Agar gel strength: A correlation study between chemical composition and rheological properties

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ABSTRACT

The relation between the chemical composition and the mechanical strength of four different agar hydrogels was particularly elucidated by comparing the results obtained by pyrolysis-gas chromatography/mass spectrometry and rheological characterization. Agar composition was studied exploiting a pyrolysis-gas chromatography/mass spectrometry approach in order to distinguish its anhydrous, galactose and glucose units. Agar gels in “as-is”, annealed and

additivated form were studied by means of amplitude, frequency and time sweep rheological tests to evaluate all the preparation approaches commonly used by conservators, also taking into account changes in the transparency via UV-vis spectroscopy.

A high percentage of anhydrous units in the polymer backbone was found to provide superior mechanical stiffness to the as-is hydrogels, but it did not seem to affect their long-term stability. The annealing process significantly improved the rheological response of galactose-rich agar hydrogels being able to promote the establishment of additional crosslinking points, whereas the additive presence showed to improve the hydrogel stiffness owing to a more structured polymer network. Moreover, the progressive reduction of the impurities and/or network defects within the hydrogels obtained by annealing, slightly increased the transparency of the hydrogels, which is an important aspect for applications in the conservation of Cultural Heritage.

Keywords: agar-agar; hydrogels; 3,6-anhydro-L-galactose; gel strength; Py-GC/MS; rheology.

1. Introduction

Gels are diffusely present in our every-day life and are used in a wide range of different applications from food to the pharmaceutical and biomedical industries. Nowadays, natural hydrocolloids extracted from different types of seaweed and bacteria, such as alginate, agar, gellan, hyaluronic acid and carrageenan, are extensively investigated to obtain targeted gels for specific purposes and with peculiar functionalities [1–7]. In particular, agar and gellan gels have gained an important role as cleaning tools in the field of conservation of Cultural Heritage, thanks to their versatility and effectiveness. Gels can be easily applied on artworks and then gently removed after a suitable application time, allowing to better control the cleaning operations and to limit the penetration of the liquid cleaning phase in the substrate; moreover, different chemicals (e.g. solvents, chelating agents and surfactants) can be easily employed to additivate gels in order to improve their performances [8–16]. Although agar gels are widely

used by conservators and in several other application fields, the correlation between their rheological properties and chemical composition is not yet deeply understood and only few works are reported.

Agar is a polysaccharide extracted from different types of red algae consisting primarily of D- and L-galactose units. Since 1956, structural studies of this natural polymer based on its fractionation by chemical and enzymatic hydrolysis were performed by Araki [21–23]. The main components of agar are agarose and a charged fraction called agaropectin. These two polysaccharides have the same monomers but different structure. The first one is a linear polymer consisting of alternating β-D-galactose and 3,6-anhydro-L-galactose units linked by glycosidic bonds, and it is the fraction that most determines the gelling properties of agar [24]. The second agar component, agaropectin, is an heterogeneous agarose consisting of the same repeating units in which some 3,6-anhydro-L-galactose rings are replaced by L-galactose-6-sulphate or by methoxy or pyruvate groups, consequently reducing the polymer gelling properties [25]. According to the literature [26–29], the type of red seaweed species, the environmental condition of seaweed growth and the physiological factors, as well as the extraction methods, strongly affect the relative proportion of the main components and consequently the agar gelling and rheological properties with both the amount of sulphates and anhydrous units playing a fundament role in affecting the final rheological characteristics of the gels.

In a previous work [30] some of the authors already reported a multi-analytical characterization of four different agar powders highlighting important compositional differences, but also some limitations of the applied analytical method. In particular, although the thermally assisted hydrolysis and methylation method (THM) used for the pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) analytical screening allowed to hydrolyse the polysaccharides and to derivatize the analytes in a single step [31], it was found to prevent the identification of the anhydrous units of galactose (3,6-anhydro-galactopyranose). The reactivity of the anhydrous part of agarose, linked to the galactose units with 1-4 glycosidic bonds, appears to be different from that of 1,6-anhydro-glucopyranose (or levoglucosan), here used as an anhydrous

standard, which under THM conditions gives the corresponding anhydrous permethylated compounds. Indeed, the identification of anhydro-galactopyranose markers and pyrolysis products deriving from the galactose units would allow a semi-quantitative evaluation of agar composition, thus highlighting how the chemical composition of different agars affects the correspondent hydrogel rheological properties. Furthermore, according to the empirical experiences of conservators, interesting changes in the mechanical response are observed with repeated annealing processes (i.e. the samples are heated and cooled several times), together with a tendency to transparency; these features should be taken into account and investigated in order to better understand the overall behaviour of agar gels.

That said, the aim of the present study was to identify a correlation between the chemical composition of four different agar gels (i.e. relative amount of anhydro-galactose and galactose units) and their behaviour in terms of gel strength and transparency. In particular, the effect of polymer concentration, annealing cycle and additive presence on the viscoelastic moduli (i.e. storage modulus G' and loss modulus G") of the gels was studied by means of amplitude, frequency and time sweep tests. Moreover, the transparency of the as-is and annealed samples was qualitatively evaluated via UV-vis spectroscopy in order to confirm the empirical experiences of conservators.

The composition analysis proved the strong variance in terms of repeating units in the investigated agars, which is an important factor to be taken into account for applications where targeted properties are required. The rheological characterization revealed the strong effect of agar composition on the strength of the prepared hydrogels, which though does not influence the stability of the hydrogel over time. In particular, a high moiety of anhydrous units in the agar backbone leads to considerably stiffer hydrogels at medium and high polymer concentrations, whereas at low concentration similar viscoelastic moduli were obtained independently on the polysaccharide composition. Above all, the annealing process was found to increase only the strength of the hydrogels prepared with galactose- and glucose-rich agars; indeed, such units somehow get in the way of the gelation mechanism of agar and

consequently, progressive annealing processes can be applied to obtain stiffer hydrogels characterized as well by a higher transparency.

2. Materials and Methods

2.1 Materials

Four different agar powders were selected: Agar Art (CTS S.r.l.) and Agar Purissimo (Bresciani S.r.l.), usually applied in the field of conservation, Agar Sigma (Sigma-Aldrich, A7002_CAS:9002-18-0), here selected as standard, and another agar powder used in the food industry (in the following named Agar Food) and imported from United Kingdom. Disodium ethylenediaminetetraacetic acid (EDTA , Merck-Millipore) and triammonium citrate (TAC, Bresciani S.r.l.) were used as additives for the gels.

2.2 Preparation of agar hydrogels

Agar powders were added to deionized water in the concentration of 1% w/v, 3% w/v and 5% w/v to replicate conservators indications. The samples were placed in a microwave operating at 700 W and brought to the boil (T = 100 °C) for a few seconds, vigorously mixed and heated again in order to allow the complete dissolution of agar powders in water. The obtained solutions were poured in circular 3D-printed moulds with a diameter of 25 mm and a height of 2.5 mm; before testing, the as-is samples were allowed to cool down at room temperature for at least 1 hour to ensure the complete and homogeneous gelation of the solution.

Hydrogels with an agar concentration of 1% w/v and 3% w/v were annealed at 100 °C to investigate the effect of this treatment on the mechanical and optical properties of the gels. One annealing cycle was applied to the as-is gels placing them in the aforementioned microwave and heating at T = 100 °C until complete “re-solubilization” of agar; then annealed gels were obtained by the same cooling procedure as the as-is samples. Note that annealed 5% w/v samples were not prepared and investigated because they were characterized by a high mechanical response already in the as-is state.

Additivated hydrogels with 1% of EDTA or TAC were similarly prepared; once the agar powder was completely dissolved after the heating step, the proper amount of additive was added and the systems vigorously mixed to ensure total solubilization and homogenization before the cooling process.

2.3 Pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS)

A multi-shot pyrolizer EGA/PY-3030D (Frontier Lab, Japan) directly connected to a GC/MS system was used. The GC was a 6890N Network GC System (Agilent Technologies, USA) with a methylphenyl-polysiloxane cross-linked 5% phenyl methyl silicone (30 m, 0,25 mm i.d., 0,25 µm film thickness) capillary column. The pyrolysis temperature was set at 400 °C, the interface temperature was 300 °C and the temperature of the injector was kept at 280 °C. The carrier gas was helium (1.0 mL/min) and split ratio was 1/20 of the total flow. The mass spectrometer coupled to the GC apparatus was a 5973 Network Mass Selective Detector (Agilent Technologies, USA). Mass spectra were recorded under electron impact at 70 eV, scan range 40-500 m/z. The interface was kept at 280 °C, ion source at 230 °C and quadrupole mass analyser at 150 °C. All instruments were controlled by Enhanced Chem Station (ver. 9.00.00.38) software. The mass spectra assignment was done with the NIST 2008 library and by comparison with literature data.

Agar powders were analysed without any preliminary or derivatization treatment. An amount of 0.2 mg of sample was placed in a stainless steel cup and inserted into the micro-furnace of the pyrolyser. For each analysis three replicas were performed.

2.4 Rheological measurements

Rheological tests were performed using a Physica MCR 301 rotational rheometer (Anton Paar GmbH, Austria) equipped with a Peltier heating system and solvent trap kit to reduce as much as possible the solvent evaporation; all measurements were carried out at a temperature of 20 ± 0.2 °C. A plate-plate geometry with a diameter of 25 mm (PP25) was used and a fixed

normal force (F_N) of 0.15 N was applied to avoid the sample slipping; the gap (d) typically varied from 2 to 3 mm depending on the sample height.

Amplitude sweep tests (AS) were initially performed on each sample to determine the linear viscoelastic region (LVER) at a fixed frequency of 1 Hz and a strain (γ) varying from 0.005 to 1%. Subsequently, the frequency-dependant response of the hydrogels was investigated by means of frequency sweep tests (FS) carried out in frequency range 0.1-80 Hz using a deformation within the LVER (0.05-0.1%). Finally, sample stability was evaluated via time sweep tests (TS), with a fixed amplitude of 0.05-0.1% and a frequency of 1 Hz, continuously measuring the viscoelastic moduli over a time period of 30 min.

Each rheological test was performed three times to ensure result reproducibility.

2.5 Optical properties

The transparency of the as-is and annealed hydrogels at a 1% w/v concentration was evaluated by means of UV-vis spectroscopy using a Perkin-Elmer lambda 9 UV/VIS/NIR Spectrophotometer; the absorbance and transmittance spectra were collected in the wavelength 400-800 nm range. The percentage transmittance values at 450 nm and 650 nm have been used to qualitatively compare the transparency of the investigated samples.

Each test was performed twice to ensure result reproducibility.

3. Results and Discussion

3.1 Pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS)

At first, pyrolysis measurements were carried out at a pyrolysis temperature of 600 °C, as suggested by a previous study of some of the authors about plant gums [32]. At this temperature the main pyrolysis peak is 2-furyl hydroxymethyl ketone, which is a marker of the anhydrous agar fraction, whereas the markers related to galactose units cannot be identified. Hence, in order to reduce the pyrolytic fragmentation and the occurring of secondary pyrolysis reactions it was decided to reduce the pyrolysis temperature to 400 °C.

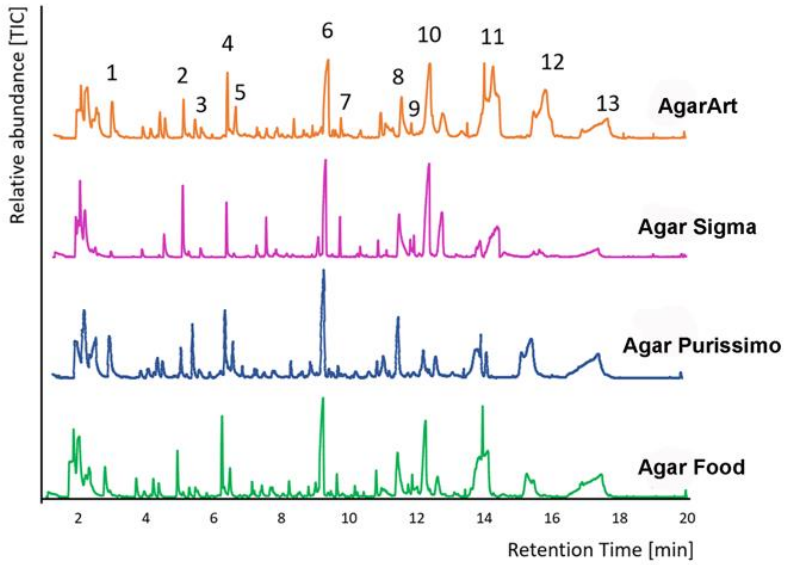

Figure 1. Pyrograms of the four agar samples.

Pyrograms of the four agar samples (Figure 1) show the presence of many pyrolysis products typical of polysaccharide materials [24,33], such as 2-furaldehyde (peak 2), 1-(2-furanyl)-ethanone (peak 4), 1,2-cyclopentanedione (peak 5) and 5-(hydroxymethyl)-2-furancarboxyaldeyde (peak 8). The latter, in particular, is considered a marker of hexose sugars [33,34] like galactose, which is the main monomer present in the polysaccharides contained in agar. Table 1 lists the main peaks obtained by Py-GC/MS and the corresponding assignments.

Table 1. Assignments of the main pyrolysis product found in the agar samples.

Peak n°	RT [min]	Assignments	MW	Main m/z
1	2.77	1-hydroxy-2-propanone	74	43, 45, 74
2	4.92	2-furaldehyde	96	96, 95, 97,67
3	5.26	2-furanmethanol	98	98, 41,53,81,97,69
4	6.24	1-(2-furanyl)-ethanone	110	95, 110, 96, 67
5	6.48	1,2-cyclopentandione	98	98,55,42,41,69

6	9.21	2-furyl hydroxymethyl ketone	126	126,95,96,67
7	9.64	levoglucosenone	126	98,96,53,68,97,42
8	11.45	5-(hydroxymethyl)-2-furaldehyde	126	97, 126,69,41
9	12.21	1-deoxy-3,6-anhydro-lyxo-hexopyranos-2-ulose	144	144,57,85,73,44
10	12,58	Anhydro-deoxy-galactopyranose	144	144,97,87,57,69
11	13.12	1,6-anydro- β-D-galactopyranose	162	60,73,43,56,70
12	15.34	1,6-anydro- β-D-glucopyranose	162	60,73,43,56,70
13	17.02	1,6-anydro- β-D-galactofuranose + 1,6-anydro- β-D-glucofuranose (coelution)	162	73,69,70,85,44,57

Among the specific pyrolysis products indicative of the various constituent units of agar, 2-furyl hydroxymethyl ketone (peak 6) was identified as the main product as well as its precursor, 1-deoxy-3,6-anhydro-lyxo- hexopyranos-2-ulose (peak 9); these molecules are the pyrolysis products of the anhydrous part of agarose. The markers of the galactose unit were identified in peak 11 (1,6-anydro-β-D-glucopyranose) and in its furanose isomer 1,6-anydro-β-D-galactofuranose (peak 13, in co-elution). Peak 12 is the pyrolysis product of glucose (1,6-anydro-β-D-glucopyranose or levoglucosan); even though glucose is not present as a structural unit in the polysaccharides of agar, compound 12 can result from the pyrolysis of free glucose or cellulosic derivatives, whose presence in agar is possibly due to an incomplete purification process of the red algae [31].

The good reproducibility of the Py-GC/MS analyses allowed to perform a semiquantitative data analysis. This was done by determining the content percentage of the anhydro-galactose, galactose and glucose units by integration of the main pyrolysis products. To this purpose peaks reported in Table 1 were integrated, with the exception of peak 13, considered as the coelution of the two anhydrous furanose derivatives of galactose and glucose. Coelution problems were also observed for other pyrolysis fragments in peaks 11 and 12, but in these two cases manual integration allows to exclude major interferences. In particular, the

percentage data reported in the form of histograms in Figure 2 were obtained by dividing the area of peaks 11 (galactose), 12 (glucose) and the sum of peaks 6 and 9, deriving from the anhydrous units of galactose, with the total area of all the main pyrolysis markers (peaks 1-12). The standard deviation calculated for each data is between 0.8 and 2.7, showing the good reproducibility of the measurements. Higher values of standard deviation (3.0-5.5) were observed in particular for peaks 11 and 12 that, as previously explained, were manually integrated to exclude interfering signals and therefore are subject to greater variability.

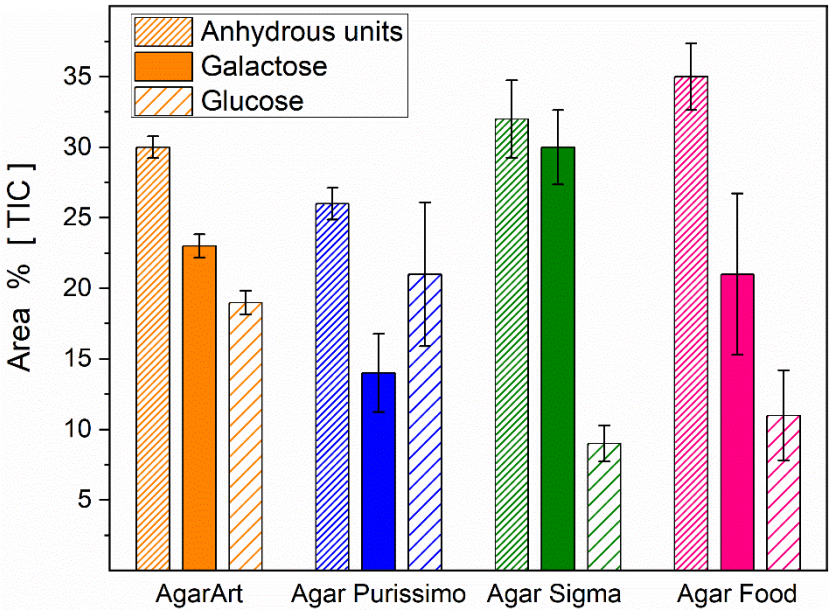

Figure 2. Percentage of anhydrous units, galactose and glucose of correspondent agar samples.

From the obtained results it is possible to point out that the purest agar is Agar Sigma, indeed considered as standard reference: the estimated percentage of anhydrous units and not-anhydrous ones are comparable (32% and 30% respectively), whereas the amount of glucose is only 9%. Agar Food exhibits a low percentage of glucose but a high percentage of anhydrous units (35%), whereas Agar Art shows a content of anhydrous units (30%) which is consistent with that of Agar Sigma, but an almost double amount of glucose (19%). Finally, the most inconsistent marker values are observed in Agar Purissimo, as already reported in a previous study [30]. Indeed, Agar Purissimo contains only 26% of anhydrous units, 14% of galactose units and a high percentage of glucose (21%).

Regarding the identification of substituted derivatives of galactose containing sulphate, methoxy or pyruvate groups, they were not detected in the pyrograms: this is probably due to coelution problems, typically occuring in the pyrolysis of polysaccharides that often generates isomeric products with very similar mass spectra. Moreover, the pyrolysis temperature, optimized in this work to identify markers of galactose and anhydro-galactose, may not be ideal to elute other products.

3.2 Rheological properties

3.2.1 As-is and annealed agar hydrogels

Figure 3 shows the rheological behaviour of Agar Purissimo hydrogels as an example, with the same trend observed for all the other samples.

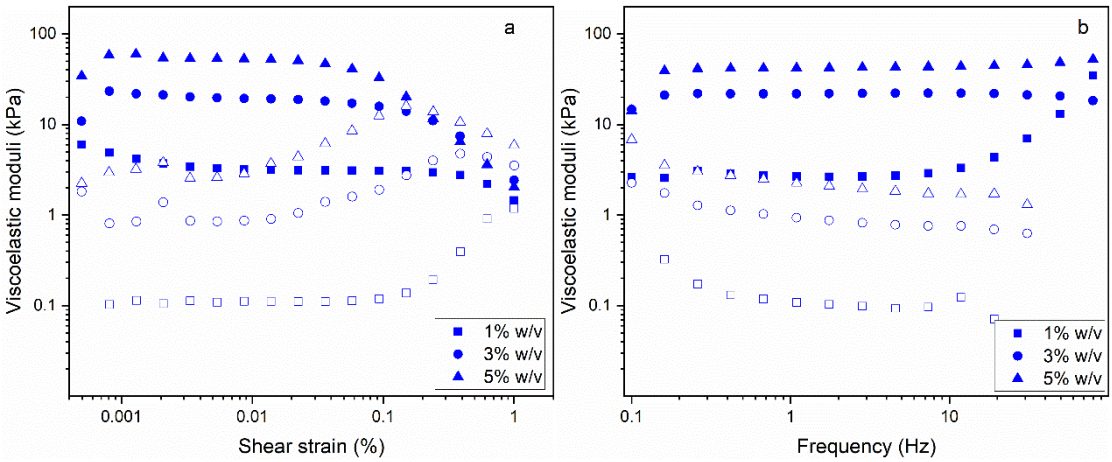

Figure 3. Amplitude sweep (a) and frequency sweep (b) curves of Agar Purissimo hydrogels at different concentrations. Solid and empty points represent the elastic modulus G' and the viscous modulus G", respectively.

The dependence of the viscoelastic moduli (i.e. G' and G") upon the applied shear strain (γ) is depicted in Figure 3-a; the region in which the moduli are strain-independent and parallel corresponds to the liner viscoelastic region (LVER). As clearly shown, a higher polymer concentration corresponds to greater moduli and to a significant reduction of the LVER, as well as to the decrease of the yield strain (i.e. critical strain value at which the moduli crossover occurs); such results can be ascribable to the increased crosslinking density and to the consequent formation of a highly structured polymer network with improved mechanical

properties, which however is able to withstand lower stress before undergoes to a deconstruction phenomenon.

Figure 3-b reports the dynamical rheological properties of the as-is gels; similarly to the AS results, the samples with a higher polymer concentration are characterized by superior viscoelastic properties. Moreover, in the whole investigated frequency range, the hydrogels show a significant predominance of the storage modulus G' above the loss modulus G", therefore indicating a strong gel behaviour which is further confirmed by the almost frequency-independency of the elastic modulus G'.

Figure 4 summarizes the rheological properties of all the as-is hydrogels; for comparison, the complex modulus G* (G* = G' + iG") has been always taken at a frequency of 1 Hz .

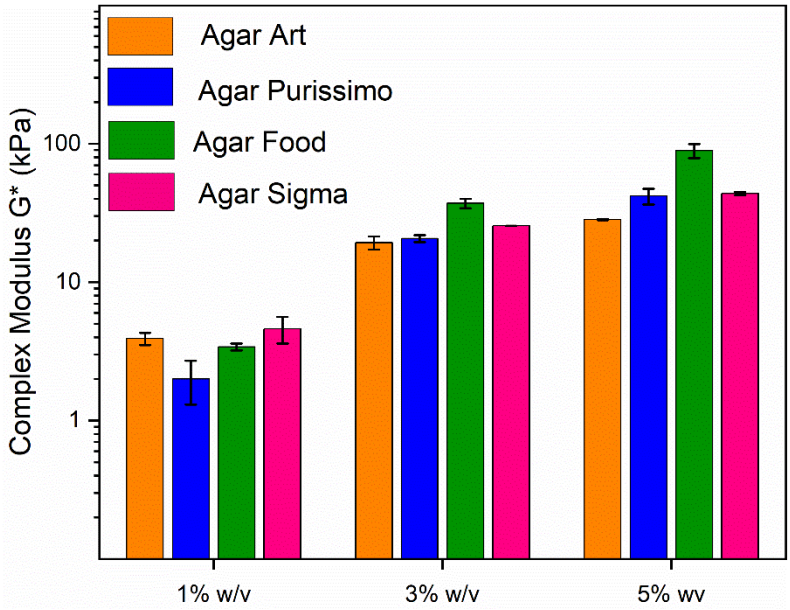

Figure 4. G* modulus of agar samples at different concentrations.

A strong increment of G* can be observed increasing the polymer concentration independently on the used type of agar, even if interesting differences related to the agar composition can be noted. In particular, whereas the agar composition plays an important role at medium and high concentration (3% w/v and 5% w/v), it does not seem to affect the hydrogel rheological response at low concentration (1% w/v); in general, taking into account the obtained composition results, it can be assumed that a high percentage of anhydrous units leads to hydrogels with greater mechanical properties, even if the presence of the non-anhydrous

moiety can somehow hinder the crosslinking process thus reducing the stiffness of the samples. More in detail, Agar Food hydrogels are characterized by the greatest moduli, which is in agreement with the high percentage of anhydrous units and the low percentage of galactose ones (35% and 21% respectively); on the contrary, Agar Art hydrogels can be considered the least mechanically performing due to the high percentage of galactose and glucose moieties (23% and 19% respectively) compared to the anhydrous units amount (30%). Agar Purissimo and Agar Sigma hydrogels show an intermediate behaviour which indeed reflects their composition, which consists in a high amount of glucose and galactose units, respectively.

Figure 5 reports the comparison between the complex modulus of the as-is and the annealed hydrogels.

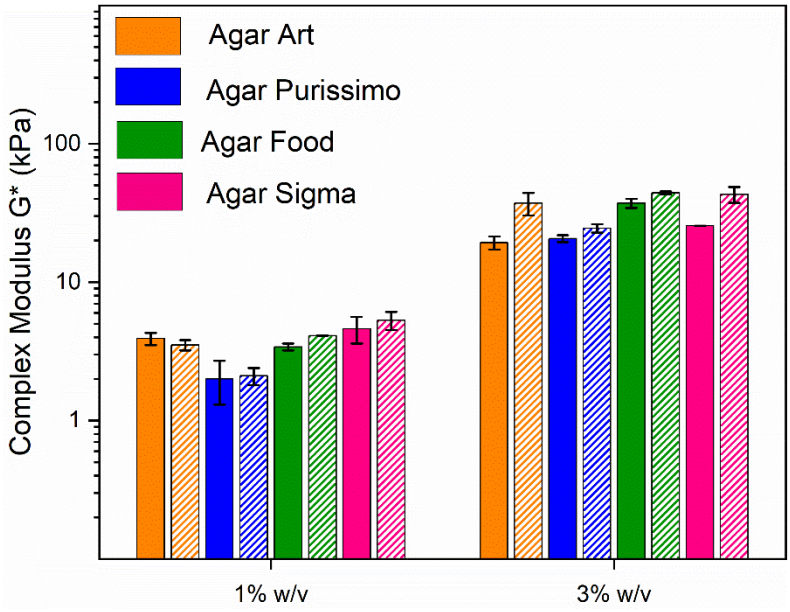

Figure 5. G* modulus of as-is (solid bars) and annealed (dashed bars) agar hydrogels at 1 and 3% w/v concentration.

As clearly shown, the annealing process has not a significant effect for the 1% w/v hydrogels but increases the moduli of the 3% w/v samples; however, the importance of such increment appears to be once again strongly dependent on the polysaccharide composition. Indeed, Agar Art and Agar Sigma, which are composed by a high percentage of galactose units compared to the percentage of anhydrous units, show an increment of the complex modulus

around 80%; on the contrary, Agar Purissimo and Agar Food, in which the galactose units are present in a significantly lower percentage than the anhydrous ones, the increment of the complex modulus is only around 20%. Bearing in mind these results, it can be assumed that the galactose units probably act as retardants/opponents of the crosslinking reaction hence reducing the mechanical properties of the agar as-is hydrogels; however, the annealing process most likely forces the breakdown of the physical network created by the polymer chains during the first gelation phenomenon and subsequently promotes the formation of additional crosslinking points between the anhydrous units (i.e. more structured network) leading to gels with improved stifness.

3.2.2 Additivated agar hydrogels

A comparison between the complex modulus G* of the as-is and additivated agar hydrogels is shown in Figure 6-a.

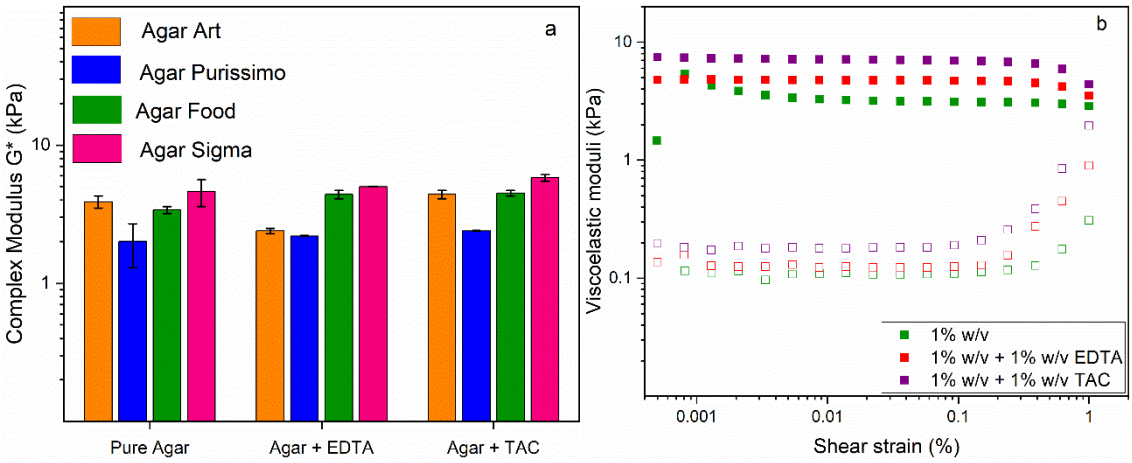

Figure 6. Comparison between G* of the standard and additivated agar hydrogels (a) and amplitude sweep curves of Agar Sigma samples (b).

The addition of EDTA and TAC leads in all cases, except one (Agar Art hydrogels additivated with EDTA), to an increase of the stiffness of the hydrogels. Agar, as most other polysaccharides, shows a pH-sensitive behaviour with the polymer chains shrinking at acidic pH values; consequently, being the used additives able to significantly decrease the pH, they probably promote the establishment of closer crosslinking points between the polymer chains

compared to the standard samples, which corresponds to a higher mechanical response. To this regard, TAC seems to have a more significant impact than EDTA in increasing the viscoelastic moduli of agar hydrogels thus leading to a denser polymer network due to the different acidity. To be noted that the evidence of the additive effect appears to depend on the agar composition since a high percentage of glucose residues is able to reduce the shrinking of the polymer chains, which in turn leads to a negligible increment of the viscoelastic properties of the gels (Agar Art and Agar Purissimo). Such hypothesis is further confirmed by the dependence of the additivated hydrogel viscoelastic moduli upon the applied shear strain, shown in Figure 6-b (Agar Sigma hydrogels). Indeed, the additivated agar hydrogels are characterized by a reduced LVER compared to the as-is samples, clearly indicating the formation of a more structured network which, despite the improved mechanical properties, is characterized by a lower yield strain.

3.3 Hydrogel stability

Figure 7 reports the time dependence of the viscoelastic moduli from a time period of 30 min for as-is Agar Art 1% w/v sample; similar behaviour was obtained for all the other samples.

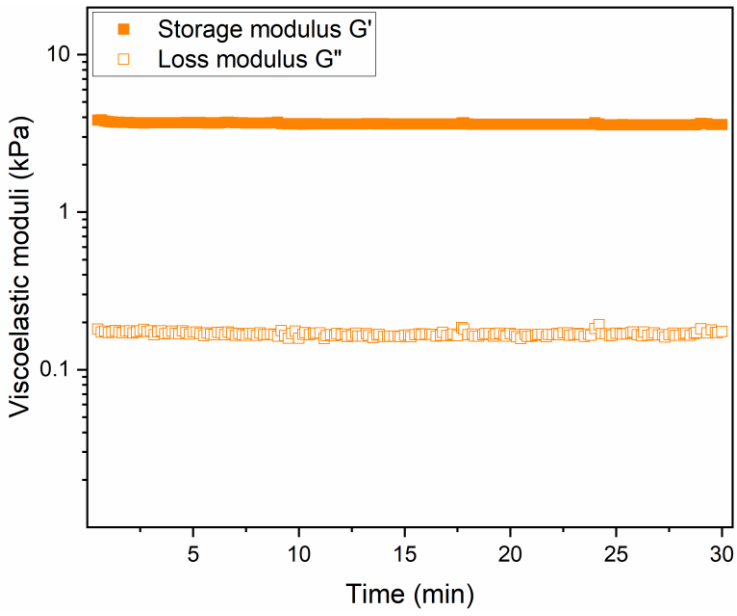

Figure 7. Time dependence behaviour of the viscoelastic moduli for Agar Art 1% w/v sample.

Hydrogel stability is a fundamental aspect from a practical point of view in Cultural Heritage conservation in order to ensure a safe applicability and an efficient cleaning effect. In particular, an increase of the hydrogel stiffness could indicate a progressive drying of the products with the risk to negatively affect their cleaning capability; on the contrary, a network structure deconstruction could lead to a decrease of the hydrogel mechanical response, consequently reducing the easy removal of the products once the conservation step is concluded [35].

No significant variations can be detected in the viscoelastic moduli of all hydrogels over the entire investigated time period (Fig. 7), proving that despite agar composition influences the gelation process it has not an important effect on the sample stability. Above all, the high stability displayed by the samples clearly indicated their suitability for cleaning and conservation purposes.

3.4 Transparency evaluation

Figure 7 shows the absorbance and transmittance spectra of 1% w/v Agar Food hydrogels. The optical behaviour of as-is and annealed samples is reported in green and blue, respectively; similar trends were observed for the other agars.

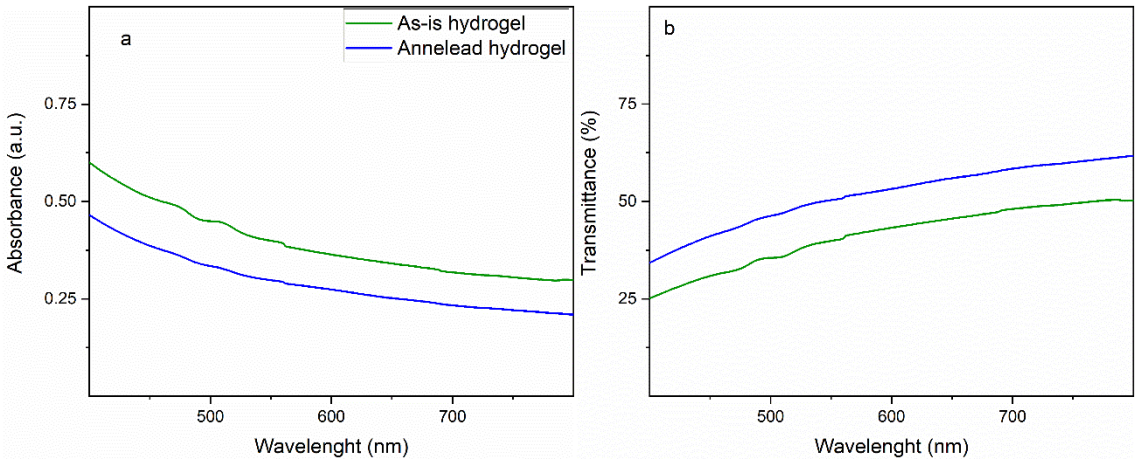

Figure 8. Absorbance (a) and transmittance spectrum (b) of as-is and annealed Agar Food hydrogels.

The absorbance spectrum is characterized by the absence of neat absorption peaks and by an increase of the absorbance as the wavelength decreases; such behaviour, according to Lambert-Beer law, indicates that the scattering is due exclusively to the presence of the polymer network, along with its defects and impurities.

Table 2 summarizes the transmittance values (%) of the as-is and annealed hydrogels; 450 nm and 650 nm were chosen as referring wavelength in the blue and red region, respectively.

Table 2. Transmittance (%) for the standard and annealed agar hydrogels.

	Transmittance (%)_450 nm		**Transmittance (%)_650 nm**	
Sample	*as-is* gels	Annealed gels	*as-is* gels	Annealed gels
Agar Art	36 ± 2	43 ± 1	46 ± 1	58 ± 2
Agar Purissimo	47 ± 1	54 ± 2	60 ± 2	61 ± 1
Agar Sigma	43 ± 1	45 ± 3	61 ± 1	62 ± 2
Agar Food	31 ± 3	41 ± 1	46 ± 2	56 ± 1

In terms of transparency, the improved optical properties are mainly due to the structural modifications and/or dissolution of both the impurities and glucose units, which consequently leads to the lowering of the network defects thus reducing the scattering of the light within the hydrogels. Considering the composition results, a good agreement with the transparency of the hydrogels was obtained. In particular, Agar Sigma hydrogels showed no increase in transparency, which is consistent with the high purity of such product; conversely, Agar Art and Agar Food, which are both characterized by a high amount of impurities, showed a considerably higher transparency after the annealing process.

4. Conclusions

In the presented work, the correlation between agar composition and the mechanical behavior (i.e. viscoelastic moduli) of the correspondent hydrogels was demonstrated and elucidated combining pyrolysis-gas chromatography/mass spectrometry and rheological results.

Despite further studies are necessary in order to obtain as much information as possible about the composition of agar in a single run measurement, for the first time the applied pyrolysis-

gas chromatography/mass spectrometry approach successfully allowed to clearly differentiate the anhydro-galactose units, which was hypothesized to be responsible for the gel strength, from the galactose structural units, as well as from the glucose impurities. The rheological response of the prepared hydrogels was found to rise as the polymer concentration increased, most likely as a consequence of the establishment of a progressively denser polymer network. In detail, anhydrous unit-rich agar samples appeared to be the mechanically most performing, confirming their role in the agar gelation mechanism; conversely, galactose structural units and glucose residues seemed to get in the way of the phenomenon reducing the hydrogel stiffness. However, the annealing process commonly employed by conservators was proved to prevail over the effect of the composition being able to promote the formation of additional crosslinking points in galactose-rich agar and allowing the establishment of a highly structured network. Moreover, transparency changes were evident in few samples characterized by an important amount of glucose residues and impurities, which were reduced by the annealing process leading to a defect-free network with a greater transparency effect.

Above all, the obtained results should be considered as an important step forward in the selection and design of targeted products for a specific purpose having proved the significant correlation between agar composition and the behaviour of the related hydrogels.

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Declaration of interest

None.

Data availability

Data will be made available on request.

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