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REVIEW PAPER

INFLUENCE OF EXTRACTION METHODS ON ULVAN WATER HOLDING CAPACITY AND RHEOLOGICAL PROPERTIES: A SYSTEMATIC REVIEW

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Abstract

Ulvan, a sulfated polysaccharide derived from green algae such as *Ulva* and *Enteromorpha* sp., possesses water-binding capability, making it a valuable natural hydrocolloid in the food sector. Physicochemical properties, such as water-holding capacity (WHC) and rheology, influence their use as thickeners, stabilizers, and gelling agents. Ulvan extraction methods, including hot water extraction (HWE), microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), enzymatic extraction (EAE), and chemical solvent extraction (CAE), have a substantial impact on these qualities. The purpose of this review is to investigate the effects of extraction procedures on the WHC and rheological properties of ulvan to maximize its use in food items. A systematic literature review (SLR) was conducted, which involved scanning publications in databases such as Google Scholar, Scopus, and ScienceDirect. According to the evaluation results, HWE and EAE retain a branched structure and high sulfate content, making them acceptable for applications requiring strong gel and high WHC. In contrast, the MAE, UAE, and CAE approaches generate linear ulvan that enhances viscosity at high concentrations. The use of UAE and EAE can improve monomer retention, although careful management is required to maintain the sulfate concentration and molecular weight.

Keywords: Green seaweed, hydrocolloids, sulfated polysaccharides

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Introduction

Ulvan is a sulfated polysaccharide extracted from the cell walls of green algae, such as *Ulva* and *Enteromorpha* sp. As a natural hydrocolloid, ulvan can bind water due to its hydroxyl groups and polyelectrolyte properties. Hydrocolloids, including ulvan, are widely used as food additives, such as thickeners, stabilizers, and gelling agents (Kraithong *et al*., 2023). The physicochemical properties of hydrocolloids, including their water-holding capacity and rheological properties, are crucial for their application in food products (Barakat *et al*., 2022).

The extraction method plays a key role in determining the characteristics of ulvan, including its molecular weight and monomer composition. The hot-water extraction (HWE) method is the most commonly used technique; however, it has drawbacks such as high energy consumption, a long time, and low yield (Jiang *et al*., 2023). Therefore, modern methods such as microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), enzyme-assisted extraction (EAE), and chemical solvent extraction (CSE) have been developed to improve extraction efficiency (André *et al*., 2023).

These different extraction methods impact the properties of ulvan, including its water-holding capacity and rheology. These properties are key to its application in the food industry. However, information on the relationship between extraction methods, water-holding capacity, and rheological properties of ulvan is limited. Therefore, an in-depth review is needed to understand the effect of extraction methods on these properties. This helps optimize the application of ulvan in various products.

Materials and methods

This systematic literature review (SLR) followed a three-step approach of planning, conducting, and reporting. The primary objective of this review was to investigate the relationship between extraction methods, water-holding capacity, and ulvan rheology. The scientific articles selected for this review were sorted from Google Scholar, Scopus, ScienceDirect, Wiley Online Library, Nature, PubMed, and CrossRef databases. Articles were selected based on three main inclusion criteria: the English language, title, and abstract, which indicated the evaluation of extraction methods, functional properties, rheology, ulvan, molecular structure, and the *Ulva* genus.

We began our research using the keywords “Ulvan” or “ulva or green algae”, and “Extraction”, and “Rheology” or “Structure” or “Molecular” or “Functional” or “WHC” or “Water holding capacity”. A total of 2053 studies were found in Google Scholar, 49 works were found in Scopus, 1539 works were found in ScienceDirect, 222 works were found in the Wiley Online Library, 32 works were found in Nature, 34 works were found in PubMed, and 1775 works were found in CrossRef. Subsequently, we performed duplication selection based on the similarity of authors, titles, and years of publication. The filtered articles were then re-selected based on

topic relevance and language. The filtered articles from the above stages were used to write the paper (Figure 1).

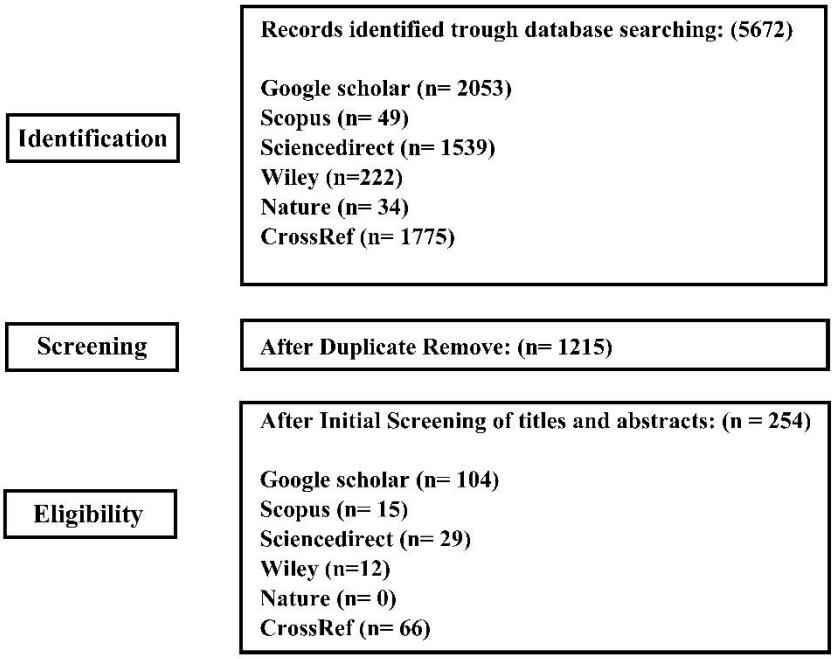

Figure 1. Preferred reporting items for systematic review diagram

Results and discussion

Ulvan

Ulvan, a marine polysaccharide, is extracted from green seaweed (*Ulva* and *Enteromorpha* sp.) and is the major structural component of cell walls (Kidgell *et al*., 2019). Ulvan content is approximately 10-30% of seaweed biomass, which is highly dependent not only on the species but also on the pretreatment, extraction (Guidara *et al*., 2021), and purification procedures. It is found outside the cell lumen and connects to cellulose, proteins, and other polysaccharides via hydrogen, metal, and ionic bonds (Wang *et al*., 2022).

Ulvan plays a structural role (Flórez-Fernández *et al*., 2023) by providing cellular rigidity and physiological adaptation to the marine environment (Anisha *et al*., 2023). The structure of ulvan is more variable than the one of sulfated polysaccharides from brown and red algae, which are affected by ecophysiological conditions. The repeating disaccharides rhamnose sulfate, glucuronic acid, iduronic acid, and xylose constitute a slightly branched and complex ulvan structure (Yaich *et al*., 2011) (Figure 2). To deeply analyze the structure of ulvan, the Performance Liquid Chromatography (HPLC) method can be used, which is considered the most effective and innovative method for the identification and quantification of

monosaccharides. On the other hand, FT-IR spectroscopy has also been used for the analysis of polysaccharides, with specific band intensities and locations in the fingerprint area, allowing for a more detailed identification of their structural components (Amor *et al*., 2021). Ulvan contains both neutral and acidic sugars with sulfate and carboxylate groups contributing to its acidity. Ulvan possesses a variety of functional and biological properties, including thickening, gelling (Anisha *et al*., 2023), and stabilizing agents (Don *et al*., 2022). These functional properties depend on the water-holding capacity and rheological behavior.

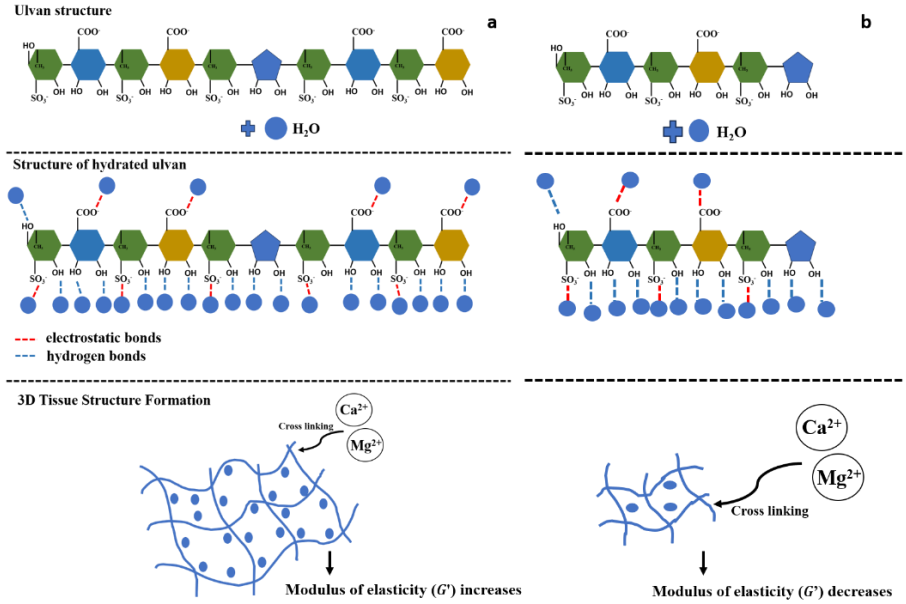

Figure 2. Ulvan molecular interactions: relationship of functional groups to gel network formation and water holding capacity. (a) Hight molecular weight and (b) Low molecular weight ulvan

Ulvan extraction method

Ulvan extraction is primarily used to isolate sulfated polysaccharides from the cell walls of green seaweeds, particularly those belonging to the genus *Ulvaceae*, and is a crucial step in the isolation and purification of such polysaccharides (Taşkiran *et al*., 2023). Various extraction methods have been used in previous studies. The conventional method involves the use of hot water (HWE) (Ibrahim *et al*., 2022). In addition, chemical extraction methods use alkalis, acids (CAE) (André e*t al*., 2023), and chelating agents, such as EDTA and sodium oxalate (Moawad *et al*., 2022). Various innovative methods have been applied to improve extraction yields, including the use of enzymes (EAE) (Guidara *et al*., 2021), microwaves (MEA) (André *et al*., 2023), ultrasonic waves (UEA) (Kazemi e*t al*., 2023), and combination methods.

Various extraction processes yield ulvan with distinct functional properties, which are closely related to its chemical and physical characteristics. For example, the sulfate content is closely related to its gelation properties (Kidgell *et al*., 2019). The sugar composition in ulvan is related to its biocompatibility and film-forming ability, while the protein content is closely related to its gelling and binding properties (Pari *et al*., 2024). Ulvan with fewer constituent monomers or short chains has a lower molecular weight, affecting its solubility and viscosity, whereas ulvan with long chains has a higher molecular weight, affecting its stability and structure-forming abilities. According to certain studies, the molecular weight of these polysaccharides is directly related to their physical properties, such as water-holding capacity (WHC) and viscosity. These two qualities, WHC and rheology, are crucial in ulvan applications across the food, cosmetic, and pharmaceutical sectors, as they significantly impact the texture, stability, and functionality of the constituents in the finished product.

Water Holding Capacity and Rheological Properties of Ulvan

Water Holding Capacity (WHC) is the ability of a material to absorb and hold water. This parameter is crucial in characterizing gels, biopolymer materials, and other applications (Costa *et al*., 2025). WHC plays a crucial role in food processing technology, particularly in terms of yield, sensory evaluation, stability, texture, and production processes. One of the important applications of ulvan as a hydrocolloid is as an emulsifier (Shao *et al*., 2016), stabilizing agent (Mo'o *et al*., 2020), and probiotics, such as in yogurt (Shah *et al*., 2023), because ulvan can increase viscosity, form gels, extend the physical stability of products, and food supplements. The combination of ulvan's ability to modify the structure and properties of WHC helps create more stable, sensorially appealing, and durable products.

The WHC of ulvan is affected by various factors, including its molecular weight, sulfate content, and monomer composition. In Ulvan, the high molecular weight is not only related to the length of the polymer chain but also to the number of active sites that can interact with water. In addition to hydroxyl (-OH) groups, which are highly hydrophilic and capable of forming hydrogen bonds with water molecules.
Several other functional groups also play a crucial role in enhancing the water absorption capability (WHC). One of them is the sulfate group (-SO_3-), which is polar and negatively charged, allowing for strong electrostatic interactions with water molecules, as well as creating more space between polymer chains due to the electrostatic repulsion between sulfate groups. In addition, carboxyl groups (–COOH) can be found on oxidized ulvan, which is acidic and polar, contributing to the formation of hydrogen bonds or ionic interactions with water molecules, especially in more alkaline or acidic environments.

High-molecular-weight polymer chains form more open three-dimensional gels or network structures, leaving ample space for water to be trapped. This structure is controlled not only by hydroxyl groups but also by cooperative interactions among nearby functional groups, such as sulfate and carboxyl groups. Although the hydroxyl group is the primary location for water binding, the addition of sulfate and carboxyl groups enhances ulvan's ability to absorb and retain water. Generally, an

increase in molecular weight corresponds to a greater number of active sites and a wider space for water absorption, thereby improving the water-holding capacity (WHC) of ulvan. Several scholars have correlated molecular weight with polysaccharide chain length, finding that longer chains have greater molecular weights than shorter ones. The chain length has a considerable impact on how it interacts with water. According to Ramaswamy *et al*. (2013), the flexibility of lengthy chains allows for easier interactions with water, which contributes to a higher WHC. This flexibility also enhances the radius of gyration and hydrodynamic volume, resulting in improved water retention.

Ulvan, a sulfated polysaccharide rich in functional groups, has a remarkable ability to absorb and retain water, which significantly influences its mechanical and rheological properties, including viscosity, gel strength, and elasticity. According to Xu *et al*. (2024), the rheology of polysaccharides is closely related to the distribution of molecular weights and the entanglements of molecular chains. This capacity is closely tied to ulvan's unique three-dimensional (3D) structure, which is formed through interactions between its functional groups, such as hydrogen bonding, ionic bonding, and cross-linking. Viscosity, defined as the resistance of a medium to slow deformation during flow, arises from friction between neighboring particles moving at different speeds and is related to the shear strain rate, or shear deformation rate, within the medium. The rheological characteristics of ulvan, as observed by previous researchers, are listed in Table 1.

The relationship between shear stress and shear rate demonstrates that the flow behavior of ulvan solution often follows a power law model. The power law asserts that a fluid's viscosity varies with shear rate and is influenced by the flow index n. This relationship can be stated by equation 1.

$$\tau = k\,\dot{\gamma}^{n} \qquad (1)$$

where τ is the shear stress, $\dot{\gamma}$ is the shear rate, k is the flow constant, and n is the flow index. In the ulvan system, the R^2 value close to 1 confirms that the flow properties match the power law model.

This means that ulvan solution, at both low and high concentrations, has a nonlinear relationship with shear stress and shear rate, with the viscosity determined by n. At low concentrations, the flow index n is close to one ($n \approx 1$), reflecting Newtonian behavior in which the viscosity remains relatively constant. This is because the ulvan molecules are widely separated, resulting in limited interactions between molecules that do not form substantial molecular networks. However, at higher concentrations, the value of n decreases, showing shear-thinning qualities. This is due to the fact that as the shear rate increases, the molecular network begins to break down, resulting in a decrease in viscosity and an increase in shear thinning. As the shear rate increases, the interactions between molecules strengthen, and the molecular network generated becomes denser, resulting in a greater drop in viscosity. Overall, the data fit to this power law revealed that ulvan has flow characteristics that can be predicted well using a power law rheological model, as evidenced by the high R^2 values.

Table 1. Rheological characteristics of ulvan.

Sample	Con (%)	Temp (°C)	Shear rate (s^{-1})	μ_{50} (mPa.s)	n	Hysteresis area (Pa. s^{-1})	G' (Pa)	G" (Pa)	γ (%)	Tan σ
Ulvan solution (Viscosity) Ulvan + sodium tetraborate (ossilation) (Yaich *et al*., 2014)	1.6	20	0.1 – 1000	7 - 10	Non- Newtonian (shear thinning)	333,57 - 2973.44	10 - 100	1-5	0.3	<1
Ulvan + $CaCl_2$ + H_3BO_3 solution (Rheology) (Toskas *et al*., 2011)	2.34	20	0.1 - 100	8000	Non- Newtonian (shear thinning)	-	8	1	-	<1
Ulvan solution (Viscosity) (Shao *et al*., 2014)	0.1 - 1	25	0 - 4000	1.5 – 3.5	Non- Newtonian (Shear-tickening)	-	-	-	-	-
Ulvan + NaCl, KCl, $CaCl_2$ (Qiao *et al*., 2016)	1 – 1.4	25	1- 600	2000 - 7500	Non- Newtonian (shear thinning)	20700 - 46000	1- 55	0.1	2	<1
Ulvan + Cinnamaldehyde emulsion (Shao *et al*., 2016)	0.1 - 4	25	0.01 to 500	0.01 – 0.05	Non- Newtonian (shear thinning)	-	-	-	-	-
Ulvan + $CaCl_2$+ H_3BO_3 (Inoubli *et al*., 2024)	1	20	1- 500	0.05	Non- Newtonian (shear thinning)	-	0.1	0.00001	-	<1
Ulvan solution (Chen *et al*., 2021)	4	25	0.1 - 100	50 - 150	Non- Newtonian (shear thinning)	-	0.08	0.001	3	<1
Ulvan + $CaCl_2$+ H_3BO_3 (Sari-Chmayssem *et al*., 2018)	0.3 (viscosity) 1 (osillasi)	20	0.01 - 1000	0.005 - 10	Non- Newtonian (shear thinning)	-	40	2		<1
Ulvan solution (viscosity) Ulvan + rice flour (Kraithong *et al*., 2024)	0.2- 0.6	25	1-100	3 - 9	Non- Newtonian (shear thinning)	-	500-600	50-60	0.1	<1

In addition, ulvan concentration also affects its consistency value. At low concentrations, ulvan molecules separate and thus do not form a strong molecular network, resulting in a low consistency K value. In contrast, at high concentrations, intermolecular interactions increase, forming a denser molecular network that enhances flow resistance and leads to higher K values. In other words, at high concentrations, ulvan better "resists" flow, as reflected by an increased K value.

In general, the change in viscosity is affected by both the consistency coefficient k value and the n value in the power law model, where an increase in k values indicates a decrease in n values. In addition to the concentration factor, the addition of multivalent ions can affect the viscosity of ulvan. Ca^{2+} ions can tighten polymer chains through ion bridges, whereas borate ions add crosslinks to strengthen the (Yaich *et al*., 2014). Although the viscosity increases owing to this strengthening of intermolecular contacts, the system still exhibits shear-thinning qualities, meaning that the viscosity decreases at high shear rates, consistent with the non-Newtonian behavior of ulvan. Interestingly, ulvan in Shao *et al*. (2016) exhibited shear thickening behavior even in the absence of multivalent ions or other polysaccharides. This phenomenon can be explained using the hydrodynamic clustering theory, which states that at low shear rates, ulvan molecules can form local aggregates, increasing viscosity as shear force is applied. This feature is especially important for applications requiring high stability, such as those in the food sector, where the shear-thickening effect can assist in stabilizing emulsions and preventing phase separation. Furthermore, combining ulvan with additives such as rice flour or cinnamaldehyde emulsions (Shao *et al*., 2016) showed stabilizing effects, indicating ulvan's potential for stabilizing emulsions in food and cosmetic formulations.

The hysteresis region of ulvan can be observed under various test conditions, including modifications to composition and test procedure, as shown in the table. The hysteresis area, measured in $Pa \cdot s^{-1}$, provides insight into the energy lost throughout the deformation cycle (Qiao *et al*., 2016), which is a crucial factor in researching the viscoelastic properties of materials. In general, the link between the hysteresis area and viscosity is heavily influenced by the material's molecular structure. Molecules that are larger, more complex, or more crosslinked tend to have higher viscosities and smaller hysteresis areas. In contrast, materials with simpler or more flexible molecular structures may exhibit lower viscosities and wider hysteresis regions. As a result, an in-depth study of the molecular structure and interactions between system components is required to explain variations in hysteresis area values in materials such as ulvan.

The varying hysteresis area values in ulvan supplemented with NaCl, KCl, $CaCl_2$, and sodium tetraborate (Table 1), although with the same ulvan content, can be attributed to the chemical and physical interactions between each additive and the ulvan molecular structure. Salts with varying charges, such as NaCl, KCl, and $CaCl_2$, can generate stronger ionic bonds between ulvan molecules. Multivalent ions such as Ca^{2+} can significantly boost crosslinking, strengthen the molecular structure, and increase the viscosity of the solution. This increases the viscosity, resulting in a smaller hysteresis area. In contrast, sodium tetraborate forms weaker hydrogen

bonds and is less robust than the ionic connections created by these salts, although it can cause crosslinking. The weaker interaction results in a wider hysteresis area, although using the same ulvan concentration. Thus, the strength of the interaction between ulvan and the additive has a greater influence on the difference in the hysteresis area than the ulvan concentration.

Sulfated polysaccharides, such as ulvan, are viscoelastic materials with both solid and liquid characteristics, making them ideal for dynamic rheological evaluation. These materials can be examined using dynamic frequency testing, which reveals important information about their mechanical properties, as their behavior changes in response to different frequencies. However, prior to completing the frequency sweep tests, an amplitude sweep is required to define the linear viscoelastic (LVE) area of the sample.

The amplitude sweep test involves altering the strain amplitude while maintaining a fixed frequency (Hz) to observe how the material reacts (Stojkov et al., 2021). This test identifies the region where the material behaves elastically and the stress-strain relationship remains linear. In the LVE region, the material responds consistently, and the observed moduli are independent of the strain amplitude, indicating that the sample is neither undergoing structural changes nor nonlinear behavior. Establishing the LVE region ensures that the ensuing frequency sweep data reflect the material's genuine rheological properties without interference from viscoelastic transitions, such as gelation or the onset of flow. This process is critical for obtaining precise and trustworthy rheological data, particularly when examining complex materials such as ulvan, which exhibit both elastic and viscous reactions.

According to previous studies, shear strain data (γ %) on ulvan samples show that these materials mostly behave in the Linear Viscoelasticity (LVE) range at low strains, between 0.1% and 5% (Qiao *et al*., 2016; Sari-Chmayssem *et al.*, 2018; Baltrusch *et al*., 2024; Kraithong *et al*., 2025). Within this range, ulvan and its blends retain predominantly elastic properties with little or no permanent changes in the material structure. This indicates that under LVE testing conditions, ulvan behaves in a stable manner and can return to its original shape once the force or stress is removed, which is typical for materials exhibiting linear viscoelasticity.

At such low stresses, the stress-distortion relationship remains linear, indicating that the ulvan material can return to its former state after being stressed without experiencing irreversible deformation. This behavior characterizes the elastic phase of the material, in which only transient deformation occurs and is restored when the testing force is stopped. Under these conditions, the material did not display the plastic or viscoplastic properties observed at higher strains (Yaich *et al*., 2014). This feature is essential in various applications in which Ulvan or its blends are utilized as materials to maintain structural stability and resist permanent deformation when subjected to stress. For example, in gel or emulsion formulation systems, the LVE requirement guarantees that the material can be treated and used without losing its structural integrity.

The frequency sweep test is another rheological method for determining the link between the testing frequency and the storage (G') and loss (G'') moduli of the

material (Stojkov *et al*., 2021). The elastic modulus (G') reflects the solid or elastic nature of the material, indicating its ability to store elastic energy, whereas the viscous modulus (G") describes its liquid or viscous nature, indicating the ability of the material to flow and dissipate heat. These changes in the G' and G" values illustrate the transition between ulvan's elastic and viscous components as the frequency varies, providing crucial insights into how the material behaves under dynamic conditions and the interactions between its structural components.

Previous research indicates that ulvan demonstrates G' > G" at concentrations of ≥ 1.6% (Yaich *et al*., 2014) without the addition of multivalent ions and ≥ 0.2% (Qiao *et al*., 2016; Sari-Chmayssem *et al*., 2018; Kraithong *et al*., 2025) when supplemented with multivalent ions or other polysaccharides. The addition of additives such as borate is theoretically significant because complex bonds occur between the borate and diol groups in ulvan (Sulastri *et al*., 2021). Adding borate raised the elastic modulus (G') of the ulvan solution, which is consistent with gelation theory (Kraithong *et al*., 2025). This shows that ulvan can be employed as a gelling agent in biomaterial applications. This trend is consistent with the observation that an increase in the storage modulus (G') indicates the strengthening of the molecular network in solution.

The tan σ (loss factor) value is one of the main indicators of the viscoelastic properties of ulvan, where all ulvan samples showed tan σ consistently lower than 1 in both shear thinning and shear thickening (Qiao *et al*., 2016; Baltrusch *et al*., 2024). A tan σ value < 1 indicates that the ulvan system is predominantly elastic (gel-like) rather than viscous (fluid-like). This is consistent with the data observed in various studies. Yaich *et al*. (2014) showed that ulvan with sodium tetraborate has a tan σ value of 0.3, which confirms the dominance of elastic properties in the ulvan polymer network. This is consistent with the results of Toskas *et al*. (2011), which showed that the addition of multivalent ions such as Ca^{2+} and H_3BO_3 increased the elastic modulus (G') and viscosity of the ulvan solution without losing its gel-like properties. Even under shear-thickening conditions, ulvan retains its gel-like characteristics with tan σ < 1, reflecting its ability to form an elastic polymer network even under conditions of increased viscosity.

The gel-like properties of ulvan, shown through a tan σ value < 1, are closely related to the presence of functional groups such as hydroxyl (-OH), sulfate (-SO_3-), and carboxyl (-COOH) (Kraithong *et al*., 2025). These groups facilitate the formation of a more stable and elastic three-dimensional polymer network through hydrogen bonding and electrostatic interactions. Owing to these elastic mechanical properties, ulvan has great potential for various practical applications. Under both shear thinning and shear thickening conditions, ulvan can maintain its mechanical stability, making it an ideal material for emulsion stabilization, biomaterials such as tissue gels, and thickeners in food and cosmetic formulations.

Relationship between extraction method on water holding capacity and rheology

Ulvan is a heterogeneous polysaccharide with distinct rheological properties and a high water-holding capacity (WHC), which is determined by its molecular weight,

chain structure, and extraction process (Xu *et al*., 2024). Ulvan's molecular weight ranges from 2.56 to 2790 kDa due to its polyelectrolyte nature, which might cause aggregation during measurement (Baltrusch *et al*., 2024). Different extraction processes produce ulvan with a variety of molecular features that directly influence its WHC and rheological properties (Table 2).

Hot Water Extraction (HWE) is a method used to produce ulvan with medium to high molecular weight, depending on the species. For instance, ulvan from *Ulva intestinalis* has a molecular weight of 28-194 kDa (Peasura *et al*., 2015; Kazemi *et al*., 2023), whereas *Ulva rotundata* yields 180-500 kDa (Robic *et al*., 2008, 2009). HWE involves heat, pressure, and partial hydrolysis to efficiently extract ulvan. Initially, water is heated to a high temperature (80-120°C), which weakens non-covalent interactions, such as hydrogen bonds and hydrophobic interactions, in the algal cell wall (Torrego-Moreno *et al*., 2026). Increased temperature also raises the kinetic energy of molecules, facilitating the dissolution of polysaccharides, such as ulvan. The pressure further accelerated the reaction, allowing ulvan to be efficiently released from the cell matrix.

During this process, glycosidic linkages in the ulvan chain undergo partial hydrolysis aided by organic acids in algae, which become more reactive at high temperatures (Li *et al*., 2024). This breaks down the long ulvan chains into smaller fragments. However, the branched structure of ulvan is preserved because monosaccharides such as rhamnose and xylose support branching and sulfate groups stabilize the structure (Beaumont *et al*., 2021). The resulting ulvan fragments can interact via ionic or hydrogen bonding to form a three-dimensional network. This network imparts unique properties to ulvan, such as increased viscosity at low concentrations (Shao *et al*., 2014) and the ability to form elastic gels with a high elastic modulus (G').

However, excessive temperatures and prolonged extraction times can lead to thermal degradation, resulting in a reduction of the molecular weight of ulvan and a diminution of its gel-forming capability (Kazemi *et al*., 2023). Nevertheless, when optimized, HWE produces ulvan fragments with stable branched structures and a high elastic modulus, which are particularly beneficial for applications in biomedicine, food additives, and sustainable material development.

In contrast, Acid Extraction (CAE), which utilizes a low pH (1.5-2), aggressively degrades ulvan, producing small molecular weight fragments (Yaich *et al*., 2014). For example, *Ulva intestinalis* extracted using CAE has a molecular weight of approximately 88 kDa (Peasura *et al*., 2015). Ulvan, with more linear chain structures created by CAE, has greater flexibility, allowing for stronger intermolecular interactions that increase viscosity at high concentrations, while exhibiting more liquid-like rheological properties at low concentrations.

Table 2. Comparison of extraction methods on WHC and ulvan rheology.

Extraction Method	Main Process	Impact on WHC	Impact on Rheology	Advantages	Limitations
Hot Water Extraction (HWE)	Heating water at a certain temperature to release the ulvan from the raw material matrix.	WHC is quite good because the moderate temperature keeps the functional groups (sulfate and carboxyl) intact.	Rheology is stable, viscosity and gelation ability are maintained because hydrogen bonds and molecular interactions are not broken.	Simple process, environmentally friendly.	Extraction time is longer than other methods.
Chemical-Assisted Extraction (CAE)	The use of chemical solvents (acids or bases) to break the ulvan bonds with the material matrix.	WHC can increase if the solvent used is gentle and of the right concentration; if it is too aggressive, WHC decreases due to degradation of the ulvan structure.	Rheology varies; viscosity can decrease if the ulvan structure is damaged, but the use of gentle solvents can maintain gel stability.	Fast process, adaptable to raw materials.	Risk of ulvan structure damage if solvent is too aggressive.
Enzyme-Assisted Extraction (EAE)	Use of enzymes (e.g. cellulase or hemicellulase) to break the bonds in the matrix and release the ulvan.	High WHC because the structure of the ulvan is preserved, allowing the ulvan to absorb more water.	Stable rheology; high viscosity due to intact molecular structure, and more consistent gelation than other methods.	Soft to the ulvan structure, resulting in high quality ulvan.	High cost for enzymes and longer process time.
Microwave-Assisted Extraction (MAE)	The use of microwave heating to accelerate the release of ulvan from the material matrix.	WHC is good if heating temperature and time are precisely controlled; high temperature or too long a time can damage the ulvan, lowering WHC.	Rheology depends on temperature and time settings; viscosity is good with proper parameter control, but decreases if molecular degradation occurs.	Fast and efficient process.	Risk of molecular degradation if temperature or time is not controlled.
Ultrasound-Assisted Extraction (UAE)	Uses ultrasonic vibrations to destroy cell structures and accelerate ulvan release.	WHC is good if the ultrasonic intensity and duration are controlled; WHC decreases if the energy is too strong or the extraction time is too long, causing damage to the ulvan molecules.	Rheology is stable if the process is well controlled; results in high viscosity and good gelation, with a more stable gel structure.	Fast process, requires less solvent.	Requires specialized equipment, risk of structure damage if intensity is not controlled

The CAE approach yields ulvan with a linear structure because acidic chemicals and mild heat degrade the branches or complex structures of ulvan while preserving the main chain (Guidara *et al*., 2019). This structure is more stable and consistent, making it ideal for applications that require viscosity or biological activity of linear ulvan.

Enzymatic Extraction (EAE) utilizes enzymes to selectively break down plant cell walls, thereby liberating ulvan without causing molecular damage (Chen *et al*., 2021). Protamex, Viscozyme L, Cellulysin, Neutrase, Flavorzyme, Ulvan-lyase, and Glucuronan-lyase all operate on the surrounding cell wall components of ulvan, including cellulose, hemicellulose, and pectin. This mechanism destroys the cell wall, while ulvan remains intact and stable. The primary advantage of EAE is its ability to preserve the ulvan's branch structure, which is essential for its functional qualities such as gelling and water-holding capacity (WHC) (Yaich *et al*., 2014). Selective enzymes, such as flavorzyme and ulvan-lyase, break down specific sections of ulvan without destroying its molecular branching, thereby providing ulvan with flexibility and stability (Wang *et al*., 2022).

Microwave-Assisted Extraction (MAE), on the other hand, uses high temperatures to speed up ulvan extraction and increase yield (Le *et al*., 2019). However, if the temperature is not closely monitored, excessive heating may result in ulvan depolymerization and the creation of a more linear structure. This depolymerization decreases the ulvan's molecular weight and functionality, whereas the development of a linear structure decreases the ulvan's capacity to gel and retain water (Tsubaki *et al*., 2016). As a result, careful temperature and time control during the MAE process are critical for maximizing the extraction yield while retaining the ideal ulvan structure.

Ultrasonic-Assisted Extraction (UAE) utilizes ultrasonic cavitation effects to accelerate ulvan extraction (Ramadhan *et al*., 2022). Mechanical energy breaks the polysaccharide bonds in ulvan, resulting in lower molecular weights and more linear structures. Combining ultrasonic and enzymatic techniques (U-EAE) accelerates depolymerization, resulting in ulvan with a lower molecular weight suitable for applications requiring high viscosity at high concentrations (Wang *et al*., 2024).

Thus, the extraction method has a substantial impact on the molecular structure of ulvan, including its molecular weight and degree of degradation, which in turn affect its water-holding capacity (WHC) and rheological behavior. Hot Water Extraction (HWE) and Enzymatic Extraction (EAE) are ideal methods for generating ulvan with branching structures, which have remarkable gel stability even at low concentrations. Chemical-Assisted Extraction (CAE), Ultrasound-Assisted Extraction (UAE), and Microwave-Assisted Extraction (MAE) are more effective at producing linear ulvan structures, which increase viscosity as concentrations increase. As a result, the extraction technique should be carefully chosen and adjusted based on the intended application, whether to enhance gel stability or increase ulvan flexibility and viscosity.

Sulfate is an essential component of ulvan, influencing its functional qualities, such as water retention capacity (WHC) and gelling ability. The extraction procedure has

a major impact on the sulfate content and characteristics of ulvan. Hot Water Extraction (HWE) has been shown to create ulvan with significant sulfate concentrations, such as 20.17% in *Ulva pertusa* (Li *et al*., 2018) and 19% in *Ulva lactuca* (Sathivel *et al*., 2008), particularly when conducted under neutral or slightly alkaline conditions with sodium oxalate. This high sulfate content promotes the formation of a stable three-dimensional network (Bu *et al*., 2026), increasing the elastic modulus (G') and improving WHC, making ulvan excellent for applications requiring high gel stability, such as in the food industry. In contrast, Chemical-Assisted Extraction (CAE) employs an acidic solution with a low pH (1.5-2), which causes sulfate breakdown and produces ulvan with a lower sulfate content of approximately 36.72%-38.35% in *Ulva intestinalis* (Peasura *et al*., 2015). Although the viscosity of ulvan increases at higher concentrations, its ability to produce elastic gels is limited owing to its linear molecular structure and decreased WHC.

Other methods, such as Enzyme-Assisted Extraction (EAE), can maintain up to 29.9% sulfate content via selective hydrolysis at low temperatures (50°C) (Reis *et al*., 2020). The relatively high sulfate content helps preserve the water-holding capacity (WHC) and rheological properties of ulvan. WHC is directly influenced by the hydrophilic nature of sulfate groups, which facilitates water retention, while the rheological behavior, such as viscosity and elasticity, benefits from the structural stability provided by the retained sulfate groups.

Microwave-Assisted Extraction (MAE), on the other hand, improves extraction efficiency, but at high temperatures of up to 200°C, ulvan depolymerization occurs, causing a significant drop in sulfate content, for example, from 5.96% at 120°C to 3.37% at 200°C for *Ulva spp* (André et al., 2023). This loss of sulfate and depolymerization weakens the ability of ulvan to form three-dimensional networks, resulting in decreased WHC and reduced elastic modulus (G'), which negatively affects its gel-forming and viscoelastic properties.

Similarly, Ultrasound-Assisted Extraction (UAE) enhances extraction efficiency but may cause sulfate degradation under high ultrasonic intensity, as observed in *Ulva intestinalis* extracted at 65°C, where the sulfate content was reduced to 18% (Kazemi *et al*., 2023). The lower sulfate concentration impairs the WHC and compromises the rheological characteristics of ulvan, such as its ability to maintain a stable viscosity or elastic gel structure, owing to the diminished interaction between polymer chains.

Thus, the extraction method must be optimized to balance the sulfate retention, WHC, and rheological parameters. HWE remains the method of choice for high-performance applications requiring strong viscoelastic gels and superior WHC, whereas EAE, MAE, and UAE may serve niche purposes where specific molecular characteristics or extraction efficiencies are desired, albeit with trade-offs in functional properties.

The main monomers of ulvan, such as rhamnose, glucuronic acid, xylose, and iduronic acid, play an important role in determining the functional properties of ulvan, including water retention capacity (WHC) and rheological properties. HWE retains considerable levels of major monomers, including rhamnose and glucuronic

acid. For example, Ulva extracted from *Enteromorpha clathrata* using HWE 100 oC for 2 h contains 10,70% rhamnose and 4.00 % glucuronic acid, *Ulva rotundata* (2h at 85 °C) contains rhamnose 50,2% and 7% xylose, and *Ulva pertusa* contains rhamnose 13 – 15%, galactose 0,64 – 1,59%, glucose < 1% and Xylose 5,53 – 6,29% (Kraithong et al., 2023). The high rhamnose content promotes the creation of a three-dimensional network via interactions between hydroxyl and sulfate groups, boosting the WHC and elastic modulus (G'). This makes the HWE-derived ulvan ideal for applications that require stable gels with high viscoelasticity. In contrast, the CAE method at low pH resulted in the destruction of the ulvan polymer structure, leading to a decrease in the content of monomers, such as glucuronic acid and xylose.

EAE, which uses specific enzymes such as cellulase and protease (Chen *et al*., 2021), was able to increase the content of certain monomers, especially rhamnose. In *Ulva spp.*, EAE produced 39.13%–59.00% rhamnose, 4.00%–14.00% glucose, and 3.4%–11.90% glucuronic acid in certain fractions, which is higher than other methods. The increased rhamnose content, along with glucose and glucuronic acid, contributes significantly to the water-holding capacity (WHC) and rheological properties of ulvan. Rhamnose, with its hydrophilic qualities, aids in water absorption, whereas glucuronic acid leads to the creation of a better gel structure, thus improving the WHC. Furthermore, as the primary component of the polysaccharide series, glucose contributes to the structural integrity of the gel. This combined change in monomer composition causes ulvan to exhibit increased viscosity and shear-thinning behavior, indicating that enzymatic extraction not only increases the monomer content but also modifies the polysaccharide structure, making it more effective for applications requiring high WHC and specific rheological properties.

Several ideas can explain the association between ulvan's change in monomer composition and an increase in its WHC and rheological properties during enzymatic extraction (EAE). According to the hydrophobic and hydrophilic interaction theory, hydrophilic compounds, such as rhamnose and glucuronic acid, enhance ulvan's capacity to interact with water. The structure of rhamnose contains hydroxyl (-OH) groups, which form hydrogen bonds with water molecules, enhancing ulvan's WHC. Glucuronic acid, which contains carboxyl groups (-COOH), facilitates gel formation through ionic interactions and the crosslinking of ulvan molecules, thereby increasing viscosity and enhancing gel-forming ability. In contrast, glucose, the major component of the polysaccharide family, creates microcrystalline structures that provide ulvan with mechanical stability, influencing its viscosity and shear-thinning behavior. Enzymatic extraction techniques utilize cellulases and proteases to break down large polysaccharides into smaller monomers, facilitating improved water interaction and viscosity modulation. As a result, a higher monomer concentration not only raises the WHC but also improves ulvan's rheological qualities, making it more appropriate for applications that require both.

The MAE method yielded variations in monomer content, depending on the extraction temperature. High temperatures (>160 °C) caused monosaccharide degradation, whereas moderate temperatures (120 °C) helped maintain the integrity of monomers, such as glucuronic acid and rhamnose (André *et al*., 2023). The

rhamnose content at different temperatures (120, 140, 160, 180, and 200°C) was 37.44 ± 0.25%, 35.31 ± 0.08%, 33.18 ± 0.27%, 32.02 ± 0.16%, and 25.55 ± 0.09%, respectively, when ulvan was extracted from *Ulva pertusa Kjellm*.

The UAE approach increases monomer availability by breaking down the structure of the ulvan complex; nevertheless, high intensity can result in reduced monomer content owing to excessive depolymerization. At 80 kHz frequency, amplitude, 1130 W power, and a constant temperature of 55°C, the resultant monomer contents were as follows: rhamnose (23.58%), glucuronic acid (7.89%), iduronic acid (4.51%), and xylose (3.59%) (Baltrusch *et al*., 2024). Combination approaches, such as UAE-EAE, yield better results in terms of monomer content, particularly rhamnose and glucuronic acid, compared to solo procedures. The combination approach resulted in greater monomer content for rhamnose (22.4%-29.5%), xylose (10.0%-13.3%), glucose (5.0%-8.2%), and galactose (0.3%-0.5%). The combination of ultrasonically assisted extraction (UAE) and enzymatic extraction (EAE) yielded a higher rhamnose content than UAE alone, due to the synergy between the two processes. UAE uses ultrasonic vibrations to break down cell membranes and liberate intracellular components, such as ulvan. However, if the extraction intensity or duration is too high, UAE may result in excessive depolymerization, thereby lowering the concentration of some monomers, notably, rhamnose. In contrast, EAE uses enzymes such as cellulases and proteases to selectively break polysaccharide bonds without altering the monomer structure, allowing rhamnose and other monomers to remain intact. The combination of these two approaches enhances the extraction efficiency because EAE opens up the polysaccharide structure, enabling enzymes to break down complex linkages more efficiently. Furthermore, EAE helps prevent excessive degradation of rhamnose, increasing its content in the extracted fraction. With more regulated extraction conditions, the UAE-EAE combination enables more optimal extraction of rhamnose without sacrificing its stability, resulting in a higher rhamnose concentration than UAE alone. These findings indicate that the extraction method has an impact not only on the amount of ulvan extracted but also on its monomer composition, which is crucial for determining the biological features and applications of ulvan.

Conclusions

Extraction methods significantly impact the molecular structure, sulfate content, and functional properties of ulvan, which are crucial for its water-holding capacity (WHC) and rheological behavior. Hot Water Extraction (HWE) and Enzymatic Extraction (EAE) maintain branching structures and high sulfate content, making them ideal for applications requiring strong gelling and WHC. In contrast, methods such as Chemical-Assisted Extraction (CAE), Microwave-Assisted Extraction (MAE), and Ultrasound-Assisted Extraction (UAE) yield more linear ulvan, which enhances viscosity at higher concentrations.

HWE is efficient but energy-intensive, with potential thermal degradation at high temperatures. EAE preserves functional properties and is ideal for viscoelastic gels, but is costly and enzyme-dependent. MAE offers fast extraction but may reduce the

sulfate content owing to high temperatures. The UAE boosts efficiency and can retain monomers when combined with EAE; however, excessive intensity may lower the molecular weight and sulfate content. CAE increases viscosity at high concentrations, but it risks degradation due to harsh conditions.

The combination of UAE and EAE shows promise for optimizing monomer retention, especially rhamnose and glucuronic acid, but requires careful control to preserve the sulfate content and molecular weight. Further research should focus on balancing these factors to enhance ulvan's industrial applications in the food, cosmetics, and biomedical fields.

Ulvan's high WHC and rheological qualities make it appropriate for use as a stabilizer in dairy- or plant-based beverages, thickening in yogurt or pudding, and as an emulsifier in sauces and mayonnaise. Its capacity to retain moisture extends the shelf life of bread products, whereas its gel-forming and binding properties improve the texture and juiciness of meat analogs. However, if ulvan has poor WHC and rheological properties, its potential shifts to the nutraceutical sector, where its bioactive components, such as its antioxidant, anti-inflammatory, and immunomodulatory properties, make it valuable for functional foods and dietary supplements that promote health and disease prevention.

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