Biomedical Applications of Marine Biopolymers in Tissue Engineering and Regenerative Medicine

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Abstract

Marine ecosystems harbor incredibly rich biodiversity, which houses an extensive variety of biological substances with abundant economic value. Tissue engineering and regenerative medicine (TERM) employ engineering and natural biotic laws for fabricating novel tissues and organs, encouraging the repair and renewal of tissues and organs impaired by trauma or disease. Biopolymers were studied extensively in regenerative medicine due to their nontoxicity, low antigenicity, feasibility to complicated shapes and scaffolds, appropriate porosity, and mechanical strength with the ability to support cell growth and proliferation. Biopolymers derived from marine sources form a part of the growing number of materials reported as suitable options in regenerative medicine due to their natural availability, biodegradability, biocompatibility, and ease of isolation. The biomedical industry is investigating marine biomaterials with specific properties for application in bone regeneration, wound dressing, wound healing, regenerative

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medicine, and drug delivery. In the present review, we have discussed some recent developments in marine biopolymers focusing on their promise for biomedical applications, specifically in TERM.

Keywords

Marine biopolymers · Biomedicine · Tissue engineering · Drug delivery · Carrageenan · Fucoidan

3.1 Introduction

Damage to tissues and organs is a serious problem caused due to accidents, infections, or illnesses. It remained an unresolved challenge to anneal the damaged organs and restore their functionality due to the shortage of organs for transplantation. In the United States, an average of 18 deaths are recorded each day due to the shortage of organs (Liu et al. 2013). The increase in organ transplantation surgeries' failure due to graft rejections has imposed another challenge to surpass this serious issue. With the failures in organ transplantation surgeries and the shortage of organs, tissue engineering strategies have become eminent in developing new alternatives to repair and replace defective organs (Mandrycky et al. 2017). With advances in material sciences, biomedicine and bioengineering technologies, researchers are fostering various approaches to provide solutions to address this serious issue. In 1993, regenerative medicine through tissue engineering (TE) was proposed to overcome the body's shortage of organs and graft rejections (Vacanti and Vacanti 2000).

Tissue engineering and regenerative medicine employ the principles of engineering and biology to fabricate fresh tissues and organs and induce the renewal of new tissues and organs destroyed by trauma or any disease. It focuses on developing supporting matrices or scaffolds where cells are seeded and cultured with certain growth regulators and bioactive compounds. These supplements promote cell growth, and the supporting scaffolds point to new organs as implants to replace the damaged tissues or organs (Kaihara and Vacanti 1999). The engineered implants adequately integrate at the implantation location, followed by cells undergoing multiplication and differentiation to produce a new extracellular matrix. Then the implanted scaffolds undergo degradation and excrete out of the body (Velasco et al. 2015). With this perception, various biodegradable polymers such as collagen, silk fibroin and chondroitin sulfate were used to develop the scaffold materials. But it is believed that these materials would interfere with the body's growth, physiology and biological roles by interacting with other cells (Grabska-Zielińska et al. 2020). The marine ecosystem harbors abundant natural substances with great economic and medicinal importance. Marine biopolymers were considered ideal polymers for developing scaffolds in supporting tissue and organ matrices. Their inertness towards the cellular processes, porosity, ability to support cell growth, nontoxicity, low antigenicity, biodegradability, and flexibility to complex shapes are suitable

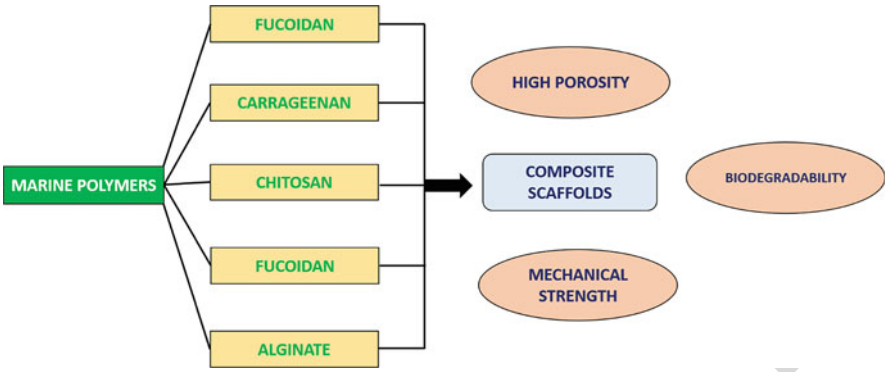

Fig. 3.1 Popular marine polymers and their desirable properties for use in TERM

materials in developing scaffolds for TERM. Chitin/chitosan, alginic acid, fucoidan, and carrageenan are marine biopolymers currently used in modern medicine and applied in TERM. Recent advancements in biomedicine focusing on potential marine biopolymers in TERM were reviewed in the following sections of this article. Some of the marine biopolymers used in TERM and their desirable properties are depicted in Fig. 3.1.

3.2 Marine Biopolymers in TERM

Regenerative medicine is an interdisciplinary field of biomedical research that integrates the laws of medicine, materials science and biology that enables the generation of organs having better function and biological structures. It combines tissue engineering and drug delivery approaches (Murphy and Atala 2014). Regenerative medicine helps implant the framework material that is the basis of recruiting the native cells into the scaffold, thereby resulting in extracellular matrix (ECM) deposition. Cell scaffold provides artificial ECM in a temporary environment that will help support the cells in adherence, infiltration, proliferation, and differentiation (Hussey et al. 2018). Cell scaffolds perform a major part in TE as they can provide early mechanical support and maintain cells present in the damaged areas aiding in cell division, metabolism and production of ECM (Mantha et al. 2019).

The properties of an ideal scaffolding material include desired mechanical strength, suitable microstructure, and biocompatibility, along with the capability to host cells and retain their biochemical processes (Salvatore et al. 2021). Cell-supporting matrices are made of both natural and synthetic biomaterials. Polymers from the natural origin are preferred as they share similarities with ECM, are chemically versatile, and have improved biological performance (Boni et al. 2018). Marine biopolymers were used in regenerative medicine for varied tissue applications, which is depicted in Fig. 3.2.

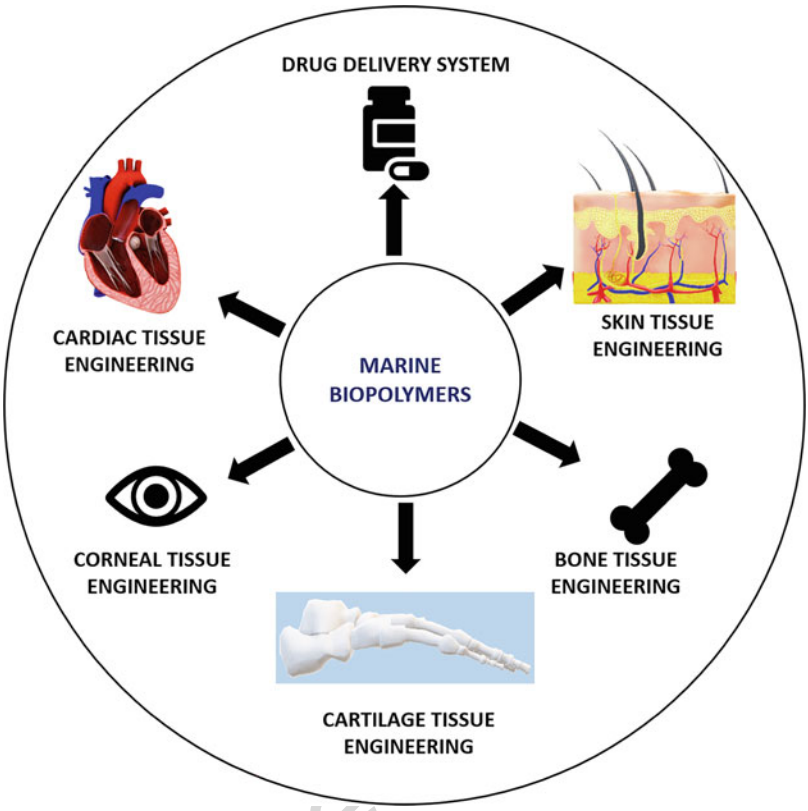

Fig. 3.2 Medical applications of marine biopolymers in tissue engineering. (Figure adapted from Kalirajan et al. 2021 and redrawn for this article)

3.3 Tissue Engineering: Perspectives of Marine Biopolymers

3.3.1 Fucoidan

Fucoidan is a polysaccharide composed of fucose and sulfate groups. It is present in various pheophycaea (brown algae) members. Fucoidan is also a component in the cell wall of red algae to protect them from external stress conditions. The commercial form of fucoidan was obtained from the seaweeds like *Cladosiphon okamuranus*, *Fucus vesiculosus*, *Laminaria japonica,* and *Undaria pinnatifida* (Fitton 2011). Other fucoidan forms were also obtained from invertebrate species, such as sea hedgehogs and sea cucumbers (Atashrazm et al. 2015). Fucoidan is primarily comprised of galactose, sulfated fucose, xylose, and uronic acids etc. (Rioux et al. 2007). Fucoidan was studied for its therapeutic activities like anti-cancer, anti-coagulant, anti-inflammatory, anti-oxidant, anti-viral, and

immunomodulatory functions (Jin and Kim 2011). Fucoidan has been used in engineering various damaged tissues and organs in the past decade. The anionic property of fucoidan, owing to the fucose and sulfate group esters, tends to induce osteoblast formations in the mesenchymal stem cells (Li et al. 2008). A composite from the chitosan-alginate-fucoidan combined improvised the bioactivity, mineralization and adsorption of proteins for bone regeneration in MG63 cells (Venkatesan et al. 2014). In another study, a fucoidan-incorporated scaffold Tcp-Fu-Ch (Tricalcium phosphate-fucoidan and chitosan) exhibited osteogenic differentiation of bone marrow cells that aided treatment for bone ailments (Puvaneswary et al. 2016). Besides the regeneration of bones, fucoidans were extensively investigated for their potential role in wound healing and dressing. Fucoidan alleviates and stimulates the heparin-binding cytokines that help to promote new blood vessel formation during wound repair. Fucoidans interact with cell signaling factors such as fibroblast growth factor (FGF) and transforming growth factor-β (TGF-β) to induce fibroblast proliferation resulting in the reconstruction of skin epidermis (O'Leary et al. 2004; Song et al. 2014). Therefore, it facilitates wound healing with nontoxic action on the tissues.

Several reports have proved that fucoidans are key regulators for wound healing. For instance, a low-molecular-weight fucoidan with 5 kDa derived from the seaweed *Undaria pinnatifida* has thickened dermal excision in a wound-healing rat model (Obluchinsksya et al. 2015). This was a dose-dependent effect where wounded animals treated with this solution showed accelerated angiogenesis and remodeled collagen. A combination of fucoidan and polyvinyl alcohol (PVA) in a 70:30 ratio showed enhanced wound healing effects within 8 days post-treatment. Higher collagen content was observed in the treatment group compared to the control with complete re-epithelialization and renewal of the epidermis at the wounded site (Feki et al. 2019). A micro chitosan-fucoidan biodegradable hydrogel created by Nakamura et al. enhanced the fibroblast growth factor 2 (FGF-2) function and secured it from deactivation. Mice injected with this hydrogel subcutaneously developed significant neovascularization at the injection site (Nakamura et al. 2008). Micro and macro 3D scaffolds based on fucoidan filled with vascular endothelial growth factor (VEGF) facilitate neovascularization by ensuring angiogenic activity in ischemic tissues (Purnama et al. 2013).

3.3.2 Carrageenan

Carrageenan (CRG) is a seaweed polysaccharide isolated from marine red algae (Rhodophyceae) bearing distinguishing structural and functional characteristics. CRG is an anionic, sulfated polygalacton comprising of interchanging linear polymers of α-1,3 D-galactose and β-(1-4)-3,6-anhydro-D-galactose bearing ester sulfates (15–40%). CRGs closely resemble glycosaminoglycans of ECM (Yegappan et al. 2018). CRGs are categorized into six types based on the percent of sulfation and their origin, such as Kappa (κ)-, Iota (ι)-, Lambda (λ)-, Mu (μ)-, Nu (ν)-, and Theta (θ)-CRGs. Kappa, Iota, and Lambda are commercially important (Cunha and

Grenha 2016). CRG is popularly utilized as gelling, emulsifying, and stabilizing agent in medical and industrial applications (Liu et al. 2015). CRG was studied for its anti-inflammatory and anti-cancer properties. CRG was further identified as a herpes and human papillomavirus inhibitor (Zhou et al. 2004; Panlasigui et al. 2003; Carlucci et al. 1999; Buck et al. 2006). Recently hydrogels based on marine biopolymers (chitosan, alginate, agarose, carrageenan) have been utilized in tissue engineering due to their abundance (Varoni et al. 2012; Vignesh et al. 2018). Some reports where CRG was utilized in regenerative medicine are presented in Table 3.1.

Hydrogels are complex and intricate framework of hydrophilic long biopolymers comprising 90–99% of water. These materials gained a reputation for their applications in tissue engineering due to excellent biological behavior like biodegradability, biocompatibility, minimum cytotoxicity, immunogenicity and ease of tuning their physical and chemical properties (Saul and Williams 2011). The 3D structure closely mimics the tissue extracellular matrix environment, providing a biocompatible environment for cell growth and proliferation (Geckil et al. 2010). Out of the CRGs available in markets, Kappa CRG, upon cooling and under the availability of cations such as K^+ and Ca^{2+} undergoes structural and mechanical transitions and aggregations led to form thermotropic and ionotropic hydrogels. CRG hydrogels are brittle under physiological conditions and have a high swelling ratio with poor mechanical stability (Thakur et al. 2016). CRG polymeric backbone is amenable to chemical alterations because hydroxyl or sulfate functional moieties are available. Some chemical modifications on CRG, such as oxidation, over sulfation, carboxymethylation, methylacrylation, and phosphorylation, have endowed the polymer with new functionalities and properties (Yegappan et al. 2018).

When mixing with functional bioactive molecules, CRGs can form an apatite layer (Kim et al. 2011). CRG hydrogels incorporated with varied proportions of rod-shaped *n*-hydroxyapatite (*n*HAP) demonstrated enhanced cell-cell communication and adherence (Morais et al. 2013; Gonzalez et al. 2017). Poly (hydroxybutyrate) (PHB) and poly(hydroxybutyrate valerate) (PHBV) nanofilaments engineered by superficial application of κ-CRG hydrogel led to improved osteoblast cell specialization and biomineralization. Compared to non-CRG fibers, CRG-coated fibers exhibit nano–microscale apatite crystals. Furthermore, CRG-coated fibers improved biomineralization upon culturing in osteosarcoma cells (Goonoo et al. 2017). Selenium nanoparticles (SeNPs) in the presence of hydroxyl, carboxyl and sulfate groups of CRG achieved excellent stability. Moreover, λ-CRG/SeNPs hydrogel showed no toxicity and displayed calcium deposits indicating biomineralization when cultured in the presence of osteoblast D1 cells (Kim et al. 2016). A blended scaffolding designed using ι-CRG, chitosan, and gelatin resulted in osteogenic delineation and rescue of adipose-derived mesenchymal stem cells (AdMSCs) because the availability of several chemical moieties such as hydroxyl, carboxyl, amine, and sulfate in the scaffolds favored cell survival (Li et al. 2015). Microvascular-like endothelial cells encapsulated inside κ-CRG hydrogel coated with the chitosan retained their functionality and phenotype, and survived a prolonged period (Mihaila et al. 2014). Collagen, hydroxyapatite and

Table 3.1 Recent biomedical applications of CRG in combination with other biomaterials in tissue engineering

S. no.	Carrageenan + other polymers	Properties observed	References
1.	Nanocomposite films of aldehyde-modified carrageenan/gelatin/halloysite nanotubes (AD-Carr/Gel/HNTs)	AD-Carr/Gel/HNTs nanocomposite films are found to be hemocompatible and nontoxic by in vitro assays	Akrami-Hasan-Kohal et al. (2020)
2.	Nano hydroxyapatite-silver—3% wt carrageenan	3% CRG addition led to higher stiffness of the hydrogel scaffold, induced better cell adhesion and proliferation and biodegradable rate	Swain et al. (2021)
3.	Poly(vinyl alcohol) (PVA)/methacrylate kappa-carrageenan (κ-CaMA) composite hydrogel encapsulated with chitooligosaccharides (COS) [PVA/κ-CaMA/COS]	PVA/κ-CaMA/COS hydrogel showed uniformly thick, highly porous 3D architecture with uniformly distributed pores, a high fluid absorption and retention capacity, mechanical stability and in vitro biocompatibility. Wound dressing resulted in tissue re-epithelization and skin tissue remodeling	Chandika et al. (2021)
4.	Carboxymethyl kappa-carrageenan + poly(vinyl alcohol)	Enhanced fibrinogen adsorption, platelet activation and blood clotting. Displayed antibacterial activity against *S. aureus* and *P. aeruginosa*; showed promise as wound dressing	Madruga et al. (2021a, b)
5.	Kappa carrageenan (κCA)-coated starch/cellulose nanofiber (CNF)	Surface coating of starch/CNF hydrogel with κCA improved mechanical properties, reduced swelling ability and degradation rate of starch/CNF, and showed blood compatibility and blood clotting ability	Tavakoli et al. (2021)
6.	Poly(vinyl alcohol) (PVA) and carboxymethyl-kappa-carrageenan (CMKC) blend poly(vinyl alcohol) (PVA) and carboxymethyl-kappa-carrageenan (CMKC) blends poly (vinyl alcohol) (PVA) and carboxymethyl-kappa-carrageenan (CMKC) blends poly(vinyl alcohol) and carboxymethyl-kappa-carrageenan (CMKC) blends	CMKC improved cytocompatibility, biodegradability, cell growth and adhesion compared to PVA fibers alone. PVA/CMKC nanofibers enhance human adipose-derived stem cells' response to osteogenic differentiation signals	Madruga et al. (2021a, b)
7.	Porous bioactive alginate/carrageenan/calcium silicate scaffolds	In BTE, the formation of hydroxyapatite crystals was observed on the surface of the scaffold after soaking in SBF solution; the presence of calcium	Sathain et al. (2021)

(continued)

Table 3.1 (continued)

S. no.	Carrageenan + other polymers	Properties observed	References
		silicate enhanced the bioactivity and mechanical properties of the scaffolds; in vitro drug release behavior of diclofenac-loaded scaffold was suitable for acute inflammation post-surgery	

κ-CRG complexed scaffold was fabricated bearing excellent bone-like innate structure. CRG components in the composite protect the nerve growth factor (NGF) by preventing its degradation and allowing sustained release to overcome frequent administration of NGF, which is critical for the repair, preservation and growth of nerves (Wang et al. 2009, 2010).

CRG contains several sulfate moieties in its structure, which imitates the biologically existing sulfated glycosaminoglycans in the ECM portion of cartilage, thus making CRG an excellent choice for the engineering of cartilage tissue (Popa et al. 2016). κ-CRG hydrocolloid in combination with iron oxide magnetic nanoparticles showed feasible properties such as increased stability, chondrocyte differentiation, cellular responses, and increase in expression of chondrocyte gene markers (Popa et al. 2012, 2016). Human adipose stem cells (HASCs) encapsulated in κ-CRG scaffold were noncytotoxic, and supported chondrocytes differentiation and proliferation in the presence of chondrogenic growth factors (Popa et al. 2015). CRG hydrogels have excellent mechanical properties and freeze-thaw stability during cryopreservation—thawing process making them excellent materials for encapsulation systems (Popa et al. 2013). κ-CRG hydrogel in the presence of human adipose-derived stem cells (hADSC) and TGF-β1 promoted chondrogenic differentiation combined with chondrocyte-related gene expression (Rocha et al. 2011). CRG, fibrin, and hyaluronic acid (HA) composite scaffold encapsulated human articular chondrocytes (HACs) have displayed better elasticity in comparison with fibrin/HA only besides promoting cartilage ECM accumulation (Fuss et al. 2000). Gelatin methacryloyl (GelMA) and methacrylated (Mκ-CRG) incorporated with nano silicates possessed multiple applications in tissue engineering. Seeding cells in GelMA and Mκ-CRG regions displayed round and diffused arrangement, a characteristic feature of chondrocytes and osteoblasts (Pereira et al. 2009). Despite several advantages of CRG in tissue engineering, few reports of adverse effects of CRG is an important drawback that requires attention when utilizing CRG for medical purposes. More in vivo research reports are needed to support the regenerative efficacy and medical application of CRG as a valid biopolymer for tissue engineering applications.

3.3.3 Chitosan

Chitosan is derived through the deacetylation of chitin which is part of the crustacean exoskeleton of Arthropod members such as crustaceans and insects. Chitosan is a natural polymer with a linear structure consisting of D-glucosamine units bonded through β(1-4)glycosidic linkages and varied *N*-acetyl-D-glucosamine (NAG) groups. The molecular mass of chitosan falls in the range of 300 to above 1000 kDa, subject to the degree of deacetylation, preparation method, and source of the polymer. Several reports have evidenced that chitosan displays increased cell attachment, division, osteoblast specialization, and mineralization (Seol et al. 2004; Di Martino et al. 2005; Senel et al. 2004). In bone tissue engineering (BTE), chitosan could be fabricated to create 3D scaffoldings with numerous ceramics and polymers of varied porosity and possessing properties such as being bioactive, biodegradable, and biocompatible with a hydrophilic surface.

These composites can mimic the natural bone function and promote cell attachment, multiplication and differentiation. Chitosan could be chemically modified by attaching functional groups, peptides or amino acids on primary amines and secondary hydroxyl groups that aid in optimization and functionalization for use in BTE (Amidi et al. 2010). Chitosan was also used extensively in promoting wound healing, and it also possesses antimicrobial activity owing to its cationic property. Chitosan activates and modulates the inflammatory cells, thereby facilitating the development of tissues. As a wound dressing component, chitosan promotes blood clotting (Alexander et al. 1996). The role of chitosan in cell division, osteoblast specialization and bone mineralization has been well recognized (Van Tienen et al. 2002). Some of the research reports where chitosan was used for TERM applications are presented in Table 3.2.

Due to its physical properties, it is possible to fabricate chitosan into several structures, such as membranes, scaffolds, 3D structures, and fibers. Chitosan-based scaffolds are produced through lyophilization or freeze drying, 3D printing, salt leaching, and electrospinning methods. Most of these fabrication techniques have no control over the scaffold geometry and architecture. The application of CS scaffolds, irrespective of fabrication methodology on load-bearing sites, is highly doubtful. Therefore, the amalgamation of CS with other natural, synthetic, and ceramic polymers has displayed increased biological activity and structural properties compared to natural CS. Scaffold porosity is crucial for cellular penetration, attachment, release of extracellular matrix elements, and bone growth (Van Tienen et al. 2002; Yang et al. 2001). Therefore, the essential criterion for optimal cell infiltration and adhesion is controlling the required pore size. The porosity of the CS scaffold is influenced by CS percentage, type of crosslinkers, temperature, and addition of nanomaterials etc., including crosslinkers like tripolyphosphate and ethyl-3 [3-dimethylaminopropyl] carbodiimide hydrochloride (EDAC) improved the pore structure and pore connections inside the scaffolds (Liu et al. 1989; Lie et al. 2003; Park et al. 2002). Adding nanoparticles to chitosan scaffolds or CS amalgamated matrices may or may not affect the pore size. Adding the Hap material has

t.1 **Table 3.2** Applications of chitosan in tissue engineering and biomedical applications when combined with different polymers for improved biological properties

t.2 S. no.	Chitosan + other polymer(s)	Properties observed	References
t.3 1	Chitosan: gelatin (Ch:Gel) cryogels	• Good biocompatibility with L929 mouse fibroblasts	Öfkeli et al. (2021)
t.4		• Neither necrosis nor foreign body rejection was witnessed	
t.5		• Nonirritant and nontoxic for bone tissue	
t.6 2	Hydroxyapatite (HAP), chitosan (CS), and antibiotic gentamicin (Gent) composite reinforced with graphene (Gr)	• Coatings had porous, uniform, fracture-free surfaces	Stevanović et al. (2020)
t.7		• Large specific areas of graphene enabled strong bonding with chitosan	
t.8		• Gentamicin addition strongly improved the antibacterial activity	
t.9		• Exhibited low cytotoxicity and good biocompatibility	
t.10 3	Chitosan (CS) + polyethylene oxide (PEO) nanofibers + Coral	• Plasma treatment of composite enhanced osteoblast performance and cell adhesion	Tabaei et al. (2021)
t.11		• Thick layers of flake-like carbonate apatite nanocrystals were covering plasma-treated coral/CS/PEO fibers	
t.12 4	Chitosan + silver nanoparticles (AgNPs)	• CS-Ag polymeric scaffolds are highly porous and biodegradable	Vaidhyanathan et al. (2021)
t.13		• Found to be cyto-compatible and supported growth of osteoblasts	
t.14		• Showed enhanced osteogenic differentiation and matrix mineralization	
t.15 5	Bioglass + chitosan (BG/CH) beads loaded with streptomycin sulfate (STRS)	• STRS loaded BG/CH beads displayed inhibition against Gram-positive and Gram-negative organisms	Al-esnawy et al. (2021)
t.16		• Beads displayed the biological activity of deposition of a layer of hydroxyapatite on their surface	
t.17 6	Injected hydrogels containing poly(*N*-isopropylacrylamide) (PNIPAAm)-based copolymer/ graphene oxide (GO) composite with different ratios to chitosan (CS)	• Displayed enhanced deposition of minerals and activity of alkaline phosphatase (ALP)	Amiryaghoubi et al. (2020)
t.18		• Upregulate the expression of the Runt-related transcription factor 2 and osteocalcin in the human dental pulp stem cells (hDPSCs)	

(continued)

Table 3.2 (continued)

S. no.	Chitosan + other polymer(s)	Properties observed	References
7	Chitosan (CS) + polycaprolactone (PCL) + zinc divalent ions	• CS/PCL/Zn scaffolds were compatible with mesenchymal cells from human ovarian follicular fluid (HOFF) and promoted osteoblast differentiation	Chandramohan et al. (2021)
		• The construct improved calcium deposition and ALP activity	
		• Osteoblast marker expression increased, such as Runx2, type-1 collagen, osteonectin (ON) and osteocalcin (OC)	
8	Chitosan (CS) + mesoporous zinc sulfate (mZS)	• Scaffolds showed improved porosity, degradation rate and biomineralization	Jindal et al. (2020)
		• Displayed antibacterial activity against *Staphylococcus aureus*	
		• Scaffold showed biocompatibility and allowed cellular attachment	
9	Chitosan (CS) + regenerated cellulose (rCL) nanofibers	• The composite showed unique pore morphology, higher compressive strength, improved biomineralization and increased pre-osteoblast cell (MC3T3-E1) viability, attachment and proliferation	Maharjan et al. (2021)

significantly reduced the porosity. Adding Zirconium to the CS scaffolds yielded better pore connectivity (Lima et al. 2013; Teimouri et al. 2015).

The connectivity among the pores of the CS scaffold occupies a critical role in transporting oxygen and nutrients, removing wastes and neovascularization. Water retention ability denotes tissue engineering scaffolds' capacity to absorb and retain water. This property is also referred to as swelling ability. The swelling ability is influenced by ionizable chemical moieties inside the scaffold and the neighboring surroundings (Chapekar 2000). CS swells through protonation of amine/imine groups which determine the swelling ability. Adding $n\mathrm{SiO_2}$ and $n\mathrm{ZrO_2}$ significantly inhibited the swelling ability of the CS scaffold (Pattnaik et al. 2011). Attachment of proteins or peptides to the TE scaffolds for a basis for ensuing cell communications, cell proliferation and spreading upon introduction into a biological system (Misra et al. 2012; Rogina et al. 2015). NH_2 and COOH functional groups of chitosan are crucial for protein adsorption. Since these functional groups are hydrophilic, they associate with proteins using their hydrophilic side chains through noncovalent interactions such as Vander Waals and electrostatic forces. Nanoparticles are frequently added to CS scaffold to increase the protein adsorption capacity, including

$n\mathrm{SiO}_2$, and $n\mathrm{ZrO}_2$ chitosan/alginate of chitosan scaffold improved the protein adsorption capacity of biocomposite scaffolds (Sowjanya et al. 2013; Pattnaik et al. 2010). Biomineralization is a process of overlay of minerals and ions accumulated on the exterior of TE scaffolds after coming in contact with a biological medium. The deposition of bone apatite material on the scaffold exterior is critical for properly integrating the polymer scaffold with the bone. Therefore, the biomineralization ability of a composite scaffold is established by exposing the materials to simulated body fluids (SBF). Chitosan is an excellent matrix for biomineralization owing to the availability of amine and carboxyl side chains.

Chitosan alone showed poor biomineralization capacity compared to CS biocomposite scaffolds which showed excellent biomineralization potential—including $n\mathrm{ZrO}_2$ to CS/$n\mathrm{SiO}_2$ scaffolds and including nBGC to CS/Gn particles increased nucleation sites, leading to enhanced apatite formation (Pattnaik et al. 2011; Peter et al. 2010). CS only exhibited marginal biomineralization, while CS combined with diopside particles enhanced the deposition of apatite due to the involvement of silanol groups (Kumar et al. 2014). CS/Alg scaffolds demonstrated enhanced biomineralization potential upon adding $n\mathrm{SiO}_2$ particles (Sowjanya et al. 2013; Jongwattanapisan et al. 2011). Biodegradation is another hallmark of tissue bioengineering scaffolds which involves the slow and gradual breakdown of biomaterial in a living system through the action of enzymes and additional bioactive elements. Biodegradation is an important property for BTE scaffolds, and the amount of degradation must be equal to that of creating new bone.

3.3.4 Role of Alginate in Wound Healing

Alginate is a hydrophilic polysaccharide found naturally and accounted for one of the copiously biosynthesized materials derived from bacteria and red seaweed. Alginate has widespread applications in the field of biomedicine. It has excellent biocompatibility and biodegradability (Hinchliffe et al. 2021). Tissue engineering utilizes alginate-based biomaterials as successful drug delivery systems and cell carriers. The alginate derivatives can be obtained by modifying various morphological and chemical properties of the native alginate (Abasalizadeh et al. 2020). Advancements in the delivery systems resulted in the advancements in scaffold materials that are often utilized to deliver drugs, growth factors, and cells for the therapeutic purpose. Such composite scaffolds will help to keep the biologically active substances and the surrounding cells intact (Rodrigo-Navarro et al. 2021). Based on the site of the implant, biomaterials will be subjected to various pH that might affect the mechanical and degradation properties along with its swelling behavior. Hence, alginate stands predominantly for its long-term stability and performance as a biomaterial in vitro. Degradation rate is directly related to the molecular weight of the biomaterial (Nicu et al. 2021). Therefore, the molecular weight of alginate-based biomaterials strongly influences the structural rigidity and degradation rate. Increased molecular weight results in decreased availability of reactive groups for hydrolysis, resulting in a much slower rate of degradation. The

degradation rate, in turn, will affect the mechanical properties inherently, which will impact the structural changes at molecular levels (Kreller et al. 2021).

U.S. FDA-approved alginate has gained popularity for several applications in nutrition supplements, regenerative medicine, and semi-permeable separation. There is an immense requirement for alginate-based biopolymers in drug delivery and TE (Thomas 2021). Understanding stem cells' relationship with alginate-based biomaterials is crucial, as stem cells are important components of regenerative medicine. Data from in vitro implantation and in vitro cytotoxicity assays suggest that alginate-based scaffolds and microcapsules have minimal or very less cytotoxicity and are histocompatible (Prasathkumar and Sadhasivam 2021). These in vitro studies have revealed information on the interactions that are adjustable between the biocomposites and platelet release-associated growth factors that will enhance the repair of the hematoma-like fracture. In addition, rat tail vertebrate was used to demonstrate the minimally invasive delivery approach through inoculation using microcomputed tomography for in situ rejuvenation of implant materials (Xue et al. 2021). These studies have proved that alginate-based biomaterials undergo degradation, thereby allowing vascularization and having negligible levels of inflammatory responses post-transplantation. Hence, alginate-based scaffolds have proved as potential carriers for cells and drugs that aid tissue regeneration (Sahoo and Biswal 2021). AU6

The application of dressing to open wounds has been followed for ages as it can prevent wounds from getting injured further or from bacterial invasion. Here, gauze is the simplest material that is used for dressing. Advantages of gauze include low cost, easy handling and great absorbent capacity (Tang 2021). But the only disadvantage of the gauze is the secondary injury it might cause while peeling off. Recent findings include the development of high-quality wound dressings that will help create an occlusive environment that is moist and will promote a rapid healing process. Such dressings include gels, sponges, and semi-occlusive or occlusive dressings (Kim et al. 2021). Alginate has found use in several wound dressings, including hydrogels, sponges and electrospun mats. These will act as prospective substrates for the repair process offering wide benefits such as hemostatic ability and the ability to form a gel by absorbing the wound exudates (Bonferoni et al. 2021).

Alginate possesses all the properties that will promote wound healing, such as optimal water vapor transmission rate, good water absorptivity, gentle anti-septic properties, conformability, biodegradability, and nontoxicity. Various studies on alginate-based wound dressings suggest that alginate has enhanced properties of wound healing by monocyte stimulation which in turn produces increased concentrations of cytokines such as TNF-α and IL-6 (Miguel et al. 2021). Cytokine generation at the site of a wound will result in the generation of pro-inflammatory factors that will result in rapid wound healing. Enhanced bioactivity of the alginate could be due to the occurrence of endotoxins inherently. It was proposed that a hydrogel formed in situ from oxidized gelatin and alginate, along with a trace of borax, displayed excellent wound-healing properties when used as a wound dressing. In this wound dressing, alginate stands for wound healing, and borax stands for its anti-septic property (Balakrishnan et al. 2021).

In addition, the alginate backbone lacks a signal sequence responsible for adherence; thereby, alginate-based wound dressings will not result in secondary injury while peeling off. Wounds having large volume exudations can be dressed with an alginate-based sponge. A moist environment will promote wound healing, and alginate-based gels will help prevent the wound from drying out and keep it moist throughout the healing process (Tunçer 2021). As the wounds provide the most favorable environment for bacterial growth by encouraging the colonization of microbes, thereby leading to delayed healing and infection, the antimicrobial properties of the wound dressings prevent bacterial growth. A combination of alginate with silver nanoparticles and chitosan will be done for making antibacterial wound dressings. A composite polysaccharide sponge comprising alginate and water-soluble chitosan is an antimicrobial and anti-adhesive wound dressing (Kharaziha et al. 2021).

3.4 Conclusions

TARE technology is a suitable substitute for conventional tissue or organ grafting techniques. Among the marine biopolymers, chitosan, carrageenan, alginate, and fucoidan were promising and are researched extensively by in situ, in vitro, and in vivo methods. Although numerous scaffolds were designed using the above polymers and several biocompatible materials, they have drawbacks such as consistent reproducibility, mechanical strength, and neovascularization issues. Utilization of novel technologies for the fabrication of scaffolds containing the above bioactive materials and inclusion of bioactive molecules followed by testing their applications in promoting the tissue engineering and repair processes in vivo systems are needed.

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References

Abasalizadeh F, Moghaddam SV, Alizadeh E, Kashani E, Fazljou SMB, Torbati M, Akbarzadeh A (2020) Alginate-based hydrogels as drug delivery vehicles in cancer treatment and their applications in wound dressing and 3D bioprinting. J Biol Eng 14(1):1–22. https://doi.org/10.1186/s13036-020-0227-7

Akrami-Hasan-Kohal M, Ghorbani M, Mahmoodzadeh F, Nikzad B (2020) Development of reinforced aldehyde-modified kappa-carrageenan/gelatin film by incorporation of halloysite nanotubes for biomedical applications. Int J Biol Macromol 160:669–676. https://doi.org/10.1016/j.ijbiomac.2020.05.222

Al-esnawy AA, Ereiba KT, Bakr AM, Abdraboh AS (2021) Characterization and antibacterial activity of streptomycin sulfate loaded bioglass/chitosan beads for bone tissue engineering. J Mol Struct 1227:129715. https://doi.org/10.1016/j.molstruc.2020.129715

Alexander H, Brunski JB, Cooper SL, Hench LL, Hergenrother RW, Hoffman AS, Yannas IV (1996) Classes of materials used in medicine. In: Biomaterials science. Elsevier, Amsterdam, pp 37–130. https://doi.org/10.1016/B978-0-08-050014-0.50007-9

Amidi M, Mastrobattista E, Jiskoot W, Hennink WE (2010) Chitosan-based delivery systems for protein therapeutics and antigens. Adv Drug Deliv Rev 62(1):59–82. https://doi.org/10.1016/j.addr.2009.11.009

Amiryaghoubi N, Pesyan NN, FathiM OY (2020) Injectable thermosensitive hybrid hydrogel containing graphene oxide and chitosan as dental pulp stem cells scaffold for bone tissue engineering. Int J Biol Macromol 162:1338–1357. https://doi.org/10.1016/j.ijbiomac.2020.06.138

Atashrazm F, Lowenthal RM, Woods GM, Holloway AF, Dickinson JL (2015) Fucoidan and cancer: a multifunctional molecule with anti-tumor potential. Mar Drugs 13(4):2327–2346. https://doi.org/10.3390/md13042327

Balakrishnan SB, Kuppu S, Thambusamy S (2021) Biologically important alumina nanoparticles modified polyvinylpyrrolidone scaffolds in vitro characterizations and it is in vivo wound healing efficacy. J Mol Struct 1246:131195. https://doi.org/10.1016/j.molstruc.2021.131195

Bonferoni MC, Caramella C, Catenacci L, Conti B, Dorati R, Ferrari F, Genta I, Modena T, Perteghella S, Rossi S, Sandri G, Sorrenti M, Torre ML, Tripodo G (2021) Biomaterials for soft tissue repair and regeneration: a focus on Italian research in the field. Pharmaceutics 13(9):1341. https://doi.org/10.3390/pharmaceutics13091341

Boni R, Ali A, Shavandi A, Clarkson AN (2018) Current and novel polymeric biomaterials for neural tissue engineering. J Biomed Sci 25(1):1–21. https://doi.org/10.1186/s12929-018-0491-8

Buck CB, Thompson CD, Roberts JN, Müller M, Lowy DR, Schiller JT (2006) Carrageenan is a potent inhibitor of papillomavirus infection. PLoS Pathog 2(7):671–680. https://doi.org/10.1371/journal.ppat.0020069

Carlucci MJ, Scolaro LA, Damonte EB (1999) Inhibitory action of natural carrageenans on herpes simplex virus infection of mouse astrocytes. Chemotherapy 45(6):429–436. https://doi.org/10.1159/000007236

Chandika P, Kim MS, Khan F, Kim YM, Heo SY, Oh GW, Kim NG, Jung WK (2021) Wound healing properties of triple cross-linked poly(vinyl alcohol)/methacrylate kappa-carrageenan/chitooligosaccharide hydrogel. Carbohydr Polym 269:118272. https://doi.org/10.1016/j.carbpol.2021.118272

Chandramohan Y, Jeganathan K, Sivanesan S, Koka P, Amritha TMS, Vimalraj S, Dhanasekaran A (2021) Assessment of human ovarian follicular fluid derived mesenchymal stem cells in chitosan/PCL/Zn scaffold for bone tissue regeneration. Life Sci 264:118502. https://doi.org/10.1016/j.lfs.2020.118502

Chapekar MS (2000) Tissue engineering: challenges and opportunities. J Biomed Mater Res 53(6):617–620. https://doi.org/10.1002/1097-4636(2000)53:6<617::AID-JBM1>3.0.CO;2-C

Cunha L, Grenha A (2016) Sulfated seaweed polysaccharides as multifunctional materials in drug delivery applications. Mar Drugs 14(3):42. https://doi.org/10.3390/md14030042

Di Martino A, Sittinger M, Risbud MV (2005) Chitosan: a versatile biopolymer for orthopaedic tissue-engineering. Biomaterials 26(30):5983–5990. https://doi.org/10.1016/j.biomaterials.2005.03.016

Feki A, Bardaa S, Hajji S, Ktari N, Hamdi M, Chabchoub N, Kallel R, Boudawara T, Nasri M, Ben AI (2019) *Falkenbergia rufolanosa* polysaccharide - poly(vinyl alcohol) composite films: a promising wound healing agent against dermal laser burns in rats. Int J Boil Macromol 144:954–966. https://doi.org/10.1016/j.ijbiomac.2019.09.173

Fitton JH (2011) Therapies from fucoidan; multifunctional marine polymers. Mar Drugs 9(10):1731–1760. https://doi.org/10.3390/md9101731

Fuss M, Ehlers EM, Russlies M, Rohwedel J, Behrens P (2000) Characteristics of human chondrocytes, osteoblasts and fibroblasts seeded onto a type I/III collagen sponge under different culture conditions. A light, scanning and transmission electron microscopy study. Ann Anat 182(4):303–310. https://doi.org/10.1016/S0940-9602(00)80002-3

Geckil H, Xu F, Zhang X, Moon S, Demirci U (2010) Engineering hydrogelsas extracellular matrix mimics. Nanomedicine 5(3):469–484. https://doi.org/10.2217/nnm.10.12

González JI, Ossa CPO (2017) Injectability evaluation of bone-graft substitutes based on carrageenan and hydroxyapatite nanorods. In: Meyers M et al (eds) Proceedings of the 3rd Pan American materials congress. The minerals, metals & materials series. Springer, Cham, pp 33–46. https://doi.org/10.1007/978-3-319-52132-9_4

Goonoo N, Khanbabaee B, Steuber M, Bhaw-Luximon A, Jonas U, Pietsch U, Jhurry D, Schönherr H (2017) κ-carrageenan enhances the biomineralization and osteogenic differentiation of electrospun polyhydroxybutyrate and polyhydroxybutyrate valerate fibers. Biomacromolecules 18(5):1563–1573. https://doi.org/10.1021/acs.biomac.7b00150

Grabska-Zielińska S, Sionkowska A, Reczyńska K, PamułaE. (2020) Physico-chemical characterization and biological tests of collagen/silk fibroin/chitosan scaffolds cross-linked by dialdehyde starch. Polymers 12(2):372. https://doi.org/10.3390/polym12020372

Hinchliffe JD, Parassini Madappura A, Syed Mohamed SMD, Roy I (2021) Biomedical applications of bacteria-derived polymers. Polymers 13(7):1081. https://doi.org/10.3390/polym13071081

Hussey GS, Dziki JL, Badylak SF (2018) Extracellular matrix-based materials for regenerative medicine. Nat Rev Mater 3(7):159–173. https://doi.org/10.1038/s41578-018-0023-x

Jin G, Kim GH (2011) Rapid-prototyped PCL/fucoidan composite scaffolds for bone tissue regeneration: design, fabrication, and physical/biological properties. J Mater Chem 21(44): 17710–17718. https://doi.org/10.1039/C1JM12915E

Jindal A, Mondal T, Bhattacharya J (2020) An *in vitro* evaluation of zinc silicate fortified chitosan scaffolds for bone tissue engineering. Int J Biol Macromol 164:4252–4262. https://doi.org/10.1016/j.ijbiomac.2020.09.018

Jongwattanapisan P, Charoenphandhu N, Krishnamra N, Thongbunchoo J, Tang IM, Hoonsawat R, Smith SM, Pon-On W (2011) *In vitro* study of the SBF and osteoblast-like cells on hydroxyapatite/chitosan–silica nanocomposite. Mater Sci Eng C 31(2):290–299. https://doi.org/10.1016/j.msec.2010.09.009

Kaihara S, Vacanti JP (1999) Tissue engineering: toward new solutions for transplantation and reconstructive surgery. Arch Surg 134(11):1184–1188. https://doi.org/10.1001/archsurg.134.11.1184

Kalirajan C, Dukle A, Nathanael AJ, Oh TH, Manivasagam G (2021) A critical review on polymeric biomaterials for biomedical applications. Polymers 13(17):3015. https://doi.org/10.3390/polym13173015

Kharaziha M, Baidya A, Annabi N (2021) Rational design of immunomodulatory hydrogels for chronic wound healing. Adv Mater 33:e2100176. https://doi.org/10.1002/adma.202100176

Kim IY, Iwatsuki R, Kikuta K, Morita Y, Miyazaki T, Ohtsuki C (2011) Thermoreversible behavior of κ-carrageenan and its apatite-forming ability in simulated body fluid. Mater Sci Eng C 31(7): 1472–1476. https://doi.org/10.1016/j.msec.2011.05.015

Kim J, Lee KY, Lee CM (2016) Selenium nanoparticles formed by modulation of carrageenan enhance osteogenic differentiation of mesenchymal stem cells. J Nanosci Nanotechnol 16(3): 2482–2487. https://doi.org/10.1166/jnn.2016.10764

Kim K, Mahajan A, Patel K, Syed S, Acevedo-Jake AM, Kumar VA (2021) Materials and cytokines in the healing of diabetic foot ulcers. Adv Ther 4:2100075. https://doi.org/10.1002/adtp.202100075

Kreller T, Distler T, Heid S, Gerth S, Detsch R, Boccaccini AR (2021) Physico-chemical modification of gelatine for the improvement of 3D printability of oxidized alginate-gelatine hydrogels towards cartilage tissue engineering. Mater Des 208:109877. https://doi.org/10.1016/j.matdes.2021.109877

Kumar JP, Lakshmi L, Jyothsna V, Balaji DR, Saravanan S, Moorthi A, Selvamurugan N (2014) Synthesis and characterization of diopside particles and their suitability along with chitosan matrix for bone tissue engineering *in vitro* and *in vivo*. J Biomed Nanotechnol 10(6):970–981. https://doi.org/10.1166/jbn.2014.1808

Li B, Lu F, Wei X, Zhao R (2008) Fucoidan: structure and bioactivity. Molecules (Basel, Switzerland) 13(8):1671–1695. https://doi.org/10.3390/molecules13081671

Li J, Yang B, Qian Y, Wang Q, Han R, Hao T, Shu Y, Zhang Y, Yao F, Wang C (2015) Iota-carrageenan/chitosan/gelatin scaffold for the osteogenic differentiation of adipose-derived MSCs *in vitro*. J Biomed Mater Res Part B Appl Biomater 103(7):1498–1510. https://doi.org/10.1002/jbm.b.33339

Lie GC, Mao Z, Shen J, Hu X, Han C (2003) Thermal dehydration treatment and glutaraldehyde cross-linking to increase the biostability of collagen–chitosan porous scaffolds used as dermal equivalent. J Biomater Sci Polym Ed 14(8):861–874. https://doi.org/10.1163/156856203768366576

Lima PAL, Resende CX, de Almeida Soares GD, Anselme K, Almeida LE (2013) Preparation, characterization and biological test of 3D-scaffolds based on chitosan, fibroin and hydroxyapatite for bone tissue engineering. Mater Sci Eng C 33(6):3389–3395. https://doi.org/10.1016/j.msec.2013.04.026

Liu B, Harrell R, Davis RH, Dresden MH, Spira M (1989) The effect of gamma irradiation on injectable human amnion collagen. J Biomed Mater Res 23(8):833–844. https://doi.org/10.1002/jbm.820230803

Liu Y, Lim J, Teoh SH (2013) Development of clinically relevant scaffolds for vascularised bone tissue engineering. Biotechnol Adv 31(5):688–705. https://doi.org/10.1016/j.biotechadv.2012.10.003

Liu J, Zhan X, Wan J, Wang Y, Wang C (2015) Review for carrageenan-based pharmaceutical biomaterials: favourable physical features versus adverse biological effects. Carbohydr Polym 121:27–36. https://doi.org/10.1016/j.carbpol.2014.11.063

Madruga LY, Balaban RC, Popat KC, Kipper MJ (2021a) Biocompatible crosslinked nanofibers of poly(vinyl alcohol)/carboxymethyl-kappa-carrageenan produced by a green process. Macromol Biosci 21(1):2000292. https://doi.org/10.1002/mabi.202000292

Madruga LY, Popat KC, Balaban RC, Kipper MJ (2021b) Enhanced blood coagulation and antibacterial activities of carboxymethyl-kappa-carrageenan-containing nanofibers. Carbohydr Polym 273:118541. https://doi.org/10.1016/j.carbpol.2021.118541

Maharjan B, Park J, Kaliannagounder VK, Awasthi GP, Joshi MK, Park CH, Kim CS (2021) Regenerated cellulose nanofiber reinforced chitosan hydrogel scaffolds for bone tissue engineering. Carbohydr Polym 251:117023. https://doi.org/10.1016/j.carbpol.2020.117023

Mandrycky C, Phong K, Zheng Y (2017) Tissue engineering toward organ-specific regeneration and disease modeling. MRS Commun 7(3):332–347. https://doi.org/10.1557/mrc.2017.58

Mantha S, Pillai S, Khayambashi P, Upadhyay A, Zhang Y, Tao O, Pham HM, Tran SD (2019) Smart hydrogels in tissue engineering and regenerative medicine. Materials 12(20):3323. https://doi.org/10.3390/ma12203323

Miguel SP, Ribeiro MP, Coutinho P (2021) Biomedical applications of biodegradable polymers in wound care. In: Kumar P, Kothari V (eds) Wound healing research. Springer, Singapore, pp 509–597. https://doi.org/10.1007/978-981-16-2677-7_17

Mihaila SM, Popa EG, Reis RL, Marques AP, Gomes ME (2014) Fabrication of endothelial cell-laden carrageenan microfibers for microvascularized bone tissue engineering applications. Biomacromolecules 15(8):2849–2860. https://doi.org/10.1021/bm500036a

Misra RDK, Girase B, Nune VKC, Xu W (2012) Cellular interactions and modulated osteoblasts functions mediated by protein adsorption. Adv Eng Mater 14(5):B247–B257. https://doi.org/10.1002/adem.201180071

Morais DS, Rodrigues MA, Silva TI, Lopes MA, Santos M, Santos JD, Botelho CM (2013) Development and characterization of novel alginate-based hydrogels as vehicles for bone substitutes. Carbohydr Polym 95(1):134–142. https://doi.org/10.1016/j.carbpol.2013.02.067

Murphy SV, Atala A (2014) 3D bioprinting of tissues and organs. Nat Biotechnol 32(8):773–785. https://doi.org/10.1038/nbt.2958

Nakamura S, Nambu M, Ishizuka T, Hattori H, Kanatani Y, Takase B, Kishimoto S, Amano Y, Aoki H, Kiyosawa T et al (2008) Effect of controlled release of fibroblast growth factor-2 from chitosan/fucoidanmicro complex-hydrogel on in vitro and in vivo vascularization. J Biomed Mater Res A 85(3):619–627. https://doi.org/10.1002/jbm.a.31563

Nicu R, Ciolacu F, Ciolacu DE (2021) Advanced functional materials based on nanocellulose for pharmaceutical/medical applications. Pharmaceutics 13(8):1125. https://doi.org/10.3390/pharmaceutics13081125

O'Leary R, Rerek M, Wood EJ (2004) Fucoidan modulates the effect of transforming growth factor (TGF)-beta1on fibroblast proliferation and wound repopulation in in vitro models of dermal wound repair. Biol Pharm Bull 27(2):266–270. https://doi.org/10.1248/bpb.27.266

Obluchinsksya ED, Makarova MN, Pozharitskaya ON, Shikov AN (2015) Effects of ultrasound treatment on the chemical composition and anticoagulant properties of dry fucus extract. Pharm Chem J 49(3):183–186. https://doi.org/10.1007/s11094-015-1250-8

Öfkeli F, Demir D, Bölgen N (2021) Biomimetic mineralization of chitosan/gelatin cryogels and in vivo biocompatibility assessments for bone tissue engineering. J Appl Polym Sci 138(14): 50337. https://doi.org/10.1002/app.50337

Panlasigui LN, Baello OQ, Dimatangal JM, Dumelod BD (2003) Blood cholesterol and lipid-lowering effects of carrageenan on human volunteers. Asia Pac J Clin Nutr 12(2): 209–214. PMID: 12810413

Park SN, Park JC, Kim HO, Song MJ, Suh H (2002) Characterization of porous collagen/hyaluronic acid scaffold modified by 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide cross-linking. Biomaterials 23(4):1205–1212. https://doi.org/10.1016/S0142-9612(01)00235-6

Pattnaik S, Nethala S, Tripathi A, Saravanan S, Moorthi A, Selvamurugan N (2011) Chitosan scaffolds containing silicon dioxide and zirconia nano particles for bone tissue engineering. Int J Biol Macromol 49(5):1167–1172. https://doi.org/10.1016/j.ijbiomac.2011.09.016

Pereira RC, Scaranari M, Castagnola P, Grandizio M, Azevedo HS, Reis RL, Cancedda R, Gentili C (2009) Novel injectable gel (system) as a vehicle for human articular chondrocytes in cartilage tissue regeneration. J Tissue Eng Regen Med 3(2):97–106. https://doi.org/10.1002/term.145

Peter M, Binulal NS, Nair SV, Selvamurugan N, Tamura H, Jayakumar R (2010) Novel biodegradable chitosan–gelatin/nano-bioactive glass ceramic composite scaffolds for alveolar bone tissue engineering. Chem Eng J 158(2):353–361. https://doi.org/10.1016/j.cej.2010.02.003

Popa E, Reis R, Gomes M (2012) Chondrogenic phenotype of different cells encapsulated in κ-carrageenan hydrogels for cartilage regeneration strategies. Biotechnol Appl Biochem 59(2):132–141. https://doi.org/10.1002/bab.1007

Popa EG, Rodrigues MT, Coutinho DF, Oliveira MB, Mano JF, Reis RL, Gomes ME (2013) Cryopreservation of cell laden natural origin hydrogels for cartilage regeneration strategies. Soft Matter 9(3):875–885. https://doi.org/10.1039/C2SM26846A

Popa EG, Caridade SG, Mano JF, Reis RL, Gomes ME (2015) Chondrogenic potential of injectable κ-carrageenan hydrogel with encapsulated adipose stem cells for cartilage tissue-engineering applications. J Tissue Eng Regen Med 9(5):550–563. https://doi.org/10.1002/term.1683

Popa EG, Santo VE, Rodrigues MT, Gomes ME (2016) Magnetically responsive hydrogels for modulation of chondrogenic commitment of human adipose-derived stem cells. Polymers 8(2): 28. https://doi.org/10.3390/polym8020028

Prasathkumar M, Sadhasivam S (2021) Chitosan/hyaluronic acid/alginate and an assorted polymers loaded with honey, plant, and marine compounds for progressive wound healing—know-how. Int J Biol Macromol 186:656–685. https://doi.org/10.1016/j.ijbiomac.2021.07.067

Purnama A, Aid-Launais R, Haddad O, Maire M, Mantovani D, Letourneur D, Hlawaty H, Le Visage C, Maire M (2013) Fucoidan in a 3D scaffold interacts with vascular endothelial growth factor and promotes neovascularization in mice. Drug Deliv Transl Res 5(2):187–197. https://doi.org/10.1007/s13346-013-0177-4

Puvaneswary S, Raghavendran HB, Talebian S, Murali MR, Mahmod SA, Singh S, Kamarul T (2016) Incorporation of fucoidan in β-tricalcium phosphate-chitosan scaffold prompts the differentiation of human bone marrow stromal cells into osteogenic lineage. Sci Rep 6(1): 1–11. https://doi.org/10.1038/srep24202

Rioux LE, Turgeon SL, Beaulieu M (2007) Rheological characterization of polysaccharides extracted from brown seaweeds. J Sci Food Agric 87(9):1630–1638. https://doi.org/10.1002/jsfa.2829

Rocha PM, Santo VE, Gomes ME, Reis RL, Mano JF (2011) Encapsulation of adipose-derived stem cells and transforming growth factor-β1 in carrageenan-based hydrogels for cartilage tissue engineering. J Bioact Compat Polym 26(5):493–507. https://doi.org/10.1177/0883911511420700

Rodrigo-Navarro A, Sankaran S, Dalby MJ, del Campo A, Salmeron-Sanchez M (2021) Engineered living biomaterials. Nat Rev Mater 6(12):1175–1190. https://doi.org/10.1038/s41578-021-00350-8

Rogina A, Rico P, Ferrer GG, Ivanković M, Ivanković H (2015) Effect of in situ formed hydroxyapatite on microstructure of freeze-gelled chitosan-based biocomposite scaffolds. Eur Polym J 68:278–287. https://doi.org/10.1016/j.eurpolymj.2015.05.004

Sahoo DR, Biswal T (2021) Alginate and its application to tissue engineering. SN Appl Sci 3(1): 1–19. https://doi.org/10.1007/s42452-020-04096-w

Salvatore L, Gallo N, Natali ML, Terzi A, Sannino A, Madaghiele M (2021) Mimicking the hierarchical organization of natural collagen: toward the development of ideal scaffolding material for tissue regeneration. Front Bioeng Biotechnol 9:258. https://doi.org/10.3389/fbioe.2021.644595

Sathain A, Monvisade P, Siriphannon P (2021) Bioactive alginate/carrageenan/calcium silicate porous scaffolds for bone tissue engineering. Mater Today Commun 26:102165. https://doi.org/10.1016/j.mtcomm.2021.102165

Saul JM, Williams DF (2011) Hydrogels in regenerative medicine. Handbook of polymer applications in medicine and medical devices. William Andrew Publishing, Norwich, pp 279–302. https://doi.org/10.1016/B978-0-323-22805-3.00012-8

Şenel S, McClure SJ (2004) Potential applications of chitosan in veterinary medicine. Adv Drug Deliv Rev 56(10):1467–1480. https://doi.org/10.1016/j.addr.2004.02.007

Seol YJ, Lee JY, Park YJ, Lee YM, Rhyu IC, Lee SJ, Han SB, Chung CP (2004) Chitosan sponges as tissue engineering scaffolds for bone formation. Biotechnol Lett 26(13):1037–1041. https://doi.org/10.1023/B:BILE.0000032962.79531.fd

Song YS, Li H, Balcos MC, Yun HY, Baek KJ, Kwon NS, Choi HR, Park KC, Kim DS (2014) Fucoidan promotes the reconstruction of skin equivalents. Korean J Physiol Pharmacol 18(4): 327–331. https://doi.org/10.4196/kjpp.2014.18.4.327

Sowjanya JA, Singh J, Mohita T, Sarvanan S, Moorthi A, Srinivasan N, Selvamurugan N (2013) Biocomposite scaffolds containing chitosan/alginate/nano-silica for bone tissue engineering. Colloids Surf B Biointerfaces 109:294–300. https://doi.org/10.1016/j.colsurfb.2013.04.006

Stevanović M, Djošić M, Janković A, Kojić V, Vukašinović-Sekulić M, Stojanović J, Odović J, Sakač MC, Yop RK, Mišković-Stanković V (2020) Antibacterial graphene-based hydroxyapatite/chitosan coating with gentamicin for potential applications in bone tissue engineering. J Biomed Mater Res A 108(11):2175–2189. https://doi.org/10.1002/jbm.a.36974

Swain S, Kwon TY, Rautray TR (2021) Fabrication of silver doped nano hydroxyapatite-carrageenan hydrogels for articular cartilage applications. bioRxiv. https://doi.org/10.1101/2020.12.31.424664

Tabaei PSE, Asadian M, Ghobeira R, Cools P, Thukkaram M, Derakhshandeh PG, Abednatanzi S, Voort PVD, Verbeken K, Vercruysse C, Declerch H, Morent R, De Geyter N (2021) Combinatorial effects of coral addition and plasma treatment on the properties of chitosan/polyethylene oxide nanofibers intended for bone tissue engineering. Carbohydr Polym 253:117211. https://doi.org/10.1016/j.carbpol.2020.117211

Tang SLP (2021) Dressings for advanced wound care. CRC Press, Boca Raton

Tavakoli S, Kharaziha M, Nemati S, Kalateh A (2021) Nanocomposite hydrogel based on carrageenan-coated starch/cellulose nanofibers as a hemorrhage control material. Carbohydr Polym 251:117013. https://doi.org/10.1016/j.carbpol.2020.117013

Teimouri A, Ebrahimi R, Emadi R, Beni BH, Chermahini AN (2015) Nanocomposite of silk fibroin–chitosan/nano ZrO2 for tissue engineering applications: fabrication and morphology. Int J Biol Macromol 76:292–302. https://doi.org/10.1016/j.ijbiomac.2015.02.023

Thakur A, Jaiswal MK, Peak CW, Carrow JK, Gentry J, Dolatshahi-Pirouz A, Gaharwar AK (2016) Injectable shear-thinning nanoengineered hydrogels for stem cell delivery. Nanoscale 8(24): 12362–12372. https://doi.org/10.1039/C6NR02299E

Thomas LV (2021) Polymeric biomaterials in tissue engineering: retrospect and prospects. In: Bhaskar B, Sreenivasa Rao P, Kasoju N, Nagarjuna V, Baadhe RR (eds) Biomaterials in tissue engineering and regenerative medicine. Springer, Singapore, pp 89–118. https://doi.org/10.1007/978-981-16-0002-9_4

Tunçer S (2021) Biopolysaccharides: properties and applications. In: Polysaccharides: properties and applications. Wiley, New York, pp 95–134. https://doi.org/10.1002/9781119711414.ch6

Vacanti CA, Vacanti JP (2000) The science of tissue engineering. Orthop Clin North Am 31(3): 351–356. https://doi.org/10.1016/s0030-5898(05)70155-3

Vaidhyanathan B, Vincent P, Vadivel S, Karuppiah P, Al-Dhabi NA, Sadhasivam DR, Vimalraj S, Saravanan S (2021) Fabrication and investigation of the suitability of chitosan-silver composite scaffolds for bone tissue engineering applications. Process Biochem 100:178–187. https://doi.org/10.1016/j.procbio.2020.10.008

Van Tienen TG, Heijkants RG, Buma P, de Groot JH, Pennings AJ, Veth RP (2002) Tissue ingrowth and degradation of two biodegradable porous polymers with different porosities and pore sizes. Biomaterials 23(8):1731–1738. https://doi.org/10.1016/S0142-9612(01)00280-0

Varoni E, Tschon M, Palazzo B, Nitti P, Martini L, Rimondini L (2012) Agarose gel as biomaterial or scaffold for implantation surgery: characterization, histological and histomorphometric study on soft tissue response. Connect Tissue Res 53(6):548–554. https://doi.org/10.3109/03008207.2012.712583

Velasco MA, Narváez-Tovar CA, Garzón-Alvarado DA (2015) Design, materials, and mechanobiology of biodegradable scaffolds for bone tissue engineering. Biomed Res Int 2015:729076. https://doi.org/10.1155/2015/729076

Venkatesan J, Bhatnagar I, Kim SK (2014) Chitosan-alginate biocomposite containing fucoidan for bone tissue engineering. Mar Drugs 12(1):300–316. https://doi.org/10.3390/md12010300

Vignesh S, Gopalakrishnan A, Poorna MR, Nair SV, Jayakumar R, Mony U (2018) Fabrication of micropatterned alginate-gelatin and k-carrageenan hydrogels of defined shapes using simple wax mould method as a platform for stem cell/induced Pluripotent Stem Cells (iPSC) culture. Int J Biol Macromol 112:737–744. https://doi.org/10.1016/j.ijbiomac.2018.02.031

Wang L, Cao J, Lei D, Cheng X, Yang Y, Hou R, Cui F (2009) Effects of nerve growth factor delivery via a gel to inferior alveolar nerve in mandibular distraction osteogenesis. J Craniofac Surg 20(6):2188–2192. https://doi.org/10.1097/SCS.0b013e3181bf04c7

Wang L, Cao J, Lei DL, Cheng XB, Zhou HZ, Hou R, Cui FZ (2010) Application of nerve growth factor by gel increases formation of bone in mandibular distraction osteogenesis in rabbits. Br J Oral Maxillofac Surg 48(7):515–519. https://doi.org/10.1016/j.bjoms.2009.08.042

Xue X, Hu Y, Deng Y, Su J (2021) Recent advances in design of functional biocompatible hydrogels for bone tissue engineering. Adv Funct Mater 31(19):2009432. https://doi.org/10.1002/adfm.202009432

Yang S, Leong KF, Du Z, Chua CK (2001) The design of scaffolds for use in tissue engineering. Part I. Traditional factors. Tissue Eng 7(6):679–689. https://doi.org/10.1089/107632701753337645

Yegappan R, Selvaprithiviraj V, Amirthalingam S, Jayakumar R (2018) Carrageenan based hydrogels for drug delivery, tissue engineering and wound healing. Carbohydr Polym 198: 385–400. https://doi.org/10.1016/j.carbpol.2018.06.086

Zhou G, Sun YP, Xin H, Zhang Y, Li Z, Xu Z (2004) *In vivo* antitumor and immunomodulation activities of different molecular weight lambda-carrageenans from *Chondrus ocellatus*. Pharmacol Res 50(1):47–53. https://doi.org/10.1016/j.phrs.2003.12.002

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Abstract

Carrageenan is a naturally occurring polysaccharide obtained from seaweeds. This chapter provides a general overview of various types of carrageenans isolated from red seaweeds regarding their suitability for different applications. Commercial applications of carrageenan include use as gelling, stabilizing, and thickening agents in foods, in pharmaceutical formulations, and a range of other industrial applications. Highlights are given on using seaweed polymers as a new generation of functional ingredients in the manufacture of food and pharmaceuticals. Applications of carrageenan in enzyme and whole-cell immobilization are also discussed.

Keywords

carrageenan; biocatalyst; food additives; encapsulation; immobilization

10

Carrageenan for Industrial Food Processing and Preservation

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10.1 Introduction

10.1.1 Historical Significance

The idea of using carrageenan started centuries ago in Ireland. It is obtained from the red seaweed species *Chondrus crispus*, or commonly called Irish Moss, a historically significant potential food thickener widely used for beer clarification and textile sizing during the nineteenth century [1]. The name carrageenan is derived from the source, *C. crispus*, which is also known as Carrageenan Moss. In Ireland, it is called Carraigin.

10.1.2 Commercial Development

During the nineteenth century, the commercialization of carrageenan was done in England in the form of whole meals, which had a great market value. Later, the refined extract was traded [2]. Production of carrageenan spread to the United States when World War II had impeded the production and trade of agar, and this has led to the increased demand for carrageenan [3]. Postwar, the production of carrageenan has increased exponentially. By the time of early 1960s, the majority of small-scale producers merged with a few large-scale industries, such as Hercules, Inc. (USA), The Copenhagen Pectin Factory Ltd. (Denmark), FMC Corp. (USA), Marine Colloids (USA), and Litex (Denmark).

10.1.3 Biological Background

Carrageenan is a group of sulfated galactans extracted from red seaweeds known as Rhodophyceae or red algae. A few of these seaweeds contain carrageenan as their

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principal structural polysaccharide [4]. The content of carrageenan in commercially exploited seaweed ranges between 30% and 60% of the dry weight; the upper limit is 70–80%. Most of the carrageenan is found in the cell wall [4]. The reproductive phase of the plant plays a key role in the synthesis of carrageenan in important red algal families. It has been found that all haploid cells take part in the synthesis of κ-family of carrageenans, whereas the diploid cells of some of the algal families synthesize the distinctive γ-family of carrageenans [5]. The composition of the carrageenan varies with the genetic specificity among algal species, owing to a balance between tetrasporophytic and gametophytic biomass in the overall population. Seasonal variations might affect this balance [6].

10.2 Production

10.2.1 Growth of Seaweeds

Development of algae will happen on a long-lived crustose base that serves as holdfast adhering to rocks, thereby sustaining the algal thallus, which resembles a fleshy plant-like structure [7]. Holdfast will promote adherence of the algae to the substrate, fixing them in any kind of salinity and degrees of immersion. Studies on *Chondrus*, *Gigartina*, and *Iridara* have revealed information on holdfasts [8].

Many factors affect the growth of the algae, including water temperature, nutrients, light, and thallus surface area. Some of the negative parameters that affect growth and productivity include diseases, wave action, and grazing organisms [9]. Thalli, at an early stage, will show exponential growth, although the growth factors are non-limiting. Soon after maturation, there will be an increase in the frond loss, thereby resulting in the linear increase of the net biomass [10]. The biomass will reach a steady state eventually, and the incidence of light will become a limiting factor, while the population dynamics will become a principal factor when algae start growing and develop into a dense standing crop [11]. During the growth season, the yield of seaweed will be around 2–3% everyday. The yield is reduced by wave action, causing frond loss, which can be substantial and accounts for one-third of the total biomass loss [12].

Numerous investigations comparing seaweeds growing in the wild and in mariculture suggest that higher growth rates will show an impact on the higher protein content but relatively lower carrageenan levels [13]. The majority of seaweeds are perennial plants. Thalli generally have a short lifespan because of exposure to environmental stress; however, the holdfast base can survive for several years [9].

The growth rate of industrially produced seaweeds may vary concerning the growth area; for instance, *Chondrus* sp. shows a good growth profile in subtidal and intertidal areas at the North Atlantic coasts of North America and Europe, and also on the West Pacific coasts of Japan and Korea [14]. The subtidal and intertidal areas at the West Pacific coasts of New Zealand and Australia, and the East Pacific coasts of North and South Americas, on the other hand, work well for *Iridaea* and *Gigartina* sp. The subtidal and some intertidal areas at the coasts of East Africa, Asia, and the Caribbean support good growth of *Eucheuma* sp. [15].

10.2.2 Farming of the Seaweed

Farming of the seaweed can be done in two ways: mariculture and artificial culture in a controlled environment. This needs a significant effort where tank cultures were used, which includes cultivating suspended algae in tanks with a sufficient water supply and nutrient adjustment [16]. In Canada, efforts to cultivate *Chondrus* were abandoned completely because of unfavorable economic conditions. One technique that is more economical is the use of film culture/greenhouse mist, which has been applied for growing brown algae with lower throughput of water and having the most efficient photosynthesis [17]. However, there is no such evidence on the cultivation of carrageenan using the same approach. Currently, the only commercial seaweed farming strategy with low labor and capital costs is mariculture [18]. The Philippines has successfully cultivated *Eucheuma*, sharing the practice of cultivation to the other neighboring countries, such as Indonesia [19].

Mariculture yield has been intensified since its introduction in the early 1970s. From 1973 to 1983, the Philippines recorded the highest rates of exports i.e. from 600 to 26,000 metric tons of *Eucheuma* rose every year, accounting for about 60% of the total world's production [19]. Demonstration using the prime growth areas in the farming of carrageenan has led to the development of various farming techniques by carrageenan producers [20]. Usually, cultivation of the carrageenan was done by native farmers in family operations. Early cultivation techniques used horizontal nets suspended by mangrove stakes at the subtidal level [21]. Seedlings of *Eucheuma* were vegetatively propagated by tying them to the horizontal nets and the monofilament lines. Proper maintenance of conditions and good management will result in the yield of 1 kg (wet weight) of thalli for a few hundred grams of seedlings [22].

10.2.3 Supply

The annual production of the carrageenan (in metric tons) has been indicated as follows: Canada, 5000 *Chondrus*; the Philippines, 26,000 *Eucheuma*; Indonesia, 4000 *Eucheuma*; South America, 7000 *Iridaea* and *Gigartina*. *Eucheuma* is majorly cultivated in the Philippines [23]. *Iridaea* is predominant and commonly grown in Chile. Whereas *Chondrus* was once the dominant source of carrageenan, it is now in limited supply because of the underdeveloped growth areas, overharvesting, and poor harvesting methods [24].

10.2.4 Harvesting Procedures

Seaweed harvesting procedures are labor-intensive and require sophisticated methods. Harvesting of *Eucheuma* is done by hand. *Iridaea* and *Gigartina* are harvested by collecting the material that is washed off ashore by the sea waves [25]. Harvesting of *Chondrus* is done using boats, and large piles of the collected seaweed are deposited on the shore. At times, other seaweeds grow by attaching to *Chondrus*, which is hence called mixed seaweed [26]. The collected seaweed will be dried up to

20–45% of the residual moisture, to reduce its weight and to improve the shelf life. Drying can be done either by exposing to the sun naturally or by using rotary air dryers [27].

10.2.5 Manufacture

Manufacturing of carrageenan comprises a series of steps, such as extraction, purification, concentration, precipitation, and drying. *Furcellaria* is also subjected to subsequent washing to remove excess color. Before extraction, seaweeds are washed in water [28]. A cold alkaline solution is used to soak the seaweeds before extraction. *Eucheuma* sp., i.e. *Eucheuma cottonii* is subjected to alkaline modification consists of soaking the seaweed in KOH solution at elevated temperature for a short period prior to processing it [29]. Sometimes seaweed is subjected to basic processing, or it may be subjected to drying, grounding, and selling the semi-purified seaweed for nonedible gelling applications [30].

Extraction is performed with a strongly alkaline solution at a temperature close to its boiling point for a period of 10–30 h, leading to the disintegration of the seaweed. This alkaline modification helps in increasing the stability of the carrageenans [31]. The extraction process when carried at lower pH and temperature, it can result in a special viscosity and loss of the gelling ability of the carrageenan [32].

Purification and Concentration Centrifugation or filtration aids in the separation of seaweed from the extraction mixture. A solution containing 1–2% of carrageenan is purified by fine filtration using porous silica [33]. The cost can be minimized by precipitating the filtered carrageenan. The final concentration after processing with a multistage vacuum evaporator will be 2–3% [34].

Precipitation of carrageenan is carried out by the addition of alcohol, such as 2-propanol. This addition of alcohol to the carrageenan solution results in the production of fibrous carrageenan as a coagulum [33]. It is then pressed and separated to remove traces of the residual moisture. Distillation helps in the later removal of alcohol. A few of the carrageenans, such as κ-type carrageenan, are precipitated by the spray method [35]. Spraying of the carrageenan solution into ice-cold 1–1.5% KCl will result in the formation of gelled fibers. Now, the coagulum is drained and subjected to repeated cycles of freezing and thawing to remove residual liquid from the gel fibers. The resulting fibers are then dewatered by pressing or centrifugation. This process is applied to carrageenans that are strongly potassium-sensitive, i.e. furcellaran-containing seaweed [36].

Drying is the step after precipitation, where the precipitated carrageenan is dried using vacuum drying cabinets or using inert gas in a closed system, which is called a convective drying system. Direct drying with air is used to dry the KCl-precipitated curd. Carrageenans dried using the roller-drying method may have high contents of non-carrageenan soluble components [37].

10.2.6 Product Standardization

The requirements of the product application mainly depend on the source of the seaweed. A product for any specific application will be selected based on seaweed

source and analytical value [38]. Product standardization is done by considering the functionality of the specific application. Three categories of commercial carrageenan are viscosity builders, gelling agents, and stabilizers. A few of the standardized tests that can be performed are stabilization efficiency, tests of gel strength, organoleptic evaluation, and viscosity (Chandan and Kilara, 2015).

10.2.7 Producers and Markets

The leading producers of carrageenan are Satin S.A. (France), Marine Colloids (US), Copenhagen Pectin Factory Ltd. (Denmark), and Litex (Denmark). Other leading producers of carrageenan are based in Portugal, Korea, Argentina, Japan, the Philippines, and Brazil. The total market volume of carrageenan in 1984 was 12,000–14,000 metric tons. In addition to this, about 2000 metric tons of alkaline-treated seaweed, especially meant for nonfood applications, were also produced. Industrial applications of the carrageenan include dairy products (chocolate milk, ice creams, and flams), water desserts (tart glazing, ready-made, and powder gels), toothpaste, and pet foods. The largest consumers of carrageenan globally are Japan, US, France, and Germany [39].

10.3 Structure

10.3.1 Primary Structure

Carrageenan belongs to a family of linear galactan polysaccharides obtained from red seaweeds, such as Phyllophoraceae, Gigartinaceae, Furcellariaceae, Hypneaceae, and Solieriaceae. Furcellariaceae carrageenan has 15–20% of the ester sulfate content and linkages of (1,3)-α-D- and (1,4)-β-D-glycosidic linkages [40]. Natural carrageenan is made up of a mixture of nonhomologous polysaccharides, and hence the terminal repeating unit means the principal disaccharide unit inside the structure. The structures of various types of carrageenans are illustrated in Figure 10.1.

10.3.2 Secondary Structure

The secondary structure of carrageenan adopts a $^4C_1$ conformation, made up of 3,6-anhydro rings that is supported by the steric repulsion of axial substituents. The entry of the molecular chain into an ordered helical conformation determines the formation of the 3,6-anhydro ring [42].

10.3.3 Tertiary and Quaternary Structure

The tertiary structure of carrageenan is of prime importance as it plays a significant role in gelation. The double-helical structure of the carrageenan can be seen in both solid and liquid phases. K-carrageenan is believed to consist of a double helical tertiary structure with inconclusive results. This inconclusiveness has led to further investigation of κ-carrageenan's tertiary structure [43].

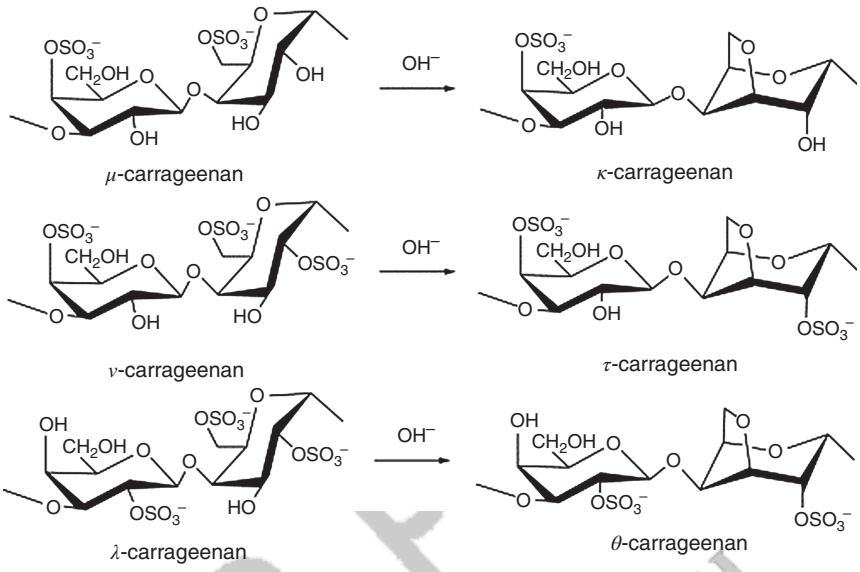

Figure 10.1 Structure of various carrageenans. Source: Ahmed et al. [41]/with permission of Elsevier.

10.3.4 Biosynthesis

Synthesis of the galactan backbone of the carrageenan takes place inside the Golgi apparatus of the cell. The cell wall is the place for sulfation by sulfotransferases. Both tetrasporic and gametophytic plants contain sulfohydrolases inside the cell wall. Their action is limited to lambda carrageenan because of its inhibitory effect [44].

10.4 Properties

10.4.1 Dispersibility and Solubility

Carrageenan is soluble in all polar solvents, especially water. At an elevated temperature, slightly higher than its gelling interval, carrageenan slowly solubilizes, resulting in the formation of a viscous solution [45]. Dispersion is an important parameter for carrageenan to become soluble as lump formation can only be avoided by efficient dissolution. Carrageenan powder must be in fine-mesh form to achieve efficient dissolution. Other methods for good dissolution are high-speed mixing or combining the carrageenan with some inert material, such as sugar before lump formation [46]. Another method is to continuously maintain the gelling conditions (temperature, cations) while in dispersion. Even then, the gelling conditions might prevent the incipient dissolution, leading to the formation of the lumps. The actual dissolution starts only after raising the temperature of the system along with agitation [47].

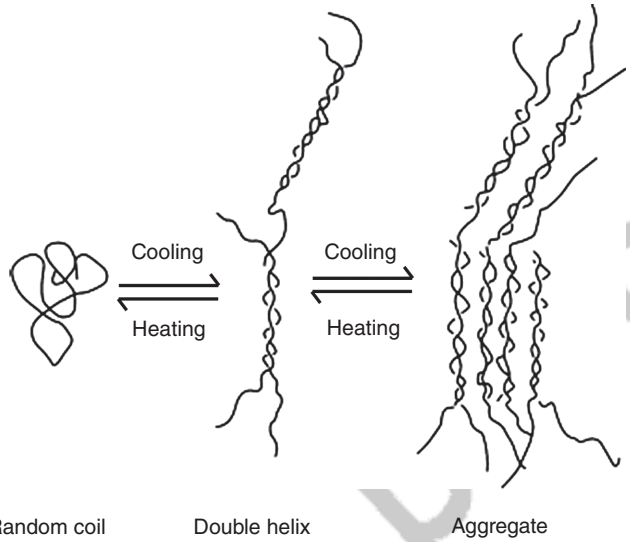

Figure 10.2 Gelation mechanism of carrageenan. Source: Williams and Phillips [49]/with permission of Elsevier.

10.4.2 Gel Formation

A few of the carrageenans can form a thermoreversible gel upon cooling of the heated solution that contains various salts. K-carrageenans from cohesive gels at the lowest polymer concentration, i.e. 0.5% in water and 0.1–0.2% in bio-colloidal systems, such as milk. The gel formation and gelation temperature mainly depend on various parameters, such as ionic and nonionic solutes, counterion concentration, polysaccharides, and carrageenan concentration but with little sensitivity [48]. The gel formation mechanism of carrageenan is illustrated in Figure 10.2.

10.5 Rheological Properties

10.5.1 Solutions

Carrageenan solutions are pseudoplastic in behavior and are non-Newtonian, i.e. shear-thinning. The rheological behavior of carrageenan is due to the decrease in the intermolecular entanglement, caused by the alignment and extension of randomly coiled molecules in the flow direction. The viscosity of the carrageenan solution depends on various parameters and decreases with the decrease in concentration, sulfation, molecular weight, and increase in the temperature, flexibility, and ionic screening effects [50].

10.5.2 Gels

K-carrageenan can be broken down with sufficient shear, and the regelling will occur only after reaching the thermal melting point. The ι-carrageenan gel will become

a viscous solution when subjected to shear stress, and this viscous flow is termed thixotropic. When the shear ceases, the gel can be returned to its original state [51].

10.5.3 Chemical Stability

The cleavage of glycosidic linkages will result in the breakdown of molecular chains by hydrolysis within the carrageenan solution. Acidic or oxidizing conditions will cause this cleavage, which also occurs over time and with an increasing temperature. I-carrageenan degrades at about half the rate of κ-carrageenan [52].

10.5.4 Interactions with Other Gums

Gel characteristics can be greatly improved by combining carrageenan with different gums. The pure form of K-carrageenan yields gels that are brittle, rigid, and generating. Resilience, softness, water retention, and cohesiveness directly affect the texture of the gel. Therefore, modification of such properties enhances the gel texture [53]. The blending of the ι and λ types of the carrageenan will achieve such properties. Locust bean gum has a synergistic effect. The minimum gelling polymer concentration can be reduced by up to one-third by mixing locust bean gum and κ-carrageenan, irrespective of the nongelling locust gum. Furthermore, this mixture has increased resilience and strength at a ratio of 1 : 2 of locust bean gum and κ-carrageenan [54].

10.5.5 Protein Reactivity

Carrageenans have received special attention to their protein reactivity compared to a range of other sulfated polysaccharides. Because of its proteinaceous nature, carrageenan is extensively used in the thickening of milk, resulting in a major position in the dairy market [55]. Strikingly, a few of the other protein products that use carrageenan include cottonseed, soybean, coconut, and peanut. Application of carrageenans in such systems will ensure the stabilization of proteins, which are sensitive to Ca^{2+} precipitation [56].

10.5.6 Entrapment

Entrapment is a mild immobilization technique that does not require modification or binding of the enzyme. Enzyme leakage can be effectively prevented by entrapment using matrices, lattice structures, or membranes [57]. Polymeric matrix entrapment is done by polymerization or cross-linking, treating the polymer solution containing the enzyme with heat or chemicals. An alternative approach is the entrapment and microencapsulation with carrageenan and polyacrylamide. In contrast to other methods, this universal method is applicable to any kind of biocatalyst. The major disadvantage of this approach is that small or large pore sizes will either lead to the leakage of the enzyme or prevent the diffusion of the substrate from reaching the biocatalyst [58].

10.5.7 Microencapsulation

Carrageenan has wide applications in microencapsulation. This method uses semipermeable polymer membranes made of carrageenan, cellulose nitrate, or nylon. Whole cells or enzymes are encapsulated within the microcapsules. Nonetheless, the pore size of the membrane lies between 1 and 100 nm, thereby preventing the leakage of contents and allows dialysis of the substrate across the membrane [59].

10.6 Applications of Carrageenan in Enzyme Immobilization

In addition to immobilizing whole cells using carrageenan matrices, the same approach can also be applied for enzyme immobilization. A few examples are elaborated in the following sections.

10.6.1 Asymmetric Synthesis Using a Nonaqueous Solution

Earlier works suggest that carrageenan is not a popular carrier for enzyme immobilization, unlike for whole cells. The first contribution to the usage of carrageenan for enzyme entrapment was reported by Kitchell et al. [60]. They have demonstrated the immobilization of an esterase by dropping the enzyme/carrageenan solution into ice-cold alcohol that is saturated with inorganic salts [61]. The inorganic salts are important for the hardening, curing, and stability of beads in organic media. Another example is the immobilization of lipase enzyme extracted from *Pseudomonas* species using the gel preparation method for the synthesis of propyl laurate. The final yield was approximately 80% when carrageenan and microemulsion-based lipase gel were used as catalysts [62].

10.6.2 Biosensors

Biosensor-based detectors offer a high sensitivity that is dependent on enzymes or whole cells that act as catalysts for the conversion of desired compounds. Biosensors that can work with nonaqueous solutions are called organic phase enzyme electrodes (OPEE) [63]. OPEEs contain a commercial-type gas diffusion amperometric electrode for oxygen as their base. Immobilized enzymes located on the top of the oxygen electrode determine the selectivity of these electrodes. Immobilization of the enzyme happens by the adsorption of the immobilized solution into a dry κ-carrageenan film, a dehydration method [64]. Electrode design is based on the enzyme or the combination of enzymes.

- Hydrogen peroxide can be determined by an electrode containing catalase enzyme.
- The presence of lecithin in foods and pharmaceutical products can be determined by an electrode containing a combination of choline oxides and phospholipase D.

Table 10.1 Overview of applications of enzymes immobilized in carrageenan.

Enzyme immobilized within carrageenan matrices	Application	Method employed	References
Glucoamylase	Ethanol production from cornstarch	Droplet	[65]
Tannase	Hydrolysis of tea tannins	Droplet	[66]
Aspartase	Immobilization studies	Droplet, gel	[67]
Lipase	Esterification of laurate to propyl laurate	Gel, emulsion	[68]
Naringinase	Debitte ring of citrus juice	Droplet	[69]
Catalase	Hydrogen peroxide determination	Dehydration	[70, 71]
Fumarase	Immobilization studies	Droplet, gel	[67]
Subtilisin	Transesterification of *N*-acetyl-L-phenylalanine esters	Dehydration	[72]

- Polyphenol content in olive oil can be determined by an electrode containing tyrosinase.

Some of the other applications of carrageenan for enzyme immobilization are summarized in Table 10.1.

10.7 Applications of Carrageenan in Whole-cell Immobilization

The data available on whole-cell immobilization using carrageenan is enormous, so only a few examples will be given.

10.7.1 Nitrogen Removal (Wastewater Treatment)

Removal of nitrogen from wastewater will always remain a challenge because it involves two major steps: nitrification and denitrification. As the nitrification bacteria are strict aerobes, and denitrification bacteria are strict anaerobes, it is hard to combine these two steps in a single wastewater removal system. Nitrogen removal may be facilitated by immobilizing *Nitrosomonas europaea* and *Pseudomonas* sp. together using κ-carrageenan, thereby maintaining an oxygen gradient within the entrapment beads [73].

10.7.2 Degradation of Morpholine

Morpholine is a compound found in most industrial effluents that need to be treated before being released into the environment. It is used in antioxidants, solvents,

optical brighteners, inhibitors of corrosion, and for the production of herbicides and drugs [74]. Morpholine undergoes a N-nitrosation reaction in the presence of nitrites, leading to the formation of the strong mutagen nitrosomorpholine. The formation of this mutagen necessitates the removal of morpholine from wastewater. Bacteria used for the degradation of morpholine are *Mycobacterium* sp. [75]. The disadvantage of this bacterium is that it grows in aggregates that will result in insufficient degradation of morpholine. This problem can be eliminated by immobilization of *Mycobacterium aurum* using κ-carrageenan, resulting in improved morpholine degradation [76].

10.7.3 Degradation of Chlorophenol Pollutants

Chlorophenol degradation was done by using microorganisms isolated from activated sludge and immobilized in κ-carrageenan by using a novel hardening method with KCl and chitosan [77]. Both aerobic and anaerobic microbial communities are combined and co-immobilized in κ-carrageenan. When fused beads are subjected to limited air conditions, these will catalyze the degradation of 2,4,6-trichlorophenol. Decontamination of pentachlorophenol from the soil will be done by immobilizing *Pseudomonas* sp. UG30 cells in κ-carrageenan [78].

10.7.4 Amino Acid Synthesis

Amino acids play a key role in animal and human nutrition as the building blocks of proteins. Since only the L-form of an amino acid can be metabolized, there is a strong interest in the production of this form for use in many medical and food applications.

10.7.4.1 L-Aspartic Acid Production

Production of L-Aspartic acid is done either by microbial fermentation or by an enzymatic reaction that occurs between fumaric acid and ammonia that is catalyzed by the L-aspartate enzyme. This method signifies the first industrial application of immobilized whole cells and was developed by Tosa et al. [79]. Early experiments were done by immobilizing *Escherichia coli* using polyacrylamide gels. Later, polyacrylamide was replaced by κ-carrageenan, as it offers a surge in the half-life of the biocatalyst and its first activity; however, in addition to these, this method was simple, cheaper, and also uses milder conditions [80].

10.7.4.2 Production of Malic Acids

Usage of L-malic acid is predominant in foods and beverages, for example, as acidulants in the majority of fruit drinks and jams. The application of malic acid has been extended towards the pharmaceutical industry to formulate amino acid infusions. Recent studies have described the production of malic acid catalyzed by fumase, with fumaric acid as the starting material [81]. This method was first developed on an industrial scale by Tanabe Seiyaku in 1974 by immobilizing *Brevibacterium ammoniagenes* in polyacrylamide gels and later using κ-carrageenan because of its earlier advantages for the production of aspartic acid. During this reaction, succinic

acid can be produced as an unnecessary byproduct that can be eliminated by treating the immobilized cells with bile extracts [82]. Later in 1983, Takata et al. [83] identified a ninefold increase in the productivity of malic acid when they immobilized cells in κ-carrageenan rather than in polyacrylamide. In particular, higher fumarase activity was observed in *Brevibacterium flavum* immobilized in κ-carrageenan than with *B. ammoniagenes* immobilized in polyacrylamide [84].

10.7.4.3 Production of L-Alanine

L-Alanine is used as a food additive and has also wider applications for medical purposes and as a flavor enhancer. First, industrial production of L-alanine was done in 1965 by using intact cells of *Pseudomonas dacunhae* in an enzymatic batch reaction, with L-aspartic acid serving as the substrate [85]. Later this method was replaced by Yamamoto et al., in 1980 by one producing L-alanine in a conventional column reactor containing immobilized cells of *P. dacunhae* in κ-carrageenan. L-aspartate produced by *E. coli* was later converted to L-alanine with aspartate B-decarboxylase. Through this mechanism, the efficient production of L-alanine is made possible with co-immobilization of *E. coli* and *P. dacunhae*, using ammonia fumarate as a substrate [86].

10.7.4.4 Production of Tryptophan

Production of tryptophan can be done by the condensation reaction between L-serine and indole, catalyzed by tryptophanase. Immobilization of the *E. coli* cells having tryptophanase activity was demonstrated in κ-carrageenan gels. Importantly, these cells are placed in the liquid-impelled reactor comprising two liquid phases in which one is an organic solvent, i.e. indole, and the other is an aqueous phase containing L-serine and a cofactor, i.e. pyridoxal phosphate [87].

Some of the applications of carrageenan in whole-cell immobilization for a variety of commercial purposes are summarized in Table 10.2.

10.8 Food Applications

The carrageenan market is dominated by food industries, especially by dairy industries. Carrageenan is used a gelling agent, for viscosity building, and stabilizing. Before using the carrageenan for any purpose, knowledge of the process and the chemical composition of the carrageenan is considered necessary.

10.8.1 Vinegar Production

Vinegar is commonly applied in the seasoning and storage of food. Vinegar can be produced by bacteria on an industrial scale. Production of vinegar in a bubble-mixed reactor by using κ-carrageenan beads as a carrier was first introduced by Osuga et al. in 1984. Vinegar can be produced for 120 days (about four months) continuously and can be extended to 460 days (about one and a half years). Improvement in the production was made by using an air-lift reactor with the provision to culture

Table 10.2 Overview of applications of whole-cell systems immobilized in carrageenan matrices.

Source	Application	Method	References
Pseudomonas dacunhae	Synthesis of L-alanine using a column reactor	Droplet	[88]
Solanum aviculare	Synthesis of carveol and carvone from (−)-limonene	Droplet	[89]
Acetobacter aceti	Production of vinegar	Droplet	[90]
Rhodotorula rubra	Conversion of *trans*-cinnamic acid into L-phenylalanine	Droplet	[72]
Streptomyces aureofaciens	Production of chlortetracycline and tetracycline	Immobilization of whole cells	[91]
Lactococcus lactis	Continuous lactic starter production	Gel beads containing mixed strains	[92]
Brevibacterium ammoniagenes	Production of L-malic acid	Emulsion, gel	[93]
Saccharomyces cerevisiae LCC3021	Beer production	Droplet	[94]
Zymomonas mobilis NRRL B14023	Ethanol production from corn	Droplet	[65]
Escherichia coli	Production of L-aspartic acid	Gel	[95, 96]
Dioscorea deltoidea	Synthesis of carveol and carvone from (−)-limonene	Gel (cubes)	[89]

Acetobacter species K1024 isolated from vinegar broth, which is a commercial source. Later, the development of immobilization of *Acetobacter suboxydans* cells in κ-carrageenan and maintaining them in a mixed tabletop bioreactor has increased the yield of vinegar [90].

10.8.2 Dairy Products

Stabilization of chocolate milk can be done by using κ-carrageenan that prevents creaming and sedimentation at a concentration of 0.02–0.03%. Stabilization of milk using carrageenan can be achieved by agitation and it starts during cooling at a temperature of 15 °C by the formation of a thixotropic gel that can withstand agitation for a limited time [97]. Non-fat milk is stabilized using κ- or ι-carrageenan (0.02–0.04%) after replacing the milk fat with vegetable fat. λ and i type carrageenan's at a percentage of 0.05% is used to stabilize imitation milk, which is made from sodium caseinate or soy protein with vegetable fat are replaced in place of milk fat. Stabilization of the

evaporated milk in opposition to the cream formed by the increased fat content was demonstrated using 0.005% of κ-carrageenan [98].

Flans or custards are milk puddings made of milk combined with gelling agents. The majority of these make use of carrageenan, while sometimes starch is supplemented with other phosphates, along with other gums, such as locust bean gum. Consistent with this observation, numerous studies have reported that hot-fillings are made of a mix of carrageenan or κ-carrageenan at a percentage of 0.20–0.35%, and all the others come under the softer gelled product [99]. On the other hand, cold fillings with 0.30–0.35% of ι-carrageenan result in gel formation, and the mixed types of carrageenans in similar amounts give the creamy appearance of the final product. This concept is particularly important when freeze-thaw stable milk gels are made with several types of carrageenan and gum mixtures supporting the level of solids, i.e., 30%. The carrageenan composition will be *i*-type or mixed type (0.20–0.30%), combined with carboxymethylcellulose (0.50%) and locust bean gum (0.25%). Nevertheless, to our knowledge, carrageenan-locust bean gums can be used to improve the texture and stabilize acidified milk gels, such as low-fat yogurt [100].

Locust bean gum and guar gum, along with carboxymethylcellulose, were used for stabilizing ice creams, as they normally contain 12% of the emulsified fat. Usage of gums in ice creams will reduce the formation of air bubbles while whipping and help in controlling the ice crystal growth, and keep a good texture. κ-carrageenan at 0.02–0.03% can be used to stabilize the ice creams against whey separation. Therefore, it is possible to speculate that texture and the stabilization of cottage cheese against creaming and whey separations were depicted by using κ-carrageenan (0.01–0.04%) with a combination of locust bean gum [101].

Carrageenans at 0.03–0.05% will greatly stabilize whipped products against protein separation and fat during the processes, such as before, during, or after freezing. Such products include whipped desserts and whipped cream, and a few of the comparable products include whipped products sold in aerosol cans containing caseinate or soy protein along with vegetable fat and whipped toppings. It is plausible that stabilization of coffee whiteners, either in liquid or frozen form, against the separation of protein and fat can be done with the addition of gums along with Lambda or mixed types of carrageenan at 0.1–03% (Chandan and Kilara, 2015).

Instant chocolate dry mixes intended for cold preparations, either with water or milk, can be stabilized against cocoa sedimentation using Lambda-type carrageenan or mixed types of carrageenans at 0.1%. Intriguingly, the mouthfeel and added body to the chocolate mixes are from the swelling of the carrageenan particles [102]. Consistent with this observation, instant milkshake mixes intended for either cold milk or water preparations are made of whipping agents and stabilizers that include 0.2% carrageenan. It stabilizes the air bubbles while shaking.

Milk puddings or cooked puddings intended for hot preparations that can be made with water or milk may hold traces of tetrasodium pyrophosphate that will result in the formation of complexes with Ca^{2+}, resulting in gel formation [103]. The addition of 0.2% carrageenan imparts water retention, leading to a creamy appearance. 0.1–0.3% of the carrageenan is used to thicken instant pie filling creams for cold preparation with water or milk [104].

10.8.3 Pre-fermentation of Milk to Produce Fresh Cheese

Industrial production of yogurt and fresh cheese was carried out by rapid acidification followed by the inoculation of the skim milk with mixed cultures of lactic acid bacteria that are immobilized. This type of inoculation with immobilized bacteria is much more effective than any other traditional batch methods [105]. Using the traditional methods, there will be a 50% reduction in fresh cheese production. Immobilization of three different strains of *Lactococcus lactis* and one strain of *Leuconostoc mesenteroides* was prepared separately in locust bean/κ-carrageenan grown in a 2 l stirred tank bioreactor [106].

10.8.4 Gelled Water Desserts

Gelled water desserts will always be at a low pH of 3.4, as they incorporate fruits or flavors. The lower range of pH makes the carrageenan unstable, and hence its residence time within the solution should be minimized. This was made possible with the addition of acid before rapid cooling, filling, and gelation [46]. For such applications, κ- and ι-types of carrageenans, along with locust bean gum, are used. Fruit suspensions made of less sugar, intended for use in diabetics, are made with carrageenan. Stability in terms of freeze/thaw is supported using a 1% mix of ι-carrageenan, along with locust gum, and carboxymethylcellulose, with a relative solubility of 30% [28].

10.8.5 Bakery Products

Carrageenan is used in the preparation of tart filling and glazing. The domestic hot preparations of the tart fillings are made of starch and mixed types of carrageenan (1–2%). Tart glazing for both industrial and domestic hot preparations is made up of gelling by κ-carrageenan, along with the locust bean gum (0.7–1.0%) [107]. Intriguingly, the gelation temperature is enhanced by adding potassium salt that makes the glaze solidify instantly. Stability in terms of freeze/thaw is kept using a 1% mix of ι-carrageenan, along with locust gum, and carboxymethylcellulose with a relative solubility of 30% [108].

10.8.6 Production of Beer

The brewing industry invests heavily in the production process. However, using immobilized yeast cells to produce beer is still under research. The typical fermentation process of beer involves the conversion of fermentable sugars into ethanol, flavor-active compounds, and carbon dioxide [109]. Time taken for the total process is five to seven days. Flavor maturation will take yet another one to three weeks. The presence of immobilized yeast may accelerate the fermentation process, as there will be a higher supply of yeast in the bioreactor. The University of West Ontario in collaboration with Labatt breweries invented a novel immobilization strategy for yeast cells in κ-carrageenan beads that supports continuous beer production using

a static mixing process. In this study, continuous production was carried out in a 50 l gas lift draft tube bioreactor with a residence time of 20 h [110].

10.8.7 Production of Ethanol

Industrial production of ethanol was carried out using a fluidized bed reactor containing immobilized cells of *Zymomonas mobilis* with glucose as a substrate. Later, this research was extended toward the use of a glass column fermenter packed with the bacteria, i.e. co-immobilized along with industrial glucoamylase in carrageenan gel beads with starch as a substrate [111]. Another approach involves the use of a packed bed tapered glass column reactor containing a carrageenan gel matrix that protects the immobilized *Saccharomyces cerevisiae* cells. However, continuous production of ethanol is made possible from pineapple cannery waste. Production of ethanol was noticed to be higher with cells immobilized in κ-carrageenan than with free cells [112].

10.8.8 Meat Products

Carrageenan can be added during meat and ham processing to enhance brine uptake, reduce cooking loss, and improve the sliceability. Brine uptake in various kinds of meat after the addition of κ-type of carrageenan and sodium tripolyphosphate was measured in sectioned ham (20–80%), sectioned poultry meat (30%), and multi-needle-injected cooked ham (40–50%) [113]. Application of the carrageenan is possible using the dispersion of carrageenan in the brine followed by either injecting or massaging the meat. Finally, the meat is cooked so that the carrageenan will be dissolved followed by gelling upon cooling. This process resulted in increased water binding and improved texture [114].

Carrageenan (0.2–1.0%) along with the locust bean gum can be used for meat and fish products. Applications of the carrageenan in fish products include the coating of the products for mechanical protection, to preserve the flavors, and for the suspension of the seasonings [115]. Furthermore, studies have demonstrated that Aspic is a savory jelly prepared from meat stock that has 0.5–1.0% carrageenan along with locust bean gum, and the rest of the ingredients include fish, meat, vegetables, and various other seasonings. Interestingly, cohesion and water binding of the meat products can be achieved by κ- or ι-type carrageenans. Interestingly, vegan meat made of casein and other vegetable proteins contained 1% of carrageenan [116].

10.9 Other Food Applications

Sugar preserves can be gelled using 0.5–1.0% of mixed types of carrageenans. Fruit drinks intended for instant cold preparations contain λ- or mixed carrageenans (0.2–0.5%). Stabilization of mousses for cold preparations is made with ι- or mixed types of carrageenans (1.2%). Sauces and salad dressings intended for cold preparations may have a mix of κ- and ι-carrageenan (0.5%). It is considered possible

that degradation of the carrageenan is protected by refrigeration and the gelling to happen eventually. In addition, clarification of beer can be done using carrageenan by precipitation or complexation of impurities in the wort that might be created by rapid heating and cooling [117].

Toothpaste when incorporated with carrageenan, helps in thickening, improved texture, effortless application, good flavor, thixotropic flow, and good rinseability. 0.7–1.2% of ι-type or weak κ-a type of carrageenan is used in toothpaste. Nevertheless, antacid preparations use a good amount of κ-carrageenan, and locust bean gum (0.2–0.5%). 1.5% of carrageenan, along with potassium salt and other gums, were previously used in the preparation of the air gels [118].

Fat separation during the processing of canned foods and a few pet foods can be done using κ-carrageenan along with locust bean gum (0.2–0.5%). The final product holds the stabilized and evenly distributed meat pieces inside the gravy, shape retention and a good body are because of the addition of the carrageenan [119]. Thickening and stabilization of abrasive suspensions, ceramic glazes, pesticide suspensions, and water-based paintings can be done using *i*-type of carrageenan (0.2–0.8%). κ-carrageenan can also be applied as an immobilizing agent to trap microorganisms. It can be used as a packaging material for columns and can be used for permeability of substrate and products. κ-Carrageenan along with potassium salt was used as a substitute for agar in microbiological applications [120].

10.10 Conclusions

Carrageenans are commercially prominent water-soluble gums that comprise the matrix material of the red seaweed, serving as analogs to that of cellulose in terrestrial plants. The versatility of the carrageenan in many industrial applications is because of its rheological properties. Toxicological studies on different types of carrageenan have revealed that there are no large differences in their effects of different forms of carrageenan prepared from different species of seaweed, such as *C. crispus*. It was proved that carrageenan is less toxic and not teratogenic. Applications of carrageenan are widespread in dairy industries, such as cottage cheese, yogurt, cream cheese, skimmed milk, and also in a few of the desserts and sweets, such as ice creams, milkshakes, custard, pie-filling, and many chocolate products. In addition to food processing, carrageenan is an excellent food additive. Applications of carrageenan can also be extended toward immobilization, entrapment, pharmaceutical, and cosmetic industries.

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References

1 Necas, J. and Bartosikova, L. (2013). Carrageenan: a review. *Veterinarni Medicina* 58 (4): 187–205.

2 Jönsson, M., Allahgholi, L., Sardari, R.R. et al. (2020). Extraction and modification of macroalgal polysaccharides for current and next-generation applications. *Molecules* 25 (4): 930.

3 Mantri, V.A., Eswaran, K., Shanmugam, M. et al. (2017). An appraisal on commercial farming of *Kappaphycus alvarezii* in India: success in diversification of livelihood and prospects. *Journal of Applied Phycology* 29 (1): 335–357.

4 Pierre, G., Delattre, C., Laroche, C., and Michaud, P. (2014). *Galactans and Its Applications. Polysaccharides*. Cham, Switzerland: Springer International Publishing.

5 Chopin, T. (1986). The red alga *Chondrus crispus* Stackhouse (Irish moss) and carrageenans: a review. *Canadian Technical Reports of Fisheries and Aquatic Sciences* 1514, v+-69.

6 Stanley, N.F. (1990). Carrageenans. In: *Food Gels* (ed. P. Harris), 79–119. Springer, Dordrecht.

7 Knutsen, S.H., Myslabodski, D.E., Larsen, B., and Usov, A.I. (1994). A modified system of nomenclature for red algal galactans. *Botanica Marina* 37: 163–169. https://doi.org/10.1515/botm.1994.37.2.163.

8 Boddy, L. (2016). Pathogens of autotrophs. In: *The Fungi* (ed. S.C. Watkinson, L. Boddy, and N.P. Money), 245–292. Academic Press.

9 Fleurence, J. and Levine, I. (ed.) (2016). *Seaweed in Health and Disease Prevention*. Academic Press.

10 Mercado, J.M., Salles, S., and Cortés, D. (2020). Ecophysiology of marine algae: factors regulating primary production and growth in natural environments. *Encyclopedia of Marine Biotechnology* 1: 341–357.

11 Turchin, P. and Batzli, G.O. (2001 Jun). Availability of food and the population dynamics of arvicoline rodents. *Ecology* 82 (6): 1521–1534.

12 Gaylord, B., Denny, M.W., and Koehl, M.A. (2003 Mar). Modulation of wave forces on kelp canopies by alongshore currents. *Limnology and Oceanography* 48 (2): 860–871.

13 Roleda, M.Y. and Hurd, C.L. (2019 Sep 3). Seaweed nutrient physiology: application of concepts to aquaculture and bioremediation. *Phycologia* 58 (5): 552–562.

14 Chapman, V. (2012). *Seaweeds and Their Uses*. Springer Netherlands.

15 Raven, J.A., Johnston, A.M., Kübler, J.E. et al. (2002). Mechanistic interpretation of carbon isotope discrimination by marine macroalgae and seagrasses. *Functional Plant Biology* 29 (3): 355–378.

16 Troell, M., Halling, C., Neori, A. et al. (2003 Oct 31). Integrated mariculture: asking the right questions. *Aquaculture* 226 (1–4): 69–90.

17 Bixler, H.J. and Porse, H. (2011 Jun). A decade of change in the seaweed hydrocolloids industry. *Journal of Applied Phycology* 23 (3): 321–335.

18 Buschmann, A.H., Camus, C., Infante, J. et al. (2017 Oct 2). Seaweed production: overview of the global state of exploitation, farming and emerging research activity. *European Journal of Phycology* 52 (4): 391–406.

19 Hayashi, L., Reis, R.P., dos Santos, A.A. et al. (2017). The cultivation of *Kappaphycus* and *Eucheuma* in tropical and sub-tropical waters. In: *Tropical Seaweed Farming Trends, Problems and Opportunities* (ed. A.Q. Hurtado, A.T. Critchley, and I.C. Neish), 55–90. Cham: Springer.

20 Sharmila R. (2014). In vitro production of an Anticancer drug Andrographolide from *Andrographis paniculata* (Doctoral dissertation). Guide(s): Subbu Rathinam KM, Department of Biotechnology, Bharathidasan University. http://hdl.handle.net/10603/215776.

21 Valderrama, D., Cai, J., Hishamunda, N., and Ridler, N. (ed.) (2013. FAO Fisheries and Aquaculture Technical Paper). *Social and Economic Dimensions of Carrageenan Seaweed Farming*. Rome: Food and Agriculture Organization of the United Nations.

22 Chapman, V.J. and Chapman, D.J. (1980). Laver or 'Nori' industry and carragheen or Irish Moss. In: *Seaweeds and Their Uses* (ed. V. Chapman and D.J. Chapman), 98–147. Dordrecht: Springer.

23 Werner, A., Clarke, D., and Kraan, S. (2004). Strategic review and the feasibility of seaweed aquaculture in Ireland. NDP Marine RTDI Desk Study Series, Marine Institute.

24 Stengel, D.B. and Connan, S. (2015). Marine algae: a source of biomass for biotechnological applications. In: *Natural Products from Marine Algae* (ed. D.B. Stengel and S. Connan), 1–37. New York, NY: Humana Press.

25 Goldman, J.C., Ryther, J.H., Waaland, R., and Wilson, E.H. (1977). *Topical Report on Sources and Systems for Aquatic Plant Biomass as an Energy Resource*. Cambridge, MA: Dynatech R/D Co.

26 O'Connor, K. (2017). *Seaweed: A Global History*. London, UK: Reaktion Books.

27 Falshaw, R., Furneaux, R.H., Wong, H. et al. (1996). Structural analysis of carrageenans from Burmese and Thai samples of *Catenella nipae* Zanardini. *Carbohydrate Research* 285: 81–98.

28 Hernandez-Carmona, G., Freile-Pelegrín, Y., and Hernández-Garibay, E. (2013 Jan 1). Conventional and alternative technologies for the extraction of algal polysaccharides. In: *Functional Ingredients from Algae for Foods and Nutraceuticals* (ed. H. Domínguez), 475–516. Woodhead Publishing.

29 Stanley, N. (1987). Production, properties and uses of carrageenan. Production and utilization of products from commercial seaweeds. *FAO Fisheries Technical Paper* 288: 116–146.

30 Khalil, H.P., Tye, Y.Y., Saurabh, C.K. et al. (2017 Apr 1). Biodegradable polymer films from seaweed polysaccharides: a review on cellulose as a reinforcement material. *Express Polymer Letters* 11 (4): 244–265.

31 Karidurganavar, M.Y., Kittur, A.A., and Kamble, R.R. (2014). Natural and synthetic biomedical polymers. In: *Polymer Synthesis and Processing* (ed. C. Laurencin and M. Deng), 1–19. Burlington, USA: Elsevier Publishers.

32 Hilliou, L., Larotonda, F.D., Abreu, P. et al. (2012 Mar 1). The impact of seaweed life phase and postharvest storage duration on the chemical and rheological properties of hybrid carrageenans isolated from Portuguese *Mastocarpus stellatus*. *Carbohydrate Polymers* 87 (4): 2655–2663.

33 Ibraheem, O. and Babatunde, O.M. (2019 Apr 8). Marine polysaccharides: extraction techniques, structural determination, and description of their biological activities. In: *Enzymatic Technologies for Marine Polysaccharides* (ed. A. Trincone), 219–272. CRC Press.

34 Chandan, R.C. and Kilara, A. (2015). Dairy processing and quality assurance. In: *Puddings and Dairy-Based Desserts* (ed. R.C. Chandan, A. Kilara, and N.P. Shah), 397–427. https://onlinelibrary.wiley.com/doi/abs/10.1002/9781118810279.ch17.

35 Meireles, F.A. (2013). Carrageenan yield and quality of *Chondrus crispus* Stackhouse (Rhodophyta) cultivated in an Integrated Multi-Trophic Aquaculture (IMTA) system (Masters dissertation). Advisor(S): Pereira LC. University of Coimbra. https://hdl.handle.net/10316/24718.

36 Tobacman, J.K. (2001). Review of harmful gastrointestinal effects of carrageenan in animal experiments. *Environmental Health Perspectives* 109 (10): 983–994.

37 Hashsham, S.A. (2007). Culture techniques. In: *Methods for General and Molecular Microbiology* (ed. T.J. Bevveridge, J.A. Breznak, G.A. Marzluf, et al.), 270–285. Washington, DC: ASM Press.

38 Herrero, M. and Ibanez, E. (2015 Jan 1). Green processes and sustainability: an overview on the extraction of high added-value products from seaweeds and microalgae. *The Journal of Supercritical Fluids* 96: 211–216.

39 Bixler, H.J. (1996). Recent developments in manufacturing and marketing carrageenan. In *Fifteenth International Seaweed Symposium* (pp. 35-57). Springer: Dordrecht.

40 Usov, A.I. (1998 Jul 1). Structural analysis of red seaweed galactans of agar and carrageenan groups. *Food Hydrocolloids* 12 (3): 301–308.

41 Ahmed, A.B., Adel, M., Karimi, P., and Peidayesh, M. (2014 Jan 1). Pharmaceutical, cosmeceutical, and traditional applications of marine carbohydrates. *Advances in Food and Nutrition Research* 73: 197–220.

42 Damonte, E.B., Matulewicz, M.C., and Cerezo, A.S. (2004 Sep 1). Sulfated seaweed polysaccharides as antiviral agents. *Current Medicinal Chemistry* 11 (18): 2399–2419.

43 Duong, T. (2013). Molecular understanding of whey protein-based matrices for the preservation of *Saccharomyces boulardii* cells in spray dried microcapsules. Doctoral dissertation. RMIT University.

44 Ciancia, M., Matulewicz, M.C., and Tuvikene, R. (2020). Structural diversity in galactans from red seaweeds and its influence on rheological properties. *Frontiers in Plant Science* 11: 1392.

45 Belitz, H.D., Grosch, W., and Schieberle, P. (2009). 4.2 Monosaccharides. In: *Food Chemistry*, 248–292. Berlin Heidelberg, Berlin: Springer.

46 King, A.H. (2019 Jul 17). Brown seaweed extracts (alginates). In: *Food Hydrocolloids* (ed. M. Glicksman), 115–188. CRC Press.

47 Whistler R, editor. *Industrial Gums: Polysaccharides and Their Derivatives. Elsevier*; 2012.

48 Tan, Y. (2003). Effect of biopolymer addition on the properties of rennet-induced skim milk gels. Doctoral dissertation. Massey University.

49 Williams, P.A. and Phillips, G.O. (2003). GUMS properties of individual gums. In: *Encyclopedia of Food Sciences and Nutrition*, 2e (ed. B. Caballero), 2992–3001. Academic press.

50 Acharya, P.C., Suares, D., Shetty, S. et al. (2018). Rheology and its implications on performance of liquid dosage forms. In: *Dosage Form Design Considerations* (ed. R.K. Tekade), 549–597. Academic Press.

51 Suresh, H., Ho, V., and Zhou, J. (2020 Aug). Rheological characteristics of soluble fibres during chemically simulated digestion and their suitability for gastroparesis patients. *Nutrients* 12 (8): 2479.

52 Tecson, M.G., Abad, L.V., Ebajo, V.D. Jr., and Camacho, D.H. (2021 May 1). Ultrasound-assisted depolymerization of kappa-carrageenan and characterization of degradation product. *Ultrasonics Sonochemistry* 73: 105540.

53 Totosaus, A. and Perez-Chabela, M.L. (2009 Mar 1). Textural properties and microstructure of low-fat and sodium-reduced meat batters formulated with gellan gum and dicationic salts. *LWT-Food Science and Technology* 42 (2): 563–569.

54 Sadar, L.N. (2004). *Rheological and Textural Characteristics of Copolymerized Hydrocolloidal Solutions Containing Curdlan Gum*. College Park, MD: University of Maryland.

55 Lisitsyn, A., Semenova, A., Nasonova, V. et al. (2021 Jan). Approaches in animal proteins and natural polysaccharides application for food packaging: edible film production and quality estimation. *Polymers* 13 (10): 1592.

56 Dima, C., Assadpour, E., Dima, S., and Jafari, S.M. (2020 May). Bioavailability of nutraceuticals: role of the food matrix, processing conditions, the gastrointestinal tract, and nanodelivery systems. *Comprehensive Reviews in Food Science and Food Safety* 19 (3): 954–994.

57 Nguyen, H.H. and Kim, M. (2017). An overview of techniques in enzyme immobilization. *Applied Science and Convergence Technology* 26 (6): 157–163.

58 Taheri-Kafrani, A., Kharazmi, S., Nasrollahzadeh, M. et al. (2021 Jul). Recent developments in enzyme immobilization technology for high-throughput processing in food industries. *Critical Reviews in Food Science and Nutrition* 61: 3160–3196.

59 Martin, A.M. (ed.) (2012). *Bioconversion of Waste Materials to Industrial Products*. Springer Science & Business Media.

60 Kitchell, B.S., Henkens, R.W., Brown, P., Baldwin, S.W. (1993). Carrageenan-immobilized esterase. US Patent No. 5262313.

61 Ghanbarzadeh, M., Golmoradizadeh, A., and Homaei, A. (2018 Jun). Carrageenans and carrageenases: versatile polysaccharides and promising marine enzymes. *Phytochemistry Reviews* 17 (3): 535–571.

62 Wang, K. (2012). *Enzyme Immobilization on Chitosan-Based Supports. Chitosan-Based Hydrogels: Functions and Applications*, 339–406. Boca Raton, FL: CRC Press, Taylor & Francis Group.

63 Yeom, S.J., Kim, M., Kwon, K.K. et al. (2018 Nov 29). A synthetic microbial biosensor for high-throughput screening of lactam biocatalysts. *Nature Communications* 9 (1): 1–2.

64 Azevedo, A.M., Prazeres, D.M., Cabral, J.M., and Fonseca, L.P. (2005 Aug 15). Ethanol biosensors based on alcohol oxidase. *Biosensors and Bioelectronics* 21 (2): 235–247.

65 Krishnan, M.S., Taylor, F., Davison, B.H., and Nghiem, N.P. (2000 Nov 1). Economic analysis of fuel ethanol production from corn starch using fluidized-bed bioreactors. *Bioresource Technology* 75 (2): 99–105.

66 Boadi, D.K. and Neufeld, R.J. (2001 May 7). Encapsulation of tannase for the hydrolysis of tea tannins. *Enzyme and Microbial Technology* 28 (7–8): 590–595.

67 Tosa, T., Sato, T., Mori, T. et al. (1979 Oct). Immobilization of enzymes and microbial cells using carrageenan as matrix. *Biotechnology and Bioengineering* 21 (10): 1697–1709.

68 Stamatis, H. and Xenakis, A. (1999 Apr 2). Biocatalysis using microemulsion-based polymer gels containing lipase. *Journal of Molecular Catalysis B: Enzymatic* 6 (4): 399–406.

69 Puri, M., Marwaha, S.S., and Kothari, R.M. (1996 Mar 1). Studies on the applicability of alginate-entrapped naringiase for the debittering of kinnow juice. *Enzyme and Microbial Technology* 18 (4): 281–285.

70 Campanella, L., Favero, G., Sammartino, M.P., and Tomassetti, M. (1999 Sep 15). Enzymatic immobilization in kappa–carrageenan gel suitable for organic phase enzyme electrode (OPEE) assembly. *Journal of Molecular Catalysis B: Enzymatic* 7 (1–4): 101–113.

71 Campanella, L., Roversi, R., Sammartino, M.P., and Tomassetti, M. (1998 Oct 1). Hydrogen peroxide determination in pharmaceutical formulations and cosmetics using a new catalase biosensor. *Journal of Pharmaceutical and Biomedical Analysis* 18 (1–2): 105–116.

72 Van de Velde, F., Lourenço, N.D., Pinheiro, H.M., and Bakker, M. (2002 Sep). Carrageenan: a food-grade and biocompatible support for immobilisation techniques. *Advanced Synthesis and Catalysis* 344 (8): 815–835.

73 Santos, V.A., Marchal, L.M., Tramper, J., and Wijffels, R.H. (1996). Modeling and evaluation of an integrated nitrogen removal system with microorganisms co-immobilized in double-layer gel beads. *Biotechnology Progress* 12 (2): 240–248.

74 Kumar R. (2018). Biological approaches for treating industrial effluents containing morpholine (Doctoral thesis). Guide (A): Kapur S. Department of Biological Sciences, BITS.

75 Coughlin, J.R. (1979). *Formation of n-Nitrosamines from Maillard Browning Reaction Products in the Presence of Nitrite*. Davis, CA: University of California.

76 Aggarwal, J., Sharma, S., Kamyab, H., and Kumar, A. (2020). The realm of biopolymers and their usage: an overview. *Journal of Environmental Treatment Techniques* 8: 1005–1006.

77 Bianca, H.F. (2017). Encapsulation of *Lactobacillus rhamnosus* GG into hybrid alginate-silica microparticles. Doctoral dissertation, Université de Lorraine.

78 Suttigarn, A. (2005). *Arsenite Oxidation by Alcaligenes faecalis Strain O1201 in Bioreactor Systems*. University of Kentucky.

79 Tosa, T., Sato, T., Mori, T. et al. (1973 Jan). Continuous production of L-aspartic acid by immobilized aspartase. *Biotechnology and Bioengineering* 15 (1): 69–84.

80 Elella, M.H., Goda, E.S., Gab-Allah, M.A. et al. (2020 Nov 5). Xanthan gum-derived materials for applications in environment and eco-friendly materials: a review. *Journal of Environmental Chemical Engineering* 9: 104702.

81 Taylor, T.M. and Doores, S.X. (2020 Nov 10). Organic acids. In: *Antimicrobials in Food* (ed. P.M. Davidson, T.M. Taylor, and J.R.D. David), 133–190. CRC Press.

82 Chibata, I., Tosa, T., and Sato, T. (1974 May). Immobilized aspartase-containing microbial cells: preparation and enzymatic properties. *Applied Microbiology* 27 (5): 878–885.

83 Takata, I., Tosa, T., and Chibata, I. (1983). Stabilization of fumarase activity of Brevibacterium flavum cells by immobilization with k-carrageenan. *Applied Biochemistry and Biotechnology* 8: 31–38. https://link.springer.com/article/10.1007/BF02798346.

84 Chibata, I. and Tosa, T. (1981). Use of immobilized cells. *Annual Review of Biophysics and Bioengineering* 10 (1): 197–216.

85 Chibata, I., Kakimoto, T., and Kato, J. (1965). Enzymatic production of L-alanine by Pseudomonas dacunhae. *Appl. Microbiol.* 13: 638–645.

86 Takamatsu, S., Umemura, I., Yamamoto, K. et al. (1982). Production of l-alanine from ammonium fumarate using two immobilized microorganisms. *European Journal of Applied Microbiology and Biotechnology* 15 (3): 147–152.

87 Mateus, D.M., Alves, S.S., and Da Fonseca, M.M. (1996 Feb). Model for the production of L-tryptophan from L-serine and indole by immobilized cells in a three-phase liquid-impelled loop reactor. *Bioprocess Engineering* 14 (3): 151–158.

88 Yamamoto, K., Tosa, T., and Chibata, I. (1980). Continuous production of l-alanine using *Pseudomonas dacunhae* immobilized with carrageenan. *Biotechnology and Bioengineering* 22 (10): 2045–2054.

89 Vaněk, T., Valterová, I., Vaňková, R., and Vaisar, T. (1999 Jul). Biotransformation of (−)-limonene using *Solanum aviculare* and *Dioscorea deltoidea* immobilized plant cells. *Biotechnology Letters* 21 (7): 625–628.

90 Osuga, J., Mori, A., and Kato, J. (1984). Acetic acid production by immobilized *Acetobacter aceti* cells entrapped in a κ-carrageenan gel. *Journal of Fermentation Technology* 62 (2): 139–149.

91 Teruel, M.A., Gontier, E., Bienaime, C. et al. (1997 Oct 1). Response surface analysis of chlortetracycline and tetracycline production with κ-carrageenan immobilized *Streptomyces aureofaciens*. *Enzyme and Microbial Technology* 21 (5): 314–320.

92 Lamboley, L., Lacroix, C., Champagne, C.P., and Vuillemard, J.C. (1997 Dec 5). Continuous mixed strain mesophilic lactic starter production in supplemented whey permeate medium using immobilized cell technology. *Biotechnology and Bioengineering* 56 (5): 502–516.

93 Tanaka, A., Tosa, T., and Kobayashi, T. (ed.) (1992). *Industrial Application of Immobilized Biocatalysts*. CRC Press.

94 Pilkington, H., Margaritis, A., Mensour, N. et al. (1999). Kappa-carrageenan gel immobilisation of lager brewing yeast. *Journal of the Institute of Brewing.* 105 (6): 398–405.

95 Pitcher, W.H. (ed.) (1980). *Immobilized Enzymes for Food Processing*. Boca Raton, FL: CRC Press.

96 Hartmeier, W. (2012). *Immobilized Biocatalysts: An Introduction*. Springer Science & Business Media.

97 Tran, M. (2017). Effect of high-pressure jet processing on the physicochemical and foaming properties of whole milk and its impact on cocoa stability in chocolate milk. Master's thesis, Penn State.

98 Blakemore, W.R. and Harpel, A.R. (2010). Carrageenan. In: *Food Stabiliser, Thickeners and Gelling Agents* (ed. A. Imeson), 72–94. New Jersey, USA: Blackwell Publishing Ltd.

99 Riaz, M.N. and Chaudry, M.M. (ed.) (2018). *Handbook of Halal Food Production*. CRC Press.

100 Imeson, A.P. (2012). *Thickening and Gelling Agents for Food*. Springer Science & Business Media.

101 Ghribi, A.M., Zouari, M., Attia, H., and Besbes, S. (2021 Jul 1). Study of protein/κ-carrageenan mixture's effect on low-fat whipping cream formulation. *LWT* 147: 111647.

102 Fallourd, M.J. and Viscione, L. (2009). Ingredient selection for stabilization and texture optimization of functional beverages and the inclusion of dietary fibre. In: *Functional and Speciality Beverage Technology* (ed. P. Paquin), 3–38. Woodhead Publishing.

103 Chandan, R.C. and Kilara, A. (2008). Puddings and dairy-based desserts. *Dairy Processing and Quality Assurance* 10: 387–410.

104 Imeson, A.P. (2009 Jan 1). Carrageenan and furcellaran. In: *Handbook of Hydrocolloids* (ed. G.O. Phillips and P.A. Williams), 164–185. Woodhead Publishing.

105 Nielsen, B., Gürakan, G.C., and Uenlue, G. (2014 Dec 1). Kefir: a multifaceted fermented dairy product. *Probiotics and Antimicrobial Proteins* 6 (3–4): 123–135.

106 Lappa, I.K., Papadaki, A., Kachrimanidou, V. et al. (2019 Aug). Cheese whey processing: integrated biorefinery concepts and emerging food applications. *Foods* 8 (8): 347.

107 Sandford, P.A. and Baird, J. (1983 Jan 1). Industrial utilization of polysaccharides. In: *Polysaccharides* (ed. G.O. Aspinall), 411–490. Academic Press.

108 Nieto, M.B. (2009). Structure and function of polysaccharide gum-based edible films and coatings. In: *Edible Films and Coatings for Food Applications* (ed. K.C. Huber and M.E. Embuscado), 57–112. New York, NY: Springer.

109 Willaert, R. and Nedovic, V.A. (2006 Aug). Primary beer fermentation by immobilized yeast—a review on flavour formation and control strategies. *Journal of Chemical Technology and Biotechnology: International Research in Process, Environmental and Clean Technology* 81 (8): 1353–1367.

110 Mensour, N.A., Margaritis, A., Briens, C.L. et al. (1997 Nov 12). New developments in the brewing industry using immobilised yeast cell bioreactor systems. *Journal of the Institute of Brewing* 103 (6): 363–370.

111 Krishnan, M.S., Nghiem, N.P., and Davison, B.H. (1999). Ethanol production from corn starch in a fluidized-bed bioreactor. In: *Twentieth Symposium on Biotechnology for Fuels and Chemicals* (ed. B.H. Davison and M. Finkelstein), 359–372. Totowa, NJ: Humana Press.

112 Nigam, J.N. (1999 Jul 2). Continuous ethanol production from pineapple cannery waste. *Journal of Biotechnology* 72 (3): 197–202.

113 DeFreitas, Z., Sebranek, J.G., Olson, D.G., and Carr, J.M. (1997 May). Carrageenan effects on thermal stability of meat proteins. *Journal of Food Science* 62 (3): 544–547.

114 Yusop, S.M., O'Sullivan, M.G., and Kerry, J.P. (2011). Marinating and enhancement of the nutritional content of processed meat products. In: *Processed Meats* (ed. J.P. Kerry and J.F. Kerry), 421–449. Sawston, Cambridge, UK: Woodhead Publishing.

115 McClements, D.J. and Grossmann, L. (2021). The science of plant-based foods: constructing next-generation meat, fish, milk, and egg analogs. *Comprehensive Reviews in Food Science and Food Safety* 20: 4049–4100.

116 Nussinovitch, A. (2019). *Cooking Innovations: Using Hydrocolloids for Thickening, Gelling, and Emulsification*. CRC Press.

117 Edwards, W.P. (2018). *The Science of Sugar Confectionery*. Royal Society of Chemistry.

118 Tobacman, J.K., Wallace, R.B., and Zimmerman, M.B. (2001 May 1). Consumption of carrageenan and other water-soluble polymers used as food additives and incidence of mammary carcinoma. *Medical Hypotheses* 56 (5): 589–598.

119 Maier, H., Anderson, M., Karl, C. et al. (1993 Jan 1). Guar, locust bean, tara, and fenugreek gums. In: *In Industrial Gums* (ed. R.L. Whistler and J.N. Bemiller), 181–226. Academic Press.

120 Kosseva, M.R. (2011 Aug). Immobilization of microbial cells in food fermentation processes. *Food and Bioprocess Technology* 4 (6): 1089–1118.

121 Patel, S. (2012 Sep). Therapeutic importance of sulfated polysaccharides from seaweeds: updating the recent findings. *3 Biotech* 2 (3): 171–185.

11

Inulin as a Prebiotic Agent in Human Nutrition and Healthcare

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11.1 Introduction

Inulin is a nonstructural storage carbohydrate that acts as an energy reservoir and osmoregulator in plants. Inulin is found in onions, *Asparagus* (*Asparagus officinalis*), garlic, leeks, chicory (*Cichorium intybus*), and Jerusalem artichoke (*Helianthus tuberosus*) (Table 11.1) [14]. Chicory and Jerusalem artichoke are used industrially to produce inulin because they contain the highest inulin content. Inulin structure can be represented as GFn, where G and F are glucosyl and fructosyl moieties. n represents fructosyl moieties with a β-(2,1) linkage [15]. The presence of this bond prevents the easy breakdown of inulin by digestive enzymes, unlike most other dietary carbohydrates. That is why inulin has a low caloric value. As inulin is undigestible by the digestive system's enzymes, it reaches the large intestine, which is responsible for the digestion of this nondigestible carbon. Inulin is generally regarded as a nutraceutical since it has both nutritional and functional attributes. Inulin is colorless, tasteless, highly soluble, and does not alter the sensory characteristics of any food product to which it is added. Because inulin is highly soluble, it is used as an additive for dairy products, such as cheese, yogurt, and flavored milk. Inulin is also regarded as a potential prebiotic agent. Several *in vitro* and *in vivo* studies have proven that inulin supplementation stimulated the growth of beneficial bacteria, such as *Bifidobacterium*, in the colon of the large intestine, thereby promoting gut microflora and mucosal immunity in the host [16]. Along with *Bifidobacterium*, various beneficial bacteria, such as *Lactobacillus*, *Faecalibacterium*, and *Anaerostipes*, became more abundant in the gut microbiome after inulin supplementation [17].

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Table 11.1 Sources, origin, and content of inulin.

Source	Origin	Content (g/100 g)	References
Smallanthus sonchifolius	Root	35	[1]
Stevia rebaudiana	Bulb	18–23	[2]
Allium sativum	Bulb	14–23	[3]
Hordeum vulgare	Grains	18–20	[4]
Cichorium intybus	Root	11–20	[5]
Helianthus tuberosus	Tuber	12–19	[6]
Asparagus sp.	Root	15	[7]
Agave sp.	Stem	12–15	[8]
Taraxacum officinale	Root	12–15	[9]
Dahlia pinata cav.	Tuber	10–12	[10]
Pfalia glomerate	Root	11.45	[11]
Allium sp.	Bulb	5–9	[12]
Arctium sp.	Root	8.3–9.9	[13]

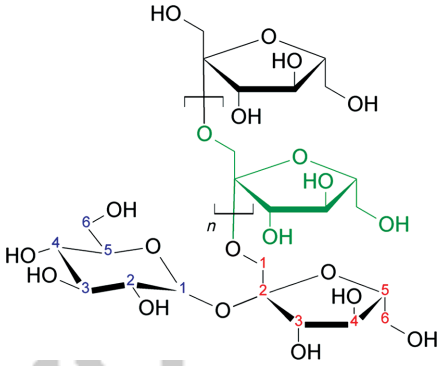
In humans, inulin intake promoted the growth of butyrate producers, such as members of Ruminococcaceae, Bifidobacteriaceae, and Lachnospiraceae [18]. Inulin fermentation in the colon of the large intestine promotes specific metabolic and physiological functions by the production of short-chain fatty acids (SCFAs), such as acetate, lactate, butyrate, and propionate. Production of SCFAs as a result of inulin fermentation improves lipid metabolism and the bioavailability of essential minerals, such as calcium, magnesium, and iron, in the body [19]. In this chapter, we intend to focus on the structure and properties of inulin, its metabolism in the host, and its relevance as a functional food and prebiotic ingredient in human nutrition, metabolism, and health to help researchers from various disciplines in understanding the functional properties and potentiality of inulin as a prebiotic agent in shaping host metabolism and health.

11.2 Biochemistry and Metabolism

Inulin is composed of a chain of up to 60 β-(2,1)-glycosidic bonded D-fructosyl subgroups and an α-(1,2) D-glucose bond at the terminal fructose moiety (Figure 11.1). The degree of polymerization critically depends on the source of the plant and the harvest period. Predominately, inulin is isolated from the fresh roots of chicory (Compositae family). The non-fractionated isolate contains fructose, glucose, sucrose, and several small oligosaccharides. Like other dietary fiber components, inulin cannot be digested enzymatically; it moves to the colon region of the large intestine, where the gut microbiome ferments and metabolizes it [20]. Unlike normal monosaccharides, which elevate sugar levels in the blood, inulin helps in controlling the blood sugar levels and managing the progression of diabetes. During

Figure 11.1 Structure of inulin.

Color Fig: **11.1**



its digestion, inulin releases SCFAs in abundance, which are absorbed by the colon and act as metabolic fuel for colonocytes. SCFAs, therefore, play an important role in maintaining the integrity of colon and gut metabolism.

11.3 Significance as a Functional Food

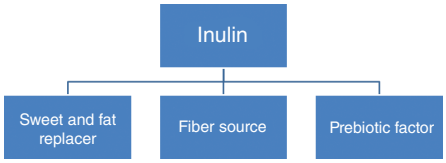
Inulin has always been a part of the human diet. Food products fortified with inulin significantly improve nutritional and organoleptic properties. Inulin is highly soluble in water, making it very relevant as an additive to fortify various food products. Inulin has a much lower caloric value than other carbohydrates. It also acts as a prebiotic agent because of its modulatory actions on the gut microbiome. Inulin fermentation in the colon promotes various metabolic and physiological functions through SCFAs (Figure 11.2). These SCFAs affect the morphology and functions of colonocytes. They stimulate mineral absorption and increase mucosal blood flow. Experimental investigations on higher animal models demonstrated a decrease in intestinal cell pH, multiplication of the mucosa cells, and differentiation with inulin supplementation. For *Clostridium difficile* infections, inulin is used in ulcerative colitis therapy [21]. Inulin also enhances digestive health and absorption of minerals, inhibits the growth of different cancers, and promotes the metabolism of lipids and carbohydrates.

11.4 Relevance of Inulin on Human Health

Inulin is fermented selectively by *Lactobacillus* and *Bifidobacterium* in the colon of the large intestine. In consequence, inulin modulates the gut microbiome and

Figure 11.2 Relevance of inulin in human health.

Color Fig: **11.2**



Color Fig: **11.3**

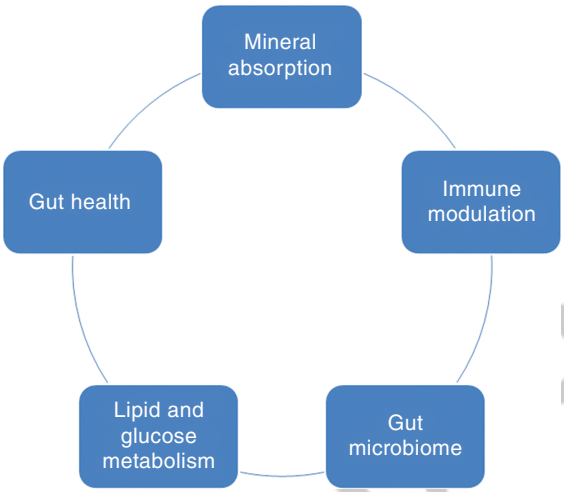


Figure 11.3 Functional and metabolic effects of inulin on human health.

leads to a reduction in the population of pathogenic microorganisms in the gut. Upon fermentation of inulin, the gut microbiome secretes certain health-promoting metabolites. Numerous studies have proven the prebiotic activity of inulin through selective fermentation. *In vitro* studies demonstrated the substantial rise in the *Bifidobacterium* population as a result of inulin fermentation [16]. The retention and survival of probiotics, such as *Lactobacillus acidophilus*, *Bifidobacterium lactis*, and *Lactobacillus casei*, was increased for the patients treated with dietary inulin fiber in *in vivo* trials [22]. The functional and metabolic effects of inulin on human health are illustrated in Figure 11.3.

11.4.1 Oral and Gut Health

Inulin is highly resistant to hydrolysis by salivary enzymes. But some oral cavity residents, such as streptococci can ferment inulin and release acids. According to Doran and Verran, inulin stimulates acidogenic bacteria in the oral cavity and reduces oral malodor. They concluded that inulin could reduce the tongue pH [23]. Inulin was also associated with an increase in the fecal load, relieving constipation and lowering intestinal pH [24]. The consumption of one gram of inulin is linked to an increase of 1.5–2.0 g in fecal weight. Inulin was also shown to alleviate constipation in elderly persons. Additional 20–40 g of chicory inulin in the daily diet resulted in constipation relief. The benefits of inulin on different gastrointestinal illnesses, such as ulcerative colitis, irritable bowel syndrome, colon cancer, inflammatory bowel diseases, and Crohn's disease, have been noted. Studies in mice and rats revealed the relevance of inulin for these disorders. A beneficial effect of inulin in ulcerative colitis patients was observed when inulin was coadministered with *Bifidobacterium longum* [25]. Similarly, supplementation of 15 g of inulin to patients suffering from Crohn's disease for 21 days improved their metabolic functions by stimulating the growth of bifidobacteria. When inulin was co-formulated with *B. lactis* and *Lactobacillus rhamnosus*, the risk of colon cancer in humans was reduced [26].

11.4.2 Influence on the Gut Microbiome

Different variables, such as food and medication consumption, impact the composition and metabolic activities of the gut microbiome. The link between gut microbiota and certain metabolic disorders has been documented by researchers. Inulin, as a selectively fermented fiber, may lead to specific modifications for the host's microbiome composition and functions. *In vitro* experiments, animal models, and studies of human volunteers examined the prebiotic properties of inulin. Inulin supplementation has been demonstrated to selectively promote *Bifidobacterium* development in the stomach [16], thereby maintaining the stability and health of the microbial host population. Along with *Bifidobacterium*, there has been an enhanced response to inulin supplementation from many butyrate-producing bacteria, such as *Eubacterium* sp. and *Faecalibacterium prausnitzii* [27]. Olive et al. showed a considerable increase in the population and sustainability of probiotic bacteria, such as *B. lactis, L. rhamnosus*, and *L. acidophilus*, following the administration of low inulin concentrations in fat-free milk [28]. Inulin might be employed as a fat substitute for non-fat milk products, and offer the same sensory features.

11.4.3 Immune Modulation

The influence of inulin on the immune system was elucidated through animal studies. Large-scale studies with human volunteers will be needed to know the full effect of inulin and its derivatives in modulating the immune system. However, animal studies could provide some insights into the immunomodulatory functions of inulin. Inulin was found to activate immune cells in Peyer's patches by promoting the production of interleukin 10 (IL-10) and natural killer (NK) cell-mediated cytotoxicity [29]. Inulin enhances IgA-mediated signaling in the ileum and caecum. Inulin primarily improves certain parameters in the gut-associated lymphoid tissue (GALT). Some studies also proved that inulin intake at low doses improved the efficacy of vaccines. Elevated levels of serum IgG and mucosal IgA antibodies were observed in mice vaccinated with *Salmonella* vaccine upon inulin feeding [30].

11.4.4 Mineral Absorption

Fermentable fiber, like inulin, can assist in maintaining mineral equilibrium in the body. Inulin lowers the intestinal pH upon fermentation by the gut microflora. Low pH in the intestine improves the bioavailability of calcium. The SCFAs produced as a result of inulin digestion influence calcium absorption in the gut. For instance, a rise in butyrate concentration stimulates the growth of cells and also increases the surface area of the absorptive region in the intestine. Inulin, in combination with oligofructose, enhanced calcium absorption by 18% in adolescent girls [31]. The action of inulin on mineral absorption was also proved in postmenopausal women and adult men [32, 33]. Further studies have shown that co-supplementation with inulin and oligofructose displayed a higher potential in calcium and magnesium absorption [34]. The effect of inulin on iron absorption was examined and evaluated in a piglet phenotype model by virtue of its resemblance in genetic makeup and its

gastrointestinal and digestive system similarity compared to humans. Inulin intake was associated with enhanced iron absorption and hemoglobin levels [35].

11.4.5 Lipid and Glucose Metabolism

Inulin can lower and stabilize the concentration of lipids and glucose in the blood. The hypocholesterolemic effect of inulin was directly related to the production of SCFAs. Chicory root-derived inulin significantly reduced the concentration of triglycerides by 40 mg/dl in serum [36]. A reduction in total serum cholesterol was observed in the study conducted by Causey et al. [50]. Changes in the profile of SCFAs were observed after inulin administration. Feeding C57BL/6J wild-type mice with inulin for two weeks resulted in higher fecal SCFA levels with no significant effect on the levels of liver and plasma lipids [37]. Inulin has unique metabolic functions that benefit people at risk of diabetes. In a randomized double-blind and crossover clinical trial conducted by Guess et al. [38], inulin was associated with a significant reduction in insulin resistance among diabetic patients. Inulin intake reduced the four glycemic indicators: fasting blood glucose (FBG), fasting insulin (FINS), glycosylated hemoglobin (HbA1c), and homeostasis model assessment-insulin resistance (HoMA-IR) among prediabetic and diabetic patients. The authors concluded that inulin supplementation has a potential impact and great clinical value in the therapy and management of prediabetes and type-2 diabetes mellitus (T2DM).

11.5 Applications in the Food Industry

Inulin is being used widely in food industries because of its functional attributes. Many food producers are showing great interest in developing novel food products with potential health benefits based on consumer requirements, such as low fat, low sugar, high fiber, and prebiotic effects (Table 11.2). As a nondigestible fiber, inulin acts as a prebiotic agent as it is selectively fermentable by the gut microflora and improves the activity of beneficial microorganisms. Inulin can be a large- or short-chain molecule. Large-chain inulin molecules are highly soluble in water and are good fat replacers, whereas short-chain inulin molecules enhance flavor and act as replacers of sucrose. Inulin is mainly used as an organoleptic and nutritional factor in food products. Inulin is a major fiber constituent. It improves the texture and taste of the food products. It is being used in breakfast items, such as cereals and certain bakery items [46]. Fortifying bakery food products with inulin improves crispiness and keeps them moist and fresh for a long time. Being soluble in an aqueous environment, inulin permits the addition of fiber to beverages and dairy products. In one study, improved bowel movements and increased stool weight were witnessed among adults fed with inulin-formulated beverages [47]. Since inulin is indigestible by the gastric and salivary enzymes, it acts as a prebiotic factor for gut microflora. Inulin is used in functional foods as a prebiotic factor for promoting beneficial bacteria in the large intestine. Low concentrations of inulin in skim milk increase the

Table 11.2 Applications of inulin in food industries.

Product	Function	Concentration (% w/v)	References
Bakery	Sugar replacer	2–15	[39]
Cereals	Fiber and prebiotic	2–25	[40]
Dairy products	Fat and sugar replacer	2–10	[41]
Desserts	Fat and sugar replacer	2–10	[42]
Table spreads	Fiber and prebiotic	2–10	[43]
Chocolate	Sugar replacer	2–30	[44]
Tablets	Fiber, prebiotic, and sugar replacer	5–100	[45]

growth, activity, and sustainability of *B. lactis*, *L. acidophilus*, and *L. rhamnosus* [28]. Inulin is also being used as a fat and sugar replacer in food products. Long-chain inulin molecules are effective fat replacers in jelly-like substances. They add texture to the product and provide a fat-like feel. Long-chain inulin molecules are also used in low-fat yogurts to improve creaminess and smoothness [48]. The addition of 15% inulin in biscuits yields good sensory properties while reducing their fat content. Short-chain inulin molecules show the properties of sugar replacers. They improve the sweetness of sucrose by up to 35%, which makes them useful as saccharose substitutes in food items. The inclusion of short-chain inulin molecules made sugar-free chocolate possible [49]. The food industry uses inulin to produce innovative foods with increased sensory and nutraceutical qualities that appeal to consumers due to their nutritional value and other health advantages.

11.6 Conclusions

Inulin is a nondigestible dietary fiber with several health benefits. It is a constituent of a normal diet. Inulin is widely used as a food ingredient in many food industries. Inulin accounts for various therapeutic functions. It acts as a fiber source, fat, and sugar replacer. Therefore, it can be used in low-calorie and fat-free food products and reduce the risk of various metabolic disorders. Finally, inulin can be used as a functional food that has immense potential to exert various health benefits.

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References

1 Castro, A., Céspedes, G., Carballo, S. et al. (2013). Dietary fiber, fructooligosaccharides, and physicochemical properties of homogenized aqueous suspensions of yacon (*Smallanthus sonchifolius*). *Food Research International* 50 (1): 392–400.

2 Lopes, S.M., Krausová, G., Rada, V. et al. (2015). Isolation and characterization of inulin with a high degree of polymerization from roots of *Stevia rebaudiana* (*Bert*.) Bertoni. *Carbohydrate Research* 411: 15–21.

3 Álvarez-Borroto, R., Ruano-Nieto, A.L., Calle-Minaca, M.R., and Lara-Fiallos, M.V. (2015). Extracción y determinación de inulina del ajo comúnautóctono (*Allium sativum*). *Revista Cubana De Química* 27 (2): 131–146.

4 Nemeth, C., Andersson, A., Andersson, R. et al. (2014). Relationship of grain fructan content to degree of polymerisation in different Barleys. *Food and Nutrition Sciences* 5: 581–589.

5 Figueira, G.M., Park, K.J., Brod, F.P.R., and Honorio, S.L. (2004). Evaluation of desorption isotherms, drying rates and inulin concentration of chicory roots (*Cichorium intybus* L.) with and without enzymatic inactivation. *Journal of Food Engineering* 63 (3): 273–280.

6 Bach, V., Clausen, M.R., and Edelenbos, M. (2015). Production of Jerusalem artichoke (*Helianthus tuberosus* L.) and impact on inulin and phenolic compounds. In: *Processing and Impact on Active Components in Food*, 97–102. Academic Press.

7 Chi, Z.M., Zhang, T., Cao, T.S. et al. (2011). Biotechnological potential of inulin for bioprocesses. *Bioresource Technology* 102 (6): 4295–4303.

8 Moreno-Vilet, L., Camacho-Ruiz, R.M., and Portales-Pérez, D.P. (2016). Prebiotic *Agave* fructans and immune aspects. In: *Probiotics, Prebiotics, and Synbiotics. Bioactive Foods in Health Promotion*, 165–179. Academic Press.

9 Kango, N. (2008). Production of inulinase using tap roots of dandelion (*Taraxacum officinale*) by *Aspergillus niger*. *Journal of Food Engineering* 85 (3): 473–478.

10 Zhu, Z., He, J., Liu, G. et al. (2016). Recent insights for the green recovery of inulin from plant food materials using non-conventional extraction technologies: a review. *Innovative Food Science & Emerging Technologies* 33: 1–9.

11 Caleffi, E.R., Krausová, G., Hyršlová, I. et al. (2015). Isolation and prebiotic activity of inulin-type fructan extracted from *Pfaffia glomerata* (Spreng) Pedersen roots. *International Journal of Biological Macromolecules* 80: 392–399.

12 Judprasong, K., Tanjor, S., Puwastien, P., and Sungpuag, P. (2011). Investigation of Thai plants for potential sources of inulin-type fructans. *Journal of Food Composition and Analysis* 24 (4–5): 642–649.

13 Lou, Z., Wang, H., Wang, D., and Zhang, Y. (2009). Preparation of inulin and phenols-rich dietary fibre powder from burdock root. *Carbohydrate Polymers* 78 (4): 666–671.

14 De Oliveira, A.J.B., Gonçalves, R.A.C., Chierrito, T.P.C. et al. (2011). Structure and degree of polymerisation of fructooligosaccharides present in roots and leaves of *Stevia rebaudiana* (Bert.) Bertoni. *Food Chemistry* 129 (2): 305–311.

15 Roberfroid, M.B. (2005). Introducing inulin-type fructans. *British Journal of Nutrition* 93 (S1): S13–S25.

16 Mulabagal, V., Wang, H., Ngouajio, M., and Nair, M.G. (2009). Characterization and quantification of health beneficial anthocyanins in leaf chicory (*Cichorium intybus*) varieties. *European Food Research and Technology* 230 (1): 47–53.

17 Le Bastard, Q., Chapelet, G., Javaudin, F. et al. (2020). The effects of inulin on gut microbial composition: a systematic review of evidence from human studies. *European Journal of Clinical Microbiology & Infectious Diseases: Official Publication of the European Society of Clinical Microbiology* 39 (3): 403–413.

18 Rowland, I., Gibson, G., Heinken, A. et al. (2018). Gut microbiota functions: metabolism of nutrients and other food components. *European Journal of Nutrition* 57 (1): 1–24.

19 Scholz-Ahrens, K.E. and Schrezenmeir, J. (2007). Inulin and oligofructose and mineral metabolism: the evidence from animal trials. *The Journal of Nutrition* 137 (11 Suppl): 2513S–2523S.

20 Wang, X. and Gibson, G.R. (1993). Effects of the *in vitro* fermentation of oligofructose and inulin by bacteria growing in the human large intestine. *The Journal of Applied Bacteriology* 75 (4): 373–380.

21 Gaskins, H.R., Mackie, R.I., May, T., and Garleb, K.A. (1996). Dietary fructo-oligosaccharide modulates large intestinal inflammatory responses to *Clostridium difficile* in antibiotic-compromised mice. *Microbial Ecology in Health and Disease* 9: 157–166.

22 Markowiak, P. and Śliżewska, K. (2017). Effects of probiotics, prebiotics, and synbiotics on human health. *Nutrients* 9 (9): 1021.

23 Doran, A.L. and Verran, J. (2007). A clinical study on the effect of the prebiotic inulin in the control of oral malodour. *Microbial Ecology in Health and Disease* 19 (3): 158–163.

24 Anderson, J.W., Baird, P., Davis, R.H. Jr., et al. (2009). Health benefits of dietary fiber. *Nutrition Reviews* 67: 188–205.

25 Furrie, E., Macfarlane, S., Kennedy, A. et al. (2005). Synbiotic therapy (*Bifidobacterium longum*/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. *Gut* 54 (2): 242–249.

26 Davani-Davari, D., Negahdaripour, M., Karimzadeh, I. et al. (2019). Prebiotics: definition, types, sources, mechanisms, and clinical applications. *Foods (Basel, Switzerland)* 8 (3): 92.

27 Ramirez-Farias, C., Slezak, K., Fuller, Z. et al. (2009). Effect of inulin on the human gut microbiota: stimulation of *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii*. *The British Journal of Nutrition* 101 (4): 541–550.

28 Oliveira, R.P.D.S., Perego, P., Oliveira, M.N.D., and Converti, A. (2011). Effect of inulin as prebiotic and synbiotic interactions between probiotics to improve fermented milk firmness. *Journal of Food Engineering* 107 (1): 36–40.

29 Kelly-Quagliana, K.A., Nelson, P.D., and Buddington, R.K. (2003). Dietary oligofructose and inulin modulate immune functions in mice. *Nutrition Research* 23 (2): 257–267.

30 Benyacoub, J., Rochat, F., Saudan, K.Y. et al. (2008). Feeding a diet containing a fructooligosaccharide mix can enhance *Salmonella* vaccine efficacy in mice. *Journal of Nutrition* 138 (1): 123–129.

31 Griffin, I.J., Hicks, P.M.D., Heaney, R.P., and Abrams, S.A. (2003). Enriched chicory inulin increases calcium absorption mainly in girls with lower calcium absorption. *Nutrition Research* 23 (7): 901–909.

32 Roberfroid, M.B. (2007). Inulin-type fructans: functional food ingredients. *The Journal of Nutrition* 137 (11): 2493S–2502S.

33 Tahiri, M., Tressol, J.C., Arnaud, Y. et al. (2003). Effect of short-chain fructooligosaccharides on intestinal calcium absorption and calcium status in postmenopausal women: a stable-isotope study. *American Journal of Clinical Nutrition* 77 (2): 449–457.

34 Coudray, C., Tressol, J.C., Gueux, E., and Rayssiguier, Y. (2003). Effects of inulin-type fructans of different chain length and type of branching on intestinal absorption and balance of calcium and magnesium in rats. *European Journal of Nutrition* 42 (2): 91–98.

35 Yasuda, K., Roneker, K.R., Miller, D.D. et al. (2006). Supplemental dietary inulin affects the bioavailability of iron in corn and soybean meal to young pigs. *The Journal of Nutrition* 136 (12): 3033–3038.

36 Ooi, L.G. and Liong, M.T. (2010). Cholesterol-lowering effects of probiotics and prebiotics: a review of *in vivo* and *in vitro* findings. *International Journal of Molecular Sciences* 11 (6): 2499–2522.

37 Mistry, R.H., Gu, F., Schols, H.A. et al. (2018). Effect of the prebiotic fiber inulin on cholesterol metabolism in wildtype mice. *Scientific Reports* 8: 13238.

38 Guess, N.D., Dornhorst, A., Oliver, N. et al. (2015). A randomized controlled trial: the effect of inulin on weight management and ectopic fat in subjects with prediabetes. *Nutrition and Metabolism* 12: 36.

39 Nieto-Nieto, T.V., Wang, Y.X., Ozimek, L., and Chen, L. (2015). Inulin at low concentrations significantly improves the gelling properties of oat protein–A molecular mechanism study. *Food Hydrocolloids* 50: 116–127.

40 Foschia, M., Peressini, D., Sensidoni, A., and Brennan, C.S. (2013). The effects of dietary fibre addition on the quality of common cereal products. *Journal of Cereal Science* 58 (2): 216–227.

41 Meyer, D., Bayarri, S., Tárrega, A., and Costell, E. (2011). Inulin as texture modifier in dairy products. *Food Hydrocolloids* 25 (8): 1881–1890.

42 Krasaekoopt, W. and Watcharapoka, S. (2014). Effect of addition of inulin and galactooligosaccharide on the survival of microencapsulated probiotics in alginate beads coated with chitosan in simulated digestive system, yogurt and fruit juice. *LWT- Food Science and Technology* 57 (2): 761–766.

43 Glibowski, P. (2010). Effect of thermal and mechanical factors on rheological properties of high performance inulin gels and spreads. *Journal of Food Engineering* 99 (1): 106–113.

44 Aidoo, R.P., Afoakwa, E.O., and Dewettinck, K. (2014). Optimization of inulin and polydextrose mixtures as sucrose replacers during sugar-free chocolate

manufacture–Rheological, microstructure and physical quality characteristics. *Journal of Food Engineering* 126: 35–42.

45 Eissens, A.C., Bolhuis, G.K., Hinrichs, W.L., and Frijlink, H.W. (2002). Inulin as filler-binder for tablets prepared by direct compaction. *European Journal of Pharmaceutical Sciences* 15 (1): 31–38.

46 Franck, A. (2002). Technological functionality of inulin and oligofructose. *The British Journal of Nutrition* 87 (Suppl 2): S287–S291.

47 Dahl, W.J., Whiting, S.J., Isaac, T.M. et al. (2005). Effects of thickened beverages fortified with inulin on beverage acceptance, gastrointestinal function, and bone resorption in institutionalized adults. *Nutrition (Burbank, Los Angeles County, Calif.)* 21 (3): 308–311.

48 Karimi, R., Azizi, M.H., Ghasemlou, M., and Vaziri, M. (2015). Application of inulin in cheese as prebiotic, fat replacer and texturizer: a review. *Carbohydrate Polymers* 119: 85–100.

49 Farzanmehr, H. and Abbasi, S. (2009). Effects of inulin and bulking agents on some physicochemical, textural and sensory properties of milk chocolate. *Journal of Texture Studies* 40: 536–553.

50 Causey, J.L., Feirtag, J.M., Gallaher, D.D. et al. (2000). Effects of dietary inulin on serum lipids, blood glucose and the gastrointestinal environment in hypercholesterolemic men. *Nutrition Research* 20 (2): 191–201.

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Abstract

Inulin is a nondigestible fiber derived from various plant sources. It is resistant to digestive enzymes and is digested by microbes in the large intestine as a result producing short-chain fatty acids (SCFAs). Inulin acts as a prebiotic agent in modulating the gut microbiome and providing various health benefits. Inulin was considered a nutraceutical agent for its extensive health benefits. It modulates the immune system, controls the progression of various tumors, increases mineral absorption, inhibits the growth of infectious agents, and improves lipid and sugar metabolism. Inulin is used in fortifying food products as a fiber source, as a prebiotic, as a fat, and as a sugar replacement in food products. In this chapter, we review the biochemical nature, significance, health benefits, and industrial applications of inulin, as documented in the scientific literature.

Keywords

inulin; prebiotic; gut microbiome; health; food applications

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اُردو غنچہ - 1

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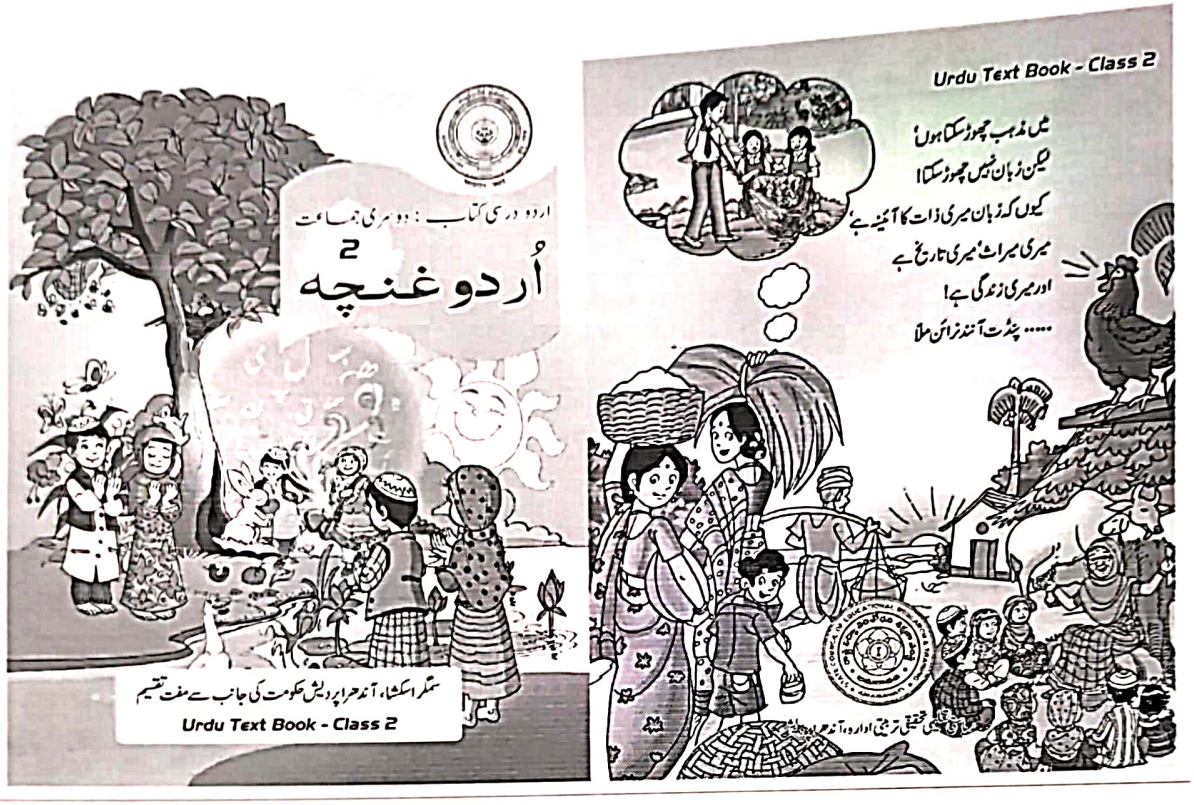

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اُردو غنچہ - 5

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