

The effect of accelerated weathering on gelatin biofilms properties

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Zásady pro vypracování

- V literární studii se zaměřte na současný stav řešené problematiky a kriticky jej zhodnoťte.
- Aplikujte technologický postup přípravy želatin z vybraných tkání obsahujících kolagen.
- Připravte litím želatinové filmy. Po stárnutí filmů testujte jejich vlastnosti.
- Výsledky měření zpracujte vhodnými metodami, proveďte diskusi a navrhněte aplikaci připravených filmů pro praxi.

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Said, N.S.; Sarbon, N.M. Physical and Mechanical Characteristics of Gelatin-Based Films as a Potential Food Packaging Material: A Review. *Membranes* 2022, 12, 442.
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ABSTRAKT

Práce se věnuje obalovým materiálům, využití biopolymerů a biodegradabilních materiálů v tomto odvětví. Shrnuje i základní poznatky z oblasti urychleného povětrnostního stárnutí. Praktickou stránkou se tato práce zbývá analyzováním vlivu urychleného povětrnostního stárnutí prostřednictvím Xenotestu na vlastnosti želatinových filmů. Přínosem není jen tato analýza, ale také popis vlastností nového druhu želatiny – jelení želatiny, a zkoumání jejího možného využití pro obalové materiály. Vliv urychleného stárnutí byl zkoumán prostřednictvím měřících metod DSC, DMA, FTIR, SEM a testováním pevnosti v tahu, bariérových vlastností, bobtnání, zákalu a indexu žlutosti. Intervaly doby stárnutí vzorků byly stanovené na 100, 200 a 300 h pod window-glass filtrem, což by v reálných podmínkách mělo odpovídat stárnutí 1, 2 a 3 měsíců produktu za okenním sklem. Ve spolupráci s německou firmou Atlas MTT GmbH bylo domluveno porovnávací měření, kdy v jejich laboratoři byly degradovány vzorky na 100 a 300h pod store-light filtrem, který simuluje osvětlení v obchodech. Degradované vzorky byly zesíťované pomocí glutaraldehydu (GA) a bylo dodrženo jeho legislativou omezené množství pro obalové materiály ve styku s potravinou, které je 2mg GA na kilogram potravin. Výše uvedenými testy byly analyzovány i vlastnosti nezesíťovaných filmů a zesíťovaných nedegradovaných filmů. Rozdíly ve vlastnostech vzorků byly prodiskutovány.

Součástí této práce je i SWOT analýza, která zhodnocuje silné (strengths = S) a slabé (weaknesses = W) stránky, příležitosti (opportunities = O) a hrozby (threats = T) tohoto výzkumu. Byla provedena na začátku experimentu a následně i na konci měření. Hlavními hrozbami tohoto výzkumu byla náročná příprava filmů, legislativní omezení a především špatná reprodukovatelnost vlastností vytvořené želatiny a filmů. Mezi silné stránky byl zařazen fakt, že se jedná o nový zbytkový produkt, který ještě není využíván a tím je přispěno k udržitelnosti a podpořena ekologie. To, že se jedná o nový materiál bylo zařazeno i mezi slabé stránky, protože jeho vlastnosti nejsou zatím prozkoumány. Jako příležitost byla zmíněna možnost náhrady syntetických obalových materiálů spolu s možností celosvětové výroby a podpoření tak cirkulární ekonomiky.

Klíčová slova: želatina, jelení želatina, síťování glutaraldehydem, urychlené povětrnostní stárnutí, termické analýzy, bobtnání, pevnost v tahu, bariérové vlastnosti, udržitelnost, zbytkové produkty

ABSTRACT

The thesis focuses on packaging materials, the use of biopolymers and biodegradable materials in this industry. It also summarizes the basic knowledge in the field of accelerated weathering. On the practical part, this work focuses on analysing the effect of accelerated weathering by Xenotest on the properties of gelatin films. The contribution is not only this analysis but also the description of the properties of a new type of gelatine, deer gelatine, and the investigation of its possible use for packaging materials. The effect of accelerated weathering was investigated through DSC, DMA, FTIR, SEM measurements and by tensile strength and barrier properties testing, swelling, haze and yellowness index. The aging intervals of the samples were set at 100, 200 and 300 h under window-glass filter, which in real conditions should correspond to 1, 2 and 3 months of aging of the product behind the window-glass. In cooperation with the German company Atlas MTT GmbH, a comparative measurement was arranged where samples were degraded in their laboratory for 100 and 300 h under a store-light filter, simulating the illumination in stores. The degraded samples were cross-linked with glutaraldehyde (GA) and complied with its legislative limit for food contact packaging materials of 2 mg GA per kilogram of product. The above tests were also used to analyse the properties of the non-crosslinked films and the crosslinked non-degraded films. The differences in the properties of the samples were discussed.

This thesis includes a SWOT analysis that evaluates the strengths (= S) and weaknesses (= W), opportunities (= O) and threats (= T) of this research. It was conducted at the beginning of the experiment and subsequently at the end of the measurement. The main threats of this research were the difficult preparation of the films, legislative restrictions and, above all, the low reproducibility of the properties of the gelatine and films produced. Among the strengths was the fact that this is a new by-product that is not yet used, therefore contributing to sustainability and supporting ecology. The fact that it is a new material was also classified as a weakness, as its properties are not yet explored. The possibility of replacing synthetic packaging materials was mentioned as an opportunity, along with the possibility of global exports to support the circular economy.

Keywords: gelatin films, deer gelatin, Glutaraldehyde crosslinking, accelerated weathering, thermal analysis, swelling, tensile testing, barrier properties, sustainability, by-products

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INTRODUCTION AND MOTIVATION

Nowadays, due to the increasing waste of synthetic polymers, research is increasingly focused on developing and improving the properties of biopolymers and biodegradable polymers. Synthetic materials are becoming more and more of an environmental problem, whether it is the extraction of raw materials or the waste after the final use of their products. Such materials have a very long degradation time and therefore threaten, burden and overwhelm nature and the entire ecosystem. [1, 2]

Biopolymers such as gelatine, starch, cellulose or soy protein are already used in the packaging industry. However, they have not yet replaced synthetic packaging, mainly because their properties are often inferior to those of synthetic materials. Synthetic materials continue to win with their low cost, versatility, ease of processing and optical properties. They also have the edge in terms of barrier properties, which are a key aspect of food packaging. [2]

Commercially used gelatins include pork and beef, but they cannot be used in countries where they are forbidden by religion because these animals are considered sacred. Because of this aspect, gelatins made from new raw materials are constantly being researched and analysed. Fish gelatins are now very much under investigation, and much research is focused on the different properties between fish species, whether freshwater or seawater, and between the species within these groups. [3–6] For example, the properties of rabbit skin gelatins have also been investigated. [7] In this thesis, the properties of gelatins from a relatively new source, deer tendon, are investigated. In the context of packaging materials, and even the effect of crosslinking on the properties of these gelatins is investigated.

As this is a biodegradable material, it is necessary to determine the conditions and degradation process before its use on packaging materials and use this to estimate its shelf-life. Subjecting synthetic material products to accelerated weathering is quite common, but biopolymers and biodegradable materials have not yet been studied as much in this area. So far, research has mainly dealt with the exposure to accelerated weathering of PLA (polylactide acid) products. [8–10] Only one paper was found that exposed gelatine to this degradation method [11], but it did not mention the raw material gelatin source and was mainly a comparison of gelatines with different silk fibre contents.

The motivation of this work is to investigate the properties of a new gelatin and to reveal the effect of crosslinking and accelerated weathering on these properties.

I THEORETICAL PART

1 PACKAGING MATERIALS IN FOOD INDUSTRY

Packaging materials are an integral part of every person's day, a large amount of material and waste, and packaging materials in the food industry represent a significant part of it. These include paper, metal or glass packaging, which are all recyclable. In addition to these options, packaging made of synthetic polymers such as high-, low- and linear low-density polyethylene (HDPE, LDPE, LLDPE), polystyrene (PS), polypropylene (PP) and polyethylene terephthalate (PET) also form a large part of the packaging. However, these types of polymers are considered to be non-degradable and are therefore being replaced by biopolymers. This relatively new group opens the door to biodegradability, but it also brings concerns and new challenges regarding their properties, such as barrier properties, water absorption or mechanical parameters, which are poor in comparison to synthetic polymers. Not only because of their good properties but also because of their low cost, synthetic packaging is still at the top, it has been reported that 46 % of global plastic waste (340 million tonnes) is generated by the packaging industry (as of year 2021). Packaging materials are particularly popular because they keep food fresh longer, thus extending its shelf-life, while preventing contamination during transport. [2, 12–15]

The downside of these synthetic packaging materials is their long decomposition time, which can be in the range of 10-20 years or 500-1000 years, and the high environmental burden. [1] In view of the increasing focus on eco-friendliness, biodegradable materials are an interesting topic, even if their properties are often not comparable to synthetic non-degradable materials. [2, 15, 16]

1.1 Biodegradable and edible packaging

Biodegradable materials, or materials from renewable sources, are decomposable by the activity of microorganisms in nature into basic building units that do not burden nature and the ecosystem. Such materials include proteins, starch, or even polylactic acid (PLA). The proteins used can be of plant or animal origin and thus differ in their basic structure and properties. What they have in common is that they are used for the production of films, either as edible coatings, where a film is applied directly to the surface of the food to ensure that its properties such as taste and colour are preserved over a longer period of time, or as packaging materials with modified properties using additives that also preserve the appearance and properties of the food while protecting it between production and use by the final consumer. [17]

When it comes to edible packaging, it does not have to be only the industrially processed ones. For example, a waffle cone for ice cream, a bread crust for soup or fruit scraps as beverage containers can also be considered edible packaging. Due to the ecological theme, the industrially processed ones bring bigger burden. Such packaging can also be adapted as anti-microbial or antioxidant using only natural substances such as polyphenols from green tea or garlic juice. It is important for edible packaging to be colourless, tasteless, odourless and safe for consumers. However, sometimes an appealing colour and taste is required. Many ingredients may not be natural to humans and some ingredients may be toxic in certain amounts, so the doses of these ingredients need to be monitored, and safe amounts combined with their effectiveness need to be used. [1]

1.1.1 Protein-based packaging

In addition to lipids and polysaccharides, proteins are also widely used, which, as already mentioned, can be of plant or animal origin. Both groups find their use. Representatives of polysaccharides include chitosan, which is formed by partial acetylation of chitin. It is an inexpensive material that is widely used for hydrogels and is noted for its biocompatibility, biodegradability and antimicrobial properties. Other polysaccharides used for film production in the food industry include pectin, starch and or sodium alginate. For protein hydrogels, gelatin, whey or zein are used. [16–18]

1.1.1.1 Plant proteins

Plant proteins are found in a plant-based food, but are generally not as essential as animal proteins. Soy protein and zein are used to form films for packaging materials.

Soy protein has very poor properties compared to synthetic polymers, but is used as a cheap and biodegradable material for edible films and coatings.

Corn zein is also used for edible coatings and has excellent film-forming, antibacterial and barrier properties and good temperature stability. However, the mechanical properties of films from this source are poor. [17, 19]

1.1.1.2 Animal proteins

The main sources of animal protein are meat, dairy products, milk and eggs. This includes proteins such as casein and collagen. Casein is a milk protein that has good film-

forming properties and offers a possibility in the research and development of edible coating materials.

Collagen is found in connective tissues and is therefore a part of by-products from meat processing in the food industry. The collagen molecule is made up of multiple fibrils twisted into a 3D spiral structure called tropocollagen. This structure makes it insoluble in water. The main amino acids are glycine, proline and lysine, with their specific content depending on the type of raw material. Partial hydrolysis of collagen with the help of enzymes results in gelatin, which is water-soluble and forms good films. It consists mainly of the amino acids glycine or alanine, and the amino acid residues proline or hydroxyproline. The production of gelatin uses bovine and porcine hides and skins, but non-traditional sources such as carp skins and deer tendons are beginning to be discovered. These gelatins are still under-explored and have great potential, if only because they could be used in products and packaging exported to countries where pork or beef products are banned for religious reasons. In the pure state, gelatin films are very wet, have low thermal stability and poor mechanical properties. [5, 17, 20–23]

1.1.2 Adjusting properties

As already mentioned, the disadvantage of biopolymers is their poor properties, especially mechanical strength and wettability, compared to commonly used synthetic polymers. Both of these properties can be influenced by the degree of crosslinking of the structure, whereby wetting decreases and strength increases with increasing level of crosslinking. The degree of crosslinking is determined by the reactivity of the side chains, the amino acid composition and the amount of free amino and carboxyl groups. The most reactive groups include hydroxyproline, serine, threonine, tyrosine, aspartic and glutamic acids, lysine and arginine. The surface charge then influences solubility and the protein-protein interaction itself. [20, 24, 25] The crosslinking process of gelatins proceeds via Schiff bases, which involves condensation of formyl groups of the aldehydes and ϵ -amino groups from lysine residues with hydroxylysine. There are two crosslinking mechanisms - intrahelical and interhelical. In the intrahelical mechanism, linkages are formed between polypeptide chains of one helix; the other mechanism involves the formation of linkages between chains of different helices, which leads to an increase in molecular weight and affects swelling and flexibility. A commonly used crosslinking agent is glutaraldehyde [26, 27] Other crosslinking methods include electrolytic crosslinking [28], using enzymes [29] or proanthocyanidins, which fall into the category of condensed tannins [30].

Other additives affecting the properties of biofilms are plasticizers which improve the flexibility of the material and thus improve processability and lower the glass transition temperature. It is critical to estimate the appropriate concentration, small amount will not provide sufficient flexibility, while high amount lead to a deterioration of barrier properties and an increase in stickiness. Glycerol is a commonly used plasticizer. [17, 31, 32] Other plasticizer that can be used are polyethylene glycols, mannitol, sorbitol.[31]

Other substances added to packaging materials include antimicrobial and antioxidant additives, which mainly help to maintain the freshness of the food, as well as stabilizers, which ensure performance and durability in different conditions. Various fillers or dyes may also be added, or blends of biopolymers are used to improve properties. [17]

1.1.3 Animal by-products

The slaughtered animals can be used mainly for meat, but there are also other parts of their bodies that have found their use over the years. In general, by-products mean all tissues and organs other than meat, for example bones, stomachs, blood or skin. [33, 34] Animal by-products can be divided into non-carcass meat (EBPs) and non-meat products (IEBPs), which can be further subdivided. EBPs are approved as safe for human consumption. IEBPs are further subdivided into edible, which mainly includes organs, and non-edible, which are intended for destruction or are transformed into usable products by further processing. These include, for example, skin, fat or bones. [33]

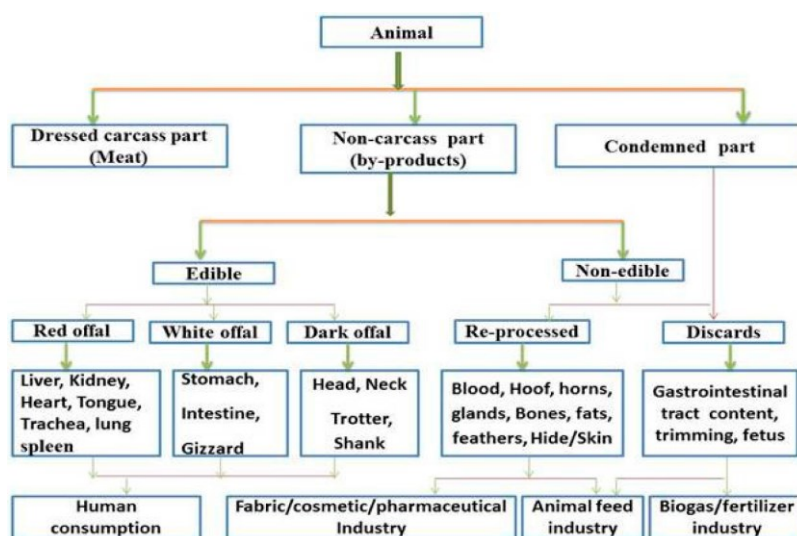


Figure 1: Schema showing different products from the animal slaughter. [33]

According to data from the State Veterinary Administration, more than 15 million tonnes of animal by-products produced by slaughterhouses, livestock farms, dairies and meat processing plants were recorded in the EU as of 2008. [34]

The area of deer farming, slaughtering and processing of meat and its products is overseen by the Federation of European Deer Farmers Association. In the Czech Republic there are almost 19,000 animals registered, in Germany the organisation registers 38,000 animals, in Sweden and the UK 30,000. Meat and by-products are sold mainly to local markets. [35]

In Scotland, about 100 000 wild deer are recorded as being shot annually, equivalent to about 3 500 tonnes. The amount of meat and products from farmed deer is around 70 tonnes (as of 2018). [36]

Gelatin is made from collagen, which is found in almost all tissues, but mostly in the skin and connective tissues. These source products fall under IEBPs. According to Global Gelatin Market, globally, pig skin and bovine skin are the most processed for gelatin production by 2025. The most common applications are then in food and nutraceuticals, with slightly less for the pharmaceutical and photographic industries. The least, about 1/5 of the volume used in food, is used for other purposes. Within food, gelatin is mostly used for the production of confectionery. In the EU, pig skins are processed more than bovine hides and the use of the final product is roughly the same in all the above areas. [37]

2 PREPARATION OF FILMS BASED ON BIOPOLYMERS

Various processing methods can be used to prepare biopolymer films. Solution casting prepares flexible films, extrusion moulding is used for material homogenization and shaping, thermoplastic processing and blending improve properties.

2.1 Solution casting

This processing method is the subject of a lot of research work and is well applicable on a laboratory scale but is disadvantageous for industrial use mainly because of the long processing times. The concentration of the solution prepared for casting is crucial for the final properties of the product and for the simplicity of processing, at high concentrations it is possible that the solution will not pour well into the mould due to its high viscosity. Too low concentrations can cause very poor mechanical properties and brittleness of the final product. Basically, a given amount of biopolymer is dissolved in water, or in another solvent. If a plasticizer is used, then it is usually added to the solution after dissolution of the polymer. Two methods can be used to crosslink the product thus produced. The first is by adding a crosslinking agent to the solution itself followed by casting into a mould. In some cases, the crosslinking is done after the film has been prepared by immersion in the crosslinking agent solution. [17, 38]

2.2 Extrusion

It is a method designed primarily for synthetic polymers, so the aim is to produce a thermoplastic starch that can be processed by this efficient and industry-proven method. The processing of starch by this method is very challenging because of its behaviour under heat, pressure and shear stress. The solution for better processability is the addition of a plasticiser, which is also applicable to other biodegradable polymers. The shape and design of the extrusion die is also a major challenge. [17] Ideal conditions are 164 °C and 313 rpm due to [39] but depends on the ratio of the fillers/copolymer. Generally, the screw speed is usually between 50 and 100 rpm. [17]

2.3 Other processing methods

Other method used to process biodegradable materials is compression moulding with ideal moulding temperature between 140 and 180 °C and pressure 5-12 MPa, where temperature range compliance is the most important. Injection moulding with temperature

between 160 and 210 °C and pressure 60-150 MPa is also used. Electrospinning and 3D printing, which allows the creation of layered materials with precisely defined shapes and are suitable for active packaging applications. [17]

3 PROPERTIES TESTING OF PACKAGING MATERIALS AND APPLICATION

For packaging materials, strength and barrier properties are most important. Other parameters investigated are moisture absorption, melting and glass transition temperatures, mechanical behaviour at changing temperatures, shelf-life prediction, transparency and possibly microscopic surface. [2, 17, 40, 41]

3.1 Physical and thermal properties

Tensile testing determines the flexibility and tensile strength of the material being tested. Most commonly, the stress at break, elongation at break and maximum force are monitored. Often all analyzed tensile properties are measured according to ASTM D882. The test consists of clamping the specimen between the jaws of the tearing machine and then moving the jaws away from each other until the specimen breaks. It is also possible to perform a compression test and an impact properties test. [42]

Differential scanning calorimetry is classified as a thermal method. There are two techniques, the first is to keep the sample and reference at the same temperature by using a heat flux where the sample is in a different chamber to the reference. The second method, which is more commonly used, consists of measuring the temperatures of the sample and reference under the same volume of heat input. The samples are in one common chamber. [43, 44]

Thermogravimetric analysis is used to evaluate the thermal properties of materials. It consists of a very precise measurement of the mass of a sample as it is heated, cooled or tempered at a single temperature. [44]

Thermo-mechanical methods include dynamic mechanical analysis, which measures mechanical properties as a function of temperature or frequency. During the measurement, the material is deformed by force or displacement in cycles, often by oscillation or bending. [45]

3.2 Chemical stability

Chemical stability is also important for packaging materials, especially for applications in the pharmaceutical, cosmetics and food industries. It should remain stable in contact with the packaged contents and not interact. [17]

3.3 Barrier properties

Gas and water permeability are very important properties for packaging materials, especially for food packaging. To preserve the properties of the packaged food, it is necessary to prevent the passage of gases and moisture through the packaging. The main gases that can pass through membranes and affect the product properties are water vapour, oxygen and carbon dioxide, therefore the barrier properties against these gases are the most investigated. [42]

3.4 Application of biopolymers

Biopolymers are, as already mentioned, increasingly being used in an attempt to replace synthetic ones in various applications. Often studied biopolymers are gelatin, collagen itself, starch, cellulose, chitosan, polylactic acid or polycaprolactone. [46]

The most common application of these materials is in the packaging and food industry. As packaging materials, they find their use in the food industry already mentioned, but also in the pharmaceutical industry. [47] In the food industry, they find their use as edible coatings, most often used to preserve the colour and flavour of fruit and vegetables. Polysaccharides can be used as stabilisers, emulsifiers, gelling agents or coagulants. [48] Gelatine can also be added to food in this way and finds its application as a binder and protein source in protein bars and as a binder in low-fat margarine. Gelatin hydrolysates are used to foam spreads and thus reduce the fat content by up to 10%. [49] Another application is in antimicrobial packaging, where essential oils are added to the polymer matrix to prolong the shelf-life. [47] Polysaccharide-based nanocomposite films are used for antimicrobial and antioxidant packaging, which consist of the incorporation of bioactive compounds and nanomaterials into polysaccharide films. The addition of these substances also improves the physical and chemical characteristics. [50]

The pharmaceutical industry uses, for example, gelatins, which are used to make capsules for easier dosing of medicines. These capsules are always made up of two parts, they can be soft capsules, which are usually filled with an oily substance and cannot be split,

and hard gelatine capsules, which are most often filled with a powdered form of the drug and can be separated. [49] Chitosan, which is non-toxic, odourless and has antimicrobial properties, is also used for drug delivery. [51]

Biopolymers are also widely used in the medical field, where they can be used as wound dressings or hydrogels for wound healing. Here, collagen or chitosan in combination with cellulose can be used. Due to their non-toxicity, biopolymers can also be used for skin grafts, scaffolds and dermal substitutes, as well as dental implants, cochlear implants, blood vessel prostheses, heart valves, joint replacements or artificial ligaments and tendons. [52]

A large area of application is cosmetics. It is mainly proteins and polysaccharides and their use in the skin care, hair care, oral care and mesotherapy. Of the proteins, collagen, elastin and keratin are mainly used. In the field of polysaccharides, chitosan, hyaluronic acid, alginate or starch are used for these purposes. Collagen is used as a moisturizing and softening agent for hair and skin. Elastin is also included in skin care products. [53]

4 ACCELERATED WEATHERING

Weathering in general is the aging of materials due to weather conditions. There are several methods to measure material ageing, but all of these methods take time. Over time, weather standards have been established, such as for example Florida standard - very warm, very sunny and humid weather and Arizona standard - extremely dry with extreme amounts of sunshine (irradiance), which also qualifies as accelerated weathering due to its extreme conditions. These are methods, along with test chambers, that are able to simulate the effect of radiation and weathering conditions over a longer time period on the material over months/years. It helps to predict the behavior of materials and products to ensure their safety or to set a limit safe period of time for which they can be used. It is also used in the development of materials in the automotive industry. Whole cars can be tested either in outdoor locations in the states listed above or directly under radiation in test chambers. Both methods, natural environment and irradiation chambers, can also be used for individual parts only. [54, 55]

4.1 History

The first high intensity radiation source for accelerated weathering came on the market in the early 20th century, and it was carbon arcs. Paints and varnishes were tested. This method was very complex to operate, did not fully correspond to sunlight and each material degraded very differently, so it was difficult to get comparative data. [55]

In 1960, the xenon arc lamp came on the market and its radiation was very close to that of the noon sun, which was further amplified by the use of "extended UV" filters that achieved wavelengths of 270-295 nm, which do not occur naturally. These lamps can be cooled with water or air. Water cooled lamps used a combination of a quartz inner filter and a borosilicate outer filter (quartz/boro). The air-cooled lamp used a combination of a single borosilicate filter. Ideal conditions were achieved only with the use of a borosilicate inner and outer filter (boro/boro), which is defined by ASTM G155 and ISO 4892-2 as a "daylight" filter. This lamp and filter system is widely used these days. [55]

Other sources of irradiation may be fluorescent lamps used for their low cost and ease of manipulation. The newest on the market are high-intensity metal halide lamps, which were developed in Japan and the EU, and the US market does not yet have testing standards for them. They are capable of irradiance in the 290-400 nm range. [55]

4.2 Natural weathering

Natural weathering can be divided into different testing groups – direct weathering, open-backed exposures, backed exposures, black box exposures, indirect exposures and natural accelerated weathering. [54]

Direct weathering is defined as direct exposure of the test product to sunlight and other weather conditions. In this method, the samples are fixed in a holder which is rotated at a fixed angle to the south side (in the case of the northern hemisphere). This testing follows different standards depending on the material. These include, for example ISO 877 Plastics, ISO 2810 Paints and Varnishes or ISO 105-B03 Textiles. [54]

Open-backed exposure consists of holding the samples by the smallest possible surface so that the backs of the samples are also exposed to the airflow in the surrounding environment. The specimens most commonly tested by this method are transparent objects such as glass or headlights but also coil coatings. [54]

Backed exposures is again an outdoor test where the sample is mounted on a substrate and the environment of its end use is simulated. Products tested by this method are most commonly PVC (polyvinyl chloride) siding, roofing membranes. Samples can be deposited at a 45° (Florida, Arizona) or 90° (Florida) angle. [54]

Black Box exposures follow ASTM Standard D4141 Method A, this is a frequently used method for coatings and automotive paint. It is a simulation of the air heat-sink characteristics of an automotive body. The structure consists of an open black metal box, with test samples placed in the opening. [54]

Indirect exposure is done under glass that absorbs radiation below 310 nm when light passes through it. It is therefore a determination of the colour fading of mainly the interior furnishings of the house. This method follows similar standards to direct weathering. [54]

Natural accelerated weathering involves the amplification of radiation using mirrors. The mirrors are placed on a support against the sun at a specific angle, each of the mirrors is rotated to reflect the beam directly onto the specimen, and the specimen is mounted on a holder against the mirrors. This test is performed to reduce the number of cycles and testing time. [54]

4.3 Laboratory weathering

Xenon arc instruments use xenon lamps that, when properly filtered, simulate UV and visible spectrum radiation. It is often used for materials that will be exposed to sunlight. These instruments are widely used for testing textiles, polymers, paints and in the automotive industry. There are two types, which are air-cooled and water-cooled lamps. [54]

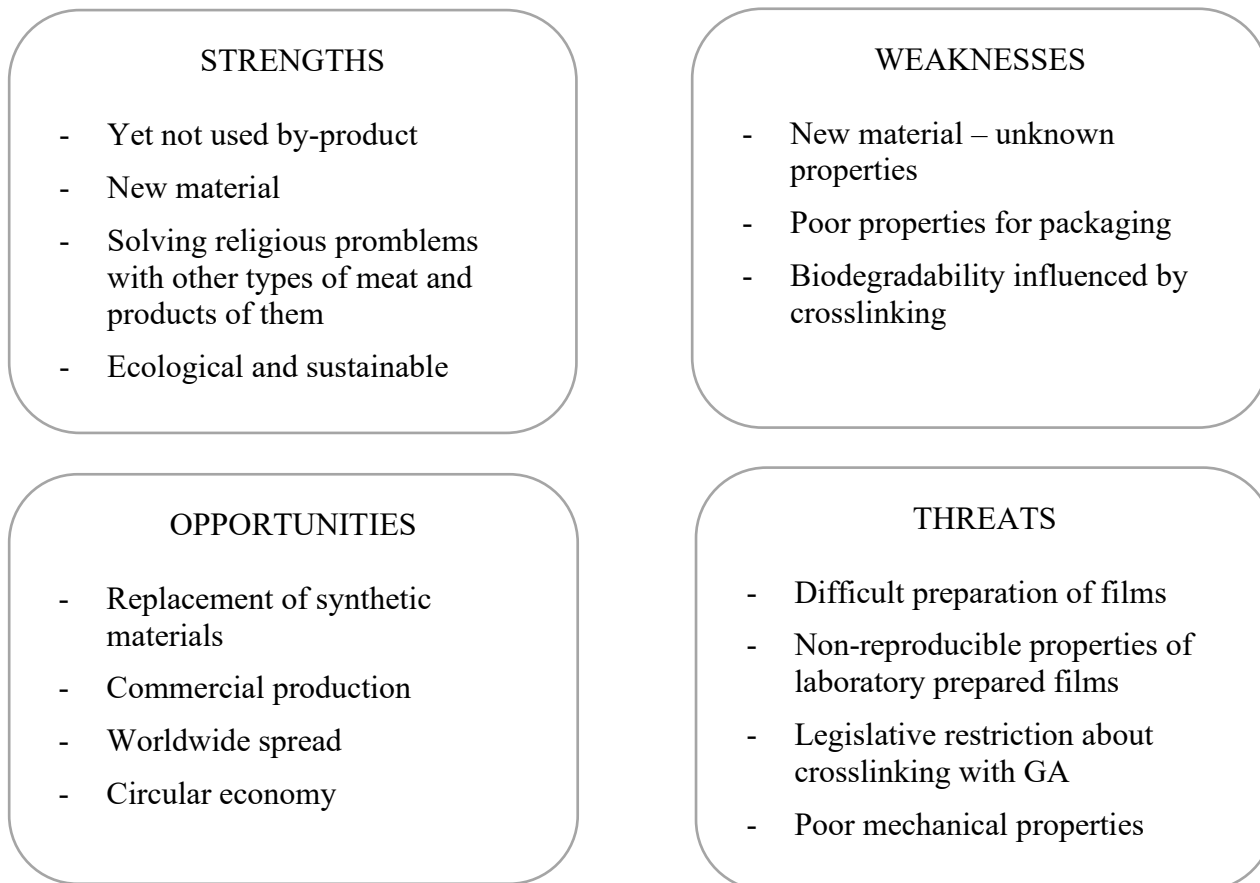
Fluorescent UV devices are used in tests where there is a need to vary light/dark cycles, temperature, humidity, water spray and control irradiation over time. Different types of fluorescent UV lamps are used in these devices - UV-B lamps and UV-A lamps. UV-B lamps emit most in the 280 to 360 nm region, UV-A lamps in the 340 to 370 nm range. [54]

Metal halide systems are considered as devices capable of full spectrum solar simulation systems. They are most commonly used by automotive manufacturers and their suppliers. [54]

Other devices are chambers that use carbon arc. The original use was to evaluate the effect of light on dyed textiles. These lamps are much stronger than natural sunlight. [54]

5 HYPOTHESES, SWOT ANALYSIS AND AIMS OF THE THESIS

5.1 SWOT analysis



5.2 Aims and hypotheses of the thesis

The aim of this work is to investigate the properties and a new type of gelatine and its potential use in the packaging industry. At the same time, the aim of this work is to evaluate the change in the properties of the prepared films due to accelerated weathering. Literature research is also included to compare the measured properties with those of already investigated gelatins or other biomaterials. The tested parameters of the prepared films will be compared with each other. Samples without crosslinker with plasticizer only will be compared with crosslinked undegraded samples and undegraded crosslinked samples with degraded for 100, 200 and 300 hours.

Finally, the work carried out will be evaluated and further procedures and suggestions for further investigations will be recommended.

Main hypotheses are:

1. The assumption is that the effect of crosslinking is significant mainly in the mechanical barrier properties, the crosslinked specimens should achieve higher tensile strength and have higher barrier properties.
2. It is also assumed that degradation will have an effect and deterioration of the tested properties will occur. Specifically, there will be an increase in gas permeability, the films will be less strong, and hazing/yellowing will occur.

II PRACTICAL PART

6 SOURCE MATERIAL, CHEMICALS, INSTRUMENTS AND WORK PROCEDURE

6.1 Source material

Deer Achilles tendons provided by the Institute of Food Technology at UTB were used as raw material for the preparation of gelatin. The tendons, already cleaned of meat and cooled to 2 °C to prevent overheating during grinding, were ground with a double-edged knife to a size of approximately 5 mm. The ground tendons were weighed into bags of approximately 700 g, vacuum-packed and shock-frozen at -80 °C.



Figure 2: A picture of grinded raw material – deer tendons.

6.2 Chemicals, instruments and devices

The chemicals used were sodium chloride (NaCl), sodium hydroxide (NaOH), 3% hydrochloric acid (HCl) solution, Protamex enzyme, petroleum ether, ethanol, glycerol, glutaraldehyde (GA), distilled water.

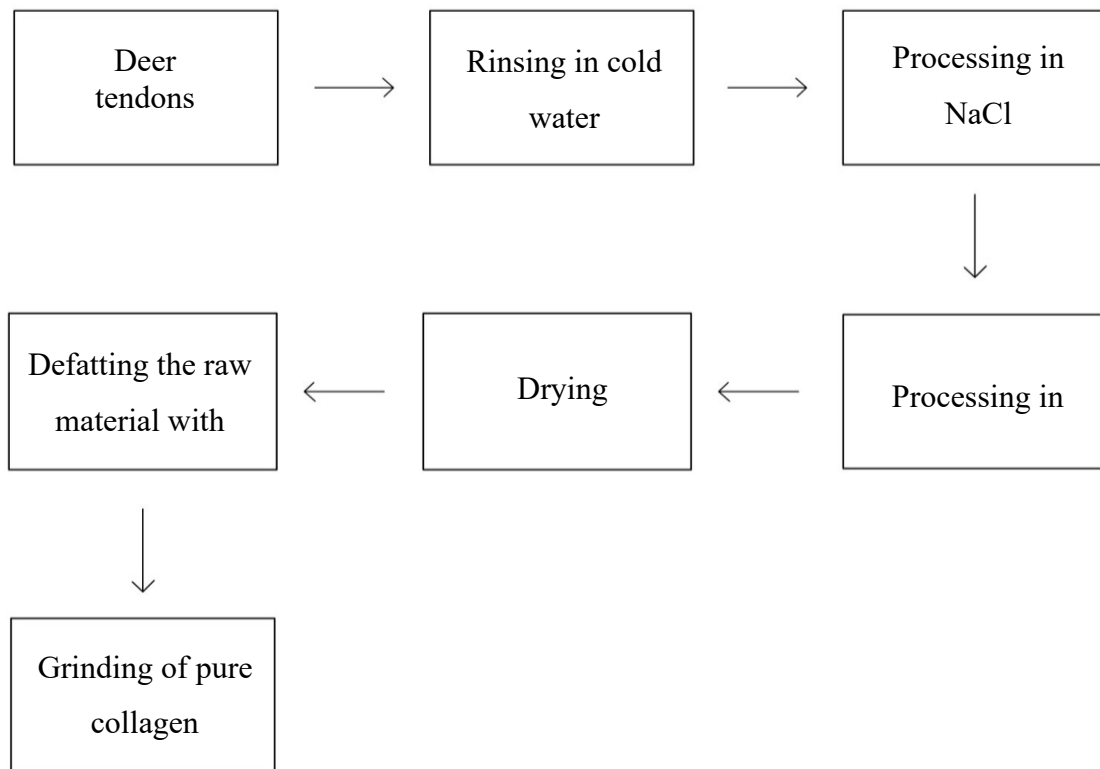
Materials used during the preparation of gelatin and films: sieve, three-layer cloth, beaker, Erlenmayer flask, glass rod, glass slide, weighing cup, silicone plates, petri dish, plate, bucket, pipette, volumetric cylinder, spatula, gloves.

Devices used during preparation of gelatin and gelatin films: Kern analytical balance, heater with magnetic stirrer IKA C-MAG HS 7, DSC1/700/1476 Mettler Toledo, shaking machine, cooler, KEYENCE VHX digital microscope, STA 449 Jupiter by NETZSCH, Alpha II by BRUKER, STEVENS-LFRA TEXTURE ANALYSER, GALDABINI Guasar 25 tensile testing, DMA 1 by METTLER TOLEDO, UltraScan PRO D65 by Hontelab.

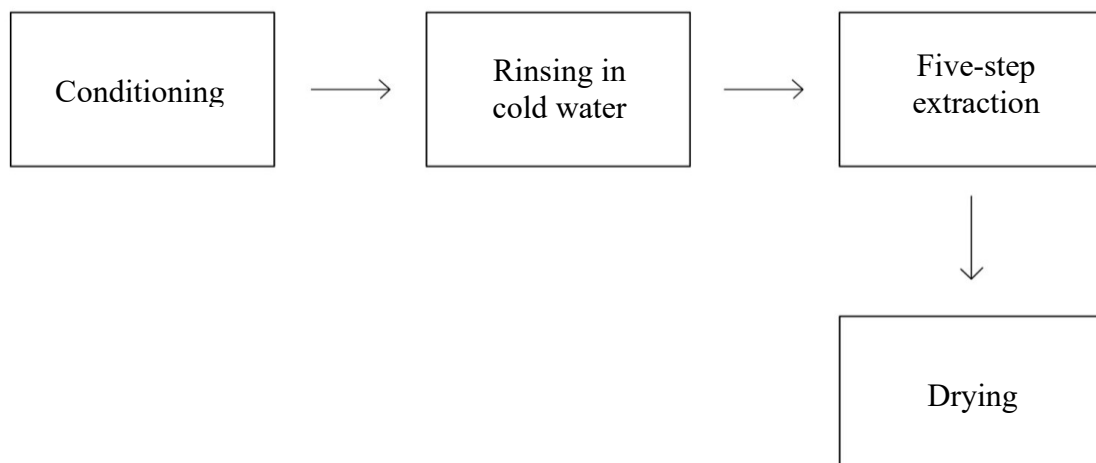
6.3 Work procedure of gelatin preparation

The procedure of the preparation of gelatin consists of two main parts, which are divided into single steps. The work procedure is from previous diploma thesis. [56]

A) Preparation of pure collagen



B) Preparation of gelatin



6.3.1 Preparation of pure collagen

First of all, about 1,5 kg of raw material (ground deer tendon) was defrosted and then the raw material was washed for about 10 minutes on a sieve with cold water. This procedure led to the removal of albumins.

In the next two steps, globulins were removed. First, the raw material was mixed in a bucket with 0.2 mol/l NaCl in a 1:6 ratio and left to process at room temperature with occasional stirring for 1.5 hours. The NaCl solution together with the first part of the globulins was filtered and the raw material was washed again with cold water.



Figure 3: Process of preparation of pure collagen.

In the following step, the raw material was mixed with 0.03 mol/l NaOH in a 1:6 ratio and worked in solution for 45 min. Further, the solution was filtered again, the raw material was washed and placed in fresh NaOH solution of the same mixing ratio. These steps were repeated three more times. After completion of this process, the raw material was placed in an air circulating dryer and left there until completely dry.

The dried material was broken and approximately 200 g of the material was placed in an Erlenmayer flask. A 1:1 mixture of petroleum ether/ethanol solvents was added to the flask at a total material ratio of 6:1. The flask was placed on a shaker for 1.5 to 2 days, with the solvent being replaced with a new one after half of a day each time. This process resulted in defatting of the raw material and obtaining pure collagen. Finally, the solvent was filtered out and the material was spread on a sheet, placed in a digester and the remaining solvent was evaporated. The pure collagen thus prepared was ground and subsequently used for the preparation of gelatin.



Figure 4: Defatted raw material ready for gelatin extraction.

6.3.2 Preparation of gelatin

For the preparation of gelatin, 70 g of pure collagen was weighed. In an Erlenmeyer flask, the raw material was mixed 1:10 with distilled water and the flask was placed on a shaker at 6 °C. After the first 20 minutes of shaking, the pH was checked and adjusted to neutral using a 3% HCl solution, to a range of 6.5 to 7. To the mixture thus prepared, Protamex enzyme was added at 0.3 wt% (on a dry weight basis of purified collagen). The flask was placed back on the shaker and the mixture was shaken for 24 hours. The pH was checked and adjusted every 30 min until the value was within the specified range twice in a row.

After 24 h, the solution from the flask, the hydrolysate, was filtered through a three-layer cloth and dried at 70 °C for approximately 24 h. The solid fraction trapped on the cloth was washed with cold water to remove the enzyme. This solid fraction was further used for extraction.

Gelatin extraction was carried out in five stages at different temperatures. The solid fraction after conditioning was placed in a beaker, topped up with distilled water at a ratio of 1:10 and placed on a cooker with a sensor for temperature control. Each time after the extraction was completed, the contents of the beaker were filtered through a three-layered cloth, and the liquid fraction was first placed in a refrigerator for 20 min and then in an air-circulating dryer. It was left in the oven at 40 °C overnight, then dried at 65 °C to dryness. The solid portion trapped on the fabric was each time returned to the beaker, topped up with distilled water in the same proportion and placed on a temperature-controlled stove. Extraction of the first fraction was carried out at 55 °C for 30 min. After the filtration was

completed, the liquid fraction, i.e. the first gelatin fraction, was heated to 85 °C for 6 min before being placed in a refrigerator. Extraction of the second gelatin fraction had the same procedure but was carried out at 65 °C for the same time.

The third fraction was extracted at 75 °C for 30 min. The liquid fraction was not subsequently heated and was placed in the refrigerator immediately after filtering. The fourth stage of extraction was carried out at 85 °C for 45 minutes, and the subsequent procedure was the same as for the third fraction. The last stage was the extraction of the glue, which was carried out at 95 °C for 120 min. Again, after filtering the liquid fraction, the procedure was the same as for the third fraction. The solid fraction trapped on the fabric was placed on a petri dish and dried at 103 °C overnight.

The weights of the hydrolysate, all fractions including the glue and the solid fraction from the end of the extraction were taken and used in the mass balance.

6.4 Work procedure of biofilms preparation

All the tested films were prepared by casting gelatin solution on a circular or rectangular plate according to the subsequent testing. For their highest gel strength results, the third and fourth gelatin fractions were used. The gel strength of these two fractions differed by only a few units, 180 and 176 Bloom respectively, so it was possible to mix them together.

6.4.1 Optimization of biofilms preparation

In the first stage, several samples of pure gelatin were prepared; these samples were very fragile. Subsequently, a plasticizer, glycerol, was added, and according to literature research, its ideal amount was found to be between 15 and 30 wt.%. In practice, this amount proved to be not satisfying. Films were then tried with different concentrations of plasticizer and with different ratios of plasticizer to gelatin. The gelatin/plasticizer percentages tested were 3/3 %, 3/5 % and 5/3 % (on solution volume).

The film containing 3% gelatin and plasticizer was stronger and could be removed from the plate but was sticky. The film with higher plasticizer content compared to gelatin was very sticky, tore and could not be peeled off the plate. The film with a higher gelatin content than plasticizer proved to be the best combination; it was strong enough for first sensorial evaluation, held its shape, and stuck minimally.



Figure 5: Dried gelatin film with optimal ratio of gelatin:glycerol.

Another parameter investigated was the effect of the type of dryer - without and with air circulation. The films were better and faster dried in the dryer with air circulation on a 40 °C.

6.4.2 Gelatin film preparation

The required amount of prepared gelatin was weighed into the beaker and the calculated amount of distilled water was added. The mixture was stirred with a glass rod and the gelatin was allowed to swell in the water for 20 min. Subsequently, the beaker was placed on a cooker with a temperature sensor and the mixture was heated to 50 °C with constant stirring and left until the gelatin was completely dissolved. In the last step, the plasticizer (glycerol), or plasticizer and crosslinker (glutaraldehyde), were added. The solution thus prepared was poured into a form and placed in the dryer for 24 h on 40 °C. If crosslinker was present, the film was left in the desiccator for another week for further possible crosslinking. This effect was confirmed by continuous tensile tests. (see in “Appendices” – Appendix P I)



Figure 6: Preparation of the gelatin films. Freshly poured gelatin solution into the forms, ready to dry.

The amount of cross-linker was added in accordance with the European Union regulation limiting the amount of glutaraldehyde in food contact products to 2 mg per 1 kg of product. After subsequent calculations, 0.03 % (per quantity of gelatin) of glutaraldehyde (GA) was added to the films.

6.5 Accelerated weathering

Xenotest Alpha+ from company ATLAS Material Testing Technology GmbH. was used to expose gelatin film samples to accelerated weathering. From the literature research, it was found that the application of accelerated weathering to gelatin samples is not well studied, therefore, the test parameters were based on the insights gained after consultation with an expert in accelerated weathering from company Atlas MTT GmbH. Test times of 100, 200 and 300 hours were chosen, corresponding to approximately 1, 2 and 3 months of natural ageing. Radiation was eliminated with a "window glass" filter to the range 300-400 nm, a humidity of 40 ± 5 %, temperature of 40 ± 5 °C and irradiance 50 ± 5 W/m² were chosen. The cooperation with the German company Atlas MTT GmbH was in addition to the consultation established in the form of comparative testing, in which the gelatin films sent were tested for 100 and 300 h at the temperature 35 ± 3 °C, with not regulated humidity, using a "store light" filter 300-800 nm (to simulate aging under supermarket illumination) and irradiance 250 ± 50 W/m². All aged samples were tested as the unaged gelatins and the results were consulted.

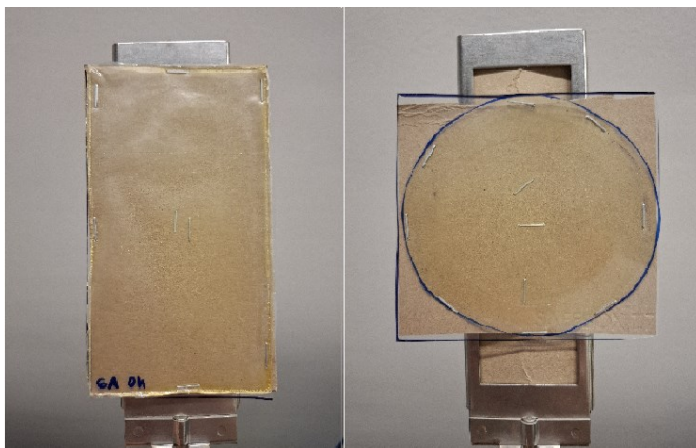


Figure 7: Samples for tensile strength and barrier properties prepared for accelerated weathering.

The specimens were mounted on paper pads covered with PET foil through which they were clamped in stainless steel machine holders. Only 5 of the 10 positions of the machine were filled at the time so that the wide gas permeability samples did not overlap, and proper irradiation of the entire sample was obtained.

6.6 Properties testing

The test specimens were conditioned at laboratory temperature and 50 % humidity for 24 h before each gas permeability and tensile strength measurement. The samples measured were un-crosslinked, crosslinked and degraded on the Xenotest.

6.6.1 Gas permeability measurement

The permeability of the gelatin films to air was investigated at laboratory temperature, i.e. 22 ± 2 °C. The pressure drop was set to 1 bar, the volume of the test chamber is 19.5 ml. The samples were 0.21 mm thick on average. The measurements followed the standard ČSN 640115, also as DIN 53 380 Part 3. Circular samples of 9 cm in diameter were cut from the prepared circular plates to provide overlaps over the test area, which corresponds to an area of 30.5 cm^2 , approximately 6 cm in diameter. The layout of the test area was as follows: under the sample, a circle of a paper towel overlapping the test area, a rubber seal around the test area, the sample, a second rubber seal around the test area.

Un-crosslinked samples containing only plasticiser (0h_Gly), crosslinked non-degraded samples (GA_0h), samples exposed to accelerated weathering for 100, 200 and 300 h (GA_100h, GA_200h, GA_300h) were tested and compared with each other. Comparison was also made between the samples degraded under the window-glass filter

and samples degraded under the store-light filter (SL_100h and SL_300h), which was provided by company Atlas MTT GmbH.

6.6.2 Gel strength

As part of the analysis of the produced gelatin, gel strength measurements were performed according to GMIA (Gelatin Manufactures Institute of America) on a STEVENS-LFRA TEXTURE ANALYSER. For the measurement, a quantity of gelatin was weighed into a prescribed container according to the chosen method to form a gel sample of 6.67% (w/w) concentration, then the gelatin was covered with distilled water and the mixture was allowed to swell for 1h. The dissolution was carried out in a water bath at 45 °C until completely dissolved, after which the solution was set aside for a while and then placed in a refrigerator for approximately 18 h. The measurements were carried out twice, with the second measurement taken at a different position from the first. The average for each fraction was then calculated from the gel strength values. For each fraction, a different measurement method was used, influenced by the available quantity of the produced product, therefore the gel strength values were normalized through appropriate conversion factors.

6.6.3 Swelling

Swelling was again tested for all prepared samples, either un-crosslinked, crosslinked or degraded. A 2x2 cm specimen was prepared from each sample. Distilled water was filled into small dishes and each sample was placed in each dish separately. After selected time intervals, the samples were dried with a cloth, weighed and their dimensions were measured. The temperature of the water was measured each time the properties of the bodies were measured, and the value was within 21.0 ± 0.5 °C. The same procedure was chosen as in the [26, 57, 58]



Figure 8: Samples of different films immersed in distilled water.

6.6.4 Tensile testing

Tensile strength was the mechanical properties parameter investigated using GALDABINI Guesar 25 machine. For this measurement, samples were prepared by pouring the solution onto a 7x12 cm plate and then drying at 40 ± 2 °C for 24 h. The solution was prepared as described in the Sample Preparation section. Test specimens 1 cm wide and 6.5 cm long were cut from the conditioned film and clamped in a tearing machine with a 3 cm gap between the jaws. The jaws were moved at a speed of 20 mm/min until the sample broke. Five bodies were measured from each sample, and the values of each measurement parameter were averaged and displayed in a table with standard deviation.

6.6.5 Vibrational characterization of functional groups

The Alpha II instrument from BRUKER was used for testing. The measurement consists of emitting a beam of light at many frequencies and recording how much of this beam was absorbed by the sample, the resulting values are given as a function of absorbance/intensity on an inverted wavelength scale in cm^{-1} , by FTIR method (Fourier transform infrared spectroscopy) The presence of chemical bonds/end groups can then be inferred from the individual peaks of the wavenumber. This is a non-destructive method, hence gelatin films were used for the measurements after gas permeability measurements. These samples were pre-dried overnight at 100 °C to eliminate excess moisture that may have masked the response of other bonds in the macromolecule chains. The sample was clamped between the beam source and the diamond tip and well attached. The measurement range was from 4000 to 400 cm^{-1} with a resolution of 4 cm^{-1} , and 32 scans were always performed.

6.6.6 Thermogravimetric analysis

The principle of this measurement is to record very accurately the change in mass of the sample as the temperature changes. In general, from this analysis, thermo-oxidative stability can then be discussed. For the analysis of gelatin samples, a temperature range of 25 °C to 600 °C with a heating rate of 10 °C/min was chosen with air as measuring medium (20ml/min). This method is destructive, therefore samples subjected to gas permeability measurements were used. Approximately 20 mg of each sample tested was weighed into small cups weighing about 135 mg. The opened cups were placed in a sample holder. The measurements were performed on an STA 449 Jupiter from Netzsch. The measurement output is a graph of the dependence of the percentage weight loss on temperature.

6.6.7 Differential scanning calorimetry

This analysis consists in comparing the temperature of the reference sample and the measured sample at the same heat flow. The result is a dependence of the heat flow on temperature, with the bending of the curve indicating the glass transition temperature, the exothermic peak indicating crystallization and the endothermic peak indicating melting of the sample. For this measurement, about 7 mg of sample was weighed into small aluminum pans, the samples were dried overnight at 100 °C to remove excess moisture. The lid of the pan was pierced prior to measurement to allow any released moisture to escape. For the measurements, the following heating and cooling procedure was chosen, always at a rate of 20 °C/min: from 25 °C to 130 °C, temperature steady state for 1 minute, cooling to -30 °C, temperature steady state again for 1 minute and reheating to 300 °C. The measurements were carried out on a Mettler Toledo instrument DSC1/700/1476.

6.6.8 Dynamic mechanical analysis

For this measurement, a temperature range of -30 to 130 °C was chosen, with a heating rate of 10 °C/min. A force of 1 N was set, with a displacement of 10 µm and a frequency of 1 Hz, the samples were measured on tension. Strip-shaped bodies of more than 10 mm in length (that is, the jaw distance) and approximately 5.5 mm in width were prepared for testing. The thickness of each body was also measured. For this measurement DMA 1 by METTLER TOLEDO was used.

6.6.9 Spectrophotometry

The yellowness index (YI), according to ASTM E313, and haze, according to ASTM D1003-21, of the samples were measured on the spectrophotometer UltraScan PRO D65 by HonterLab. Samples after gas permeability measurements were used. After calibration of the instrument, an uncross-linked sample was placed in front of the radiation source and stored as a standard. Measurements were taken at full transmission with the largest opening. The final value of haze and YI is an average of measuring three different spots on the sample.

6.6.10 Scanning electron microscope

The structure of the gelatin samples was imaged on a VHX Digital microscope from KEYENCE. The magnifications used were: 80x, 200x and 400x. The sample had to be placed on a white plate, otherwise the microscope scanned the structure of the black plate

due to the transparency of the samples. Under the lowest magnification (20x), a suitable area was selected and then recorded under the other magnifications.

7 RESULTS AND DISCUSSION

7.1 Barrier properties

For the measurement of barrier properties, air was selected as the measured gas. It was chosen because it is most appropriate for the application targeted in this work - the replacement of polyethylene bags. Most publications are interested exclusively in vapour permeability and oxygen permeability. [5, 21, 59–61]

For sample GA_100h, only one sample was measured because the others could not be removed from the Xenotest holder in one piece. For sample GA_300h, only two samples were measured, the others had cracks, and the pressure drop during measurement was not stable, so to avoid damaging the sensor, these damaged samples were not measured. Also, two samples each from the German laboratory (SL_100h and SL_300h) were measured. This was only a comparative measurement with a different type of radiation filter and due to the workload of the laboratory it was not possible to degrade more samples.

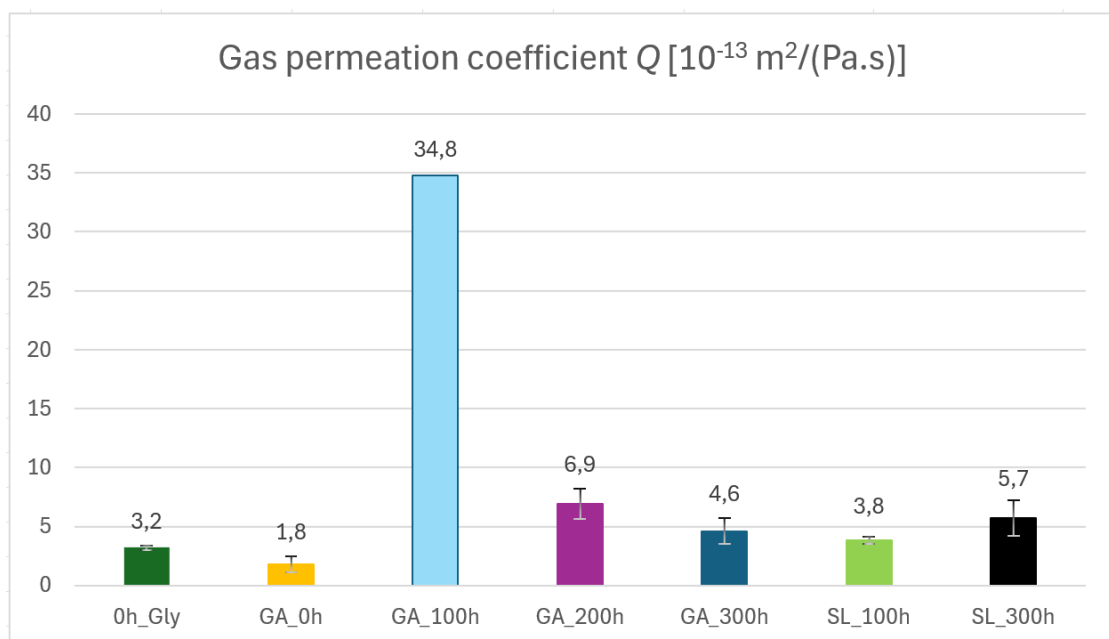


Figure 9: Column graph with values of gas permeation coefficient Q. Measured samples were: 0h_Gly – non-degraded sample without crosslinking agent, GA_0h – non-degraded crosslinked sample, GA_100h, 200h and 300h – crosslinked samples degraded for 100, 200 and 300 h under window-glass (WG) filter, SL_100h and 300h – crosslinked samples degraded under store-light (SL) filter for 100 and 300 h.

As can be seen from the graph, the crosslinking has reduced the permeability, with a drop of almost a half. The extreme increase in the permeation coefficient for sample GA_100h may be due to poor crosslinking of the samples, which would be consistent with

all the other tested samples sticking to the holder due to temperature and humidity. Many defects were observed in the film surface when measured under the microscope. The other degraded samples reach higher permeability than the non-degraded sample GA_0h, so the effect of degradation can be considered, which may have caused the chains to break down and therefore ease the path for gases through the membrane.

As already mentioned, in the publications, the focus is mainly on oxygen permeability, in [60] they investigated the effect of modifying chitosan with gelatin and starch, in both cases there was a decrease in permeability compared to chitosan alone, which in some cases has barrier properties comparable to ethylene vinyl alcohol (EVOH) and polyvinylidene chloride films. Reference [61] investigated the difference in barrier properties between gelatin and emulsion film, the emulsion film had up to five times higher oxygen permeability, it is discussed that this effect may be due to the presence of water which disrupts the structure and allows oxygen to pass through more easily. The barrier properties of gelatin films are generally poorer than those of synthetic polymers. [21]

7.2 Gel strength

Table 1: Values of gel strength for different fractions of gelatin.

	Gel strength [Bloom]
1. fraction	not stiff
2. fraction	133
3. fraction	180
4. fraction	176
glue	144

Gel strengths were determined for each extracted gelatin fraction, where the first fraction did not solidify, therefore no value was measured. The highest value can be seen for the 3rd and 4th fractions, these values are very similar, therefore these two fractions were mixed and used for the production of gelatin films. The second fraction of gelatin was used in a collaboration in another thesis where such strength was not required for its purpose. Gel strength is a standard analysis of gelatins, for example, a value of 147 Bloom was measured for gelatine from giant squid. [62] In [63] the gel strengths of different gelatins are compared, with porcine gelatine reaching the highest values, about 300 Bloom, followed by bovine gelatine with a value of 225 Bloom.

Gelatins can be divided into three groups according to gel strength - high, medium and low bloom gelatins with values of and 200 Bloom, values between 120 and 200 Bloom and values below 120 Bloom. Products made from such gelatins tend to be less coloured and odourless. They are advantageous for use in industrial production, because less gelatin is needed to achieve the same desired product properties than would be needed for a lower gel strength gelatin. [49]

7.3 Tensile testing

As part of this work, the specimens were subjected to tensile strength measurements, as the determination of this property is critical for packaging materials. Measured samples were: with only plasticizer (0h_Gly), with plasticizer and crosslinking agent (GA_0h), crosslinked samples degraded by accelerated weathering with window-glass filter (GA_100h; GA_200h and GA_300h) and samples degraded by accelerated weathering with store-light filter (SL_100h; SL_300h) in a German laboratory. The results of sample GA_100h must be taken with reservation, the tensile properties were measured on the sample after the gas permeability measurement, due to the stresses from this measurement the results of the tensile test may no longer be reliable.

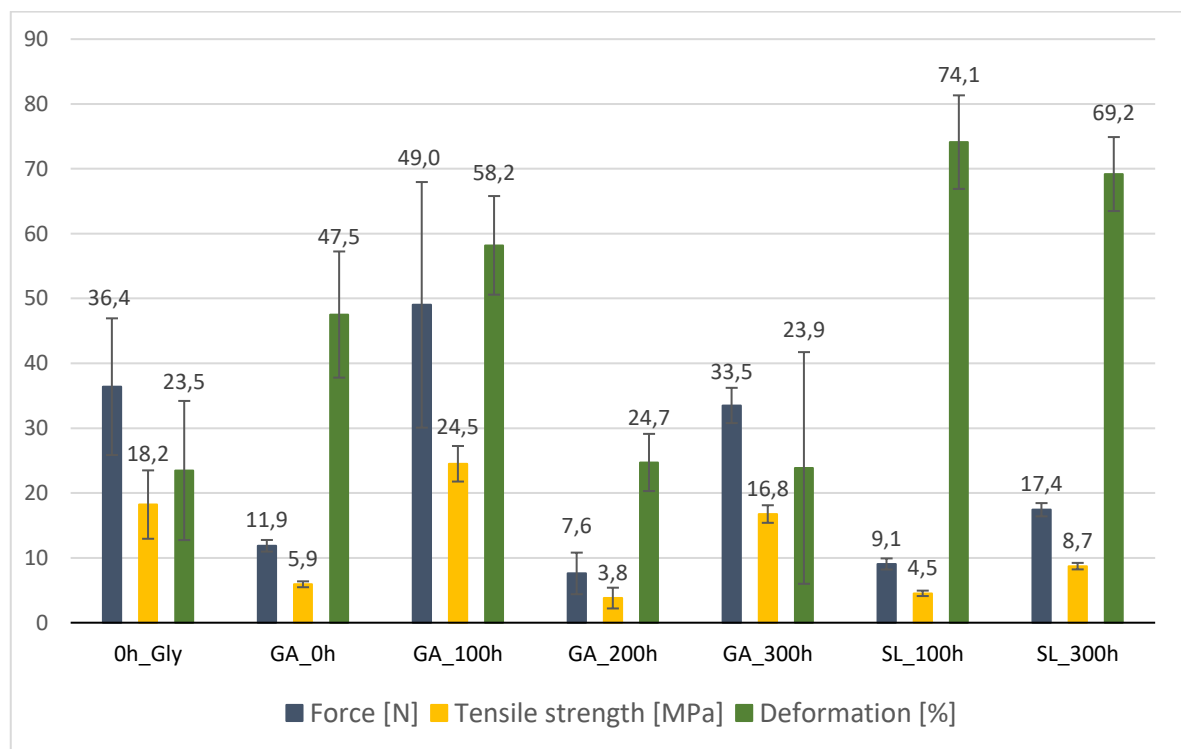


Figure 10: Values of force necessary for breaking of the sample [N] (blue), tensile strength [MPa] (yellow) and deformation (elongation) at break [%] (green).

It is very difficult to find a trend in the graph that can be used to clearly express the effect of crosslinking or degradation. The cross-linked sample GA_0h has almost four times lower tensile strength but increased deformation at break. In contrast, GA_100h is almost five times stronger in tensile strength, but the elongation at break is not significantly different. The specimen degraded to 200 h has the lowest tensile strength of all measured specimens. The effect of accelerated ageing could be considered here, but the specimens degraded at 300 h (GA_300h) have five times higher tensile strength and comparable breaking strain. For the samples degraded under the store-light filter the opposite tendency can also be observed, i.e. the more degraded sample has a higher tensile strength, both samples again have statistically comparable elongation at break. It is possible that the increase in the strength of the 100 h degraded sample (GA_100h) may be due to crosslinking after exposure to temperature and radiation. The same situation could be happening for the samples from Germany.

The tensile strength values of the un-crosslinked gelatine are comparable to the results of fish gelatine in [28] and beef gelatine in [24]. The fish gelatine measured in [4], on the other hand, reaches low values, around 2 MPa, after crosslinking and addition of chitosan the values increased to about 8 MPa. In reference [32, 64] the effect of the amount of plasticizer on the tensile strength is investigated, from the results it can be concluded that the tensile strength decreases with increasing amount of plasticizer but the value of maximum elongation increases. The tensile strength of rabbit skin gelatine and pork gelatine is over 30 MPa. [7] In [21], the effect of moisture on the elongation of films of different types of gelatins was investigated. It can be summarized that the higher the moisture content of the films, the more elongation they achieved. The addition of chitosan may result in a stiffening and an increase in the force required to break the specimens, but on the other hand the elasticity and maximum deformation of the specimens decreases. [20] The addition of silk fibres to the gelatine matrix has the same effect. [11]

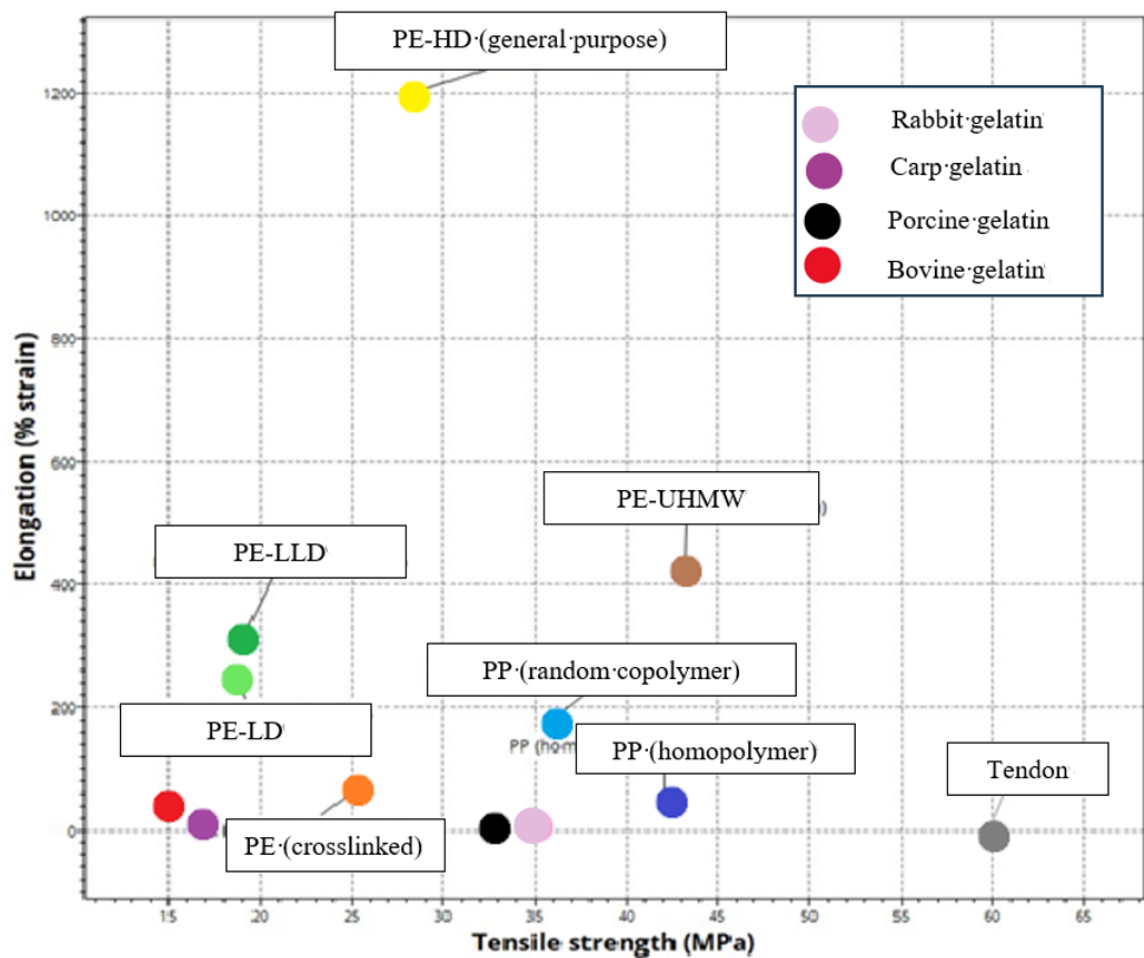


Figure 11: Graph generated by Granta EduPack 2024 R2 with added values of elongation vs. tensile strength of gelatins discussed in literature.

The above graph was generated in Granta EduPack 2024 R2, which stores a database of various materials and their properties. Using this program, a graph of tensile strength and elongation at break for materials used commercially for packaging materials was generated. Additionally the values for gelatins from literature were added. Tensile strength values up to 20 MPa can be seen for low density polyethylene (PE-LD) and linear low density polyethylene (PE-LLD). Samples 0h_Gly and GA_300h are close to these values. GA_100h is close to the value of cross-linked PE. The tensile strength value of crosslinked non-degraded sample GA_0h cannot be compared with these materials. Only samples degraded under a store-light filter achieve similar elongation values to cross-linked polyethylene.

7.4 Swelling

From the following graphs, it is possible to see the change in thickness, area and weight as a function of the time the samples were left in water.

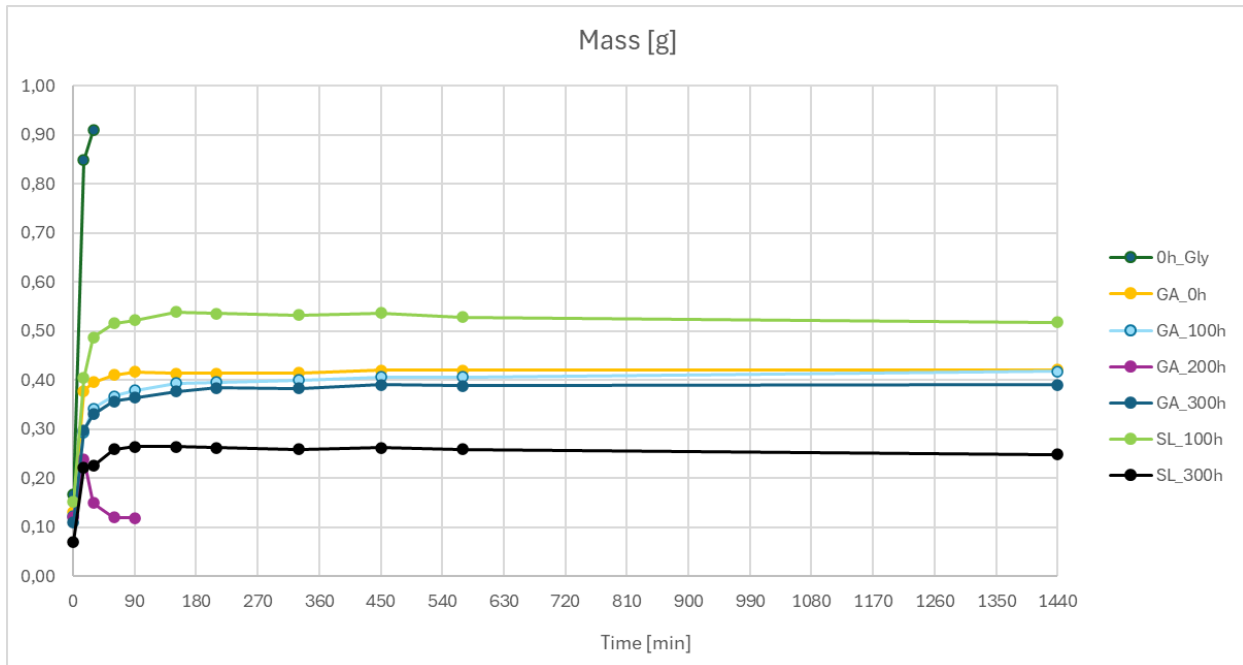


Figure 12: Graph of changing the mass [g] depending on swelling time [min]. Time 1440 min corresponds to 24 h.

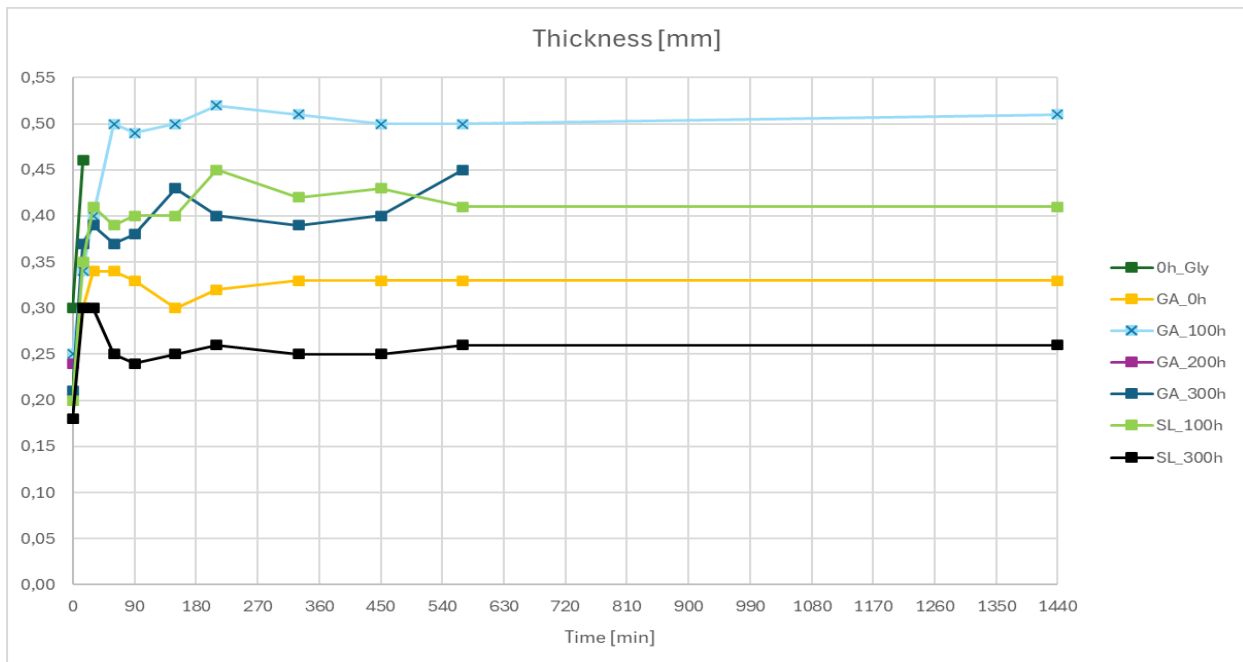


Figure 13: Graph of change of the thickness of the samples during time of swelling.

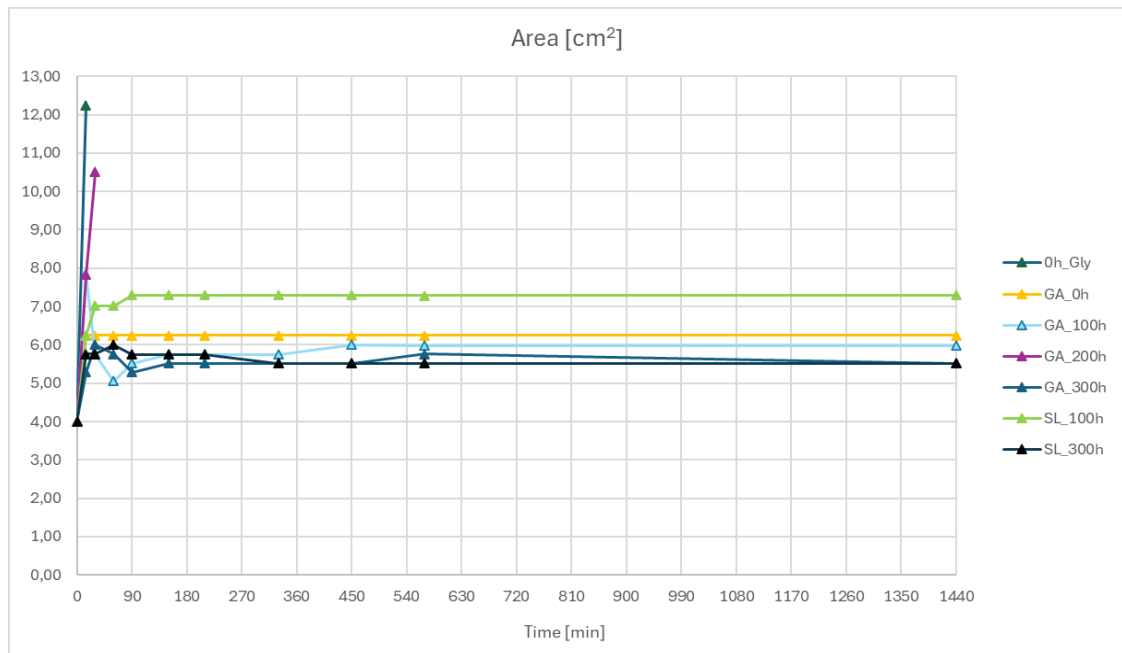


Figure 14: Graph of change of the samples area in increasing time of swelling.

Most publications focus primarily on the percentage change in weight, where the original weight is subtracted from the weight of the swollen sample, the whole divided by the original weight, and multiplied by 100. [30, 57, 65, 66]

Table 2: Values procentual swelling index of measured samples. There are: non-crosslinked sample (0h_Gly), crosslinked sample (GA_0h), crosslinked samples degraded under WG filter (GA_100h, 200h and 300h) and crosslinked samples degraded under SL (SL_100h and 300h)

	Swelling [%]					
Time [h]	15 min	30 min	1.5 h	5.5 h	1 day	1 week
0h_Gly	410.7	447.8	dissolved	dissolved	dissolved	dissolved
GA_0h	186.9	200.5	216.1	214.5	219.7	dissolved
GA_100h	141.6	181.9	212.4	229.8	244.6	dissolved
GA_200h	98.5	23.9	-1.3	dissolved	dissolved	dissolved
GA_300h	173.4	203.9	235.0	252.2	259.3	dissolved
SL_100h	165.9	220.2	243.4	250.3	240.2	dissolved
SL_300h	216.4	220.2	275.8	268.5	252.8	dissolved

The non-crosslinked sample was dissolved first, the crosslinked sample swelled half as much, and a similar trend was observed in [26,65]. The GA_100h sample swelled less than the undegraded sample at first, but after 5.5 h it started to absorb more water. The GA_200h sample did not swell and started to dissolve immediately, after 90 min it had disintegrated so much that it could no longer be weighed, and after 24 h it was almost completely

dissolved. GA_300h and SL_100h behave very similarly, with SL_300h showing a greater water absorption in the first measurement, after which the value stabilises and the same behaviour as the other samples is observed.

The surface area of the measured samples did not change significantly, except for the uncrosslinked sample 0h_Gly, which doubled in size due to water absorption into the structure, and the sample GA_200h, which probably did not have such a dense structure. The change in thickness occurred mainly during the first 90 minutes, with no major changes thereafter.

From the water absorption, one could also consider the biodegradability of the crosslinked samples, since dissolution occurs after a certain, relatively short time. Even so, this question calls for a more thorough investigation.

7.5 Vibrational characterization of functional groups

In the measured spectrum, the individual wavenumber ranges correspond to certain bonds and amide peaks. Fourier transform infrared spectroscopy method is used.

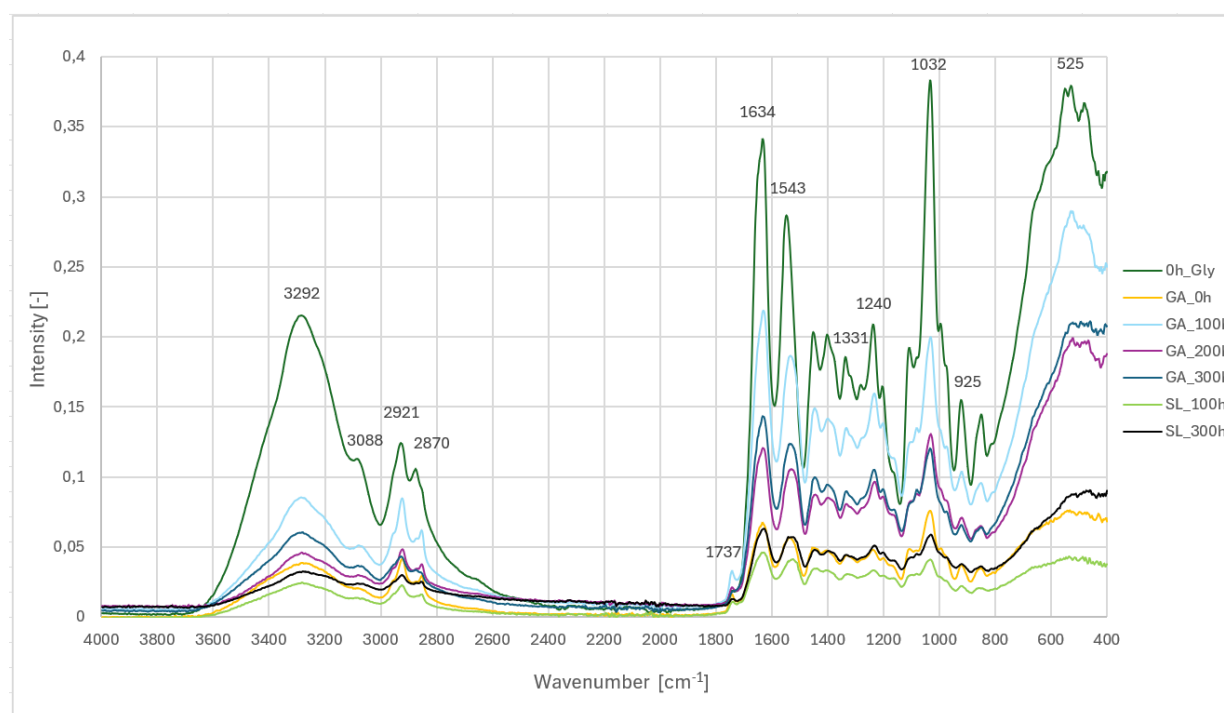


Figure 15: Graph of FTIR measurement (Wavenumber [cm^{-1}] vs. Intensity [-]) with values of peaks displayed.

The first broad peak can be seen in the range $3500 - 3200 \text{ cm}^{-1}$, which corresponds to the peak of Amide A and corresponds to N-H bond stretching and hydrogen bonding. [7, 32,

67–73] For gelatin films modified by the addition of, for example, chitosan, this peak shifts to higher numbers. [4, 30] Differences can also be seen based on the different gelatin source material, for fish gelatin such as used in [4] the peak is at 3285 cm^{-1} , for gelatin extracted from rabbit skin the peak for Amide A is found around 3400 cm^{-1} , as well as for porcine gelatin [7] And for chicken gelatin the Amide A peak can also appear around lower values, specifically around 3275 cm^{-1} . [73] In [70], they characterized residues from the production of beef gelatin capsules, for which the Amide A peak was in the range of $3277\text{--}3281\text{ cm}^{-1}$. Crosslinking also has an effect, as discussed in [68, 69], where crosslinking results in a shift to higher values of about 20 cm^{-1} . No significant shift is seen in the spectrum, the cause may be due to the masking of the peaks characterizing crosslinking, by the peaks for gelatin and glycerol. [70]

The next range is the Amide B peak, which is generally in the range $3080\text{--}2899\text{ cm}^{-1}$, corresponding to the stretching of asymmetric CH_2 bonds. [73] In [70], a region for this bond is reported in the range $2930\text{--}2926\text{ cm}^{-1}$, as is also in [71]. Even slightly smaller values are given by [72], i.e. 2920 cm^{-1} . In [7] the value of this peak is around 2930 cm^{-1} for rabbit gelatin and 2927 cm^{-1} for porcine gelatin. In the above spectrum, according to these publications, this corresponds to the peak shown at 2921 cm^{-1} . In [31], this peak is shown at a higher value, 2943 cm^{-1} . In the measured spectrum, even for this peak, there is no shift between samples

A region for Amide I and stretching of $\text{C}=\text{O}$ bonds is reported in the range $1700\text{--}1600\text{ cm}^{-1}$. [59, 74] Values around 1636 and 1657 cm^{-1} are given for rabbit and pork gelatin, respectively. [7] With similar values, this peak is also commented for chicken gelatin [71], and for beef gelatin a peak at 1629 cm^{-1} [70] and starch-modified beef gelatin at 1631 cm^{-1} [72]. This peak reaches a high intensity in all of these publications, and this is also the case for the above spectrum. There is also a small shift of the peaks of the crosslinked samples towards higher values, which was also observed for fish and beef gelatin with zein. [59, 68]

For the bending of N-H bonds a range of $1580\text{--}1500\text{ cm}^{-1}$ is reported, Amide II [59, 73] The spectrum for bovine gelatin showed a peak at 1541 cm^{-1} [59], fish 1537 cm^{-1} [68]. Sample 0h_Gly, which is not cross-linked, shows a peak at 1543 cm^{-1} and there is a noticeable shift of the peaks of the degraded samples to lower values, for GA_100h there was a shift to 1522 cm^{-1} , GA_200h to 1508 cm^{-1} , GA_300h to 1520 cm^{-1} . For the samples degraded in cooperation with the German company Atlas MTT GmbH. under the store-light filter (SL_100h and SL_300h) there was no shift.

At lower wavelengths, the N-H bonds bend and the C-N bonds stretch. This corresponds to an Amide III peak in the range $1350\text{--}1200\text{ cm}^{-1}$. [70–73] In the above graph, the peak for these bonds for the 0h_Gly sample is at 1240 cm^{-1} , with no significant shift for the samples degraded under the store-light filter and the crosslinked sample without degradation (GA_0h). While the degraded samples show a tendency of a slight shift towards higher values differing by a maximum of 6 units. Other bonds with response in the range $1150\text{--}1000\text{ cm}^{-1}$ are C-O-C and C-O. [67, 72] Source [65] states that the range $1200\text{--}900\text{ cm}^{-1}$ corresponds to C-O, C-C and C-O-H bonds, while stretching of C-O-C bonds is attributed to values as high as 860 cm^{-1} and 765 cm^{-1} . In [4], the spectrum shows a peak at 1043 cm^{-1} , which the authors attribute to vibrational stretching of the C=O bond and deformation of the O=H bond, which may be due to the interaction of gelatin and glycerol. The peak at 1032 cm^{-1} in [70] is attributed to glycerol. The measured spectra show the vibrational response at 1032 cm^{-1} for the un-crosslinked sample 0h_Gly, while for the other samples there is no significant shift, at most by 2 units.

7.6 Thermogravimetric analysis

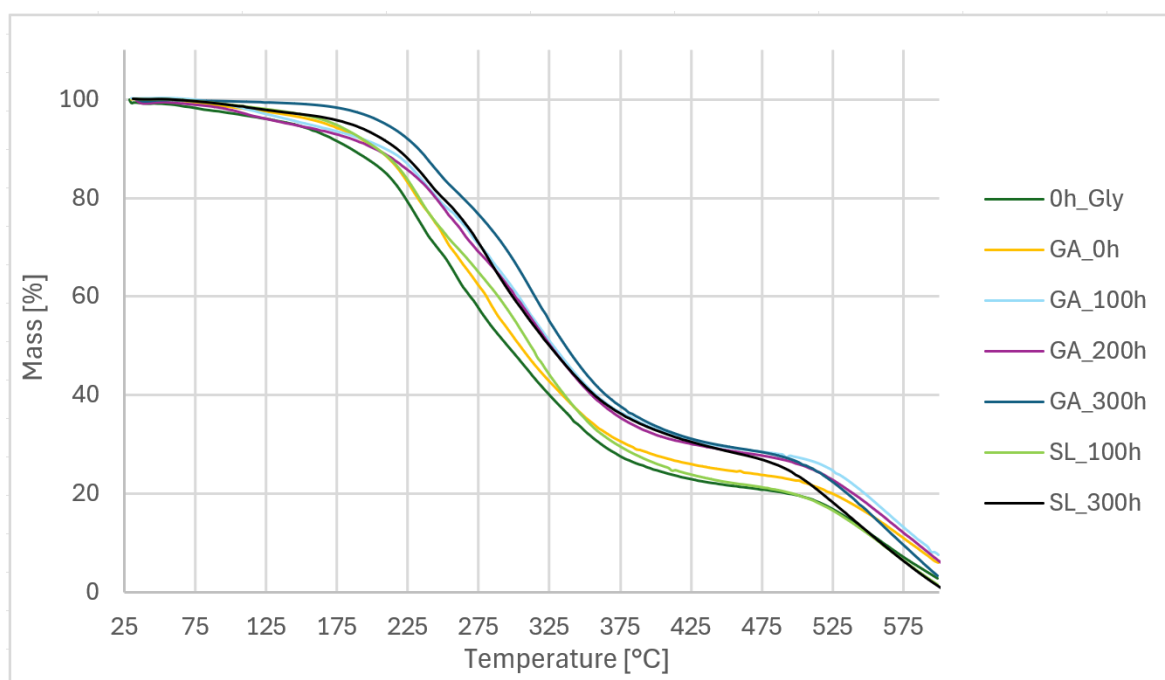


Figure 16: TGA measurement: mass [%] dependance on temperature [°C]. Interval of temperatures choosed for this measurement was 25–600 °C.

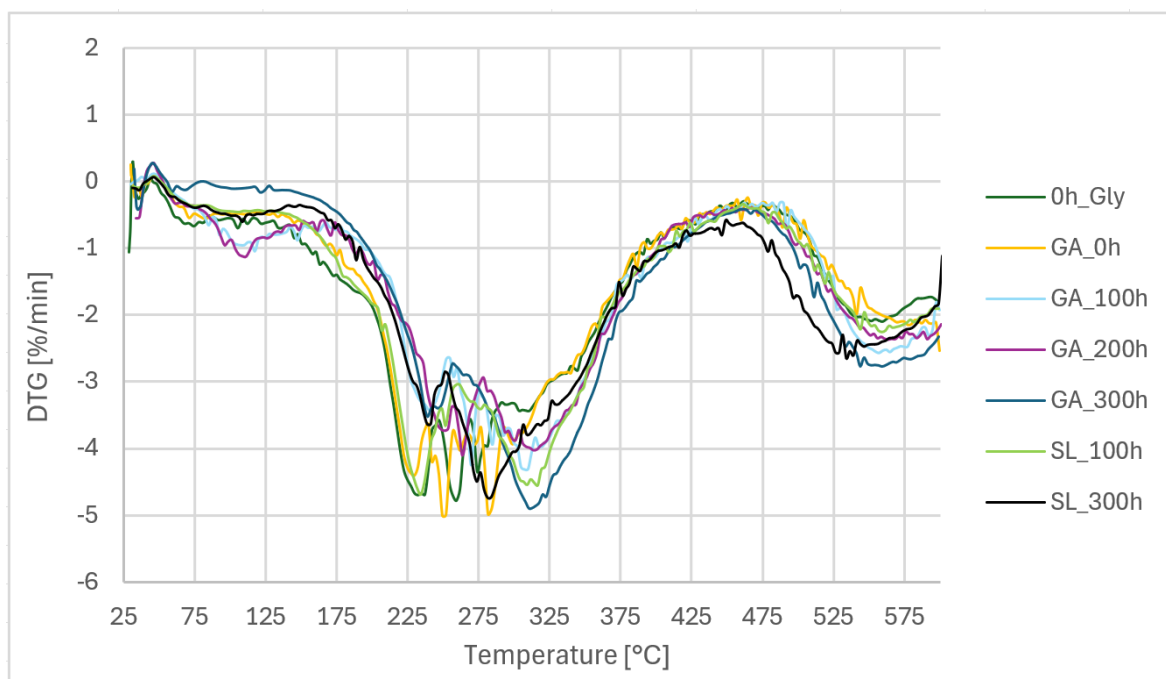


Figure 17: Derivations of TGA curves [%/min] displayed in dependence on temperature [°C]

Three temperature ranges [75] were selected and the following table summarises the percentage mass loss within these intervals and the residual mass at the end of the measurements.

Table 3: Mass loss [%] of different samples in three main intervals of degradation (25-200 °C, 200-450 °C and 450-600 °C). In the table is also residual mass after measurement.

Sample	Mass loss [%]			Residual mass [%]
	25-200 °C	200-450 °C	450-600 °C	
0h_Gly	12.92	65.27	18.90	2.91
GA_0h	9.46	65.78	18.77	5.99
GA_100h	8.71	60.79	21.89	8.61
GA_200h	9.93	61.23	22.74	6.10
GA_300h	3.80	66.62	26.29	3.29
SL_100h	9.11	68.45	20.84	1.60
SL_300h	6.99	64.36	27.71	0.94

In the first temperature interval, almost all samples show a decrease in mass of about 10 %, with the exception of only a 3% decrease in the degraded sample GA_300h and a half decrease relative to the others in the sample SL_300h. This initial drop is associated with moisture loss and glycerol degradation, with bound water leaving above about 130 °C. [3,

25, 29, 70, 75] All samples were equally dried (100 °C, overnight) prior to this measurement, so it can be considered that the crosslinked samples did not contain as much moisture, while the two most degraded samples under different filters in this range lost the minimum amount of mass, thus containing less water

The second temperature range, at which significant weight loss occurs, is attributed to the breakdown of proteins such as elastin or collagen and the degradation of the gelatin itself. The portion of collagen that was not degraded has carbonized. [3, 29, 70, 75] A smaller weight loss in this range is observed for samples degraded at 100 and 200h (GA_100h, GA_200h), which may be due to better crosslinking of the samples. The largest weight loss is observed for the SL_100h sample. Three peaks can be seen in the derivative curve in this region, which could correspond to the degradation of three different chain lengths

At temperatures above 450 °C, the larger matrix molecules are subsequently degraded and oxidized, leading to depolymerization of the gelatin and disintegration of the sample. [29] A relatively high loss compared to the other samples can be observed for sample SL_300h, where almost all of the remaining material was degraded, as only less than one percent of the original mass remained after this temperature phase.

7.7 Differential scanning calorimetry

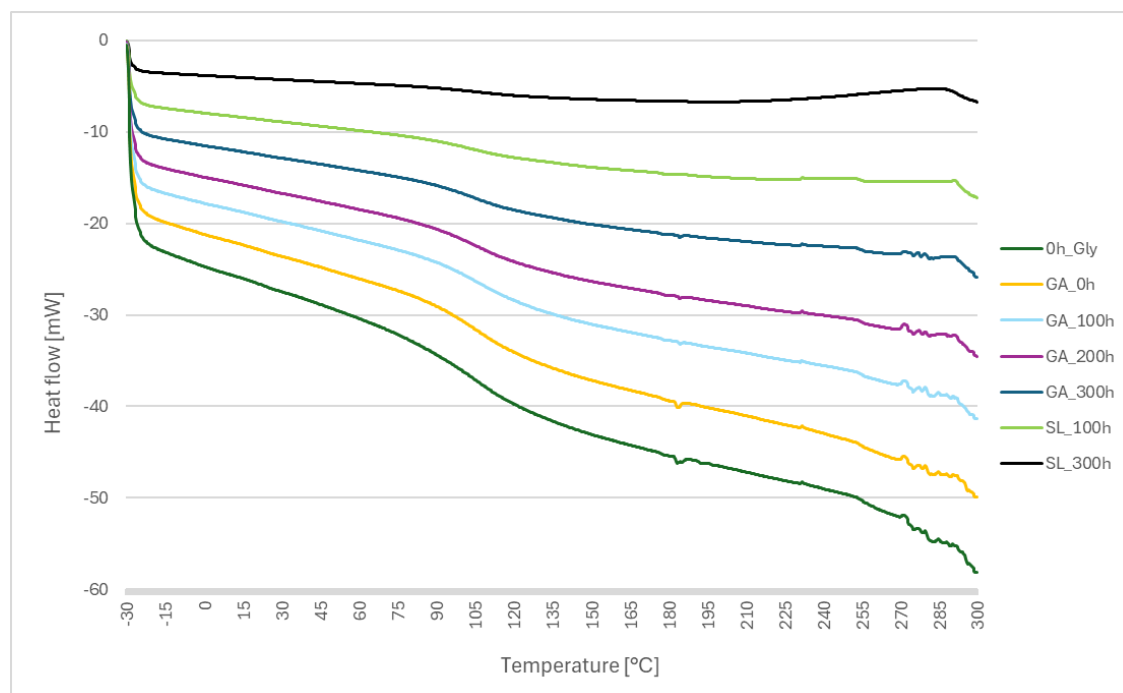


Figure 18: DSC graph of heat flow [mW] due to temperature [°C]. It is second heating scan in which range of the temperature was -30-300 °C.

Table 4: Values of glass transition temperature T_g and melting temperature T_m read from the graph (Figure 18).

Sample	T_g [°C]	T_m [°C]
0h Gly	75.37	271.35
GA_0h	98.28	227.03
GA_100h	114.71	186.79
GA_200h	114.25	186.07
GA_300h	107.95	185.12
SL_100h	107.23	
SL_300h	109.77	

The graph shows the second heating curves, from which the glass transition temperatures for the individual samples can be read. This temperature is most often shown as an axis-like bend in the curve and the value is read from the middle part. [3, 4, 20, 61, 64, 74, 76] The values read from the individual curves are summarized in Table 4, it can be seen that the glass transition temperature (T_g) is lowest for the uncrosslinked sample, the crosslinking increased the temperature. Further increases occurred for the 100 and 200 h degraded samples, and a slightly smaller increase in T_g compared to the undegraded sample is also seen for the samples degraded under the store-light filter (SL_100h, SL_300h). These values are higher than, commercial PLA [76] and fish gelatin [3, 4, 61], for beef gelatin was T_g lower [20]. Similar values were reached by the measured agarose. [74] In [64] the effect of plasticizer on this temperature was investigated, commercial bovine gelatin was used and T_g was measured to be around 140 °C, when glycerol was added this temperature was reduced. Cross-linked chains are much less mobile, so a higher temperature is needed to cross the 'frozen' state that characterises T_g . Samples subjected to accelerated weathering have this temperature so much higher, probably because of the additional crosslinking by heat and irradiation.

The melting temperature (T_m) is caused by the melting of the crystalline parts in the polymer matrix, and this temperature is strongly dependent on the crosslinking of the polymer structure. [28] It is also dependent on the addition of the plasticizer, and this temperature shifts to higher values with increasing plasticizer content. [64] The melting temperature can also be influenced by the proline and hydroxyproline content, where at higher values of these amino acids the T_m is higher, beef and pork containing over 20 mol%, fish only around 15 mol%. [3] From research by our Institute of Polymer Engineering in collaboration with the Institute of Food Technology, the content of these amino acids in deer

tendon gelatins was found to be over 100 g/kg. The Table 4 shows a decrease in this temperature, first due to crosslinking and then probably due to degradation, where long molecules were broken down, thus allowing them to move more easily, resulting in a lower melting point.

7.8 Dynamic mechanical analysis

The following graph plots the dependence of the storage modulus E' , and the $\tan \delta$, which is the ratio of the loss and storage modulus, on temperature.

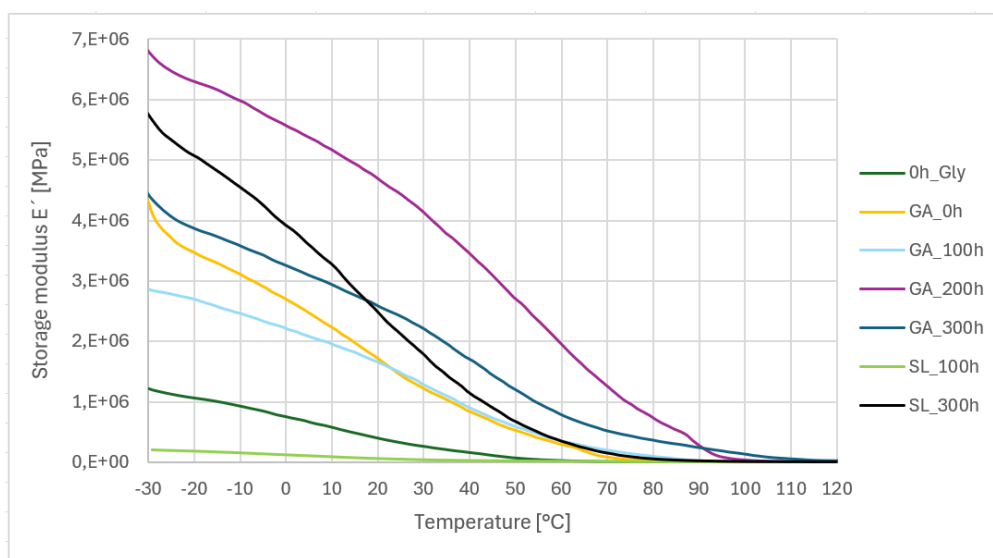


Figure 19: Stiffnes of films defined by storage modulus [MPa] depending on tepmerature [°C]. Temperature interval was -30-130 °C.

The storage modulus defines stiffnes, and stiffness of all samples decreases with increasing temperature, but the lowest stiffness (lowest modulus value) can be observed for the sample degraded under the store-light filter for 100 h and the uncrosslinked sample, so they are the least resistant to deformation, while their $\tan \delta$ values reach the highest values. The GA_100h sample reaches a value of about 3000 GPa, and at a temperature of about 30 °C its strength decreases to the initial strength of the uncrosslinked sample and the SL_100h sample. Samples GA_0h and SL_300h show a sharper drop in modulus and loss of stiffness already around 5 °C. For sample GA_300h, this break is only evident around 25 °C. Sample GA_200h has the highest strength and resistance to deformation over the entire temperature range measured. Thus, it can be said that the sample is able to resist small deformations even at lower and higher temperatures, but at a single large applied force, as was used in the tensile

test, the strength of the sample is small. The structure is therefore able to withstand smaller stress forces and retain its stiffness. [77]

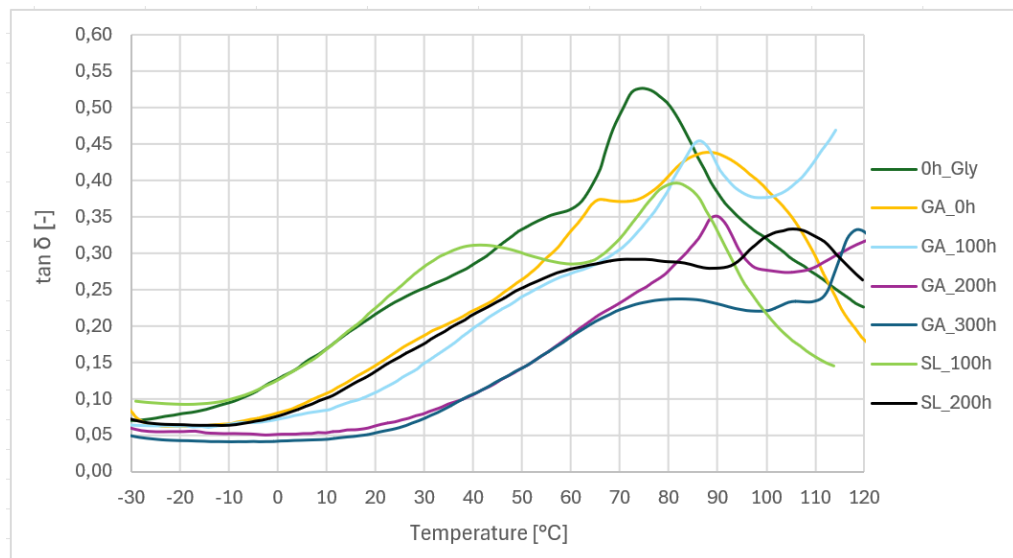


Figure 20: Dependence of $\tan \delta$ on temperature [°C] with same range of temperatures like in (Figure 19).

From the $\tan \delta$ peak it is possible to read the approximate glass transition temperature, and often more of these temperatures can be observed than, for example, on a DSC. [78] In general, this parameter characterises the behaviour of a material, with zero values corresponding to a fully elastic material (solid-like behaviour), and values tending towards infinity to fully viscous behaviour (liquid-like). [79] The glass transition temperatures from this graph already correspond more closely to the temperatures measured by DSC, and reach rather slightly higher values – 0h_Gly = 55 and 75 °C, GA_0h = 65 and 88 °C, GA_100h = 60 and 85 °C, GA_200h = 90 °C, GA_300h = 80; 105 and 118 °C, SL_100h = 40 and 80 °C, SL_300h = 70 and 105 °C. The reason for the higher and more values is probably due to the fact that the DSC measurement was only analysed from the second heating record. Two different temperatures may be a result of the different segments mobility. [80]

7.9 Spectrophotometry

Haze and yellowness index were measured on the samples and these values were compared between the samples.

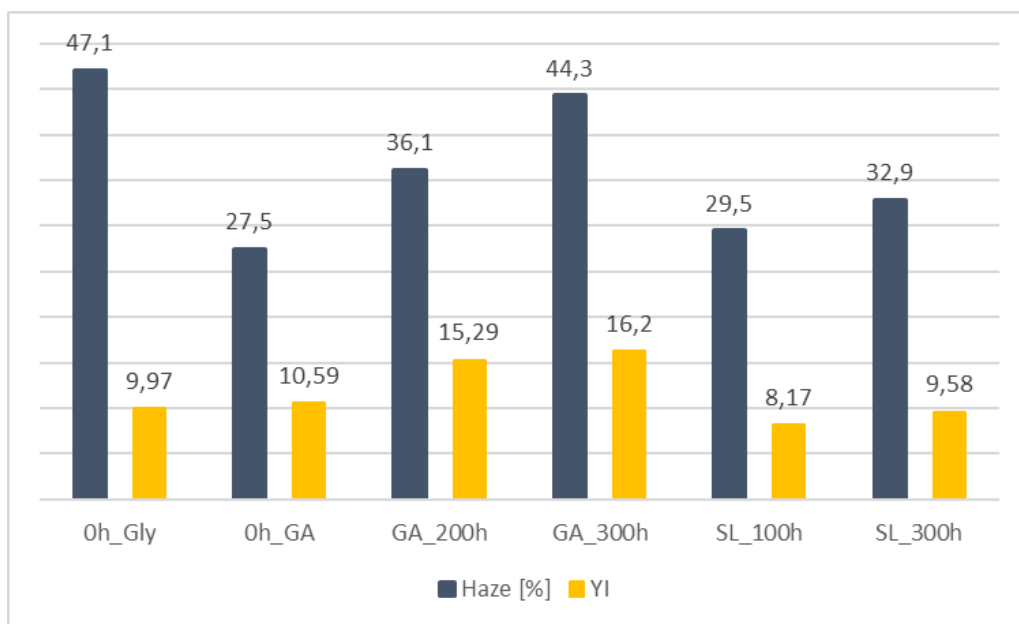


Figure 21: Graph with values of haze and yellowness index (YI) for different samples: non-crosslinked (0h_Gly), crosslinked non-degraded (GA_0h), crosslinked degraded (GA_100h, 200h and 300 h under WG filter, SL_100h and 300h under SL filter).

A clear reduction in haze due to sample crosslinking can be observed, with almost half the decrease between the un-crosslinked and crosslinked samples. At the same time, the effect of degradation is evident, with haze increasing with more degradation time. The samples degraded under the store-light filter did not show such an increase in opacity, and even a sample degraded for 300 h under these conditions has less haze than a sample degraded for 200 h under the window-glass filter. The transparency of the films also varies with the source material [63], with, for example, beef and pork gelatins being more transparent than carp skin gelatins.

As for YI, there is an increasing tendency for sample crosslinking and degradation. The samples degraded under the store-light filter show lower YI values than the un-crosslinked sample, but even for these samples an increase is seen with longer degradation time. Most publications focus mainly on the overall color change and the yellow color parameter b^* alone. In [70] there is a significant yellowing due to the addition of the crosslinker. The addition of plasticizer also increases this parameter. [7]

7.10 Scanning electron microscope



Figure 22: 80x (left), 200x (middle) and 400x (right) magnified non-crosslinked sample – 0h_Gly



Figure 23: 80x (left), 200x (middle) and 400x (right) magnified crosslinked non-degraded sample – GA_0h



Figure 24: 80x (left), 200x (middle) and 400x (right) magnified crosslinked sample degraded under WG filter for 200 h – GA_100h



Figure 25: 80x (left), 200x (middle) and 400x (right) magnified crosslinked sample degraded under WG filter for 100 h – GA_200h

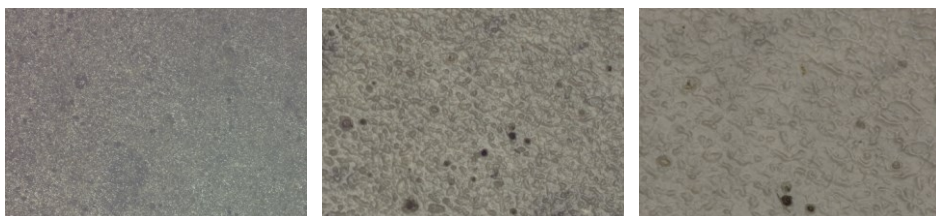


Figure 26: 80x (left), 200x (middle) and 400x (right) magnified crosslinked sample degraded under WG filter for 300 h – GA_300h



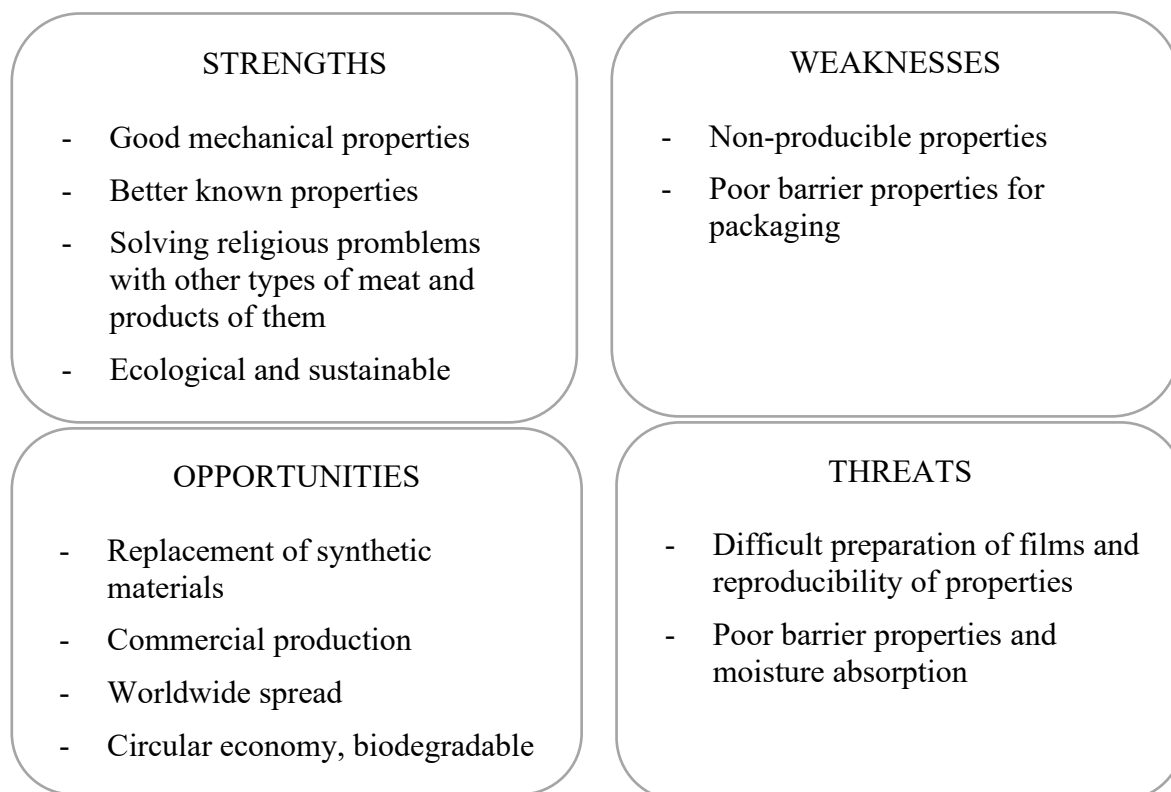
Figure 27: 80x (left), 200x (middle) and 400x (right) magnified crosslinked sample degraded under SL filter for 100 h – SL_100h



Figure 28: 80x (left), 200x (middle) and 400x (right) magnified crosslinked sample degraded under SL filter for 300 h – SL_300h

If the un-crosslinked and the crosslinked sample are compared, it can be seen in the highest magnification images that the crosslinking has homogenized and the surface is smoother, such an effect is also described in [71] and in [6] after chitosan modification. The appearance of the surface therefore depends on the interaction of the gelatin with the crosslinker. [30] Due to the effect of crosslinking, it is possible to fill the porous structure, which can be seen in the images in [75]. Due to the effect of crosslinking, it is possible to see in the images a microstructure consisting of a network of twisted wires, the same phenomenon has been observed in [4, 28] Subsequently, due to accelerated weathering, it is possible to observe an increasing appearance of anomalies, dark spots, sinkholes and, in the case of GA_300h, extreme wrinkling of the surface. The surface of sample SL_100h is comparable to the undegraded crosslinked sample GA_0h. The surface of sample SL_300h is comparable to that of GA_200h, which may account for the less aggressive degradation under the store-light filter than under the window-glass filter.

8 SWOT ANALYSIS AND BENEFITS OF THIS THESIS



This work has contributed to a closer examination of the properties of the new gelatins, opened the door for further investigation of the effect of accelerated weathering on the properties of biomaterial films, and helped to optimize the process of preparing gelatin films. At the same time, this research has raised additional questions and limitations that need to be answered and addressed by further investigation. Specifically, the issue of biodegradability of crosslinked materials, while the swelling test outlined that this property might not be affected, there is a need to subject such materials to proper, standards regulated, testing.

A major obstacle is the homogeneity and reproducibility of the gelatins and films formed from them. Whether it is multiple extraction, in which the human factor may have been involved, it may also be variability in the raw material and errors in the actual preparation, storage and conditioning of the films. A big question and suggestion for further investigation is the cross-linking process, this process needs to be investigated more closely and the ideal conditions for good cross-linking of films clarified. Another possibility is the use of other crosslinkers, where legislation would not prevent their addition in larger quantities, and thus could improve the properties.

CONCLUSION

In this thesis, the effect of accelerated ageing on the properties of gelatine films was mainly investigated. The properties of the samples were also compared with each other within the non-crosslinked/crosslinked structure. Furthermore, a comparative measurement with a different radiation filter in the Xenotest was performed, this measurement was carried out in cooperation with the German laboratory of Atlas MTT GmbH.

The observation of the effect of weathering is not clear- conclusive because there were other effects such as human error, different batch of raw material etc. The un-crosslinked samples containing only the plasticizer showed a clear much higher water absorption, as the test swelling resulted in complete dissolution of the sample within 90 minutes. The effect of crosslinking was evident in this test, as the crosslinked sample did not soak up nearly as much water and did not change significantly in weight after 30 minutes. The effect of degradation is overshadowed by the possible addition crosslinking process due to heat during irradiation of the samples in the Xenotest. If comparisons are made only within degraded samples, the degradation time has an effect. Even by sensorial evaluation, the degraded samples were more brittle with a higher degradation time, the crosslinked samples appeared to be stronger compared to the non-crosslinked samples.

From the results of barrier properties and tensile strength measurements, the unpredicted behaviour of the sample degraded for the least time is observed. This specimen achieves the highest tensile strength, 24.5 MPa, and also has the highest air permeation coefficient, $34.8 \cdot 10^{-13} \text{ m}^2 \cdot \text{Pa}^{-1} \cdot \text{s}^{-1}$. Another anomaly is the behavior of the sample degraded for 200 h, reaching the lowest tensile strength of 3.8 MPa, and the swelling test resulted in immediate dissolution. On the other hand, the barrier properties of this sample came out as expected, being higher than those of the undegraded sample.

It was assumed that degradation would be evident in the FTIR measurements, even here no significant change in the sample structure was shown to be due to degradation. Thermal degradation provided differences only in the first phase, in which moisture loss occurs, with the crosslinked samples containing less of this moisture. The degraded samples contained even less moisture than the non-degraded samples, again supporting the theory of subsequent crosslinking.

The effect of crosslinking was evident from the increased value of the glass transition temperature from both the DSC evaluation and the bending of the DMA $\tan \delta$ curves. The

only sign that can be considered as a sign of degradation is the reduced values of this temperature in samples degraded for 300 h.

Crosslinking has an effect on the haze of the gelatin films, with a decreasing the values, while at the same time the effect of degradation is evident by increasing haze and yellowness index. Similarly, degradation and crosslinking can also be seen in the SEM images.

It would certainly be beneficial to build on this work, if only because of the increasing demand for biodegradable materials and the use of a not yet processed by-product of meat production - deer tendons.

BIBLIOGRAPHY

- [1] ABDULLA, S. F., R. SHAMS a K. K. DASH. Edible packaging as sustainable alternative to synthetic plastic: a comprehensive review. *Environmental Science and Pollution Research* [online]. 2024. ISSN 1614-7499. Available from: doi:10.1007/s11356-024-32806-z
- [2] PERERA, K. Y., A. K. JAISWAL a S. JAISWAL. Biopolymer-based sustainable food packaging materials: challenges, solutions, and applications. *Foods* [online]. 2023, 12(12). ISSN 2304-8158. Available from: doi:10.3390/foods12122422
- [3] CHIOU, Bor-S., R.J. AVENA-BUSTILLOS, P. J. BECHTEL, S. H. IMAM, G. M. GLENN a W. J. ORTS. Effects of drying temperature on barrier and mechanical properties of cold-water fish gelatin films. *Journal of Food Engineering* [online]. 2009, 95(2), 327–331. ISSN 02608774. Available from: doi:10.1016/j.jfoodeng.2009.05.011
- [4] CHEN, H., X. LAN, Q. ZHANG, H. LIU, H. REN, M. DU a J. TANG. Improving the comprehensive properties of gelatin films by transglutaminase and chitosan. *Food Hydrocolloids* [online]. 2024, 151, 109854. ISSN 0268005X. Available from: doi:10.1016/j.foodhyd.2024.109854
- [5] TYUFTIN, A. A. a J. P. KERRY. Gelatin films: Study review of barrier properties and implications for future studies employing biopolymer films. *Food Packaging and Shelf Life* [online]. 2021, 29, 100688. ISSN 22142894. Available from: doi:10.1016/j.fpsl.2021.100688
- [6] JEYA SHAKILA, R., E. JEEVITHAN, A. VARATHARAJAKUMAR, G. JEYASEKARAN a D. SUKUMAR. Comparison of the properties of multi-composite fish gelatin films with that of mammalian gelatin films. *Food Chemistry* [online]. 2012, 135(4), 2260–2267. ISSN 03088146. Available from: doi:10.1016/j.foodchem.2012.07.069
- [7] MA, L., H. YANG, M. MA, X. ZHANG a Y. ZHANG. Mechanical and structural properties of rabbit skin gelatin films. *International Journal of Food Properties* [online]. 2018, 21(1), 1203–1218. ISSN 1094-2912. Available from: doi:10.1080/10942912.2018.1476874
- [8] ALFARO, M. E. C., S. L. STARES, G. M. DE OLIVEIRA BARRA a D. HOTZA. Effects of accelerated weathering on properties of 3D-printed PLA scaffolds. *Materials Today Communications* [online]. 2022, 33, 104821. ISSN 23524928. Available from: doi:10.1016/j.mtcomm.2022.104821
- [9] ALFARO, M. E. C., S. L. STARES, G. M. DE OLIVEIRA BARRA a D. HOTZA. Estimation of shelf life of 3D-printed PLA scaffolds by accelerated weathering. *Materials Today Communications* [online]. 2022, 32, 104140. ISSN 23524928. Available from: doi:10.1016/j.mtcomm.2022.104140
- [10] KAYNAK, C. a B. SARI. Accelerated weathering performance of polylactide and its montmorillonite nanocomposite. *Applied Clay Science* [online]. 2016, 121–122, 86–94. ISSN 01691317. Available from: doi:10.1016/j.clay.2015.12.025
- [11] SHUBHRA, Q.i T. H., A. K. M. M. ALAM, M. A. KHAN, M. SAHA, D. SAHA, J. A. KHAN a M. A. QUAIYYUM. The Preparation and characterization of silk/gelatin biocomposites. *Polymer-Plastics Technology and Engineering* [online]. 2010, 49(10), 983–990. ISSN 0360-2559. Available from: doi:10.1080/03602559.2010.482074
- [12] WU, Feng, Manjusri MISRA a Amar K. MOHANTY. Challenges and new opportunities on barrier performance of biodegradable polymers for sustainable packaging. *Progress in Polymer Science* [online]. 2021, 117. ISSN 00796700. Available from: doi:10.1016/j.progpolymsci.2021.101395

- [13] AGARWAL, A., B. SHAIDA, M. RASTOGI a N. B. SINGH. Food Packaging Materials with Special Reference to Biopolymers-Properties and Applications. *Chemistry Africa* [online]. 2023, 6(1), 117–144. ISSN 2522-5758. Available from: doi:10.1007/s42250-022-00446-w
- [14] LIGHTSTEAD, A. *Packing with robots* [online]. 15. duben 2025 [vid. 2025-04-22]. Available from: <https://www.pwrpack.com/pwr-insights/food-packaging-materials/>
- [15] ALI, M. Q., N. AHMAD, M. A. AZHAR, M. S. A. MUNAIM, A. HUSSAIN a A. A. MAHDI. An overview: exploring the potential of fruit and vegetable waste and by-products in food biodegradable packaging. *Discover Food* [online]. 2024, 4(1). ISSN 2731-4286. Available from: doi:10.1007/s44187-024-00117-4
- [16] ZHAO, M., P. HAN, H. MU, S. SUN, J. DONG, J. SUN, S. LU, Q. WANG a H. JI. Food packaging films from natural polysaccharides and protein hydrogels: A comprehensive review. *Food Chemistry: X* [online]. 2025, 25. ISSN 25901575. Available from: doi:10.1016/j.fochx.2025.102174
- [17] SINGH, S., M. HABIB, E. S. RAO, Y. KUMAR, K. BASHIR, S. JAN a K. JAN. A comprehensive overview of biodegradable packaging films: Part I—sources, additives, and preparation methods. *Discover Food* [online]. 2025, 5(1), 1–20. ISSN 2731-4286. Available from: doi:10.1007/s44187-025-00303-y
- [18] AMIN, U., M. KHAN, Y. MAJEED, M. REBEZOV, M. KHAYRULLIN, E. BOBKOVA, M. A. SHARIATI, I. M. CHUNG a M. THIRUVENGADAM. Potentials of polysaccharides, lipids and proteins in biodegradable food packaging applications. *International Journal of Biological Macromolecules* [online]. 2021, 183, 2184–2198. ISSN 01418130. Available from: doi:10.1016/j.ijbiomac.2021.05.182
- [19] ZHAO, M., P. HAN, H. MU, S. SUN, J. DONG, J. SUN, S. LU, Q. WANG a H. JI. Food packaging films from natural polysaccharides and protein hydrogels: A comprehensive review. *Food Chemistry: X* [online]. 2025, 25. ISSN 25901575. Available from: doi:10.1016/j.fochx.2025.102174
- [20] GÓMEZ-ESTACA, J., M.C. GÓMEZ-GUILLÉN, F. FERNÁNDEZ-MARTÍN a P. MONTERO. Effects of gelatin origin, bovine-hide and tuna-skin, on the properties of compound gelatin–chitosan films. *Food Hydrocolloids* [online]. 2011, 25(6), 1461–1469. ISSN 0268005X. Available from: doi:10.1016/j.foodhyd.2011.01.007
- [21] AVENA-BUSTILLOS, R.J., B. CHIOU, C.W. OLSEN, P.J. BECHTEL, D.A. OLSON a T.H. MCHUGH. Gelation, oxygen permeability, and mechanical properties of mammalian and fish gelatin films. *Journal of Food Science* [online]. 2011, 76(7). ISSN 0022-1147. Available from: doi:10.1111/j.1750-3841.2011.02312.x
- [22] CHENG, S. H. a N. M. SARBON. Chicken gelatin films: rheological properties of film forming solutions and film characterisation as influenced by starch incorporation. *International Food Research Journal*. 2020, 6(27).
- [23] ACHET, D. a X. W. HE. *Determination of the renaturation level in gelatin films*. 1994.
- [24] DE CARVALHO, R. A. a C. R. F. GROSSO. Properties of chemically modified gelatin films. *Brazilian Journal of Chemical Engineering* [online]. 2006, 23(1), 45–53. ISSN 0104-6632. Available from: doi:10.1590/S0104-66322006000100006
- [25] BEPPU, M.M., R.S. VIEIRA, C.G. AIMOLI a C.C. SANTANA. Crosslinking of chitosan membranes using glutaraldehyde: Effect on ion permeability and water absorption. *Journal of Membrane Science* [online]. 2007, 301(1–2), 126–130. ISSN 03767388. Available from: doi:10.1016/j.memsci.2007.06.015
- [26] CATALINA, M., G. E. ATTENBURROW, J. COT, A. D. COVINGTON a A. P. M. ANTUNES. Influence of crosslinkers and crosslinking method on the properties of

- gelatin films extracted from leather solid waste. *Journal of Applied Polymer Science* [online]. 2011, 119(4), 2105–2111. ISSN 0021-8995. Available from: doi:10.1002/app.32932
- [27] YU, Zhe, Jingmin WU, Ting ZHANG, Chi CHEN, Yun MA, Hongxiang LIU, Bor Sen CHIOU, Fei LIU a Jian LI. Improving the crosslinking of collagen casing and glutaraldehyde by facilitating the formation of conjugate structure via pH. *Collagen and leather* [online]. 2024, 6(1). ISSN 27316998. Available from: doi:10.1186/s42825-024-00172-8
- [28] SANTOS, Jaqueline P., Vanessa M. ESQUERDO, Catarina M. MOURA a Luiz A.A. PINTO. Crosslinking agents effect on gelatins from carp and tilapia skins and in their biopolymeric films. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* [online]. 2018, 539, 184–191. ISSN 09277757. Available from: doi:10.1016/j.colsurfa.2017.12.018
- [29] DE OLIVEIRA, R., C. VINICIUS TONICOLLI RIGUETO, M. ROSSETO, D. DAL CASTEL KREIN, K. SOUSA GOMES, R. APARECIDA LOSS a A. DETTMER. Influence of enzymatic crosslinking on Tambaqui (*Colossoma macropomum*) skin gelatin film properties. *Polymer Bulletin* [online]. 2024, 81(5), 4539–4556. ISSN 0170-0839. Available from: doi:10.1007/s00289-023-04924-0
- [30] KIM, Sungwoo, Marcel E. NIMNI, Zhi YANG a Bo HAN. Chitosan/gelatin-based films crosslinked by proanthocyanidin. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* [online]. 2005, 75B(2), 442–450. ISSN 1552-4973. Available from: doi:10.1002/jbm.b.30324
- [31] CAO, N., X. YANG a Y. FU. Effects of various plasticizers on mechanical and water vapor barrier properties of gelatin films. *Food Hydrocolloids* [online]. 2009, 23(3), 729–735. ISSN 0268005X. Available from: doi:10.1016/j.foodhyd.2008.07.017
- [32] NOR, M. H. M., N. N. M. NAZMI a SARBON. Effects of plasticizer concentrations on functional properties of chicken skin gelatin films. *International Food Research Journal*. 2017, 24(5), 1910–1918.
- [33] ALAO, B., A. FALOWO, A. CHULAYO a V. MUCHENJE. The Potential of animal by-products in food systems: Production, prospects and challenges. *Sustainability* [online]. 2017, 9(7), 1089. ISSN 2071-1050. Available from: doi:10.3390/su9071089
- [34] DUBEN, J. *Vedlejší živočišné produkty, co to je?* [online]. 29. 7. 2008 [vid. 2025-05-02]. Available from: https://www.svsr.cz/vedlejsi_zivocisne_produkty_co_to_je/
- [35] *Federation of European deer farmers association* [online]. 2025 [seen 2025-05-01]. Available from: <https://www.fedfa.com/fedfa-members/>
- [36] DEER WORKING GROUP. Wild venison and food safety. *The management of wild deer in Scotland: deer working group report*. 2020, 15.
- [37] Gelatin market analysis - segment forecasts till 2025. not dated.
- [38] CATALINA, M., G. E. ATTENBURROW, J. COT, A. D. COVINGTON a A. P.M. ANTUNES. Influence of crosslinkers and crosslinking method on the properties of gelatin films extracted from leather solid waste. *Journal of Applied Polymer Science* [online]. 2011, 119(4), 2105–2111. ISSN 00218995. Available from: doi:10.1002/app.32932
- [39] ALAM, M. S., S. PATHANIA a A. SHARMA. Optimization of the extrusion process for development of high fibre soybean-rice ready-to-eat snacks using carrot pomace and cauliflower trimmings. *LWT* [online]. 2016, 74, 135–144. ISSN 00236438. Available from: doi:10.1016/j.lwt.2016.07.031
- [40] AGARWAL, A., B. SHAIDA, M. RASTOGI a N. B. SINGH. Food packaging materials with special reference to biopolymers-properties and applications.

- Chemistry Africa* [online]. 2023, 6(1), 117–144. ISSN 2522-5758. Available from: doi:10.1007/s42250-022-00446-w
- [41] PICKETT, J. E. Introduction to polymer weathering, stabilization, and testing. In: *Service Life Prediction of Polymers and Coatings: Enhanced Methods* [online]. B.m.: Elsevier, 2020, s. 1–18. ISBN 9780128183670. Available from: doi:10.1016/B978-0-12-818367-0.00001-1
- [42] DIN, M. I., T. GHAFAR, J. NAJEEB, Z. HUSSAIN, R. KHALID a H. ZAHID. Potential perspectives of biodegradable plastics for food packaging application-review of properties and recent developments. *Food Additives & Contaminants: Part A* [online]. 2020, 37(4), 665–680. ISSN 1944-0049. Available from: doi:10.1080/19440049.2020.1718219
- [43] NETZSCH. *Diferenciální skenovací kalorimetrie* [online]. [seen 2025-04-04]. Available from: <https://analyzing-testing.netzsch.com/cs/produkty/diferencialni-skenovaci-kalorimetr-dsc-diferencialni-termicky-analyzator-dta>
- [44] METTLER TOLEDO. *Webinar – Fundamentals of TGA analysis* [online]. [seen 2025-05-01]. Available from: https://www.mt.com/gb/en/home/library/on-demand-webinars/lab-analytical-instruments/Thermogravimetric_Analysis.html
- [45] METTLER TOLEDO. *Webinar – DMA Principles and Basic Applications* [online]. [seen 2025-05-05]. Available from: https://www.mt.com/de/en/home/library/on-demand-webinars/lab-analytical-instruments/Dynamic_Mechanical_Analysis.html
- [46] MOHAN, S., O. S. OLUWAFEMI, N. KALARIKKAL, S. THOMAS a S. P. SONGCA. Biopolymers – Application in nanoscience and nanotechnology. In: *Recent Advances in Biopolymers* [online]. B.m.: InTech, 2016. Available from: doi:10.5772/62225
- [47] SIVAKANTHAN, S., S. RAJENDRAN, A. GAMAGE, T. MADHUIJITH a S. MANI. Antioxidant and antimicrobial applications of biopolymers: A review. *Food Research International* [online]. 2020, 136. ISSN 09639969. Available from: doi:10.1016/j.foodres.2020.109327
- [48] BARANWAL, J., B. BARSE, A. FAIS, G. L. DELOGU a A. KUMAR. Biopolymer: A sustainable material for food and medical applications. *Polymers* [online]. 2022, 14(5), 983. ISSN 2073-4360. Available from: doi:10.3390/polym14050983
- [49] SCHRIEBER, R. a H. GAREIS. *Gelatine handbook: Theory and industrial practice*. 2007.
- [50] CHEN, X., X. DING, Y. HUANG, Y. ZHAO, G. CHEN, X. XU, D. XU, B. JIAO, X. ZHAO a G. LIU. Recent advances in polysaccharide-based nanocomposite films for fruit preservation: construction, applications, and challenges. *Foods* [online]. 2025, 14(6). ISSN 2304-8158. Available from: doi:10.3390/foods14061012
- [51] OPRIŞ, O., C. MORMILE, I. LUNG, A. STEGARESCU, M.-L. SORAN a A. SORAN. An overview of biopolymers for drug delivery applications. *Applied Sciences* [online]. 2024, 14(4). ISSN 2076-3417. Available from: doi:10.3390/app14041383
- [52] KALPANA MANIVANNAN, R., N. SHARMA, V. KUMAR, I. JAYARAJ, S. VIMAL a M. UMESH. A comprehensive review on natural macromolecular biopolymers for biomedical applications: Recent advancements, current challenges, and future outlooks. *Carbohydrate Polymer Technologies and Applications* [online]. 2024, 8. ISSN 26668939. Available from: doi:10.1016/j.carpta.2024.100536
- [53] MENDOZA-MUÑOZ, N., G. LEYVA-GÓMEZ, E. PIÑÓN-SEGUNDO, M. L. ZAMBRANO-ZARAGOZA, D. QUINTANAR-GUERRERO, M. L. DEL PRADO AUDELO a Z. URBÁN-MORLÁN. Trends in biopolymer science

- applied to cosmetics. *International Journal of Cosmetic Science* [online]. 2023, 45(6), 699–724. ISSN 0142-5463. Available from: doi:10.1111/ics.12880
- [54] ATLAS MATERIAL TESTING SOLUTION. *Weathering testing guidebook*. 2001.
- [55] PICKETT, J. E. Weathering of plastics. In: *Handbook of Environmental Degradation Of Materials: Third Edition* [online]. B.m.: Elsevier Inc., 2018, s. 163–184. ISBN 9780323524735. Available from: doi:10.1016/B978-0-323-52472-8.00008-3
- [56] DVOŘÁK, J. Bc. *Vedlejší produkty ze zpracování zvěřiny jako netradiční zdroj kolagenu*. B.m., 2022. Tomas Bata University in Zlín.
- [57] LEE, K. Y., J. SHIM a H. G. LEE. Mechanical properties of gellan and gelatin composite films. *Carbohydrate Polymers* [online]. 2004, 56(2), 251–254. ISSN 01448617. Available from: doi:10.1016/j.carbpol.2003.04.001
- [58] KIM, S., M. E. NIMNI, Z. YANG a B. HAN. Chitosan/gelatin-based films crosslinked by proanthocyanidin. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* [online]. 2005, 75B(2), 442–450. ISSN 1552-4973. Available from: doi:10.1002/jbm.b.30324
- [59] AHAMMED, S., F. LIU, J. WU, M. N. KHIN, W. H. YOKOYAMA a F. ZHONG. Effect of transglutaminase crosslinking on solubility property and mechanical strength of gelatin-zein composite films. *Food Hydrocolloids* [online]. 2021, 116, 106649. ISSN 0268005X. Available from: doi:10.1016/j.foodhyd.2021.106649
- [60] WANG, H., F. DING, L. MA a Y. ZHANG. Edible films from chitosan-gelatin: Physical properties and food packaging application. *Food Bioscience* [online]. 2021, 40, 100871. ISSN 22124292. Available from: doi:10.1016/j.fbio.2020.100871
- [61] NILSUWAN, K., S. BENJAKUL a T. PRODPRAN. Properties, microstructure and heat seal ability of bilayer films based on fish gelatin and emulsified gelatin films. *Food Biophysics* [online]. 2017, 12(2), 234–243. ISSN 1557-1858. Available from: doi:10.1007/s11483-017-9479-2
- [62] GIMÉNEZ, B., J. GÓMEZ-ESTACA, A. ALEMÁN, M.C. GÓMEZ-GUILLÉN a M.P. MONTERO. Physico-chemical and film forming properties of giant squid (*Dosidicus gigas*) gelatin. *Food Hydrocolloids* [online]. 2009, 23(3), 585–592. ISSN 0268005X. Available from: doi:10.1016/j.foodhyd.2008.07.003
- [63] NINAN, G., J. JOSEPH a Z. ABUBACKER. Physical, Mechanical, and Barrier Properties of Carp and Mammalian Skin Gelatin Films. *Journal of Food Science* [online]. 2010, 75(9). ISSN 0022-1147. Available from: doi:10.1111/j.1750-3841.2010.01851.x
- [64] RIVERO, S., M.A. GARCÍA a A. PINOTTI. Correlations between structural, barrier, thermal and mechanical properties of plasticized gelatin films. *Innovative Food Science & Emerging Technologies* [online]. 2010, 11(2), 369–375. ISSN 14668564. Available from: doi:10.1016/j.ifset.2009.07.005
- [65] BIGI, A., G. COJAZZI, S. PANZAVOLTA, K. RUBINI a N. ROVERI. Mechanical and thermal properties of gelatin films at different degrees of glutaraldehyde crosslinking. *Biomaterials* [online]. 2001, 22(8), 763–768. ISSN 01429612. Available from: doi:10.1016/S0142-9612(00)00236-2
- [66] CATALINA, M., G. E. ATTENBURROW, J. COT, A. D. COVINGTON a A. P. M. ANTUNES. Influence of crosslinkers and crosslinking method on the properties of gelatin films extracted from leather solid waste. *Journal of Applied Polymer Science* [online]. 2011, 119(4), 2105–2111. ISSN 0021-8995. Available from: doi:10.1002/app.32932
- [67] MING YENG, Ch., S. HUSSEINSYAH a S. SUNG TING. Chitosan-corn cob biocomposite films by crosslinking with glutaraldehyde. *BioResources*. 2013, 8(2), 2910–2923.

- [68] FAN, Hui Yin, Daniel DUQUETTE, Marie Josée DUMONT a Benjamin K. SIMPSON. Salmon skin gelatin-corn zein composite films produced via crosslinking with glutaraldehyde: Optimization using response surface methodology and characterization. *International Journal of Biological Macromolecules* [online]. 2018, 120, 263–273. ISSN 18790003. Available from: doi:10.1016/j.ijbiomac.2018.08.084
- [69] GONENC, Ilknur a Ferhunde US. Effect of glutaraldehyde crosslinking on degree of substitution, thermal, structural, and physicochemical properties of corn starch. *Starch - Stärke* [online]. 2019, 71(3–4). ISSN 0038-9056. Available from: doi:10.1002/star.201800046
- [70] IAHNKE, Aline Oliveira e Silva, Liana STOLL, Anelise Stein BELLÉ, Plinho Francisco HERTZ, Alessandro de Oliveira RIOS, Hubert RAHIER a Simone Hickmann FLÔRES. Gelatin capsule residue-based films crosslinked with the natural agent genipin. *Packaging Technology and Science* [online]. 2020, 33(1), 15–26. ISSN 0894-3214. Available from: doi:10.1002/pts.2481
- [71] ERGE, Aydın, Büşra Zeynep GÜLER a Ömer EREN. Optimization and characterization of biodegradable films from chicken gelatin crosslinked with oxidized phenolic compounds. *Food Chemistry* [online]. 2024, 438. ISSN 18737072. Available from: doi:10.1016/j.foodchem.2023.137923
- [72] TONGDEESOONTORN, W., L. J. MAUER, S. WONGRUONG, P. SRIBURI a P. RACHTANAPUN. Mechanical and physical properties of cassava starch-gelatin composite films. *International Journal of Polymeric Materials* [online]. 2012, 61(10), 778–792. ISSN 0091-4037. Available from: doi:10.1080/00914037.2011.610049
- [73] PROKOPOVÁ, A., P. MOKREJŠ, R. GÁL, J. PAVLAČKOVÁ a A. HURAJOVÁ. Characterization of poultry gelatins prepared by a biotechnological method for targeted changes at the molecular level. *International Journal of Molecular Sciences* [online]. 2024, 25(2). ISSN 1422-0067. Available from: doi:10.3390/ijms25020916
- [74] SHAMSURI, A. a R. DAIK. Utilization of an ionic liquid/urea mixture as a physical coupling agent for agarose/talc composite films. *Materials* [online]. 2013, 6(2), 682–698. ISSN 1996-1944. Available from: doi:10.3390/ma6020682
- [75] RODRIGUES, Fabiana T., Virginia C. A. MARTINS a Ana M. G. PLEPIS. Porcine skin as a source of biodegradable matrices: alkaline treatment and glutaraldehyde crosslinking. *Polímeros* [online]. 2010, 20(2), 92–97. ISSN 1678-5169. Available from: doi:10.1590/S0104-14282010005000013
- [76] GREGOROVA, A. Application of differential scanning calorimetry to the characterization of biopolymers. In: *Applications of Calorimetry in a Wide Context - Differential Scanning Calorimetry, Isothermal Titration Calorimetry and Microcalorimetry* [online]. B.m.: InTech, 2013 [seen 2025-05-05]. Available from: doi:10.5772/53822
- [77] BARNES, S. a S. COTTS. *Understanding storage and loss modulus with TA instruments* [online]. 19. 12. 2024 [seen 2025-05-05]. Available from: <https://www.tainstruments.com/understanding-storage-and-loss-modulus-with-ta-instruments/>
- [78] *Termická analýza Excellence Dynamická mechanická analýza Kompletní charakterizace materiálu Systém STAR e. nedatováno.*
- [79] *Tan delta* [online]. [seen 2025-05-05]. Available from: <https://analyzing-testing.netzsch.com/cs/know-how/glosar/tan-delta>
- [80] PERKASA, D. P., ERIZAL, D. DARMAWAN a A. RASYID. Effect of gamma irradiation on mechanical and thermal properties of fish gelatin film isolated from Lates calcarifer scales. *Indonesian Journal of Chemistry* [online]. 2013, 13(1), 28–35. ISSN 14119420. Available from: doi:10.22146/ijc.21322

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LIST OF ABBREVIATIONS

0h_Gly	un-crosslinked non-degraded sample
GA_0h	crosslinked non-degraded sample
GA_100h	crosslinked sample degraded for 100 h under window-glass filter
GA_200h	crosslinked sample degraded for 200 h under window-glass filter
GA_300h	crosslinked sample degraded for 300 h under window-glass filter
SL_100h	crosslinked sample degraded for 100 h under store-light filter
SL_300h	crosslinked sample degraded for 300 h under store-light filter
GW filter	glass-window filter
SL filter	store-light filter
T _g	glass transition temperature
T _m	melting point temperature
GA	glutaraldehyde
YI	yellowness index
DSC	differential scanning calorimetry
DMA	dynamic mechanical analysis
FTIR	Fourier transform infrared spectroscopy
TGA	thermogravimetric analysis
SEM	scanning electron microscope

APPENDICES

Appendix P I: Graph of tensile strength measured for evaluation of crosslinking dependence on time

APPENDIX P I: GRAPH OF TENSILE STRENGTH MEASURED FOR EVALUATION OF CROSSLINKING DEPENDENCE ON TIME

Graph of tensile strength measured for evaluation of crosslinking dependence on time. Samples: X – un-crosslinked sample, 0,03% - sample crosslinked with 0,03 % GA, UL 0,03 % - sample crosslinked with 0,03 % GA with possibility of a subsequent crosslinking during time period, the same sense of sample names applies for the rest of the measured samples.

