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DESIGNING A TRANSPARENT HYDROGEL-BASED SOIL SUBSTITUTE FOR PRECISION AGRICULTURE APPLICATIONS

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ABSTRACT

Aim of the study
The aim of this research was to develop transparent hydrogel soils to monitor the development of the root system.

Material and methods
Polysaccharide hydrogels were made from gellan gum, sucrose and glycerine with universal medium. Absorbance testing, microscopic observation and thermal analysis (TG, DSC, DMA) were used to assess the primary qualities of the hydrogels. Observation of mould growth on the substrate was also carried out and the effect of the developed substrate on plant growth was analysed.

Results and conclusions
The research has shown that to obtain the good transparency it is crucial to use a minimum amount of gellan gum. The hydrogels show good resistance to cyclic loading and adequate thermal stability. Plants growing in the obtained transparent soil (substrate) developed in a similar way to the ones in natural soil. Transparent soil is a highly versatile medium due to its ability to adapt the hydration level of the environment to the needs of the plant, and to facilitate monitoring of plant growth, development of the root system and appropriate selection of seedlings.

Keywords: hydrogels, root system, agriculture

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INTRODUCTION

Long-term droughts and their accompanying reduced water availability are an inevitable consequence of the increase in average air temperature as a result of climate change. The impact of the increased Earth's temperature is manifested, among other things, in an increased frequency of extreme weather and climate events such as storms, windstorms, fires, or prolonged droughts. Global warming translates into a change in conditions previously found in terrestrial and oceanic ecosystems. This, in turn, based on the hypothesis of an increase in temperature of 2%, could result in more than doubling the climatic geographic range of approximately 8% of vertebrates, 18% of insects, and 16% of plants out of the 105,000 species studied (Salehi-Lisar and Bakhshayeshan-Agdam, 2016; IPCC, 2022). The problem of access to water and its diminishing pool in the environment is being studied by many groups of scientists (Biswas et. al., 2025; Yin et. al., 2025). Water content and availability in the environment vary over time, while there is a trend toward increasingly poor water availability for agriculture, also due to overconsumption, particularly in arid and semi-arid areas (Eliasson et al., 2003).

As a consequence, the climatic conditions in many parts of the world will no longer be conducive to agriculture. A reduction in agricultural land will also occur as a result of rising water levels. Another factor compounding this difficulty is the ever-increasing population, which could double before 2100 (Aydinalp and Cresser, 2008; United Nations, 2019). This requires measures to adapt agricultural productivity to new climatic conditions (Manyi-Loh et al., 2018). Adaptation to observed climate change should first include a solution to the problem of water scarcity. This is the main stress factor that limits plant growth. The root system plays a key role in the uptake of water and nutrients, and thus in determining the growth of the entire plant. A well-developed and effectively functioning root system can therefore ensure a sufficient supply of water to the plant even under water scarcity conditions. For this reason, it makes sense to direct agriculture towards plants, which, due to their structure, better assimilate soil resources. The selection of crop species can be carried out by phenotyping root systems in medium other than soil (Bengough et al., 2011). The development of quick and easy methods to visualise the root system of plants should focus on improving substrates with high light throughput. The development of a soil substitute with mechanical properties similar to those of the soil combined with high transparency could prove to be a significant achievement in root system phenotyping. A common feature of all soils is their opacity. The lack of transmission of visible light makes it impossible to observe deformations and processes within the soil without the use of a special apparatus.

The solution could be transparent soils, i.e. synthetic substitutes for natural soils that are permeable to visible light (Downie et al., 2012). Transparent soils are two-phase media consisting of a solid part that forms the soil skeleton and a liquid phase that fills it, with a matching refractive index (RI). The degree of transparency depends on the presence of impurities and air in the system (Morris et al., 2012; Yuan et al., 2019). A fundamental consideration in the design of transparent soils is to achieve a similarity to natural soil. Most often, similarity does not necessarily include all the characteristics of natural soil but only those that are relevant to the specific use of transparent soil. In geotechnical modelling, physical properties such as compressive strength, fracture toughness, and other properties related to deformation and force are most significant, while for botanical applications, similarity of physical and chemical properties is key (Zhang et al., 2022). The introduction of transparent soils in agricultural production allows the development of the root system to be precisely controlled and adapted to the needs of the soil. There are many proposals and formulations for transparent soils. They can be divided in terms of the material of the main matrix. The most popular are silica gels, silica powders, and hydrogels. The process should focus on the proportion of ingredients, the pore size, and the balance between transparency and mechanical properties. Now researchers are trying to find the cheapest possible solutions, hence the great popularity of hydrogels (Wang et al., 2021; Li et al., 2023). Currently, material engineering tools allow the development of transparent substrates dedicated to specific soil types. The aim of the research undertaken was to develop hydrogel transparent soils suitable for monitoring the development of the root system.

Hydrogels are a class of polymers with a high water content combined with cross-links that can be

both physical and chemical in nature. They are materials formed from hydrophilic polymers that have the ability to absorb water. This property determines their mechanical performance. In addition, polymeric gels are semisolid systems with a low degree of cross-linking, combining small amounts of solid particles dissolved or suspended in relatively large amounts of liquids (Ganji et al., 2010; Omidian and Park, 2010; Laftah et al., 2011). The cross-linking process aims to stabilise the polymers, resulting in the elongation of the polymer chain and the formation of a cross-linked structure. This results in the transformation of the liquid polymer into a solid or gel and, at the same time, increases the molecular weight of the polymer. Polymer chains that make up the hydrogel network can be linked by chemical bonds or their structure can be maintained by molecular links, additional ionic forces, hydrogen bonds, or hydrophobic interactions (Maitra and Kumar Shukla, 2014; Lu et al., 2018).

Research in transparent systems allows us to better understand the processes taking place in the root system, ion migration, etc. (e.g., continuous UV-vis measurement of ion concentration in the system). Research in transparent soils contributes to a better understanding of the water cycle in the environment.

MATERIALS AND METHODS

Materials and their manufacturing methods

For the production of polysaccharide hydrogels, gellan gum was used (Gelzan™ CM Sigma Aldrich, Germany). Additional ingredients were sucrose (M-Clarity™ Sigma Aldrich) and glycerine (M-Clarity™ quality level = MQ300, Sigma Aldrich). As part of the work, a universal medium was added (MS Murashige and Skoog basal medium, CA, USA). For the cross-linking of hydrogel substrates, $CaCl_2$ was used.

Two variants of hydrogel growing medium were prepared as part of the study: hydrogel in continuous form and spheres of approximately 2 mm in size. The first substrate preparation step was the same for both hydrogels. The beaker with demineralised water was placed on a magnetic stirrer while being heated. When the solution reached a temperature of 50°C (+/–2°C), the sugars sucrose and glycerine were added, followed by gellan gum. The elevated temperature prevented the solution from cross-linking too quickly and from maintaining a sufficiently low viscosity. Table 1 shows the types of specimens made, together with their nomenclature, shapes, and concentrations. Additionally, Figure 1 shows the fabrication scheme for both types of substrates. The second step of preparing the transparent substrates was different for the two options:

– a continuous hydrogel was obtained by introducing the MS plant nutrient into a continuously stirring solution of sugars and gellan gum at 50°C (+/–2°C), followed by $CaCl_2$ salt in an amount corresponding to 0.6 wt% of the solution;
– a hydrogel in the form of spheres was obtained by dropping a solution of sugars and gellan gum into a $CaCl_2$ solution of 3 wt% with MS medium at 25°C (+/–2°C). The spherical shape of the resulting sample is shown in Figure 2.

Table 1. Manufactured types of hydrogel substrates (source: Authors' own elaboration)

No.	Determination of sample	Mass percentage share (%)			Type of shape
		Gellan gum	Sucrose	Glycerine	
1	10_GG	1.	0	0	Sphere, block
2	10_GG_S5	1.0	5	0	Sphere, block
3	10_GG_G5	1.0	0	5	Sphere, block
4	10_GG_S3_G2	1.0	3	2	Sphere, block

GG – gellan gum
S – sucrose (corresponding number indicates concentration)
G – glycerine (corresponding number indicates concentration)

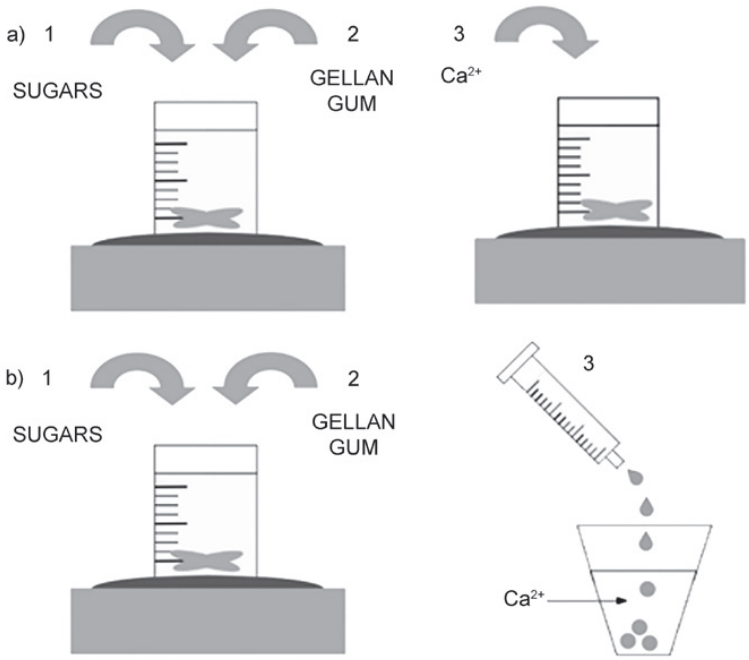

Fig. 1. Schematics of substrate fabrication: a) in continuous form and b) in spherical form (source: Authors' own elaboration)

a) b)

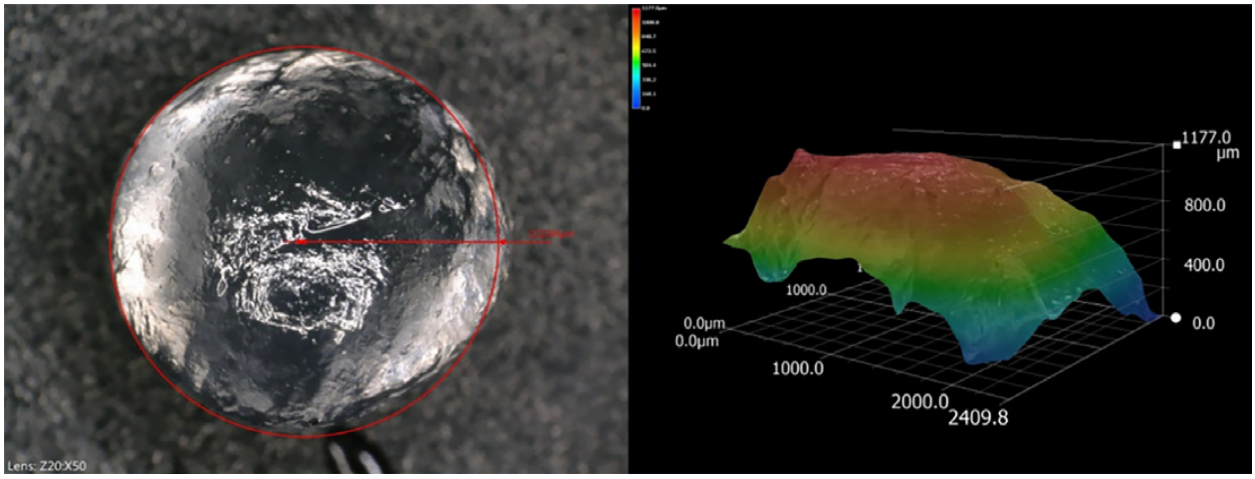

Fig. 2. Microscopic photograph of a) a hydrogel sphere with b) surface analysis (source: photo by Authors)

Analytical methods

A Phillips PU-8750 UV/VIS spectrophotometer with a dedicated UV-permeable optical glass cuvette was used to test the transparency of the samples. Plant breeding requires illumination with a wavelength in the range 380–710 nm, i.e. light in the photosynthetically active radiation range. Light of 450 nm was chosen for the study because it belongs to the maximum absorption range of chlorophyll A and has little interference with light from the UV range. The test

was repeated 6 times on each type of sample. Microscopic analysis was conducted using a Keyence VHX-5000 digital microscope that has a universal objective with a maximum magnification of 200. A microscope was used to observe the extent of mould development. Thermogravimetric (TG) analysis was carried out between 30 and 300°C to observe the dehydration of the hydrogel. The study was conducted using Netzsch's TG 209 F1 Libra analyser coupled to FTIR and TA 550. TG testing was performed on samples weighing 10–12 mg. Differential scanning calorimetry (DSC) measurements were performed using a DSC 204 F1 Phoenix differential scanning calorimeter, from Netzsch. Measurements were carried out in air atmosphere. The reference sample was an empty 40 µm aluminium crucible. The established programme involved a dynamic measurement, for which the temperature increased from 0°C to 300°C at a rate of 5°C/min. The weight of the samples was approximately 10 mg. The viscoelastic properties of the hydrogel were determined by a dynamic mechanical analysis (DMA) test in an isothermal run at 30°C. The amplitude and frequency were: 20 µm and 1 Hz. The shape of the test samples was that of a cylinder with a diameter of 15 mm and a height of 7 mm. Each thermal analysis test was repeated 3 times for each sample. Thermal analysis methods describe the quality of manufactured hydrogels very well. Since hydrogel structures base their spatial structure on different types of water bonds, it is possible to determine the strength of the bonds, the types of bonds in the hydrogel, and the hydrogel's compactness (ability to remain in the given shape) by analysing the dehydration process and the energy consumed for it. Statistical calculations (including calculation of mean, standard deviation, and regression) were applied to all methods. Statistical calculations were applied according to the needs arising from the interpretation of the application of the developed hydrogel soil. Specific temperatures were selected for analysis, and their results and deviations are presented in tables.

To assess the effect of glycerine and sucrose on mould growth, an experiment was carried out to grow moulds on three substrates that differed in the amount and type of sugar. Substrate A contained sucrose alone, substrate B contained sucrose and glycerine in a 1 : 1 ratio and substrate C contained glycerine. To transfer the mould spores to the dishes, three plates and the mould source were placed in a closed-circuit container and local ventilation was activated for 12 hours. The source of the mould was then removed and the dishes were left in the container for a period of 10 days. To visualise changes in the transparency of the produced types of hydrogels, an organoleptic test (direct observation) was applied by leaving the hydrogel blocks against a background of printed text. The blurred text indicates a decrease in transparency with time. The diameter of the hydrogel block was 15 mm and the test time was 3 months. The evaluation also included an experiment with plants to verify the long-term effect of the substrate on the development of the plant root system and a preliminary determination of the lifetime of the substrate. For the test, we chose two plants that show high root growth in a short period of time, so it was easy to observe changes and possible complications in the growth process.

RESULTS

Absorbance

The absorbance results for the samples made and the two reference samples are summarised in Table 2.

Table 2. Results of absorbance measurements (source: Authors' own elaboration)

No.	Determination	Average absorbance (-)	Standard deviation (-)
1	10_GG	1.02	0.14
2	10_GG_S5	0.48	0.10
3	10_GG_G5	0.88	0.12
4	10_GG_S3_G2	0.71	0.07
5	distilled water + nutrient solution	0.07	0.01
6	distilled water	0.06	0.03

As the absorbance increases, the amount of light transmitted decreases, so the transparency of the substrate decreases (Fig. 3). Sucrose produces the effect of significantly increasing the degree of transparency. The addition of glycerine also improves transparency, but to a lesser degree. The absorbance results obtained indicate that the addition of 5% sucrose reduces the absorbance of the GG/saccharose system, by as much as 50% compared to samples of pure GG hydrogel (even taking into account the extreme absorbance values of the obtained standard deviations). The research conducted refers to the results obtained by (Tang et al., 2001). Transparency is also affected by the type of sugar. Comparison with a mixture of sucrose and glycerine in the substrate, while maintaining a total concentration of 5% sugar, indicates that glycerine is less effective in improving the transparency of the system. The addition of micro- and macroelements to the solution only slightly reduces the transparency of the solution.

The results confirm the high light permeability of the hydrogel substrates, which is a basic assumption of the project, and indicate that the components determining the absorbance are gellan gum and sugar. At 450 nm for polysaccharides, scattering is possible as a cause of reduced transparency. In addition, scattering may be affected by air bubbles present in the hydrogel, which are centres of scattering.

TG

Samples of hydrogels and their components in pure form (gellan gum, sucrose, glycerine) were subjected to thermogravimetric analysis, based on which their degradation temperatures were determined. The degradation temperature was set as the one at which the mass loss is 1% of the initial mass. For glycerine, this temperature is 413°C. In the case of sucrose, the same weight loss already occurs at 216°C. The onset of gellan gum decomposition occurs at approximately 225°C. Decomposition of all components occurs above 225°C, therefore any weight loss processes at lower temperatures is only related to water loss. Figure 4 shows the average TG curves from the final hydrogel test. Gellan gum hydrogels without sugars (10_GG) and hydrogels with only one sugar added (10_GG_S5 and 10_GG_G5) were selected for the test.

Hydrogels are mainly composed of water, so the process that has the greatest impact on the change in the sample mass during heating is dehydration. The speed and completeness of the dehydration process depend on the number and type of bonds by which water is bound to the structure of the gelling agent (gellan gum).

In stage I, marked on the diagram under standard conditions, water occurring in the free (weakly bound) state gradually passes from the liquid state to the gas-

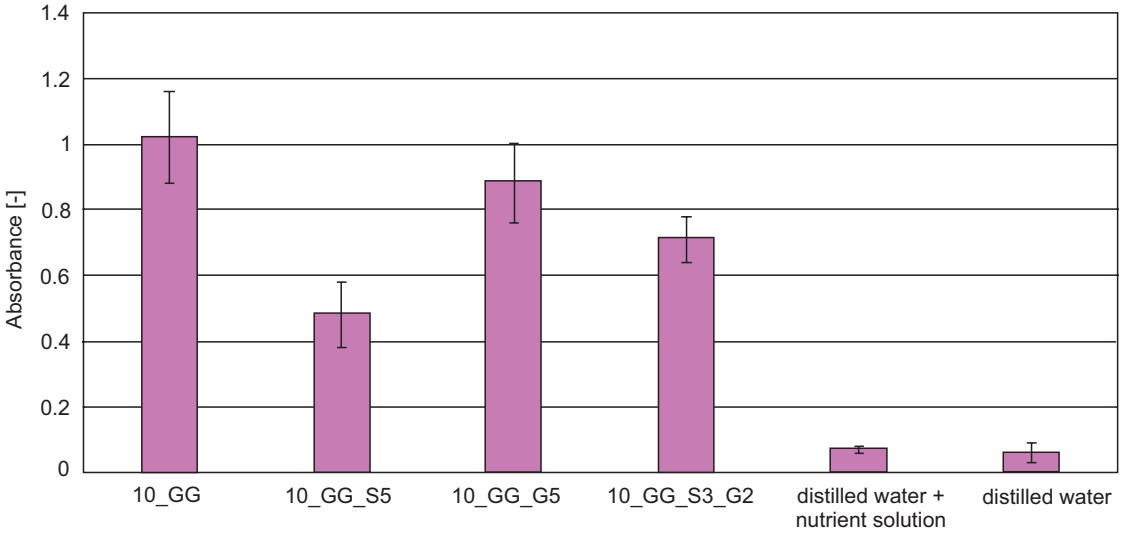

Fig. 3. Absorbance values for individual samples (source: Authors' own elaboration)

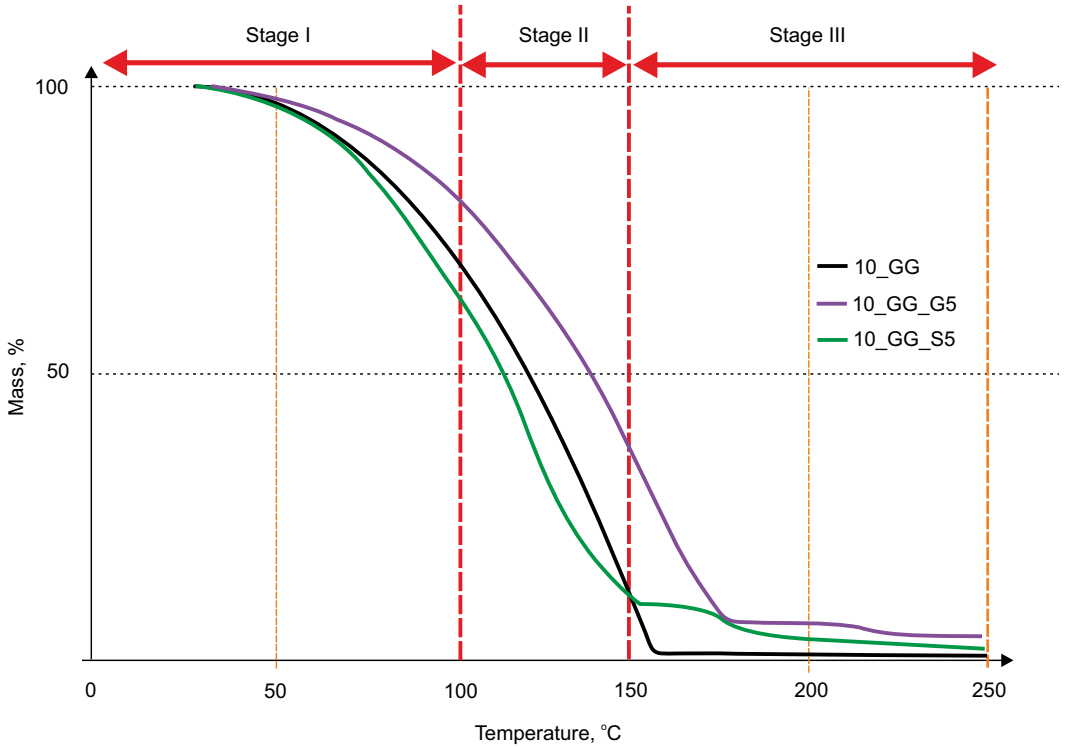

Fig. 4. TG curves for hydrogels (source: Authors' own elaboration)

eous state. The dynamics of the water evaporation process depend on the constituent substances of the hydrogel: for the sample containing sucrose in the given temperature range, the process occurs faster than for the control sample containing only gellan gum, while the addition of glycerine significantly slows down the process. This relationship can be expressed numerically by the areas under the individual curves up to a temperature of 100°C. These are, respectively:

– for 10_GG:

$$\int_{30}^{100}\left(-0.0066\cdot x^2+0.3748\cdot x+94.095\right)=$$
$$=6151.39\pm 302.34\left(\%\cdot {}^{\circ}\mathrm{C}\right)$$

– for 10_GG_S5:

$$\int_{30}^{100}\left(-0.0054\cdot x^2+0.2971\cdot x+95.11\right)=$$
$$=6258.11\pm 318.21\left(\%\cdot {}^{\circ}\mathrm{C}\right)$$

– for 10_GG_G5:

$$\int_{30}^{100}\left(-0.0037\cdot x^2+0.2151\cdot x+96.11\right)=$$
$$=6505.37\pm 331.17\left(\%\cdot {}^{\circ}\mathrm{C}\right)$$

The size of the area under the graph is a measure of the amount of water lost as a function of temperature. At this stage, the water evaporation process manifested by weight loss of the samples concerns only the loose water and the surface water, which are not structurally bound up with the gellan gum. The smaller the area under the graph, the greater the mass loss, and therefore the greater the amount of structurally unbound water in the hydrogel. For the sucrose-containing hydrogel, the surface area is 1.7% larger, while it is 5.8% larger for glycerine.

Stage II of the dehydration of the hydrogel structure is indicated in the figure. The weight loss is single-stage, and the curve assumes a near-linear shape. This trend was observed in all replicates of each type

of sample tested. The control sample (10_GG) loses mass (water) at a slightly lower rate than the sample containing additional sucrose. The relationship from the first step also applies to the sample containing glycerine, which decomposes much more slowly than the other hydrogels. The fact that complete evaporation of the water was not completed at 100°C indicates that mechanisms exist to slow down the process. These are probably the weak bonds between water and gellan gum (hydrogel network formed, hydrogen bonds) present in all samples.

In stage III, the change in the rate of water evaporation observed for samples containing sucrose and glycerine, manifested as a change in the angle of the curve on the graph, indicates the presence of a mechanism to slow down water evaporation with even greater efficiency than in stage II. When the chemical composition of the hydrogels is taken into account, it can be expected that the mechanism is the strong bonds between water and gellan gum, formed in the presence of sugars, the breaking of which requires more heat than for the evaporation of loose and weakly bound water. Table 3 summarises the thermal analysis of all samples.

Comparison of the temperatures at which complete dehydration of the samples is achieved indicates that the presence of sugars has a stabilising effect on the hydrogel structure. Furthermore, the results of the study suggest that glycerine shows a stronger stabilising effect, as 96% of the hydrogel mass loss occurs at around 255°C, while for sucrose it is only 210°C. The greatest change in hydrogel mass occurs in the temperature range 100–170°C.

DSC

The same hydrogels were subjected to DSC testing as those in the TG test. The average results are summarised in Figure 5.

Sample 10_GG underwent a rapidly accelerating endothermic transformation at approximately 100°C, which reached a maximum intensity at 103.3°C, after which the process became less intense. The flattening of the curve indicative of the end of the transformation occurred at 160°C. The total energy effect accompanying the process was 1954 J/g. The endothermic process observed in the graph is dehydration.

The curves obtained for samples 10_GG_G5 and 10_GG_S5 are similar in shape to the endothermic evaporation of water. In their case, the peak is relatively narrow (short-lived), indicating that this transformation (evaporation/dehydration) occurs much faster than in the control sample. The maximum of the transformation peak is shifted by several degrees toward higher temperatures, and the dehydration process ends as early as 130°C. The enthalpy of the conversion process varies depending on the sugar added. The peak maximum for 10_GG_S5 is shifted by 5.8°C towards higher temperatures relative to sample 10_GG. The dehydration of this sample is accompanied by the highest enthalpy value of the transformation. For samples containing only glycerine, a shift of the peak towards higher temperatures is also evident, but it is smaller at around 2 degrees. Analysis of the DSC curves leads to the conclusion that both glycerol and sucrose have a stabilising

Table 3. Summary of data related to the thermal stability of substrates and samples (source: Authors' own elaboration)

	Samples		Temperature [°C]					
			100	160	216	225	250	413
Mass loss (%)	Substrates	Glycerine	0	0	0	0	0	1
		Gellan gum	0	0	0	1	15	–
		Saccharose	0	0	1	6	59	72
	Hydrogels	10_GG	80	99	99	99	99	–
		10_GG_G5	65	76	94	95	96	–
		10_GG_S5	71	89	96	97	98	–

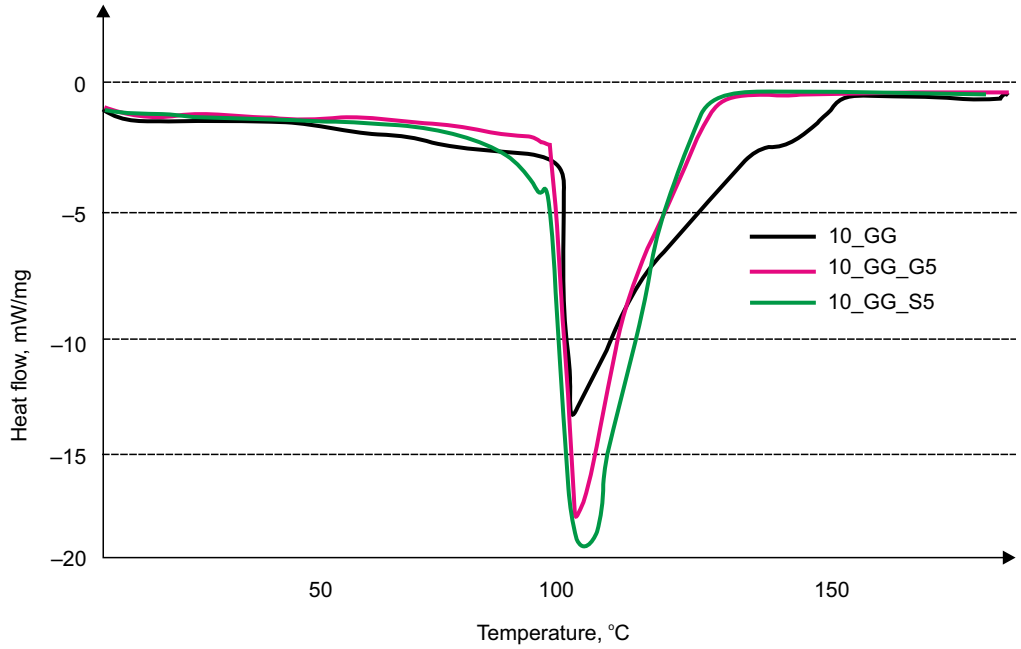

Fig. 5. DSC curves for hydrogels (source: Authors' own elaboration)

effect on the structure of gellan gum. The effect of sucrose is more efficient. The higher-energy effect may be a consequence of the extra heat energy absorbed by the hydrogel, which was used to break the secondary bonds between the sucrose and gellan gum chains.

A key issue affecting the properties of the hydrogel appears to be the amount of bound, loose, and free water in the system. Calcium ions derived from calcium chloride constitute a crosslinking agent that introduces additional, relatively strong (ionic) bonds compared to the other bonds present in the hydrogel between the chains. This leads to an increase in the amount of bound water and thus the activation energy of the dehydration process increases. Some tested sugars have a similar effect – the more functional groups derived from sugars, the more direct connections to the polymer network.

DMA

DMA testing was carried out for samples 10_GG, 10_GG_S5 and 10_GG_G5 and the results are shown in Figures 6 and 7. The values for the selected number of cycles are collected in Table 4.

For all of the samples tested, an increase in the storage modulus E' is observed as the number of cycles increases. The sucrose sample presents higher values than the reference sample (10_GG), while the glycerine sample presents lower values. The smallest change in the hydrogel;s retaining modulus was observed for the samples containing glycerine, demonstrating that its presence slows down the loss of water from the hydrogel under cyclic dynamic stress.

The magnitude of the storage modulus E' for hydrogels is related to the degree of cross-linking of the material/hydrogel. The results of the test indicate that the stiffness of the hydrogel is also influenced by the type and amount of sugars it contains. Analysis of the graph indicates that glycerine reduces the stiffness of the hydrogel. In this case, glycerine is a plasticiser that reduces the cohesive force between the chains. A hydrogel containing glycerine in its composition is characterised by a higher mobility of the polymer chains and thus higher elasticity. Sucrose has the opposite effect to that of glycerine – its addition reduces the elasticity of the hydrogel. The disaccharide stabilises the gellan gum chains by increasing the coefficient of viscous friction between their chains. The increase in the value of the retaining modulus of the hydrogel

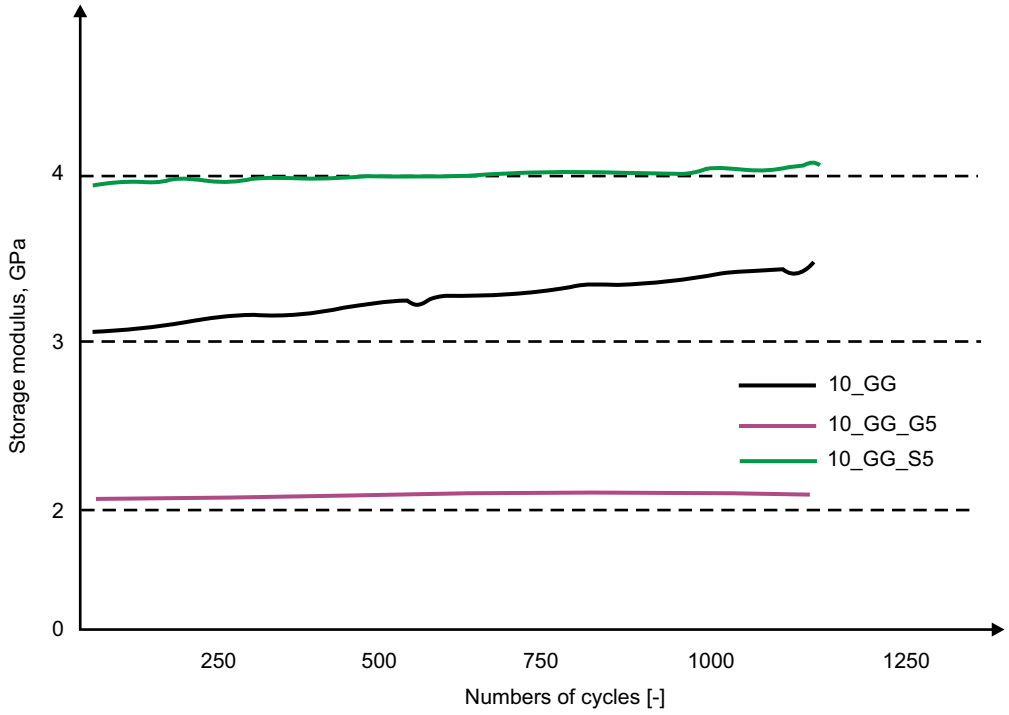

Fig. 6. Storage modulus curve for the hydrogels tested (source: Authors' own elaboration)

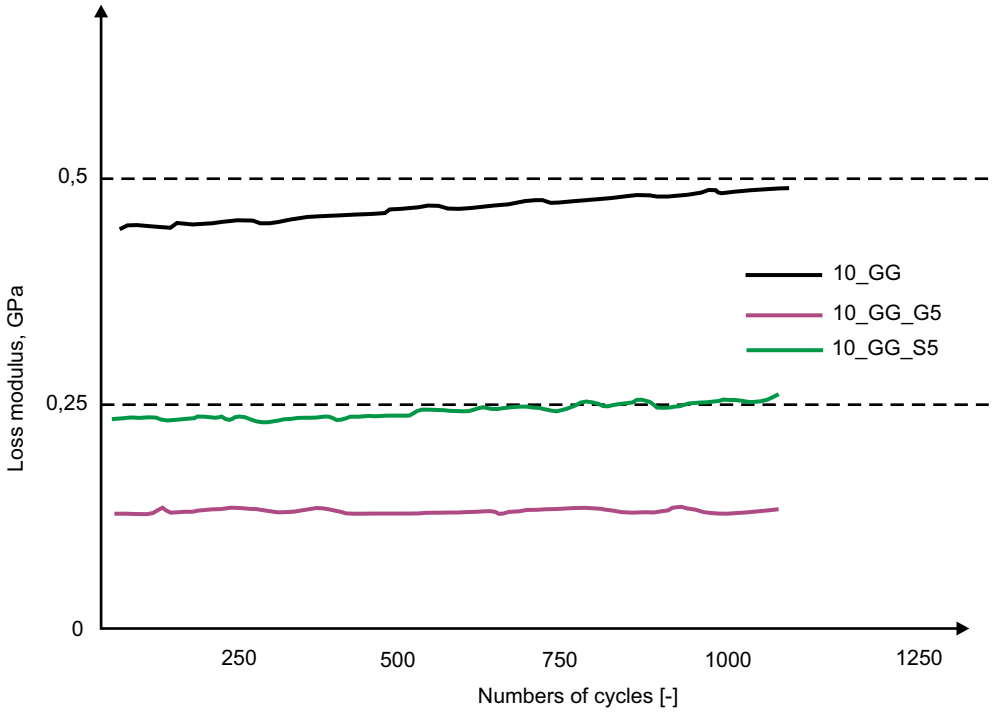

Fig. 7. Loss modulus curve for the hydrogels tested (source: Authors' own elaboration)

Table 4. Modulus values for different numbers of cycles (source: Authors' own elaboration)

Sample name	100 cycles		500 cycles		750 cycles		1000 cycles	
	E' (GPa)	E" (GPa)	E' (GPa)	E" (GPa)	E' (GPa)	E" (GPa)	E' (GPa)	E" (GPa)
10_GG	3.07 ± 0.27	0.42 ± 0.08	3.30 ± 0.29	0.45 ± 0.09	3.37 ± 0.33	0.48 ± 0.11	3.45 ± 0.35	0.49 ± 0.11
10_GG_S5	3.95 ± 0.31	0.24 ± 0.05	4.02 ± 0.32	0.24 ± 0.05	4.08 ± 0.34	0.25 ± 0.06	4.11 ± 0.35	0.25 ± 0.05
10_GG_G5	2.13 ± 0.21	0.12 ± 0.04	2.23 ± 0.22	0.12 ± 0.04	2.29 ± 0.24	0.12 ± 0.03	2.28 ± 0.24	0.12 ± 0.03

with the number of cycles passed is most likely due to the gradual loss of water. As a result of dehydration, the distances between the gellan gum chains, as well as their mobility, decrease.

The gradual increase in the value of the loss modulus E" is indicative of an increase in the energy dissipated during successive deformation cycles. The viscosity of the hydrogels increases slightly, which is probably related to the loss of water, as the polymer chains are approaching each other. For all samples, the conservative modulus was significantly higher than the loss modulus, suggesting that for the test parameters adopted in the hydrogel, elastic properties predominate over viscous properties.

Investigating the effect of added sugars on mould growth

Tests for mould growth on substrates A, B, and C showed that the mould covered the sample that contained only sucrose as a sugar source (substrate A) to the greatest extent. The mould occupied 100% of the area tested. The sample containing sucrose and glycerine (substrate B) was covered by mould at around 49%, while the sample containing only glycerine at only 18% (substrate C) (Fig. 8).

The qualitative and quantitative diversity of the moulds that cover the samples is related to the type of sugar the medium contains. The experimental results indicate that sucrose is more conducive to microbial growth than glycerine. As the sucrose content of the medium decreases, the degree of sample coverage by the mould decreases. This is probably directly related to the structure of the sugar – sucrose, unlike glycerol, is a disaccharide that breaks down to fructose and glucose, monosaccharides that are easily absorbed by fungi. Furthermore, the differences in the types of moulds that cover each substrate indicate that sucrose and glycerol favour the growth of specific, few types of moulds, while a mixture of both sugars makes the substrate an environment conducive to the growth of numerous types of moulds (Figs 9–11).

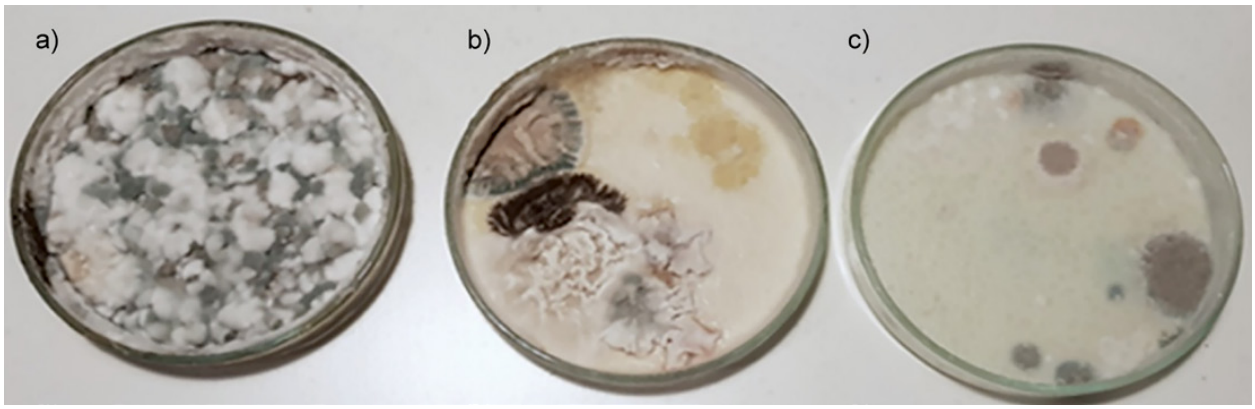

Fig. 8. Appearance of samples containing, from left: a) sucrose, b) sucrose and glycerine, c) glycerine after 14 days of experimentation (source: photo by Authors)

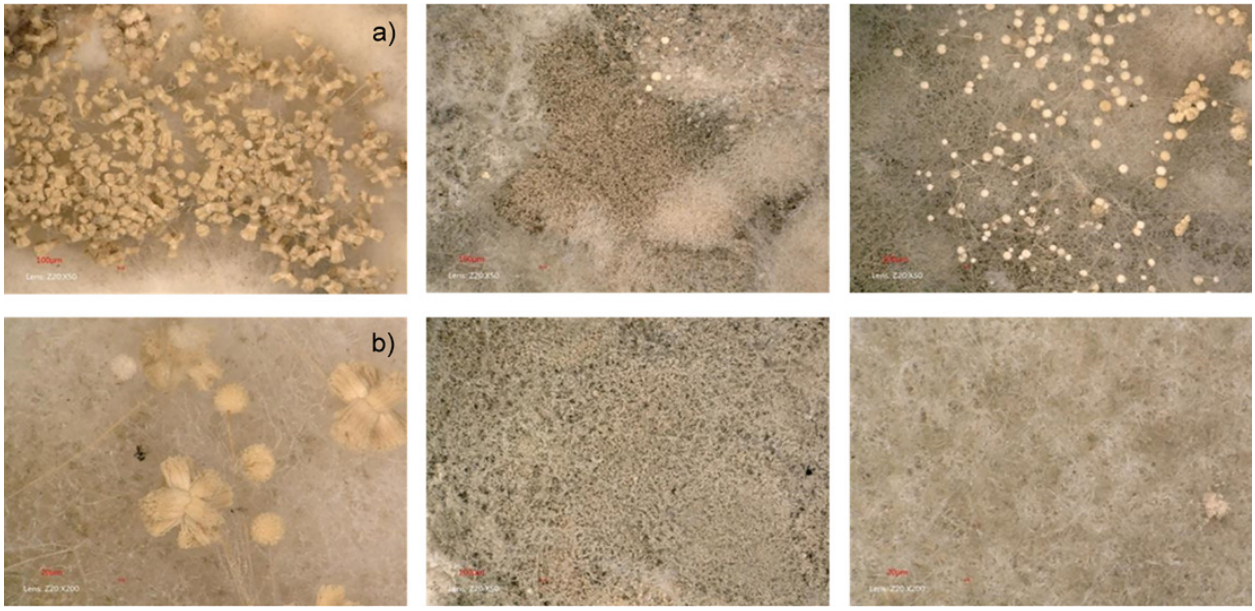

Fig. 9. Mould growing on sucrose-containing medium at 50x (a) and 200x magnification (b) (source: photo by Authors)

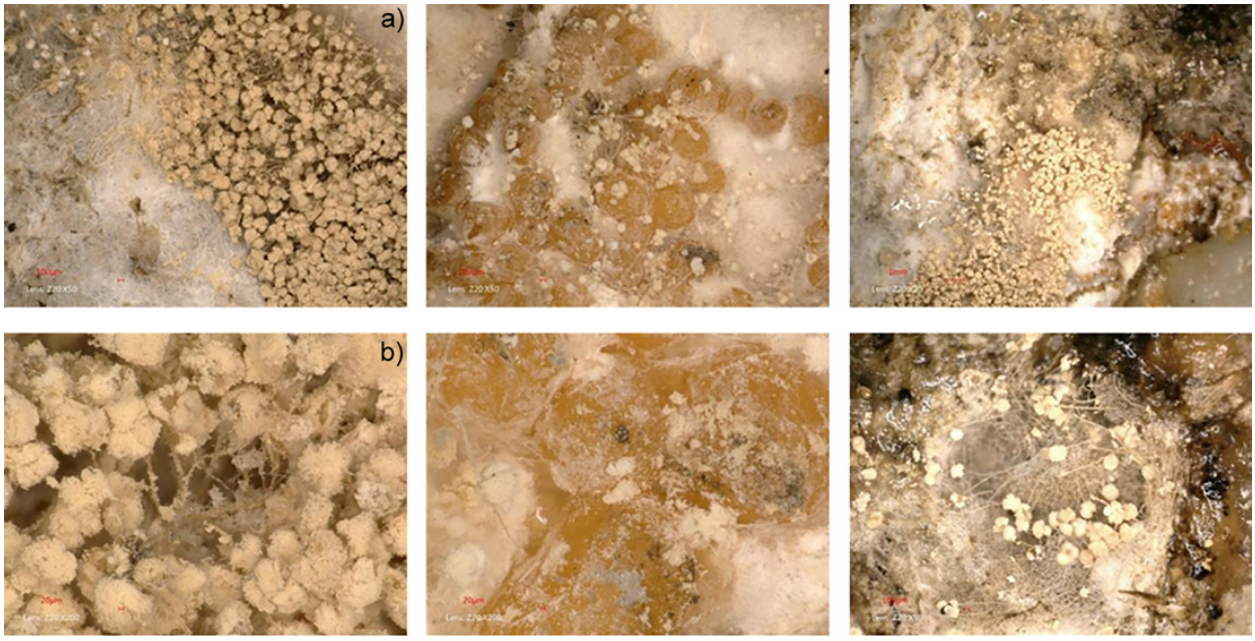

Fig. 10. Mould growing on a medium containing sucrose and glycerol at 50x (a) and 200x magnification (b) (source: photo by Authors)

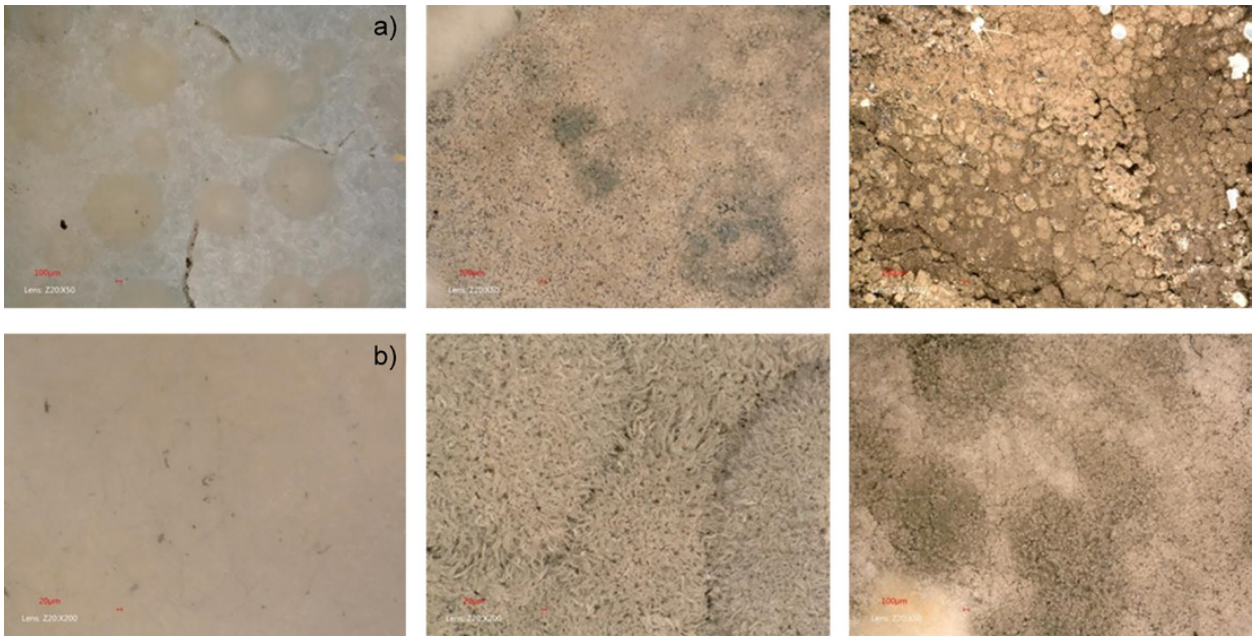

Fig. 11. Mould growing on medium containing glycerol at 50x (a) and 200x magnification (b) (source: photo by Authors)

Sample one is entirely overgrown with Cladosporium species, but Aspergillus, a species characterised by distinct, bright conidiophores, is also locally present.

Grey and dark grey-coloured colonies with large, clear, and dark spores probably belong to Bipolaris. In sample C, spores are present as uniform orange-coloured swollen vesicles. This species probably belongs to Eurotium, a bagworm for which spherical fruiting bodies are a characteristic feature. Also noticeable is the species present on the first sample, Aspergillus. White, woolly fungal colonies showing similarity to Arthrinium species are visible in the sample. Residual amounts of these are also found in sample C. The white wrinkled colonies are probably representatives of the Cladosporium and Circinella species, which has a darker colour in the central part of the colony.

Sample three appears to be predominantly covered with Botrytis species, which form rough, brownish conidiophores.

Testing the transparency of hydrogel substrates over time

Investigations of the physical properties of the substrate over a period of 3 months showed that all samples lost their original degree of transparency with the passage of time (Figure 12). For samples A (10_GG) and C (10_GG_S3_G2), the transparency was already impossible to capture after 7 weeks, while for sample B (10_GG_S5) it was already after 4 weeks. Sample D (10_GG_G5) had the highest and also the longest-lasting transparency.

The presence of microorganisms can be observed in all samples. For samples A, B, and C, outbreaks did not appear until week 7 with varying sizes – in sample B, with its high sucrose content, the outbreaks were the largest, and in sample A they were the smallest. In sample D, the microorganisms appeared the latest, but their development was much more intense than in the other samples. The results of the experiment indicate an important role for the concentration of the gelling agent on the biological stability. The concentration of gellan gum determines the amount of loosely bound water and free water in the structure. The disruption of the structure by additives impedes circulation and aeration, which favours the growth of certain bacterial strains.

A second factor influencing microbial resistance is the concentration and type of sugar in the medium. The higher availability of sucrose in sample B resulted in more intensive microbial growth than in sample C.

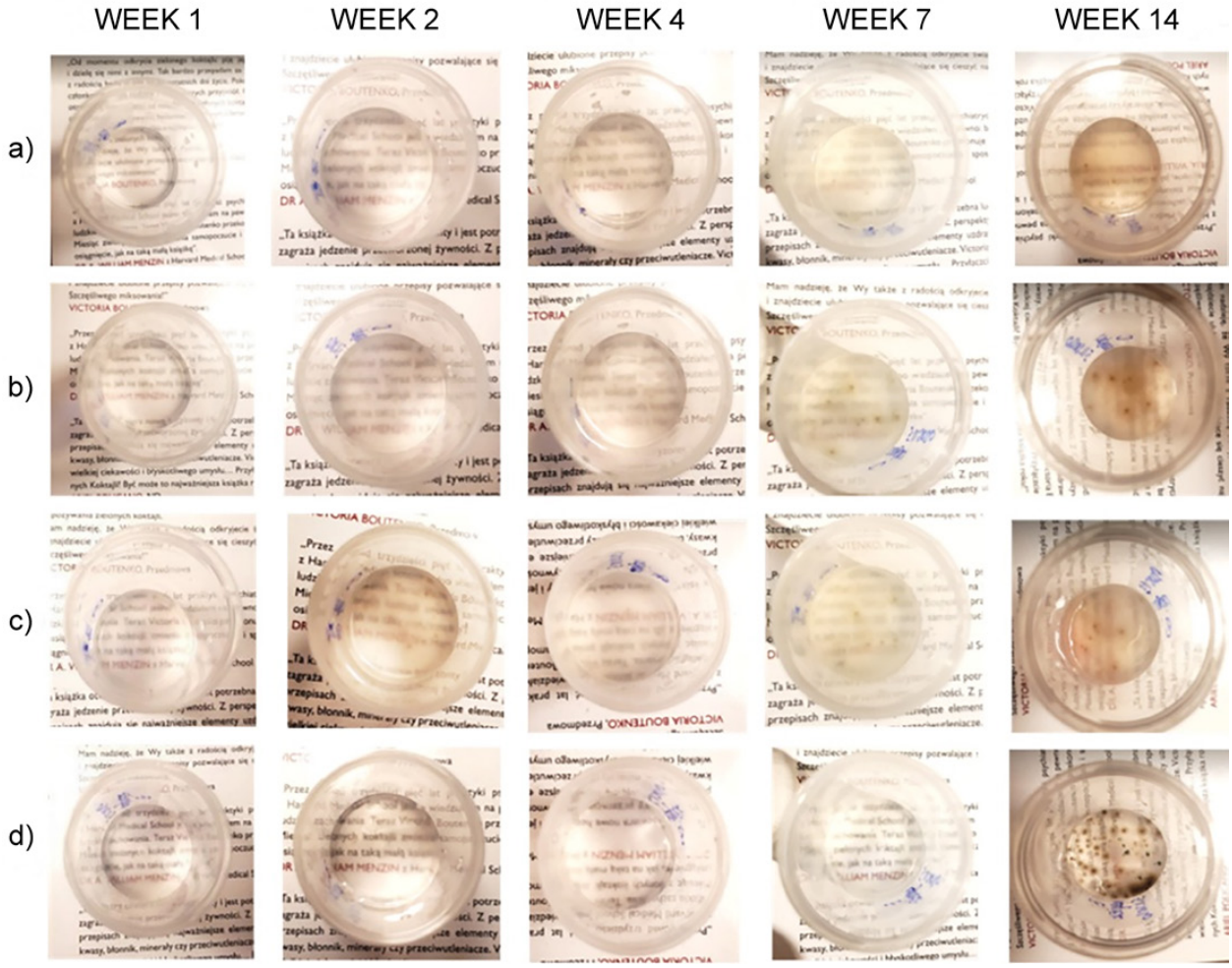

Fig. 12. Comparison of changes in hydrogel appearance over time for: a) 10_GG, b) 10_GG_S5, c) 10_GG_S3_G2, d) 10_GG_G5 (source: photo by Authors)

On the other hand, a comparison of samples B and D indicates that sucrose is the sugar preferred by bacteria and that a decrease in sucrose in favour of glycerol results in a lower microbial population.

Gellan gum itself, as a polysaccharide, can also provide a carbon source. However, it is much more difficult to access microorganisms than sucrose and glycerol, because of the chemical complexity of the substance.

Root system formation test

Investigations into the development of the root system over a 60-day period in the tested media determined the feasibility of using hydrogel media as soil for plants (Figs 13, 14). Figure 13 shows that the Crasulla ovata plant in the water died from putrefactive decay. No deterioration was observed in the condition of the plant grown in the hydrogel medium. The cultivation of hydroponic plants without controlled water circulation in the system makes it impossible to grow many species of plants. A medium consisting of hydrogel spheres, on the other hand, is a versatile medium because of its ability to control the amount of liquid present in the system on an ongoing basis.

The effect of hydrogel medium and water on water loving plants was also analysed using Philodendron scandens as an example (Fig. 14). In this case,

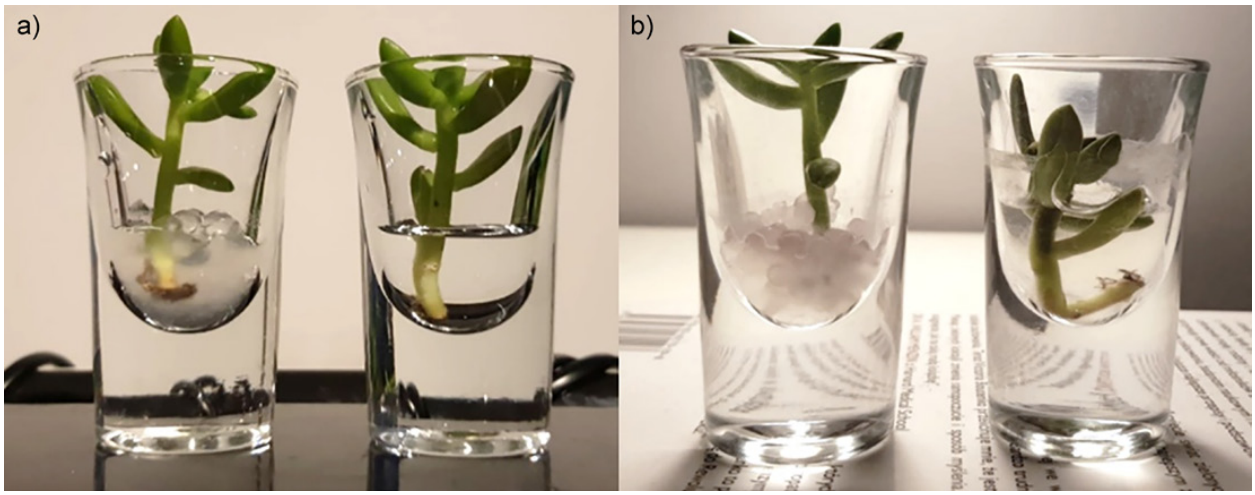

Fig. 13. Crasulla ovata cultured in hydrogel and water on day 1 (a) and after 60 days (b) (source: photo by Authors)

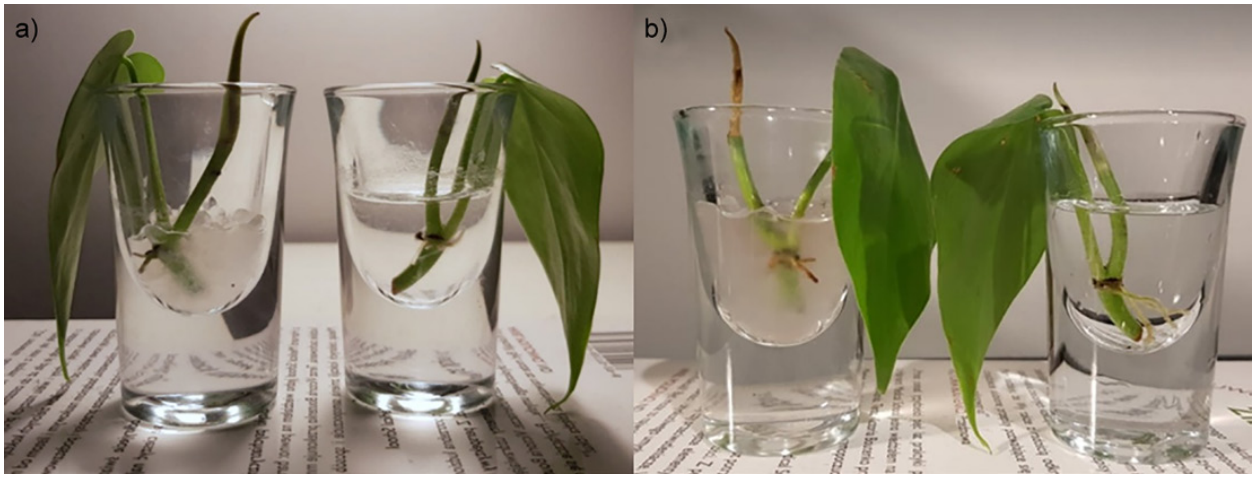

Fig. 14. Philodendron grown in hydrogel and water on day 1 (a) and after 60 days (b) (source: photo by Authors)

the development of both the plant grown in water and the hydrogel was not affected. However, a comparison of the two plants indicated significant differences in the way the root system developed. The root system of a plant grown in water is a typical bundle root system with three adventitious roots of considerable length. The root system of a plant grown in hydrogel medium is also a bundled system, but the differentiation of the adventitious roots is pronounced. The longest is several times shorter than the adventitious roots of the plant cultured in water, while its diameter is about 1,000 μm, which in turn is much higher than the diameter achieved by the other plant, which is about 600 μm (measured from microscopic images – Fig. 15). Growing plants in a homogeneous medium with low viscosity results in an unnatural development of the root system. The use of heterogeneous hydrogel media eliminates this problem and allows for a good reproduction of natural conditions.

Fig. 15. Comparison of the root system of Philodendron grown in water (a) and hydrogel (b) (source: photo by Authors)

DISCUSSION

In a transparent soil application, the transparency, mechanical strength, and biological stability must be carefully matched because a balance between mechanical strength and transparency is required to ensure the hydrogel water retainability without collapsing. The structure of the hydrogel influences how nutrients are released and the mechanical properties of the hydrogel affect how easily plant roots can penetrate and grow within the matrix (Ma et al., 2019). Achieving high transparency in a hydrogel often involves using hydrophilic polymers with low crystallinity and low refractive index that match the water. However, these same factors can sometimes lead to poor mechanical strength. Introducing cross-links between polymer chains can enhance mechanical strength and elasticity, but reduce transparency by increasing light scattering (Zhang et al., 2017).

Our research has shown that the transparency of a hydrogel depends on the amount and type of constituent substances. To the greatest extent, light transmittance is determined by the hydrogel matrix itself. It is therefore crucial to use a minimum amount of gellan gum, which is also sufficient to obtain a stable and robust hydrogel structure. In fact, gellan gum is a natural biomaterial (Zia et al., 2018; Palumbo et al., 2020). As an exopolysaccharide, it has many advantages, which undoubtedly include interesting physicochemical properties and noncytotoxicity (Bacelar et al., 2016). At this point, it is worth mentioning that gellan gum is stable during heating, has a high melting point, high clarity, biocompatibility, and strong gelling ability (Giavasis et al., 2000; Dave and Gor, 2018). Furthermore, it has been suggested that a gellan gum-based hydrogel platform can be extensively chemically modified and subject to mechanical adjustment of its properties (Xu et al., 2018; Muthukumar et al., 2019; Sworn and Stouby, 2021). In addition to gellan gum, commonly used microbial polysaccharides include dextran, alginate, or xanthan (Ahmad et al., 2015). Furthermore, our research shows that an improvement in light transmission through the hydrogel can be achieved by introducing sugar into the system. Various physical and chemical cross-linking methods have been presented during the formation of polysaccharide-based hydrogels. This is because there is variability in the molecular structures of hydrogels. This is related to the formation of poly-saccharide-based hydrogels in different forms, including solid, as a microparticle, a hydrogel membrane, or encapsulated soil (Giavasis et al., 2000). The effect of improved light transmission observed in the study may be due to the interaction of sugar with the polymer chains. The improvement in optical properties is smaller the greater the total amount of sugar and the greater the ratio of sucrose to glycerol. The most favourable, from the point of view of system transparency, is the introduction of sucrose into the system at a concentration of 5%, which is the generally accepted minimum to ensure optimal development conditions for plants. This is because gels using gellan gum appear transparent when the ionic strength and polymer concentration are low. In contrast, increasing the ionic strength results in a more opaque gel, which is thought to be an effect of intermolecular aggregation. Thus, restoration of gel transparency is possible by adding sucrose (Ahmad et al., 2015). In addition, previous studies on the effects of fructose and sucrose on the gelation temperature, clarity, and texture properties of gellan gels have shown that the gelation temperature of gellan solutions increases when sucrose is added, while the inclusion of fructose and sucrose contributes to a significant increase in gel clarity (Tang et al., 2001; Bayarri et al., 2002; Han et al., 2024).

The gellan gum-containing hydrogels obtained show good resistance to high-frequency cyclic loading. The test conditions adopted simulated operation in the most demanding environment in which a hydrogel can be used, i.e. 30°C. The stresses developed in the hydrogel during cyclic loading cause the material to strengthen as a result of water loss. The plants growing in transparent soil developed in a physiological manner, not unlike the development in soil. The substrate stabilises the plant in the soil without restricting its growth. Transparent soil is a highly versatile medium due to its ability to adapt the level of hydration of the environment to the needs of the plant, which means it can be used to grow a wide range of plants. An important aspect is the amount and type of nutrients, as these are supplied with the water introduced into the system after the hydrogel has been given its final form. In this way, macronutrients, micronutrients, vitamins, and hormones can be individually selected to suit the

plant's stage of development and dynamically changing requirements. The experiment was not conducted under aseptic conditions to provide a reliable simulation of the expected operating conditions. Thus, it is possible to estimate the length of time for which the substrate retains satisfactory properties. For the sample most resistant to microbial attack, this is not longer than 50 days. The limited lifespan is a problem that can be overcome by regularly replacing the substrate. The used hydrogel is a substance that presents no disposal problems, as it is easily biodegradable under the influence of fungi and microorganisms. In the final stage, it decomposes into CO_2 and water. Furthermore, the hydrogel form (spheres approximately 2 mm in size) allows the process to be carried out non-invasively, so that the root system of the plants remains intact (Ma et al., 2019; Wei et al. 2019). Similar findings on root growth in hydrogel substrates were reported in a paper (Downie et al., 2012). Our study compared two ways of obtaining hydrogels and determined the natural time of use of the material as soil; additionally, the composition was adjusted to optimise the effectiveness of the substrate and the time of use needed in the initial phase of plant growth (seedling).

Hydrogels, with their unique ability to absorb and retain large amounts of water, have garnered significant attention for agricultural applications (Fuchs et al., 2020). However, the synthesis of some hydrogels can be expensive, hindering their widespread adoption (Sanllehí and Barceló, 2024) and scaling up hydrogel production from laboratory to industrial levels can be challenging (Alonso et al., 2021). Their application circulates around improving soil water retention, reducing the frequency of irrigation, and mitigating the effects of drought stress on crops. This is particularly valuable in arid and semi-arid regions (Omar and Alsharaeh, 2024). Hydrogels are the most promising in promoting germination and early seedling growth (Prisa and Guerrini, 2022). The possibility of monitoring the development of the root system is an important aspect from the point of view of plant cultivation units. For plants growing in nutrient- and water-poor soils, it is essential to have a well-developed root system. Therefore, solving the problem of increasing food demand is considered by increasing the efficiency of plant cultivation (Bengough et al., 2011; Xie et al., 2024).

Research is underway on new hydrogel materials with improved optical, mechanical, and biological properties that will better mimic the natural soil environment. Combining transparent soils with advanced imaging techniques will provide even more detailed information about soil processes. Transparent soils can become an important tool in precision agriculture, allowing one to optimise crops and minimise the negative impact of agriculture on the environment.

CONCLUSIONS

Methods and techniques of materials engineering, as well as knowledge of the physicochemical conditions of the substances used to create transparent substrates, allow the development of hydrogel soils targeted to address specific plant requirements. When transparent soil is introduced as a highly versatile medium, it allows to precisely control the hydration level of the environment and match it to the needs of the plant, therefore, the substrate can be used to grow many species of plants. Hydrogel substrates allow for precise control of the amount and type of nutrients that are delivered to the system after the hydrogel is given its final form. Thus, macronutrients, micronutrients, vitamins, and hormones can be individually selected according to the plant's stage of development and dynamically changing requirements. Interventions can be made at any step in the development of the root system, for example, by replacing the substrate. The study tested a transparent hydrogel based on gellan gum in 2 forms (spheres and blocks) for use in agriculture. The hydrogel was modified with additives to improve its transparency and resistance to microorganisms while remaining biodegradable. The form of hydrogel (spheres about 2 mm in size) allows the process to be carried out non-invasively, so that the root system of plants remains intact. The work succeeded in obtaining a transparent soil that remains transparent for an extended period of time after manufacture, is resistant to microorganisms, and does not lose its mechanical properties. In the course of the work, the optimal concentration of additives that modified the hydrogel was determined. The introduction of transparent soils will allow monitoring of plant growth, development of the root system, and appropriate selection of seedlings. In precision farming, a compre-

hensive management system is an important element, adapting specific agrotechnical elements to changing conditions in different parts of the field. The process depends not only on the current state of plant development, but also on the properties of the soil. The development of transparent hydrogels and their use as transparent soils represents a significant advance with broad-reaching consequences. The transparent hydrogel soil offers a powerful tool to revolutionise agricultural practices, advance sustainable development goals, and shape future research directions. By supporting interdisciplinary collaboration and integration with advanced technologies, transparent soils have the potential to transform our understanding of plant-soil interactions and contribute to a more sustainable and food-secure future.

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ZAPROJEKTOWANIE TRANSPARENTNEGO SUBSTYTUTU GLEBY NA BAZIE HYDROŻELU DO ZASTOSOWAŃ W PRECYZYJNYM ROLNICTWIE

ABSTRAKT

Cel pracy
Celem opisanych badań było opracowanie przezroczystych gleb hydrożelowych do monitorowania rozwoju systemu korzeniowego.

Materiał i metody
Hydrożele polisacharydowe wytworzono z gumy gellan, sacharozy i gliceryny przy użyciu podłoża uniwersalnego. Do oceny podstawowych właściwości hydrożeli wykorzystano testy absorbancji, obserwacje mikroskopowe i analizę termiczną (TG, DSC, DMA). Przeprowadzono również obserwację wzrostu pleśni na podłożu oraz przeanalizowano wpływ opracowanego podłoża na wzrost roślin.

Wyniki i wnioski
Badania wykazały, że dla uzyskania dobrej przezroczystości kluczowe jest zastosowanie minimalnej ilości gumy gellan. Hydrożele wykazują dobrą odporność na obciążenia cykliczne oraz odpowiednią stabilność termiczną. Rośliny rosnące w otrzymanej przezroczystej glebie rozwijały się w podobny sposób, jak w glebie. Gleba transparentna jest bardzo uniwersalnym podłożem ze względu na możliwość dostosowania poziomu uwodnienia środowiska do potrzeb roślin oraz monitorowania ich wzrostu, rozwoju systemu korzeniowego i odpowiedniej selekcji sadzonek.

Słowa kluczowe: hydrożele, system korzeniowy, rolnictwo