Hygrothermal ageing of dry gelatine adhesive films

Microstructure-property relationships

Mosleh, Yasmine; van Die, Mees; Gard, Wolfgang; Breebaart, Iskander; van de Kuilen, Jan-Willem; Van Duin, Paul; Poulis, Johannes A.

DOI
10.1016/j.ijadhadh.2024.103654

Publication date
2024

Document Version
Final published version

Published in
International Journal of Adhesion and Adhesives

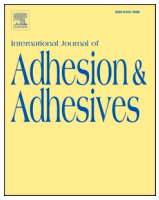
Citation (APA)
Mosleh, Y., van Die, M., Gard, W., Breebaart, I., van de Kuilen, J.-W., Van Duin, P., & Poulis, J. A. (2024). Hygrothermal ageing of dry gelatine adhesive films: Microstructure-property relationships. *International Journal of Adhesion and Adhesives*, *131*, Article 103654. https://doi.org/10.1016/j.ijadhadh.2024.103654

International Journal of Adhesion & Adhesives 131 (2024) 103654

Contents lists available at ScienceDirect

International Journal of Adhesion and Adhesives

journal homepage: www.elsevier.com/locate/ijadhadh



Hygrothermal ageing of dry gelatine adhesive films: Microstructure-property relationships

Yasmine Mosleh [a,b,*], Mees van Die [a], Wolfgang Gard [b], Iskander Breebaart [c], Jan-Willem van de Kuilen [b,d], Paul van Duin [c], Johannes A. Poulis [a]

[a] Structural Integrity and Composites, Department of Aerospace Structures and Materials, Faculty of Aerospace Engineering, Delft University of Technology, Delft, the Netherlands
[b] Biobased Structures and Materials, Department of Engineering Structures, Faculty of Civil Engineering and Geosciences, Delft University of Technology, Delft, the Netherlands
[c] Department of Conservation & Science, Rijksmuseum, Amsterdam, the Netherlands
[d] Wood Technology Group, TU Munich, Munich, Germany

ARTICLE INFO

Keywords:
Hygrothermal ageing
Gelatine
Adhesive
Cultural heritage
Triple helix

ABSTRACT

Gelatine adhesives, also known as animal glues, are collagen-based water-soluble biopolymers derived from vertebrate connective tissues. One of the various fields in which gelatine adhesives are widely used is the conservation of cultural heritage such as decorated furniture and panel paintings. It is observed that, with time, the failure in these objects often occurs along the adhesive bondlines. Given the moisture and temperature sensitivity of these adhesives, obtaining knowledge of their long-term behaviour, when exposed to climate variations, is pivotal. Here, the influence of hygrothermal ageing (exposure to a combination of elevated temperature and relative humidity (RH) cycling) on the microstructure and macroscopic properties of four different types of gelatine adhesives is investigated. These adhesives were selected from different animal origins namely bovine, rabbit, and fish with different Bloom strengths. It was observed that ageing cycles interfere with the most critical structural feature of protein chains namely triple helices. A clear decay in triple helix content at the micro-scale, determined by Differential Scanning Calorimetry (DSC) and X-Ray Diffraction (XRD) techniques, was observed which had implications on the macroscopic properties of these adhesives such as reduction of strain to failure and toughness (strain energy density to failure). The rate of decay in properties was revealed to be the highest in the adhesives with the lowest triple helix content. This study provides a scientific view of microstructure-property relationships in gelatinous adhesives as a function of environmental ageing, and stipulates the underlying mechanism of the degradation of mechanical properties as the loss of structural triple helices, regardless of the animal origin.

1. Introduction

Gelatine-based adhesives, also known as animal glues, are proteinaceous natural polymers which have been used in the making of historic objects for centuries and even since antiquity [1,2]. Evidence of the use of animal glue goes back to the 4th millennium BC in Europe [3]. Historically, animal glues were used by artists and craftsmen as adhesives, consolidants, varnishes, binding media, and filler materials in artefacts such as decorative wooden furniture, panel and mural paintings, canvas linings, bookbinding, medieval illuminations, musical instruments, costumes and hats, and pottery repair [4–6]. In contemporary times, animal glues are used widely in restoration and conservation practices for both organic and inorganic materials, and more specifically for wooden objects [4].

Gelatine-based animal glues are derived from the skin, bone, or connective tissues of mammalian or fish species through the hydrolysis process. Gelatine is a water-soluble semi-crystalline biopolymer derived from animal collagen. During the gelling and subsequent drying of gelatine, the random protein coils undergo partial renaturation back into triple helices existing in the original collagen molecular structure. These triple helices in gelatine act as physical cross-links leading to a continuous 3D network structure [4,7–9]. The triple-helix content which

* Corresponding author. Structural Integrity and Composites, Department of Aerospace Structures and Materials, Faculty of Aerospace Engineering, Delft University of Technology, Delft, the Netherlands.
E-mail address: y.mosleh@tudelft.nl (Y. Mosleh).

https://doi.org/10.1016/j.ijadhadh.2024.103654
Received 24 January 2023; Received in revised form 28 December 2023; Accepted 12 February 2024
Available online 13 February 2024

is linked to the degree of renaturation is an important physical characteristic of animal glues, dominating their thermal and mechanical performance [10–13]. The triple-helix content of animal glues can be influenced by many factors such as animal origin (defining amino acid profile e.g., hydroxy proline content), molecular weight distribution, concentration, glue drying rate and temperature, and moisture content [14–18]. As a consequence of their molecular structure and water solubility, animal glue-bonded joints are reversible, even after centuries. This reversibility of animal glues distinguishes them from modern synthetic adhesives, particularly in restoration practices of wooden objects. However, animal glues are hygroscopic and susceptible to environmentally induced degradation. Fluctuations in ambient relative humidity (RH) and temperature lead to continuous shrinkage and expansion of the adhesives, e.g. in the wooden bonded joints, which can cause the development of micro-cracks within the adhesive and/or at the interface layer. Prior studies (among which an extensive study on 17th-century furniture and panel paintings from the collection of the Rijksmuseum, The Netherlands) demonstrated that the failure in these objects mostly occurred in the adhesive lines joining the wooden panels together [19]. Fig. 1 illustrates this type of failure in a decorated cabinet with marquetry. In this figure, the development of a vertical crack along the adhesive joint can be clearly observed. The inspection of the fracture surface indicates a combination of cohesive (within adhesive and/or wood) and adhesive failure across the bonded joint surface. Such crack development is believed to be the result of a lack of climate control in Castle Amerongen. In a previous study, the indoor climate fluctuations in Castle Amerongen were monitored between the years 2003–2005 during which the temperature variations were measured between 15 and 25 °C, and RH between 50% and 75% [20]. Such climate variations are believed to be the cause of the development of such vertical cracks and the degradation of bondlines due to shrinkage and expansion of wood when exposed to changes in environmental relative humidity. However, the other twin of the same decorated cabinet manufactured by van Mekeren is currently in a well-preserved state in climate-controlled rooms at Rijksmuseum for public display, in which no signs of degradation have been observed so far. The case of the two identical cabinets of van Mekeren is a clear example showing the effect of environmental parameters on the ageing and degradation of historic objects, and hence the importance of climate control for their preservation.

Consequently, understanding the ageing behaviour of animal glues when exposed to a changing climate is vital for designing more durable formulations as well as providing the conservators with more informed choices of adhesive type for their conservation/restoration practices. Moreover, knowledge of long-term structural alterations and mechanical properties of animal glues is necessary for establishing/relaxing guidelines for climate control inside the display rooms of the museums for the preservation of objects which particularly contain animal glues. Bridarolli et al. [21] investigated the mechanical performance of several animal glues from mammalian and fish origins used in conservation practices at different temperatures and RHs. They performed a combination of dynamic mechanical analysis and tensile tests on a variety of animal glues within a range of relative humidities and temperatures allowing the construction of phase diagrams to predict the behaviour of glues in different environments. Furthermore, it was shown that sample preparation and the thermal and hygroscopic history of the material may affect the mechanical properties of the glues.

Mosleh et al. [22] studied the effect of RHs on thermal behaviour (glass transition temperature, denaturation temperature and enthalpy). Moreover, they observed a correlation between the moisture sensitivity of animal glues (from fish and mammalian origins) and their triple helix content. However, systematic data and scientific analysis on the ageing phenomena of animal glues are yet scares. Moreover, knowledge of the long-term physical and mechanical behaviour of animal glues is necessary for establishing/relaxing guidelines for climate control inside the display rooms of museums across the world for the preservation of objects containing animal glues.

In this paper, four different prevalently used animal glues of bovine, rabbit, and fish origin were chosen and their hygrothermal ageing behaviour was investigated. Adhesive films were prepared via a solution casting method and underwent an accelerated hygrothermal ageing protocol inside a controlled climate chamber. This accelerated ageing protocol entailed a combination of raised temperature (34 °C) and RH cycling (between 30% and 80% RH) for two and 4 weeks. To monitor the role of the structural changes during ageing, Differential Scanning Calorimetry (DSC), and X-Ray Diffraction (XRD) techniques were employed. Tensile experiments were performed to monitor the mechanical properties of the adhesives as a function of ageing and also to elucidate the interplay between microstructural changes and macroscopic properties of the adhesives. Moreover, the rate of decay for different adhesives was investigated.

Fig. 1. The development of a vertical crack along the adhesive joint of the panels in a decorated cabinet door. Jan van Mekeren, The Netherlands, c. 1700. Oak door: 110 × 80 × 3.5 cm, located in Castle Amerongen (The Netherlands), courtesy of René Gerritsen.

2. Materials

2.1. Gelatine adhesives (animal glues)

Four different types of animal glues commonly used in the restoration practice of wooden objects were studied. The assessed adhesives are all commercial products. The choice of these adhesives was made in close consultation with cabinet restorers of the Rijksmuseum. They include bovine bone glue, bovine skin glue, rabbit skin glue, and fish glue. Bovine bone adhesive (article number O6300 and CAS number 9000-70-8), bovine skin glue with (article number O6300), and rabbit glue (article number O6302) were all sourced from Labshop (Twello) B. V. (Apeldoorn, the Netherlands). Fish glue (series number 63080 and CAS number 9000-70-8) was obtained from Kremer Pigmente in powder form. MERGAL KM90 pesticide was added to the aqueous solutions of the adhesives to prevent biodegradation.

The gel (Bloom) strength of the adhesives was measured according to the GME method [23] using a Brookfield CT3 texture analyser equipped with an AOAC plunger (diameter: 12.70 mm, plane surface, and sharp edge). The Bloom strength values of bovine bone, Rabbit skin, bovine skin, and fish were measured as 169 ± 2.5, 261± 4.0, 306± 4.5, and 786± 12.0 respectively.

2.2. Sample preparation

A solution casting method was used to manufacture the thin adhesive

films with a thickness of around 0.24 ± 0.02 mm. During this process, 40 g of dry adhesive powder was dissolved in 200 mL of demineralised water. To avoid biological decay of the adhesive films, 0.1 ml of Mergal KM90 was added to the adhesive solution and was then stirred during 60 min au bain marie, at 60 °C using a magnetic stirrer. Subsequently, the adhesive solution (16 ml) was homogenously injected into a 10 × 10 cm Teflon mould using a syringe as illustrated in Fig. 2.

Subsequently, the injected adhesive films were left to cool and dry at room temperature (23 °C) and a controlled environmental RH of 50% in an environmental climate chamber (Weis WK-3340/70) for at least 48 h.

3. Experimental methods

3.1. Hygrothermal ageing protocol

Hygrothermal ageing of the adhesive films was performed in a controlled environment of a climate chamber Weiss WK-3340/70. The accelerated ageing protocol entailed a combination of a raised constant temperature of 34 °C and cyclic RH which varied between 30% and 80%. Preliminary equilibrium moisture content measurements on the adhesive films were performed to determine the duration of the cyclic RH when varied from 30% to 80% and back in the climate chamber. For this purpose, the adhesive films were conditioned and acclimatised at 30% RH for 24 h until their weight was constant. Subsequently, the RH of the climate chamber was increased to 80% and the weight of the adhesive films was measured every 2 h. Fig. 3 demonstrates representative curves (out of three measurements on each adhesive type) of moisture absorption when dogbone adhesive films were humidified from 30% to 80% RH (Fig. 3 left) and dehumidified from 80% to 30% RH (Fig. 3 right).

As observed for each half cycle, all adhesives reach an equilibrium moisture content after 8 h in the climate chamber. Hence, one full cycle of RH (from 30% to 80% RH and back) was set to 16 h.

The choice of 30% and 80% as minimum and maximum RHs was based on possible extreme climate conditions. Indoor RH conditions in the winter and summer could in extreme cases reach 30% (with indoor heating in the winter) and 80% (in hot and humid summer) respectively, without humidity control. 34 °C can be a plausible high indoor temperature in the summer (e.g. in rooms without climate control in the summer, depending on the geographic location), and yet 34 °C is below the glass transition temperature of the adhesives, and hence it was chosen for the ageing protocol in this study.

Hygrothermal ageing was performed for 2 weeks (21 cycles) and 4 weeks (42 cycles) using the automatic chamber program. During the ageing program, the adhesive film samples were hung on paper clips from the racks in the climate chamber, for better exposure to the environment.

3.2. Physical characterisation methods

3.2.1. Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry is one of the most important techniques to measure thermal transitions in the structure of the proteinaceous adhesives before and after ageing experiments. A TA Instrument DSC 250 differential scanning calorimeter was used to measure the glass transition temperature (T_g), denaturation temperature (T_d), and the denaturation enthalpy (ΔH_d) of adhesive films. Therefore, the four types of animal glue films were first acclimatised at 23 °C and 50% RH (standard conditions) before testing. Subsequently, hygrothermally aged adhesive films, after two weeks and four weeks of ageing, were again acclimatised at standard conditions for 24 h prior to DSC measurements.

Adhesive film samples were cut into small pieces, each weighing about 8 mg and were placed into hermetically sealed containers using Tzero aluminium pans, and an empty pan was used as a reference. For the measurements, the samples were heated and cooled. In the heating step, the samples were heated from 10 °C to 150 °C at 10 °C/min; they were then maintained at 150 °C for 5 and then cooled from 150 °C to room temperature at 10 °C/min. All the measurements were performed in triplicate.

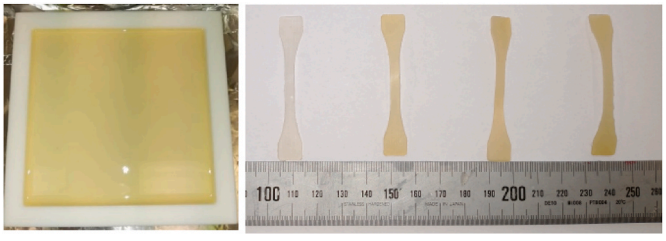


Fig. 2. Cast gelatine adhesive film in a 10 × 10 cm Teflon mould; adhesive films cut into dogbone-shaped samples for tensile testing.

3.2.2. X-ray diffraction (XRD)

X-ray diffractograms of the adhesive films were measured for 2θ between 3 and 60° at 0.1° intervals and a speed of 1°/min using a Rigaku MiniFlex 600 with a NaI scintillator detector. A CuKα radiation source was used (I = 15 mA, U = 40 kV). To study the effect of hygrothermal ageing on the crystalline microstructure content of the adhesive films, the measurements were performed on the adhesive films before and after ageing. Prior to measurements, both unaged and aged (for 2 and 4 weeks) adhesive samples were conditioned in a controlled environmental chamber set at 23 °C and 50 % RH for 24 h. XRD measurements were performed in triplicate and the average representative curves are used in the comparative graphs shown in the following sections. All the measurements were performed in triplicate.

3.3. Mechanical tests

3.3.1. Uniaxial tension

Uniaxial tensile tests based on the ISO 527-2 standard were performed to measure the mechanical properties of different adhesive films such as Young's modulus, tensile strength, strain to failure, and strain energy density to failure (toughness). For the measurements, a standard tensile INSTRON machine (model 3365) equipped with a 1 kN load cell was used. The tensile test specimens were cut into a dogbone shape with dimensions of 4 mm (gage width) × 15 mm (gage length), and 0.24 ± 0.02 mm (thickness), using a cutting die and a stamper. The strain rate was set to 10% min^{-1}. All the specimens, both aged and unaged, were conditioned for 24 h at a temperature of 23 °C and 50% RH prior to testing. For each adhesive type, 10 specimens were tested. Young's modulus (E) was calculated as the linear part of the stress-strain tensile curve between $\varepsilon_1 = 0.05\%$ and $\varepsilon_2 = 0.25\%$. Tensile strength (σ_{max}) was measured as the maximum stress, which for these materials is also stress at break. Strain to failure ($\varepsilon_{failure}$) is reported as the strain at break. Strain energy density to failure (J/m^3) was calculated as the area under the stress-strain curve up to failure. All the experiments on aged and unaged adhesives are performed on at least three different samples.

4. Results and discussion

4.1. Effect of hygrothermal ageing on triple helix content investigated by DSC and XRD measurements

Animal glue is an impure form of gelatine. Gelatine is a thermoplastic biopolymer consisting of crystalline or ''ordered'' and amorphous polypeptide chain domains. These 'ordered domains' consist of aggregates or bundles of (partially) renatured triple helices that form upon cooling and drying of the adhesive film through kinematically as well as thermodynamically controlled processes. The amorphous phase consists of colloidal chains forming single helices [24]. Partially renatured triple

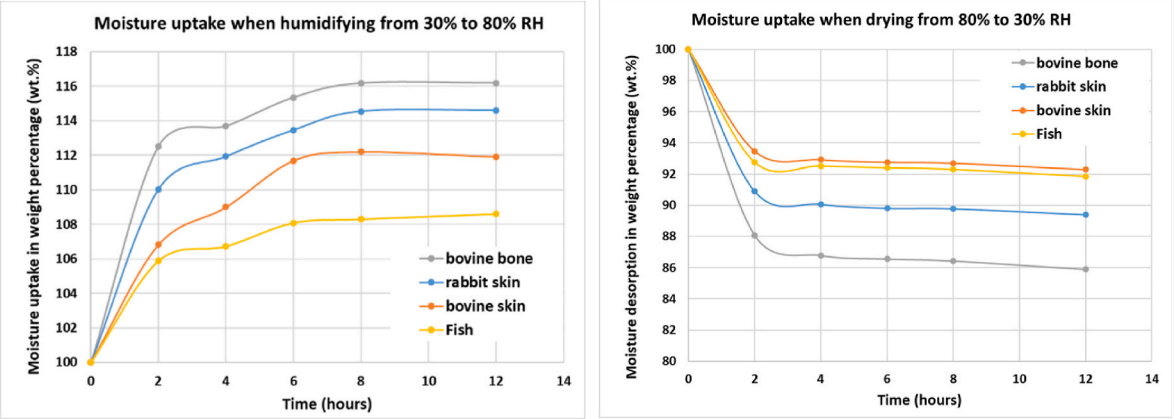


Fig. 3. Representative curves of moisture uptake of the adhesives when humidified from 30% to 80% RH (left), and moisture desorption when dried from 80% to 30% RH (right).

helices act as physical cross-links or crystalline domains. These microstructural features affect the mechanical and physical properties of the films at the macroscale [11,12,18].

Ageing often occurs at different material scales. To be able to identify ageing pathways starting at the micro level, DSC measurements were employed to quantify the possible changes in the triple helix content of the adhesives. Prior studies demonstrated that denaturation enthalpy is associated with the amount of triple helix content in gelatines [7,11,12]. The DSC technique can be utilised for measuring glass transition temperature (T_g), denaturation temperature (T_d), and denaturation enthalpy of the gelatine-based animal glues. Both unaged and hygrothermally aged adhesive films were acclimatised at room temperature and 50% RH prior to testing. Heating DSC thermograms of unaged and aged animal glues are illustrated in Fig. 4.

The first step-wise change in the heating thermogram of the animal glues represents the glass transition temperature at which the adhesive films change state from glassy and stiff to soft and rubbery. The glass transition is an important physical parameter that affects the mechanical properties of the adhesives such as elastic modulus and their tendency to creep when used in a wooden joint or canvas lining [25]. The glass transition temperature is followed by the denaturation temperature, T_d, the peak temperature of the endothermic peak. The denaturation temperature range (above 80 °C) is associated with the transition from the helix-coil conformation of the polypeptide chains in the animal glue towards an amorphous state. The denaturation enthalpy (ΔH_d), which is the area associated with this endothermic peak, is related to the triple helix content in the protein chains [12].

Table 1 summarises the values for T_g, T_d, and ΔH_d before and after hygrothermal ageing. As observed in Fig. 4e–f, ageing causes a systematic increase in T_g and T_d values for all the different adhesives. This can be attributed to dehydration or loss in water content of the adhesives during the ageing process [26]. Another possibility is related to the formation of chemical cross-linking between amorphous polypeptide chains [24]. On the contrary, ΔH_d of all four adhesives systematically decreases through ageing. This decrease indicates that the crystals in the animal glue structure get disrupted during the ageing procedure.

The decrease in denaturation enthalpy seems more severe in bone adhesives after 4 weeks compared to other adhesive types suggesting a faster rate of decay in bone glues.

Moreover, the bone glue shows the lowest enthalpy of denaturation whilst the highest denaturation enthalpy is related to fish glue; this is in line with the trend in the Bloom strength of these adhesives.

The X-ray diffraction technique is yet another technique that is employed for the quantification of triple helix content. Fig. 4 shows the representative X-ray diffraction patterns of different adhesives before and after hygrothermal ageing. Characteristic diffraction peaks at angles of 2θ~8°, and 2θ~21° are attributed to the 'crystalline' triple-helix structure, and the amorphous phase with free single-helix chains, respectively [10]. Table 2 summarises the ratio of peak areas (A_c/A_a) in order to compare the content of the 'crystalline' structure in each adhesive before and after ageing. The calculated A_c/A_a is the lowest for bone adhesive and highest for fish glue which is in line with the DSC measurements showing the lowest enthalpy of denaturation for bovine bone glue and the highest for fish glue. Moreover, similar to the trend observed in DSC measurements, the ratio associated with the triple helix content reduced after ageing in all four different adhesives.

The XRD data is in good agreement with the DSC measurements; both demonstrating the significant loss of microstructural order or degree of crystallinity after hygrothermal ageing in gelatine-based adhesives. This loss of structural order after exposure to raised temperature (34 °C) and cyclic RH can stem from the hygroscopicity of gelatine polymer chains rendering them highly sensitive to environmental humidity. When exposed to high RH, water acts as a plasticizer leading to higher mobility in polypeptide chains and in conjunction with a raised temperature, the helix-coil transition to random coil can occur, interfering with and reducing the microstructural order and triple helix content in these adhesives as proposed and illustrated in Fig. 5.

4.2. *Effect of ageing on mechanical properties of adhesive films*

The uniaxial tensile properties of the adhesive films such as Young's modulus (E), maximum stress (σ_{max}), strain to failure ($\varepsilon_{failure}$), and energy density up to failure (calculated as the area under the tensile stress-strain curve up to $\varepsilon_{failure}$), before and after ageing, are summarised in Table 3. Fig. 6 (top) illustrates the representative tensile stress-strain curves of four different adhesives. The gelatine-based adhesive films, at dried state, show a typical elastic-plastic behaviour as previously demonstrated [11,22]. The tensile experiments were performed at standard conditions (23 °C, 50% RH), underlining that the adhesive films were below their glass transition temperature (as indicated by DSC experiments) and thus in their glassy state. Fig. 6 (bottom) demonstrates the effect of ageing on the tensile behaviour of bovine skin glue as an example. The detailed values are presented in Table 3. The typical E, σ_{max}, and $\varepsilon_{failure}$ are illustrated in Fig. 6.

As shown in Table 3, Young's modulus (E) is almost constant for different adhesives and ageing hardly affected the stiffness despite differences in triple helix content in different adhesive types. Young's modulus also remained unaffected by the reduction of triple helix content (demonstrated by DSC and XRD results) as a function of ageing. Though triple helices act as physical cross-links which can increase stiffness, the experiments are performed at 23 °C and 50% RH in which adhesives are below glass transition temperature. Hence, the amorphous

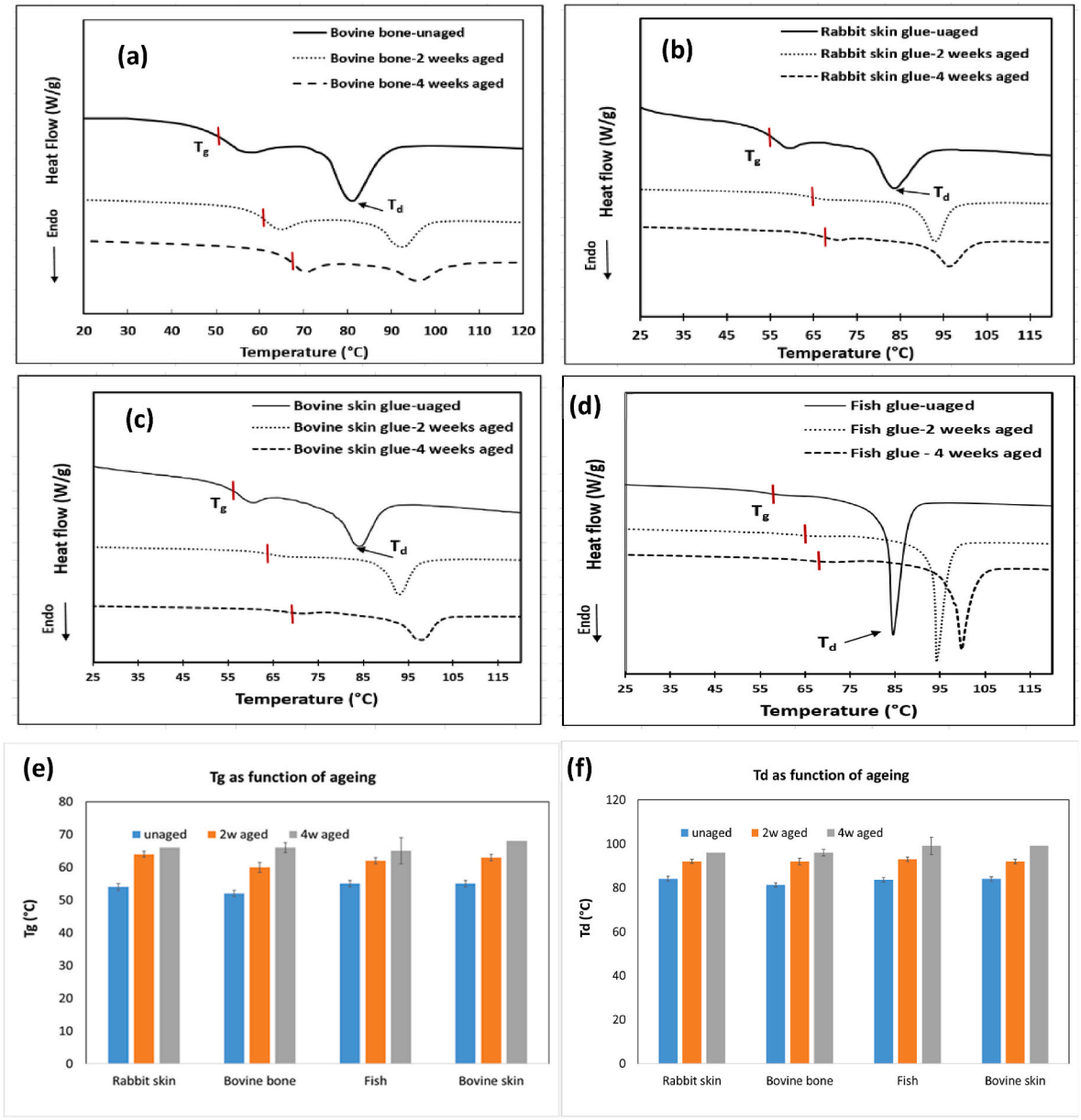


Fig. 4. (a–d) Heating DSC thermograms of unaged as well as 2 and 4 weeks hygrothermally aged animal glues after being acclimatised at 23 °C and 50% RH, prior to testing. (e–f) The increasing values of T_g and T_d of different adhesives with ageing, the error bars represent standard deviation.

Table 1
Tabulated values of glass transition temperature (Tg), denaturation temperature (T_d), and denaturation enthalpy (ΔH_d) of different adhesive films preconditioned at 23 °C and 50% RH before and after 2 and 4 weeks of hygrothermal ageing.

		T_g (°C)	T_d (°C)	ΔH_d (J/g)
Bovine bone	Unaged	52.0 ± 1.5	81.0 ± 1.0	18.5 ± 0.5
	2w-aged	60.0 ± 0.2	92.0 ± 0.0	8.3 ± 1.0
	4w-aged	66.0 ± 0.5	96.0 ± 1.0	6.5 ± 0.5
Rabbit skin	Unaged	54.0 ± 1.0	84.0 ± 0.6	23.0 ± 1.0
	2w- aged	64.0 ± 0.5	92.0 ± 0.5	14.0 ± 0.9
	4w-aged	66.0 ± 0.0	96.0 ± 0.0	13.0 ± 0.5
Bovine skin	Unaged	55.0 ± 1.0	84.0 ± 1.0	25.5 ± 3.0
	2w- aged	63.0 ± 0.5	92.0 ± 0.5	20.0 ± 1.0
	4w-aged	68.0 ± 0.0	99.0 ± 0.0	17.0 ± 1.0
Fish	Unaged	55.0 ± 1.0	84.0 ± 1.5	42.0 ± 2.0
	2w- aged	62.0 ± 0.5	93.0 ± 1.0	31.2 ± 1.1
	4w-aged	65.0 ± 0.5	99.0 ± 0.0	27.5 ± 0.5

Table 2
The ratio between the areas of diffraction peaks for crystalline ($2\theta = 8°$) and amorphous ($2\theta = 21°$) structure in gelatine-based animal glues (A_c/A_a) before and after hygrothermal ageing.

Ac/Aa	Bovine bone	Rabbit skin	Bovine skin	Fish
Unaged	0.33 ± 0.02	0.39 ± 0.06	0.52 ± 0.06	0.73 ± 0.01
2 weeks-aged	0.22 ± 0.05	0.34 ± 0.02	0.46 ± 0.01	0.64 ± 0.10
4 weeks-aged	0.19 ± 0.01	0.22 ± 0.03	0.43 ± 0.01	0.62 ± 0.10

phase within adhesives is in its glassy state with minimum segmental movements, and when loaded within the elastic region, the cross-link or crystalline content hardly affects Young's modulus. A similar observation was made in a previous study on porcine-derived gelatine adhesives with different triple helix content. It was observed that Young's modulus was unaffected by the triple helix content at a temperature below the glass transition [11].

What distinguishes the tensile behaviour of different adhesives is

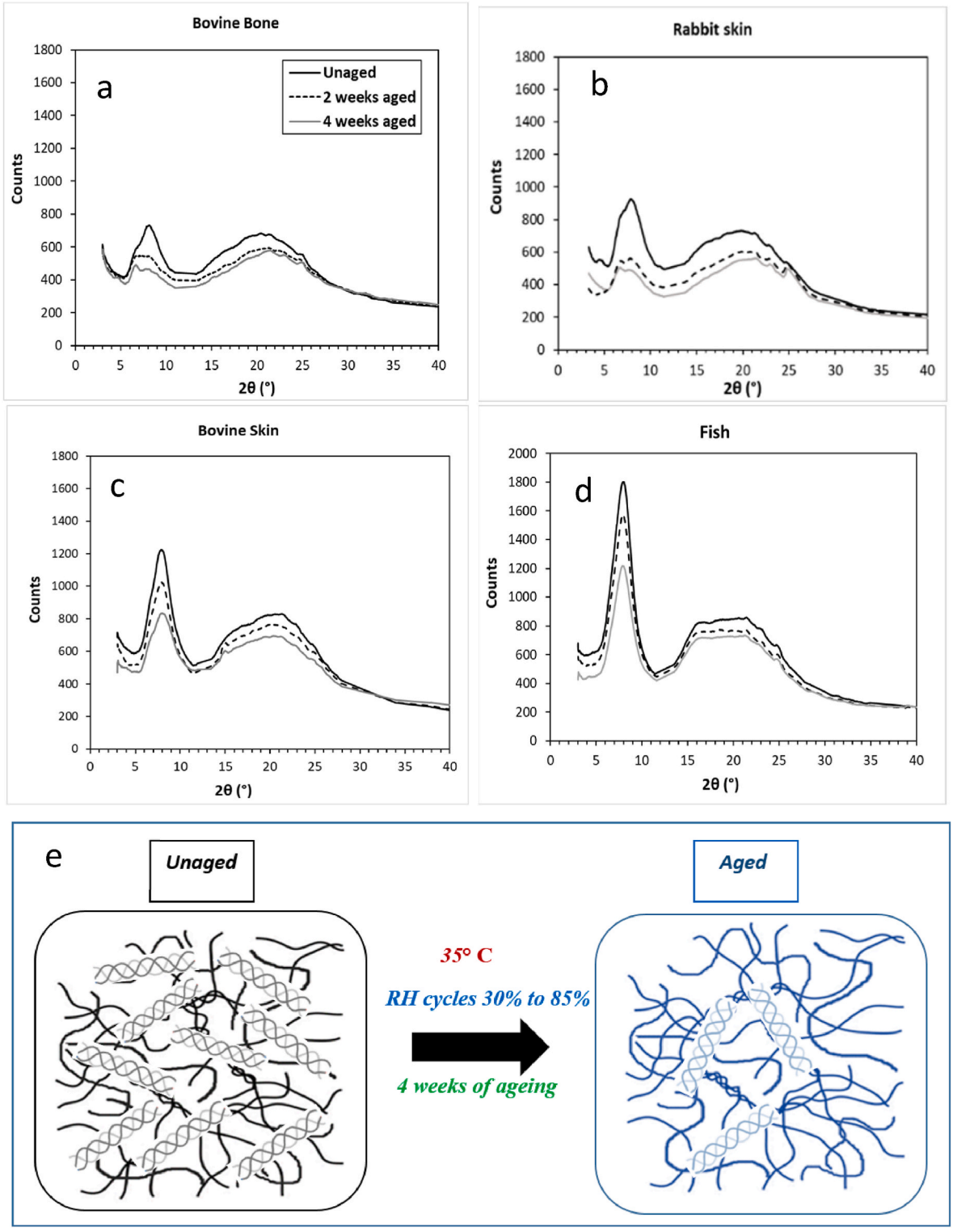


Fig. 5. (a–d) Comparative XRD diffractogram patterns of different adhesive films as a function of ageing time (2 and 4 weeks) and conditioned at 23 °C and 50% RH; (e) A proposed illustration of structural changes in gelatine-based animal glue before and after hygrothermal ageing, demonstrating the loss of triple helix content in aged samples.

their flexibility, strain to failure, and strain energy density to failure (toughness). These values are the lowest for bone glue with strain to failure around 4% and strain energy density of 1.7 MPa. The highest strain to failure and strain energy to failure (toughness) are demonstrated by fish glue with values up to 10%, and 6.3 MPa, respectively. Bovine skin glue shows the highest tensile strength (σ_{max} around 90 MPa) whilst the other adhesives show similar strength values at around 80 MPa. The observed trend in strain energy density to failure, and strain to failure of unaged adhesives seems to be linked to the triple helix content within the microstructure. This is in line with previous findings of research indicating a strong correlation between triple helix content in porcine adhesive films with higher Bloom number and tensile strain to

Table 3
Values for Young's modulus (E), maximum stress (σ_{max}), strain to failure ($\varepsilon_{failure}$), and strain energy density to failure, for different unaged and aged adhesive films, were obtained from tensile experiments at 23 °C and 50% RH.

		σ_{max} (MPa)	$\varepsilon_{failure}$ (%)	E (GPa)	Strain Energy density to failure (MPa)
Bovine bone	Unaged	78 ± 10	4.5 ± 1.0	2.7 ± 0.2	1.7 ± 0.8
	2w-aged	50 ± 15	2.3 ± 0.5	2.0 ± 0.4	0.5 ± 0.3
	4w-aged	Too brittle	Too brittle	Too brittle	Too brittle
Rabbit skin	Unaged	81 ± 3	6.5 ± 1.0	2.4 ± 0.1	3.2 ± 0.8
	2w-aged	68 ± 5	4.0 ± 2.4	2.3 ± 0.2	2.25 ± 0.60
	4w-aged	deformed	deformed	deformed	deformed
Bovine skin	Unaged	92 ± 8	6.0 ± 1.0	2.8 ± 0.7	4.0 ± 0.9
	2w-aged	93 ± 8	4.8 ± 1.3	2.9 ± 0.1	3.5 ± 1.0
	4w-aged	82 ± 8	4.6 ± 1.6	2.8 ± 0.1	2.9 ± 1.4
Fish	Unaged	82 ± 5	10.0 ± 2.0	2.6 ± 0.2	6.3 ± 1.2
	2w-aged	92 ± 2	8.0 ± 2.0	2.7 ± 0.7	5.6 ± 2.2
	4w-aged	90 ± 8	6.5 ± 1.2	2.6 ± 0.5	5.2 ± 1.1

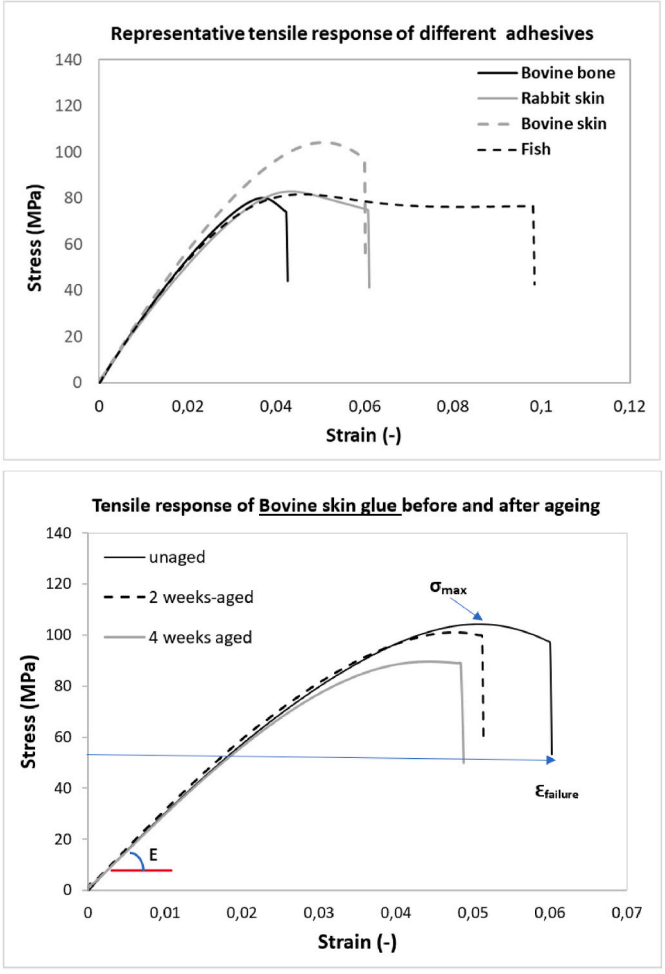


Fig. 6. Typical tensile response of different adhesives at 23 °C, 50% RH (Top), the effect of ageing on tensile response of bovine skin glue (bottom).

failure [10,11].

Similarly, it can be observed that the value of strain to failure, and strain energy density to failure of the different adhesive types demonstrates a systematic decrease as a function of ageing time. This is in agreement with the reduction of triple helix content in the adhesive films as a function of the number of ageing cycles. Moreover, as the number of hygrothermal cycles increased, bovine bone glues became so brittle that they could not be handled nor mechanically tested after 4 weeks of ageing. The loss of adhesive toughness (strain energy density to failure) and strain to failure as a function of ageing is hypothesised here as a result of microstructural changes, namely loss of triple helices at the micro-scale.

Seemingly, in contrast to stiffness, the strain to failure and toughness in these adhesives are affected by the level of triple content when surpassing the elastic region. This can be due to different mechanisms that coexist. On one hand, we postulate that these triple helices act as denser and more compact regions around which the propagating crack path deflects leading to larger absorbed energy and subsequently higher toughness. On the other hand, based on DMTA (Dynamic Mechanical Thermal Analysis) results on the same adhesives presented in a previous paper by the authors [22], the adhesives with higher triple helices namely fish and skin glues likely have higher average molecular weight whilst bone glue has a low average molecular weight as it was observed that the slope of decay of storage modulus during the glassy-rubbery transition was the highest for bone glue and the lowest for fish and skin glues. Lower average molecular weight is associated with higher chain ends and fewer chain entanglements. This can result in lower strain to failure, toughness, and fracture energy within the polymer system [22]. Moreover, the triple helices act as physical cross-links or some type of inter/intra chain entanglements which form a three-dimensional network within the polymer matrix, improving elasticity and strain to failure. Loss of these physical networks results in loss of toughness and an increase in brittleness.

It should be noted that the continuous shrinkage and expansion of the adhesives during hygrothermal cycling caused significant curling in rabbit skin glue dog bone samples hindering the tensile test measurements after 4 weeks of ageing. Hence, fish and bovine skin glue demonstrate better durability than adhesives from bovine bone and rabbit skin origin. The toughness of the adhesive is an important parameter, particularly when used in bonding wooden parts. Wood is a hygroscopic material. Fluctuations in ambient RH and temperature lead to continuous shrinkage and expansion of the wood as well as in the adhesive bondlines within the joints. Discrepancies in the dimensional changes between the wood and the adhesive over time can cause the development of micro-cracks within the adhesive and/or at the interface with the wooden substrate. Hence, the tougher and more ductile adhesives can be potentially a better choice when the same adhesive bondline thickness is used. However, in such practices, other considerations such as the open time of the glue (for completing the glueing procedure) are equally important and can also affect the conservator's choice of the adhesive type.

Fig. 7 (Top) compares the rate of decay of triple helices by plotting the normalised denaturation enthalpy obtained from DSC as a function of ageing time. As observed, bovine bone glue with the lowest Bloom number degrades faster than Fish and bovine skin glues. Fig. 7 (Bottom) demonstrates the rate of decay in adhesive toughness; fish and bovine skin glues demonstrate superior toughness as well as slower decay in toughness.

5. Conclusions

This paper provides an understanding of the effect of hygrothermal ageing on the microstructure and macroscopic properties of four different gelatine adhesives, also known as animal glues, from mammalian and fish origins. The adhesives investigated in this study are bovine bone glue, bovine skin glue, rabbit skin glue, and fish glue. Thin

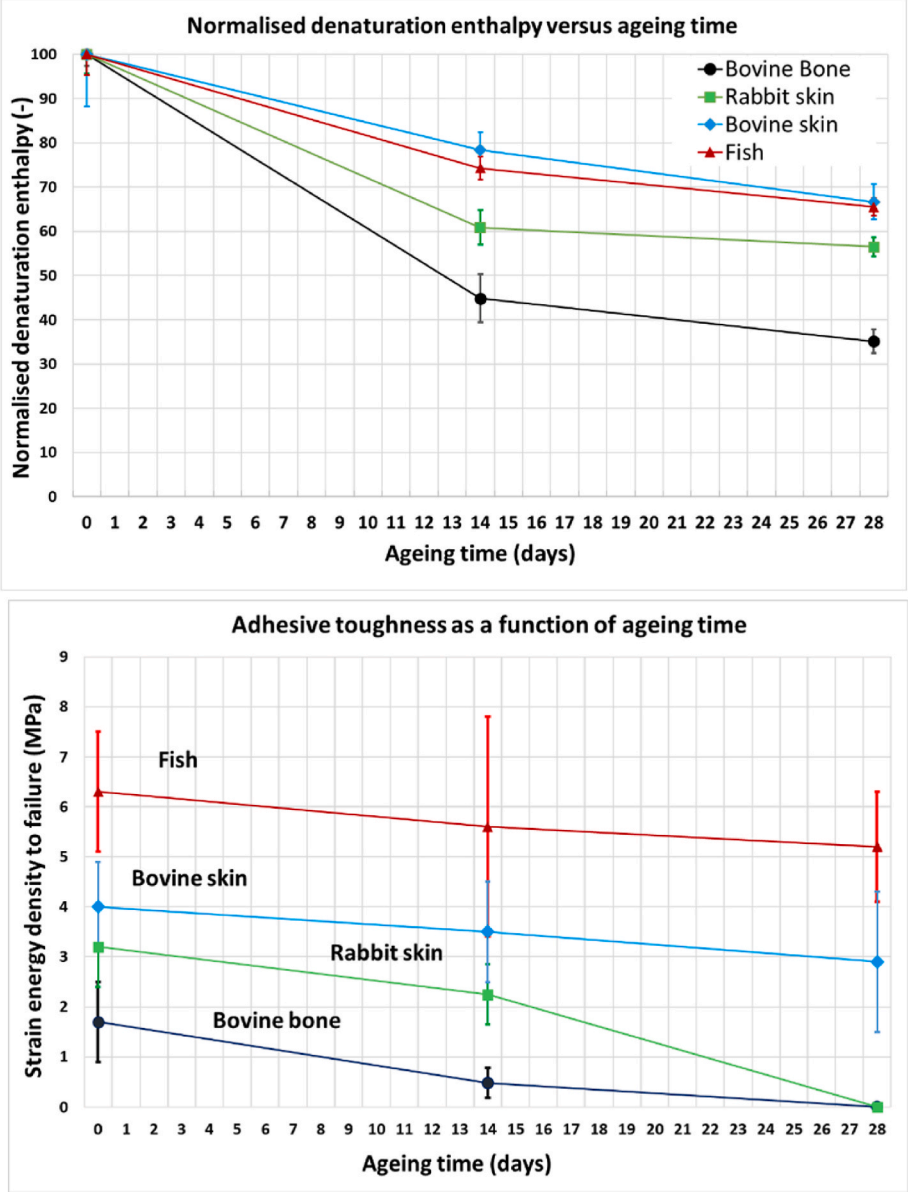


Fig. 7. (Top) The rate of degradation of triple helices as a function of ageing time. As observed, bovine bone glue with the lowest Bloom number degrades faster than Fish and bovine skin glues; (Bottom) demonstrating the rate of decay in adhesive toughness. The error bars represent the standard deviation.

adhesive films were produced via the solution casting method in controlled standard climate conditions (23 °C and 50% RH). Subsequently, the adhesive films underwent hygrothermal ageing, a combination of raised temperature (34 °C) and cycled RH (altering between 30% and 80%), for two and four weeks.

DSC technique was utilised to measure T_g, T_d, and denaturation enthalpy of adhesives before and after ageing. It was observed that ageing causes a systematic increase in T_g and T_d values for all different adhesives. This is hypothesised to be due to possible water loss after many ageing cycles. On the contrary, denaturation enthalpy, which relates to triple helix content, systematically decreased for all four different adhesives as a function of ageing. This decrease in triple helix content was also confirmed by the XRD technique. It is inferred that ageing induces changes in microstructural arrangements and essentially triple helices undergo conformational changes to the amorphous phase. It was also, observed that the rate of decay and loss of microstructural order was more significant in bovine bone glue, whilst the rate of decay in triple helix content of bovine skin and fish glue was shown to be less affected.

Tensile experiments on different adhesive films before and after ageing were performed. Different adhesives demonstrated similar stiffness values at standard conditions. However, it was observed that the toughness of the adhesive (strain energy density to failure) as well as strain to failure were affected by the triple helix content. Moreover, the values of strain to failure and strain energy density to failure of the different adhesive types demonstrate a systematic decrease as a function of ageing time which can clearly be related to a loss of triple helix arrangements. As the number of hygrothermal cycles increased, bovine bone glue became so brittle that it could not be mechanically tested and significant curling in rabbit skin glue dog bone samples was also observed after 4 weeks of ageing. The rate of decay in adhesive toughness was the highest in bovine bone and rabbit glues and the lowest for bovine skin and fish glues.

Previous investigations on 17th-century furniture and panel paintings from the collection of the Rijksmuseum (Amsterdam, The Netherlands) demonstrated that the failure in wooden artefacts mostly occurred in adhesive lines and fracture surfaces demonstrating a mixture of adhesive and cohesive failure. Given that, the question has been posed

as to how far museums can relax regulations for indoor climate control without damaging their artefact collections. The results of this study are meant to provide a deeper understanding of structural changes that may occur in animal glues as a function of environmental ageing. They demonstrate how environmental loading affects the microstructure of the protein-based adhesives and what the implications are of these microstructural changes for the mechanical performance of these adhesives e.g. their toughness when placed in a wooden joint and experiencing gradual shrinkage and expansion with movements of the wooden substrate. The generated data on the durability of animal glues can assist conservators in making a more informed selection of the type of adhesive they use for their restoration practices. The obtained knowledge and extracted data on the ageing of these adhesives can assist the development of durability modelling and lifetime prediction of (historic) objects/bonded assemblies containing animal glues. Nonetheless, it must be noted that this investigation is limited to the ageing of the adhesive films. As the next step, further investigation on the ageing of the wooden bonded assemblies is recommended by the authors. Moreover, the fundamental understanding of the ageing of gelatine materials generated here is relevant to other research fields such as food design, packaging, and medicine.

CRediT authorship contribution statement

Yasmine Mosleh: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Writing – original draft, Writing – review & editing. **Mees van Die:** Data curation, Formal analysis. **Wolfgang Gard:** Funding acquisition, Project administration, Resources, Writing – review & editing. **Iskander Breebaart:** Methodology, Resources. **Jan-Willem van de Kuilen:** Funding acquisition, Writing – review & editing. **Paul van Duin:** Funding acquisition, Supervision, Writing – review & editing. **Johannes A. Poulis:** Funding acquisition, Project administration, Supervision, Writing – review & editing.

Declaration of competing interest

Authors declare no conflict of ineterst.

Data availability

Data will be made available on request.

Acknowledgements

This study was part of the NICAS (Netherlands Institute for Conservation, Art and Science), the research project MUON (Multi-Scale and Uncertainty Approach for the Analysis of the Aging of timber Art Objects adhesively bonded by Animal Glues), a collaboration between the Technical Universities of Delft and Eindhoven and the Rijksmuseum, and was financially supported by the Netherlands Organization for Scientific Research/Netherlands Institute for Conservation, Art and Science (NWO/NICAS) with Grant number 628.007.034 5443.

References

[1] Pearson C. Animal glues and adhesives. In: Handbook of adhesive technology, revised and expanded. CRC Press; 2003.
[2] Bleicher N, Kelstrup C, Olsen JV, Cappellini E. Molecular evidence of use of hide glue in 4th millennium BC Europe. J Archaeol Sci 2015;63:65–71.
[3] Solazzo C, Courel B, Connan J, Van Dongen BE, Barden H, Penkman K, Taylor S, Demarchi B, Adam P, Schaeffer P, Nissenbaum A, Bar-Yosef O, Buckley M. Identification of the earliest collagen- and plant-based coatings from neolithic artefacts (nahal hemar cave, Israel). Sci Rep 2016;6:31053.
[4] Schellmann NC. Animal glues: a review of their key properties relevant to conservation. Stud Conserv 2007;52:55–66.
[5] Hackney S. Paintings on canvas: lining and alternatives. Tate papers; 2004.
[6] Dupont AL. Study of the degradation of gelatin in paper upon aging using aqueous size-exclusion chromatography. J Chromatogr A 2002;950:113–24.
[7] Achet D, He XW. Determination of the renaturation level in gelatin films. Polymer 1995;36:787–91.
[8] Djabourov M. Architecture of gelatin gels. Contemp Phys 1988;29:273–97.
[9] Ross-Murphy SB. Structure and rheology of gelatin gels: recent progress. Polymer 1992;33:2622–7.
[10] Bigi A, Panzavolta S, Rubini K. Relationship between triple-helix content and mechanical properties of gelatin films. Biomaterials 2004;25:5675–80.
[11] Mosleh Y, de Zeeuw W, Nijemeisland M, Bijleveld JC, van Duin P, Poulis JA. The structure–property correlations in dry gelatin adhesive films. Adv Eng Mater 2021; 23:1–8.
[12] Bigi A, Cojazzi G, Panzavolta S, Rubini K, Roveri N. Mechanical and thermal properties of gelatin at different degrees of glutaraldehyde crosslinking. Biomaterials; 2001. p. 763–8.
[13] Aguirre-Alvarez G, Foster T, Hill SE. Impact of the origin of gelatins on their intrinsic properties. J Foodserv 2012;10:306–12.
[14] Gornall JL, Terentjev EM. Universal kinetics of helix-coil transition in gelatin. Phys Rev E 2008;77:031908.
[15] Eysturskard J, Haug IJ, Ulset AS, Draget KI. Mechanical properties of mammalian and fish gelatins based on their weight average molecular weight and molecular weight distribution. Food Hydrocolloids 2009;23:2315–21.
[16] Eysturskard J, Haug IJ, Ulset AS, Joensen H, Draget KI. Mechanical properties of mammalian and fish gelatins as a function of the contents of α-chain, β-chain, and low and high molecular weight fractions. Food Biophys 2010;5:5–9.
[17] Staroszczyk H, Pielichowska J, Sztuka K, Stangret J, Kołodziejska I. Molecular and structural characteristics of cod gelatin films modified with EDC and TGase. Food Chem 2012;130:335–43.
[18] Yakimets I, Wellner N, Smith AC, Wilson RH, Farhat I, Mitchell J. Mechanical properties with respect to water content of gelatin films in glassy state. Polymer 2005;46:12577–85.
[19] Ekelund S, Van Duin P, Jorissen A, Ankersmit B, Groves RM. A method for studying climate-related changes in the condition of decorated wooden panels. Stud Conserv 2018;63:1–10.
[20] Sulaiman R, Schellen HL, Hensen JLM. Pilot study on indoor climate investigation and computer simulation in historical museum building: Amerongen Castle, The Netherlands. J Des Built Environ 2010;7:75–94.
[21] Bridarolli A, Freeman A, Fujisawa N, Łukomsk M. Mechanical properties of mammalian and fish glues over a range of temperature and humidity. J Cult Herit 2022;53:226–35.
[22] Mosleh Y, van Die M, Gard W, Breebaart I, van de Kuilen JW, van Duin P, Poulis JA. Gelatine adhesives from mammalian and fish origins for historical art objects conservation How do microstructural features determine physical and mechanical properties? J Cult Herit 2023;63:52–60.
[23] GME monograph for the testing of edible gelatine, downloaded from https://www.gelatine.org.in.publications.&.downloads.
[24] Duconseille A A, Wien F, Audonnet F, Traore A, Refregiers M, Astruc T, Sante-Lhoutellier V. The effect of origin of the gelatine and ageing on the secondary structure and water dissolution. Food Hydrocolloids 2017;66:378–88.
[25] Poulis JA, Seymour K, Mosleh Y. The creep performance of bio-based and synthetic lining adhesives at different environmental conditions. Int J Adhesion Adhes 2022; 114:103119.
[26] Gonzalez LG, Wess TJ. The effects of hydration on the collagen and gelatine phases within parchment artefacts. Heritage Science 2013:14.