Chemical and Process Engineering: New Frontiers, 2024, 45(1), e49
DOI: 10.24425/cpe.2023.147408

Biobased hydrogels as functional platforms for biocatalysis, nutrition and medical applications

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Presented at 24th Polish Conference of Chemical and Process Engineering, 13–16 June 2023, Szczecin, Poland.

Article info:
Received: 11 May 2023
Revised: 20 August 2023
Accepted: 06 September 2023

Abstract
Biobased hydrogels are three-dimensional polymeric matrices with a unique high water-holding capacity, which are mainly obtained from polysaccharides and proteins. Such a variety of natural polymer structures offers a range of hydrogel products with interesting physicochemical and biological properties. Nowadays, these matrices are already used in many industrial and environmental fields, which is considered extremely important. Moreover, the literature on the subject is constantly expanding, especially in areas of scientific research. The main purpose of this article is to briefly review the current development of matrix composition and properties of hydrogels of natural origin, considered as functional platforms in three application areas, primarily in biocatalysis, nutrition and medicine. The description of individual issues in the present article is supported by examples of case studies described in our previously published research papers, as well as considered in current projects of our research group.

Keywords
bioengineering, functional biomaterials, natural hydrogels, applications

1. INTRODUCTION

Currently, the eyes of the whole world are turned towards the dizzying degeneration of the natural environment, caused mainly by the rapidly ever-increasing rate of production and consumption of various commercial products. As a result of both the industrial manufacturing processes and the worldwide use of the goods produced, countless amounts of waste and pollution are generated, posing a huge threat to the environment. In particular, the chemical, nutrition and medical sectors produce millions of tons of by-products, and waste effluents contributing to the high carbon footprint (Naidu et al., 2021; Pandey, 2021; Sharma et al., 2022). Therefore, effective minimization of these adverse effects is crucial for regaining balance in the co-existence of mankind and nature. One of the possibilities of tackling this challenge is to leverage the achievements of bioengineering in providing sustainable, eco-friendly solutions to the production of biocatalysts and biomaterials with improved applicability. In this regard, one of the rapidly developing directions is the application of natural-derived hydrogels.

Hydrogels are hydrophilic materials that form three-dimensional structures based on cross-linked polymer chains and water bound in the voids arising between them. They can be obtained from compounds of synthetic and natural origin and create homopolymeric materials as well as hybrid matrices in the form of co-polymer blocks or interpenetrating networks based on blends of two or more substrates (Ahmed, 2015). These materials have gained wide popularity due to their desired physicochemical properties. The most important include porosity, considerable permeability for water-soluble compounds, ability to gas exchange, flexibility, tunable stiffness, favourable absorption and swelling properties, as well as the ability to maintain their three-dimensional structure thanks to water retained between the polymer chains forming the network (Ahmed, 2015; Chamkouri and Chamkouri, 2021). Adding biodegradability and biocompatibility to these features makes in particular hydrogels of natural origin the materials of greatest interest in sustainable and environmentally safe applications (Bercea, 2022; Kaczmarek et al., 2020).

Hydrogel-based materials have been known for many years. According to the Web of Science (WoS) bibliographic data (keywords: *hydrogel*, search scope: *Topic – title, abstract, author keywords, and Keywords Plus*), the research exploration of these structures began in the early 20th century. The first documented mention in this field was J.M. van Bemmelen's article from 1902, concerning research on the influence of temperature on the structure of the silicic acid hydrogel (van Bemmelen, 1902). As shown in Figure 1A, in the decades between 1900 and 1959, the results of several to a dozen studies were reported. They are mostly concerned with the preparation methods and determination of the physicochemical properties of inorganic hydrogels (Foote and Saxton, 1916; Hurd and Merz, 1946; Simon and Feher, 1931). However, the beginning of the significant research development on hydrogels defined as hydrophilic polymeric materials dates back to the 1960s.

At that time, the papers referred to detailed characterization of these materials and were primarily focused on permeation and rheological properties (Arakawa, 1962; Refojo, 1965; Watase and Arakawa, 1968). The 1970s were a real bloom of research interest in the practical application of hydrogels (Cowsar et al., 1976; Gilding et al., 1978; Peppas and Merrill, 1977; Weschsler and Wilson, 1978) which has been developing dynamically until today (Figure 1A). Moreover, in these years, the same tendency for hydrogel research in the category of biomaterials science was noticed as well (Figure 1B). To demonstrate the ever-growing interest in biobased hydrogels in terms of practical applications a variety of different commercial sectors using these materials should be mentioned, like regenerative medicine, pharmacy, catalysis, diagnostics, agriculture, environment protection, food processing, or cosmetic industry (Bercea, 2022; Kapusta et al., 2023; Klein and Poverenov, 2020; Qureshi et al., 2022; Salehipour et al., 2023). However, the most commercially prevalent hydrogel products on the market are those for medical applications. These include wound dressings (e.g., Suprasorb®G, Neoheal®Hydrogel, NU-GEL^{TM}, Purlion®, INTRASITE®Gel), soft contact lenses (e.g., Airsoft^{TM}, Gentle 59, Miacare^{TM} 1day) scaffolds imitating natural extracellular matrix (e.g., Biogelx^{TM}, Corning®Matrigel®matrix, Corning®PuraMatrix^{TM} peptide hydrogel), injectable hydrogels for tissue regeneration (e.g., Gelrin C^{TM}, Mebiol®Gel) and drug delivery implants (e.g., Cervidil®, SUPPRELIN®LA) (Aswathy et al., 2020).

According to the report 'Hydrogel Market: Global Industry Trends, Share, Size, Growth, Opportunity and Forecast 2023-2028' published by IMARC Group, the worldwide hydrogel market reached the US$ 26.2 Billion in 2022. Looking forward, they expect that it will reach US$ 36.9 Billion by 2028, with a 5.7% growth rate (CAGR) during 2023-2028. Currently, the main driving force that stimulates such dynamic development of the hydrogel market is the constantly growing demand for sustainable biodegradable materials. This is due to the increasing environmental concerns and the need to reduce the environmental impact of synthetic hydrogel products. Therefore, there is ever-growing interest in obtaining functional hydrogel matrices based on readily available, naturally originated polymers – mainly proteins, polysaccharides and their derivatives. In the presented context, the most commonly studied raw material of protein origin is gelatine, with a typical peptide sequence of Gly-X-Y, where Gly is glycine and X and Y are any other amino acids (Labus et al., 2020). Its strands contain between 50 and 1000 units that form a left-handed helical structure and can be easily cross-linked to form a hydrogel. However, due to their easy accessibility and wide structural diversity, polysaccharides are the most frequently used biopolymers for the production of hydrogels. The most popular ones include alginate, chitosan, dextran, κ-carrageenan, agarose and pectins (Figure 2). Their additional advantage is a significant number of reactive functional groups, which can effectively act as centers that enable the formation of various types of interactions/links between polymer chains resulting in the creation of stable 3D hydrogel structures.

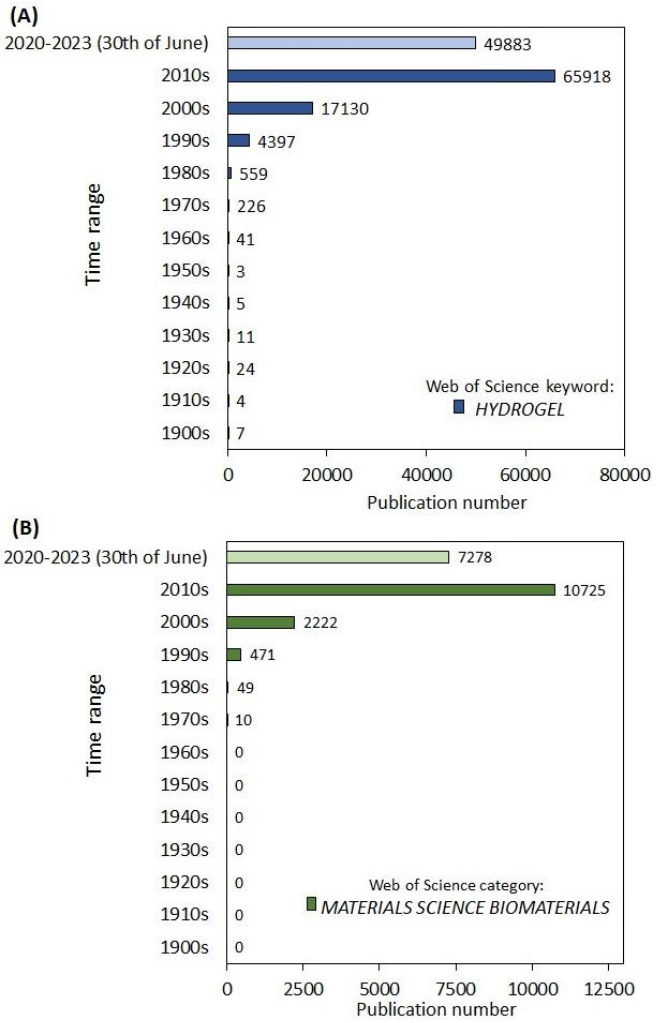


Figure 1. Bibliographic data from the Web of Science database (A) number of papers per decade searched for the keyword *hydrogels* in the search scope: *Topic – title, abstract, author keywords, and Keywords Plus*; (B) number of papers on hydrogels assigned per decade to the research category: *Material Science Biomaterials*.

In the current work, we present the contribution of our research group to the development of the indicated bioprocess engineering approach by providing functional biobased hydrogel materials dedicated to specified applications in industrial biocatalysis, food technology and medicine. After providing a brief literature background on each topic, we present particular case studies in the given field based on the overview of our previously published or currently considered research. The main aim of each application presented was to apply readily accessible biopolymers for hydrogel system creation, with the maximum simplification of the used manufacturing procedures while maintaining the highest quality and functionality of the obtained products.

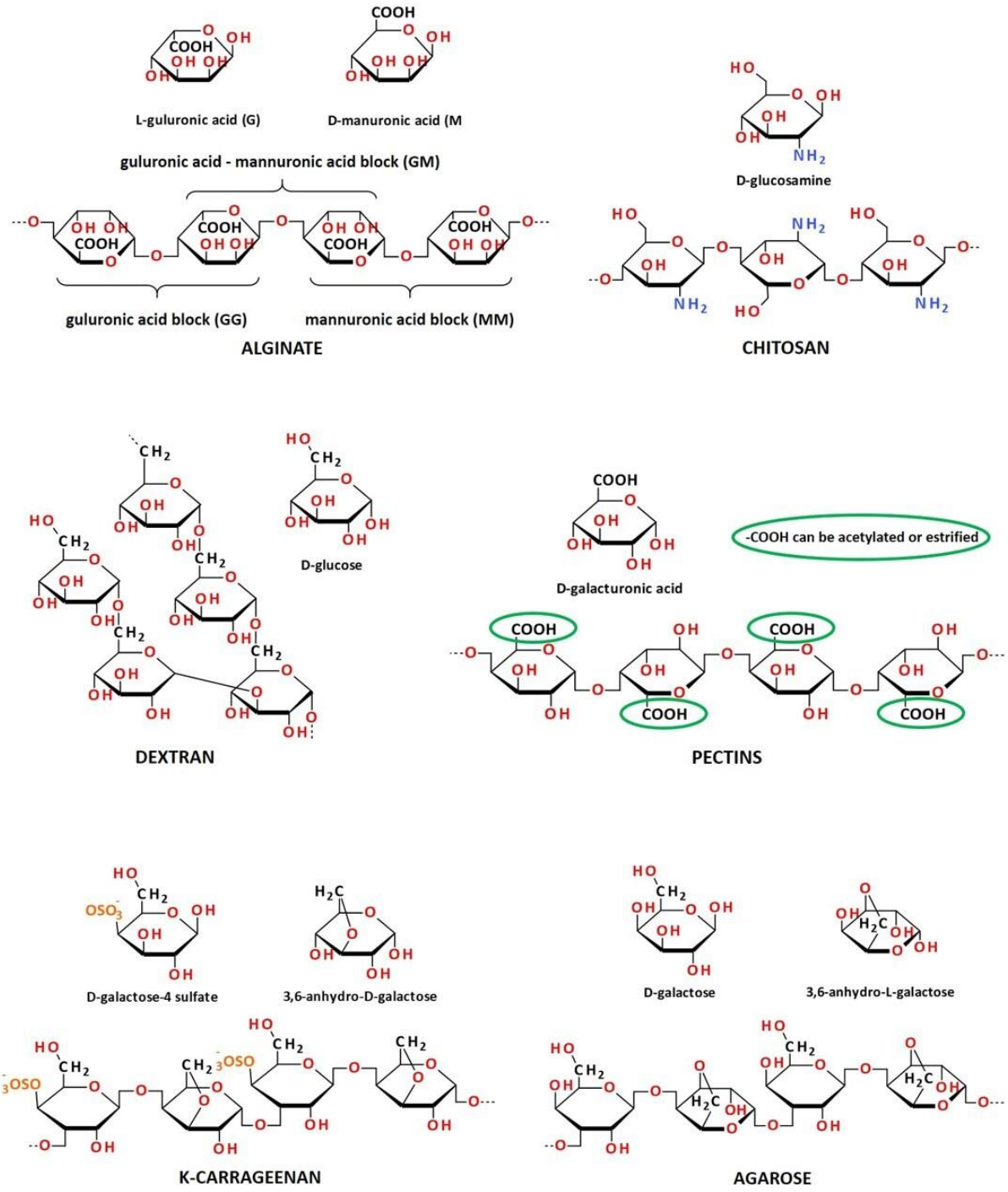


Figure 2. Chemical structures of selected polysaccharides most often used for hydrogel production and their basic building blocks.

2. BIOBASED HYDROGELS IN BIOCATALYSIS

2.1. Brief overview of current applications

In catalysis, the most popular application of naturally-derived hydrogels is the creation of long-lasting biocatalysts with high enzymatic efficacy and enhanced operational stability (Bilal and Iqbal, 2019; Maroufi et al., 2022; Salehipour et al., 2023; Tan et al., 2021). These materials are favourable carrier candidates for enzyme immobilization due to good permeability, biocompatibility, biodegradability, flexibility, mass transfer properties, non-toxicity and wide accessibility (Bercea, 2022; Kaczmarek et al., 2020). In addition, the presence of multiple reactive groups (e.g., amino, carboxylic, and/or hydroxyl moieties) in biopolymers makes them highly susceptible to modification and cross-linking with different chemical compounds. Therefore, the functional properties of the matrices can be easily adapted to the requirements of a given biocatalytic process. Single enzymes as well as multienzyme

systems can be immobilized in hydrogels (Tan et al., 2021). Among others, biopolymers such as alginate, chitosan, cellulose, agarose, guar gum, agar, carrageenan, dextran, xanthan, pectins, gelatine are used to fabricate hydrogel-based carriers (Bilal and Iqbal, 2019; Nikoshvili et al. 2023). The most common method of retaining enzymes in hydrogels is *in situ* encapsulation/entrapment during the formation of the cross-linked biopolymer matrix (Imam et al., 2021; Maroufi et al., 2022). In this case, the biocatalyst molecules are physically confined between the network-forming polymer chains, which results in maintaining their native conformation responsible for catalytic activity. From other immobilization methods, the covalent bonding and adsorption on the previously produced material are used, as well as additional chemical cross-linking to protect the enzyme from leaching from the support (Maroufi et al., 2022; Nikoshvili et al. 2023). Due to the growing pro-ecological awareness, there is intensification of research efforts focused on diverse applications of biohydrogel-assisted enzymes in food, environmental, pharmaceutical, medical and energy sectors (Bilal and Iqbal, 2019; Tan et al., 2021).

The following application is constructing highly selective and sensitive biosensors by using enzyme-loaded biobased hydrogels as a key detection element (Roquero and Katz, 2022; Tan et al., 2021; Tavakoli and Tang, 2017). Reliable determination of trace amounts of the target compounds is particularly important in medical diagnostics for selective detection of specific metabolites, as well as in monitoring the level of hazardous chemicals in post-production wastewater and water reservoirs. Among others, glucose, lactate, cholesterol, urea, or hydrogen peroxide can be assayed using such biosensing systems (Nagamine et al., 2019; Roquero and Katz, 2022; Tavakoli and Tang, 2017).

Naturally derived hydrogels can also be used to produce colourimetric screening tests relevant to biocatalytic studies. Most often, they are designed to quickly and easily determine enzymes of the desired catalytic activity in multi-component solutions (e.g., post-culture fluids). However, they can also be used to estimate favourable process conditions (i.e., pH) or to determine the specificity of substrates, activators or inhibitors of the biocatalyst tested.

The assays described here consist of a substrate immobilized in a hydrogel matrix, which reacts with the target analyte – an enzyme in the sample, producing a visual effect in the form of a change in matrix colour. For example, these tests have been used to detect the following enzymes: diacetylchitobiose deacetylase involved in the biosynthesis of glucosamine, an important polysaccharide that builds cartilage tissue (Sun et al., 2022), galactosidase and glucuronidase secreted by *Escherichia coli* in a test to detect this bacterium and its contamination of water samples (Gunda et al., 2016), alkaline phosphatase, which is an important marker of diseases of the liver, bone tissue and endocrine system (Wang et al., 2022), or β-galactosidase (Labus, 2018) responsible for the hydrolysis of lactose in dairy products, enabling the consumption of food products by people with lactose intolerance. In the examples discussed, substrates were immobilized in gelatine (Labus et al., 2018; Sun et al., 2022), agarose (Gunda et al., 2016), and alginate (Wang et al., 2022) matrices. Another known application of the phenomenon of color change due to enzymatic activity is the method of agar plates in which a substrate typical for the detection of a microorganism producing a specific enzyme is trapped in the agar. This method was used to identify laccase-producing microorganisms by preparing agar plates containing guaiacol, tannic acid (Fu et al., 2013) or ABTS (Kiiskinen et al., 2004).

2.2. Research contribution to the development of current trends

2.2.1. Matrices for immobilization of biocatalysts

In our previous study '*Comparative Study on Enzyme Immobilization Using Natural Hydrogel Matrices – Experimental Studies Supported by Molecular Model Analysis*' (Labus et al., 2020), we focused on testing two natural hydrogels (alginate and gelatine) as carriers for immobilization of invertase from *Saccharomyces cerevisiae* (EC 3.2.1.26). We proposed a novel approach in this paper. We tested the applicability of initial molecular insight into structural characteristics for estimation of the suitability of given matrices for enzyme immobilization. The concept of the performed research is presented in Figure 3.

At first, using structural models of both materials and invertase, we performed the molecular analysis of such hydrogel-biocatalyst systems. As a result, we have obtained a preliminary view that the gelatine-bound enzyme has a higher potential for efficient deployment. In order to confirm our theoretical considerations, the standard experimental studies on production conditions of immobilized invertase preparations were performed. In this regard, different concentrations of primary cross-linking agents (2–30% w/v of $CaCl_2$ and 0.25–4.0% microbial transglutaminase for alginate and gelatine, respectively) were tested. After that, it was determined whether post-immobilization additional cross-linking step with glutaraldehyde (concentration range 0.5–4.0% v/v, process time 5–60 min) is necessary to obtain stable invertase entrapped in alginate and gelatin-based hydrogel matrix. As a result, 2.0% glutaraldehyde and a cross-linking time of 30 min were selected for the alginate applied as a carrier. When gelatine-based material was used, a concentration of 0.5% glutaraldehyde and treatment for 10 minutes was sufficient to obtain a stable invertase preparation. In order to select the most favourable hydrogel-invertase system, the characteristic features of both preparations were determined. The results obtained in the cited study (Labus et al., 2020), with a comparison to the native form of the tested enzyme, are summarized in Table 1.

Table 1. Selected features of invertase immobilized in alginate and gelatine-based hydrogels in comparison to the native form of the enzyme (Labus et al., 2020).

Factor	Native invertase	Alginate entrapped invertase	Gelatine entrapped invertase
Activity[a], U/mL	19 550	8 383	3 801
Immobilization yield[b], %	–	42.9	19.4
Enzyme leakage[c] (4 °C, pH 4.5, 24h), %	–	5.5	0.0
Thermostability[c] (60 °C, 1h), %	42	60	70
Operational stability[c] (after 10 consecutive cycles), %	–	55	95
Storage stability[c] (4 °C, 28 days), %	32	70	82

[a] – one unit of enzyme activity (1 U) was defined as 1 µg of glucose formed within 1 min of sucrose hydrolysis catalyzed by invertase;
[b] – immobilization yield was determined as the activity of bound enzyme divided by the activity of the native form of the enzyme, expressed in %;
[c] – relative activity determined in defined conditions and expressed in %.

According to the higher thermal, operational and storage stability and complete retention of enzyme in the hydrogel network, the gelatine-based material was found to be the most preferred choice for invertase immobilization. In this way, the experimental findings justified our theoretical assumptions. Moreover, the suitability of the proposed preliminary structural analysis on a molecular level as a valuable tool for the pre-selection of preferable hydrogel matrices for the immobilization of a given enzyme was confirmed as well.

2.2.2. Screening colourimetric plate assays for biocatalytic purposes

In a recent paper (Labus and Maniak, 2023), we reported the development of sensitive and selective well-plate assays for the determination of enzyme catalytic properties. We have considered two approaches to produce reliable colorimetric tests for various screening applications in biocatalysis. In both cases, the supporting material was gelatine hydrogel, but enriched with varied reactive components – substrate or enzyme. For examination of the applicability of the considered systems, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate) sodium salt (ABTS) as the substrate and laccase from *Trametes versicolor* (EC 1.10.3.2) as the enzyme were used, respectively. The operating principle of the provided test was based on the conversion of a transparent ABTS into a green-blue coloured product in a reaction catalyzed by laccase, which resulted in a rapid visual response (Figure 4).

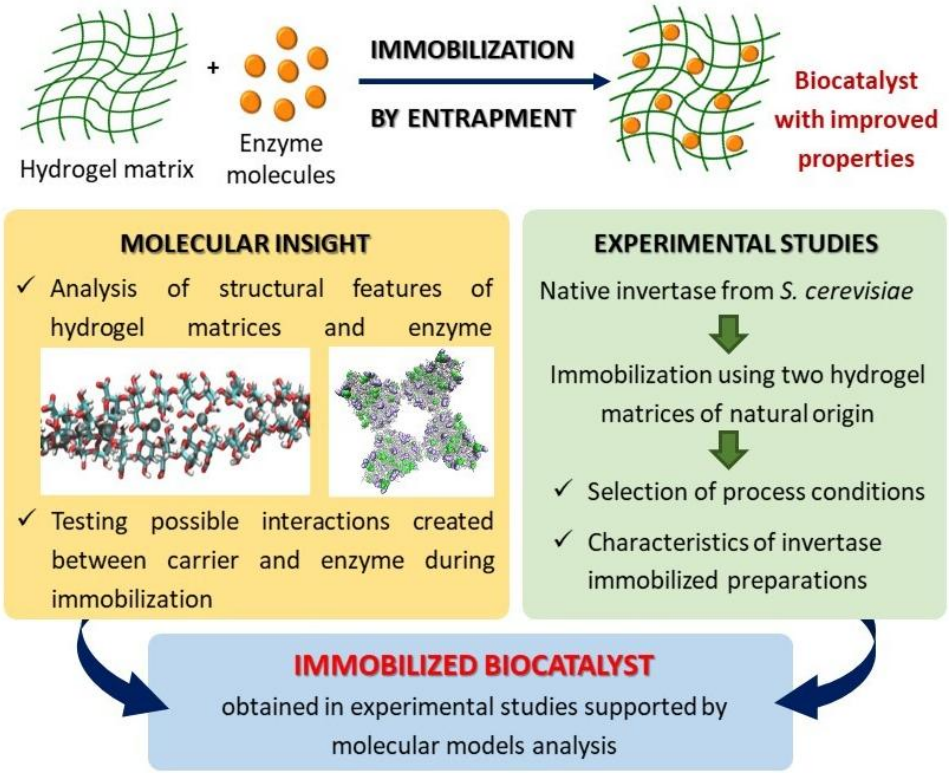


Figure 3. Research concept of a comparative study on enzyme immobilization using natural hydrogel matrices (graphical abstract modified with permission from (Labus et al., 2020)).

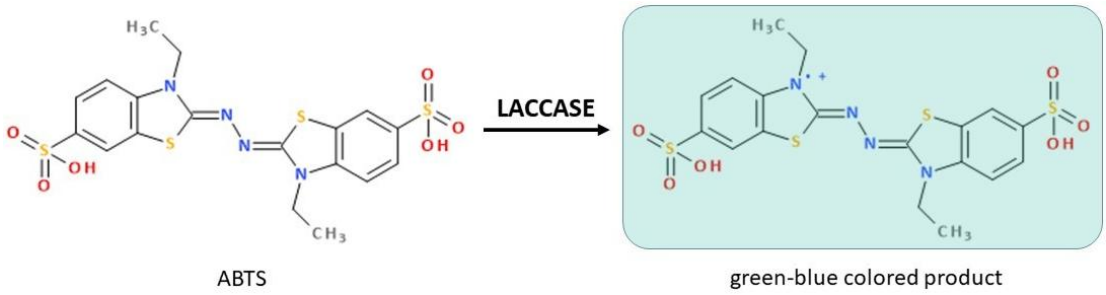


Figure 4. The oxidation of ABTS catalysed by laccase (modified with permission from (Labus and Maniak, 2023)).

The scheme of possible operation modes of the well-plate assay developed within this study is presented in Figure 5. The first approach concerned using the ABTS immobilized in gelatine-based hydrogel for visual detection of laccase in aqueous samples (Figure 5, blue pathway). We examined the performance of this test under various conditions of concentration, time and pH. As a result, we obtained the colourimetric test that can detect laccase in the concentration range of 2.5–100 mg/L in at least 20 minutes; is reliable at pH 3.0–6.0 and preserves high stability and functionality under storage conditions (4 °C, 30 days). It was also experimentally confirmed that the proposed analytical tool can be effectively used to determine the presence of trace amounts of laccase in a liquid microbial culture (white-rot fungus *Cerrena unicolor*).

The second approach involved the construction of reversed detection system (Figure 5, red pathway). Here the hydrogel-confined laccase was used for screening substrate specificity and potential inhibitory compounds of this enzyme. In the case of the presented studies, ABTS, syringaldazine and guaiacol were tested as substrates and sodium azide as a potential inhibitor. Only 10 minutes of the assay were needed to obtain an evident visual response for the strength of enzyme action on the given substrates. Whereas, testing the sodium azide in the range of 0.5–100 mg/L showed a distinct inhibition effect in 20 min for 0.5 mg/L and total inhibition for $\geq$ 75 mg/L.

Using differentiated approaches presented by our group, multifunctional colourimetric plate assays for screening purposes in biocatalysis have been developed. These tests can be adapted also to other enzyme systems. This is a huge advantage in the case of rapid enzymological preliminary studies aimed at searching for an enzyme with the desired catalytic properties. The only limitation is finding a substrate that will be converted to a coloured product and will be targeted for a given biocatalyst.

3. BIOBASED HYDROGELS IN FOOD INDUSTRY

3.1. Brief overview of current applications

Hydrogels are widely used in the food industry as functional components (Prado and Prado, 2021). Depending on the application, they can fulfill the role of the texture modifier, gelling agent, moisture improver, water holding, or film formation (Zhang et al., 2020). Edible hydrogels can be divided

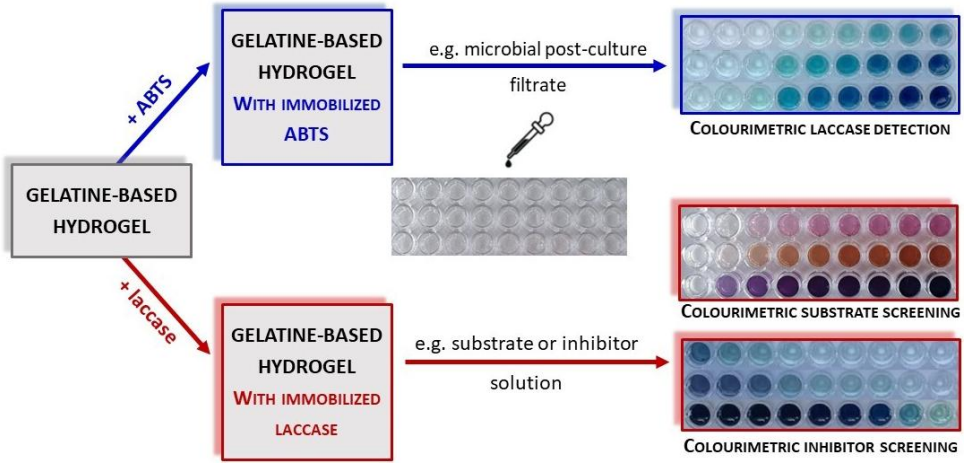


Figure 5. Scheme of operation of different versions of hydrogel-based colourimetric tests using laccase as a model biocatalyst (graphical abstract modified with permission from (Labus and Maniak, 2023)).

by origin: plant-derived (pectin, gum arabic, konjac glucomannan, guar gum), algal-derived (carrageenan, agar, alginate), animal-derived (gelatine) and microbial-derived (gellan gum, xanthan gum) (Zha et al., 2021; Zhang et al., 2020). The gel formation occurs by heating and subsequent cooling of the compound or by introducing particular ions (usually divalent cations) and chemicals or by pH adjustment (Zhang et al., 2020). The selection of hydrogel depends on the intended use and the food production process. The hydrogel structure takes the form of fluid gel (dispersed hydrogel particles in a non-hydrogel mixture) or gellies – gel networks with diverse textures: flexible or hard (Zhang et al., 2020). The fluid gels are utilized as emulsion and foam stabilizers (yoghurts, sauces, cocktails, ice cream) and texture modifiers (dairy products, plant drinks, sweets). The gels are mainly used in the confectionery and meat industry as a gelling agent, improving moisture and protecting the contents from the external environment as a gel coating (Zhang et al., 2020). The hydrogels are particularly often used for low-caloric products as fat replacers (Razavi and Behrouzian, 2018) and food formation through the 3D printing process (Lv et al., 2023).

Hydrogels are also alternatives to commonly used materials for food packaging systems. The usage of biobased hydrogels aims to reduce the production of non-biodegradable waste and create a more eco-friendly food industry (Ali and Ahmed, 2018). Among the advantages of this application are biodegradability, swelling properties, non-toxic reagent usage in the production process and the ability to develop smart packaging systems (Batista et al., 2019; Manzoor et al., 2022). They commonly take the form of films, coatings and nanofibers (Manzoor et al., 2022). Extrusion, electrospinning, spraying, wrapping, solvent casting and casting by air are some of the used methods (Ali and Ahmed, 2018; Manzoor et al., 2022). Materials with absorbent properties are especially in demand, because the moisture or exudates inside the package of food are associated with an increase in water activity (A_w) (Batista et al., 2019). The limitation of A_w by hydrogel usage may result in the reduction of the growth of microorganisms and the extension of shelf-life (Batista et al., 2019). However, efforts are made to ensure that the resulting materials fulfill similar mechanical properties to commonly used synthetic polymers (Ali and Ahmed, 2018).

3.2. Research contribution to the development of current trends

3.2.1. Edible coatings

One of the current research considered in our group is the development of edible hydrogel coatings extending the durability of fresh fruits. In this scope, we have been focused on the creation of Ca-alginate hydrogel thin films enriched with various active agents for the prolongation of the shelf-life of freshly sliced apples. The coating procedure was performed according to the scheme provided in Figure 6.

Briefly, peeled apple slices were coated with a small amount of an aqueous solution of sodium alginate (2% w/v) and glycerin (1% v/v) containing essential oils (1% v/v) and/or ascorbic acid (2% w/v). Then, immersed in a cross-linking bath (5–10% w/v aqueous solution of calcium chloride) for a few minutes. Finally, the apple pieces surrounded by one or more layers of functionalized Ca-alginate coating were dried at atmospheric pressure and ambient temperature. Ascorbic acid has been used as a common anti-browning agent. Lemongrass, thyme, rosemary, clove, black pepper, marjoram and eucalyptus essential oils have been tested to ensure the microbiological purity of the tested samples.

Preliminary studies have shown that the addition of ascorbic acid to pure alginate observably delayed the browning process of coated apple slices. Furthermore, the separate addition of essential oils of lemongrass, thyme, clove and marjoram has resulted in effective protection of pieces of fresh apple from mould infection for no less than 13 days (Figure 7).

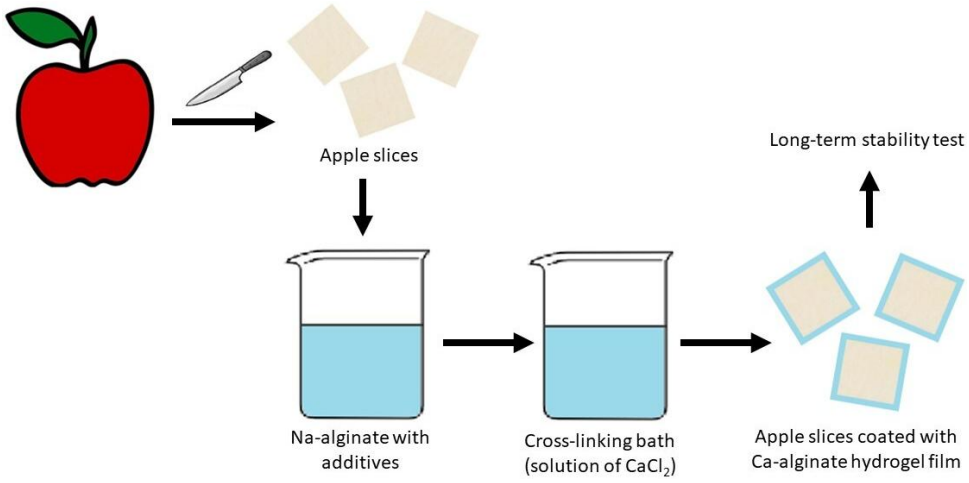


Figure 6. Scheme of coating freshly sliced apple slices with an alginate hydrogel coating enriched with functional additives.

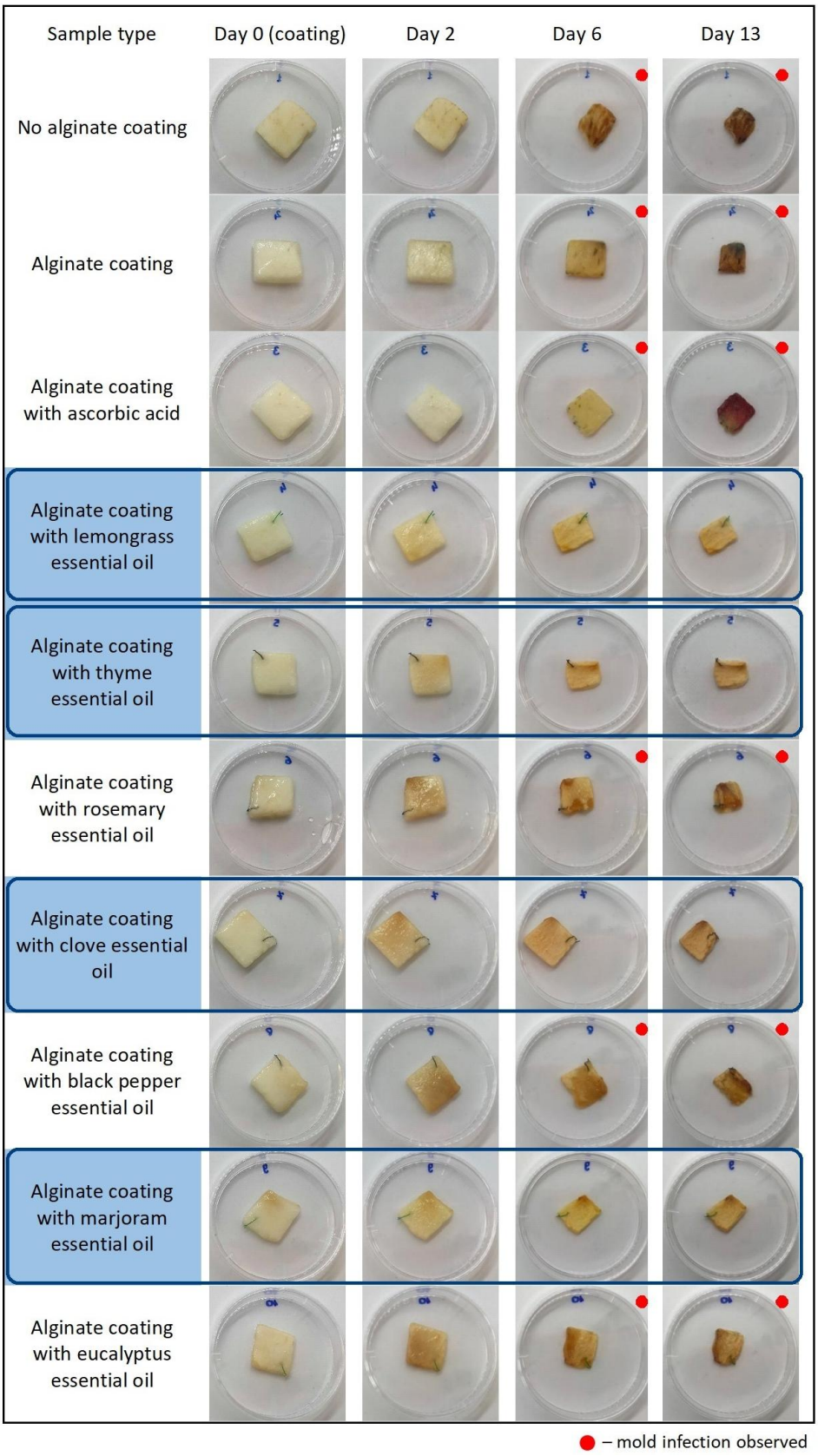


Figure 7. The effect of separate addition of ascorbic acid and various plant essentials on the functionality of the alginate-based edible hydrogel coatings tested for prolonging the durability of freshly cut apple pieces.

In the next step, the effect of the simultaneous addition of ascorbic acid and selected essential oils on the functionality of the edible alginate hydrogel coating as a protective agent with a dual antimicrobial-antioxidant activity was tested. The condition of the samples has been visually tested for over 1 month (Figure 8). As can be observed, the best result has been obtained for the combination of ascorbic acid with clove essential oil. This blend efficiently protected fresh apple slices from browning and microbial contamination and ensured the lowest water loss in the tested sample.

Although the effect of the coating enriched with a mixture of ascorbic acid and clove essential oil was very promising, the observed weight loss of the sample during storage was still not acceptable. Therefore, it was checked whether placing more layers of Ca-alginate film on freshly cut apple slices would eliminate this adverse effect. As can be observed (Figure 9), the proposed solution was successful and two layers of enriched hydrogel effectively protected the sample from noticeable dehydration.

The studies carried out so far were aimed at the preliminary screening of edible hydrogel coating formulations based on Ca-alginate enriched with active 'green' additives and were not published elsewhere. This research is continued towards developing edible coating materials with unique functionality based solely on natural, non-animal ingredients that can be tailored to given food product demands.

3.2.2. Functional vegan food products

The next research topic recently considered in our group was the development of novel vegan hydrogel formulations enriched with favourable nutritional and health-

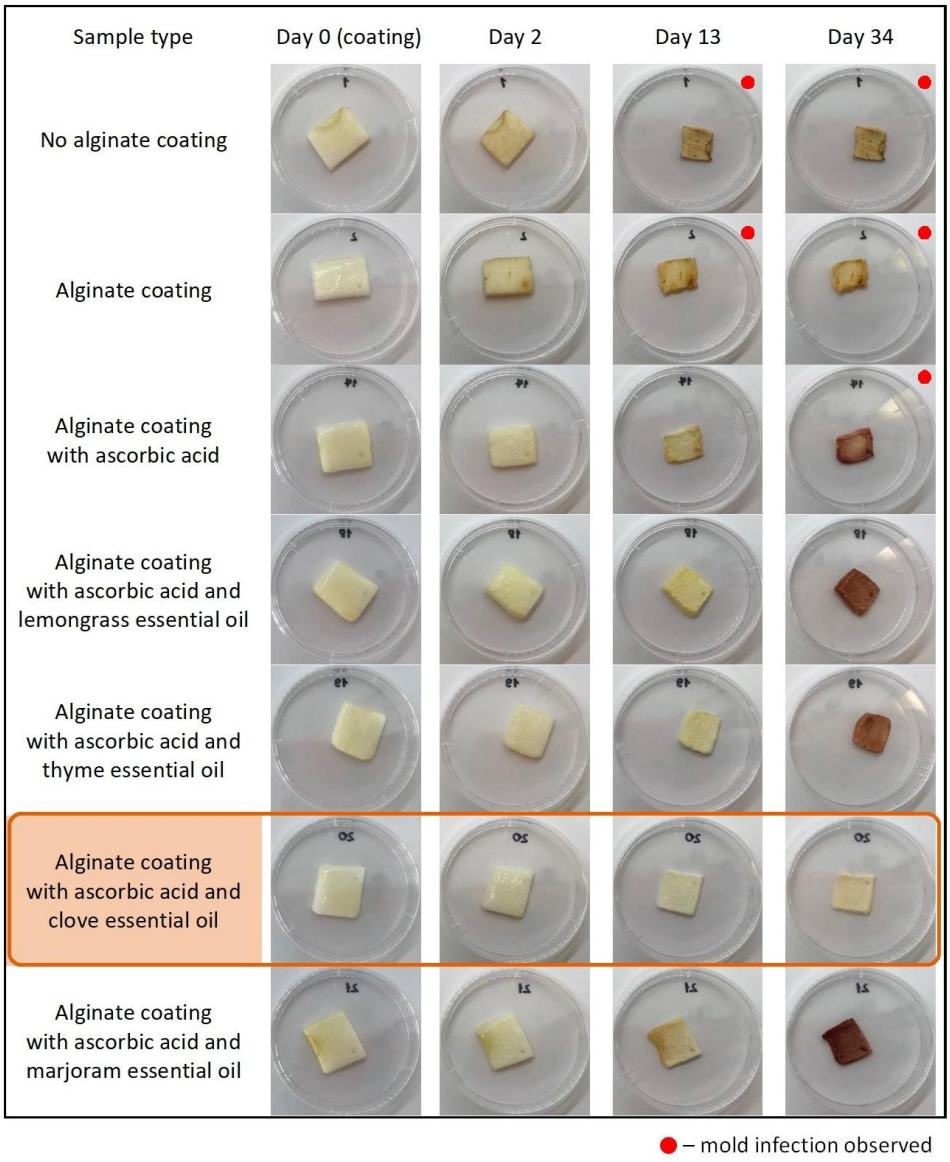


Figure 8. Effect of combining ascorbic acid with selected essential oils on the functionality of the alginate-based edible hydrogel coatings tested for prolonging the durability of freshly cut apple pieces.

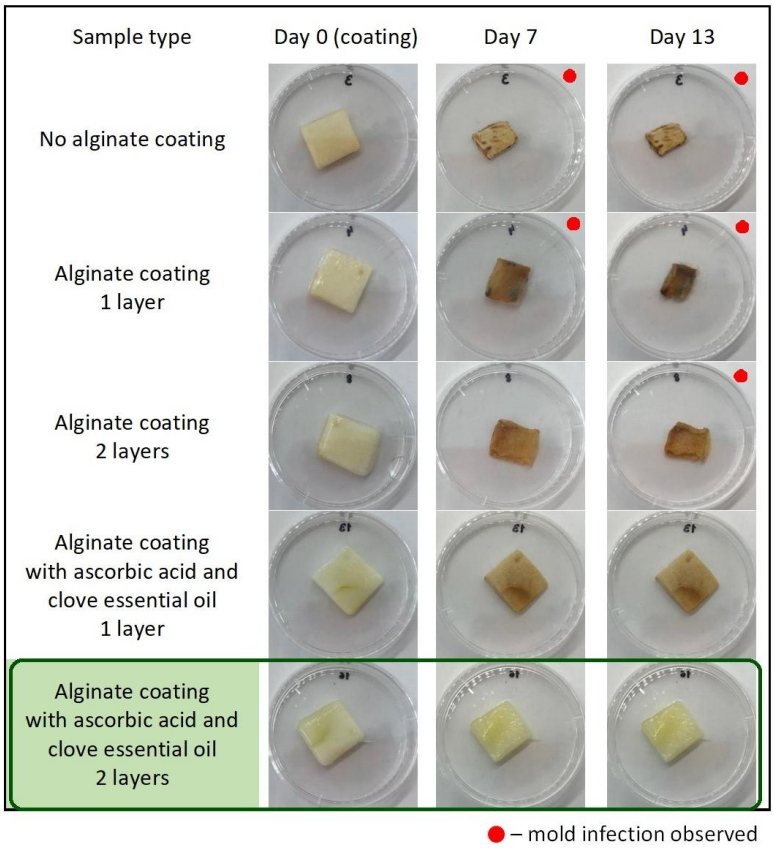


Figure 9. The influence of the number of Ca-alginate hydrogel coating layers on the effectiveness of extending the shelf-life of freshly cut apple pieces.

promoting bio-additives. For that purpose, a variety of non-animal natural-originated polymers (sodium alginate, κ-carrageenan, agar, pectin, gellan gum, and konjac gum) were tested in preliminary studies to obtain the materials with desired texture and consistency for food application. Then, the selected ones (sodium alginate and κ-carrageenan) were enriched with various blends of the following superfood additives: maca root, acai berries, spirulina, chlorella, moringa and matcha leaves. The received bio-functionalized hydrogel formulations ensure the supplementation of the appropriate amount of protein, polyphenols, antioxidants, vitamins and other micro- and macroelements in a vegan diet. Hence, these materials might be used in the manufacturing of functional health-promoting food products based on natural, non-animal components. The general concept of research performed is shown in Figure 10.

In the study, the production conditions of materials based on sodium alginate and κ-carrageenan were selected, including the concentrations of individual reagents as well as the time and temperature of cross-linking. The content of protein, reducing sugars, polyphenols and antioxidants in the tested superfood extracts was also determined (Table 2).

Then, hydrogel formulations containing blends of additives composed in such a way as to supplement the deficits of compounds necessary for supplementation in a meat-free diet were developed using sodium alginate (Table 3) and κ-carrageenan (Table 4).

Table 2. Bio-functional composition of plant and microalgae water extracts at a concentration of 5 g/l of initial superfood powder.

Raw material	Proteins [g/L]	Reducing sugars [g/L]	Polyphenols [g/L]	Antioxidants [g/L]
Moringa	0.949	0.525	0.007	0.172
Maca	0.239	0.514	0.000	0.188
Matcha	1.704	0.275	0.047	0.069
Acai	0.189	1.636	0.000	0.186
Spirulina	0.241	0.000	0.007	0.184
Chlorella	0.274	0.000	0.004	0.186

The new hydrogel formulations enriched with blends of plants or microalgae developed in the presented study ensure the delivery of a proper amount of nutrition ingredients (vitamins, micro and macroelements) in a meat-free diet. Hence, received edible materials might successfully be used in the production of functional vegan food products. Among others, possible applications involve targeted dietary supplements, edible coatings or animal product substitutes.

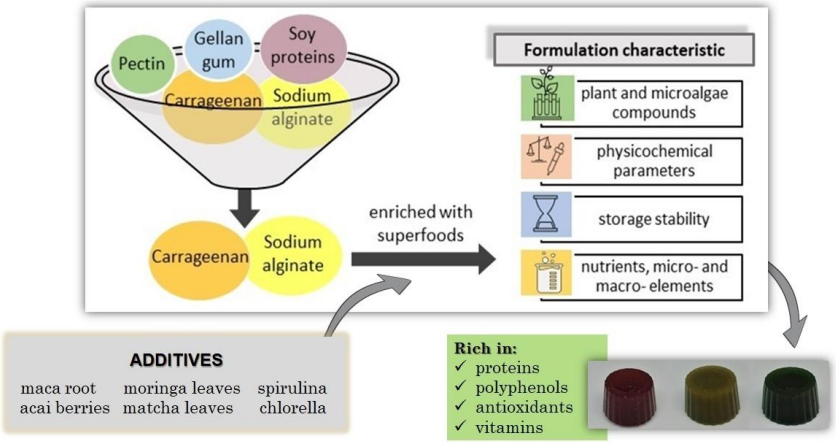
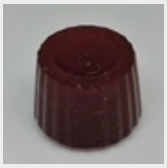


Figure 10. The general concept of research on the development of vegan hydrogel formulations enriched with bioactive superfood additives.

Table 3. Formulations of food jelly based on sodium alginate with superfood additives rich in bioactive ingredients.

Formulation number	Blend composition	Hydrogel	Ingredient composition per jelly (average mass 9.1 g)
1	• sodium alginate 1.5% • acai 50 g/l • matcha 5 g/l • maca 5 g/l • moringa 5 g/l		Calcium 0.43 mg Vit. B_2 0.03 µg Vit.B_3 0.09 mg B_{12} 0.00 µg Zinc 0.00 mg Iron 0.02 mg
2	• sodium alginate 1.5% • acai 50 g/l • moringa 5 g/l • chlorella 5 g/l		Calcium 0.84 mg Vit. B_2 1.75 µg Vit. B_3 0.10 mg Vit. B_{12} 0.00 µg Zinc 0.12 mg Iron 0.02 mg
3	• sodium alginate 1.5% • acai 50 g/l • spirulina 5 g/l • chlorella 5 g/l • maca 5 g/l		Calcium 1.01 mg Vit. B_2 3.32 µg Vit. B_3 0.015 mg Vit. B_{12} 0.06 µg Zinc 0.12 mg Iron 0.11 mg
4	• sodium alginate 1.5% • acai 50 g/l • moringa 5 g/l • spirulina 5 g/l		Calcium 0.33 mg Vit. B_2 1.63 µg Vit. B_3 0.09 mg Vit. B_{12} 0.06 µg Zinc 0.00 mg Iron 0.08 mg
5	• sodium alginate 1.5% • acai 50 g/l • moringa 5 g/l • spirulina 5 g/l		Calcium 0.45 mg Vit. B_2 1.60 µg Vit. B_3 0.01 mg Vit. B_{12} 0.06 µg Zinc 0.00 mg Iron 0.08 mg
6	• sodium alginate 1.5% • acai 50 g/l • moringa 5 g/l • spirulina 5 g/l		Calcium 0.45 mg Vit. B_2 0.03 µg Vit.B_3 0.09 mg Vit. B_{12} 0.00 µg Zinc 0.00 mg Iron 0.02 mg

Table 4. Formulations of food jelly based on κ-carrageenan with superfood additives rich in bioactive ingredients.

Formulation number	Blend composition	Hydrogel	Ingredient composition per jelly (average mass 8.7 g)
1	• κ-carrageenan 1.5% • acai 50 g/l • matcha 5 g/l • maca 5 g/l • moringa 5 g/l		Calcium 0.43 mg Vit. B_2 0.03 µg Vit.B_3 0.09 mg Vit. B_{12} 0.00 µg Zinc 0.00 mg Iron 0.02 mg
2	• κ-carrageenan 1.5% • acai 50 g/l • moringa 5 g/l		Calcium 0.27 mg Vit. B_2 0.03 µg Vit.B_3 0.09 mg Vit. B_{12} 0.00 µg Zinc 0.00 mg Iron 0.02 mg
3	• κ-carrageenan 1.5% • acai 50 g/l • spirulina 5 g/l • moringa 5 g/l		Calcium 0.33 mg Vit. B_2 1.63 µg Vit.B_3 0.09 mg Vit. B_{12} 0.06 µg Zinc 0.00 mg Iron 0.08 mg
4	• κ-carrageenan 1.5% • maca 5 g/l • spirulina 5 g/l		Calcium 0.22 mg Vit. B_2 1.6 µg Vit. B_3 0.01 mg Vit. B_{12} 0.06 µg Zinc 0.00 mg Iron 0.06 mg
5	• κ-carrageenan 1.5% • moringa 5 g/l • maca 5 g/l • matcha 5 g/l		Calcium 0.16 mg Vit. B_2 0.03 µg Vit.B_3 0.09 mg Vit. B_{12} 0.00 µg Zinc 0.00 mg Iron 0.00 mg
6	• κ-carrageenan 1.5% • spirulina 5 g/l		Calcium 0.06 mg Vit. B_2 1.6 µg Vit.B_3 0.01 mg Vit. B_{12} 0.00 µg Zinc 0.00 mg Iron 0.06 mg

4. BIOBASED HYDROGELS IN MEDICINE

4.1. Brief overview of current applications

Hydrogels show high similarity to components in the extracellular matrix, tissues and biological fluids in a mammalian body. They are recognized as 3D materials that may provide the necessary conditions for growth and cell metabolism. The hydrogel matrix may be designed to have appropriate characteristics covering mechanical resistance, mass transfer, high water sorption and retention, biocompatibility and biodegradability which are extremely desired in the medical sector (Caló and Khutoryanskiy, 2015; Binaymotlagh et al., 2022).

Regenerative medicine covers aspects of the development and application of new approaches to treating tissues to restore their lost functions due to aging or damage. In the case of natural hydrogels, the most popular application is manufacturing dressing materials with improved functionality. Such products should provide a supportive environment for wound healing, and present specific properties, e.g., maintain a moist environment and tissue temperature, allow gas exchange, stimulate angiogenesis, should be easy to remove and must be free from microbial and chemical contamination, non-toxic and non-allergenic (Dhivya et al., 2015). For example, the expected properties were provided by the chitosan-konjac glucomannan hydrogel that showed good antibacterial and adhesion properties, shortened the time of healing and presented high biocompatibility *in vivo* tests (Chen et al., 2018). In other research (Gao et al., 2019), the oxidised

sodium alginate was used to form a bioglass composite hydrogel with an adipic acid dihydrazide modifier. Such hydrogel dressing revealed dual adhesiveness to wound and implantable materials which may prevent dressing dislocation and enhance wound healing by stimulating of angiogenesis process. Another example is self-healing pH-responsive hydrogel composed of cationic guar gum and boric acid, which present muco-adhesive properties beneficial for wound healing and drug delivery systems (Madhavikutty et al., 2023). This biobased hydrogel is *in situ* cross-linkable, non-cytotoxic and presents high potential to be used as an effective mucosal protectant biomaterial.

The property to mimic extracellular matrix conditions has made hydrogels exceptionally useful tools for constructing 3D tissue models in place of the animal cell models currently used in tissue engineering. This is an alternative to 2D animal models in studying basic cellular processes, testing drug toxicity and efficacy, and monitoring processes in healthy and pathological tissues (Park and Park, 2016). Natural polymers including alginate, cellulose, collagen, chitosan, fibrin, gelatine or hyaluronic acid are biocompatible, biodegradable and promote natural processes but are characterized by rather weak mechanical properties, easy degradation, and not uniform structure (Tibbitt and Anseth, 2009). However, some of them meet the criteria for 3D tissue model construction. For instance, nanocellulose and alginate cross-linked with calcium ions form a hydrogel model of cartilage (Aswathy et al., 2020). Another example is the development of biomaterials with tunable mechanical properties based on hyaluronic acid hydrogels obtained via the 'click chemistry' method (Piluso et al., 2011). Materials obtained in this way preserve their histocompatibility and can be used for cell culture and soft tissue regeneration.

Natural hydrogels are also widely used as controlled drug delivery systems (Cao et al., 2021; Li and Mooney, 2016; Merino et al., 2015). These materials possess unique physical and chemical properties (e.g., high water-holding capacity, flexible, soft texture and low interphase tension in contact with biological fluids) to design controlled drug administration. In such systems, the mechanism of drug release can be controlled through changes in the gel structure like swelling/deswelling, dissolution, or degradation in response to some external stimulus (e.g., temperature, pH, light) (Li and Mooney, 2016; Merino et al., 2015). With the use of natural hydrogel-based drug carriers, insulin, avidin, calcitonin, BMP2 (recombinant human bone morphogenic protein 2), becaplermin, testosterone, polyhexamide, L-DOPA (3,4-dihydroxy-L-phenylalanine), plasmid DNA, growth and immunomodulatory factors, hydrophobic anticancer drugs, stem cells and many other therapeutic agents can be delivered (Cao et al., 2021; Li and Mooney, 2016).

Due to favourable physicochemical and biological properties, natural hydrogels are promising matrices for biosensing applications in medicine. Their combination with sensing molecules, such as enzymes, cells, nucleic acids, antigens, antibodies or other label compounds makes them powerful candidates for the construction of highly sensitive biosensors (Tavakoli and Tang, 2017). For example, peptide hydrogels are of great significance in this field. Among others, they are used in the targeted detection of tumor biomarkers and viruses in biological solutions with an ultra-sensitive detection limit of pmol/L (Binaymotlagh et al., 2022).

Other biomedical applications of biobased hydrogels that are intensively developed include superabsorbent hygiene products (e.g., diapers, sanitary napkins, underwear, feminine hygiene products, and adult incontinence products) produced especially with the use of cellulose-derived materials (Enawgaw et al., 2021; Haque and Mondal, 2018). They show a great ability to retain fluids and separate them away from the skin, preventing diaper dermatitis and ensuring the comfort of wearing.

4.2. Research contribution to the development of current trends

4.2.1. Colourimetric diagnostic test for lactose intolerance

One of the potential medical applications of biobased hydrogels considered in our research group was the development of a colourimetric test for the qualitative and quantitative determination of β-galactosidase (lactase, EC 3.2.1.23) (Labus, 2018). This enzyme is essential for the proper digestion of the milk sugar present in various dairy nutrition products. Therefore, the proposed solution could be used as a diagnostic assay for lactose intolerance.

Analogously to visual screening tests produced for enzymological purposes (Subsection 2.2.2), also in this case the principle of test operation was based on the conversion of a transparent substrate confined in a hydrogel matrix into a coloured product in a reaction catalyzed by a given enzyme. Here, ONPG (ortho-nitrophenyl-β-D-galactopyranoside) immobilized in a gelatine matrix was used for the colour detection of β-galactosidase in the tested samples (Figure 11).

Applying such a test, it is possible to effectively determine the enzyme in a concentration over 0.6 mg/L in a pH range of 4.0–9.0 with a fast visual response time (from 1 to 30 minutes). Moreover, the developed product enables initial quantification of β-galactosidase based on the intensity of the obtained colour and preserves specific properties at least for 90 days of storage at 4 °C. Due to the high selectivity and sensitivity, the assay developed could be a subject of great interest in medical diagnostics. It may constitute a so-called negative response test where the lack of colour would suggest further evaluation through lactose intolerance.

4.2.2. Wound dressings with antimicrobial properties

In other studies, we initially tested the antimicrobial properties of hydrogel materials made of Ca-alginate enriched with functional additives of plant origin (acai berries, moringa roots and matcha leaves) against two pathogenic bacterial

strains – *Staphylococcus aureus* (G(+) bacteria) and *Escherichia coli* (G(-) bacteria). For that purpose, a Petri-dish bacterial resistance test was performed. Firstly, carpet inoculation of liquid bacterial cultures from the logarithmic growth phase was performed on an agar-solidified Mueller-Hinton medium. Then, the disc of tested hydrogel (d = 1.0 cm) was placed in the centre of the Petri dish (d = 5.0 cm). The microbial culture was incubated at ambient temperature and after 24 h obtained results were verified. If a bright zone appeared around the hydrogel disc, it indicated that bacterial cells were sensitive to the functional components of the biobased formulation examined. For comparative purposes, a reference hydrogel containing the antibiotic (chloramphenicol) was also tested and additional control culture was made without any additives. The experimental results are depicted in Figure 12. Regardless of the alginate material functionalized with different natural additives used in the examination, there was no observed desired action on *E. coli* growth inhibition. However, it was noticed that alginate hydrogel enriched with a blend of acai berry and moringa root extracts gave a similar high bacteriostatic effect on *S. aureus* as usage of the same hydrogel containing the chloramphenicol. Therefore, this material may be a promising natural alternative for the targeted treatment of bacterial infections caused by this strain.

The presented studies are currently being extended towards the development of ready-to-use hydrogel dressings with improved properties, that due to their natural origin and biodegradability will meet the requirements of a sustainable circular economy.

5. CONCLUSIONS

Undoubtedly, nature is the inspiration for modern biomaterials used in a variety of commercial branches. Therefore, this is also the main reason for the dynamic growth of interest in biobased hydrogels. The significant benefit of this group of materials is their multi-functionality resulting from the tunability of physicochemical properties, which can be precisely tailored to the requirements of a particular application. Natural hydrogels are used with huge success in many sectors. However, they are of greatest importance mainly in biocatalysis, the food industry and medicine.

The article describes particular case studies considered in our research group, which is preceded by a brief literature overview of the most relevant applications of biobased hydrogels in these three fields. In the area of catalysis, we have developed quick and simple screening tests for enzymology applications based on gelatin hydrogels functionalized with target reactive compounds (Labus and Maniak, 2022). We

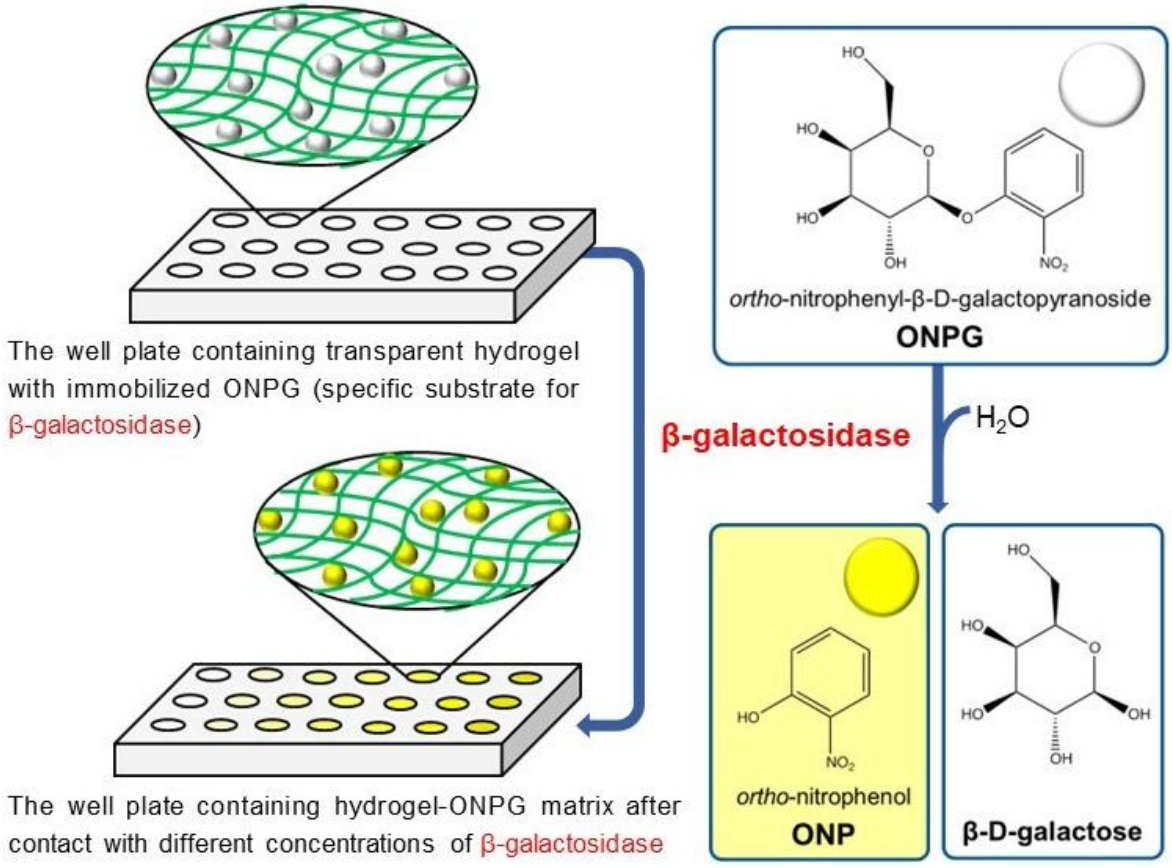
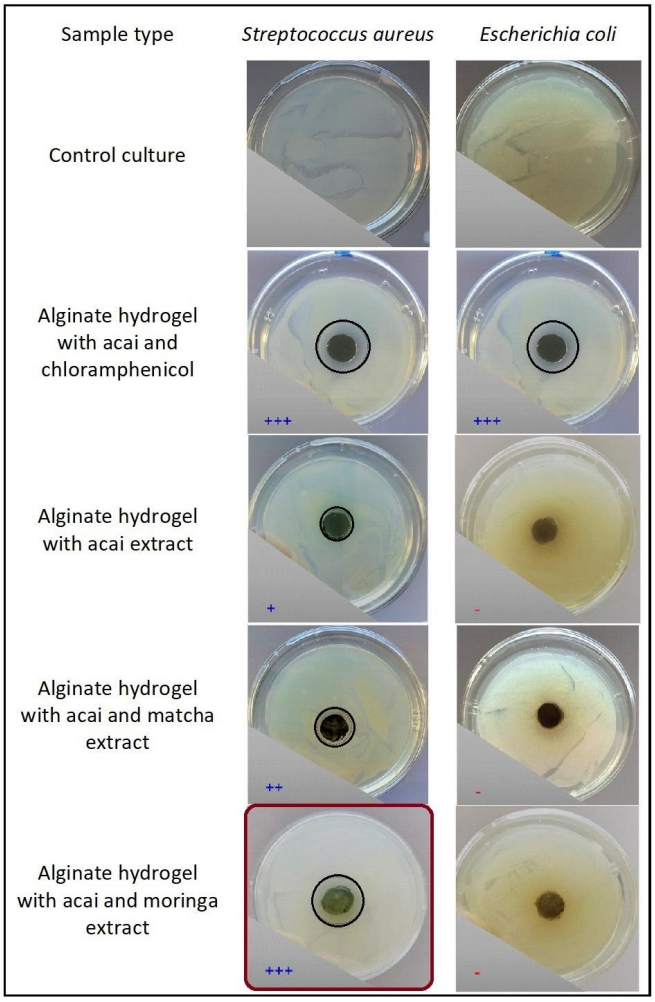


Figure 11. Scheme of operation of hydrogel-based colourimetric tests for β-galactosidase (graphical abstract modified with permission from (Labus, 2018).



Growth inhibition zone: black circle; effect: strong + + +; medium ++; slight +; no effect –

Figure 12. Antimicrobial properties of alginate-based hydrogel formulations containing superfood additives rich in bioactive ingredients.

also researched the use of natural hydrogel matrices to obtain immobilized biocatalysts with improved catalytic properties (Labus et al., 2020). In the food processing field, we considered obtaining edible hydrogel coatings based on alginate enriched with plant essential oils increasing the shelf life of freshly cut fruit and novel vegan hydrogel formulations with favourable nutritional and health-promoting bioadditives. In the case of medical applications we tested the antimicrobial properties of naturally-derived hydrogel materials containing plant extracts according to their utility to create soft wound dressings with improved bio-functionality. We also provided a highly selective and sensitive colourimetric assay for β-galactosidase detection, which could be of great interest in the medical diagnostic for the determination of lactose intolerance (Labus, 2018).

These case studies represent practical examples where readily accessible biopolymers are used for the simple creation of functional hydrogel systems for particular applications. Importantly, they fit well into the current trends in chemical engineering development towards the intensification of the use of bioengineering tools in manufacturing processes by the principles of a sustainable circular economy.

AUTHOR'S DECLARATION

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

ACKNOWLEDGEMENTS

Research case studies overviewed in this article are outcomes published previously under the research project granted by the National Science Centre of Poland (2015/19/D/ST8/01899) or obtained as part of the current Authors' statutory research activity at the Department of Micro, Nano and Bioprocess Engineering, Faculty of Chemistry, Wrocław University of Science and Technology (Poland).

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