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Highlights

Gelatin barrier properties underlines variability of the results and units in literature.

Literature data for the gelatin barrier properties was normalized.

Ceramic and infrared detecting methods are suitable for gelatin WVP measurements.

For infrared detecting method optimal conditions are required for further studies.

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Review

Gelatin films: study review of barrier properties and implications for future studies employing biopolymer films

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Abstract

Production of conventional packaging materials is now recognised as having had a major impact on world pollution. Edible/Biodegradable/Compostable (EBC) films may offer sustainable alternatives to some conventionally-used packaging materials. One of the largest protein groups available for EBC materials is bovine gelatin. Knowledge and control of gelatin barrier properties is essential if it has any potential of becoming an industrial packaging material. Review of the relevant literature demonstrated that data for gelatin barrier properties generated was generally incomparable owing to a lack of experimental standardisation. Some standard approaches are adopted for further study, particularly, to reach a point where recommendations can be made about industrial use of gelatin as a packaging material.

This review investigated barrier properties of bovine-derived gelatin films and factors affecting them for potential future industrial application. Bovine gelatin barrier properties were normalized to the same units and were dependent on film thickness, production methods employed, film composition, relative humidity, plasticizer content and nature, gelatin source and testing methods used. Literature comparison for barrier properties underlined high variability in results. It is suggested, and highly recommended that future studies carried out by researchers investigating EBC films should employ the use of standard units to express

water vapor permeability (WVP) and oxygen permeability (OP) values as *g x mm (or µm)/m^2 d atm (or kPa)* and *cm^3 mm (or µm)/m^2 d atm (or kPa*), respectively. Further research is necessary to compare results under controlled test conditions.

1. Global environmental threats of packaging waste

Climate change, over-exploitation of resources and environmental pollution are some of the major issues facing humanity this century (Plastic – the Facts, 2017). Among other industries, the packaging industry has come under the spotlight in recent times as more focus has been brought to bear on pollution of the oceans by non-biodegradable forms of packaging. As a consequence of this and the pressure to address the growing volumes of packaging waste generated by societies all over the world, efforts are being made to look at why and how we use packaging around our products, with a renewed focus on effectively reducing, reusing, recycling and recovering (as a last resort) waste packaging materials, with particular focus on recycling. The recycling focus around present and future usage of both conventional packaging and novel packaging materials is now guided by both the Bio Economy and the Circular Economy measures. There are different types of biopolymers which can be considered as bioplastics. There are edible films, which usually consist of protein (as for example gelatin) or polysaccharide (as for example alginate) and which can be consumed within the packed food product. The other example of bioplastics is compostable plastics which can be divided to different groups by its biodegradation properties according to different composability standard (at different temperature and relative humidity, solubility) as such as PLA (polylactide), cellulose, starch based composites and others which can be natural sourced or synthetic polymers containing usually ether OH-groups which can be subjected to biodegradation. It has to be added that for biopolymers it is not possible to add those materials which have the same structure as oil sourced and which synthons have biological origin as such as for example bio-polyethylene terephthalate (bio-PET, partly made from sugar cane) because its structure is identical as for conventional PET and thus it is not compostable or a bioplastic.

On Fig. 1 schematically represented paradigm for bio-plastics shift from a linear to a circular economy. In circular economy wastes will be a resource.

Figure 1. Bioplastic circular economy conception (adopted from European bioplastics, 2016).

Consequently, there is growing interest in the adoption of packaging materials and formats, based on conventional materials like plastics, but modified in a manner where the whole packaging system is comprised of a single material for ease of recycling, but which should address all of the fundamental rules required to ensure that it addresses containment, protection, preservation, information supply, convenience, legal requirements, in addition to environmental requirements, all in an economical manner.

Another approach builds on a vast wealth of knowledge that has accumulated over the past 40 years on research pertaining to the use of bioplastics from natural sources in the manufacture of packaging materials. In the vast majority of cases, these materials have been hydrocolloid in nature and therefore, based on protein and/or polysaccharide systems. From the vast body of research carried out in this area, it can be concluded that bio-polymer materials have a great capacity to form films with great structural integrity and strength upon forming, have good to excellent optical qualities, some of them have good gas barrier properties and can be cast or extruded as per the limited number of trials undertaken to date. However, it is also understood that such unaltered films are hydrophilic, especially edible films, and have a propensity to absorb water, thereby undermining all of the aforementioned properties over time, especially when employed to wrap semi-moist or moist products or when placed within environments of high humidity. Consequently, research needs to be directed at solving this particular issue, as well as standardising the fundamental properties associated with such materials, which is difficult owing to the many factors which can play a role in influencing the final properties of the resulting films.

While numerous biopolymers have been examined for their ability to form packaging films, one of the most studied to date has been bovine gelatine.

The objective of this study was to review barrier properties of different bovine gelatin films depending on its content and control method used, and to normalize and standardise this data to common units in order to make them comparable to each other and to make this applicable for future control methods of compostable films barrier properties.

2. Gelatin film production

Edible packaging is produced primarily by two main approaches: dry and wet methods (Nur Hanani et al., 2012). The wet method consists of employing film-forming compounds solubilized or dispersed in pure water (e.g. gelatin), acidified water (e.g. chitosan) or water mixed with a co-solvent (e.g. ethanol for wheat gluten or corn zein), which is then poured

(called casting) or alternatively, sprayed on to a surface in order to create a film (or sheets depending on the thickness) or to produce a coating.

Conversely, the dry method presents three ways to melt the edible base. These are: hot pressing, compression moulding and extrusion. The dry method is solvent-free, has a small processing footprint, can produce small volumes and is faster than wet method manufacture, and can be easily submitted to scale up. Extrusion is the primary process used in the manufacture of plastics, and it has been shown to be suitable for the manufacture of EBC films (Nur Hanani et al., 2012, Kerry & Tyuftin, 2017). For instance, Nur Hanani et al., (2012) used extrusion to produce bovine-derived gelatin films and they asserted that the extrusion process is suitable for the manufacture of EBC films rather than having to use the traditional casting method. The authors pointed to the fact that this was an important finding as it demonstrated to the plastics industry that biopolymeric materials could be extruded successfully. Guerrero et al., (2010) compared three production methods for biopolymer-based films: compression, casting and freeze-drying coupled with compression (Fig. 2).

Figure 2. EBC films production scheme (adopted from Guerrero et al., 2010).

The authors reported that the film created by the compression method showed better mechanical properties compared to the other test methods. To date, however, the casting method remains the most common way to produce EBC films at laboratory level (Sobral et al., 2001; Cao, Yang & Fu, 2009; Clarke et al., 2016; Carvalho & Grosso, 2004).

In order to obtain functional films, it is necessary to add plasticizers. The IUPAC definition for plasticizers is "*a substance or material incorporated in a material (usually a plastic or elastomer) to increase its flexibility*, *workability or distensibility*" (Vieira et al., 2011). Plasticizers enhance polymer-chain-flexibility and resistance to fracture thereby, reducing hardness, brittleness, tension of deformation, density and viscosity. Plasticizers increase the intermolecular space, thereby decreasing intermolecular forces. The plasticizers that are the most commonly used in EBC films are: glycerol, sorbitol, ethylene glycol, vegetables oils, lecithin waxes, mono, di and oligosaccharides and amino acids (Nur Hanani et al., 2013; Vieira et al., 2011), but others like propylene glycol, sucrose, fatty acids and monoglycerides also exist. Small and hydrophilic molecules with more than one polar group, but which are not too close to each other show great capacity to serve as plasticizes.

The type and quantity of plasticizer used in film forming solutions affect film formation and the resulting properties of the manufactured film. It has to be added that treatment of film-

forming solution with vacuum and/or ultrasound before pouring and drying can effect films' mechanical and barrier properties, because these treatments can provoke loose of low molecular weight plasticizer (e.g. glycerol) and water, as well.

3. Gelatin films structure and mechanical properties

According to Wang et al. (2007), the use of gelatin between 4 and 8% in water-based solutions produces films which possess excellent physical properties, such as; tensile strength, elongation at break, puncture strength, tear strength, acid resistance, alkali resistance, relatively low oxygen permeability and oil permeability. The partial hydrolysis of collagen allows for the production of gelatin.

Collagen is derived from animal sources and is typically found as a component of connective tissue in larger animals. Collagen is a fibrous protein formed by triple helix molecules stabilized by hydrogen and hydrophobic bonds. The physical organization of collagen is one where it appears to be tube- or bar-shaped and this structure is permitted by the presence of many glycine–proline–hydoxyproline sequences which characterizes the primary structure of collagen. The addition of more bar-like molecules forms a fibre which is held in structure by covalent bonding (Nur Hanani, Roos & Kerry, 2014).

Gelatin production results from the denaturation of collagen proteins which causes the destruction of secondary and tertiary structure of collagen and consequently, the loss of bar-shape organization and most of the helical structures. The final quality of the gelatin produced is dependent upon a number of factors; the initial collagen used, pretreatment steps employed (acid-based (type A gelatin) or alkaline-based (type B gelatin)), temperatures employed, pH fluctuations and extraction time. Gelatin with higher gel strength is obtained when more α chains are present within the gelatin and this is only possible by strictly controlling the parameters discussed above. Consequently, gelatin (Fig. 3) can be defined as a mixture of α chain, β chain and single and double unfolded chains. Gelatin can be produced as granulate or powder and is water soluble, tasteless and odourless.

Figure 3. Representative gelatin structure (adopted from Ramos et al., 2016)

Bovine, pig, poultry and fish (cod, haddock, squid…) are well-suitable and studied sources of gelatin that can be used to form films or coatings by both employing casting and extrusion

methods. There is an increasing interest in seafood- and poultry-based sources of gelatin because of previous concerns about BSE (Bovine Spongiform Encephalopathy), but primarily as these forms find a broader religious acceptance in food usage and because they are relatively untapped supply streams of gelatin (Sarbon, Badii & Howell, 2013; Nazmi, Isa & Sarbon, 2007). Different studies assert that fish and poultry can be efficient alternatives to bovine- and porcine-derived gelatin. For example, Sarbon, Badii & Howell (2013) showed that chicken and bovine gelatin amino acid compositions were very similar, especially in relation to the content of amino acid (proline and hydroxyproline) (Table 1), glycine and alanine, all of which can improve the gel modulus, strength and stability (owing to the higher gelling and melting temperatures). Conversely, fish gelatin is low amino acid content, mainly by an absence of proline and hydroxyproline as shown in Table 1, thereby producing a low gel modulus and weaker gel network (Haug, Draget & Smidsrod, 2004).

Table 1. Amino acid comparisons of chicken, bovine and fish (cold-water fish) gelatin (adopted from Norland Prod. Inc., 1999–2001 as stated in Haug, Draget & Smidsrod, 2004; Sarbon, Badii & Howell, 2013).

Amino acid	Chicken gelatin (%)	Bovine gelatin (%)	Fish gelatin (%)[*]
Alanine	10.1 ± 0.02	8.4 ± 0.10	11.2
Glycine	33.7 ± 0.02	37.1 ± 0.11	34.7
Hydroxyproline	12.1 ± 0.02	10.7 ± 0.11	6,0
Proline	13.4 ± 0.10	12.7 ± 0.14	9.6

[*]Tolerance level was not represented.

Edible films produced from gelatin sources have already been tested for commercial application and Ramos et al., (2016), Clarke et al., (2016, 2017), Reid et al., (2017), Murrieta-Martínez et al., 2018) have reported on some interesting examples:

- gelatin blended with chitosan in order to maintain the red colour of beef steaks,
- gelatin and thyme essential oil to reduce the bacterial growth on chicken tenderloin,
- gelatin with carboxymethylcellulose and potassium sorbate to improve the shelf-life of bacon,
- gelatin and chitosan coating to reduce the microbial spoilage and lipid oxidation,
- gelatin,starch and glycerol films to reduce weight loss in Red Crimson grapes,
- gelatin coated films with incorporated natural sourced antimicrobials to prolong shelf life of beef steaks and to control blown pack spoilage.

Bovine gelatin remains one of the most commonly used sources of gelatine and among its many other uses, it can be employed to produce excellent protein-based, edible films. It exists in large quantities as by-product from the slaughter of cattle. As reported in numerous studies previously (Ciannamea et al., 2018; De Carvalho & Grosso, 2006; Kanmani & Rhim, 2014; Figueroa-Lopez et al., 2018; Sobral et al., 2001; Wang et al., 2015), bovine gelatin-based, edible films present good packaging related properties. The most important properties described for packaging material are: barrier properties, thermal behaviour, mechanical properties, transparency, odour emission, oil and gas resistance. Mechanical properties of gelatin films are dependent upon the plasticizer content used, gelatin origin, environment and other parameters, particularly of a processing nature.

4. Gas barrier properties of gelatin films as reported in experimental trials

4.1 Water vapor barrier properties

The water vapor permeability of packaging materials is a critical parameter to understand in terms of shelf-life stability of food products as water vapor penetration across packaging boundaries affects microbial growth, product texture and functionality, overall chemical stability and pack fogging (if the vapor condenses) (Kerry & Tyuftin, 2018).

It is understood that gelatin films naturally possess a high water permeability (Nur Hanani, , Roos & Kerry, (2014). For this reason, numerous attempts have been made to increase gelatin barrier properties and study their WVP. As for common oil-based barrier polymer films, knowledge of barrier properties for gelatin-based EBC films are essential in order to satisfy packaging requirements for the food product in question. For the reasons described previously, the water vapor permeability of EBC packaging materials, including gelatin, must be clearly known and tightly controlled during film production (Kerry & Tyuftin, 2018).

Water vapor permeability is the flux of molecules through a material normalized to a pressure gradient and can be expressed as WVP using the following Eq. 1 (Basics of Barriers – II, 2007).

Equation 1: *Permeation = (volume or mass of a gas x material thickness) / (test area of a film x test time x pressure)*

The other parameter related to water permeation is water vapor transmission rate (WVTR) which can be expressed by Eq. 2 (Basics of Barriers – II, (2007):

Equation 2: *Transmission Rate = (volume or mass of a gas) / (test area of a film x test time)*

WVP is more commonly used in the scientific literature, whereas WVTR can be usually found in technical data and specification sheets for packaging materials utilised in industrial settings. Scientific literature published to date for bovine gelatin presents data which has been published using a diverse experimental range. There is possible to convert units one to another (McHugh & Krochta, 1994), but in most cases it causes difficulties and, consequently, cannot be easily compared to each other. In order to define and compare water permeability properties for all relevant gelatin films reported in the scientific literature WVP data were recalculated and normalized to similar units and expressed as; $g\ mm/m^2\ d\ atm$ (Annex 1, supporting material). The calculations were checked three-times and were additionally checked on a WVP permeability on-line calculator developed by Abbot, (2019). Unfortunately, in some cases, clear units were not presented in research papers and consequently, we had to correspond with the relevant authors of these scientific publications directly, in order to obtain the required information. Therefore, Table 2 presents the normalised WVP values for all bovine–derived gelatin films manufactured and reported in scientific papers stemming from extensive review of the scientific literature and where the lack of WVP properties for gelatin films without employing additives was observed.

Table 2. Normalized WVP property comparison of gelatin films produced employing different; biopolymer sources, plasticizer type and concentration, thickness values, relative humidities, temperatures and test methods.

Bovine gelatin content in film	Plasticizer type and its content	Thickness, µm	WVP *, *g mm/m² d atm*	Test relative humidity (RH)	Temperature	ASTM	Reference
Gelatin 10%	Glycerol 4.5% to gelatin	80.0 ± 4.0	481.5 ± 7.30	50%	25°C	E96	Carvalho et al., (2008)
Gelatin 1%	Sorbitol 15% to gelatin	43 ± 9	413.406	100%	22°C	E96	Sobral et al., (2001)
	Sorbitol 45% to gelatin		705.222				
	Sorbitol 65% to gelatin		924.084				
Gelatin 1,5%	Sorbitol, 25% to gelatin	72 ± 2	1310 ± 133	55%	25°C	E96	Thomazine et al., (2005)
Gelatin 12%	Malic acid 20% to gelatin	21.0 ± 0.6	0.5 ± 0.05	50%	25°C	E96	Cao et al., (2009)
	Polyethylene glycole 20% to gelatin	23.2 ± 0.7	3.3 ± 0.15				
	Sorbitol 20% to gelatin	23.7 ± 0.4	0.7 ± 0.05				
	Ethylene glycol 20% to gelatin	22.4 ± 0.7	5.6 ± 0.05				
	Diethylene glycol 20% to gelatin	23.0 ± 0.8	4.7 ± 0.05				
	Triethylene glycol 20% to gelatin	22.5 ± 0.5	4.6 ± 0.07				
	Ethanolamine 20% to gelatin	23.2 ± 0.7	3.0 ± 0.02				
	Diethanolamine 20% to gelatin	21.5 ± 0.5	2.4 ± 0.05				
	Triethanolamine 20% to gelatin	23.4 ± 0.5	3.1 ± 0.05				
Gelatin	Glycerol 0.25% water	56.5 ± 7.43	10993.8 ± 531.96		23 ± 2°C	E96	Nur Hanani et al., (2012)
		43.0 ± 9.50	7802.0 ± 531.96				
		40.8 ± 5.36	6383.5 ± 106.39	50%			
		50.3 ± 3.79	9023.0 ± 106.39				
		46.9 ± 4.15	9976.5 ± 623.15				
		55.0 ± 4.06	7014.7 ± 373.89				
Gelatin	Glycerol 0.2% (to gelatin)	20.7 ± 3.2	4063.1 ± 137.80	50%	23 ± 2°C	E96	Nur Hanani et al., (2013)
	Glycerol 0.5%	23.3 ± 2.2	4352.9 ± 15.20				
	Glycerol 0.8%	21.1 ± 6.6	3959.7 ± 45.60				
	Glycerol 1.1%	23.4 ± 2.5	4869.7 ± 122.60				
Gelatin 5%	Glycerol 33% to gelatin	57 ± 6	37.6 ± 3.42	50%	23 ± 2°C	E96	Clarke et al., (2016)
Gelatin 3.3%	Sorbitol 2% to solution	N/A	26438.5 ± 1225.63	50%	25°C	E96	Kanmani & Rhim, (2014)
Gelatin	Glycerol 40% to gelatin	125 ± 25	2.8 ± 0.33	35%	23°C	F1249	Ciannamea et al., (2018)**
			14.7 ± 1.18	50%			
			77.8 ± 0.48	70%			
			196.1 ± 0.89	90%			
Gelatin	Glycerol 30% to gelatin	58 ± 4	2188.62	65%	25°C	E96	Martucci & Ruseckaite, (2010)***
Gelatin (**only fish gelatin**) 20%		None	1630 ± 300	50%	25°C	F1249	Yi et al., (2006)
Gelatin 1,3%	Glycerol, 55% to gelatin	98 ± 2	1610 ± 261	55%	25°C	E96	Thomazine et al., (2005)
Gelatin 6.7%	Glycerol, 0% to gelatin	50	4860	75 ± 3%	25°C	E96	Avena-Bustillos, (2006)
(Casting method)							

*Initial values for WVP calculations are given in Annex 1 of supporting materials (the average film thickness value was taken for the calculations if it was necessary).

**In the paper results are given in *g mm/m² d* in Table and *g mil/m² d* in the text. From the communication with the authors the right values must be read in their Table as *g mil/m² d atm* which were normalized as given in Annex 1.

***Authors stated film thickness of 0,58 ± 0,04 µm, but it should be a mistake due to it too thin for studies reported and should be read as in mm.

From assessment of the data presented in Table 2, it is possible to make an attempt to understand why we have such a large variability in the results. Variability stemmed from the wide variety of film production methods employed, gelatin content and gelatin source used, test conditions and standard methods implemented. High WVP values are confirmed in almost all gelatin film studies when compared to those values reported for conventional oil-based plastic films with high water vapor barrier, such as; polyethylene terephthalate (PET) (WVP of 0.01 - 0.02 *g mm/m² d atm*) or oriented polypropylene (OPP) (WVP of 0.006 – 0.007 *g mm/m² d atm*), common materials used in food packaging.

Among the studies presented in Table 2, two WVP studies which were comparable for gelatin films were those presented by Ciannamea et al., (2018) and Clarke et al., (2016), who reported WVP values for gelatin films of 14.7 ± 1.18 and 37.6 ± 3.42 *g mm/m^2 d atm*, respectively. In spite of comparability, similarity of test conditions and approximately similar glycerol content used in film formulations, these results were obtained through the use of two different methods of analysis, ASTM F1249 (Infrared detecting method, Mocon Permatran equipment) and E96 (cup method which is a gravimetric technique using a desiccant in a cup with the sample sealed over the top and placed in a humidity cabinet at a set temperature/humidity and the weight gain measured over time), and employing two different film production methods (dry- and wet-methods). Another difference between both studies resides in the film thickness generated for experimental samples; which was about 70 µm thicker in the Ciannamea et al., (2018) study.

The lowest WVP values (in other words, the highest barrier properties) for gelatin films were reported by Cao, Yang, & Fu (2009). In Cao et al., (2009), WVP values for gelatin films were lower when malic acid and sorbitol were added as plasticizers to film forming solutions. For other plasticizers used, WVP values were higher. This demonstrates the importance of plasticiser selection when producing EBC films. It is interesting that WVP data for Cao, Yang, & Fu (2009) reported for gelatin films with sorbitol as a plasticizer (gelatin 12%, sorbitol 20%) of 0.7 ± 0.05 g *mm/m^2 d atm @ 50% rh* is far different that the data obtained by Thomazine et al., (2005) who reported WVP value of 1310 ± 133 *g mm/m^2 d atm @ 55% rh* with quite similar sorbitol content of 25%. This difference could be explaned by the different film thickness used (about 3 times thicker for Thomazine et al., (2005) and different gelatin content, but the WVP data shows too high difference for several orders. Data of Thomazine et al., (2005) can be compared to data reported by Sobral et al., (2001) where film content is more similar (gelatin 1%, sorbitol 15%) and WVP difference is not as high as for Cao, Yang, & Fu (2009).

The WVP values determined for gelatin films by Carvalho, Grosso & Sobral (2008) were analysed using the gravimetric method (E-96) (films were produced by casting method as in Clarke et al., (2016), but presenting a higher WVP value of 481.5 ± 7.3 *g mm/m^2 d atm @ 50% rh*, than that reported by Clarke et al., (2016), 37.6 ± 3.42 *g mm/m^2 d atm @ 50% rh* and Ciannamea et al., (2018), 14.72 *g mm/m^2 d atm @ 50% rh*. The differences determined between test samples can be attributed to variation in experimental film thicknesses and gelatin content. Another reason for WVP differences reported by these authors for gelatin films studied can be attributed to the glycerol concentration used in film forming solutions for

316 the manufacture of the respective gelatin films employed. Carvalho, Grosso & Sobral (2008)
317 employed 4.5% glycerol against 40% employed by Ciannamea et al., (2018). However, much
318 higher WVP values (up to 4869.7 *g mm/m^2 d atm*) were reported by Nur Hanani et al., (2013)
319 who used a glycerol content closer, but lower, to that employed by Carvalho, Grosso & Sobral
320 (2008) but obtained a 10-fold difference in WVP values compared to that reported by
321 Carvalho, Grosso & Sobral (2008). The values presented by these authors contradict the
322 theory of Bourlieu et al., (2009) (who reported increases in WVP values with increasing
323 hydrophilic film thickness), as film thickness employed in the Nur Hanani et al., (2013) study
324 was approximately four-times thinner than that employed in the study by Carvalho et al.,
325 (2008) and consequently, should have had lower WVP values. The reason that such
326 dramatically different WVP values existed between the studies of Carvalho et al., (2008) and
327 Nur Hanani et al., (2013) may also be due to the differences in the methodology employed to
328 measure WVP in the respective studies. In fact it can be different content of plasticiser used
329 of two authors, but WVP test methods can also have effect. The cup testing method (E96),
330 employed by both authors, while affordable and easy to use, is sensitive to operator variance
331 and has a 20% variability associated with the test, in lab to lab assessments, as reported by
332 Mocon (Mocon, 2014).
333 The lower amount of plasticizer used in Nur Hanani et al., (2013) (0.25% glycerol to gelatin)
334 should have, in fact, improved barrier properties, which it clearly did not, employing a 0.8%
335 glycerol content. According to Sobral et al., (2001) plasticizer usage decreases network
336 density. Hygroscopic plasticizers, such as sorbitol and glycerol, increase the water content of
337 films, thereby causing mobility of molecules and enhancement of water permeation. The
338 results of Nur Hanani et al., (2013) can be compared to Avena-Bustillos (2006), who reported
339 WVP of 4860 *g mm/m^2 d atm @ 75% rh*.
340 Yi et al., (2006) produced gelatin films using fish-derived gelatin and analysed the WVP
341 properties of these films using the F1249 standard MOCON Permatran 3/31 method, a method
342 similarly used by Ciannamea et al., (2018) in their studies. Interestingly, the values generated
343 by Yi et al., (2006) were higher than those generated by Ciannamea et al., (2016) (at 50% RH
344 respectively), yet similar to those reported by Nur Hanani et al., (2012, 2013) (same
345 magnitude) for beef-derived gelatin. It is important to point out that in drawing the comparison
346 between studies conducted by Nur Hanani et al., (2012, 2013) and that of Yi et al., (2006), it
347 is being done in the absence of vital information from the Yi et al., (2006) publication, namely
348 film thickness values and all plasticiser information. The highest WVP value presented for
349 gelatin film was reported by Kanmani & Rhim, (2014). The film composition presented in

their study is comparable to that reported by Sobral et al., (2001) who utilized sorbitol as the plastisizer source but employed at a much lower concentration of 2% (Kanmani & Rim, 2014), against a level of 15% employed by Sobral et al., (2001). No information was presented by Kanmani & Rhim., (2014) with respect to film thicknesses employed, but even with this omission, it is difficult to explain why such high WVP values were generated when compared to all other relevant studies. In more details plasticizer type and/or amount effect on physical properties of gelatin-based films including WVP was reported in Bergo et al., (2013); Andreuccetti et al., (2009); Jongjareonrak et al., (2006); Thomazine et al., (2005); Vanin et al., (2005).

In short, Table 2 clearly indicates the variations recorded for WVP values determined for gelatin films manufactured in different ways and the inconsistencies that exist within the presented data and between different studies. Table 2 also clearly highlights the multiple factors at play in generating this data where some research papers report unrealistic WVP values. Factors impact upon the result variations presented in relevant studies, such as; gelatin and ingredient sources, film manufacturing method, glycerol type and level used, film thicknesses employed, relative humidity levels during testing, WVP testing methodologies utilised and in some cases even errors and expression of WVP units.

4.2 Method selection for WVP studies of EBC films

From analysis of data generated from numerous scientific studies, it is fair to say that there is a deficit in knowledge and understanding with respect to issues pertaining to both the generation and analysis of data generated from permeability studies assessing either WVP or gas barrier properties of packaging or potential packaging materials. In an attempt to inform future studies investigating any physical properties associated with gelatin, like that of WVP, or indeed any form of biopolymer-based film, a number of recommendations can be made which might aid in the generation of more meaningful information within this research area and help make data generated in studies more comparable, and therefore, more scientifically useful. To begin and remaining with WVP values, data generated from studies should be expressed in standard scientific units such as *g x mm (or µm)/m^2 d atm (or kPa)* which can then be easily converted and compared with data generated in the scientific literature and can be used by an industrial personal for EBC films quality control. For early studies conducted with WVP, the method of choice was typically the E96 method which is commonly referred to as

the cup method as previously described. It consists of a cup filled with a salt saturated solution that creates a specific RH value or, with a desiccant creating an environment with 0% RH (Fig. 4).

Figure 4. Gravimetric ASTM E96 cup test method (adopted from Delgado et al., 2018).

The cup is then placed inside a climate chamber with controlled humidity and temperature and weighted periodically until the weight reaches equilibrium. ASTM E96 remains a suitable method for testing poor to medium barrier film materials. The disadvantage of a cup method is that it has a low accuracy and typically requires a highly qualified operator to operate the test equipment in order to minimise testing error.

Recently, Versaperm LLC, UK developed a more accurate instrument called the Versaperm MKVI (Fig. 5) for WVP measurement and operates in accordance with BS ISO 15016-1 (Versaperm brochure, 2013). With this method the test film sample is clamped within a chamber with moist air introduced on one side of the film and a high accuracy ceramic-based RH sensor on the other.

F**igure 5**. Versaperm equipment for hydrophilic films testing. RH can be controlled from 0 to 100% (Versaperm brochure, 2013).

The sensor side of the film is typically the dryer side and is flushed with dry air or N_2 down to the target low RH anticipated and which does not need to be zero like that required for the E96 method. At the target RH, the dry air or gas flow is terminated, and the moisture passing through the sample is allowed to increase on the sensor side of the film. When the RH level stabilises, the time-based gradient of the RH increase is then calculated. This is proportional to the permeation rate. The process is repeated until results are reproducible, essentially showing that the test film has reached a stable equilibrium against the testing environment created during the experimental test. This method is recommended for WVP assessment of EBC films and is significantly faster and more accurate than the gravimetric method (E96). Additionally, the equipment is computer-controlled and consequently, this dispenses with the necessity of having to employ a highly experienced operator to run the equipment. The ceramic-based sensor method was specially developed for films possessing low WVP values. In the case of gelatin-

based films, which are very sensitive to RH and have extremely high VWP values, this method should be used over the E96 test method.

The other method used widely in the packaging industry is the ASTM F1249 method which can be used on a MOCON Permatran module which has the highest performance and accuracy of all WVP test methods. ASTM F-1249, commonly referred to as the infrared detecting method, is that followed by MOCON (USA) in the development and application of all of their analytical test equipment for WVP measurement and consequently, is considered to be the gold standard for WVP assessment within the packaging industry. The MOCON PERMATRAN 3/33 module is primarily used to measure the WVP properties of oil-based packaging materials possessing high-to-medium barrier properties. The MOCON cell scheme present in the PERMATRAN 3/33 module is shown in Fig. 6.

Figure 6. MOCON Permatran 3/33 module scheme and its view (Stevens, 2014).

The film divides the cell as a membrane across the two chambers; the first chamber presents pure N_2 gas which passes through a water well, thereby reaching a predetermined humidity (35–90% RH or 100% RH (if a wet sponge is used). This creates a humidity gradient. In the second chamber, dry N_2 gas is introduced and flows through this half of the cell. The water vapor carried by the gas permeates through the film and this is carried by the dry N_2 gas flow to the infrared detector. Permeated water molecules are identified by an infrared sensor (75-300 μm wavelength) which compares the amplitude of electrical signal generated from a calibration film placed in the machine and the IR signal produced from the test film sample, thereby determining the film's transmission rate. The ASTM F1249 method presents higher precision, repeatability, accuracy and sensitivity for high barrier films compared to the ASTM E96 method which is heavily influenced by operator knowledge and implementation (MOCON white paper, (2017).

As can be observed in Table 2, this test method has been utilised for gelatin films already. However, this method was originally designed for conventional plastic and plastic-based laminates and not for EBC biopolymer films. MOCON Permatran module has several handles (valves) with the help of which different gas flow rates for four cells and RH can be varied and for each film those values have to be adjusted for the best results.

Thus, owing to the necessity to adjust test cells and gas flow rates for EBC films, further research is required in order to optimise this test method for such films. Additionally, research

is also required to test and contrast various WVP methods using biopolymer materials, like gelatin, which are manufactured in a state of control so as to assess their ability to produce meaningful and repeatable data.

4.3 Oxygen barrier properties of gelatin films as reported in experimental trials

Like moisture, the presence of oxygen in food packs, for the most part, negatively impacts on the quality and shelf-life of food products. With the exception of living and respiring foods, like non-processed horticultural produce, most food products are oxygen-sensitive. For example, the presence of oxygen in food packs can lead to various oxidation reactions which can result in off-flavour production, loss of nutritional value and colour changes (Kerry & Tyuftin, 2017), in addition to promoting the growth of aerobic spoilage microbes. Gelatin-based films typically present medium to high oxygen barrier properties and are very comparable to some synthetic polymers, typically possessing lower OTR values than these conventionally used plastic-forming polymers.

Similar to WVP, oxygen permeability (OP) and oxygen transmission rate (OTR) can be defined by Eq. 2, where gas volume is used. In order to compare OP results for gelatin films studied by numerous research groups previously, OP was normalized to ASTM standards and expressed in $cm^3\ \mu m/m^2\ d\ kPa$. As for WVP reported earlier, calculations were checked on permeability using the on-line calculator developed by Abbot, (2019).

The reviewed scientific literature pertainning to OP values for gelatin films is shown in Table 3.

Table 3. Normalized OP property comparison of gelatin films produced employing different; biopolymer sources, plasticizer type and concentration used, thickness values, relative humidities, temperatures and test methods.

Bovine gelatin content	**Plasticizer type and its content**	**Thickness, µm**	**OP*, $cm^3\ \mu m/m^2\ d\ kPa$**	**RH**	**Temperature**	**ASTM**	**Reference**
Gelatin	Glycerol 0.25% water	56.5 ± 7.43 43.0 ± 9.50 40.8 ± 5.36 50.3 ± 3.79 46.9 ± 4.15 55.0 ± 4.06	2461.5 ± 184.62 430.8 1230.8 1107.7 3923.1 ± 115.38 2400.0 ± 161.54	50 +/- 5%	23 ± 2 °C	Not ASTM method	Nur Hanani et al. (2012)
Gelatin	Glycerol 0.2% gelatin 0.5%gelatin 0.8%gelatin 1.1% gelatin	20.7 ± 3.2 23.3 ± 2.2 21.1 ± 6.6 23 .4 ± 2.5	19.5 ± 0.41 56.0 ± 0.81 90.2 ± 6.50 136.8 ± 9.75	50 ± 5%	23 ± 2 °C	Not ASTM method	Nur Hanani et al., (2013)
Gelatin 5%	Glycerol 33% gelatin	57 ± 4	70.6	50%	23 ^{0}C	D-3985, MOCON instrument	Clarke et al., (2016)
Gelatin	Glycerol 40% of gelatin	125 ± 25	6.1 ± 1.00 20.2 ± 3.20 104.3 ± 7.60 1908 ± 372	35% 50% 70% 90%	23°C	D-3985, MOCON instrument	Ciannamea et al., (2018)
Gelatin (**only fish**) 20%	-	none	68492.5 ± 13126.10	50%	25°C	D-3985, MOCON instrument	Yi et al., (2006)

[*]Initial values for OP calculations are given in Annex 2 of the supporting material (the average film thickness value was taken for the calculations if it was necessary).

From the scientific literature, there appears to be more uniformity with respect to OP values for gelatin-based films, than has been reported for WVP (previously described above), even if fewer papers have been published in the area. Comparable OP data for gelatin films was reported by Clarke et al., (2016) 70.6 $cm^3\ \mu m/m^2\ d\ kPa$ and Ciannamea et al., (2018) 20.2 ± 3.2 $cm^3\ \mu m/m^2\ d\ kPa$ and were of a similar magnitude employing the same test method, RH and similar glycerol content. The difference in OP values can be attributed to differences in film thickness. Nur Hanani et al., (2013) who studied the OP of gelatin films, similarly to Clarke at al., (2016) and Ciannamea at al., (2018) at RH of 50%, reported a value of 19.5 ± 0.41 $cm^3\ \mu m/m^2\ d\ kPa$, and used a non-ASTM OP testing method developed by Papkovsky et al. (2010), a different film production method to the other studies presented in Table 3, and created a thinner film using a lower glycerol content of just 0.2%. All of the studies carried out by Nur Hanani et al., (2012, 2013) employed the use of a twin-screw extruder to produce all experimental films, instead of the usual laboratory casting method, thereby making the films more industrial in form. This approach, while closest to industrial practice for plastic film formation, can allow bubble formation in the polymer matrix and because of its continuous nature, introduce variation in film thickness which can affect OP and WVP. This may explain differences presented between the Nur Hanani et al., (2012, 2013) studies and those where similar experimental conditions were used, however, it must be stated that such issues were not observed at the time of manufacture and testing for the Nur Hanani et al., (2012, 2013) studies. The lack of consistent film structure as determined from microscopic evaluation of test films was reported as an explanation for the high OP values reported by Nur Hanani et al., (2012).

When the studies presented in Table 3 are compared and contrasted, film thickness emerges as an important factor where other experimental parameters are controlled. It has been shown that the OP values for hydrophilic films, increase with increasing film thickness (Park & Chinnan, 1995).

The OP values reported by Ciannamea et al., (2018) again demonstrates the importance of test method adoption in terms of RH conditions employed during film testing. Specifically, for Ciannamea et al., (2018), OP follows the same trend reported for WVP, so it increases with increasing RH, thereby causing an increase in the free volume of films and in gelatin chain mobility owing to the plasticizing effect of water, as demonstrated clearly by Nur Hanani et al., (2013).

Yi et al., (2006) reported the highest OP values for gelatin films (68492.47 ± 13126.1 *cm^3 µm/m^2 d kPa)*. These authors employed fish-derived gelatin to form films. Yi et al., (2006) tested the OP properties of films using the ASTM D3985 standard method followed by the MOCON OxTran module. It is possible that the same reasons for high OP values for extruded gelatin films, as reported by Nur Hannai et al., (2012, 2013) may equally apply to those manufactured by Yi et al., (2006).

Similar to those comments made previously for WVP studies, OP units should be expressed in a manner which allows for conversion and comparison with other studies presented in the scientific literature (e.g. *cm^3 µm (or mm)/m^2 d kPa* (or *atm*). From the limited number of studies conducted employing gelatin, it would appear that the application of the ASTM D3985 method, known as MOCON method, seems promising for the application to gelatin films where a low RH is used, however, additional research is required to assess OP film values using this method in situations where films are held at higher RH values. The other OP detecting method reported in Nur Hanani et al. (2012), and developed by Papkovsky et al., (2000) utilizing a phosphorescent sensor, should also be compared to other analytical methods.

5. Challenges in the development of gelatin films

Currently gelatin films are well studied in terms of their chemical and mechanical properties and have been proposed as future bioplastic packaging materials for a wide range of packaging applications, including that for food and beverage products. One of the major challenges to achieving this is through circumventing issues which these materials possess in terms of poor water vapor barrier properties. Numerous literature sources highlight this challenge. Several approaches have been proposed to counteract this physical film weakness and include; addition of fatty acids (Hagenmater & Shaw, 1990; Wang et al., 2009), composite blends with different inorganic/organic additives (Syahida et al., 2020), employment of emulsions (Zhang et al., 2020; Wang et al., 2009), manipulation of plasticizer content (Sobral et al., 2001; Nur Hanani et al., 2013) and lamination of gelatin films with other polymer films (Tyuftin et al., 2020; Martucci & Ruseckaite, 2010; Pereda et al., 2011). If biopolymer films, like those made from gelatin and other similar protein and polysaccharide sources, are to replace or partially replace conventional plastics in commercial situations, then the problem of poor water and WVP barrier properties associated with such materials must be solved.

6. Conclusion

Among other biopolymers bovine gelatin is well studied for film forming properties and as a packaging material for different food products. Gelatin films have good mechanical properties and it is a promising sustainable alternative for conventional oil-based films which has large variations in formulations and production methods depending on target property for edible materials packaging industry.

The attempt to normalize literature data for the gelatin barrier properties was carried out. Gelatin WVP properties depend on film thickness, film composition, relative humidity, plastizer used, gelatin source and ASTM test method used. Literature review comparison for the barrier properties underlines high variability of the results. In some cases where the same conditions were used there are variability of the results either for WVP or for OP. Ceramic and IR WVP detecting methods are suitable for gelatin WVP measurements and additional research in its application for edible films is required. Difference of OP can be partially explained on the test method used, source of the gelatin, film production method and films structure changes. Largest variability also can be associated with complexity of the application different values by the researchers and lack of standardization for WVP and OP measurements.

Gelatin barrier properties results can be comparable in one batch of experiment for one author of paper but in many cases can't be comparable to the other literature sources due to lack of results compatibility and the absence of method standardization. Besides different values used in scientific literature it is more favourable to use similar standard units in all journals. When standard units used it will be much easier to compare the results to each other for researchers working in different scientific disciplines (Physical Chemistry or Food Packaging and Food Science). From the industrial point of view scientific literature comparison of OP or WVP values for packaging films with the data obtained from the industrial or laboratory trials usually causes difficulties for R&D and QC personal who are trained to compare standard units' data from technical data sheets (TDS) and the data from permeability measurement equipment. Time to time mistakes occur in papers due to units' complexity for young researchers who can make mistakes in units' comparison. Journal reviewers have to keep more attention to the units used in papers.

Further research is necessary for the gelatin films barrier properties studies in order to compare the results for different ASTM test methods with similar film thinness, finding new testing conditions in order to control barrier properties parameters not only in scientific research, but for film production in industry addressing such research for the increasing demands from packaging market focusing on improving of edible and compostable films properties.

Annex 1,2. Supplementary data

Supplementary data to this article can be found online at:

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Figure 1. Bioplastic circular economy conception (adopted from European bioplastics, 2016).

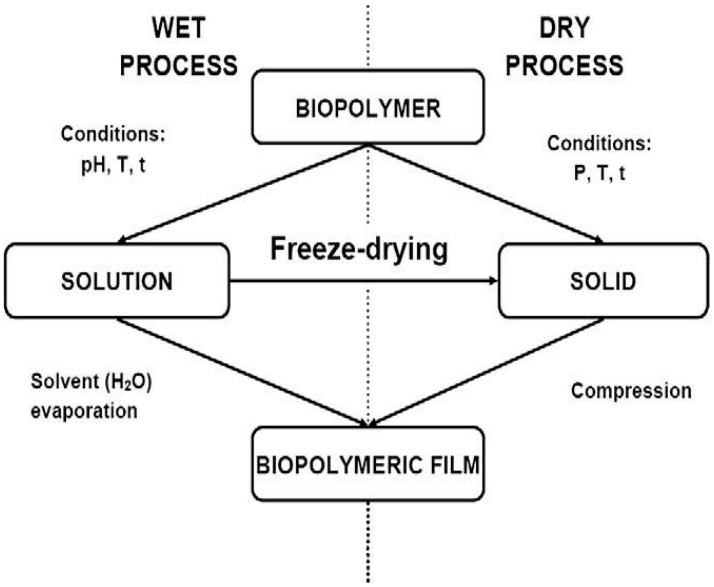


Figure 2. EBC films production scheme (adopted from Guerrero et al., 2010).

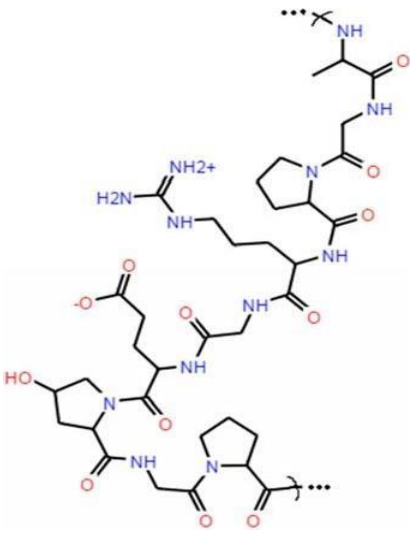


Figure 3. Representative gelatin structure (adopted from Ramos et al., 2016)

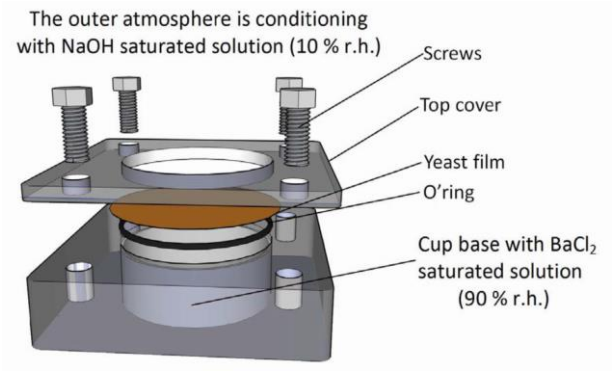


Figure 4. Gravimetric ASTM E96 cup test method (adopted from Delgado et al., 2018).

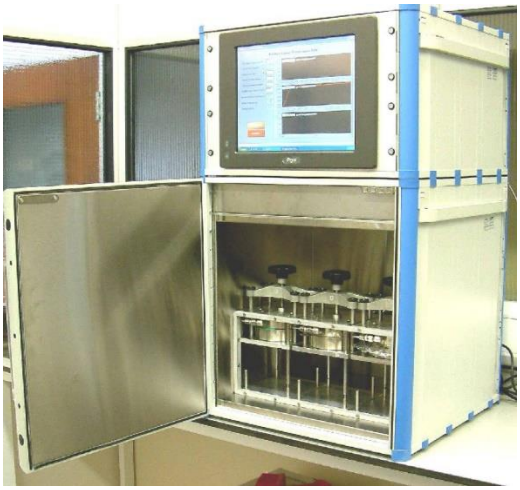


Figure 5. Versaperm equipment for hydrophilic films testing. RH can be controlled from 0 to 100% (Versaperm brochure, 2013).

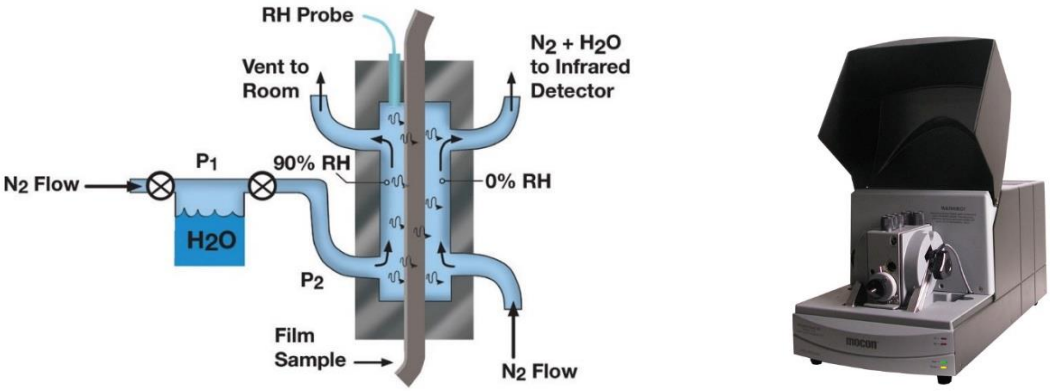


Figure 6. MOCON Permatran 3/33 module scheme and its view (Stevens, 2014).