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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.

Review 1 Gelatin films: study review of barrier properties and implications for 2 future studies employing biopolymer films 3 Andrey A. Tyuftin, Joe P. Kerry^{*} 4 Food Packaging Group, School of Food and Nutritional Sciences, 5 University College Cork, 6 Western Road, Cork, Ireland 7 8 9 10 11 *Corresponding author 12 Tel: +353 (0)21 4903798 13 Fax: +353 (0)21 4276318 14 E-mail: Joe.Kerry@ucc.ie 15 16 *Keywords*: gelatin films, film structure, water vapor permeability, oxygen permeation, barrier 17 property comparison, wvtr method selection

18

19 Abstract

20 Production of conventional packaging materials is now recognised as having had a major impact on world pollution. Edible/Biodegradable/Compostable (EBC) films may offer 21 22 sustainable alternatives to some conventionally-used packaging materials. One of the largest 23 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 24 material. Review of the relevant literature demonstrated that data for gelatin barrier properties 25 generated was generally incomparable owing to a lack of experimental standardisation. Some 26 standard approaches are adopted for further study, particularly, to reach a point where 27 recommendations can be made about industrial use of gelatin as a packaging material. 28

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 36 water vapor permeability (WVP) and oxygen permeability (OP) values as $g \ x \ mm \ (or \ \mu m)/m^2$ 37 $d \ atm \ (or \ kPa)$ and $\ cm^3 \ mm \ (or \ \mu m)/m^2 \ d \ atm \ (or \ kPa)$, respectively. Further research is 38 necessary to compare results under controlled test conditions.

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40 1. Global environmental threats of packaging waste

Climate change, over-exploitation of resources and environmental pollution are some of the 41 major issues facing humanity this century (Plastic – the Facts, 2017). Among other industries, 42 the packaging industry has come under the spotlight in recent times as more focus has been 43 44 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 45 generated by societies all over the world, efforts are being made to look at why and how we 46 use packaging around our products, with a renewed focus on effectively reducing, reusing, 47 recycling and recovering (as a last resort) waste packaging materials, with particular focus on 48 recycling. The recycling focus around present and future usage of both conventional packaging 49 and novel packaging materials is now guided by both the Bio Economy and the Circular 50 Economy measures. There are different types of biopolymers which can be considered as 51 52 bioplastics. There are edible films, which usually consist of protein (as for example gelatin) or 53 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 54 55 different groups by its biodegradation properties according to different composability standard (at different temperature and relative humidity, solubility) as such as PLA (polylactide), 56 57 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 58 59 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-60 61 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. 62

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

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

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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.

76 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 77 78 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 79 of research carried out in this area, it can be concluded that bio-polymer materials have a great 80 capacity to form films with great structural integrity and strength upon forming, have good to 81 excellent optical qualities, some of them have good gas barrier properties and can be cast or 82 extruded as per the limited number of trials undertaken to date. However, it is also understood 83 that such unaltered films are hydrophilic, especially edible films, and have a propensity to 84 absorb water, thereby undermining all of the aforementioned properties over time, especially 85 when employed to wrap semi-moist or moist products or when placed within environments of 86 87 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 88 89 difficult owing to the many factors which can play a role in influencing the final properties of the resulting films. 90

91 While numerous biopolymers have been examined for their ability to form packaging films,

92 one of the most studied to date has been bovine gelatine.

93 The objective of this study was to review barrier properties of different bovine gelatin films 94 depending on its content and control method used, and to normalize and standardise this data 95 to common units in order to make them comparable to each other and to make this applicable 96 for future control methods of compostable films barrier properties.

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98 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 sheetsdepending on the thickness) or to produce a coating.

Conversely, the dry method presents three ways to melt the edible base. These are: hot pressing, 105 compression moulding and extrusion. The dry method is solvent-free, has a small processing 106 footprint, can produce small volumes and is faster than wet method manufacture, and can be 107 easily submitted to scale up. Extrusion is the primary process used in the manufacture of 108 plastics, and it has been shown to be suitable for the manufacture of EBC films (Nur Hanani et 109 al., 2012, Kerry & Tyuftin, 2017). For instance, Nur Hanani et al., (2012) used extrusion to 110 111 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 112 authors pointed to the fact that this was an important finding as it demonstrated to the plastics 113 industry that biopolymeric materials could be extruded successfully. Guerrero et al., (2010) 114 compared three production methods for biopolymer-based films: compression, casting and 115 116 freeze-drying coupled with compression (Fig. 2).

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

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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 124 plasticizers is "a substance or material incorporated in a material (usually a plastic or 125 elastomer) to increase its flexibility, workability or distensibility" (Vieira et al., 2011). 126 Plasticizers enhance polymer-chain-flexibility and resistance to fracture thereby, reducing 127 hardness, brittleness, tension of deformation, density and viscosity. Plasticizers increase the 128 intermolecular space, thereby decreasing intermolecular forces. The plasticizers that are the 129 most commonly used in EBC films are: glycerol, sorbitol, ethylene glycol, vegetables oils, 130 lecithin waxes, mono, di and oligosaccharides and amino acids (Nur Hanani et al., 2013; Vieira 131 et al., 2011), but others like propylene glycol, sucrose, fatty acids and monoglycerides also 132 exist. Small and hydrophilic molecules with more than one polar group, but which are not too 133 close to each other show great capacity to serve as plasticizes. 134

135 The type and quantity of plasticizer used in film forming solutions affect film formation and 136 the resulting properties of the manufactured film. It has to be added that treatment of filmforming 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.

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142 **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 155 156 destruction of secondary and tertiary structure of collagen and consequently, the loss of barshape organization and most of the helical structures. The final quality of the gelatin produced 157 158 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 159 160 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 161 parameters discussed above. Consequently, gelatin (Fig. 3) can be defined as a mixture of α 162 chain, β chain and single and double unfolded chains. Gelatin can be produced as granulate or 163 powder and is water soluble, tasteless and odourless. 164

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

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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 171 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 172 as these forms find a broader religious acceptance in food usage and because they are relatively 173 untapped supply streams of gelatin (Sarbon, Badii & Howell, 2013; Nazmi, Isa & Sarbon, 174 2007). Different studies assert that fish and poultry can be efficient alternatives to bovine- and 175 porcine-derived gelatin. For example, Sarbon, Badii & Howell (2013) showed that chicken 176 and bovine gelatin amino acid compositions were very similar, especially in relation to the 177 content of amino acid (proline and hydroxyproline) (Table 1), glycine and alanine, all of which 178 179 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 180 proline and hydroxyproline as shown in Table 1, thereby producing a low gel modulus and 181 weaker gel network (Haug, Draget & Smidsrod, 2004). 182

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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 (%) ^{1⊁86} |
|----------------|---------------------|--------------------|----------------------------------|
| 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 |

187 ^{*}Tolerance level was not represented.

188

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-

191 Martínez et al., 2018) have reported on some interesting examples:

- gelatin blended with chitosan in order to maintain the red colour of beef steaks,

193 - 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,
- 197 gelatin, starch and glycerol films to reduce weight loss in Red Crimson grapes,
- 198 gelatin coated films with incorporated natural sourced antimicrobials to prolong shelf
- life of beef steaks and to control blown pack spoilage.

200 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 201 quantities as by-product from the slaughter of cattle. As reported in numerous studies 202 previously (Ciannamea et al., 2018; De Carvalho & Grosso, 2006; Kanmani & Rhim, 2014; 203 Figueroa-Lopez et al., 2018; Sobral et al., 2001; Wang et al., 2015), bovine gelatin-based, 204 edible films present good packaging related properties. The most important properties 205 described for packaging material are: barrier properties, thermal behaviour, mechanical 206 properties, transparency, odour emission, oil and gas resistance. Mechanical properties of 207 208 gelatin films are dependent upon the plasticizer content used, gelatin origin, environment and other parameters, particularly of a processing nature. 209

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

212 **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).

217 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)
- 229 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):
- 231 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.

266 Table 2. Normalized WVP property comparison of gelatin films produced employing different; biopolymer 267 sources, plasticizer type and concentration, thickness values, relative humidities, temperatures and test methods.

| Bovine gelatin content in film | n Plasticizer type and its content | Thickness, µm | WVP *, g mm/m ² 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 Sorbitol 45% to gelatin Sorbitol 65% to gelatin | 43 ± 9 | 413.406 705.222 924.084 | 100% | 22°C | E96 | Sobral et al., (2001) |
| Gelatin 1,5% | Sorbitol, 25% to gelatin | 72 ± 2 | $1310\ \pm 133$ | 55% | 25°C | E96 | Thomazine et al., (2005) |
| Gelatin 12% | Malic acid 20% to gelatin Polyethylene glycole 20% to gelatin Sorbitol 20% to gelatin | 21.0 ± 0.6 23.2 ± 0.7 23.7 ± 0.4 | 0.5 ± 0.05 3.3 ± 0.15 0.7 ± 0.05 | 50% | 25°C | E96 | Cao et al., (2009) |
| | Ethylene glycol 20% to gelatin Diethylene glycol 20% to gelatin | 22.4 ± 0.7 23.0 ± 0.8 22.5 ± 0.5 | 5.6 ± 0.05 4.7 ± 0.05 4.6 ± 0.07 | | | | |
| | Ethanolamine 20% to gelatin Diethanolamine 20% to gelatin Triethanolamine 20% to gelatin | 22.5 ± 0.5 23.2 ± 0.7 21.5 ± 0.5 23.4 ± 0.5 | 4.6 ± 0.07 3.0 ± 0.02 2.4 ± 0.05 3.1 ± 0.05 | | | | |
| 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 | $\begin{array}{c} 10993.8 \pm 531.96\\ 7802.0 \pm 531.96\\ 6383.5 \pm 106.39\\ 9023.0 \pm 106.39\\ 9976.5 \pm 623.15\\ 7014.7 \pm 373.89 \end{array}$ | 50% | $23 \pm 2^{\circ}C$ | E96 | Nur Hanani et al., (2012) |
| Gelatin | Glycerol 0.2% (to gelatin) Glycerol 0.5% Glycerol 0.8% Glycerol 1.1% | $\begin{array}{c} 20.7 \pm 3.2 \\ 23.3 \pm 2.2 \\ 21.1 \pm 6.6 \\ 23.4 \pm 2.5 \end{array}$ | $\begin{array}{c} 4063.1 \pm 137.80 \\ 4352.9 \pm 15.20 \\ 3959.7 \pm 45.60 \\ 4869.7 \pm 122.60 \end{array}$ | 50% | $23 \pm 2^{\circ}C$ | E96 | Nur Hanani et al., (2013) |
| Gelatin 5% | Glycerol 33% to gelatin | 57 ± 6 | 37.6 ± 3.42 | 50% | $23\pm2^{\circ}C$ | 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 | $\begin{array}{c} 2.8 \pm 0.33 \\ 14.7 \pm 1.18 \\ 77.8 \pm 0.48 \\ 196.1 \pm 0.89 \end{array}$ | 35% 50% 70% 90% | 23°C | F1249 | Ciannamea et al., (2018) ^{**} |
| 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 | $75\pm3\%$ | 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 $g mil/m^2 d atm$ 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.

268 269 270 271 272 273 274 From assessment of the data presented in Table 2, it is possible to make an attempt to 275 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, 276 test conditions and standard methods implemented. High WVP values are confirmed in almost 277 278 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 279 of 0.01 - 0.02 g mm/m² d atm) or oriented polypropylene (OPP) (WVP of 0.006 - 0.007 g 280 $mm/m^2 d atm$), common materials used in food packaging. 281

282 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 283 WVP values for gelatin films of 14.7 ± 1.18 and 37.6 ± 3.42 g mm/m² d atm, respectively. In 284 spite of comparability, similarity of test conditions and approximately similar glycerol content 285 used in film formulations, these results were obtained through the use of two different methods 286 of analysis, ASTM F1249 (Infrared detecting method, Mocon Permatran equipment) and E96 287 (cup method which is a gravimetric technique using a desiccant in a cup with the sample 288 sealed over the top and placed in a humidity cabinet at a set temperature/humidity and the 289 290 weight gain measured over time), and employing two different film production methods (dryand wet-methods). Another difference between both studies resides in the film thickness 291 generated for experimental samples; which was about 70 µm thicker in the Ciannamea et al., 292 (2018) study. 293

The lowest WVP values (in other words, the highest barrier properties) for gelatin films were 294 reported by Cao, Yang, & Fu (2009). In Cao et al., (2009), WVP values for gelatin films were 295 lower when malic acid and sorbitol were added as plasticizers to film forming solutions. For 296 other plasticizers used, WVP values were higher. This demonstrates the importance of 297 plasticiser selection when producing EBC films. It is interesting that WVP data for Cao, Yang, 298 299 & Fu (2009) reported for gelatin films with sorbitol as a plasticizer (gelatin 12%, sorbitol 20%) of 0.7 \pm 0.05 g mm/m² d atm @ 50% rh is far different that the data obtained by 300 Thomazine et al., (2005) who reported WVP value of 1310 \pm 133 g mm/m² d atm @ 55% rh 301 with quite similar sorbitol content of 25%. This difference could be explaned by the different 302 303 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 304 305 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, 306 307 & Fu (2009).

The WVP values determined for gelatin films by Carvalho, Grosso & Sobral (2008) were 308 analysed using the gravimetric method (E-96) (films were produced by casting method as in 309 Clarke et al., (2016), but presenting a higher WVP value of $481.5 \pm 7.3 \text{ g mm/m}^2 \text{ d atm } @$ 310 50% rh, than that reported by Clarke et al., (2016), 37.6 ± 3.42 g mm/m² d atm @ 50% rh and 311 Ciannamea et al., (2018), 14.72 g mm/m² d atm @ 50% rh. The differences determined 312 between test samples can be attributed to variation in experimental film thicknesses and 313 gelatin content. Another reason for WVP differences reported by these authors for gelatin 314 films studied can be attributed to the glycerol concentration used in film forming solutions for 315

316 the manufacture of the respective gelatin films employed. Carvalho, Grosso & Sobral (2008) employed 4.5% glycerol against 40% employed by Ciannamea et al., (2018). However, much 317 higher WVP values (up to 4869.7 g $mm/m^2 d atm$) were reported by Nur Hanani et al., (2013) 318 who used a glycerol content closer, but lower, to that employed by Carvalho, Grosso & Sobral 319 320 (2008) but obtained a 10-fold difference in WVP values compared to that reported by Carvalho, Grosso & Sobral (2008). The values presented by these authors contradict the 321 theory of Bourlieu et al., (2009) (who reported increases in WVP values with increasing 322 hydrophilic film thickness), as film thickness employed in the Nur Hanani et al., (2013) study 323 324 was approximately four-times thinner than that employed in the study by Carvalho et al., (2008) and consequently, should have had lower WVP values. The reason that such 325 dramatically different WVP values existed between the studies of Carvalho et al., (2008) and 326 Nur Hanani et al., (2013) may also be due to the differences in the methodology employed to 327 measure WVP in the respective studies. In fact it can be different content of plasticiser used 328 of two authors, but WVP test methods can also have effect. The cup testing method (E96), 329 330 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).

The lower amount of plasticizer used in Nur Hanani et al., (2013) (0.25% glycerol to gelatin) should have, in fact, improved barrier properties, which it clearly did not, employing a 0.8% glycerol content. According to Sobral et al., (2001) plasticizer usage decreases network density. Hygroscopic plasticizers, such as sorbitol and glycerol, increase the water content of films, thereby causing mobility of molecules and enhancement of water permeation. The results of Nur Hanani et al., (2013) can be compared to Avena-Bustillos (2006), who reported WVP of 4860 g mm/m² d atm @ 75% rh.

Yi et al., (2006) produced gelatin films using fish-derived gelatin and analysed the WVP 340 properties of these films using the F1249 standard MOCON Permatran 3/31 method, a method 341 similarly used by Ciannamea et al., (2018) in their studies. Interestingly, the values generated 342 by Yi et al., (2006) were higher than those generated by Ciannamea et al., (2016) (at 50% RH 343 respectively), yet similar to those reported by Nur Hanani et al., (2012, 2013) (same 344 345 magnitude) for beef-derived gelatin. It is important to point out that in drawing the comparison between studies conducted by Nur Hanani et al., (2012, 2013) and that of Yi et al., (2006), it 346 is being done in the absence of vital information from the Yi et al., (2006) publication, namely 347 film thickness values and all plasticiser information. The highest WVP value presented for 348 gelatin film was reported by Kanmani & Rhim, (2014). The film composition presented in 349

350 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), 351 against a level of 15% employed by Sobral et al., (2001). No information was presented by 352 Kanmani & Rhim., (2014) with respect to film thicknesses employed, but even with this 353 omission, it is difficult to explain why such high WVP values were generated when compared 354 to all other relevant studies. In more details plasticizer type and/or amount effect on physical 355 properties of gelatin-based films including WVP was reported in Bergo et al., (2013); 356 Andreuccetti et al., (2009); Jongjareonrak et al., (2006); Thomazine et al., (2005); Vanin et 357 358 al., (2005).

In short, Table 2 clearly indicates the variations recorded for WVP values determined for 359 gelatin films manufactured in different ways and the inconsistencies that exist within the 360 presented data and between different studies. Table 2 also clearly highlights the multiple factors 361 at play in generating this data where some research papers report unrealistic WVP values. 362 Factors impact upon the result variations presented in relevant studies, such as; gelatin and 363 ingredient sources, film manufacturing method, glycerol type and level used, film thicknesses 364 employed, relative humidity levels during testing, WVP testing methodologies utilised and in 365 some cases even errors and expression of WVP units. 366

367

368 **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 369 370 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 371 372 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, 373 374 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 375 376 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 377 378 expressed in standard scientific units such as $g \times mm (or \mu m)/m^2 d atm (or kPa)$ which can then be easily converted and compared with data generated in the scientific literature and can be 379 used by an industrial personal for EBC films quality control. For early studies conducted with 380 WVP, the method of choice was typically the E96 method which is commonly referred to as 381

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).

385

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

387

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.

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

400

The sensor side of the film is typically the dryer side and is flushed with dry air or N₂ down to 401 the target low RH anticipated and which does not need to be zero like that required for the E96 402 method. At the target RH, the dry air or gas flow is terminated, and the moisture passing 403 through the sample is allowed to increase on the sensor side of the film. When the RH level 404 stabilises, the time-based gradient of the RH increase is then calculated. This is proportional to 405 the permeation rate. The process is repeated until results are reproducible, essentially showing 406 that the test film has reached a stable equilibrium against the testing environment created during 407 the experimental test. This method is recommended for WVP assessment of EBC films and is 408 409 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 410 to employ a highly experienced operator to run the equipment. The ceramic-based sensor 411 method was specially developed for films possessing low WVP values. In the case of gelatin-412

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

The other method used widely in the packaging industry is the ASTM F1249 method which 415 416 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 417 method, is that followed by MOCON (USA) in the development and application of all of their 418 analytical test equipment for WVP measurement and consequently, is considered to be the 419 gold standard for WVP assessment within the packaging industry. The MOCON 420 PERMATRAN 3/33 module is primarily used to measure the WVP properties of oil-based 421 packaging materials possessing high-to-medium barrier properties. The MOCON cell scheme 422 423 present in the PERMATRAN 3/33 module is shown in Fig. 6.

424

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

426

The film divides the cell as a membrane across the two chambers; the first chamber presents 427 pure N₂ gas which passes through a water well, thereby reaching a predetermined humidity 428 (35–90% RH or 100% RH (if a wet sponge is used). This creates a humidity gradient. In the 429 second chamber, dry N₂ gas is introduced and flows through this half of the cell. The water 430 431 vapor carried by the gas permeates through the film and this is carried by the dry N₂ gas flow to the infrared detector. Permeated water molecules are identified by an infrared sensor (75-432 300 µm wavelength) which compares the amplitude of electrical signal generated from a 433 calibration film placed in the machine and the IR signal produced from the test film sample, 434 thereby determining the film's transmission rate. The ASTM F1249 method presents higher 435 436 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 437 white paper, (2017). 438

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.

449

450 **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 451 the quality and shelf-life of food products. With the exception of living and respiring foods, 452 like non-processed horticultural produce, most food products are oxygen-sensitive. For 453 454 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 & 455 Tyuftin, 2017), in addition to promoting the growth of aerobic spoilage microbes. Gelatin-456 based films typically present medium to high oxygen barrier properties and are very 457 comparable to some synthetic polymers, typically possessing lower OTR values than these 458 conventionally used plastic-forming polymers. 459

460 Similar to WVP, oxygen permeability (OP) and oxygen transmission rate (OTR) can be defined 461 by Eq. 2, where gas volume is used. In order to compare OP results for gelatin films studied by 462 numerous research groups previously, OP was normalized to ASTM standards and expressed 463 in $cm^3 \mu m/m^2 d kPa$. As for WVP reported earlier, calculations were checked on permeability 464 using the on-line calculator developed by Abbot, (2019).

465 The reviewed scientific literature pertaining to OP values for gelatin films is shown in Table466 3.

| 467 | Table 3. Normalized OP property comparison of gelatin films produced employing different; biopolymer sources, |
|-----|--|
| 468 | 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 μm/m ² d kPa | RH | Temperature | ASTM | Reference |
|--|---|--|--|--------------------------|------------------------|--------------------------------|---------------------------|
| Gelatin | Glycerol 0.25% water | $56.5 \pm 7.43 \\ 43.0 \pm 9.50 \\ 40.8 \pm 5.36 \\ 50.3 \pm 3.79 \\ 46.9 \pm 4.15 \\ 55.0 \pm 4.06$ | $\begin{array}{c} 2461.5\pm184.62\\ 430.8\\ 1230.8\\ 1107.7\\ 3923.1\pm115.38\\ 2400.0\pm161.54 \end{array}$ | 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 | $\begin{array}{c} 20.7 \pm 3.2 \\ 23.3 \pm 2.2 \\ 21.1 \pm 6.6 \\ 23.4 \pm 2.5 \end{array}$ | $\begin{array}{l} 19.5 \pm 0.41 \\ 56.0 \pm 0.81 \\ 90.2 \pm 6.50 \\ 136.8 \pm 9.75 \end{array}$ | $50 \pm 5\%$ | $23 \pm 2 \ ^{\circ}C$ | Not ASTM method | Nur Hanani et al., (2013) |
| Gelatin 5% | Glycerol 33% gelatin | 57 ± 4 | 70.6 | 50% | 23 °C | D-3985, MOCON instrument | Clarke et al., (2016) |
| Gelatin | Glycerol 40% of gelatin | 125 ± 25 | $\begin{array}{c} 6.1 \pm 1.00 \\ 20.2 \pm 3.20 \\ 104.3 \pm 7.60 \\ 1908 \pm 372 \end{array}$ | 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) |

469 *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 471 for gelatin-based films, than has been reported for WVP (previously described above), even if 472 fewer papers have been published in the area. Comparable OP data for gelatin films was 473 reported by Clarke et al., (2016) 70.6 $cm^3 \mu m/m^2 d kPa$ and Ciannamea et al., (2018) 20.2 ± 474 3.2 $cm^3 \mu m/m^2 d kPa$ and were of a similar magnitude employing the same test method, RH 475 and similar glycerol content. The difference in OP values can be attributed to differences in 476 film thickness. Nur Hanani et al., (2013) who studied the OP of gelatin films, similarly to 477 Clarke at al., (2016) and Ciannamea at al., (2018) at RH of 50%, reported a value of 19.5 \pm 478 0.41 $cm^3 \mu m/m^2 d kPa$, and used a non-ASTM OP testing method developed by Papkovsky et 479 al. (2010), a different film production method to the other studies presented in Table 3, and 480 created a thinner film using a lower glycerol content of just 0.2%. All of the studies carried out 481 by Nur Hanani et al., (2012, 2013) employed the use of a twin-screw extruder to produce all 482 experimental films, instead of the usual laboratory casting method, thereby making the films 483 more industrial in form. This approach, while closest to industrial practice for plastic film 484 485 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 486 487 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 488 observed at the time of manufacture and testing for the Nur Hanani et al., (2012, 2013) studies. 489 The lack of consistent film structure as determined from microscopic evaluation of test films 490 was reported as an explanation for the high OP values reported by Nur Hanani et al., (2012). 491 When the studies presented in Table 3 are compared and contrasted, film thickness emerges as 492

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). 502 Yi et al., (2006) reported the highest OP values for gelatin films (68492.47 \pm 13126.1 *cm*³ 503 $\mu m/m^2 d kPa$). These authors employed fish-derived gelatin to form films. Yi et al., (2006) 504 tested the OP properties of films using the ASTM D3985 standard method followed by the 505 MOCON OxTran module. It is possible that the same reasons for high OP values for extruded 506 gelatin films, as reported by Nur Hannai et al., (2012, 2013) may equally apply to those 507 manufactured by Yi et al., (2006).

Similar to those comments made previously for WVP studies, OP units should be expressed in 508 a manner which allows for conversion and comparison with other studies presented in the 509 scientific literature (e.g. $cm^3 \mu m (or mm)/m^2 d kPa$ (or *atm*). From the limited number of studies 510 conducted employing gelatin, it would appear that the application of the ASTM D3985 method, 511 known as MOCON method, seems promising for the application to gelatin films where a low 512 RH is used, however, additional research is required to assess OP film values using this method 513 in situations where films are held at higher RH values. The other OP detecting method reported 514 in Nur Hanani et al. (2012), and developed by Papkovsky et al., (2000) utilizing a 515 phosphorescent sensor, should also be compared to other analytical methods. 516

517

518 **5.** Challenges in the development of gelatin films

519 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 520 521 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 522 523 water vapor barrier properties. Numerous literature sources highlight this challenge. Several approaches have been proposed to counteract this physical film weakness and include; addition 524 525 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., 526 527 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., 528 2020; Martucci & Ruseckaite, 2010; Pereda et al., 2011). If biopolymer films, like those made 529 from gelatin and other similar protein and polysaccharide sources, are to replace or partially 530 replace conventional plastics in commercial situations, then the problem of poor water and 531 WVP barrier properties associated with such materials must be solved. 532

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- 535

536 **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. 542 Gelatin WVP properties depend on film thickness, film composition, relative humidity, 543 544 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 545 conditions were used there are variability of the results either for WVP or for OP. Ceramic and 546 IR WVP detecting methods are suitable for gelatin WVP measurements and additional research 547 in its application for edible films is required. Difference of OP can be partially explained on 548 the test method used, source of the gelatin, film production method and films structure changes. 549 550 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. 551

Gelatin barrier properties results can be comparable in one batch of experiment for one author 552 553 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 554 555 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 556 557 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 558 559 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 560 from technical data sheets (TDS) and the data from permeability measurement equipment. 561 Time to time mistakes occur in papers due to units' complexity for young researchers who can 562 make mistakes in units' comparison. Journal reviewers have to keep more attention to the units 563 used in papers. 564

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. 570 571

Annex 1,2. Supplementary data

- 572 Supplementary data to this article can be found online at:
- 573

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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).