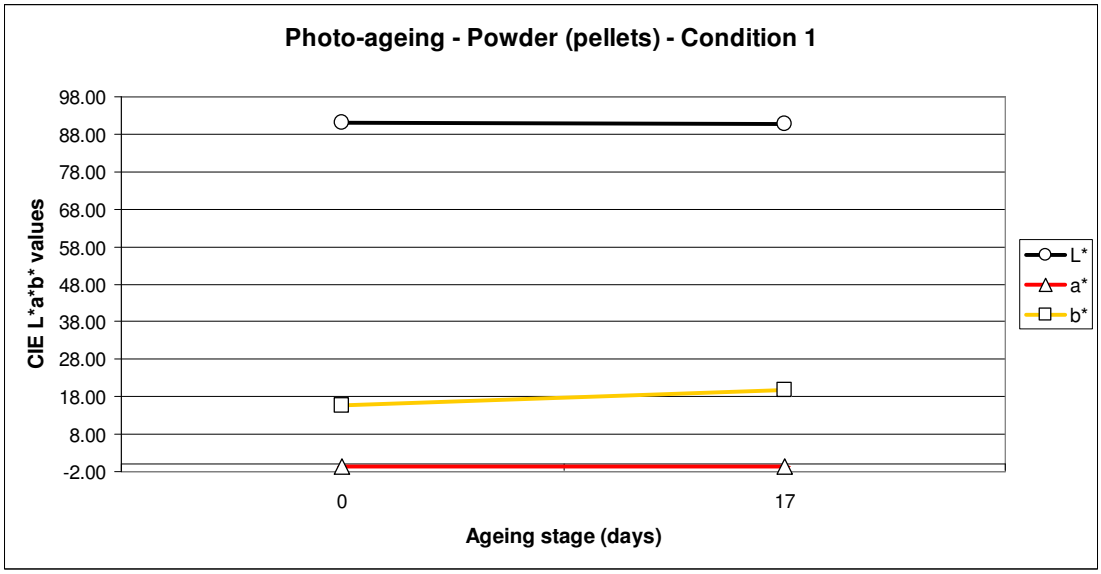
Figure 17. Visual and colour changes in photo-aged prisms in condition 5 (100% RH, sample exposed to internal atmosphere, pH 4).

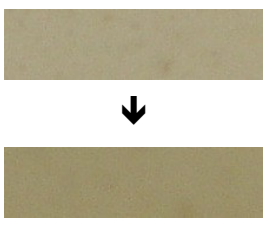




- Lightening; surface more opaque, almost notched
- $\Delta E_{ab} = 10.38$

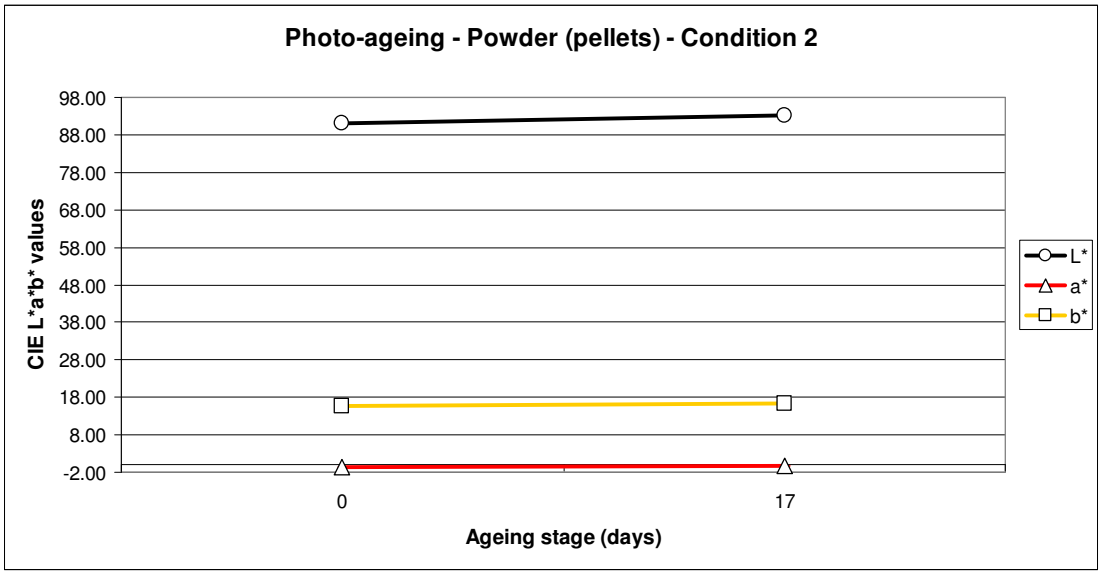
Figure 18. Visual and colour changes in photo-aged prisms in condition 6 (100% RH, sample exposed to internal atmosphere, pH 10).

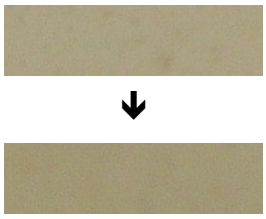




- Slight yellowing
- $\Delta E_{ab} = 3.96$

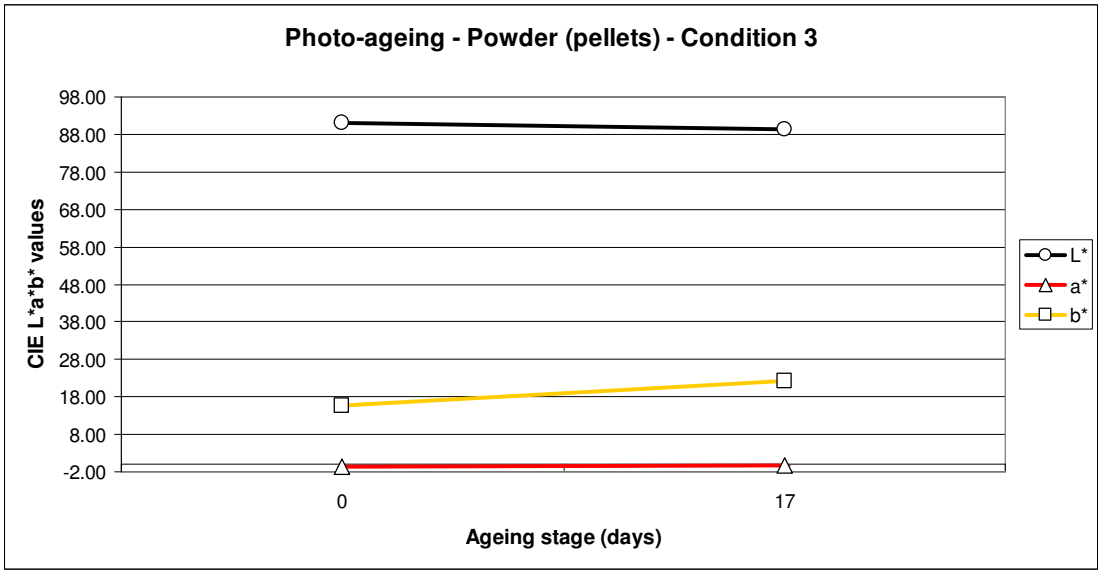
Figure 19. Visual and colour changes in photo-aged powder in condition 1 (≤ 20% RH, sample exposed to external atmosphere).

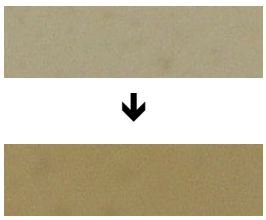




- No significant change
- $\Delta E_{ab} = 2.28$

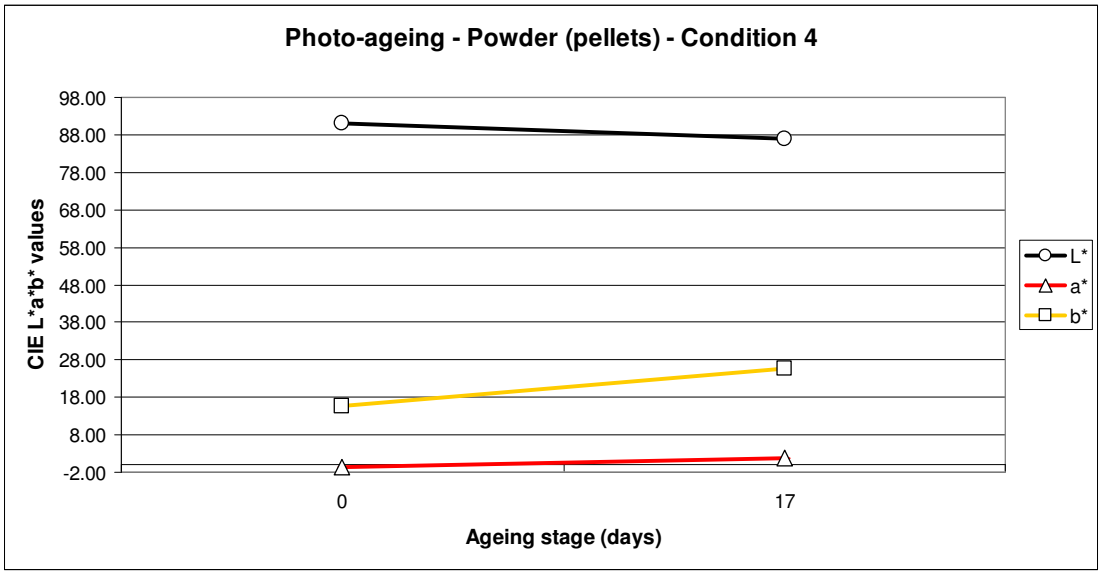
Figure 20. Visual and colour changes in photo-aged powder in condition 2 (100% RH, sample exposed to internal atmosphere, pH ≤ 5.5).

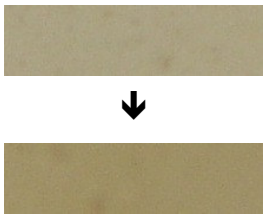




- Slight yellowing
- $\Delta E_{ab} = 6.75$

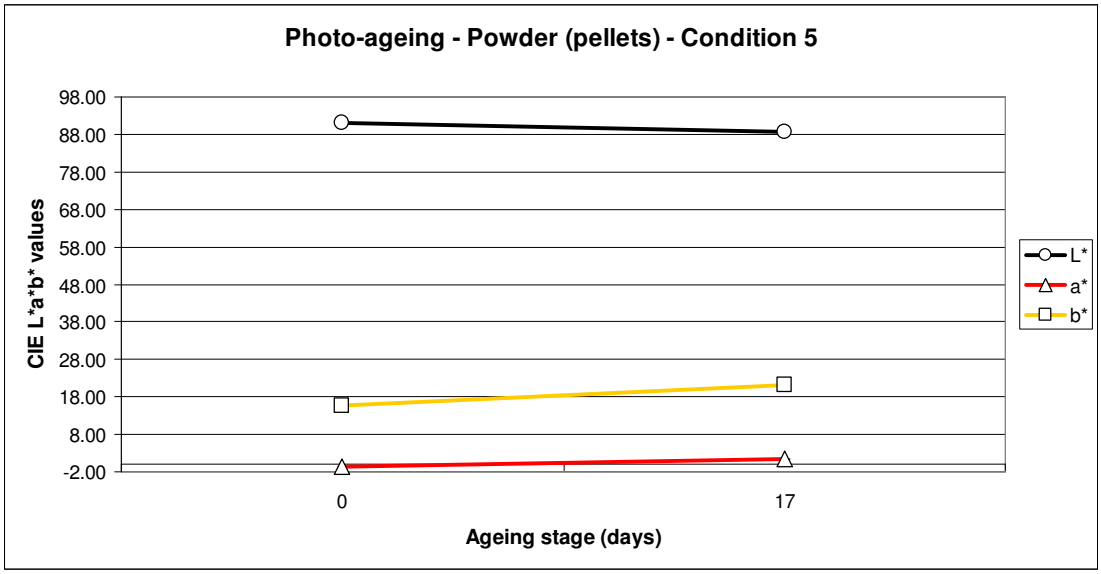
Figure 21. Visual and colour changes in photo-aged powder in condition 3 (≤ 20% RH, sample exposed to internal atmosphere).

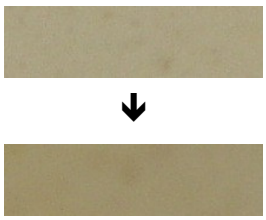




- Yellowing
- $\Delta E_{ab} = 10.92$

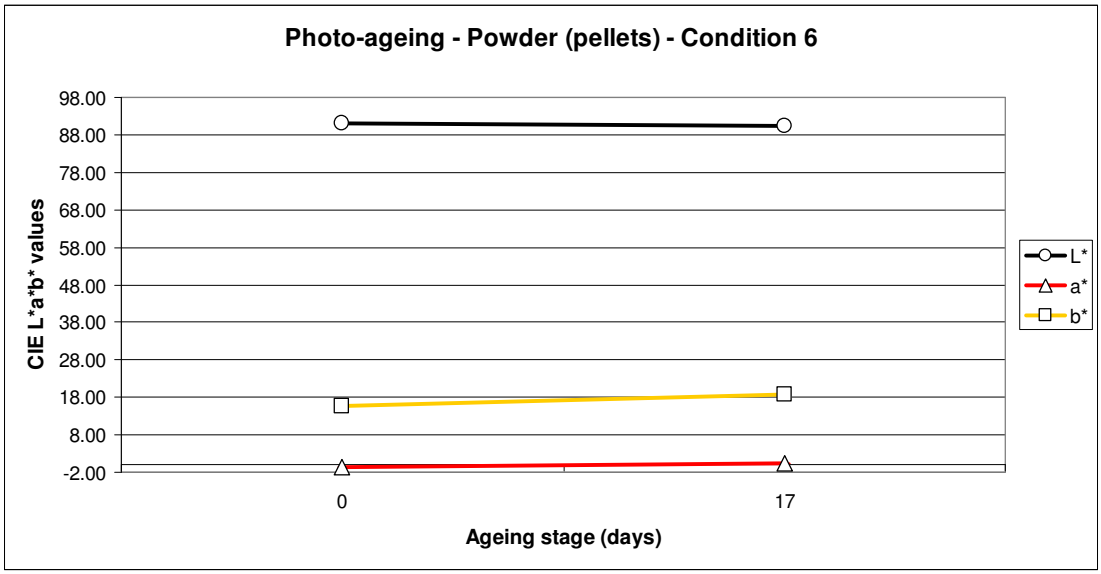
Figure 22. Visual and colour changes in photo-aged powder in condition 4 (≤ 20% RH, sample exposed to internal hypoxic atmosphere).

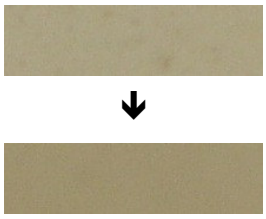




- Slight yellowing
- $\Delta E_{ab} = 6.03$

Figure 23. Visual and colour changes in photo-aged powder in condition 5 (100% RH, sample exposed to internal atmosphere, pH 4).





- No significant change
- $\Delta E_{ab} = 2.95$

Figure 24. Visual and colour changes in photo-aged powder in condition 6 (100% RH, sample exposed to internal atmosphere, pH 10).

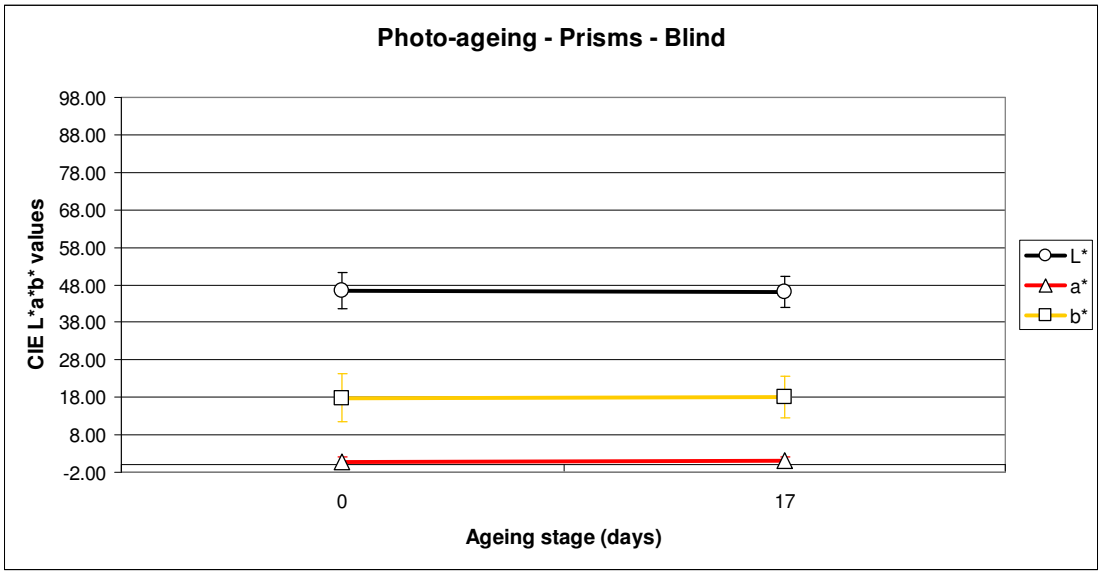


Figure 25. Colour changes in photo-aged prisms in blind condition.

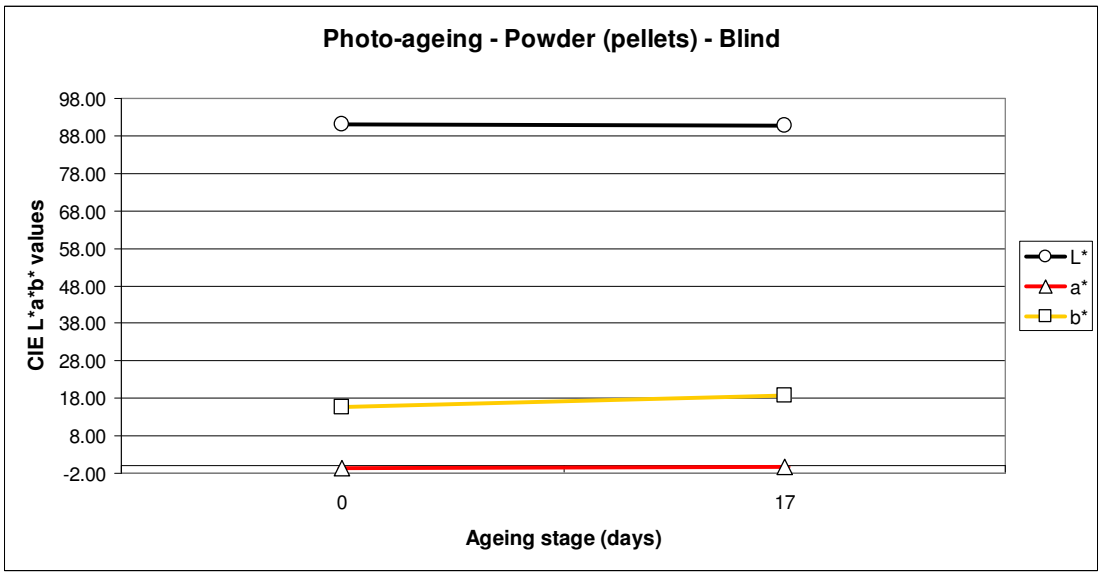
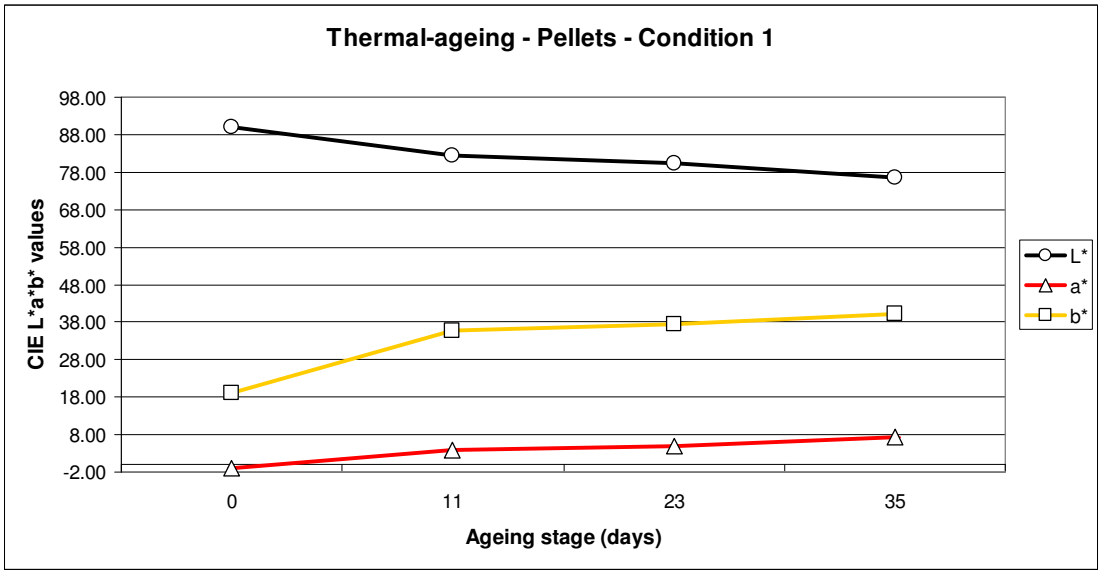
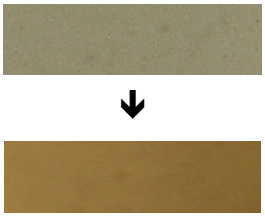


Figure 26. Colour changes in photo-aged powder in blind condition.

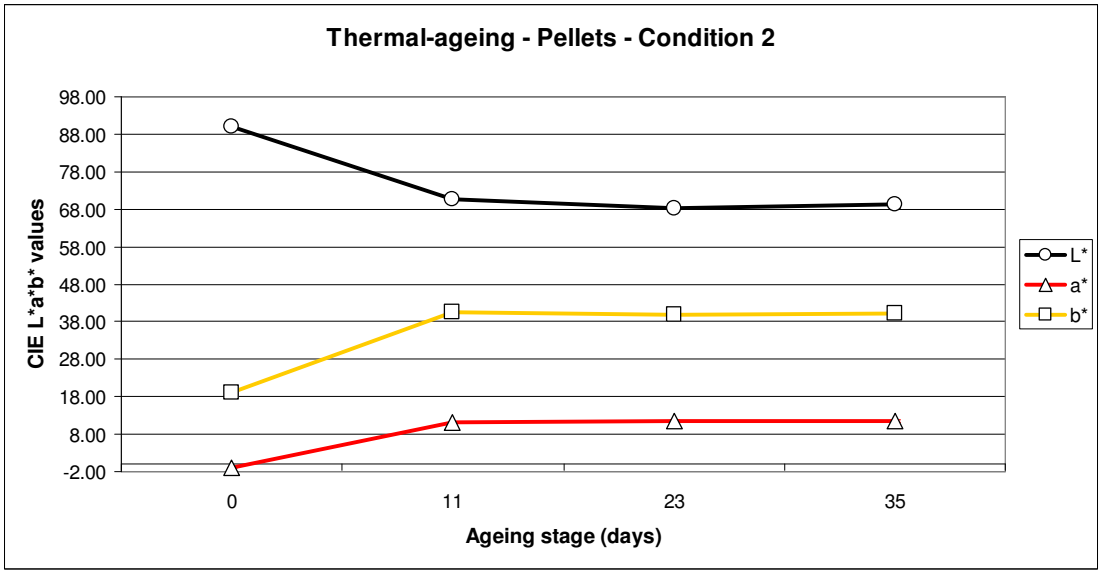
Advanced investigation:

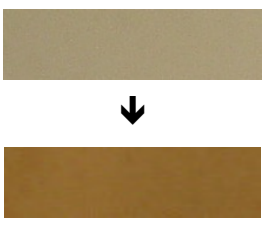




- Darkening, reddening, yellowing
- $\Delta E_{ab} = 26.12$

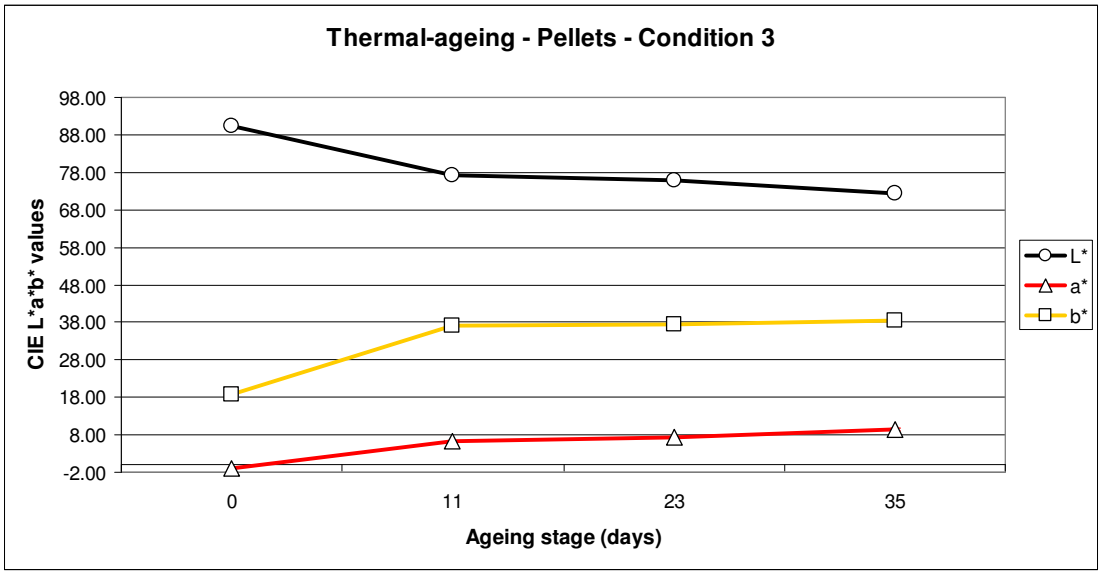
Figure 27. Visual and colour changes in thermal-aged pellets in condition 1 (≤ 20% RH, sample exposed to external atmosphere).

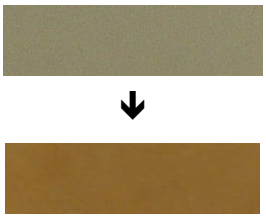




- Darkening, reddening, yellowing
- $\Delta E_{ab} = 32.28$

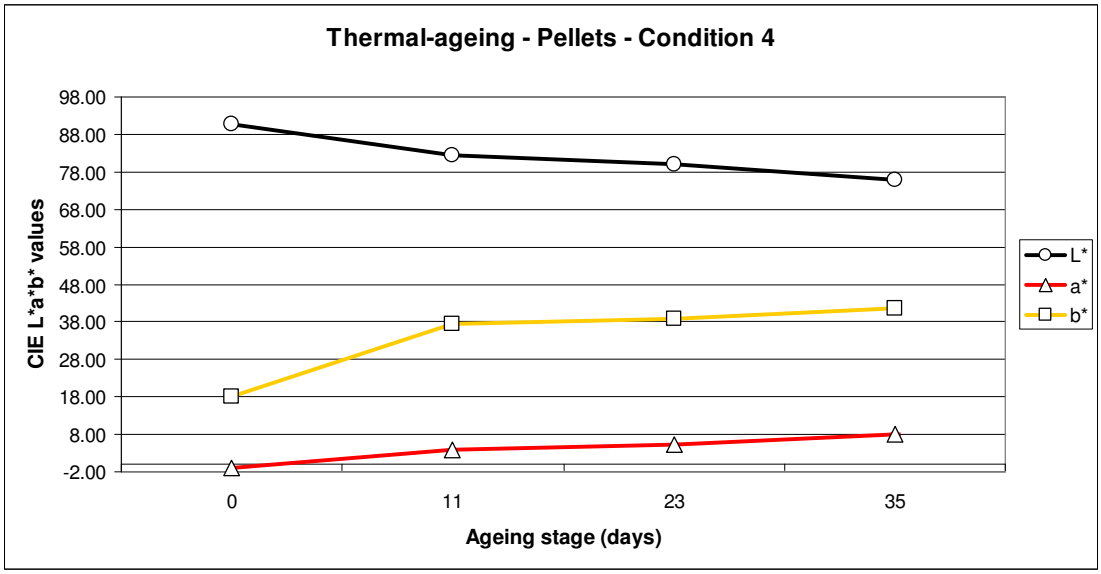
Figure 28. Visual and colour changes in thermal-aged pellets in condition 2 (100% RH, sample exposed to internal atmosphere, pH ≤ 5.5).

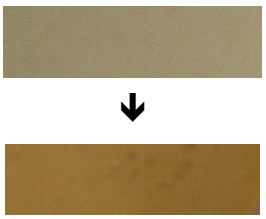




- Darkening, reddening, yellowing
- $\Delta E_{ab} = 28.75$

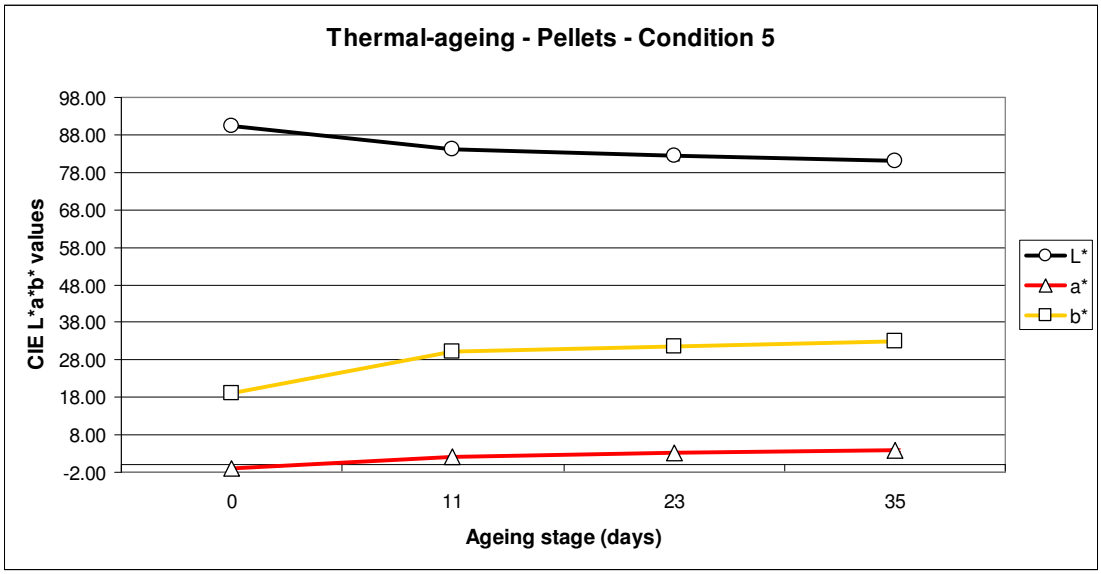
Figure 29. Visual and colour changes in thermal-aged pellets in condition 3 (100% RH, sample immersed in liquid, pH ≤ 5.5).

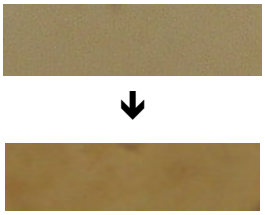




- Darkening, reddening, yellowing
- $\Delta E_{ab} = 29.23$

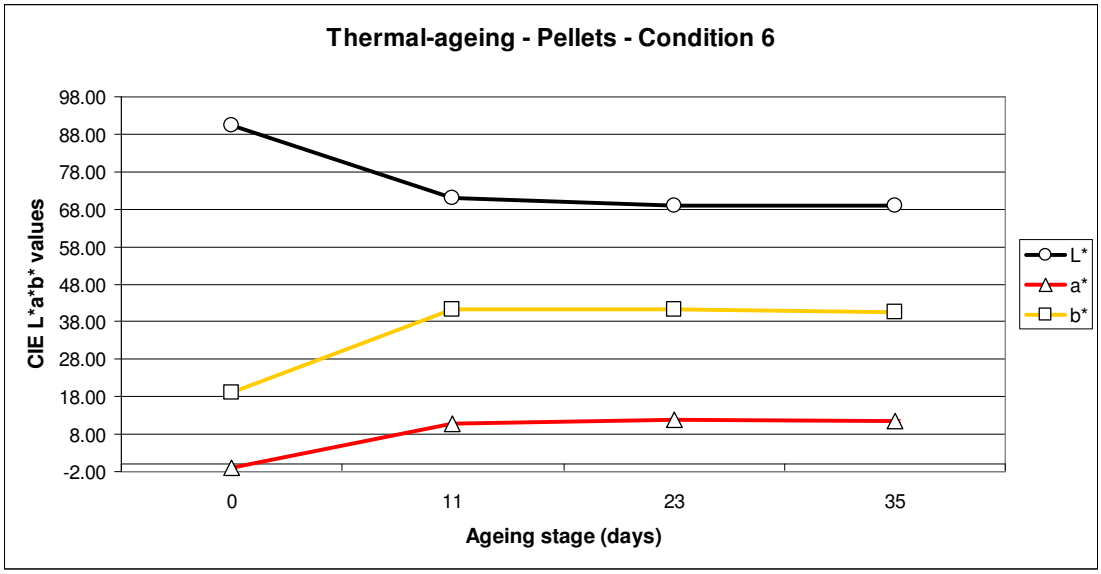
Figure 30. Visual and colour changes in thermal-aged pellets in condition 4 (≤ 20% RH, sample exposed to internal atmosphere).

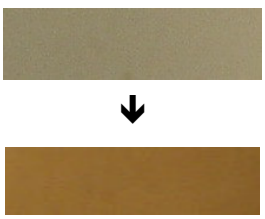




- Slight darkening, slight reddening, slight yellowing
- $\Delta E_{ab} = 17.40$

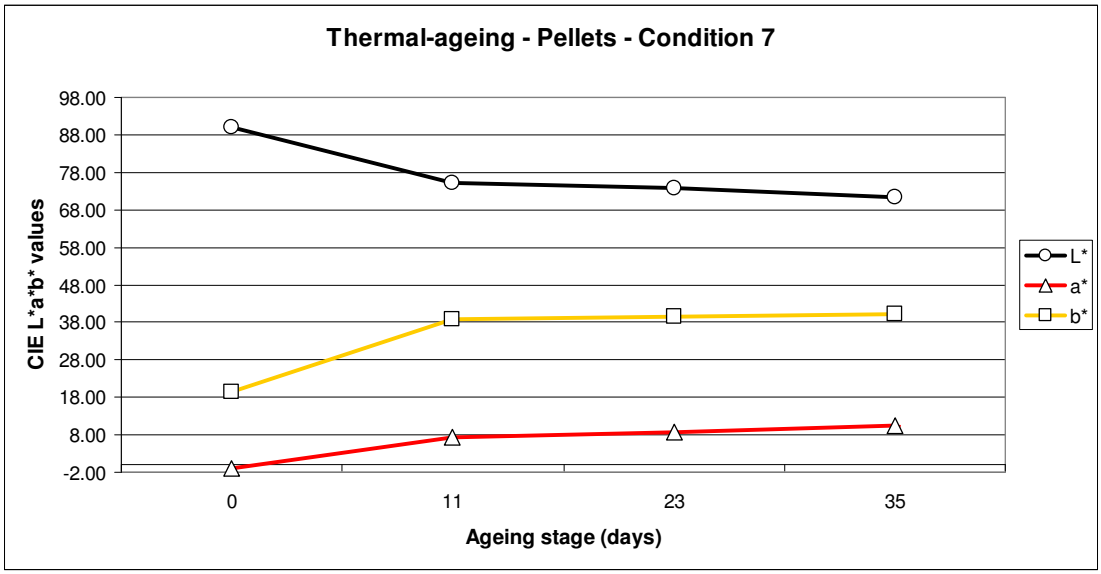
Figure 31. Visual and colour changes in thermal-aged pellets in condition 5 (≤ 20% RH, sample exposed to internal anoxic atmosphere).

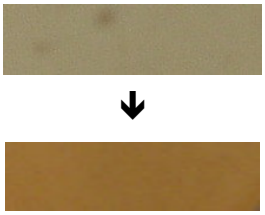




- Darkening, reddening, yellowing
- $\Delta E_{ab} = 32.80$

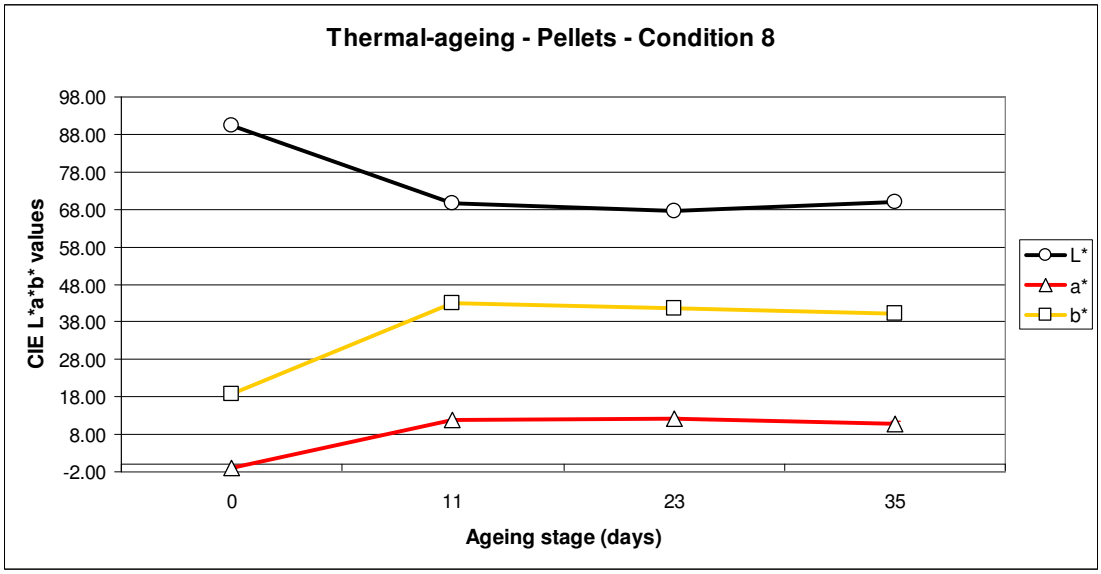
Figure 32. Visual and colour changes in thermal-aged pellets in condition 6 (100% RH, sample exposed to internal atmosphere, pH 3).

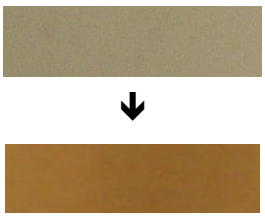




- Darkening, reddening, yellowing
- $\Delta E_{ab} = 30.13$

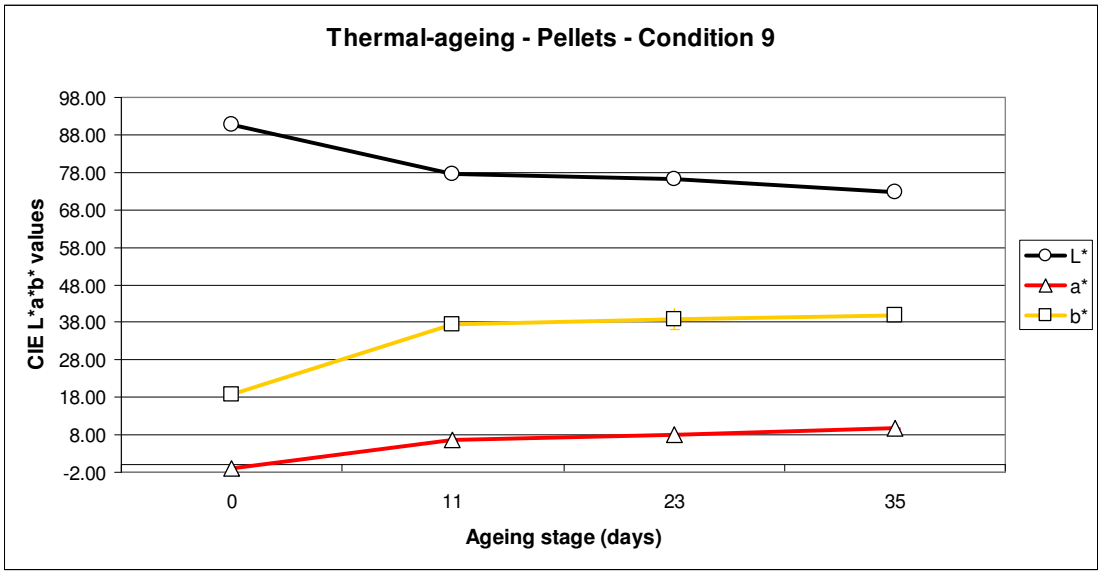
Figure 33. Visual and colour changes in thermal-aged pellets in condition 7 (100% RH, sample immersed in liquid, pH 3).

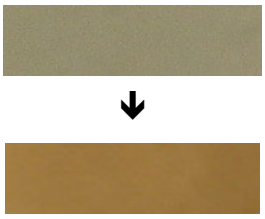




- Darkening, reddening, yellowing
- $\Delta E_{ab} = 31.64$

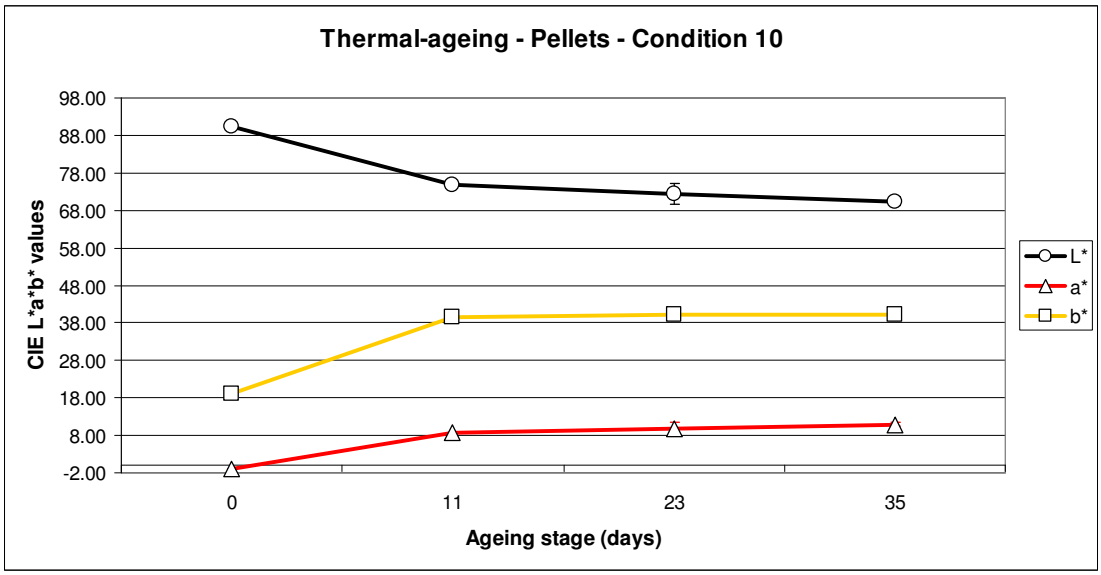
Figure 34. Visual and colour changes in thermal-aged pellets in condition 8 (100% RH, sample exposed to internal atmosphere, pH 5).

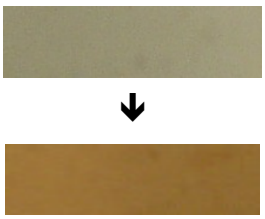




- Darkening, reddening, yellowing
- ΔE_{ab} = 29.51

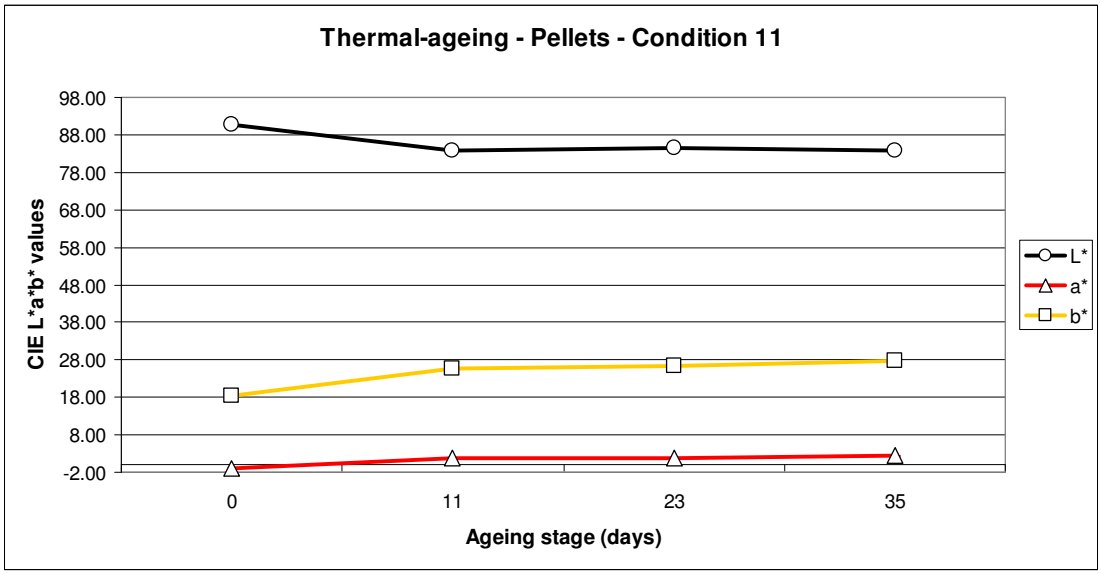
Figure 35. Visual and colour changes in thermal-aged pellets in condition 9 (100% RH, sample immersed in liquid, pH 5).

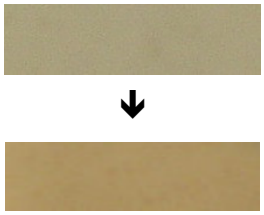




- Darkening, reddening, yellowing
- ΔE_{ab} = 31.34

Figure 36. Visual and colour changes in thermal-aged pellets in condition 10 (100% RH, sample exposed to internal atmosphere, pH 10).





- Slight yellowing; cracks on the surface
- $\Delta E_{ab} = 12.18$

Figure 37. Visual and colour changes in thermal-aged pellets in condition 11 (100% RH, sample immersed in liquid, pH 10).

A.2 ATR-FTIR spectroscopy.

Preliminary investigation:

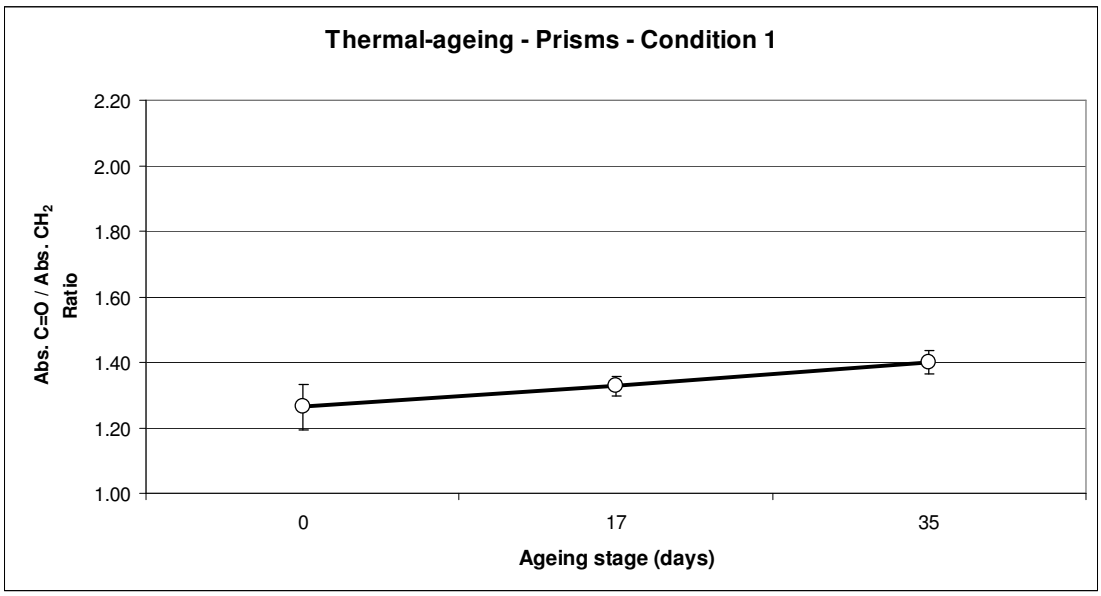


Figure 38. C=O group absorbance change in thermal-aged prisms in condition 1 (≤ 20% RH, sample exposed to external atmosphere).

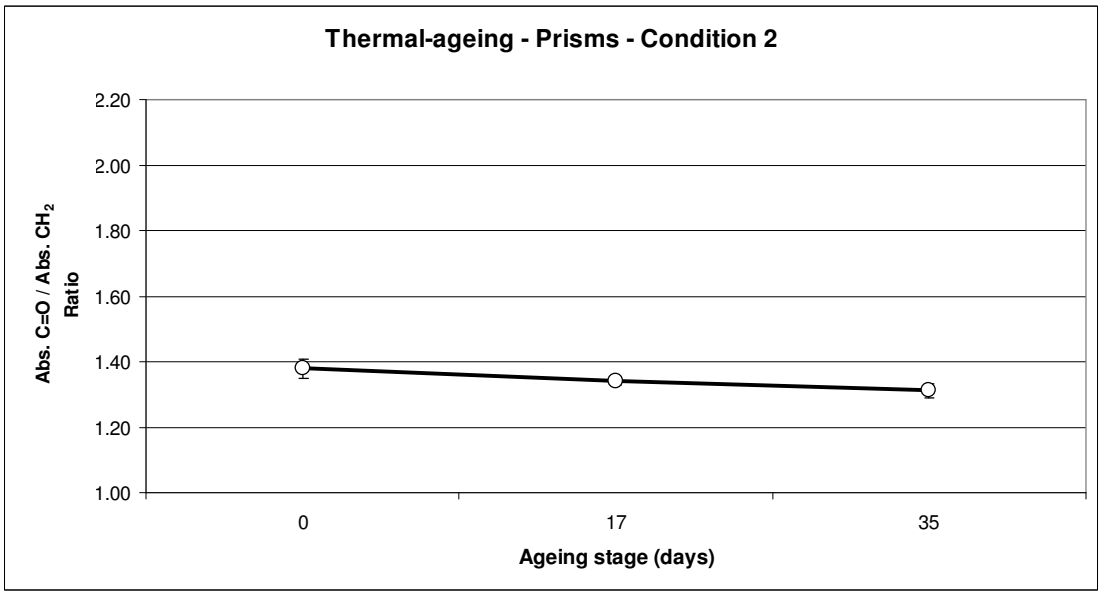


Figure 39. C=O group absorbance change in thermal-aged prisms in condition 2 (100% RH, sample exposed to internal atmosphere, pH ≤ 5.5).

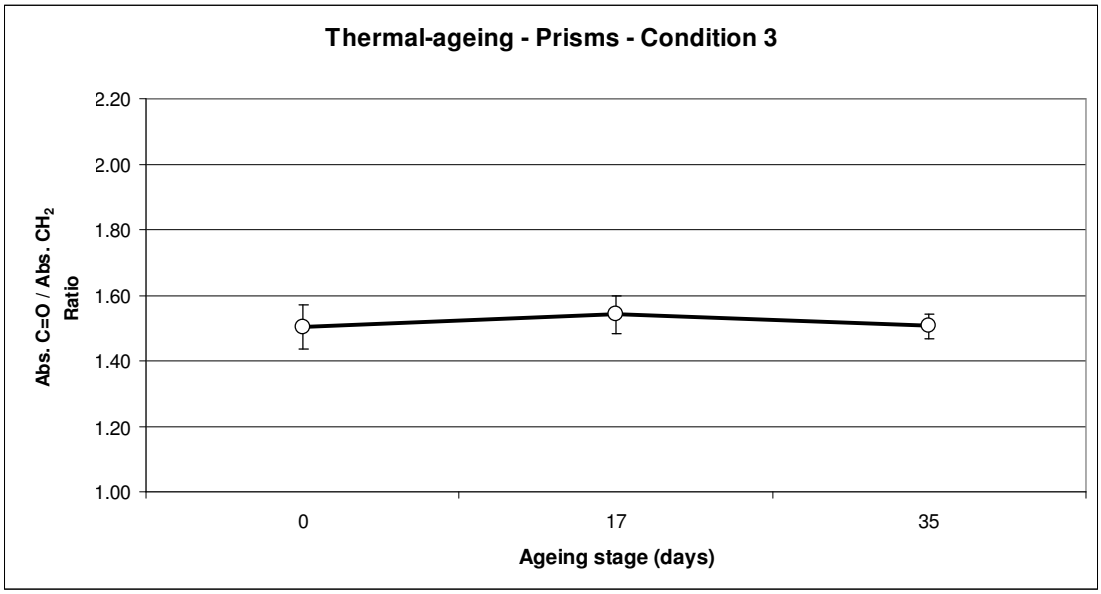


Figure 40. C=O group absorbance change in thermal-aged prisms in condition 3 (≤ 20% RH, sample exposed to internal atmosphere).

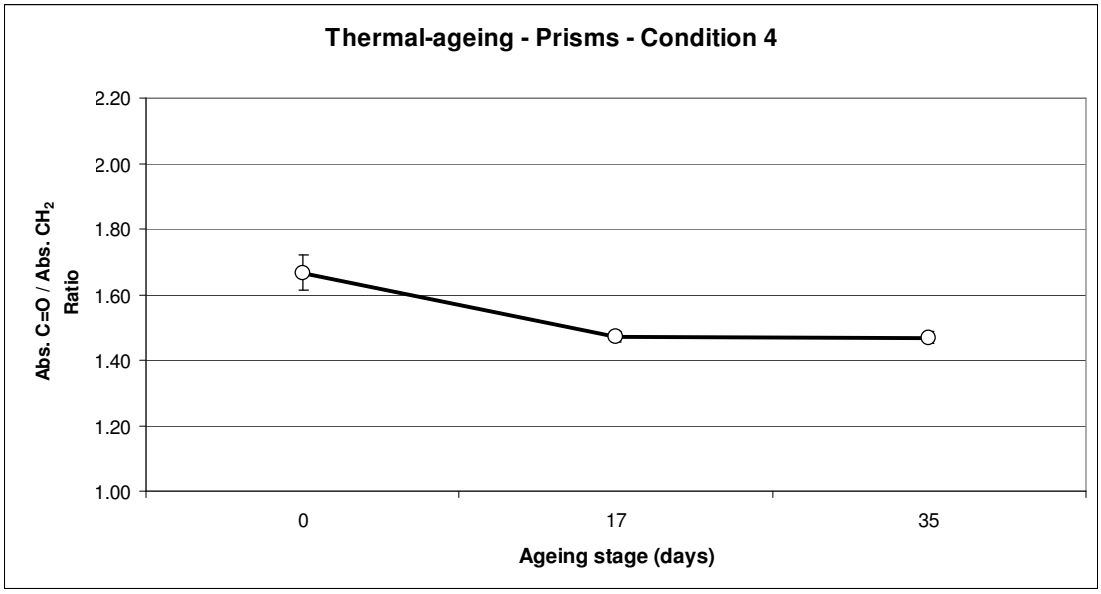


Figure 41. C=O group absorbance change in thermal-aged prisms in condition 4 (≤ 20% RH, sample exposed to internal hypoxic atmosphere).

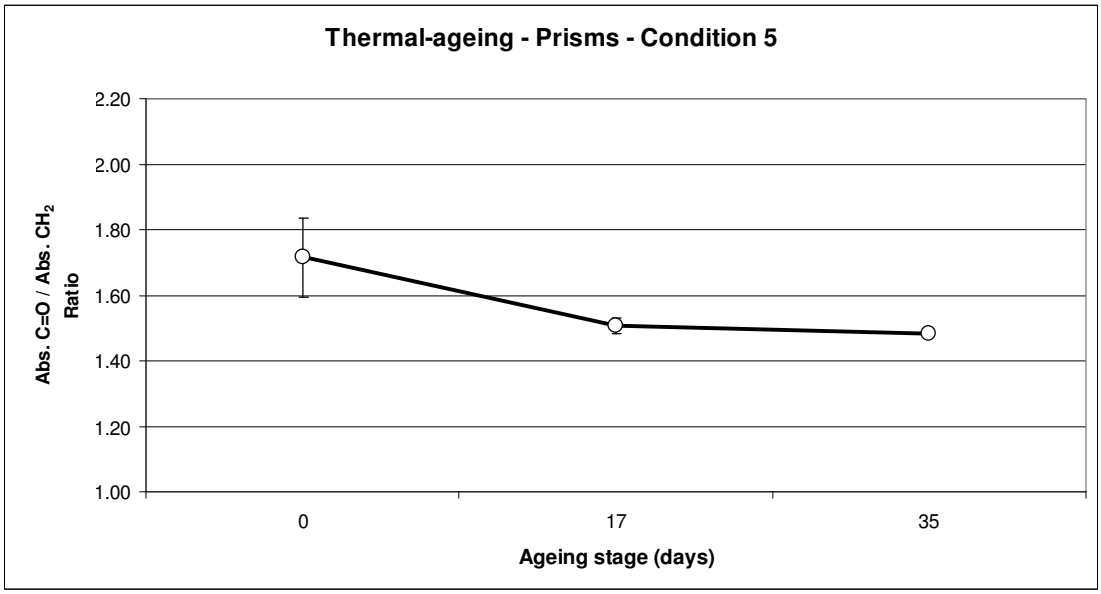


Figure 42. C=O group absorbance change in thermal-aged prisms in condition 5 (100% RH, sample exposed to internal atmosphere, pH 4).

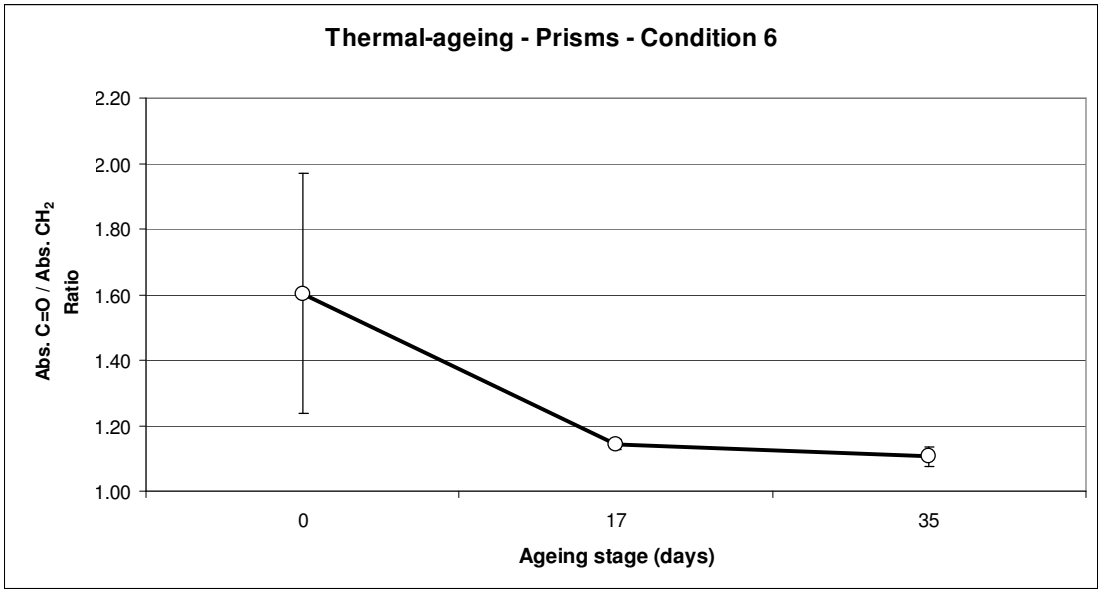


Figure 43. C=O group absorbance change in thermal-aged prisms in condition 6 (100% RH, sample exposed to internal atmosphere, pH 10).

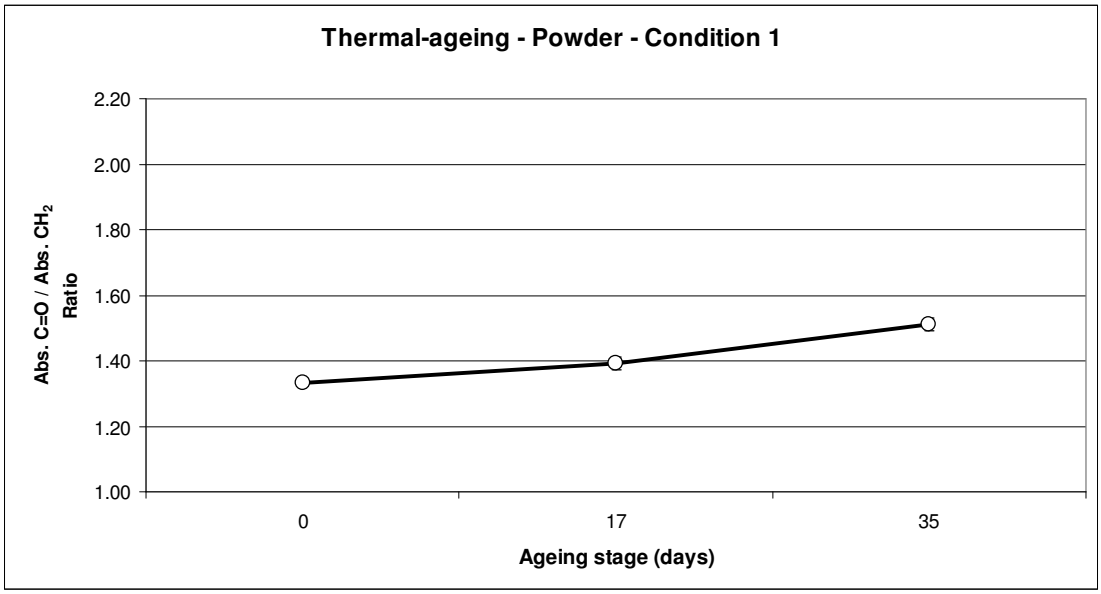


Figure 44. C=O group absorbance change in thermal-aged powder in condition 1 (≤ 20% RH, sample exposed to external atmosphere).

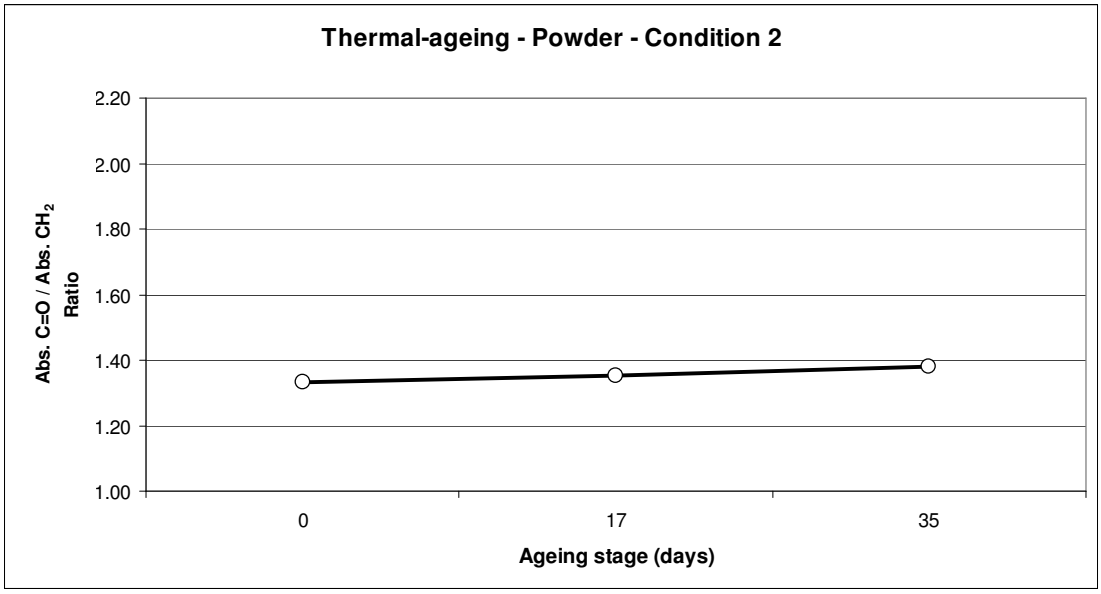


Figure 45. C=O group absorbance change in thermal-aged powder in condition 2 (100% RH, sample exposed to internal atmosphere, pH ≤ 5.5).

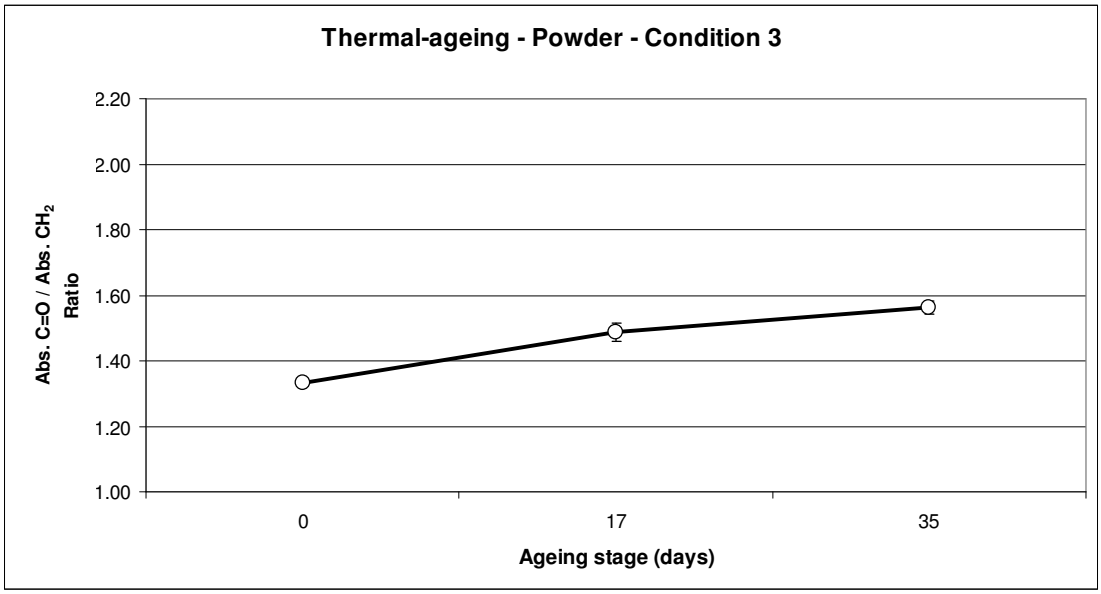


Figure 46. C=O group absorbance change in thermal-aged powder in condition 3 (≤ 20% RH, sample exposed to internal atmosphere).

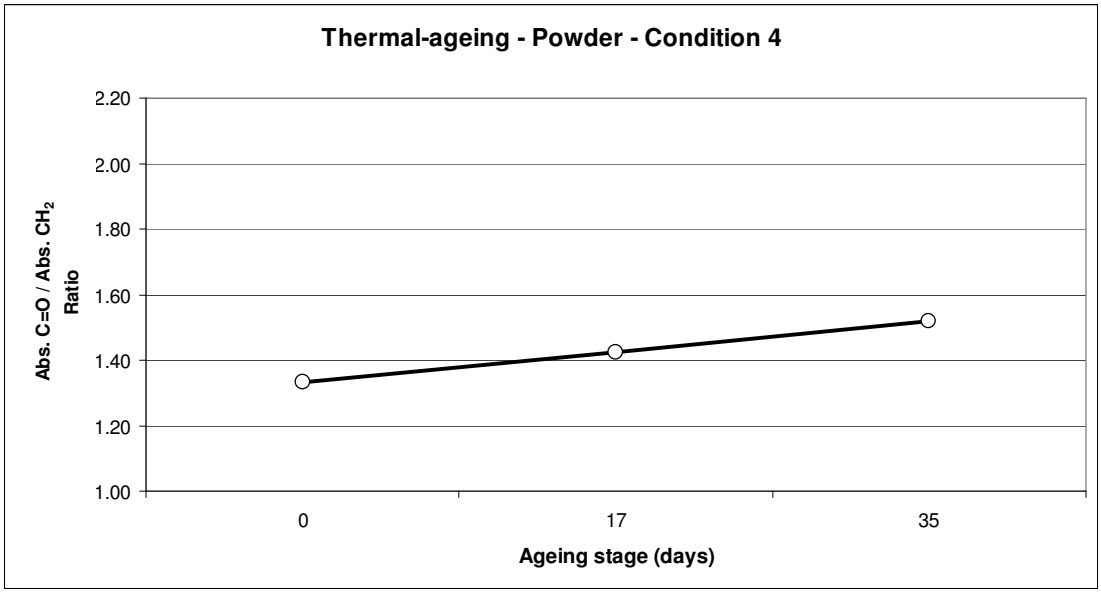


Figure 47. C=O group absorbance change in thermal-aged powder in condition 4 (≤ 20% RH, sample exposed to internal hypoxic atmosphere).

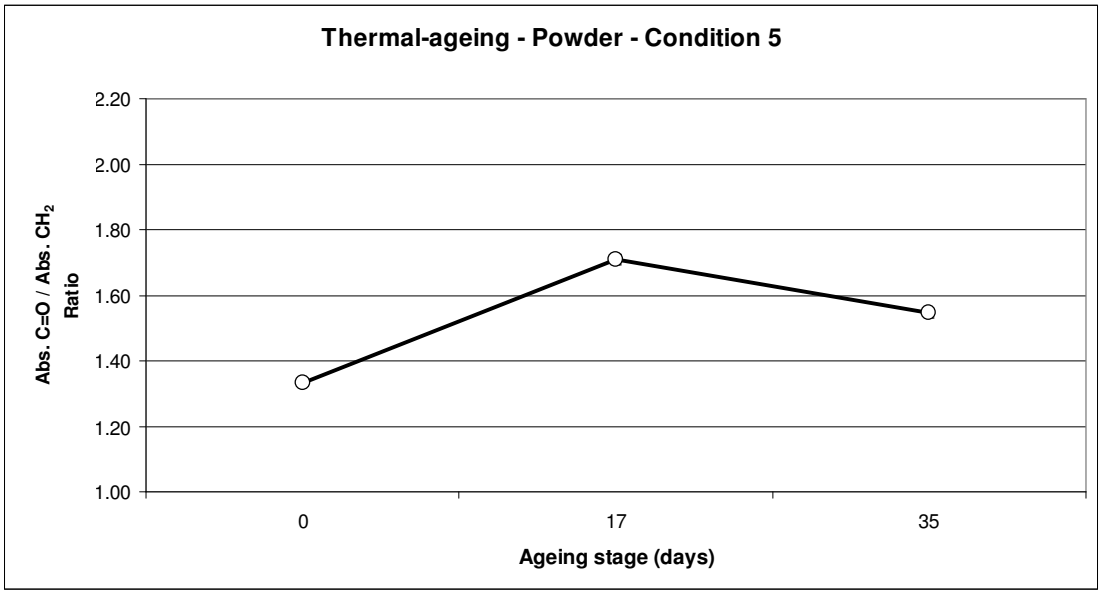


Figure 48. C=O group absorbance change in thermal-aged powder in condition 5 (100% RH, sample exposed to internal atmosphere, pH 4).

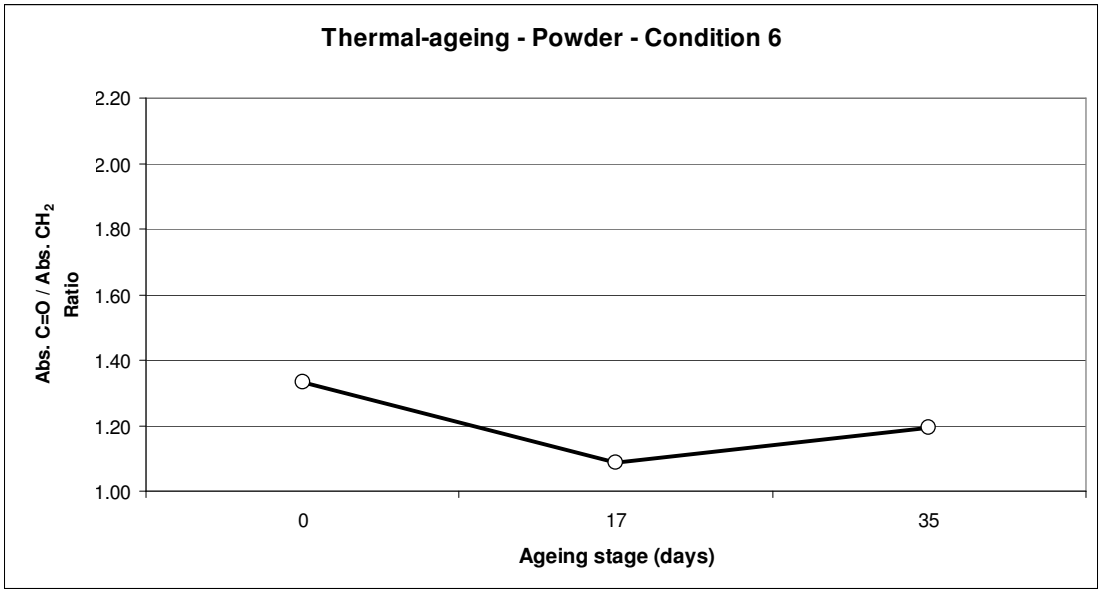


Figure 49. C=O group absorbance change in thermal-aged powder in condition 6 (100% RH, sample exposed to internal atmosphere, pH 10).

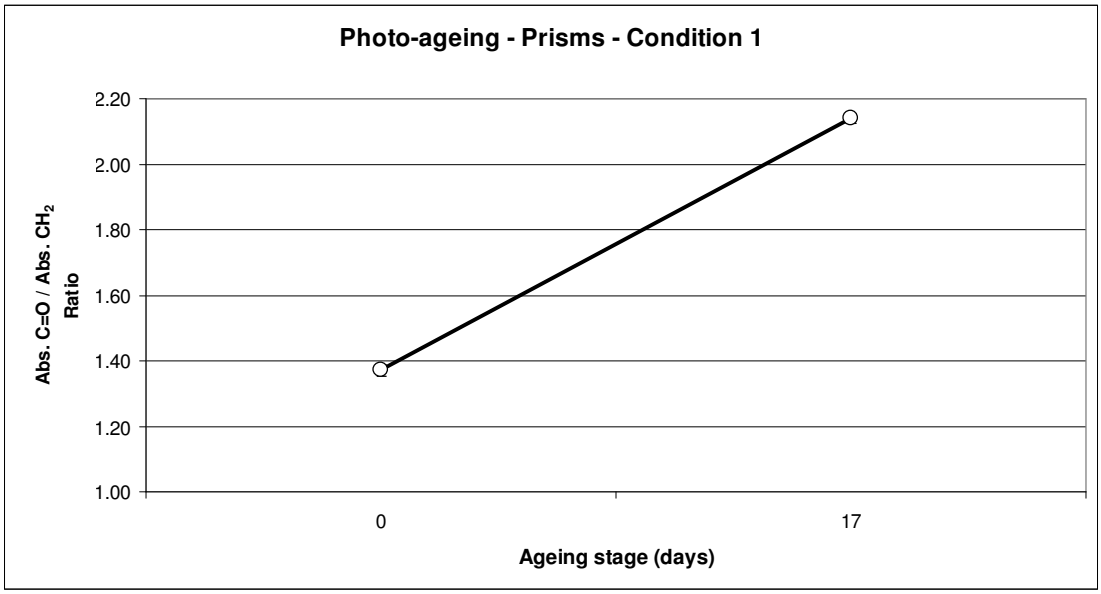


Figure 50. C=O group absorbance change in photo-aged prisms in condition 1 (≤ 20% RH, sample exposed to external atmosphere).

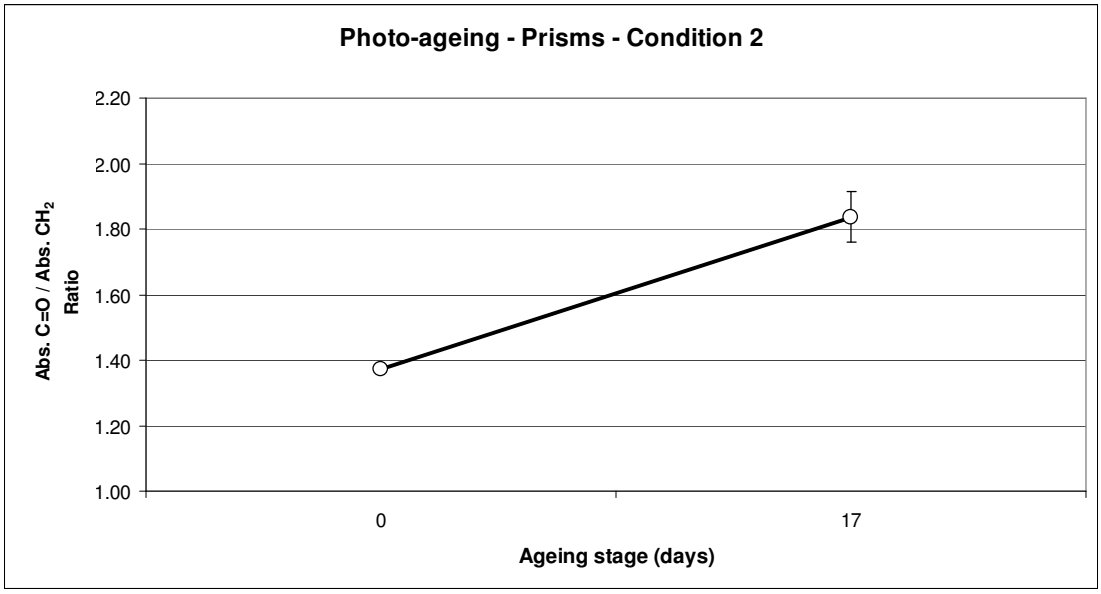


Figure 51. C=O group absorbance change in photo-aged prisms in condition 2 (100% RH, sample exposed to internal atmosphere, pH ≤ 5.5).

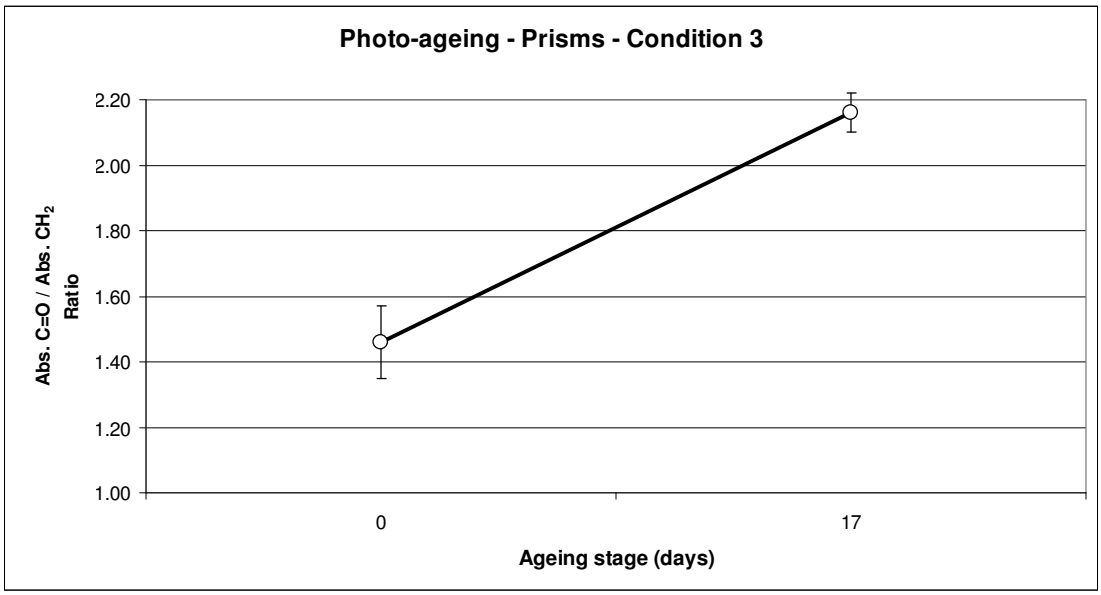


Figure 52. C=O group absorbance change in photo-aged prisms in condition 3 (≤ 20% RH, sample exposed to internal atmosphere).

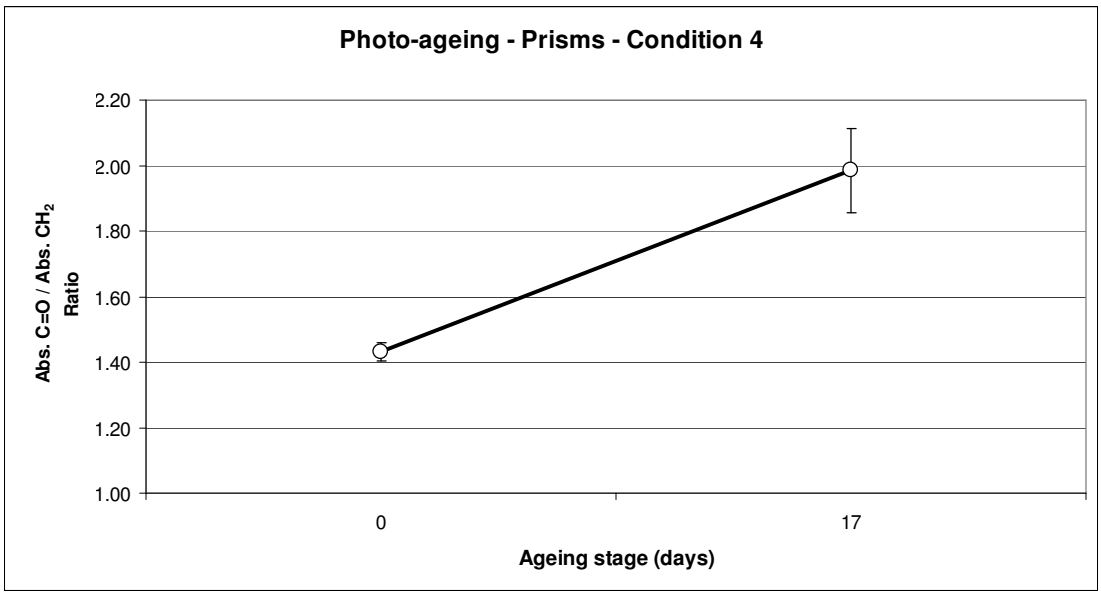


Figure 53. C=O group absorbance change in photo-aged prisms in condition 4 (≤ 20% RH, sample exposed to internal hypoxic atmosphere).

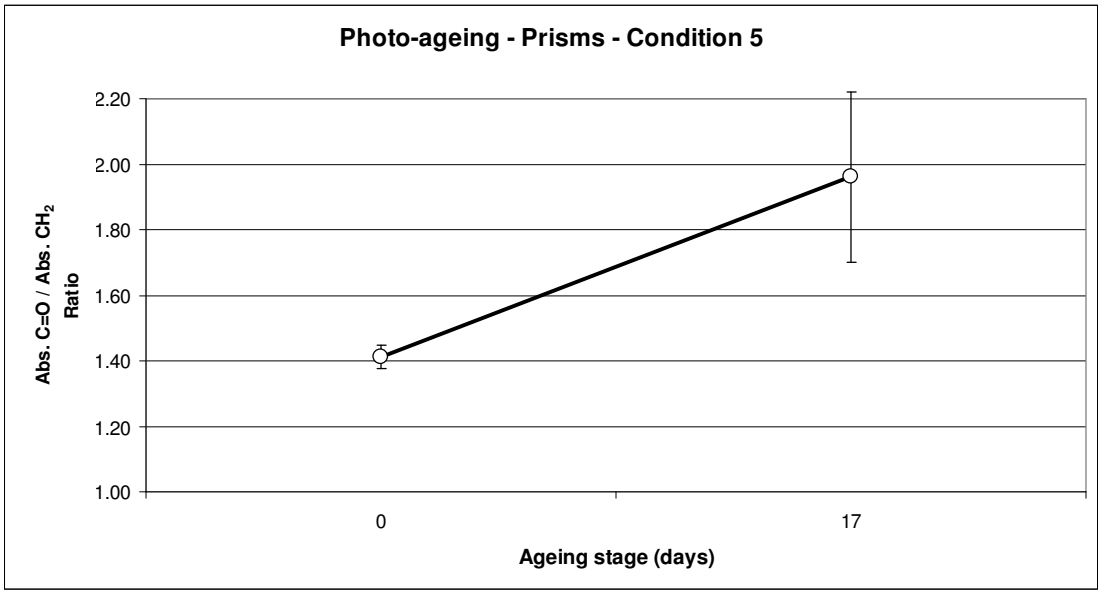


Figure 54. C=O group absorbance change in photo-aged prisms in condition 5 (100% RH, sample exposed to internal atmosphere, pH 4).

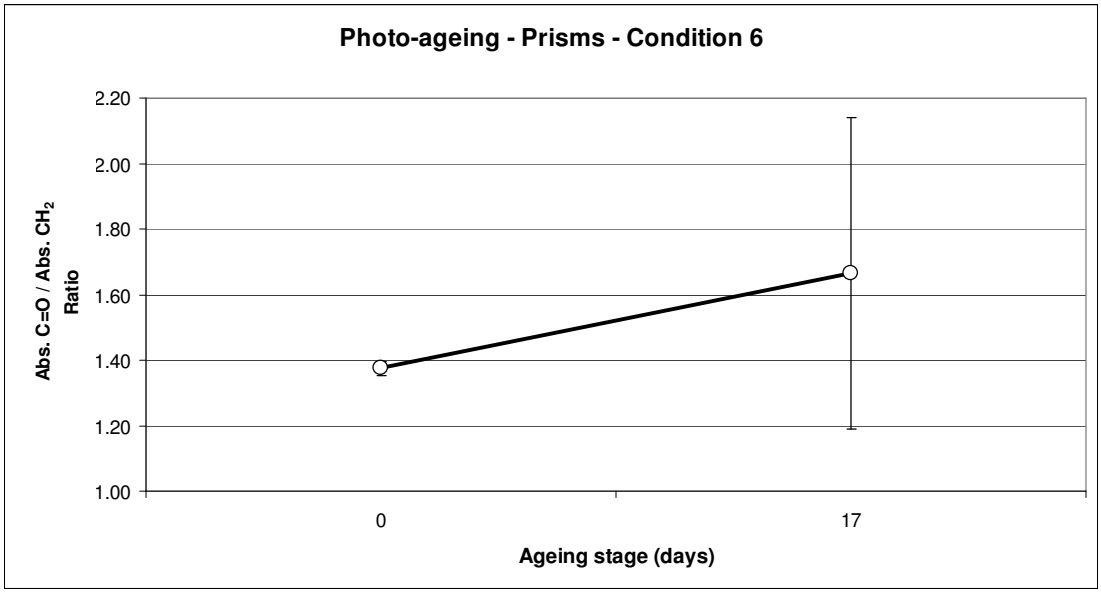


Figure 55. C=O group absorbance change in photo-aged prisms in condition 6 (100% RH, sample exposed to internal atmosphere, pH 10).

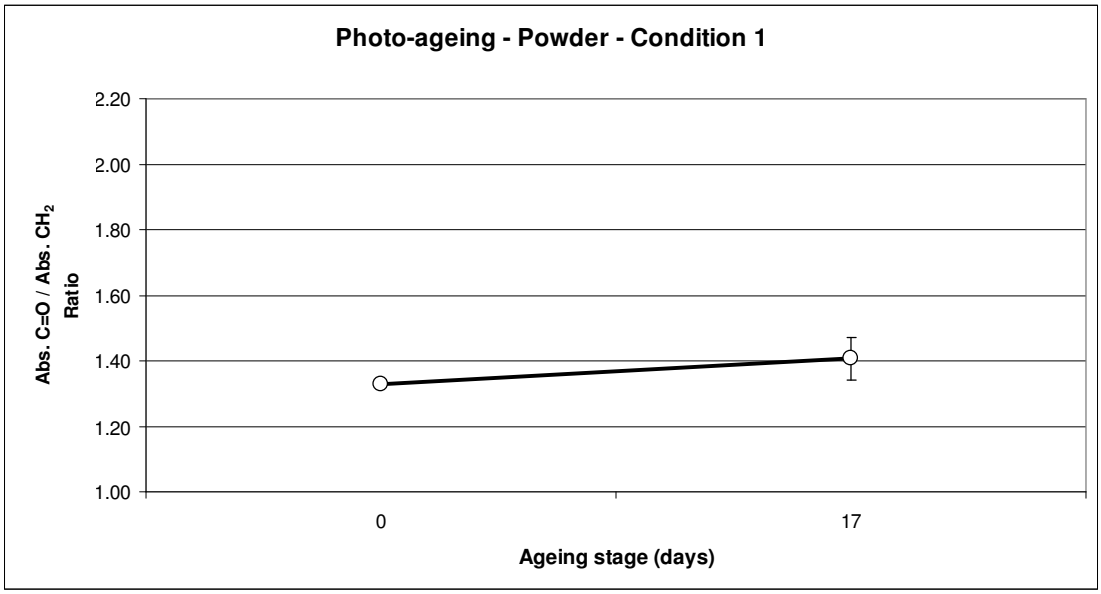


Figure 56. C=O group absorbance change in photo-aged powder in condition 1 (≤ 20% RH, sample exposed to external atmosphere).

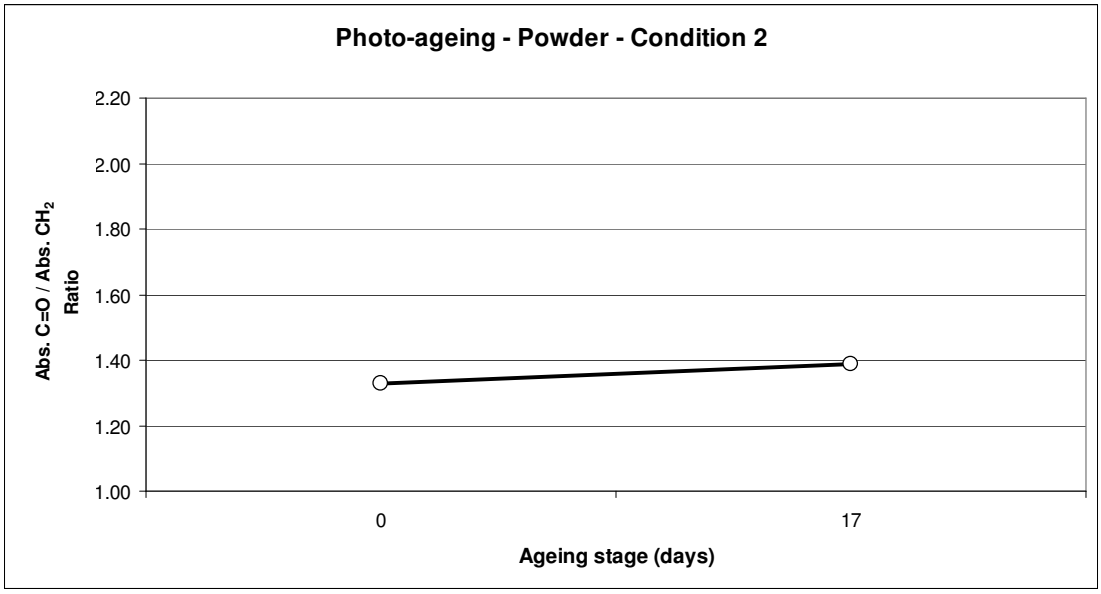


Figure 57. C=O group absorbance change in photo-aged powder in condition 2 (100% RH, sample exposed to internal atmosphere, pH ≤ 5.5).

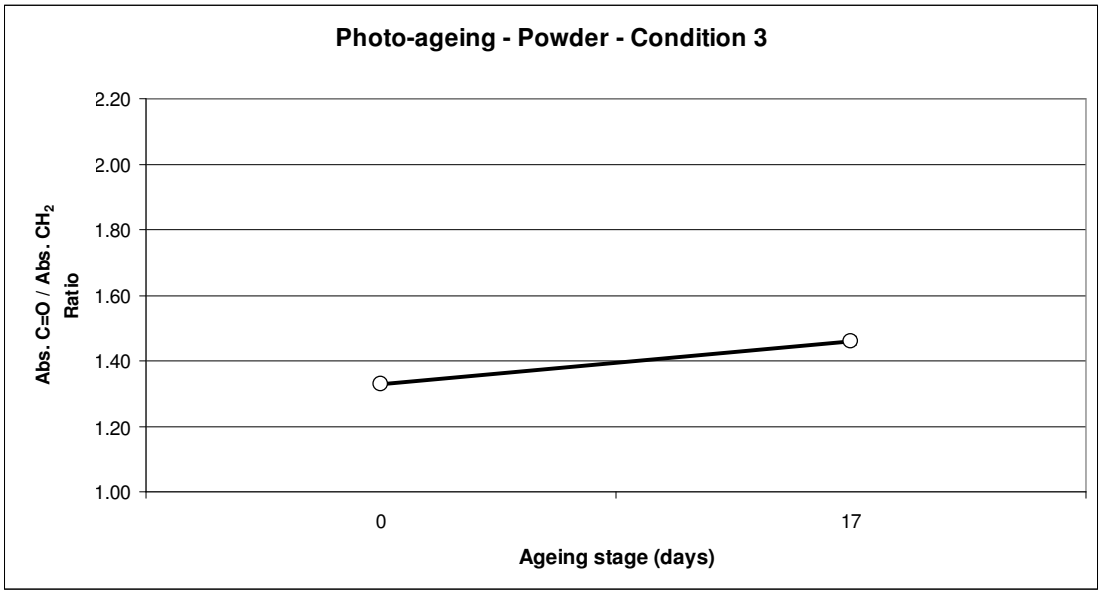


Figure 58. C=O group absorbance change in photo-aged powder in condition 3 (≤ 20% RH, sample exposed to internal atmosphere).

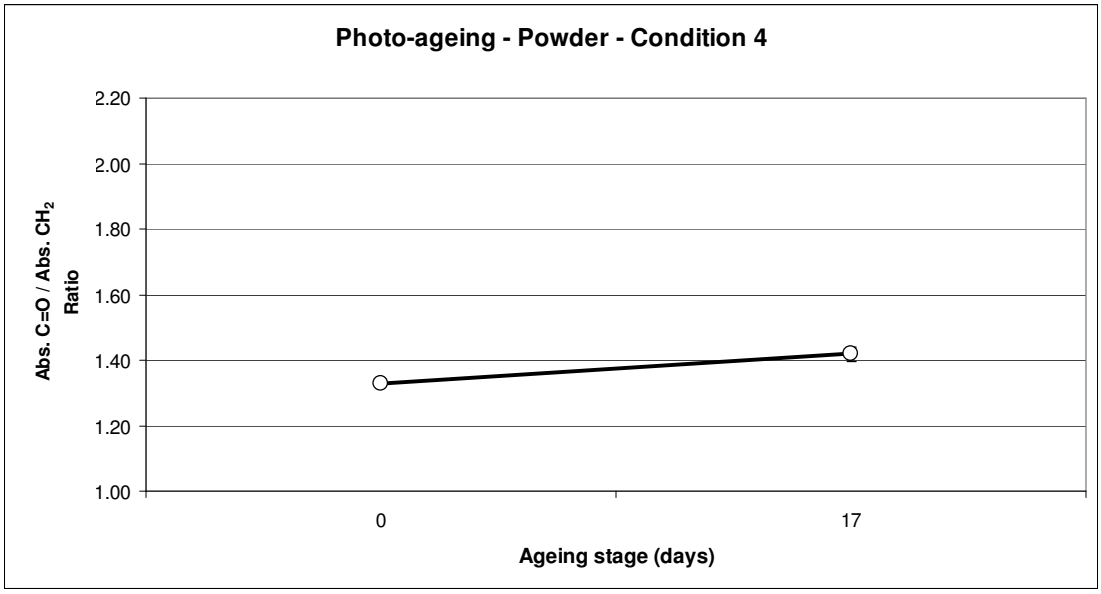


Figure 59. C=O group absorbance change in photo-aged powder in condition 4 (≤ 20% RH, sample exposed to internal hypoxic atmosphere).

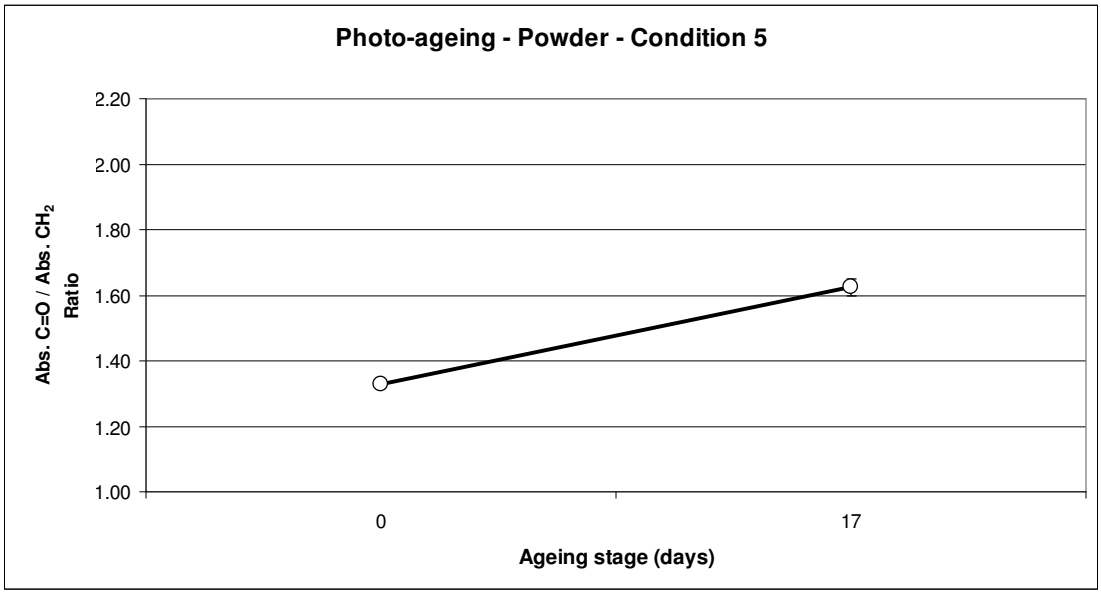


Figure 60. C=O group absorbance change in photo-aged powder in condition 5 (100% RH, sample exposed to internal atmosphere, pH 4).

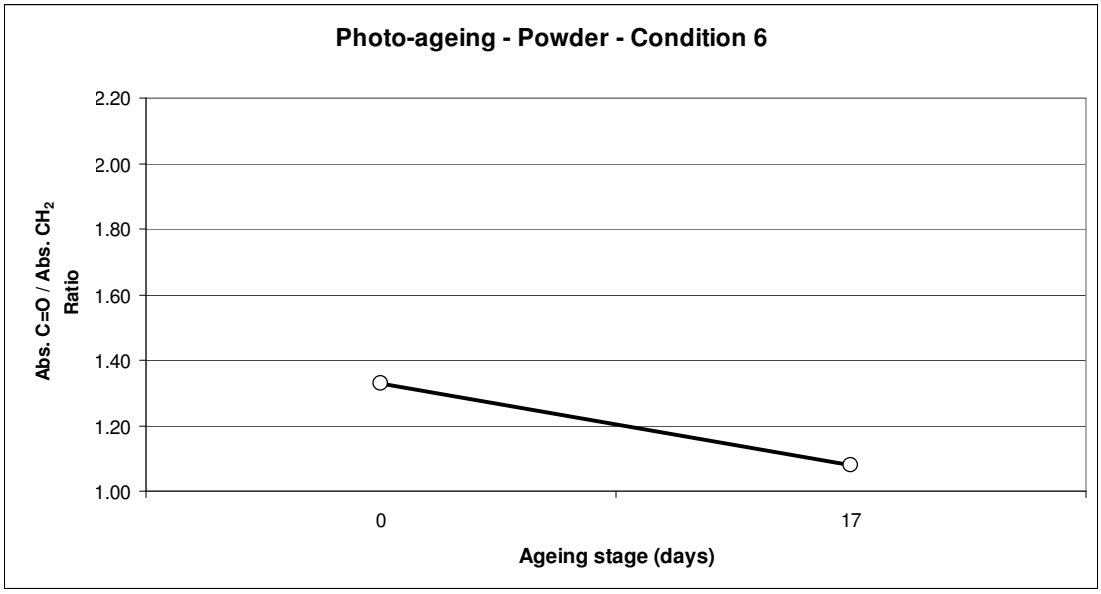


Figure 61. C=O group absorbance change in photo-aged powder in condition 6 (100% RH, sample exposed to internal atmosphere, pH 10).

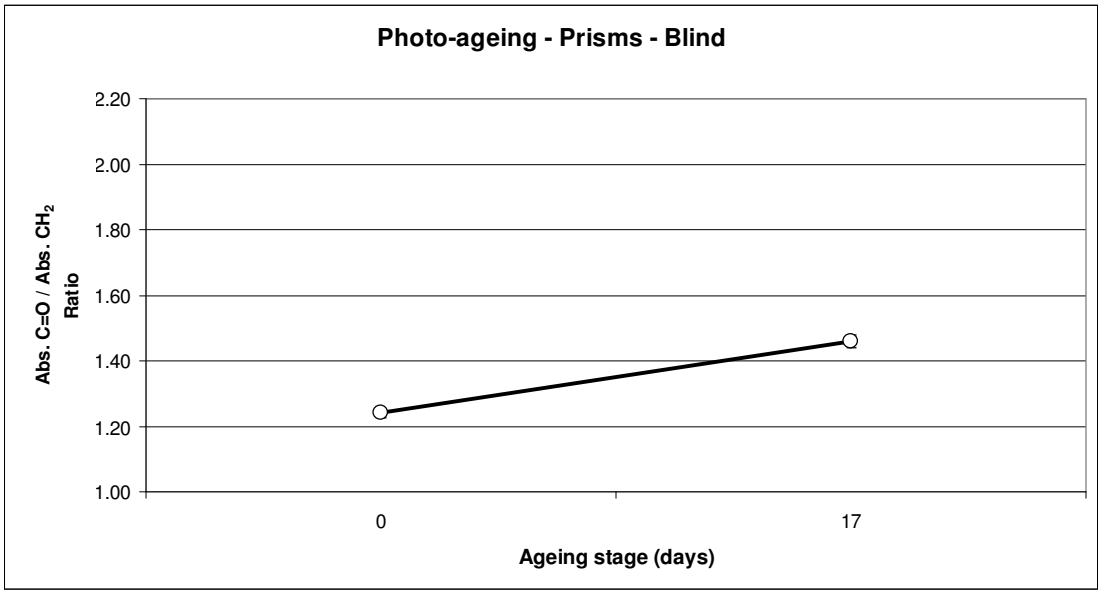


Figure 62. C=O group absorbance change in photo-aged prisms in blind condition.

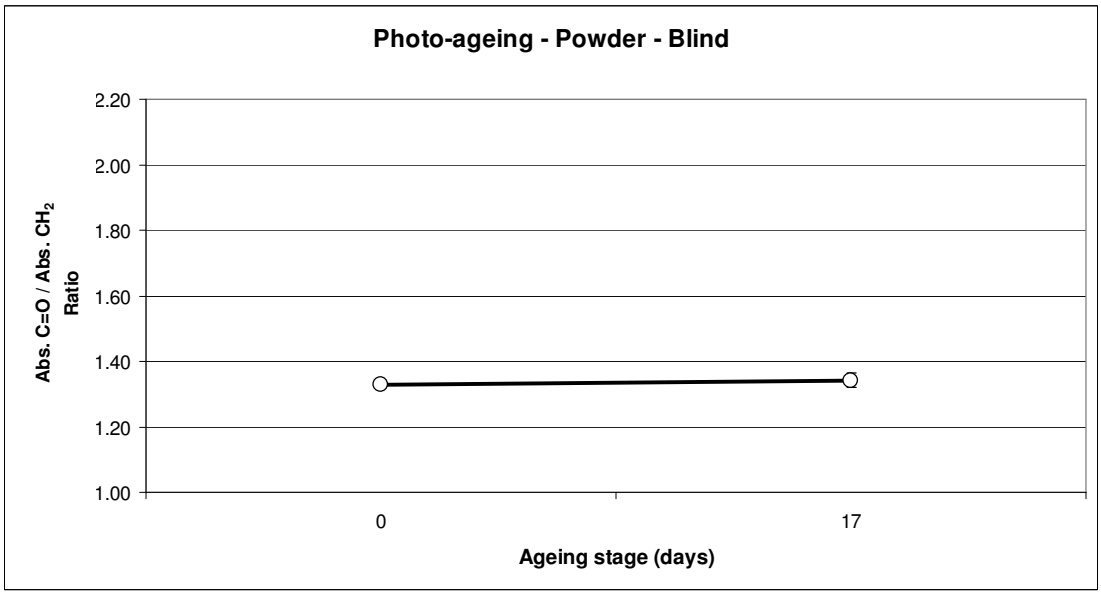


Figure 63. C=O group absorbance change in photo-aged powder in blind condition.

Advanced investigation:

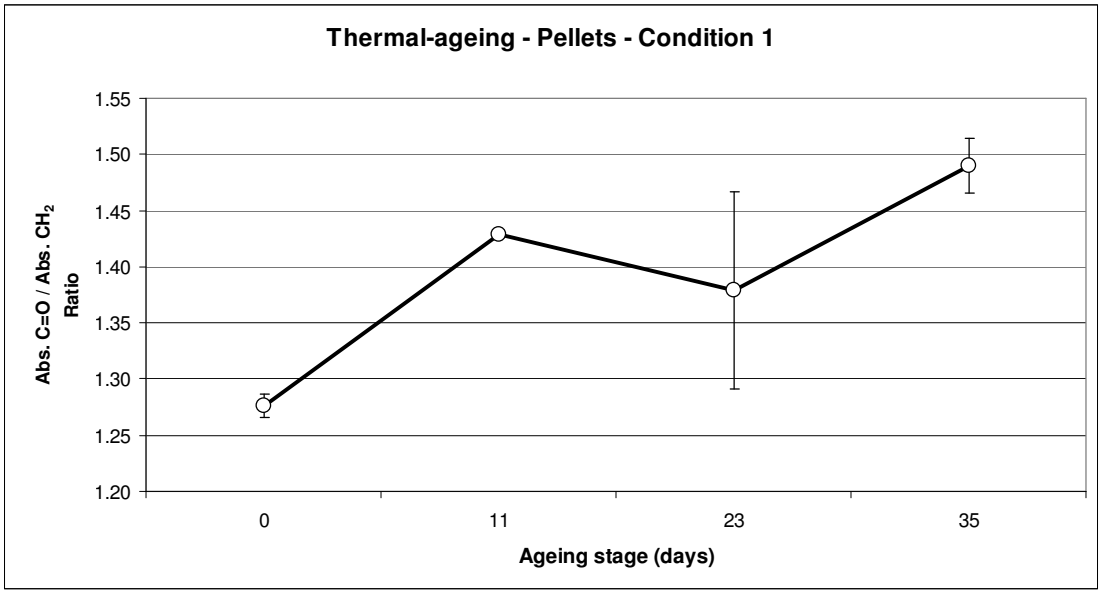


Figure 64. C=O group absorbance change in thermal-aged pellets in condition 1 (≤ 20% RH, sample exposed to external atmosphere).

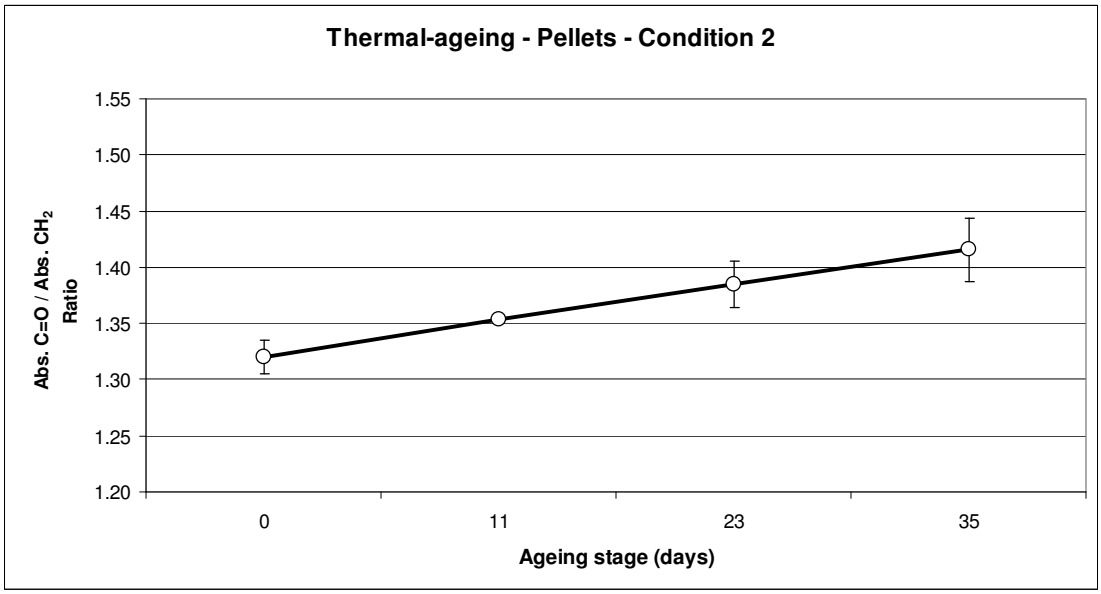


Figure 65. C=O group absorbance change in thermal-aged pellets in condition 2 (100% RH, sample exposed to internal atmosphere, pH ≤ 5.5).

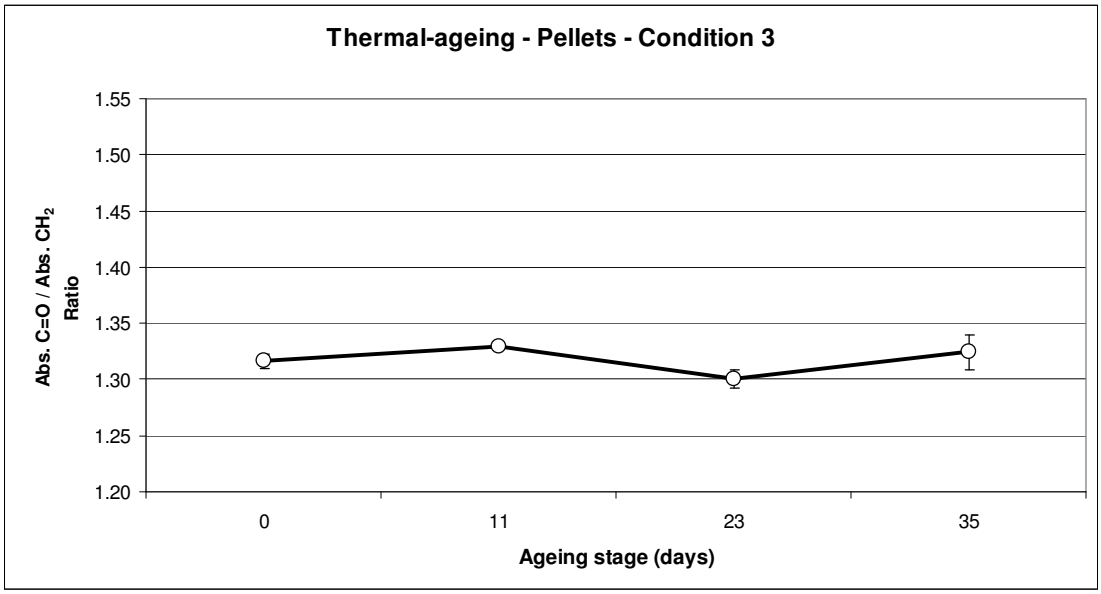


Figure 66. C=O group absorbance change in thermal-aged pellets in condition 3 (100% RH, sample immersed in liquid, pH ≤ 5.5).

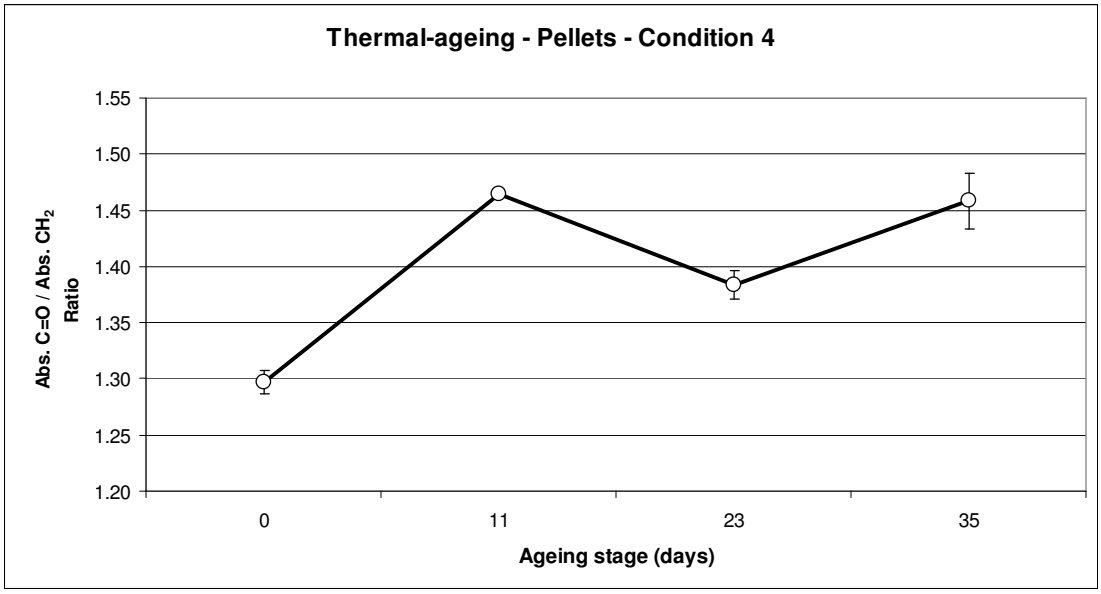


Figure 67. C=O group absorbance change in thermal-aged pellets in condition 4 (≤ 20% RH, sample exposed to internal atmosphere).

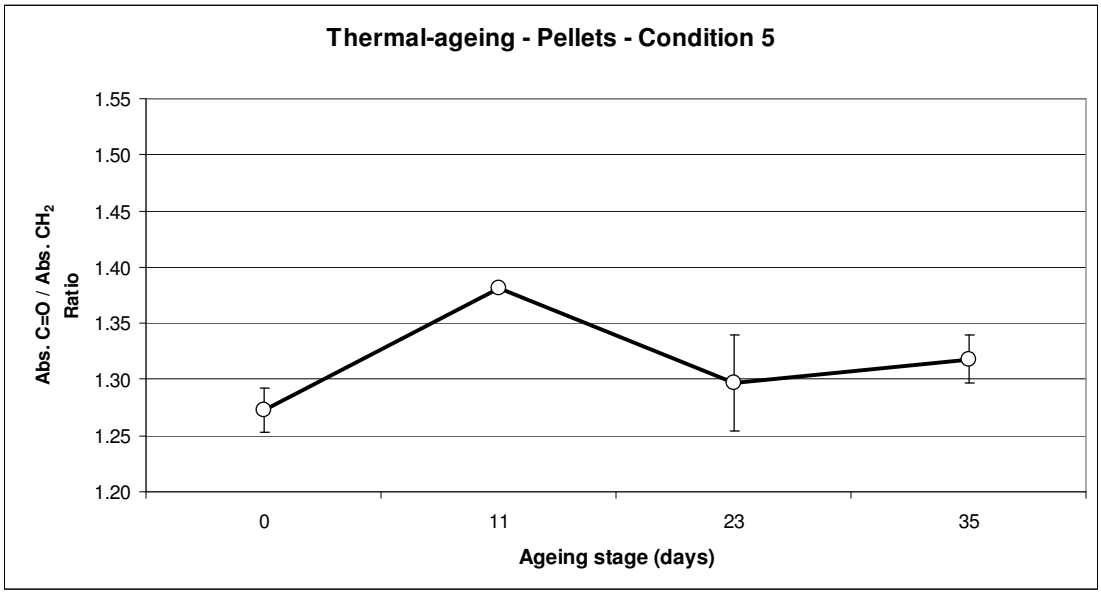


Figure 68. C=O group absorbance change in thermal-aged pellets in condition 5 (≤ 20% RH, sample exposed to internal anoxic atmosphere).

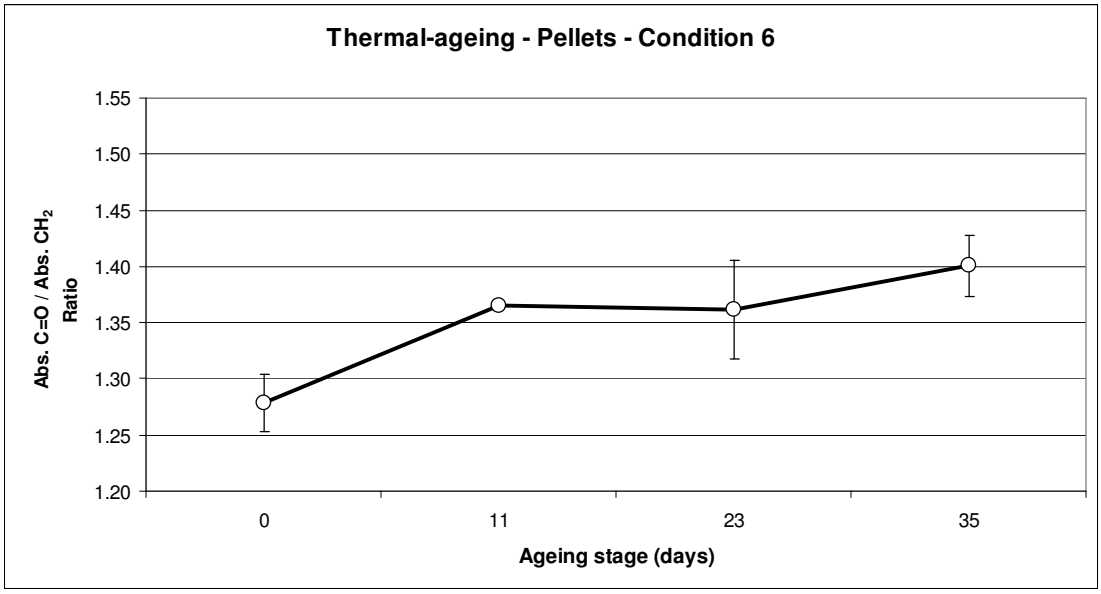


Figure 69. C=O group absorbance change in thermal-aged pellets in condition 6 (100% RH, sample exposed to internal atmosphere, pH 3).

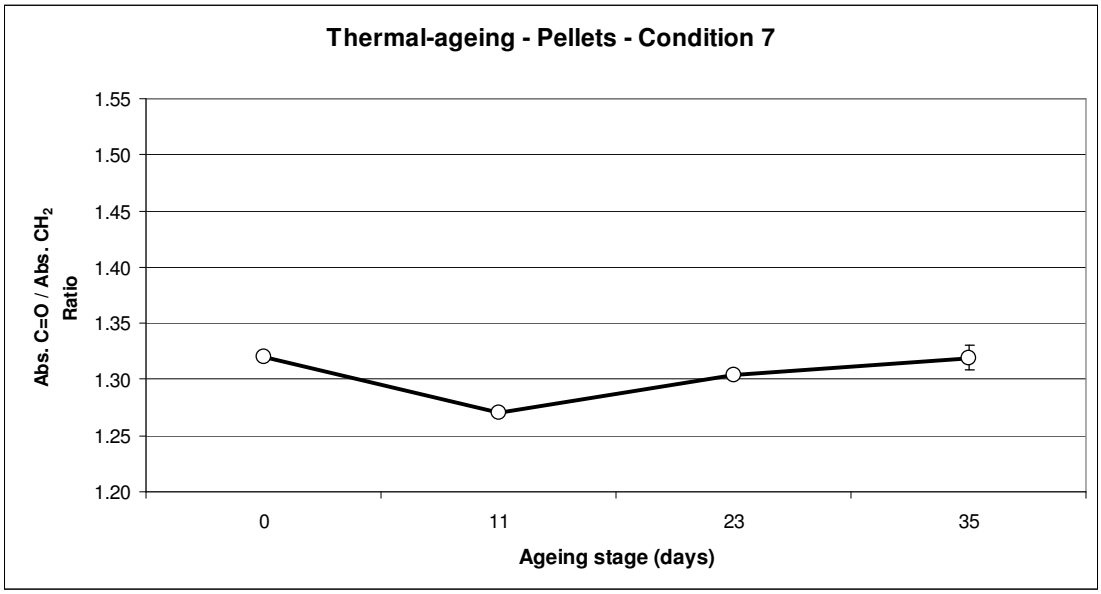


Figure 70. C=O group absorbance change in thermal-aged pellets in condition 7 (100% RH, sample immersed in liquid, pH 3).

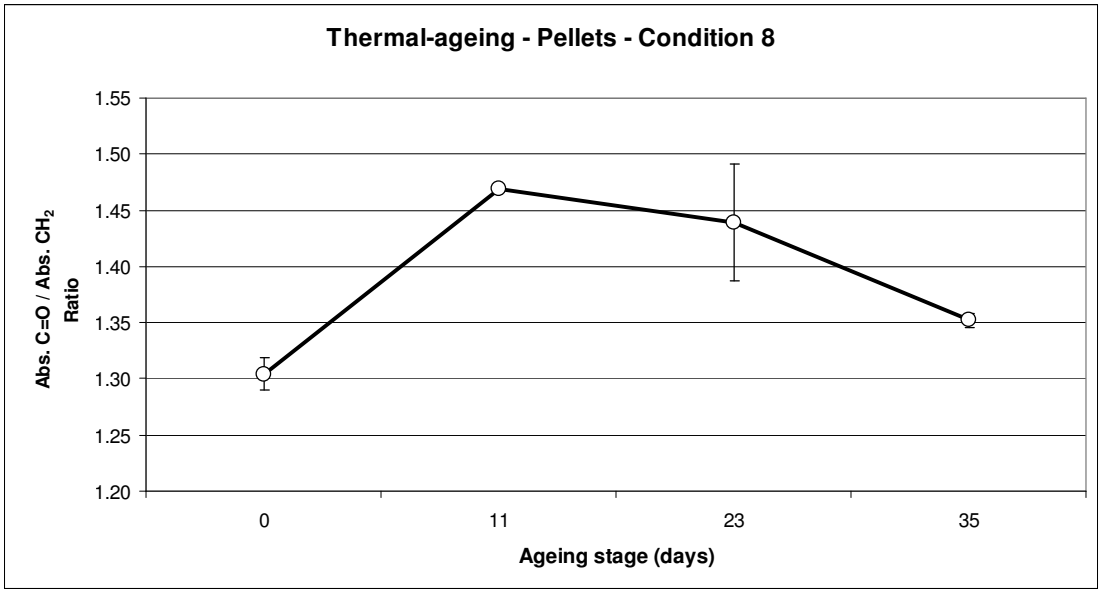


Figure 71. C=O group absorbance change in thermal-aged pellets in condition 8 (100% RH, sample exposed to internal atmosphere, pH 5).

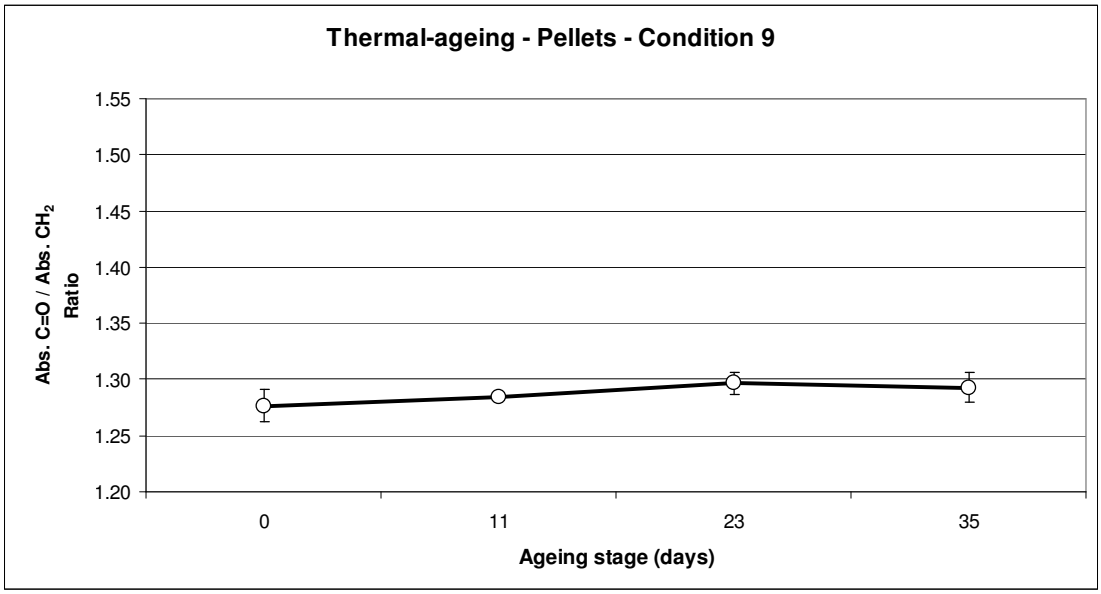


Figure 72. C=O group absorbance change in thermal-aged pellets in condition 9 (100% RH, sample immersed in liquid, pH 5).

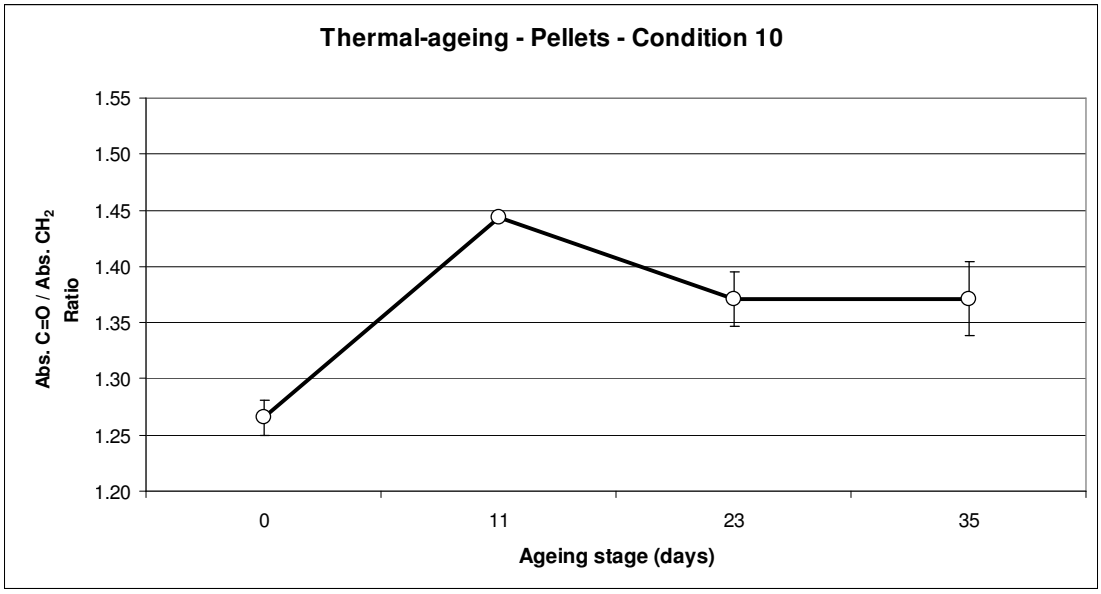


Figure 73. C=O group absorbance change in thermal-aged pellets in condition 10 (100% RH, sample exposed to internal atmosphere, pH 10).

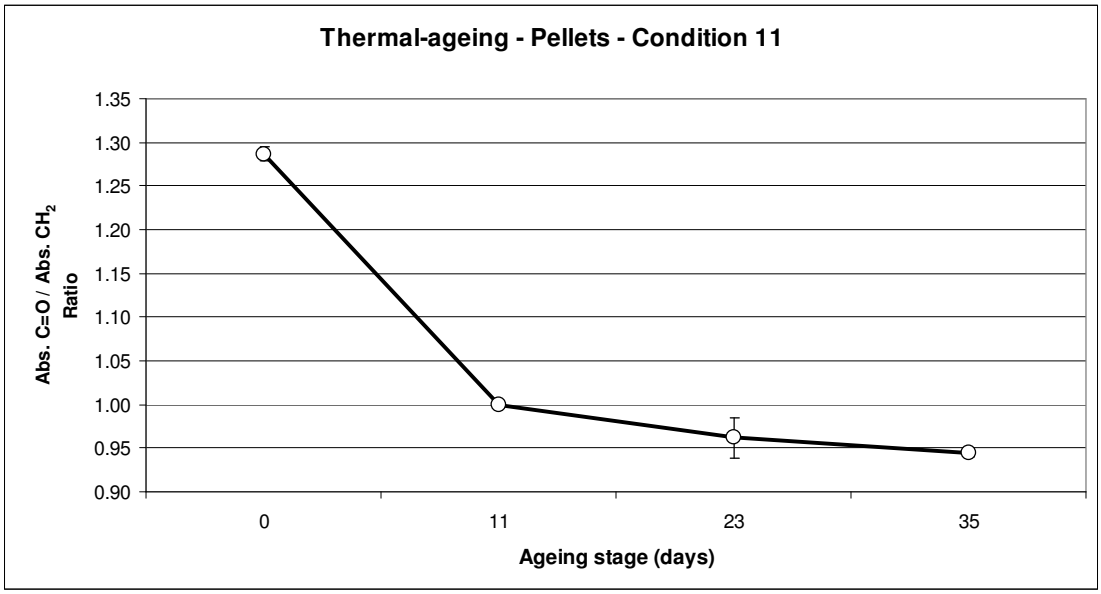


Figure 74. C=O group absorbance change in thermal-aged pellets in condition 11 (100% RH, sample immersed in liquid, pH 10).
The range is different from the one used for the other graphs.

A.3 FT-Raman spectroscopy.

Preliminary investigation:

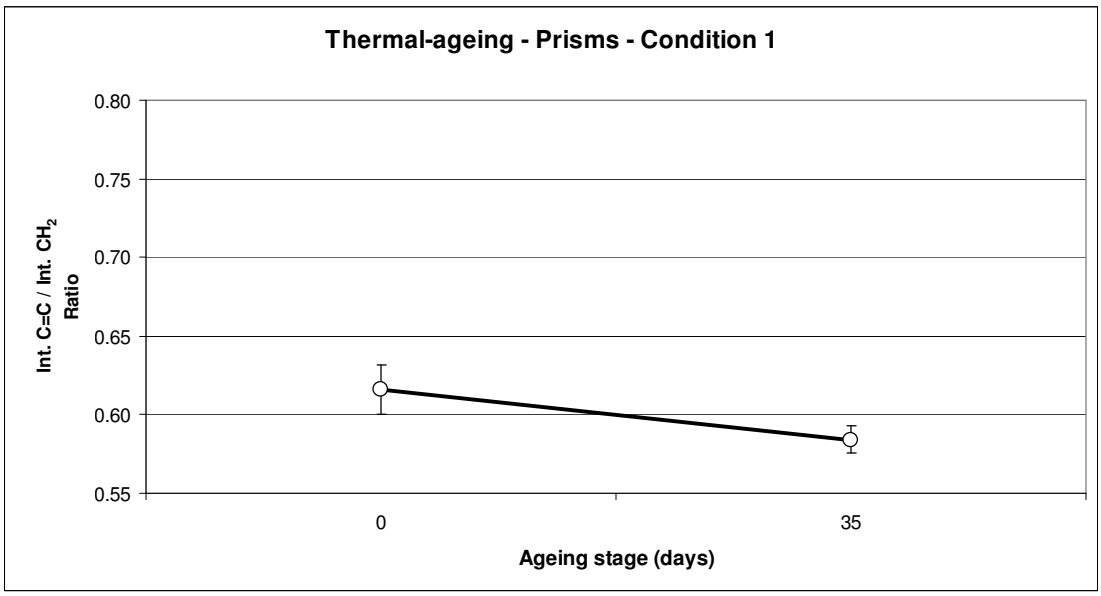


Figure 75. C=C group intensity change in thermal-aged prisms in condition 1 (≤ 20% RH, sample exposed to external atmosphere).

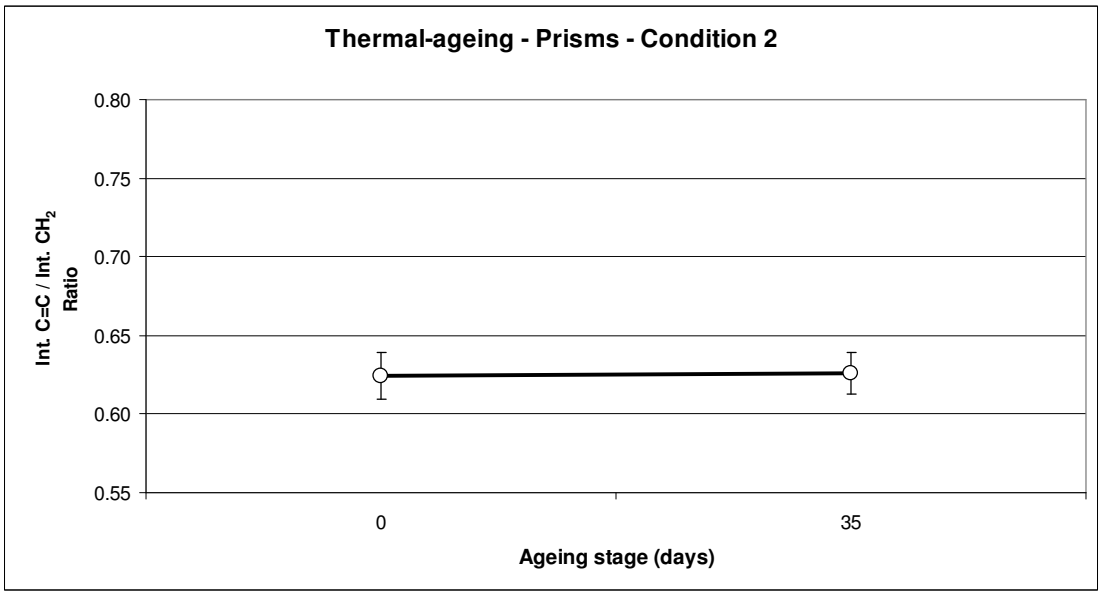


Figure 76. C=C group intensity change in thermal-aged prisms in condition 2 (100% RH, sample exposed to internal atmosphere, pH ≤ 5.5).

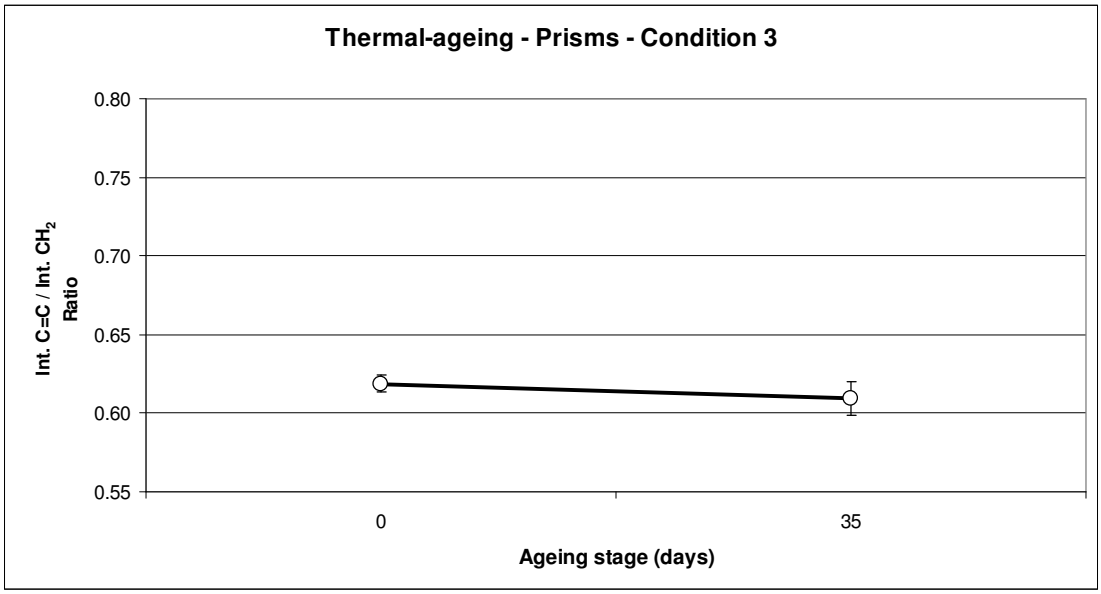


Figure 77. C=C group intensity change in thermal-aged prisms in condition 3 (≤ 20% RH, sample exposed to internal atmosphere).

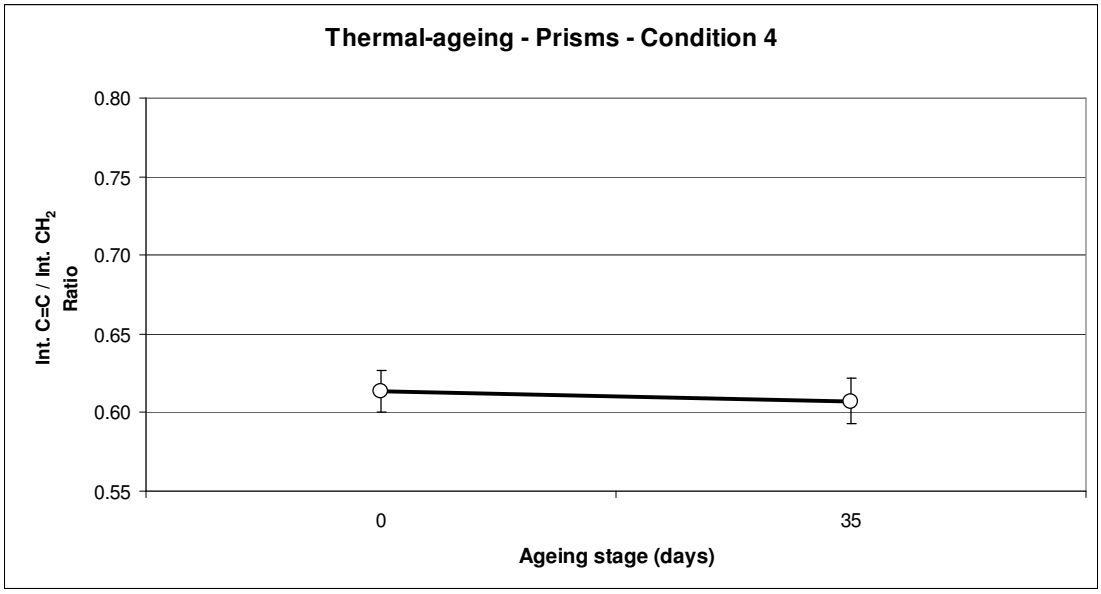


Figure 78. C=C group intensity change in thermal-aged prisms in condition 4 (≤ 20% RH, sample exposed to internal hypoxic atmosphere).

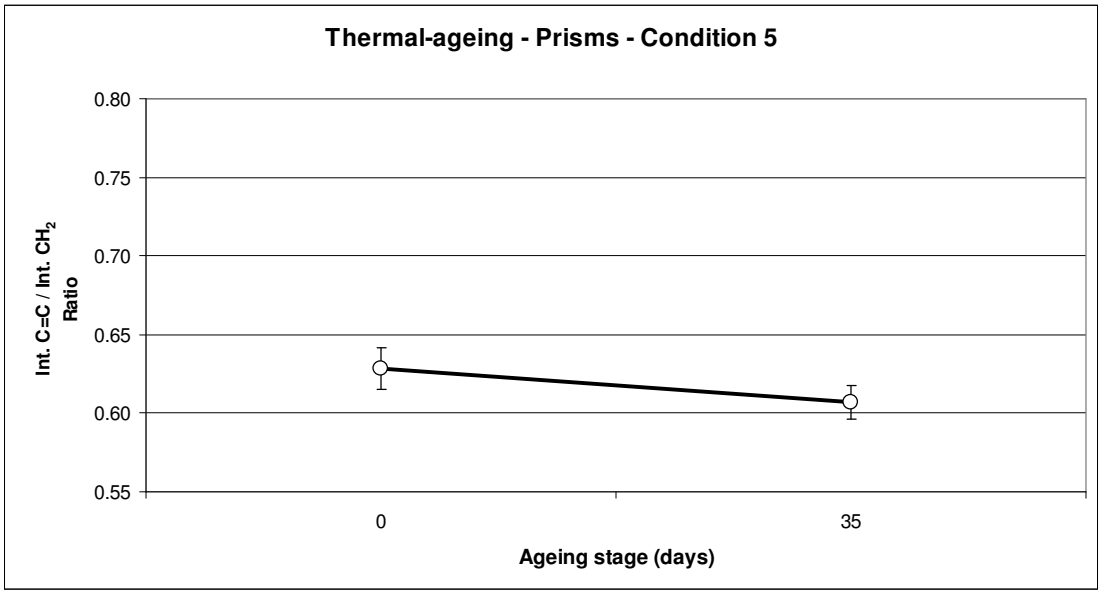


Figure 79. C=C group intensity change in thermal-aged prisms in condition 5 (100% RH, sample exposed to internal atmosphere, pH 4).

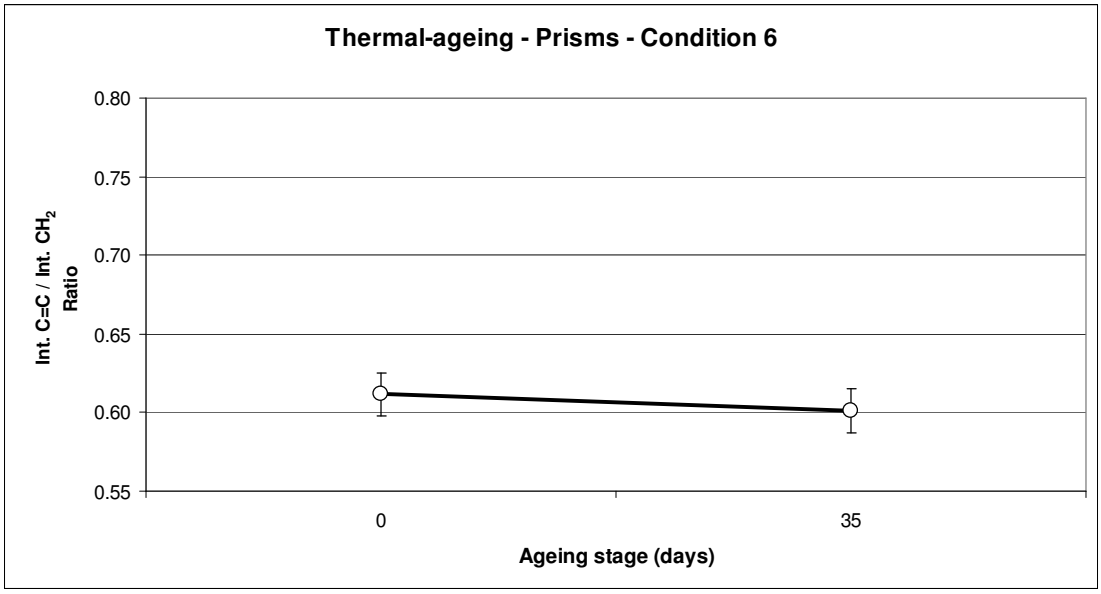


Figure 80. C=C group intensity change in thermal-aged prisms in condition 6 (100% RH, sample exposed to internal atmosphere, pH 10).

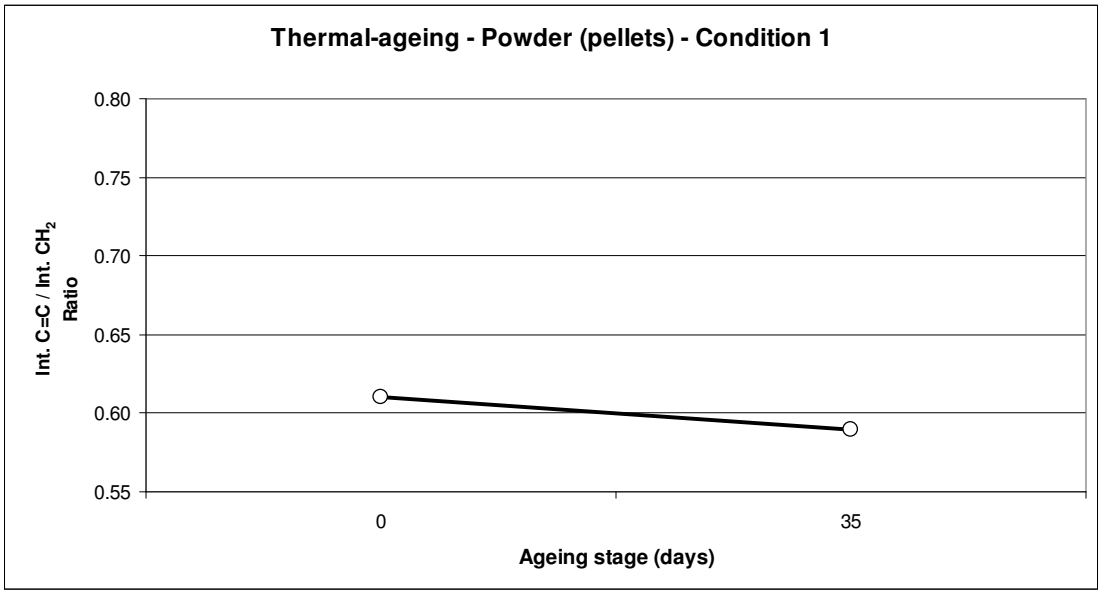


Figure 81. C=C group intensity change in thermal-aged powder in condition 1 (≤ 20% RH, sample exposed to external atmosphere).

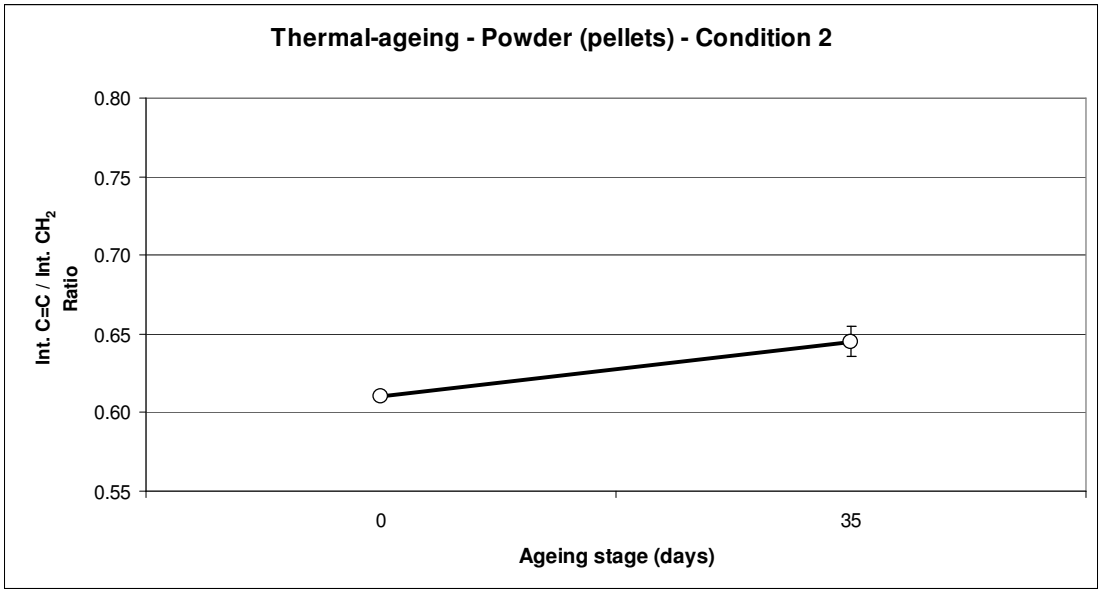


Figure 82. C=C group intensity change in thermal-aged powder in condition 2 (100% RH, sample exposed to internal atmosphere, pH ≤ 5.5).

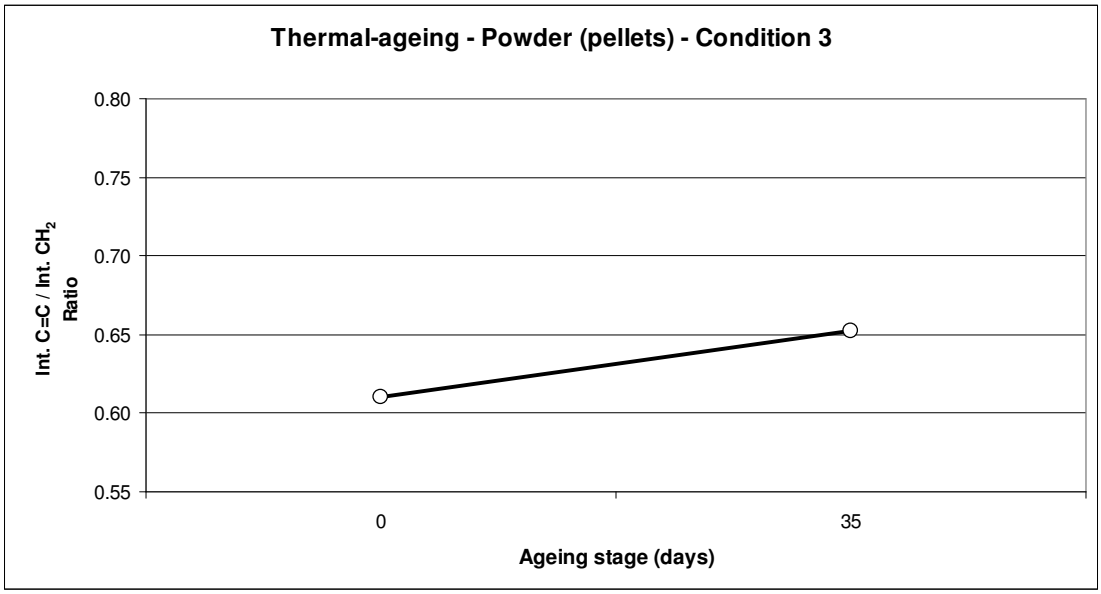


Figure 83. C=C group intensity change in thermal-aged powder in condition 3 (≤ 20% RH, sample exposed to internal atmosphere).

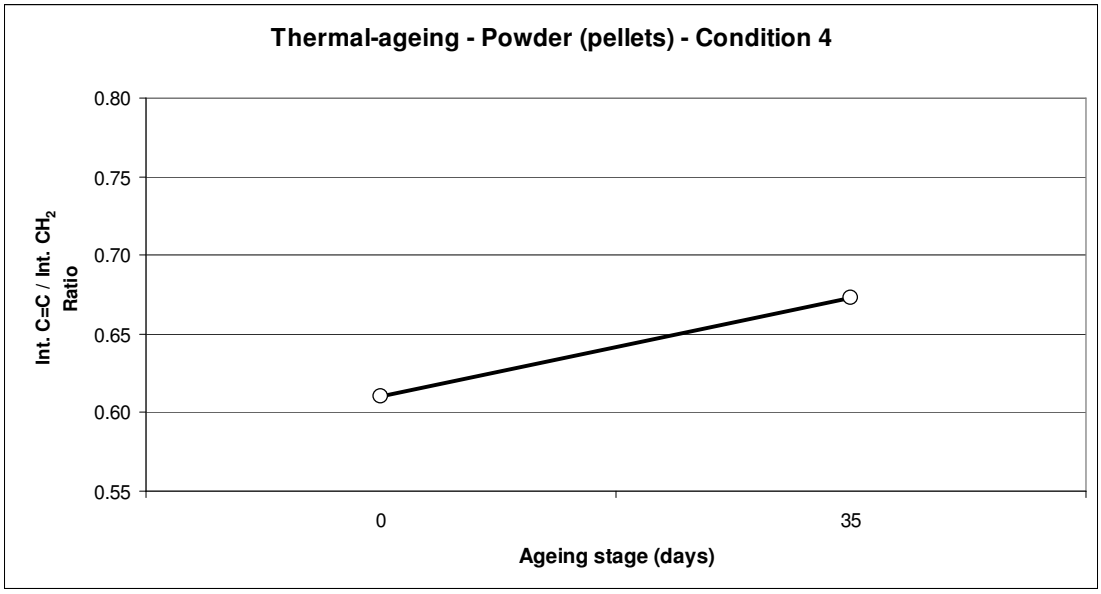


Figure 84. C=C group intensity change in thermal-aged powder in condition 4 (≤ 20% RH, sample exposed to internal hypoxic atmosphere).

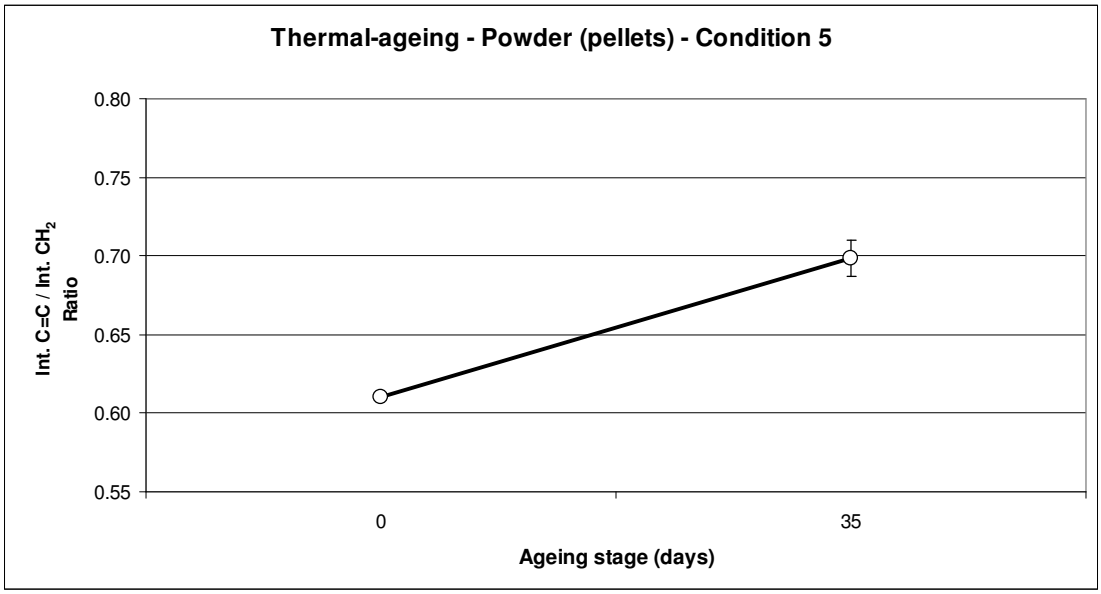


Figure 85. C=C group intensity change in thermal-aged powder in condition 5 (100% RH, sample exposed to internal atmosphere, pH 4).

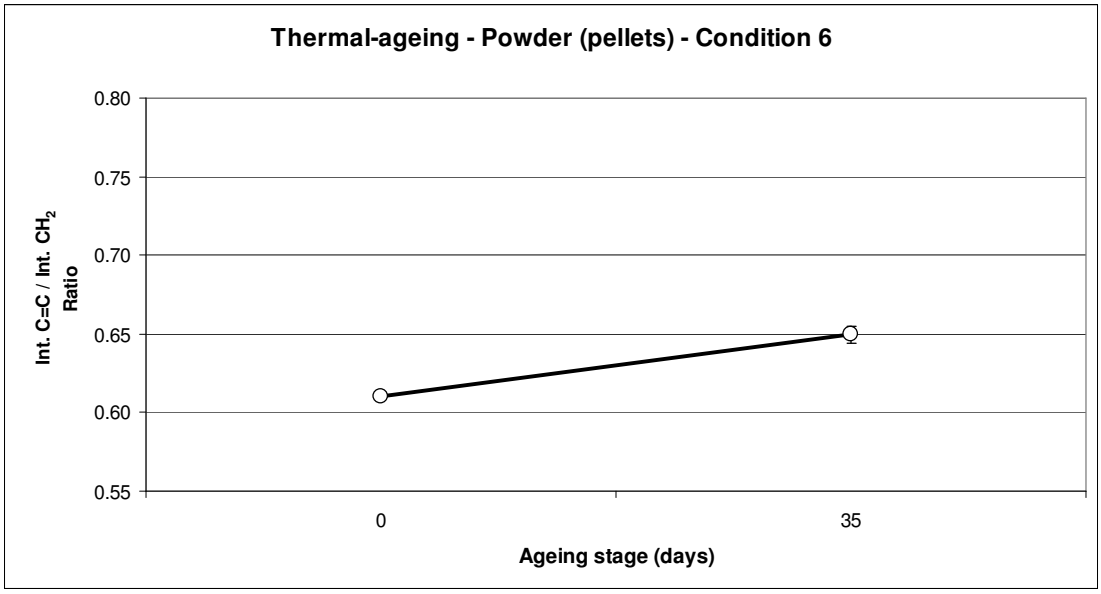


Figure 86. C=C group intensity change in thermal-aged powder in condition 6 (100% RH, sample exposed to internal atmosphere, pH 10).

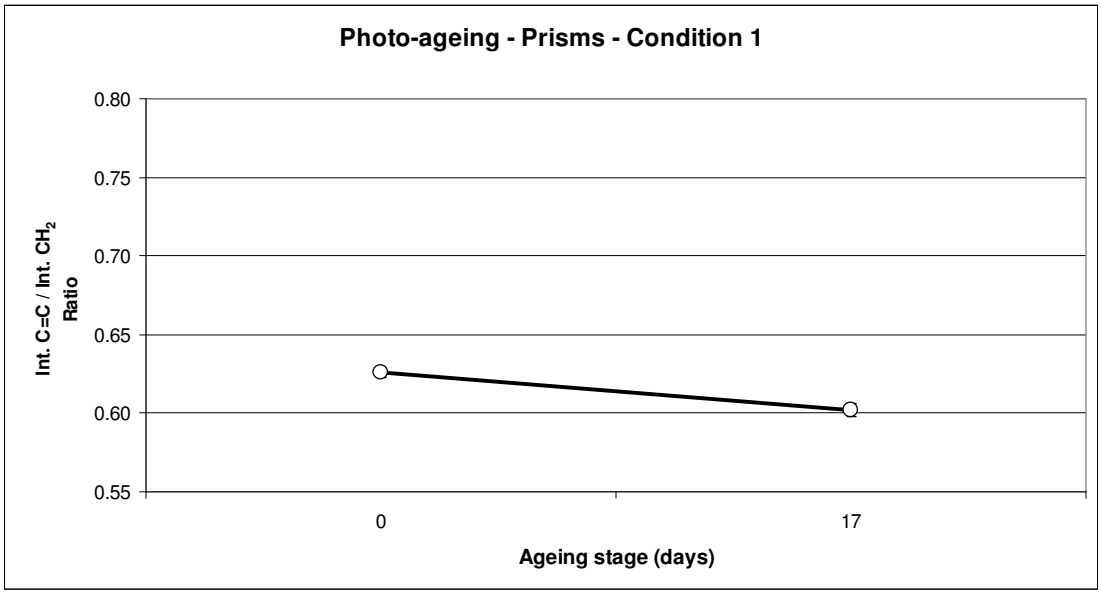


Figure 87. C=C group intensity change in photo-aged prisms in condition 1 (≤ 20% RH, sample exposed to external atmosphere).

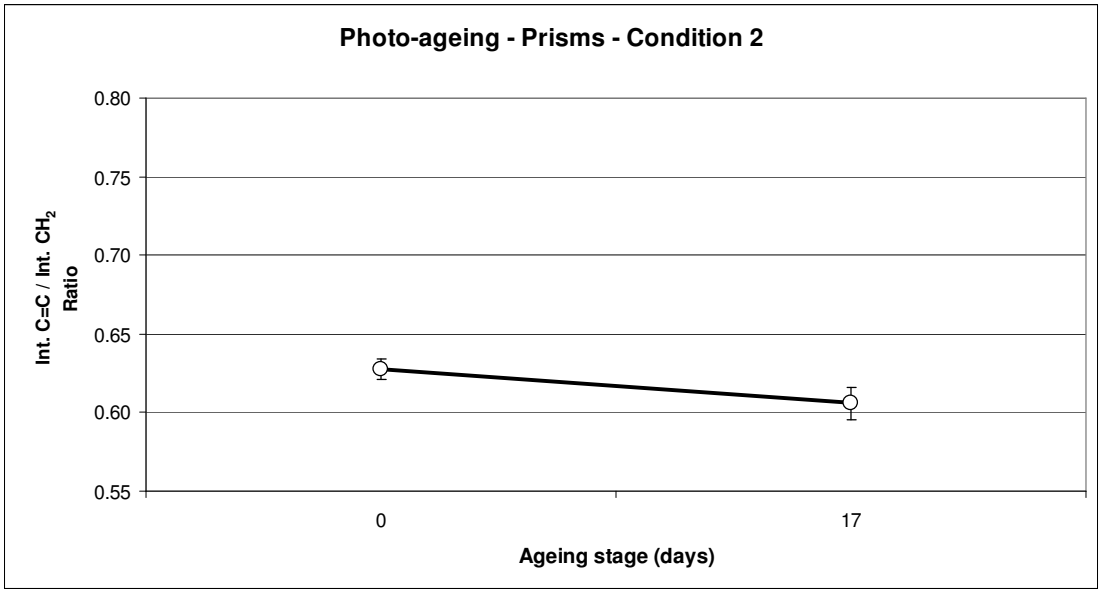


Figure 88. C=C group intensity change in photo-aged prisms in condition 2 (100% RH, sample exposed to internal atmosphere, pH ≤ 5.5).

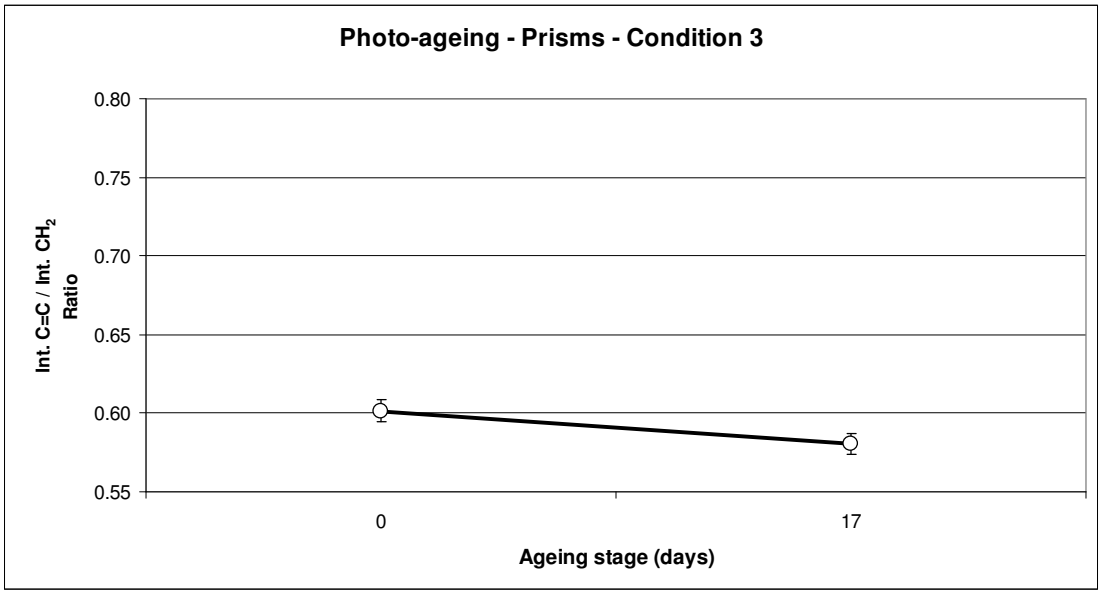


Figure 89. C=C group intensity change in photo-aged prisms in condition 3 (≤ 20% RH, sample exposed to internal atmosphere).

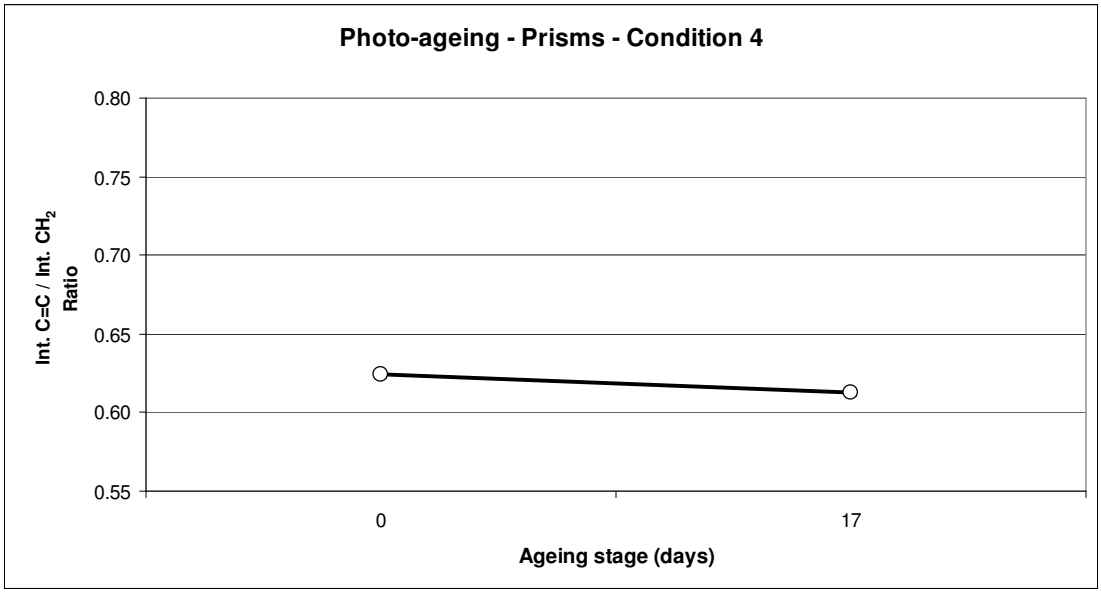


Figure 90. C=C group intensity change in photo-aged prisms in condition 4 (≤ 20% RH, sample exposed to internal hypoxic atmosphere).

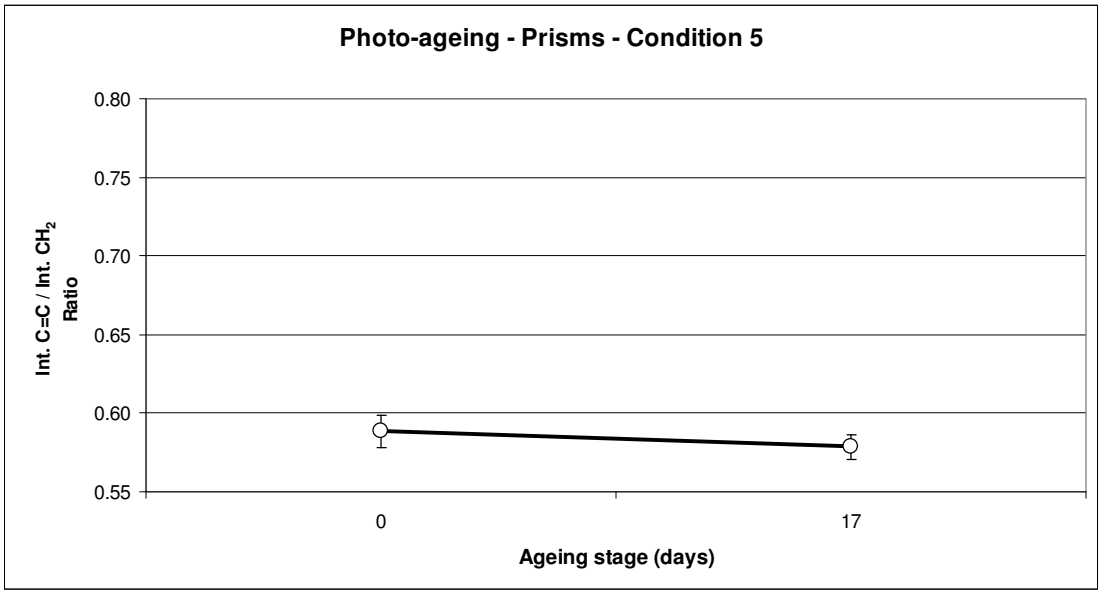


Figure 91. C=C group intensity change in photo-aged prisms in condition 5 (100% RH, sample exposed to internal atmosphere, pH 4).

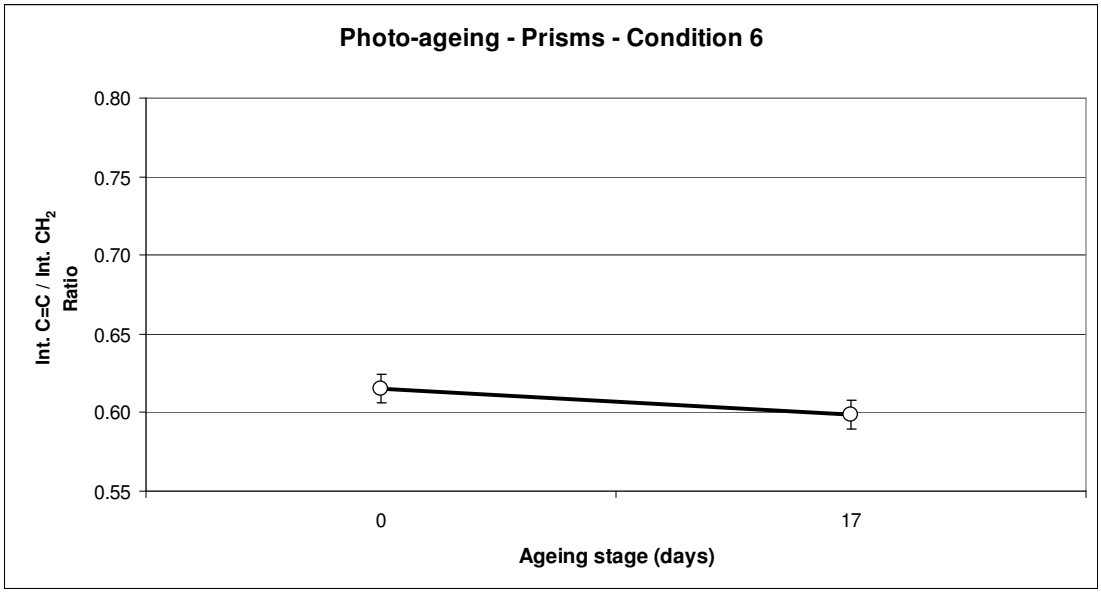


Figure 92. C=C group intensity change in photo-aged prisms in condition 6 (100% RH, sample exposed to internal atmosphere, pH 10).

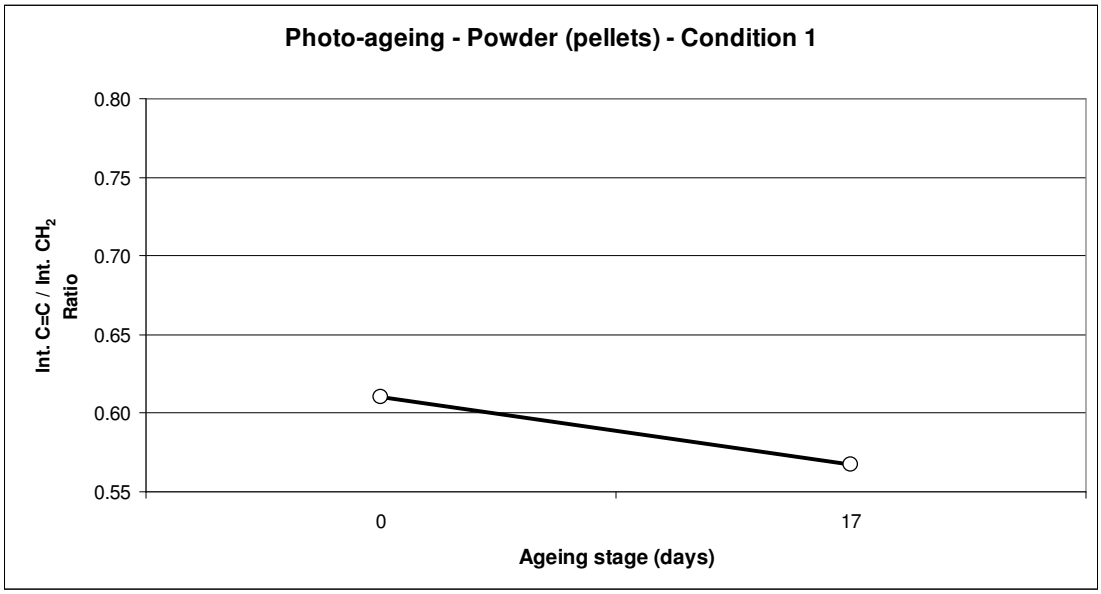


Figure 93. C=C group intensity change in photo-aged powder in condition 1 (≤ 20% RH, sample exposed to external atmosphere).

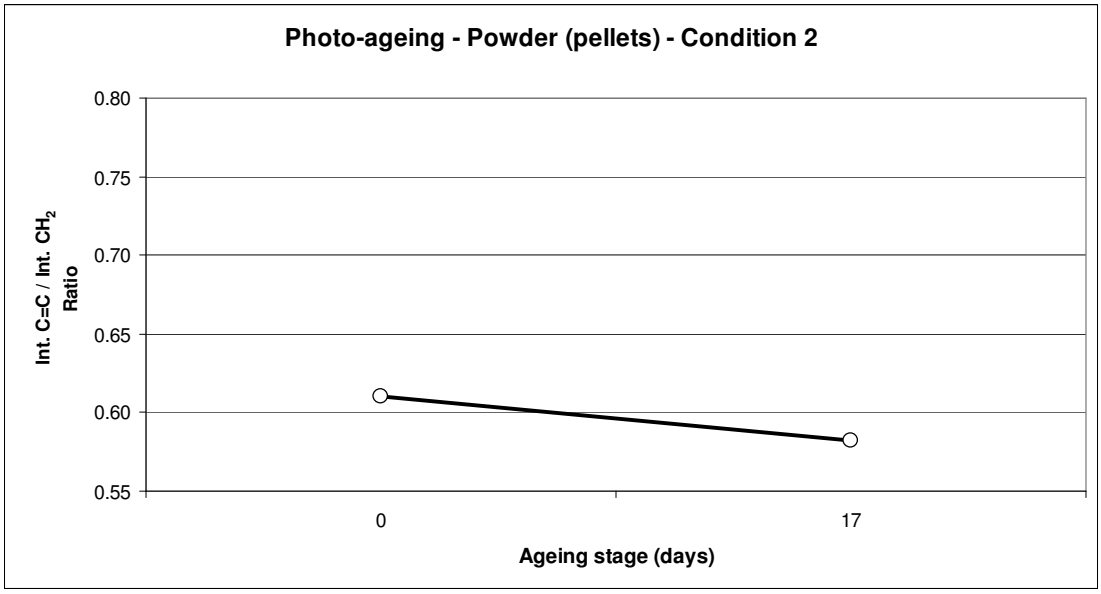


Figure 94. C=C group intensity change in photo-aged powder in condition 2 (100% RH, sample exposed to internal atmosphere, pH ≤ 5.5).

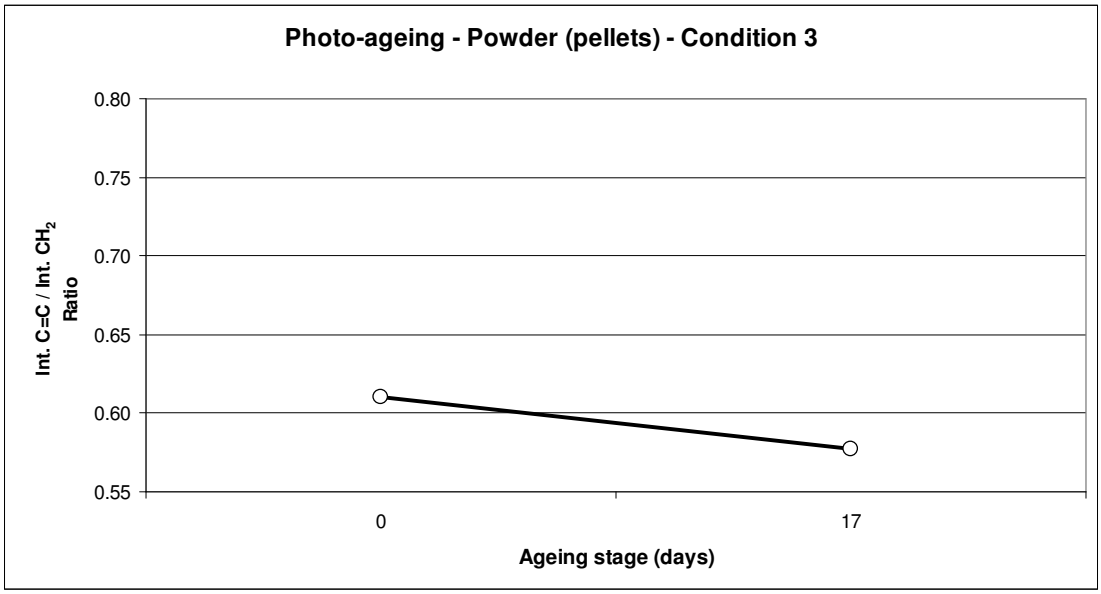


Figure 95. C=C group intensity change in photo-aged powder in condition 3 (≤ 20% RH, sample exposed to internal atmosphere).

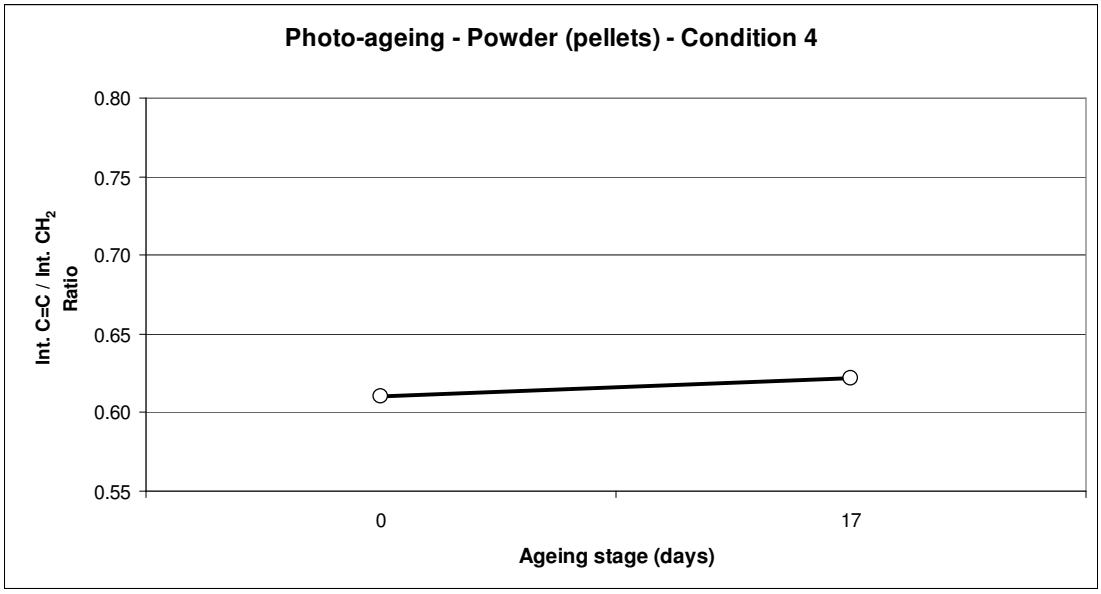


Figure 96. C=C group intensity change in photo-aged powder in condition 4 (≤ 20% RH, sample exposed to internal hypoxic atmosphere).

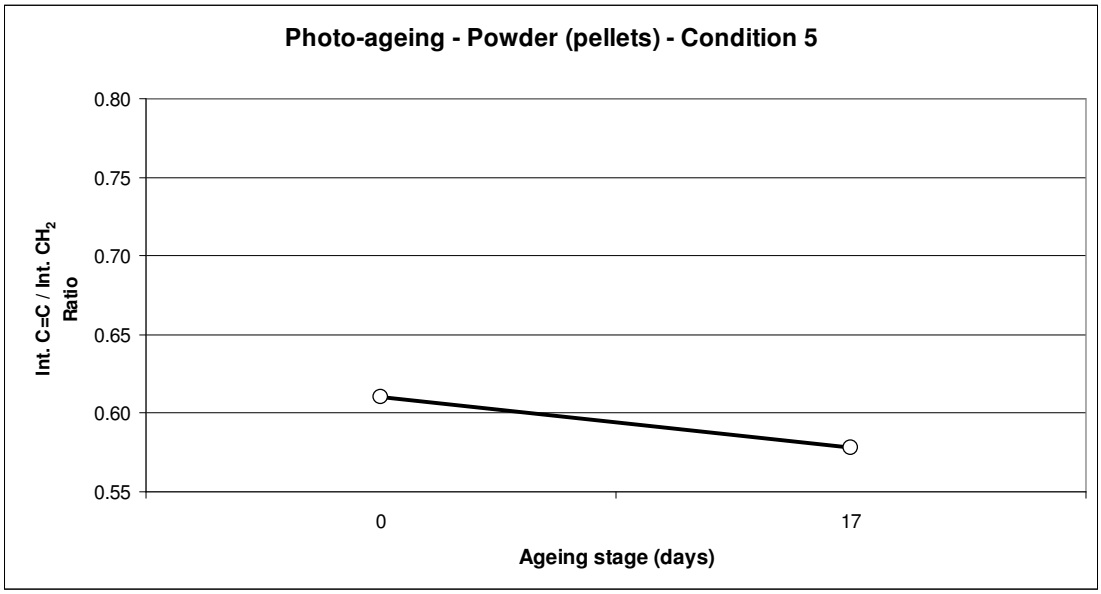


Figure 97. C=C group intensity change in photo-aged powder in condition 4 (≤ 20% RH, sample exposed to internal hypoxic atmosphere).

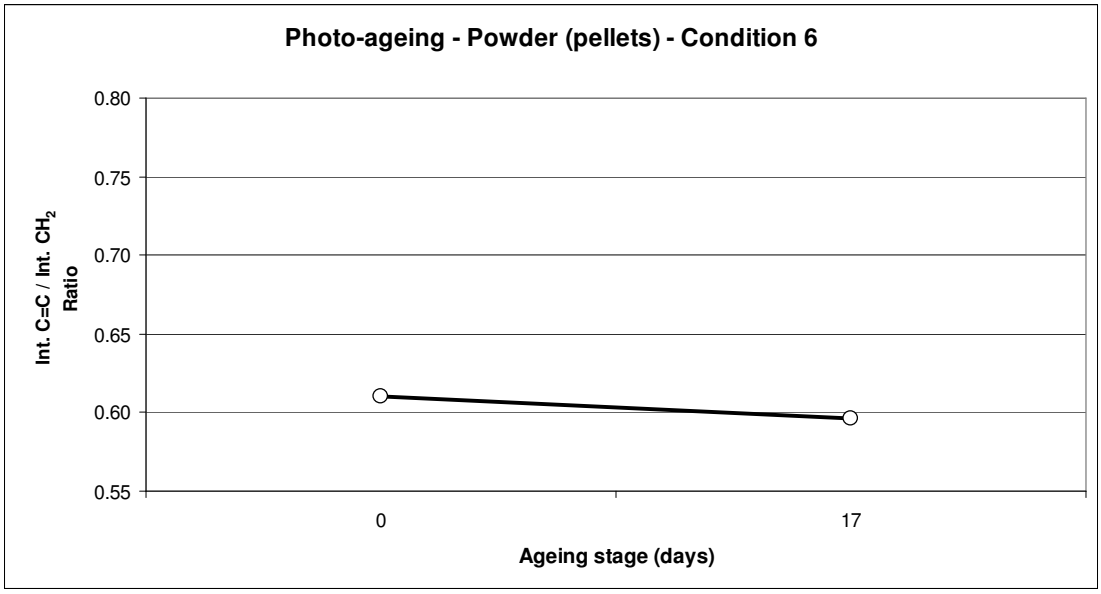


Figure 98. C=C group intensity change in photo-aged powder in condition 6 (100% RH, sample exposed to internal atmosphere, pH 10).

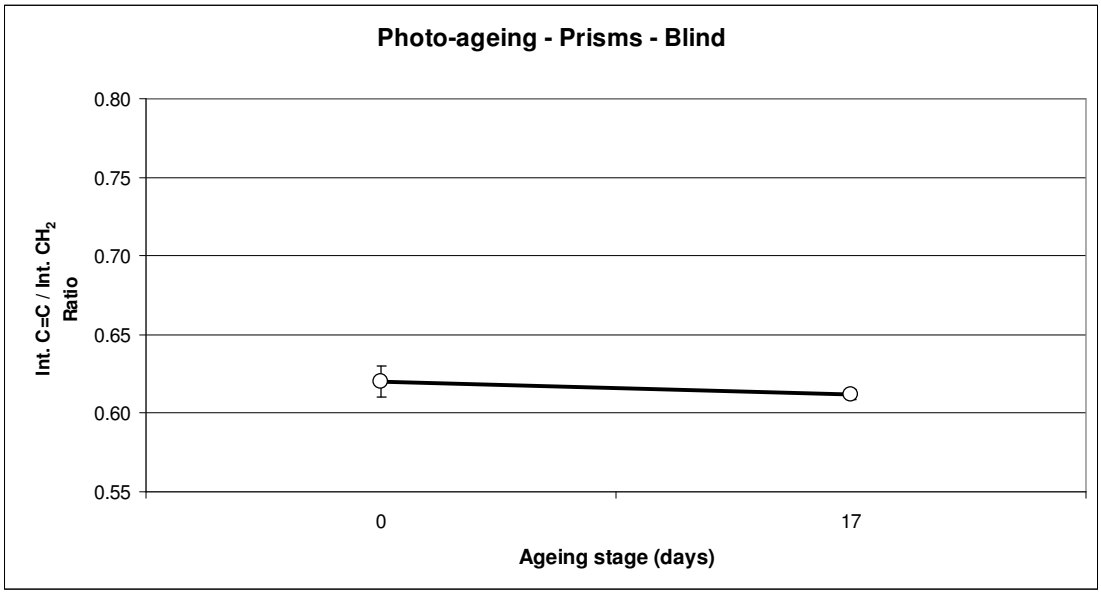


Figure 99. C=C group intensity change in photo-aged prisms in blind condition.

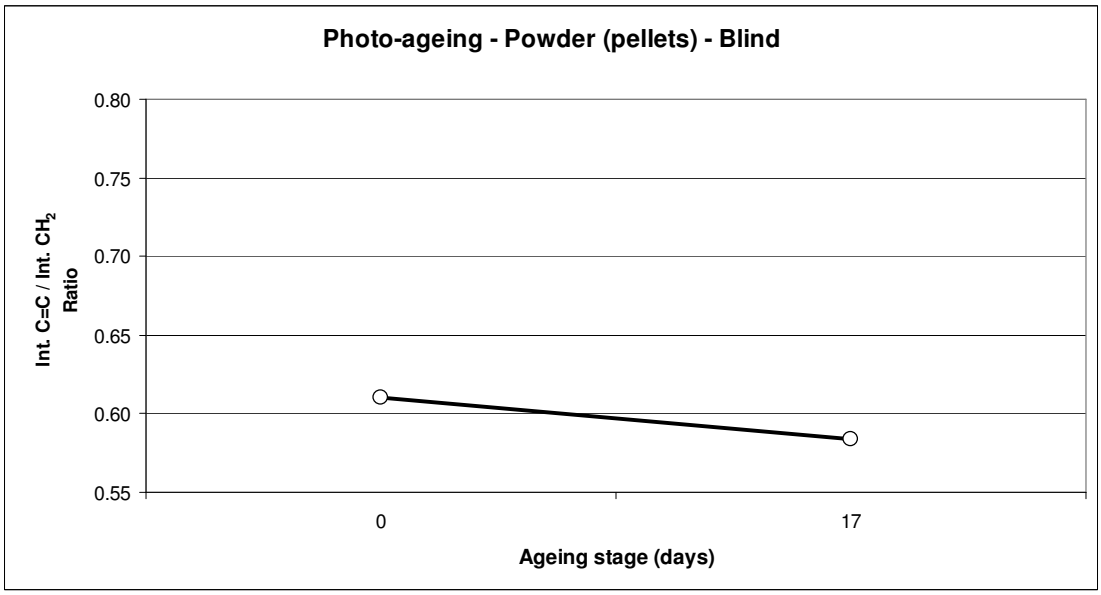


Figure 100. C=C group intensity change in photo-aged powder in blind condition.

Advanced investigation:

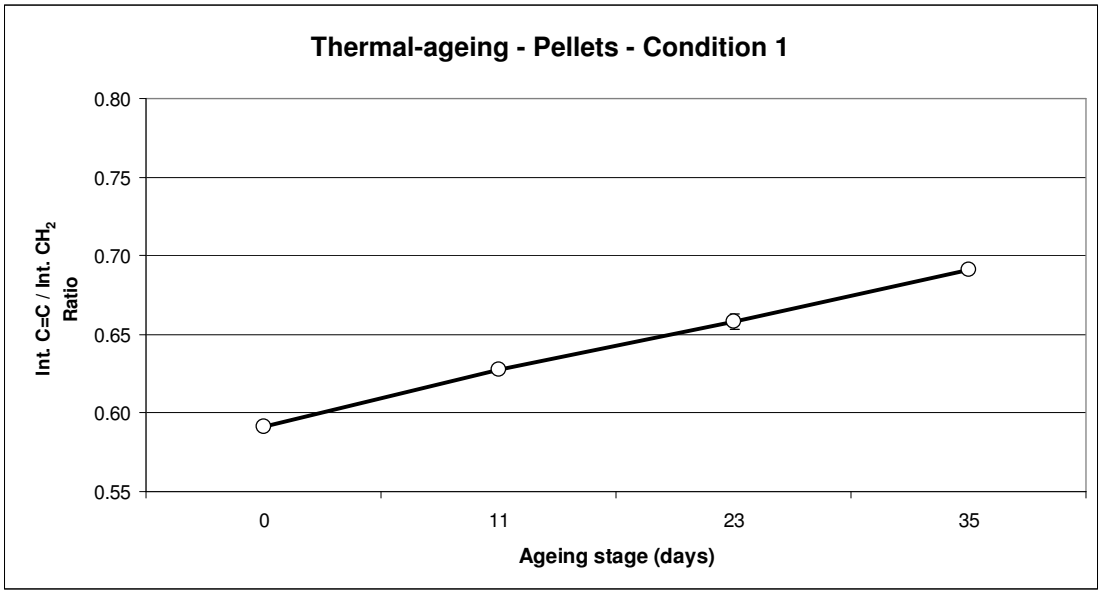


Figure 101. C=C group intensity change in thermal-aged pellets in condition 1 (≤ 20% RH, sample exposed to external atmosphere).

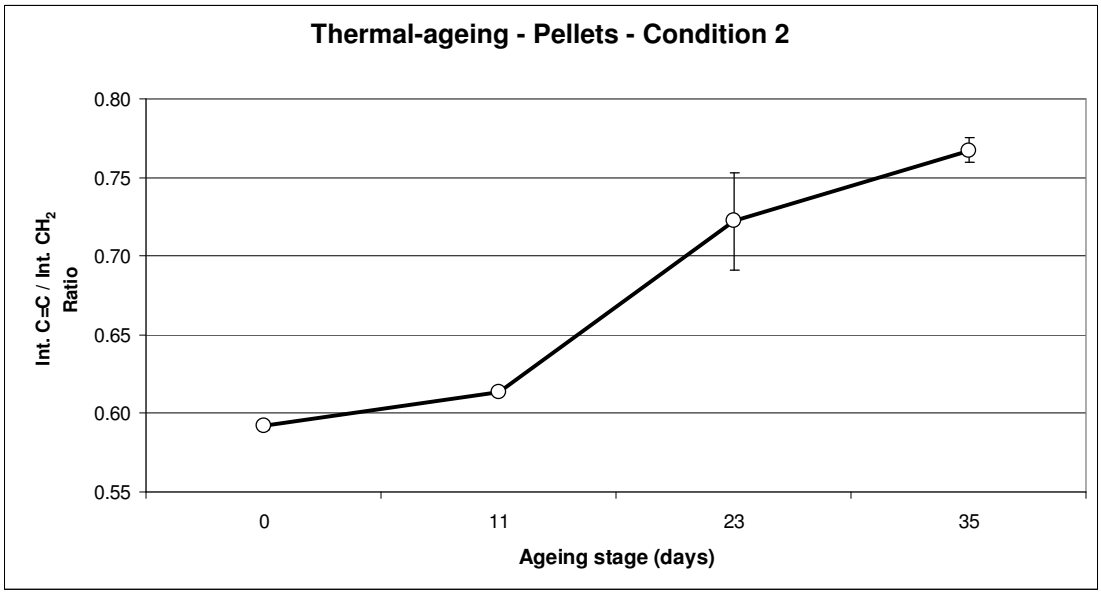


Figure 102. C=C group intensity change in thermal-aged pellets in condition 2 (100% RH, sample exposed to internal atmosphere, pH ≤ 5.5).

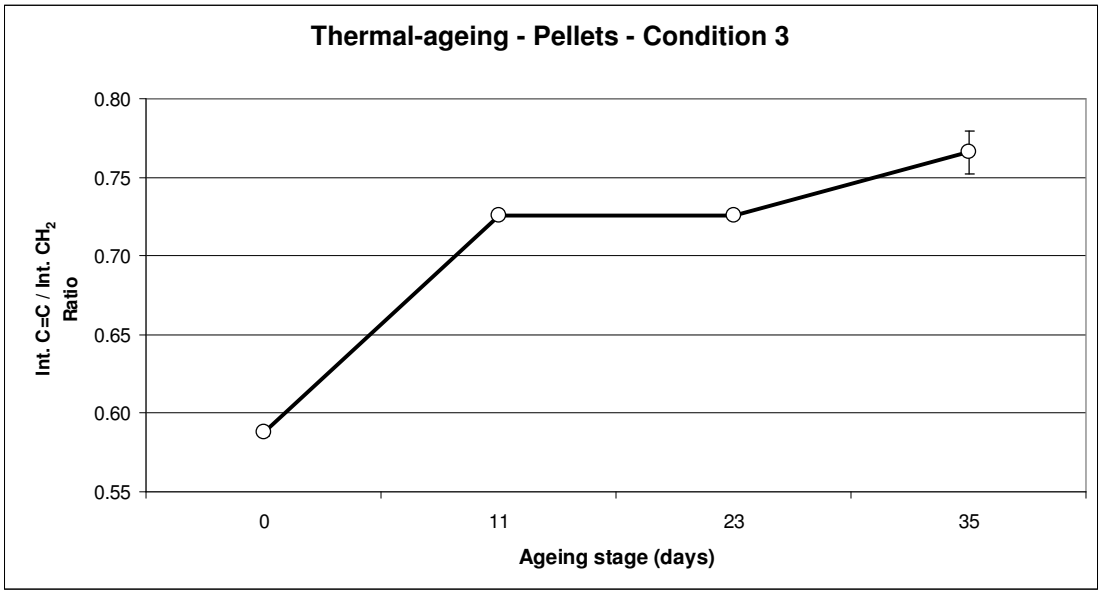


Figure 103. C=C group intensity change in thermal-aged pellets in condition 3 (100% RH, sample immersed in liquid, pH ≤ 5.5).

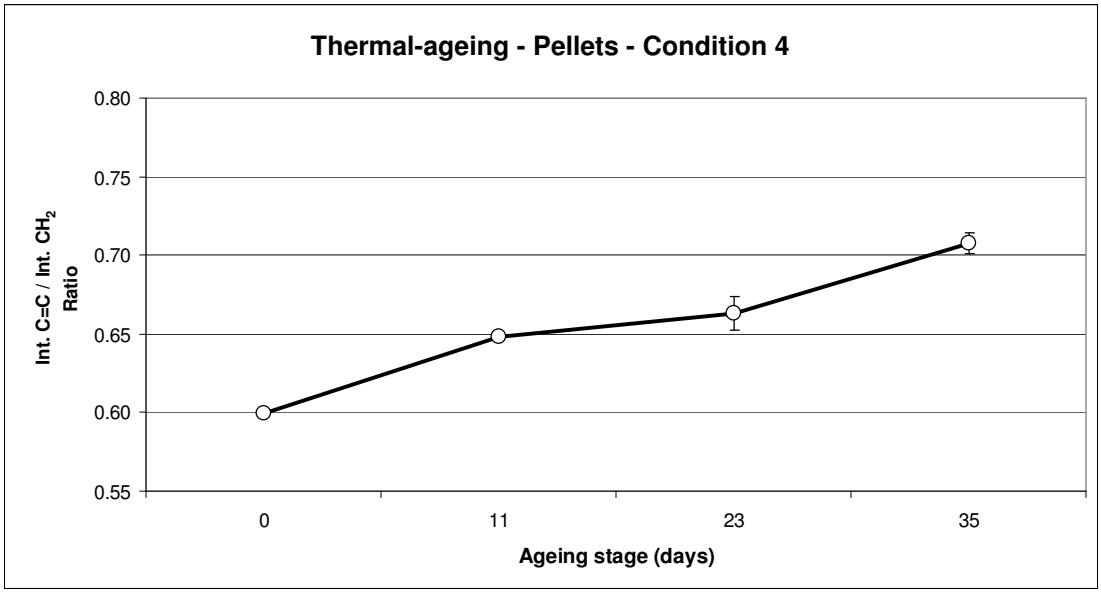


Figure 104. C=C group intensity change in thermal-aged pellets in condition 4 (≤ 20% RH, sample exposed to internal atmosphere).

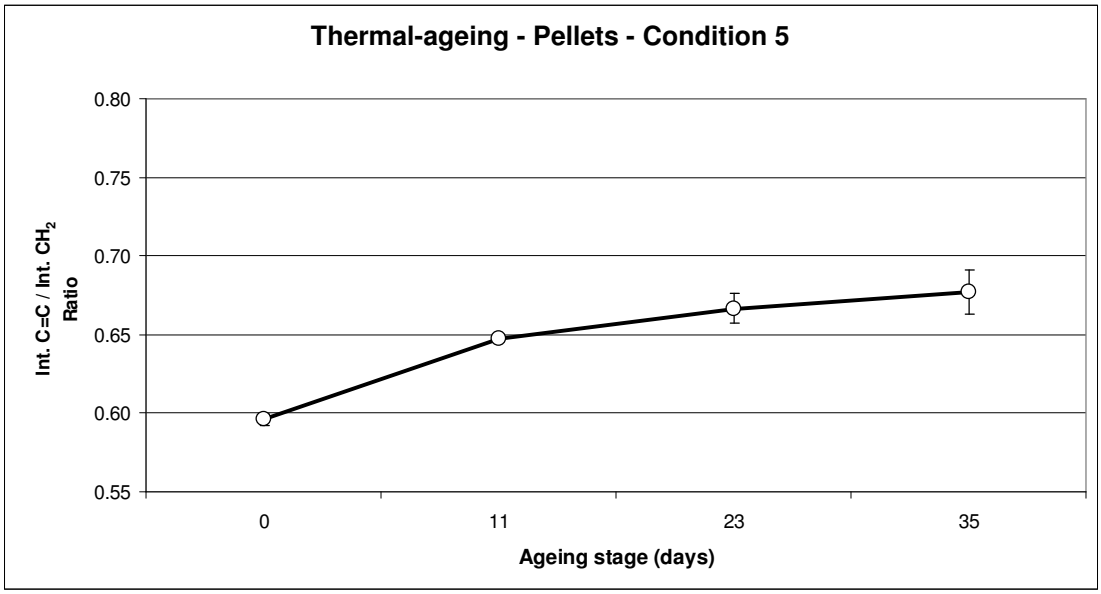


Figure 105. C=C group intensity change in thermal-aged pellets in condition 5 (≤ 20% RH, sample exposed to internal anoxic atmosphere).

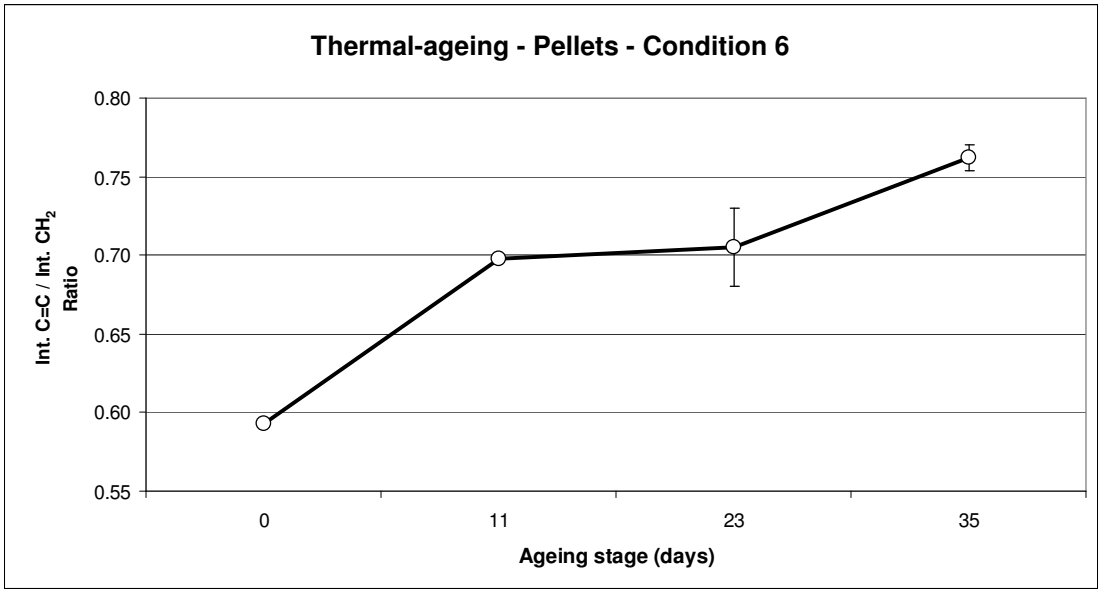


Figure 106. C=C group intensity change in thermal-aged pellets in condition 6 (100% RH, sample exposed to internal atmosphere, pH 3).

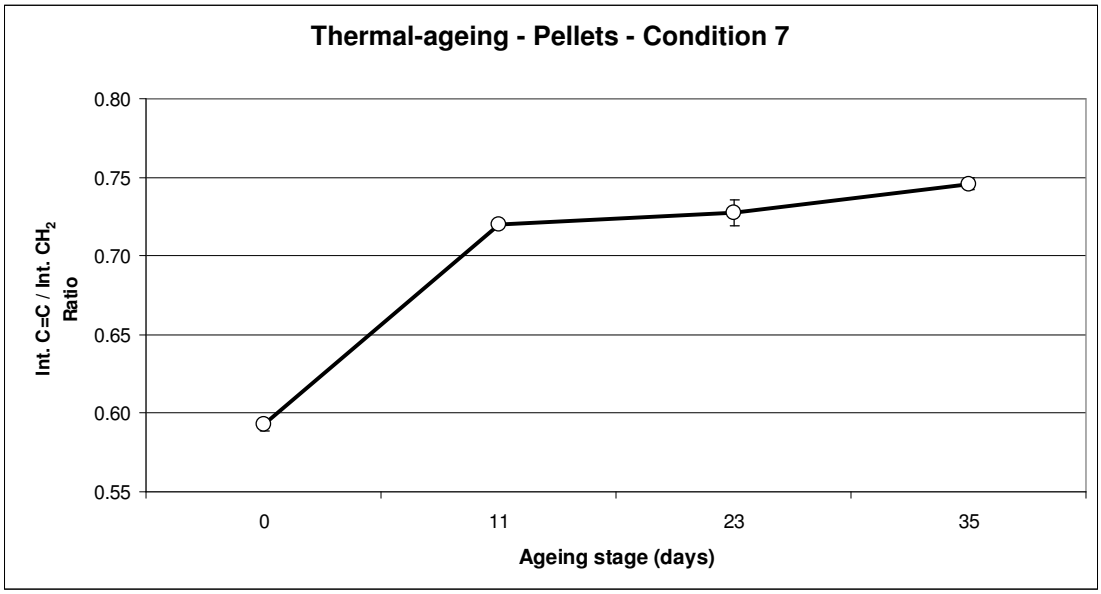


Figure 107. C=C group intensity change in thermal-aged pellets in condition 7 (100% RH, sample immersed in liquid, pH 3).

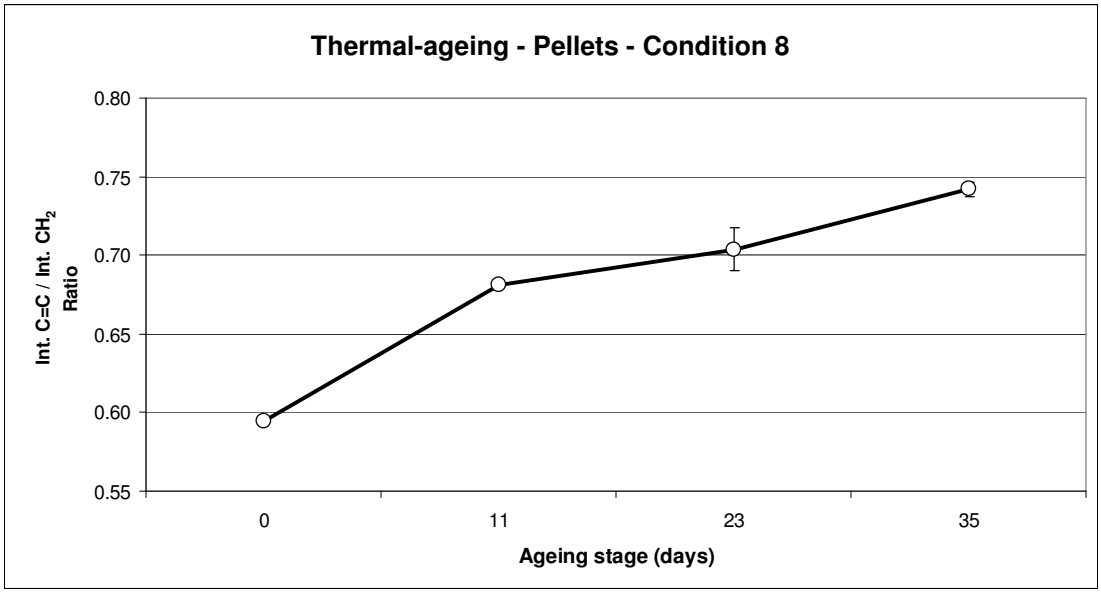


Figure 108. C=C group intensity change in thermal-aged pellets in condition 8 (100% RH, sample exposed to internal atmosphere, pH 5).

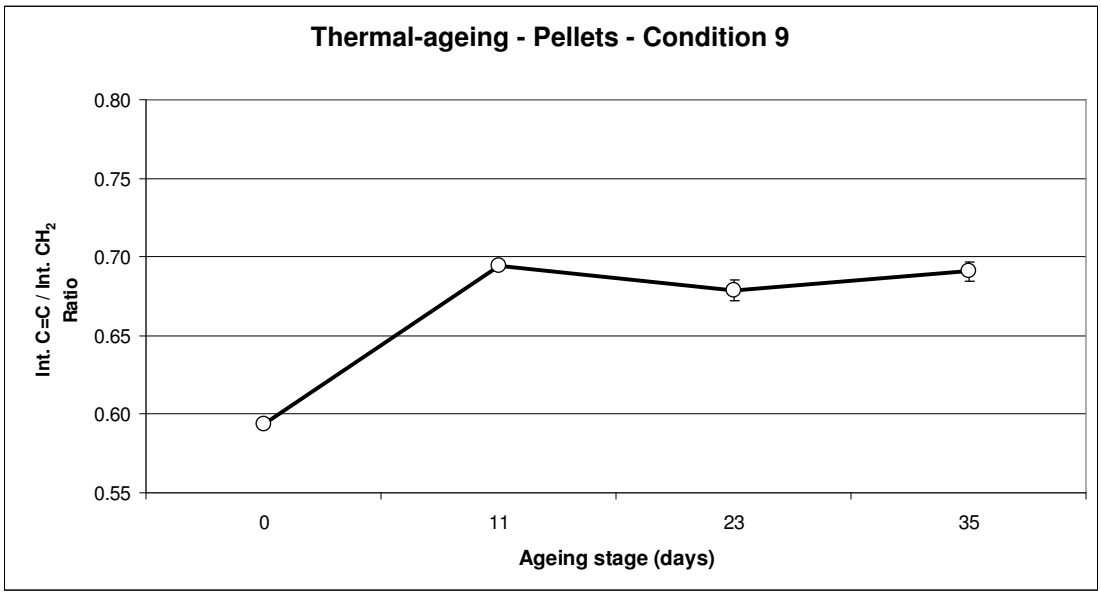


Figure 109. C=C group intensity change in thermal-aged pellets in condition 9 (100% RH, sample immersed in liquid, pH 5).

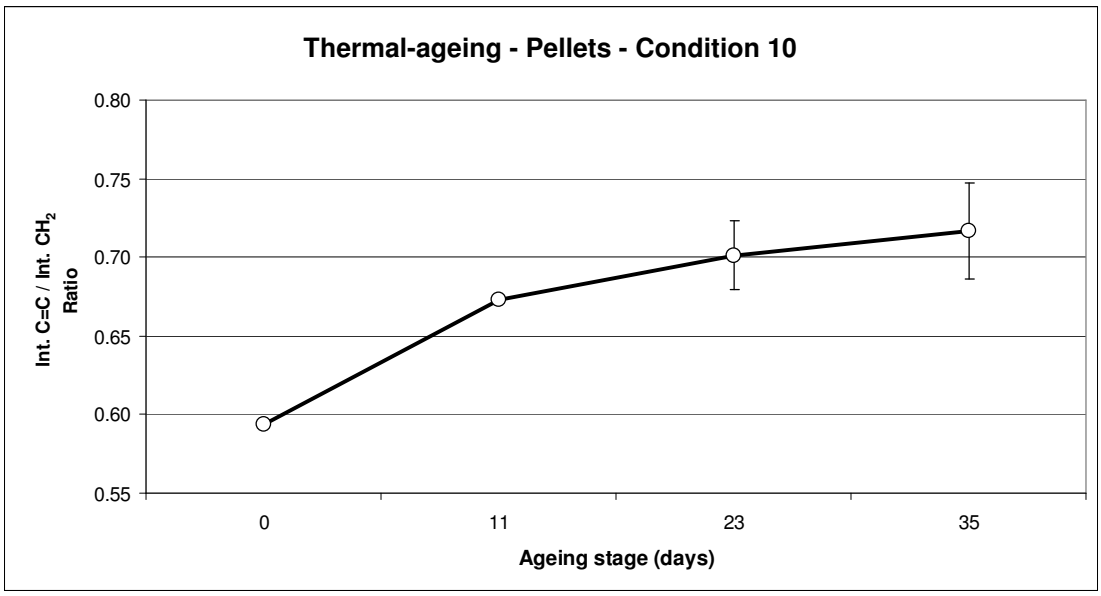


Figure 110. C=C group intensity change in thermal-aged pellets in condition 10 (100% RH, sample exposed to internal atmosphere, pH 10).

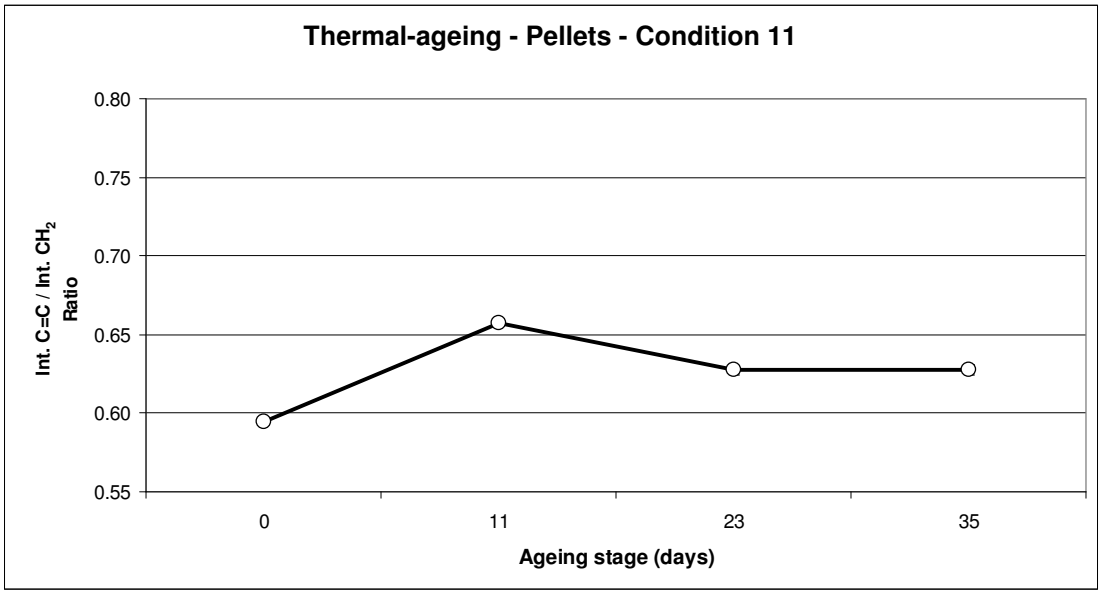


Figure 111. C=C group intensity change in thermal-aged pellets in condition 11 (100% RH, sample immersed in liquid, pH 10).

A.4 Oxygen measurement.

Advanced investigation:

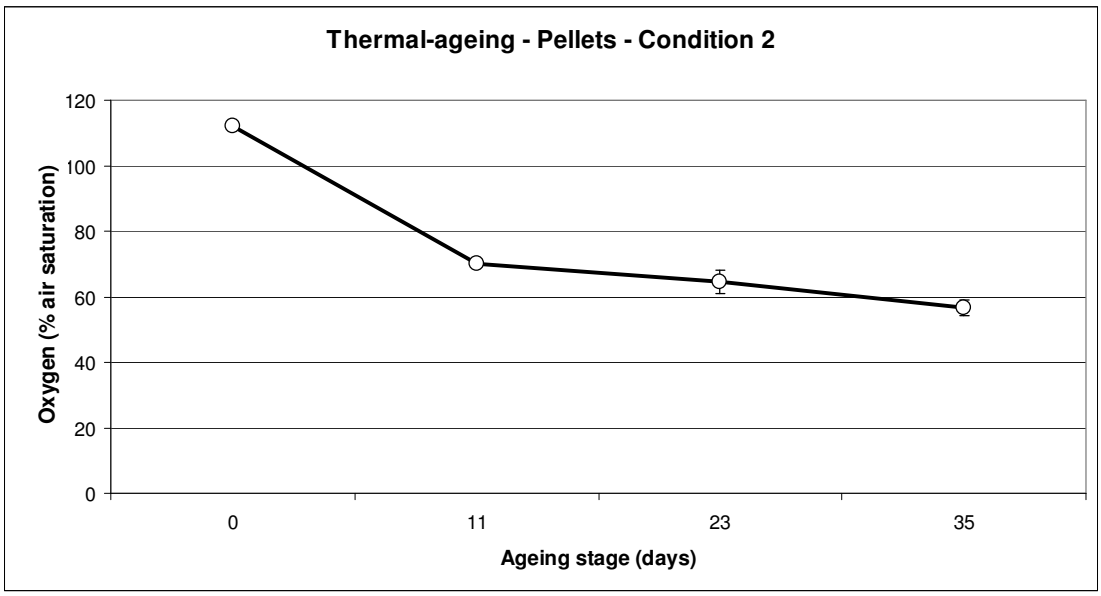


Figure 112. Oxygen consumption trend in thermal-aged pellets in condition 2 (100% RH, sample exposed to internal atmosphere, pH ≤ 5.5).

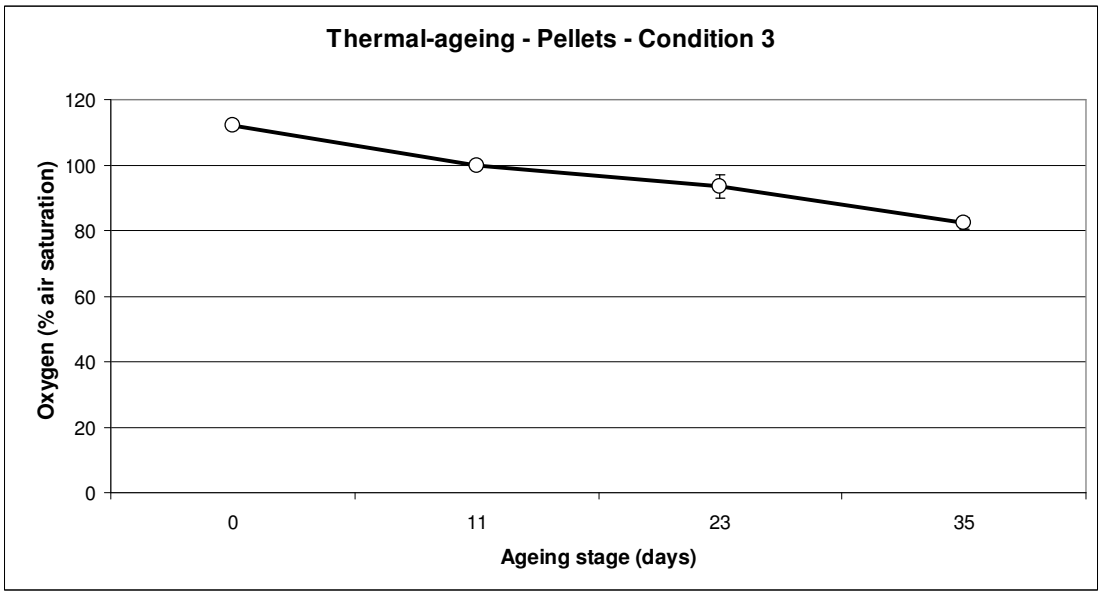


Figure 113. Oxygen consumption trend in thermal-aged pellets in condition 3 (100% RH, sample immersed in liquid, pH ≤ 5.5).

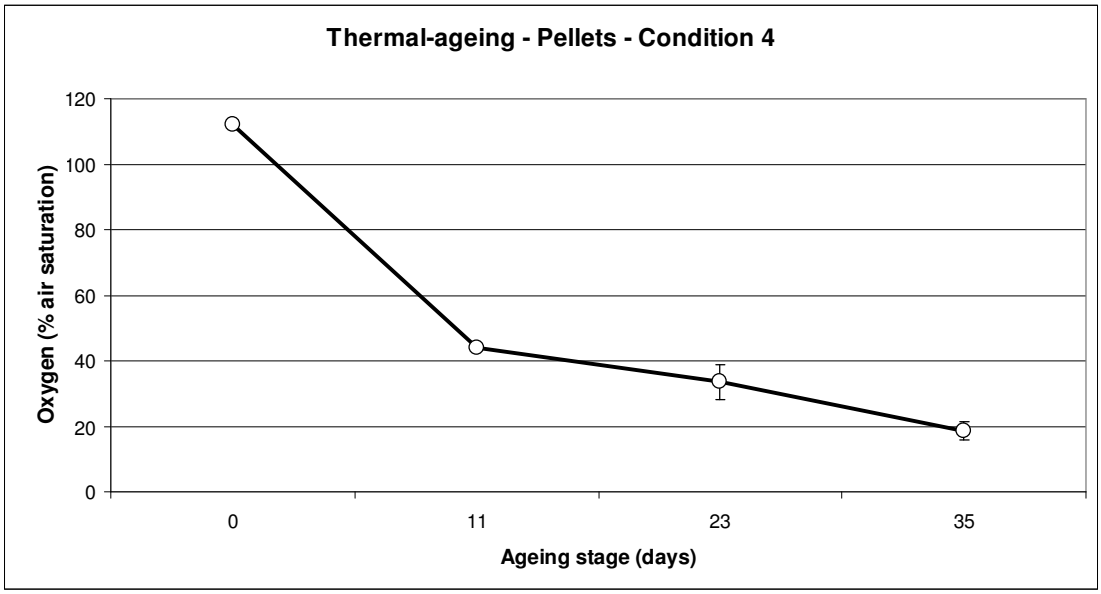


Figure 114. Oxygen consumption trend in thermal-aged pellets in condition 4 (≤ 20% RH, sample exposed to internal atmosphere).

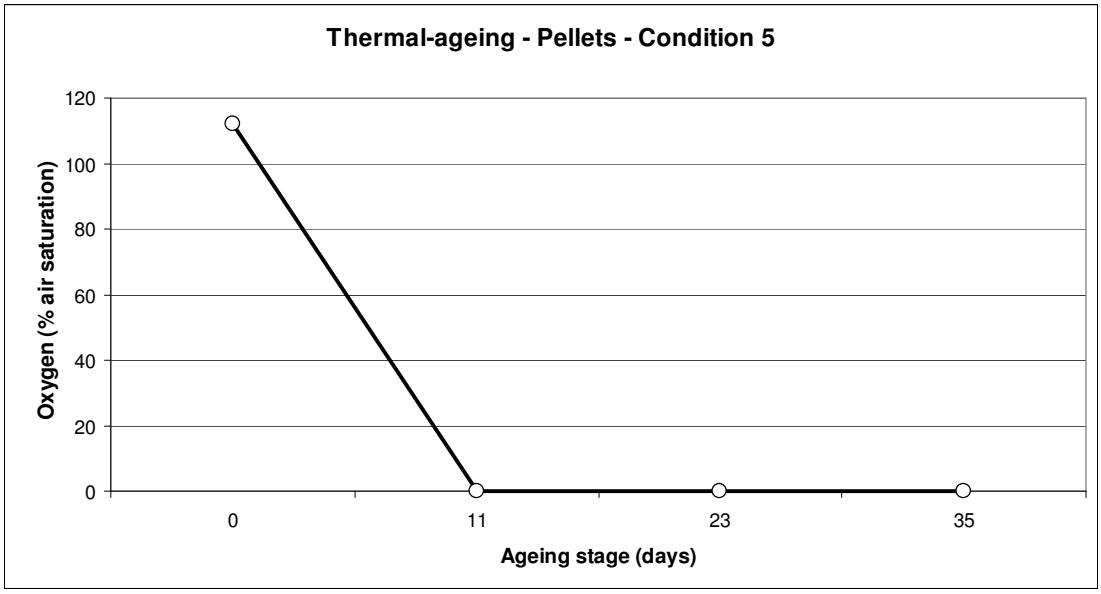


Figure 115. Oxygen consumption trend in thermal-aged pellets in condition 5 (≤ 20% RH, sample exposed to internal anoxic atmosphere).

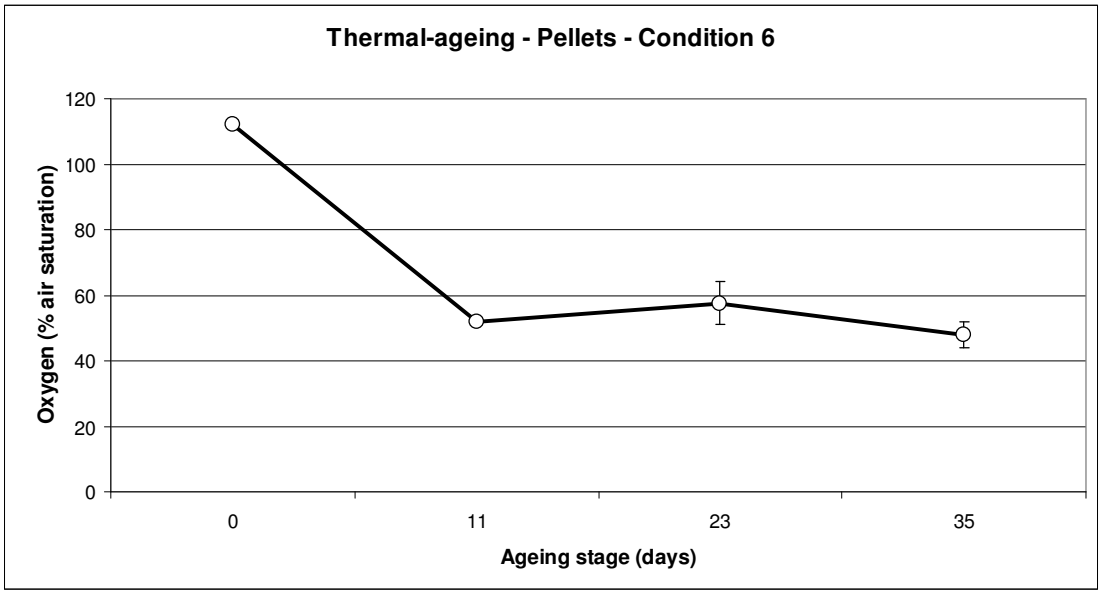


Figure 116. Oxygen consumption trend in thermal-aged pellets in condition 6 (100% RH, sample exposed to internal atmosphere, pH 3).

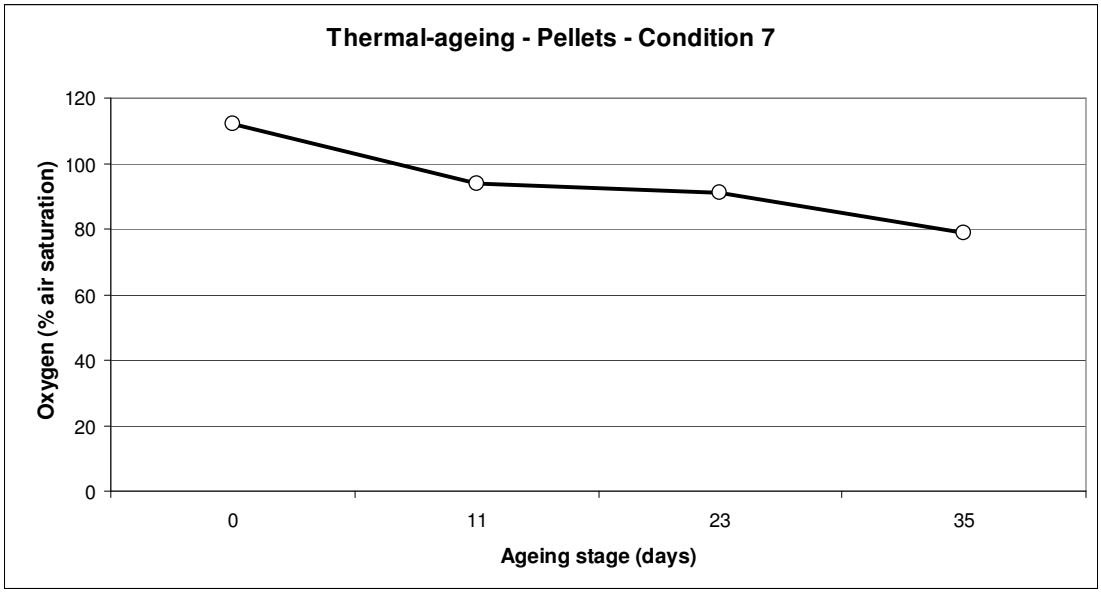


Figure 117. Oxygen consumption trend in thermal-aged pellets in condition 7 (100% RH, sample immersed in liquid, pH 3).

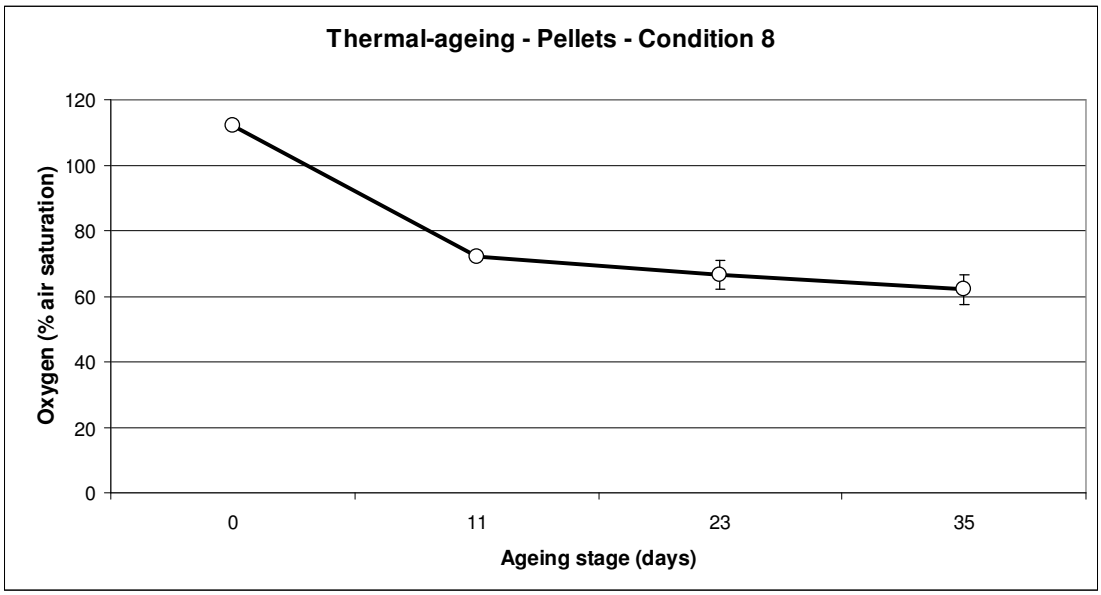


Figure 118. Oxygen consumption trend in thermal-aged pellets in condition 8 (100% RH, sample exposed to internal atmosphere, pH 5).

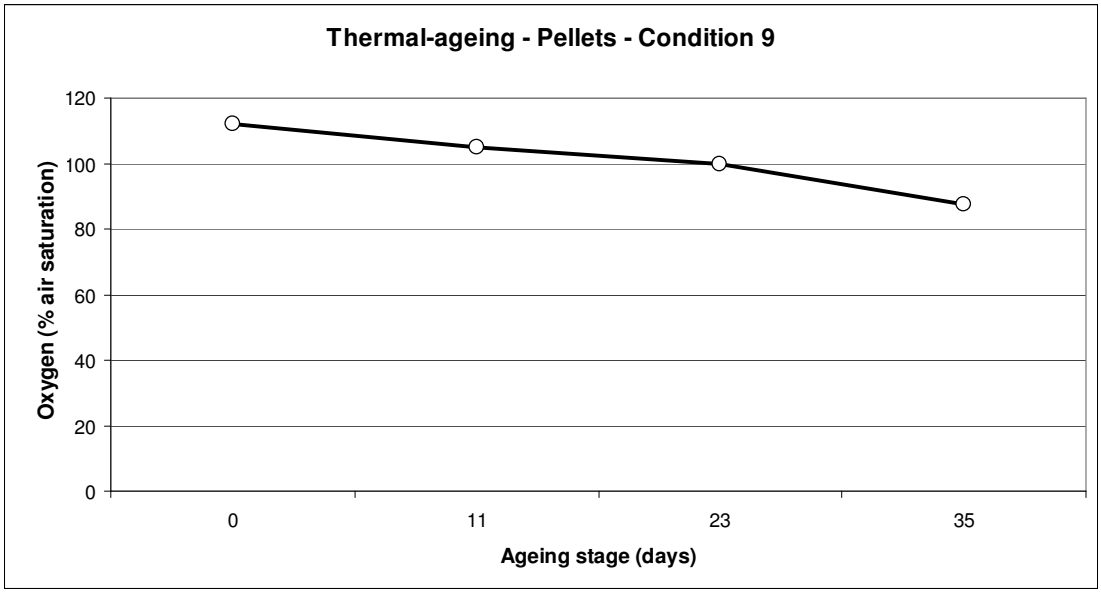


Figure 119. Oxygen consumption trend in thermal-aged pellets in condition 9 (100% RH, sample immersed in liquid, pH 5).

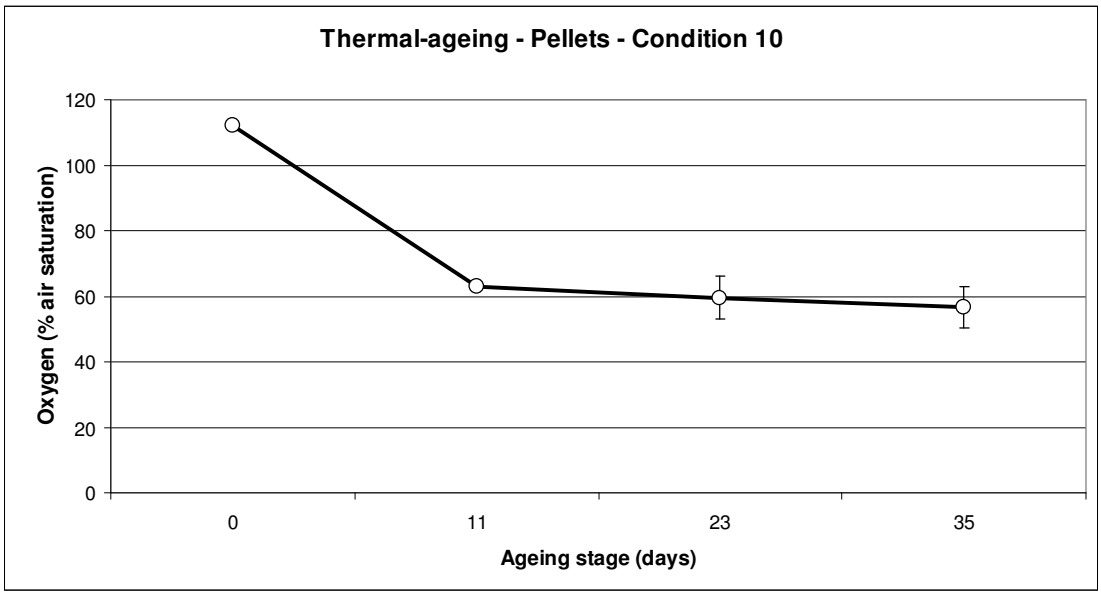


Figure 120. Oxygen consumption trend in thermal-aged pellets in condition 10 (100% RH, sample exposed to internal atmosphere, pH 10).

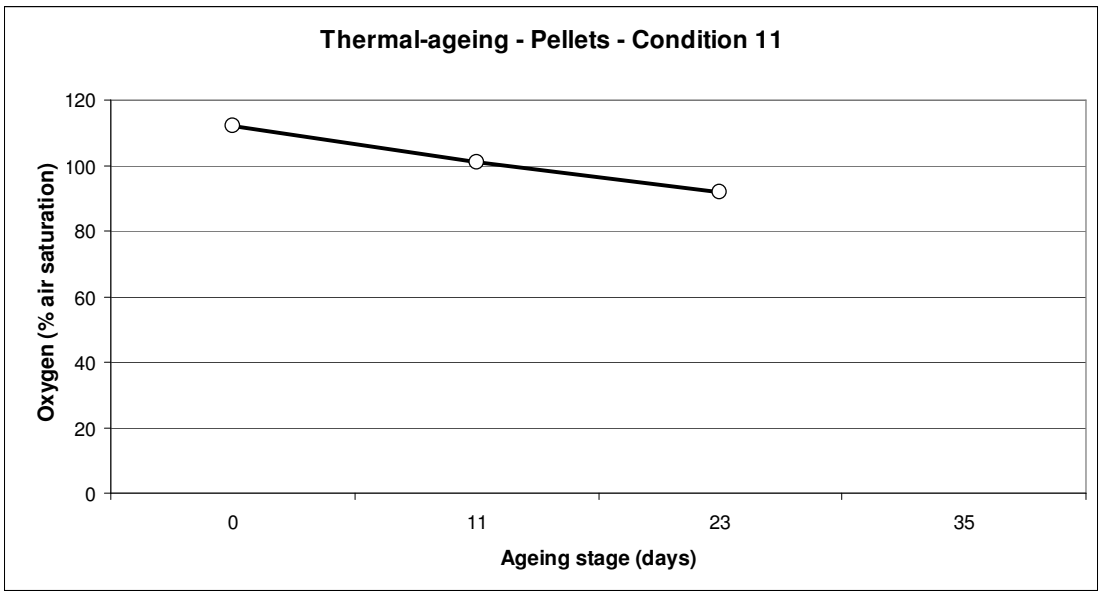


Figure 121. Oxygen consumption trend in thermal-aged pellets in condition 11 (100% RH, sample immersed in liquid, pH 10).

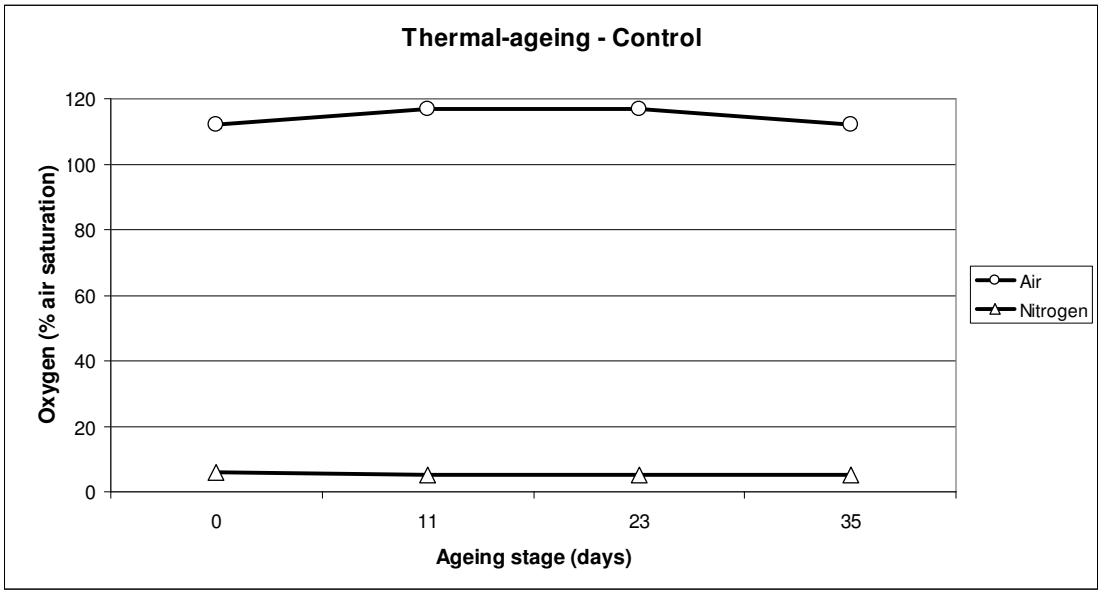


Figure 122. Oxygen concentration trend in thermal-aged control samples.

A.5 Headspace analysis.

Advanced investigation:

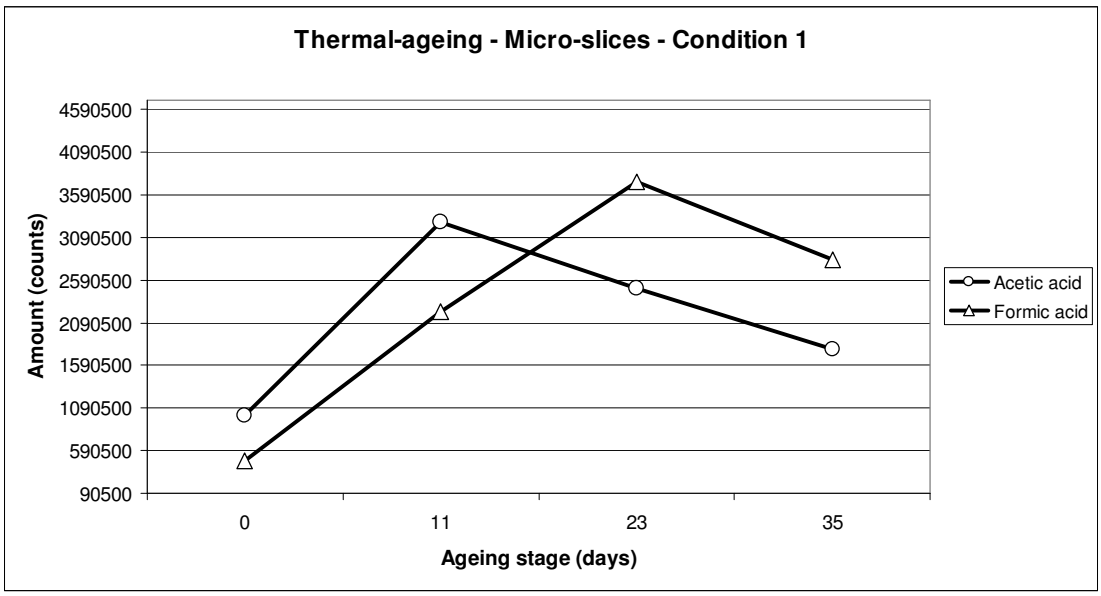


Figure 123. Off-gassing trend in thermal-aged micro-slices in condition 1 (≤ 20% RH, sample exposed to external atmosphere).

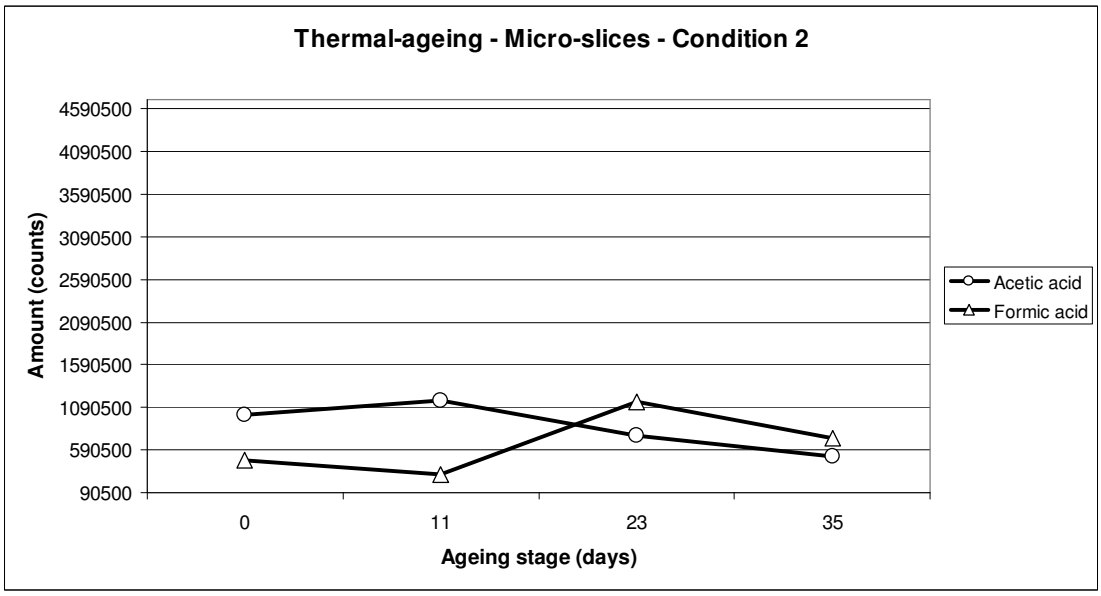


Figure 124. Off-gassing trend in thermal-aged micro-slices in condition 2 (100% RH, sample exposed to internal atmosphere, pH ≤ 5.5).

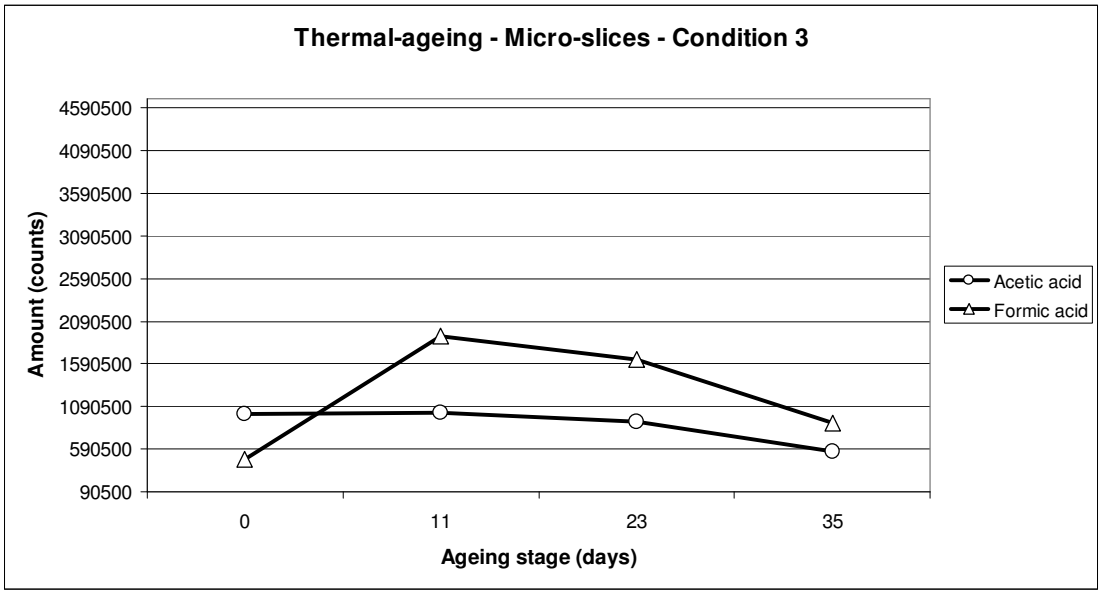


Figure 125. Off-gassing trend in thermal-aged micro-slices in condition 3 (100% RH, sample immersed in liquid, pH ≤ 5.5).

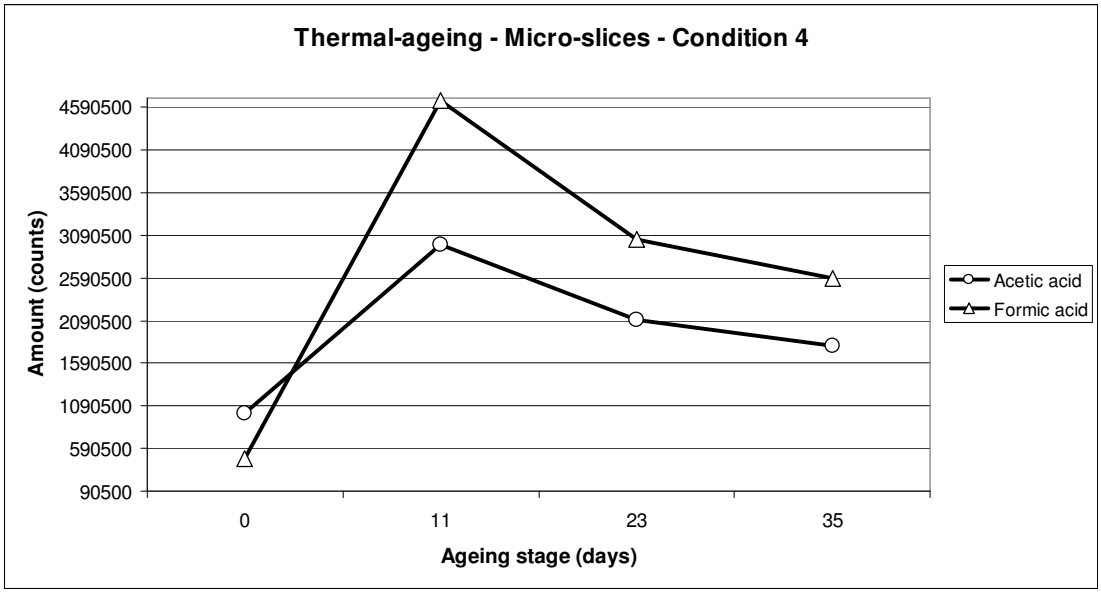


Figure 126. Off-gassing trend in thermal-aged micro-slices in condition 4 (≤ 20% RH, sample exposed to internal atmosphere).

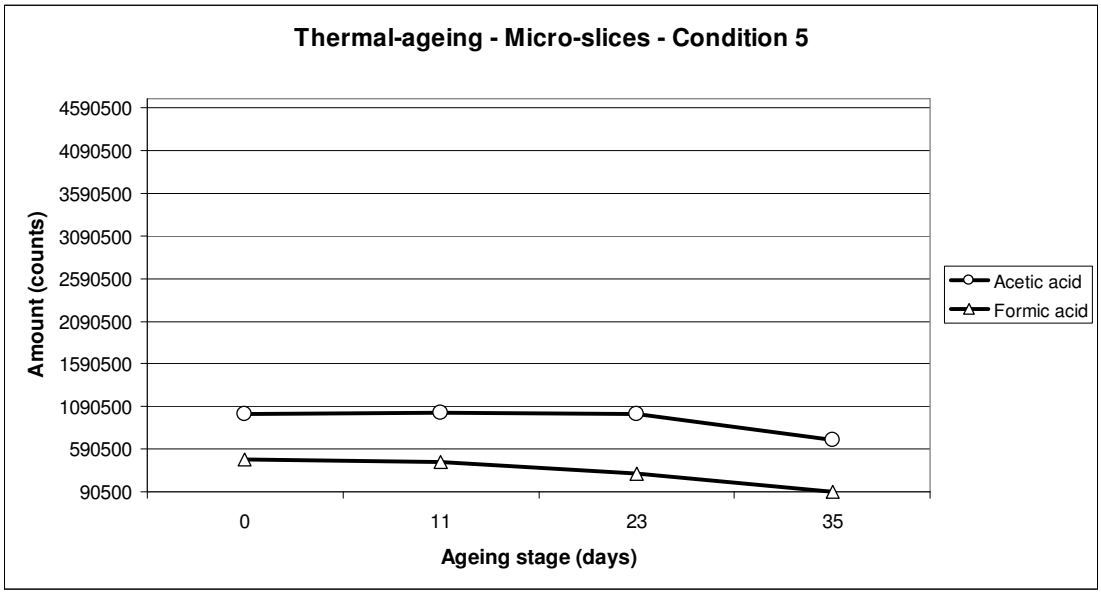


Figure 127. Off-gassing trend in thermal-aged micro-slices in condition 5 (≤ 20% RH, sample exposed to internal anoxic atmosphere).

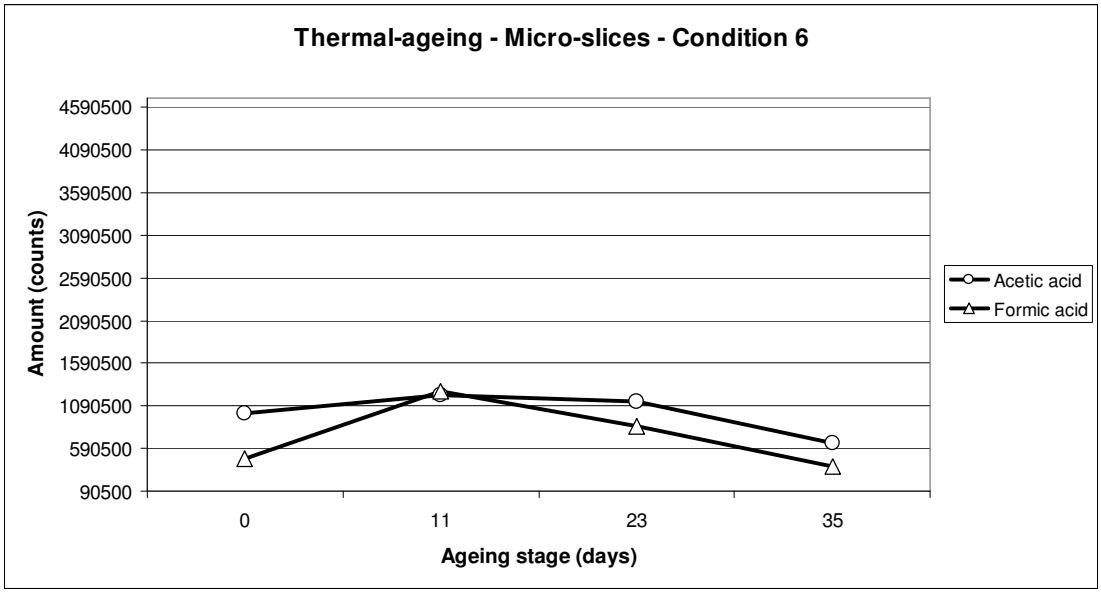


Figure 128. Off-gassing trend in thermal-aged micro-slices in condition 6 (100% RH, sample exposed to internal atmosphere, pH 3).

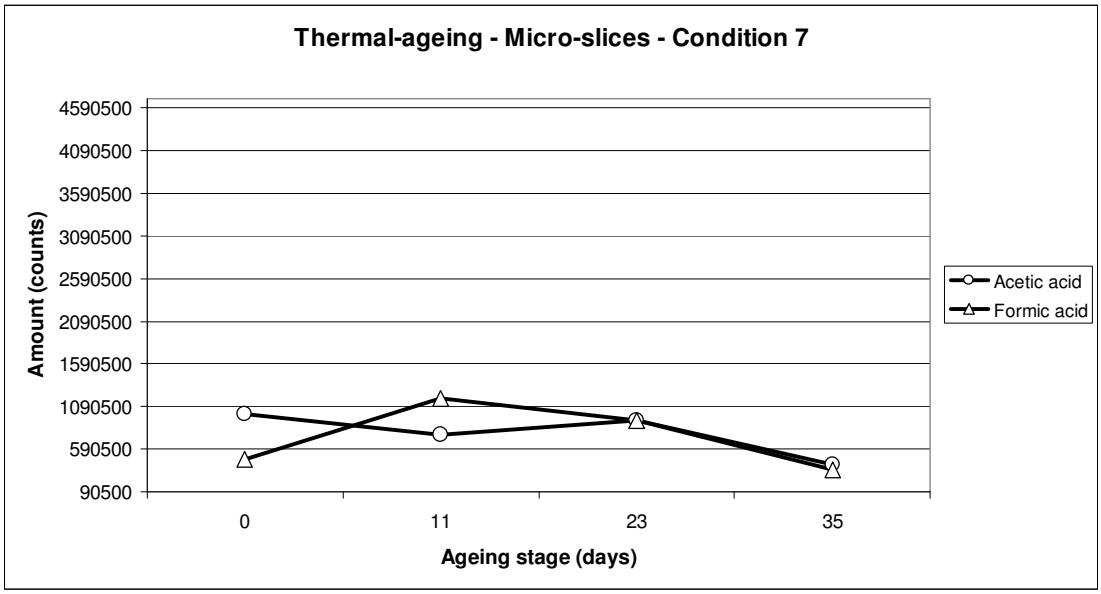


Figure 129. Off-gassing trend in thermal-aged micro-slices in condition 7 (100% RH, sample immersed in liquid, pH 3).

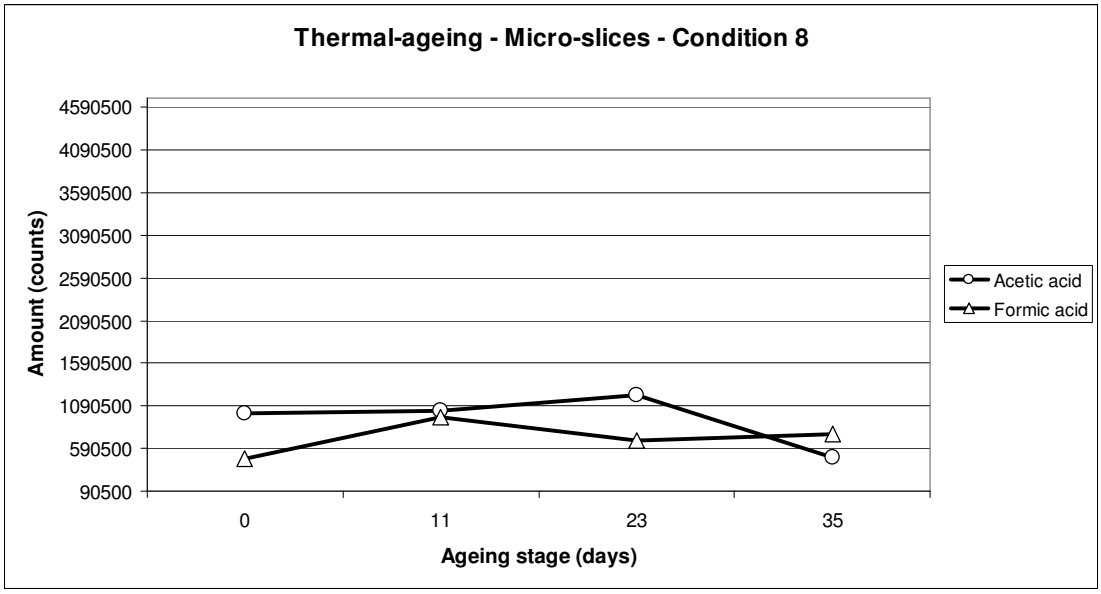


Figure 130. Off-gassing trend in thermal-aged micro-slices in condition 8 (100% RH, sample exposed to internal atmosphere, pH 5).

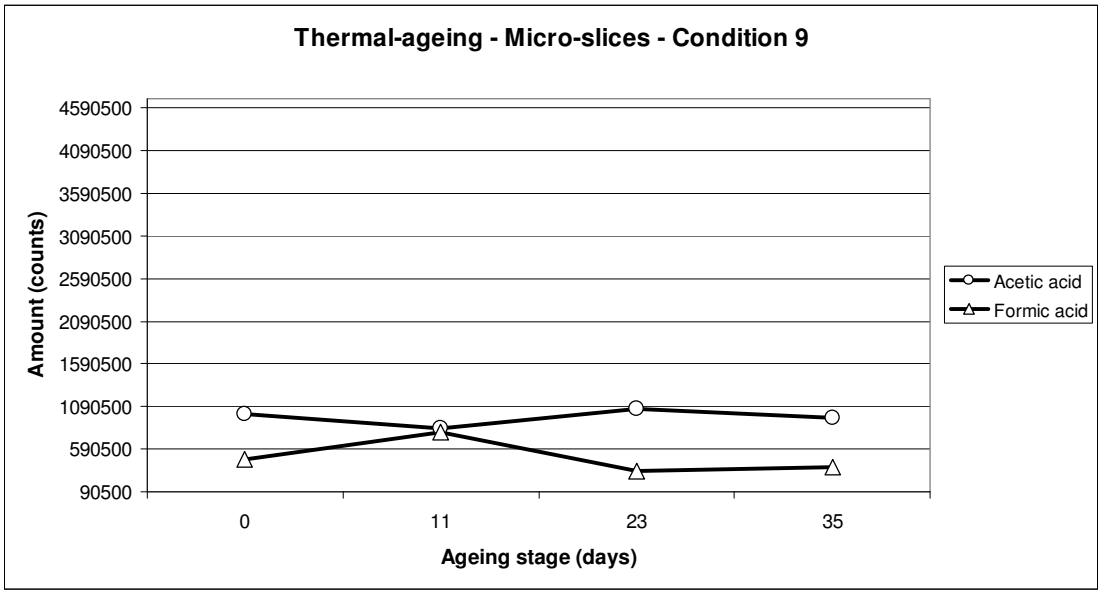


Figure 131. Off-gassing trend in thermal-aged micro-slices in condition 9 (100% RH, sample immersed in liquid, pH 5).

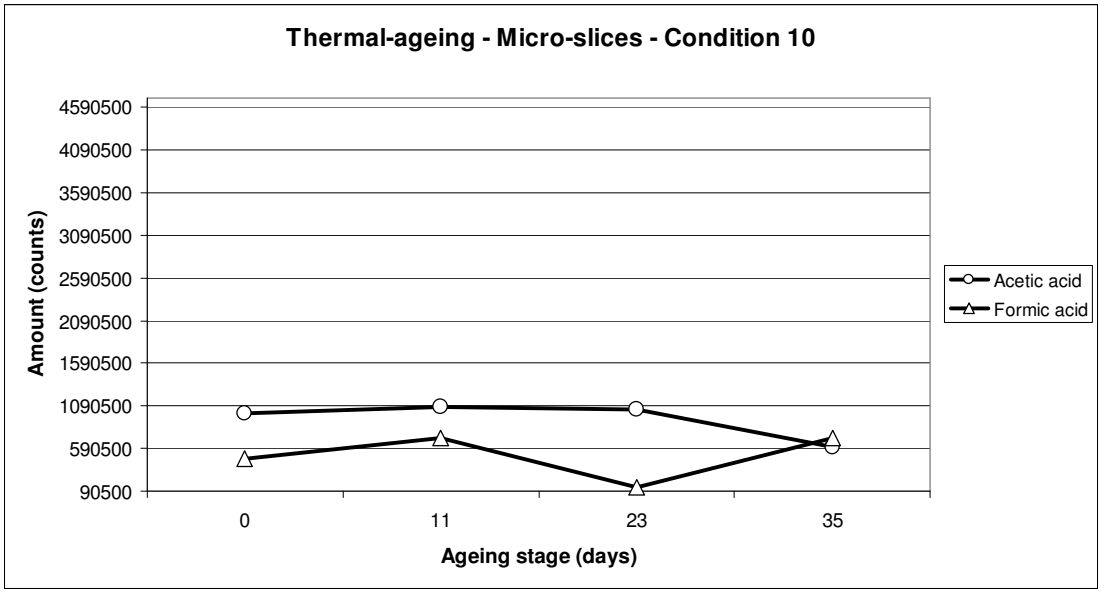


Figure 132. Off-gassing trend in thermal-aged micro-slices in condition 10 (100% RH, sample exposed to internal atmosphere, pH 10).

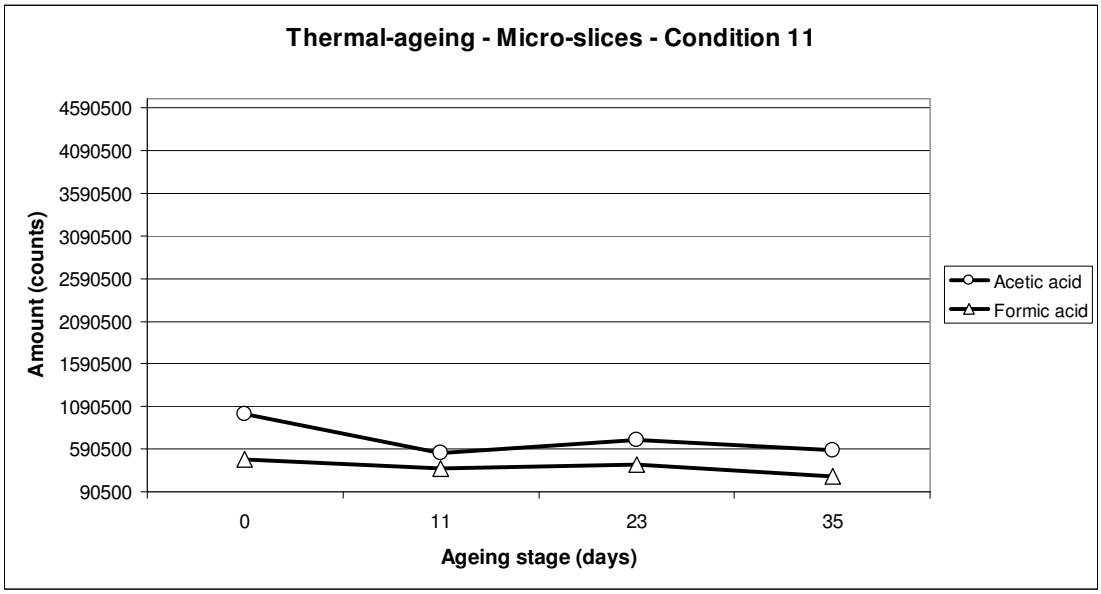


Figure 133. Off-gassing trend in thermal-aged micro-slices in condition 11 (100% RH, sample immersed in liquid, pH 10).

B. ACTIVITIES ACCOUNT.

In this appendix an account of activities, which have taken place during the PhD project period, is presented.

B.1 Teaching activities and other forms of dissemination of knowledge.

27th – 28th September 2007: presentation of the first research report at the first EPISCON annual workshop, in Oviedo (Spain).

9th – 10th October 2008: presentation of the second research report at the second EPISCON annual workshop, in Amsterdam (Netherlands).

19th November 2008: delivery of a lecture about "Amber and infrared spectroscopy" at the School of Conservation, in Copenhagen (Denmark).

2nd – 3rd December 2008: presentation of the own PhD research project at the "1st Scandinavian Conservation PhD Student Colloquium", in Gothenburg (Sweden).

28th – 29th May 2009: presentation of the third research report at the third EPISCON annual workshop, in Budapest (Hungary).

B.2 Participation in courses.

4th September 2006 – 26th January 2007: participation in core courses on Science for Conservation at the University of Bologna, in Ravenna (Italy).

7th – 13th October 2007: participation in courses on Synchrotron Radiation and Neutrons for Cultural Heritage Studies at the ESRF, in Grenoble (France).

17th – 24th January 2008: participation in course on Statistics at the School of Conservation, in Copenhagen (Denmark).

B.3 Participation in conferences and seminars.

<u>As poster presenter</u>
12th – 16th May 2008: poster presentation about the own PhD research project at the "37th International Symposium on Archaeometry", organized by Università di Siena, in Siena (Italy).

<u>As listener</u>
24th June 2009: "Seminar on electronic lab notebooks", organized by Contur Software, at University of Copenhagen, in Copenhagen (Denmark).

10th – 11th May 2007: "Conservation Science 2007", organized by ICON (The Institute of Conservation), at Politecnico di Milano, in Milan (Italy).

31st January 2007: "Science and cultural heritage – diagnostics: training and profession", organized by Italian Association Experts in Diagnosis for Cultural Heritage, at Università di Roma "La Sapienza", in Rome (Italy).

B.4 Stays abroad.

22nd – 26th October 2007: Raman Confocal Microscopy tests on Baltic amber samples at the University of Bologna, in Bologna (Italy).

10th – 13th March 2009: Confocal Profilometry tests on Baltic amber samples at Nanofocus AG, in Oberhausen (Germany).