Marine Biochemistry
Applications

Edited by

Se-Kwon Kim

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Marine Biochemistry

This book provides the latest comprehensive methods for isolation and other novel techniques for marine product development. Furthermore, this book offers knowledge on the biological, medical, and industrial applications of marine-derived medicinal food substances.

There has been a tremendous increase in the products derived from marine organisms for commercial application in industries every year. Functional foods of medicinal value are particularly in demand as new technology allows the stabilization of natural ingredients and their availability in pure forms to solve various human diseases. Marine flora and fauna have essential elements and trace minerals that nurture various hormones produced in the endocrine system to regulate the respective metabolisms, thereby providing a safe and healthy life to humans.

The overall presentation and clear demarcation of the contents by worldwide contributions is a novel entry point into the market of medicinal foods from the sea. The exploration of marine habitats for novel materials are discussed throughout the book.

The exploration and exploitation of the biochemistry of sea flora and fauna are limited, and this book extends the research possibilities into numerous marine habitats.

Various approaches for extracting and applying the flora and fauna are discussed. This book will be of value to researchers, marine biotechnologists, and medical practitioners, due to the vast information, as well as industrial and medical applications of marine substances all in one place.

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Contents

Preface

Over 70% of the earth is covered by water. The ocean is a habitat for several living organisms. The current book will provide an overview of these species, including their biochemistry, culture, and application, to better understand their use.

Furthermore, several tons of seaweed are grown for commercial purposes all over the world. Moreover, seaweed is an essential resource for a country's economic growth, and the diversity and structure of seaweeds and their applications are discussed. In addition, there is the discussion of marine-derived carbohydrates, alginate, chondroitin sulfate, astaxanthin, fucoxanthin, ulvan, and lipids.

The rising global temperature poses a severe threat to humanity. Some of the chapters indirectly aid in resolving these problems such as seaweeds, sustainable resources for food packaging, toxic issues with marine products of animals, and the transformation of marine products derived from marine-derived organisms. It is shown how marine-derived fungi can catalyze the reduction of organic compounds.

This book will be helpful to novice students, graduate students, marine biologists, those working in biotechnology, and industrialists. I am grateful to all of the people who contributed to this book.

Se-Kwon Kim

Acknowledgments

Dr. Jae-Chul Kim is the chairman, president, and founder of the Dongwon group. The company was established in 1969 to explore and utilize the oceans and marine resources. Chairman Kim is the pioneer in the deep-sea fishing boats in South Korea. He started tuna fishing in 1958 as the first mate of Korea's first deep-sea fishing vessel.

After graduating from National Fisheries University of Busan, he jumped into tuna fishing, and the university was renamed Pukyong National University. Further, he became the captain of a fleet of deep-sea fishing boats and a new tuna fishing method. He developed and succeeded in catching large amounts of tuna in the South Pacific and Indian Oceans.

He founded Dongwon Industries in 1969 at the age of 35 and started tuna fishing as the first president. After that, he built a tuna processing plant and started to produce canned food. He immersed himself in business management in earnest based on the experience he learned in the sea from his youth.

Dongwon Group has successfully expanded its business from the fishery industry as the primary industry to the manufacturing industry as the secondary industry, and financial services as the tertiary industry. Currently, Mr. Kim runs 30 affiliated companies.

To contribute to social welfare, he also established the Dongwon Educational Foundation. He devoted himself, his heart, and his soul to nurturing competent people who are the backbone of our society. He has provided scholarships to numerous college students and grants R&D funds for researchers. He recognized the great value and potential of underutilized marine resources from his youth. He dedicated himself to publishing technical books emphasizing the scientific importance of marine life and related research.

With his help, this book has been published, providing readers with how the scientific values of marine life can enhance human health and wellbeing.

I want to thank him sincerely for his support in publishing this book.

Se-Kwon Kim
Distinguished Professor
Dept. of Marine Science and Convergence Engineering
Hanyang University ERICA, South Korea

Editor

Se-Kwon Kim, PhD, is a distinguished professor at Hanyang University and Kolmar Korea Company. He worked as a distinguished professor in the Department of Marine Bio Convergence Science and Technology and director of Marine Bioprocess Research Center (MBPRC) at Pukyong National University, Busan, South Korea.

He earned an MSc and a PhD at Pukyong National University and conducted his postdoctoral studies at the Laboratory of Biochemical Engineering, University of Illinois, Urbana-Champaign, Illinois, USA. Later, he became a visiting scientist at the Memorial University of Newfoundland and University of British Colombia in Canada.

Dr. Kim served as president of the Korean Society of Chitin and Chitosan from 1986 to 1990 and the Korean Society of Marine Biotechnology from 2006 to 2007. As an acknowledgment of his research, he won the best paper award from the American Oil Chemists' Society in 2002. Dr. Kim was also the chairman for the Seventh Asia-Pacific Chitin and Chitosan Symposium, which was held in South Korea in 2006. He was the chief editor of the Korean Society's *Fisheries and Aquatic Science* from 2008 to 2009. In addition, he is a board member of the International Society of Marine Biotechnology Associations (IMBA) and International Society of Nutraceuticals and Functional Food (ISNFF).

His major research interests are investigation and development of bioactive substances from marine resources. His immense experience of marine bio-processing and mass-production technologies for the marine bioindustry is the key asset of holding majorly funded marine bio projects in Korea. Furthermore, he expanded his research field to the development of bioactive materials from marine organisms for their applications in oriental medicine, cosmeceuticals, and nutraceuticals. To date, he has authored more than 750 research papers, 70 books, and 120 patents.

Contributors

Bakrudeen Ali Ahmed Abdul
Department of Biochemistry
PRIST Deemed University
Thanjavur, India

Snezana Agatonovic-Kustrin
A.P. Arzamastsev Department of Pharmaceutical and Toxicological Chemistry
I.M. Sechenov
First Moscow State Medical University
Sechenov University
Moscow, Russian Federation

Ahmad Homaei
Department of Biology
Faculty of Marine Science and Technology
University of Hormozgan
Bandar Abbas, Iran

Hari Eko Irianto
Research and Development Center for Marine and Fisheries Product Processing and Biotechnology
and
Sahid University
Jakarta, Indonesia

Sougata Jana
Department of Pharmaceutics
Gupta College of Technological Sciences
Asansol, India
and
Department of Health and Family Welfare
Directorate of Health Services
Kolkata, India

Kannan Kamala
Department of Pharmacology
Saveetha Dental College and Hospitals
Saveetha Institute of Medical and Technical Sciences
Chennai, India

Hui-Jing Li
Weihai Marine Organism and Medical Technology Research Institute
Harbin Institute of Technology
Weihai, China

Kenji Nakajima
Laboratory of Biochemistry
Department of Nutrition
Graduate School of Koshien University
Takarazuka, Japan

Ngo Dang Nghia
Nha Trang University
Nha Trang, Vietnam

Nidhi Pareek
Department of Microbiology
School of Life Sciences
Central University of Rajasthan
Ajmer, India

Mauro Sérgio Gonçalves Pavão
Laboratory of Biochemistry and Cellular Biology of Glycoconjugates
Program of Glycobiology
Institute of Medical Biochemistry
Federal University of Rio de Janeiro
Rio de Janeiro, Brazil

Suman Samaddar
Research Institute BGS Global Institute of Medical Sciences
Bangalore, India

I. Wickramasinghe
Department of Food Science and Technology
Faculty of Applied Sciences
University of Sri Jayewardenepura
Nugegoda, Sri Lanka

Morteza Yousefzadi
Department of Marine Biology
Faculty of Marine Science and Technology
University of Hormozgan
Bandar Abbas, Iran

10 Marine Chondroitin Sulfate and Its Potential Applications

Hari Eko Irianto and Giyatmi

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CONTENTS

10.1 INTRODUCTION

Over the past few decades, various types of diseases have emerged in the world, including degenerative diseases, infectious diseases and diseases caused by improper behavior. At the beginning of 2020, the world community was shocked by the COVID-19 pandemic that hit almost all countries regardless of developed, developing and poor status. In addition, degenerative diseases are still a problem, such as diabetes mellitus, stroke, coronary heart disease, cardiovascular disease, obesity, dyslipidemia, osteoporosis, prostatitis and osteoarthritis (Suiraoka, 2012). Pharmacists and herbalists as well as scientists from other relevant fields including medical experts and marine biotechnologists are working hard to explore bioactive compounds from terrestrial and marine resources and then develop them into affordable, effective and efficient drugs to treat those diseases.

One of the bioactive compounds, i.e. marine chondroitin sulfate, has attracted the attention of scientists from various backgrounds to study individually or collaboratively. Chondroitin sulfate is a naturally occurring biomolecule that can be found widely in almost all invertebrates and vertebrates, including humans, and the many biological processes that involve it (Volpi, 2009). Chondroitin sulfate is a supplement that can help delay the course of osteoarthritis while also reducing inflammation and discomfort. Joint function improves as a result of this. Chondroitin sulfate is frequently combined with glucosamine. The prevalence of osteoarthritis in various areas, growing awareness towards joint health, development of innovative chondroitin sulfate combination products, etc., has a favorable influence on the growth of the global chondroitin sulfate market. Religious and cultural barriers to chondroitin sulfate usage, particularly in Middle Eastern nations, are some of the reasons restricting the worldwide chondroitin sulfate market's growth (Transparency Market Research, 2017), especially chondroitin obtained from non-

resources and then develop them into affordable, effective and efficient drugs to treat those diseases.

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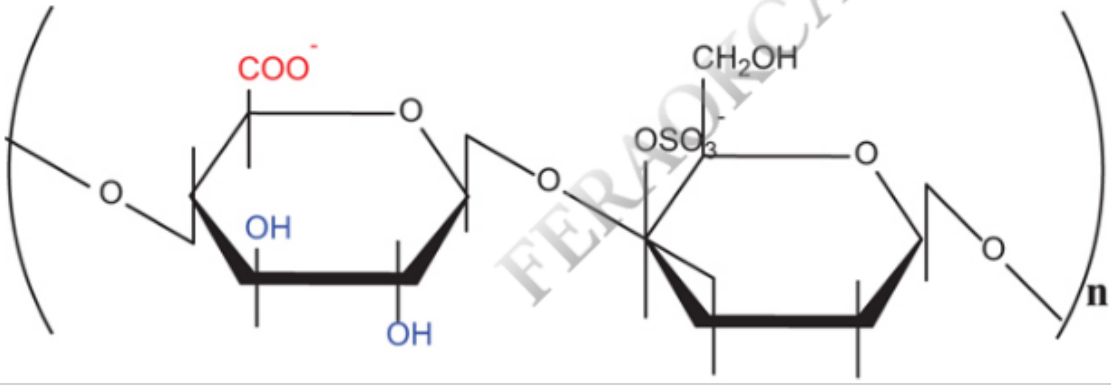
In 2020, the global chondroitin sulfate market was valued at US$1.17 billion and is expected to grow at a compound annual growth rate (CAGR) of 3.0% from 2020 to 2028. The expected increase in demand for nutritional products and prevalence of osteoarthritis are driving the market growth. Nutraceuticals dominated the market in 2020, accounting for more than 36.0% of worldwide sales. In the nutraceutical sector, sodium chondroitin sulfate is commonly used as a dietary supplement for the treatment of osteoarthritis and joint discomfort. Sodium chondroitin sulfate is also utilized as thickeners, additives, forming agents and preservatives in nutritional supplements and food items, as well as in health food and animal feed (Grand View Research, 2021). The rising acceptance of nutraceutical products that meet important nutritional needs, as well as the availability of new chondroitin sulfate compositions, can be linked to the rise of the chondroitin sulfate market. Furthermore, growing incidence and prevalence of osteoarthritis are expected to add to the worldwide chondroitin sulfate market's growth. By age 85, an estimated 40% of Americans would have symptoms of osteoarthritis in at least one hand. Furthermore, among men and women aged 45 years and over in the United States, the incidence of symptomatic knee osteoarthritis was 5.9% and 13.5%, as well as 7.2% and 18.7%, respectively (Medgadget, 2021).

Exploratory studies on the use of chondroitin sulfate have been carried out quite intensively so that future application opportunities may be wider, efficient, effective, efficacious and targeted. This chapter discusses potential applications of chondroitin sulfate for drug and nutraceutical purposes.

10.2 CHONDROITIN SULFATE

Chondroitin sulfate is a natural polymer that belongs to the glycosaminoglycans family of macromolecules with a high molecular weight (10,000–50,000 Da) that is a component of cartilage and connective tissue (Maccari et al., 2010; Konovalova et al., 2019; EC Huskisson, 2008). Glycosaminoglycans are polysaccharide molecules that are polymers of disaccharide units made up of various monosaccharides. The structure of glycosaminoglycan compounds, which is frequently dominated by disaccharide compounds, is more stressed in their categorization (Wikanta et al., 2002). There are several kinds of glycosaminoglycans, which are typically classified into four categories: (1) hyaluronic acid or hyaluronan, (2) keratan sulfate, (3) heparan sulfate/heparin and (4) chondroitin sulfate/dermatan sulfate (Krichen et al., 2018).



Da) that is a component of cartilage and connective tissue (Maccari et al., 2010; Konovalova et al., 2019; EC Huskisson, 2008). Glycosaminoglycans are polysaccharide molecules that are polymers of disaccharide units made up of various monosaccharides. The structure of glycosaminoglycan compounds, which is frequently dominated by disaccharide compounds, is more stressed in their categorization (Wikanta et al., 2002). There are several kinds of glycosaminoglycans, which are typically classified into four categories: (1) hyaluronic acid or hyaluronan, (2) keratan sulfate, (3) heparan sulfate/heparin and (4) chondroitin sulfate/dermatan sulfate (Krichen et al., 2018).

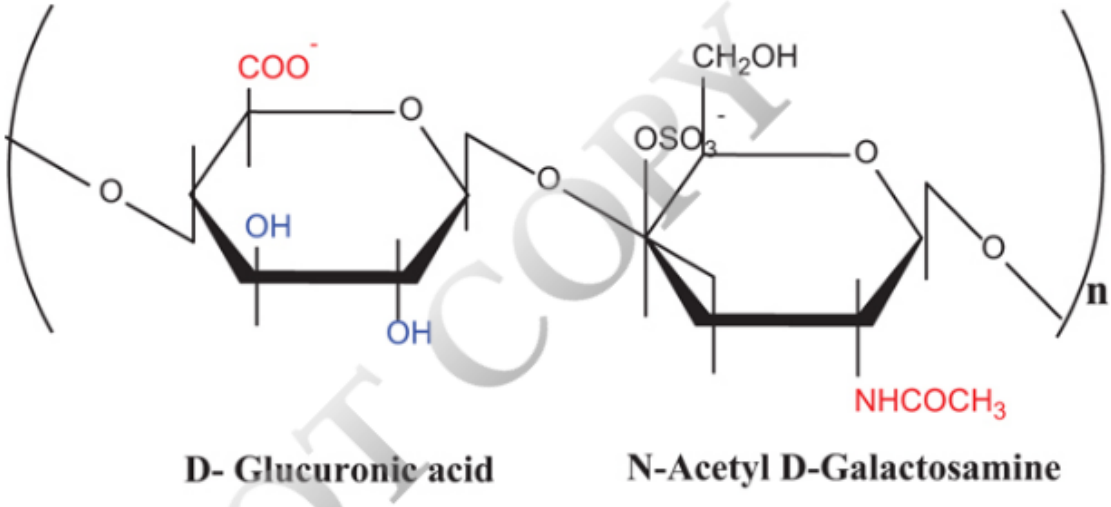


FIGURE 10.1 Chemical structure of chondroitin sulfate.

Chondroitin sulfate is a linear, complex, sulfated, polydispersity polysaccharide (Maccari et al., 2010). It is made up of glucuronic acid (GlcA) and N-acetylgalactosamine (GalNAc) repeating disaccharide units (Figure 10.1), with a sulfate group at position 4 or 6 of the GalNAc residue. Various amounts of repeated 4-sulfate and 6-sulfate disaccharides can be found in chondroitin sulfate chains having various sulfate group configurations at certain hydroxyl groups (Lee et al., 1998, Tamura et al., 2009)). In addition, chondroitin sulfate as a polysaccharide with a linking tetrasaccharide at the reducing terminal and a major moiety, called a repeating disaccharide region, belongs to the glycosaminoglycans (GAG) family. The fundamental component of the repeating disaccharide region is N-acetyl galactosaminyl glucuronic acid (βGalNAc-βGlcA), which is complemented by a distinct pattern of sulfate groups in certain hydroxyl groups (Tamura et al., 2009).

There are four major kinds of chondroitin sulfate polysaccharides: (1) chondroitin sulfate A (CS-A), (2) chondroitin sulfate C (CS-C), (3) chondroitin sulfate D (CSD) and (4) chondroitin sulfate E (CS-E). Each subtype is distinguished by a unique sulfation pattern. CS-A has a GalNAc 4-O(oxygen)-sulfate residue, CS-C has a 6-Osulfate residue, CS-D has a 2-O sulfated GlcA residue, and CS-E has a GlcNAc 4,6-O-disulfate residue (Li et al., 2020).

10.3 MARINE SOURCES OF CHONDROITIN SULFATE

Chondroitin sulfate is produced in vertebrates and invertebrates as part of proteoglycan molecules (Lamari and Karamanos, 2006). According to the source, the global market of chondroitin sulfate, also known as sulfated glycosaminoglycan, is divided into bovine, porcine, poultry, shark and synthetic. In 2020, bovine dominated the market, accounting for more than 35.0% of worldwide sales. Sharks have been designated as an endangered species, posing a threat to the industry's future growth. Sodium chondroitin sulfate from sharks, on the other hand, is preferable. A two-stage fermentation-based technique is used to manufacture synthetic sodium chondroitin sulfate (Grand View Research, 2021). However, due to concerns over bovine spongiform encephalopathy (BSE) and other reasons, exploration of microorganisms and marine species as potential sources of chondroitin sulfate has been carried out. Potential producers of chondroitin sulfate include sponges, sea cucumbers, squids, mollusks, invertebrates and mostly cartilaginous debris from fish (shark, salmon, ray, etc.) (Vázquez et al., 2013). Marine sources of chondroitin sulfate have been explored by scientists around the world, as shown in Table 10.1.

Chondroitin sulfate has been extracted from shark cartilage and stingray bone by 2.37% and 1.47%, respectively (Hanindika et al., 2014). Another observation reported that chondroitin sulfate in shark fin and ray cartilage were 15.05

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Chondroitin sulfate has been extracted from shark cartilage and stingray bone by 2.37% and 1.47%, respectively (Hanindika et al., 2014). Another observation reported that chondroitin sulfate in shark fin and ray cartilage were 15.05 and 7.49% accordingly. Fourier transform infrared spectroscopy (FTIR) of the potassium bromide pellet technique revealed that the spectrum of dry chondroitin sulfate samples extracted from various cartilage sources including shark fin and ray cartilage showed peaks at 857 and 826 cm^{-1} wave numbers, which were used to identify chondroitin-4-sulfate and chondroitin-6-sulfate each. Thus, the spectra of all extracts showed that the two cartilage samples consisted of chondroitin-4-sulfate and chondroitin-6-sulfate (Garnjanagoonchorn et al., 2007). Research on spotted dogfish (*Scyliorhinus canicula*) exhibited that head waste is the best source of chondroitin sulfate production compared to skeleton and fins (Blanco et al., 2015). Chondroitin sulfate extracted from skate (*Raja clavata*) (rays also known as skates) was 15% (w/w) yield (Murado et al., 2010).

Fucosylated chondroitin sulfates were identified in sea cucumbers, particularly *Stichopus tremulus* (Western Indian Ocean), *Pearsonothuria graeffei* (Indo-Pacific), *Isostichopus badionotus* (Western Atlantic), and *Holothuria vagabunda* (Norwegian coast). Fucosylated chondroitin sulfate is a structurally unique glycosaminoglycan discovered in the body wall of sea cucumbers, with a chondroitin sulfate backbone and connected fucose sulfate or non-sulfate side chains. This molecule has a broad range of biological action and plays a vital function in keeping the integrity of the body wall. The polysaccharides isolated from *S. tremulus*, *P. graeffei*, *I. badionotus*, and *H. vagabunda* were 7.0%, 11.0%, 9.9% and 6.3% by weight, respectively. *S. tremulus*, *P. graeffei* and *H. vagabunda* had higher levels of fucosylated chondroitin sulfates, whereas *I. badionotus* had more abundant fucan (Chen et al., 2011; Myron et al., 2014). Fucosylated chondroitin sulfates have also been isolated from *Acaudina molpadioides* (Hu et al., 2014), *Holothuria forskali* (Panagos et al., 2014), *Paracaudina chilensis* and *Holothuria hilla* (Ustyuzhanina et al., 2020).

Preparation of chondroitin sulfate from salmon nasal cartilage was also carried out by Takeda et al. (1998), Han et al. (2000), Kobayashia et al. (2017), and

Tatara et al. (2015). Cartilage has the main extracellular component in the form of the proteoglycan chondroitin sulfate (Kobayashia et al., 2017). Chondroitin sulfate is reported to be commercially produced by enzymatication of salmon nasal cartilage (Goto et al., 2011).

TABLE 10.1

Exploration of Marine Chondroitin Sulfate Sources

Marine Biota		Organ Parts	References
Shark	Silky shark (*Carcharinus falciformes*)	Fin bone	Hanindika et al. (2014)
	Black-shark (Galeus melastomus)	Cartilage	Konovalova et al.)
	Spotted dogfish (*Scyliorhinus canicula*)	Head, Skeleton and Fins	Blanco et al. (2015)
	Shark	Cartilage, by-products from shark fin soup restaurant	Garnjanagoonchorn et al. (2007)
	Blacktip shark (*Carcharhinus limbatus*)	Cartilage	Wikanta et al. (2000)
Ray	Stingray (*Raja* sp.)	Cartilage	Hanindika et al.)
	Paleedged/Sharpnose Stingray (*Dasyatis zugei*)	Cartilage	Garnjanagoonchorn et al. (2007)
	Northern stingray (*Raja hyperborean*)	Cartilage	Konovalova et al.)
	Skate (*Raja clavata*)	Cartilage	Murado et al.)
Sea cucumber	*Stichopus tremulus* *Pearsonothuria graeffei* *Isostichopus badionotus* *Holothuria vagabunda*	Body wall Body wall Body wall Body wall	Chen et al. (2011) Chen et al. (2011) Chen et al. (2011) Chen et al. (2011)
	Acaudina molpadioides	Body wall	Hu et al. (2014)
	Holothuria forskali	Body wall	Panagos et al. (2014)
	Paracaudina chilensis *Holothuria hilla*	Body wall Body wall	Ustyuzhanina et al. (2020) Ustyuzhanina et al. (2020),
Cuttlefish	Pharaoh cuttlefish (*Sepia pharaonis*)	Cuttlebone	Hanindika et al. (2014)
Squid	Diamond squid (*Thysanoteuthis rhombus*)	fins, arms, skin, head, eyes, and mantle	Tamura et al.)
Salmon	Atlantic salmon (*Salmo salar*)	Cartilaginous tissue	Konovalova et al.)
	Salmon (no scientific names)	Nasal cartilage	Han et al. (2000); Kobayashia et al. (2017); Tatara et al. (2015); Goto et al. (2011); Takeda et al. (1998)
Tuna	Bluefin tuna (*Thunnus thynnus*)	Skin	Krichen et al. (2018)
Sturgeon	Sturgeon (no scientific name)	Bone	Maccari et al. (2010)
	Pomacea sp.	Tissues	Nader et al. (1984)

Mollusca	*Tagelus gibbus*,	Tissues	Nader et al. (1984).
	Anomalocardia brasiliana	Tissues	Nader et al. (1984).
	Mud snail (*Cipangopaludina chinensis*)	Tissues	Lee et al. (1998)
	Clams (*Anodonta anodonta*)	Body tissues	Volpi and Maccari (2005)
Sponges	*Polymastia janeirensis*,	Sponge tissues	Maia et al. (2016)
	Echinodictyum dendroides,	Sponge tissues	Maia et al. (2016)
	Dragmacidon reticulatum	Sponge tissues	Maia et al.)
Crocodile	no scientific name	Cartilage (trachea, hyoid, sternum and rib)	Garnjanagoonchorn et al. (2007)

Chondroitin sulfate was also isolated from sturgeon bone by extracting a single polysaccharide from the bone at a concentration of 0.28–0.34% for dry tissue and identifying it as chondroitin sulfate. These polymers were found to include 55% monosulfate disaccharides at position 6 GalNAc, 38% monosulfate disaccharides at position 4 GalNAc, and 7% unsulfate disaccharides after specific chondroitinase and high performance liquid chromatography (HPLC) separation of the resulting repeat unsaturated disaccharides. As a bony fish, sturgeon can be a source of chondroitin sulfate, even though it is usually discarded following ovary retrieval (Maccari et al., 2010), while the skin of bluefin tuna (*Thunnus thynnus*) was used as raw material to produce chondroitin sulfate (Krichen et al., 2018).

Chondroitin sulfate has been extracted from various tissues of diamond squid (*Thysanoteuthis rhombus*). The non-edible skin, head and eyes of diamond squid can be used as an alternative source of chondroitin sulfate with the appropriate sulfate concentration. Approximately 14 kg of diamond squid having 380 g skin, 780 g head and 290 g eyes (wet weight) obtained 238, 386 and 47 mg of pure chondroitin sulfate from these tissues, respectively (Tamura et al., 2009).

The use of mollusk species as source of chondroitin sulfate is possible. Chondroitin sulfate has already been isolated from three mollusk species, including *Pomacea* sp., *Tagelus gibbus* and *Anomalocardia brasilianu*. Bivalves (*Tagelus* and *Anomalocardia*) and gastropods (*Pomacea* sp.) have different chondroitin sulfate structures. Chondroitin sulfate from *Pomaces*, for example, has no disaccharide 6-sulfate or disulfate (Nader et al., 1984). Chondroitin sulfate extracted from mud snails (*Cipangopaludina chinensis*) contain chondroitin 4-, 6- and O- sulfates (Lee et al., 1998). Glycosaminoglycans from the bodies of giant freshwater clams *Anodonta anodonta* are detected in around 0.6 mg/g dry tissue and are made up of chondroitin sulfate (about 38%), non-sulfate chondroitin (about 21%), and heparin (about 41%) (Volpi and Maccari, 2005).

Chondroitin sulfates are also isolated from marine sponges, such as *Polymastia janeirensis, Echinodictyum dendroides, and Dragmacidon reticulatum* (Maia et al., 2016), while chondroitin sulfate can be extracted from a nematode of *Caenorhabditis elegans* as well (Dierker et al., 2016).

10.4 EXTRACTION OF MARINE CHONDROITIN SULFATE

Basically, the extraction techniques developed for chondroitin sulfate rely on chemical hydrolysis of the tissue to break the proteoglycan core, followed by protein removal to recover the glycosaminoglycans (Abdallaha et al., 2020). Modern chondroitin sulfate production methods are multi-stage extraction procedures. Defatting of raw materials, alkaline and enzymatic hydrolysis, sedimentation of chondroitin sulfate from solution, further purification of the preparation and drying are the key steps of chondroitin sulfate production (Konovalova et al., 2019). Currently, there are three techniques for extracting chondroitin sulfate, i.e. alkali, enzyme and ultrasonic. The alkali technique was easy; however, the high concentration of alkali caused chondroitin sulfate breakdown, reducing its biological activity. The enzyme technique considerably increased chondroitin sulfate purity, but the yield was about the same as the alkali method, with the drawbacks of high enzyme cost and long extraction time. When compared to the alkali and enzyme methods, the ultrasonic technique considerably extended the extraction time, but the yield and purity of chondroitin sulfate were not significantly improved (He et al., 2014; Syed et al., 2017).

Improvements in extraction methods through a combination of enzymatic and chemical hydrolysis, selective

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Improvements in extraction methods through a combination of enzymatic and chemical hydrolysis, selective precipitation, and membrane technology resulted in the development of a fast and highly efficient method (15% w/w) with minimal use of reagents and high purity for the resulting chondroitin sulfate (I = 99%) (Murado et al., 2010). The following describes the methods that have been applied experimentally to the extraction of chondroitin sulfate from mollusks and cartilage.

The extraction method employed to extract chondroitin sulfate from mud snail was first by removing the shell and then three days defatting with acetone. Defatted snail powder (4 g) was dissolved in 40 ml of 0.05 M Na_2CO_3 (pH 9.2) buffer, added 2 ml subtilisin and stirred 200 rpm at 60°C for 2 days. The mixture was cooled to 4°C, added 5% trichloroacetic acid and centrifuged at 8000xg for 20 minutes. The supernatant was added with three times volume of 5% potassium acetate with ethanol, kept overnight at 4°C and then centrifuge at 10000xg for 30 minutes. The precipitate was washed with alcohol, dissolved in 40 ml of 0.2 M NaCl and centrifuged at 8000xg for 30 minutes. The supernatant was mixed with 0.5 ml of 5% cetylpyridinium chloride and centrifuged at 8000xg for 15 minutes. Finally, the precipitate was dissolved in 10 ml of 2.5 M NaCl, added with five volume ethanol and centrifuged at 10000xg for 30 minutes (Lee et al., 1998).

In addition, the method used to isolate chondroitin sulfate from fish cartilages, particularly cuttlebone, ray and shark cartilages, were by soaking the raw material in papain solution for 24 hours to free the remaining muscle tissue and then dried. The cartilage is ground and added with distilled water and acetic acid to maintain acidity at pH 4.5. After warming the mixture in an oven at 37°C for 7 hours, it was filtered using filter paper and the obtained solution was centrifuged. The resulting supernatant was added with 3% w/v cetylpyridinium chloride in 0.8 M NaCl, then put in the freezer for 10 minutes and centrifuged at 5,000 rpm for 30 minutes. Subsequently, 2 M NaCl solution was added and centrifuged again at 5,000 rpm for 30 minutes. The supernatant obtained was added with methanol, and centrifuged at 5,000 rpm for 15 minutes at 4°C. The precipitate was added with 95% ethanol and centrifuged at 3,000 rpm for 15 minutes. The precipitate was removed and then dried at room temperature (Hanindika et al., 2014). A similar method was employed with chondroitin drying using freeze drier and the yield was 6.06% (Sulityowati et al., 2015).

Optimal chondroitin sulfate recovery and purification from cartilage waste of *S. canicula* was obtained at 58°C and pH 8.5 for enzymatic hydrolysis, and 0.53–0.64 M NaOH and 1.14–1.20 volumes of ethanol for chemical treatment. Furthermore, head wastes were discovered to be the most prospective source of chondroitin sulfate synthesis from *S. canicula*. The ultrafiltration and diafiltration procedure was used to prepare extracts from the alkaline hydroalcoholic treatment at 30 kDa molecular weight cut-off for differential retention of chondroitin sulfate and concurrent rejection of protein components (Blanco et al., 2015).

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The process incorporating biological enzymolysis, mixed microbial fermentation and contemporary biological separation has been used to extract chondroitin sulfate from shark cartilage. The biological enzymolysis technology employed the enzyme compound system consisting of one or more combinations of papain, trypsin, neutral protease, pepsin, and flavorzyme. Fermented strains for mixed microbial fermentation include mostly *Aspergillus oryzae*, *Bacillus psychrosaccharolyticus* and *Bacillus subtilis* (Xuan et al., 2020).

An available review informs that enzymes that have been employed for chondroitin sulfate, including alcalase, papain, neutrase, bromelain, acid protease, actinase and pepsin (Abdallaha et al., 2020). Alcalase is a well-known endoprotease that has a high capacity to hydrolyze a variety of marine substrates (Vázquez et al., 2018).

Chondroitin sulfate quality can be improved by employing protein isoelectric point. After washing and steam boiling the cartilage for extraction, NaOH and NaCl are added to the mixture. For the enzymatic procedure, pancreatin was added, followed by deproteination and concentration. Primary sedimentation and pH value adjustment were used to eliminate impurity protein, and secondary sedimentation was used to get chondroitin sulfate crude product. After initial sedimentation, precise pH correction is required, followed by removing impurity proteins mixed in chondroitin sulfate. The chondroitin sulfate obtained has a purity of above 97% (Nanjing University of Science and Technology, 2008).

In recent years, high-intensity pulsed electric fields (PEF) technology has emerged as a growing focus of worldwide, where it has the potential to be applied to the production of chondroitin sulfate. PEF technology is a very effective method for preserving and processing a wide range of food products without significantly compromising their quality features (Syed et al., 2017). As the electrolyte, the material is placed in a chamber. To operate on the material, instantaneous pressure was produced between the two electrodes (Yin et al., 2006). PEF is widely used to extract active chemicals from natural products because of its non-thermal performance, speed, efficiency, low power consumption and low pollution. The PEF extraction system has been designed with a triangular pulse power waveform, an oscilloscope used to directly view the output voltage, a 40–3000 Hz adjustable frequency and a 70 mL processing pipe volume (He et al., 2014).

10.5 QUALITY AND SAFETY

The quality of chondroitin sulfate is influenced by the processing techniques applied to produce it. Different extraction and purification processes may bring about different consequences on the structural characteristics and properties of chondroitin sulfate, resulting in extracts with varying degrees of purity, limited biological effects, contaminants causing safety and reproducibility issues and unknown origin (Volpi, 2019). Of course, those aspects can have serious implications for consumers of end products, including pharmaceuticals, dietary supplements and nutraceuticals related to the traceability of chondroitin sulfate and the statement of the true origin of the active compounds and its content.

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Symptomatic medicines for osteoarthritis such as acetaminophen and nonsteroidal anti-inflammatory drugs (NSAIDs) are only effective in alleviating symptoms. Unfortunately, these drugs can have serious adverse effects in certain people and are sometimes contraindicated (Utami et al., 2012). Chondroitin sulfate, alone or in combination with glucosamine, has been employed for the treatment of osteoarthritis that is better tolerated at the gastrointestinal, cardiovascular, and renal levels than NSAIDs and cyclooxygenase 2 inhibitors (COXIBs) (Rubio-Terrés et al., 2020). One of its primary advantages for the treatment of aging patients with comorbidities is its safety profile. If the financial implications are ignored, there is no limit to its use in osteoarthritis patients. However, caution should be applied while selecting the kind and formulation of chondroitin sulfate (Henrotin et al., 2010).

The LD-50 of chondroitin sulfate extracted from shark cartilage using mice intraperitoneally is 60.8 mg/20 g body weight and after extrapolating to mice orally, the LD-50 value is 21.3 g/kg body weight which can be classified as a "Practically Non-Toxic" compound, according to the table for classification of toxic properties (Lestari et al., 2000). In addition, Chondroitin along with glucosamine for osteoarthritis drugs is reported to have a high safety profile (Narvy and Vangsness, 2010).

However, the administration level of chondroitin sulfate for therapeutic purposes should be of concern. The frequency of side effect incidences in arthritis patients reported in the consumption range of 800–1,200 milligrams per day (mg/day) was in most cases equivalent to that of the placebo group, which was not administered the drug. The most common adverse effects in the chondroitin sulfate group were gastrointestinal adverse effects, which occurred at a lower incidence than in the placebo group (BfR, 2018).

Regarding commercial products, only 5 of the 16 chondroitin sulfate samples from throughout the world had more than 90% chondroitin sulfate and matched the label. Maltodextrin or lactose were the predominant impurities in the remaining 11 samples with poor chondroitin sulfate. Those chondroitin sulfate preparations originate from various sources, and they all obviously fail to fulfill label requirements. As a result, more efficient and strict standards for both licensing and quality control of raw ingredients, as well as the final pharmacological formulations derived from them, should be implemented (Cunhaa et al., 2015). Because some nutraceuticals have poor chondroitin sulfate quality, stricter quality control regulations should be implemented to ensure the manufacture of high-quality products for nutraceutical use and to protect customers from low-quality, ineffective and potentially dangerous products (Volpi, 2009).

For evaluating the quality of chondroitin sulfate raw materials used in nutritional supplements, the capillary isotachophoretic (cITP) technique is recommended as an alternative to HPLC or CZE (capillary zone electrophoresis). Estimation of chondroitin sulfate origin, estimation of sulfatation degree of chondroitin sulfate and its homogeneity, estimation of chondroitin sulfate molecular mass and molecular weight dispersion, determination of chondroitin sulfate purity and detection of chondroitin sulfate degradation products are all possible with the cITP method

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10.6 POTENTIAL APPLICATIONS OF MARINE CHONDROITIN SULFATE

Chondroitin sulfates can have varying chain lengths (Mr: 5,000–50,000) (Peter, 2012) or sulfation degrees depending on their origin, which has a significant impact on their biological characteristics. Dermatan sulfate is a chondroitin sulfate isomer. It also includes -L-iduronic acid, which is produced via epimerization, and N acetyl-D-galactosamine, which is mainly sulfated at position 4. It also includes -L-iduronic acid, which is produced via epimerization, and N acetyl–d-galactosamine, which is mainly sulfated at position 4 (Volpi, 2009). Chondroitin is hydrophilic and water soluble, forming a viscous fluid similar to sodium hyaluronate. Chondroitin sulfate is important for the structural and functional integrity of joints, as it is the major constituent of GAGs in articular cartilage. Chondroitin is known to help maintain joint viscosity, stimulate cartilage repair and inhibit enzymes that degrade cartilage (Zaki, 2013).

Chondroitin sulfate has important properties that influence its application, including biocompatible, anti-inflammatory, biodegradable, non-immunogenic and non-toxic (Abdallaha et al., 2020). Due to these properties, chondroitin sulfate has broad prospective applications in various fields.

10.6.1 Potential Applications for Therapeutic Agents

Chondroitin sulfate is a substance that is already present in the human body. This substance is believed to have a function to draw water and nutrients into human cartilage so that human cartilage remains healthy and supple (Joseph, 2021). The use of this substance is usually aimed at overcoming health problems as therapeutic agents such as pain in the joints and is also commonly used as an antithrombotic, an ischemic heart disease treatment, and an extravasation therapy agent along with hyperlipidemia (Archiando, 2020). The diversity of species and tissues causes chondroitin sulfate to have a heterogeneous structure and physico-chemical profile, which is responsible for the various and specific activities of this macromolecule (Volpi, 2019). The Indonesian Agency for Drug and Food Control through the decree of HK.00.05.23.3644 of 2004 stipulates that the maximum limit of chondroitin sulfate that can be consumed from dietary supplements is 1200 mg per day.

10.6.1.1 Osteoarthritis

Osteoarthritis is a degenerative illness that affects the elderly and is the most prevalent kind of arthritis. Affected people may have severe pain and functional impairment as a result of the condition. Treatments for osteoarthritis are easy to obtain, but the outcomes are uncertain and even controversial as for chondroitin sulfate and glucosamine (Utami et al., 2012; van Blitterswijk et al., 2003; Agiba, 2017; Fernández-Martín et al., 2021). The use of chondroitin sulfate to treat osteoarthritis has been around for a long time. Glucosamine and chondroitin sulfate should be useful in

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Most treatment algorithms do not account for the use of glucosamine and chondroitin sulfate, the long-standing outpatient adjuvant being acetaminophen, followed by NSAIDs (including the newer COX-2 inhibitors), steroid or hyaluronic injections, physical therapy and joint replacement (Schenck, 2000). Several studies suggested that nutraceutical supplementation of individuals with knee/hip osteoarthritis may improve pain severity and physical function (Aghamohammadi et al., 2020; Šimánek et al., 2005). Because osteoarthritis is a slow-progressing illness, evidence of disease change following an intervention may take years to appear (Agiba, 2017).

Chondroitin sulfate is reported as a promising substance for the treatment of osteoarthritis of the knee. Depending on the assessment technique (model, dose and duration) and the source of chondroitin sulfate (origin and quality), various findings have been reported. In mild knee osteoarthritis, clinical data suggests a slow-acting effect on symptoms (Henrotin et al., 2010). In the total group of individuals with osteoarthritis of the knee, however, glucosamine and chondroitin sulfate, alone or in combination, did not significantly decrease pain. According to preliminary research, glucosamine and chondroitin sulfate together may be helpful in the subgroup of individuals with moderate-to-severe knee discomfort (Clegg et al., 2006). Classified as slow-acting medicines, chondroitin sulfate and glucosamine sulfate relieve pain and partially restore joint function in osteoarthritis patients (Bottegoni et al., 2014).

Even in meta-analyses, glucosamine and chondroitin have shown mixed efficacy in reducing knee pain and increasing joint function associated with osteoarthritis, however there is some evidence to suggest that these treatments may help delay disease progression radiographically (Narvy and Vangsness, 2010). Chondroitin sulfate lowers the levels of pro-inflammatory cytokines and transcription factors that play a role in inflammation. By blocking hydrolytic enzymes and limiting the oxidation of lipids and proteins, glucosamine sulfate improves cartilage specific matrix components and inhibits collagen degradation in chondrocytes (Bottegoni et al., 2014).

In comparison to their individual effects, the combination of glucosamine and chondroitin sulfate may be less effective. The validity and mechanism of this new finding are unknown, although it might be linked to changes in glucosamine absorption. Satisfied radiographic criteria Grade 2 knees may represent a more potentially responsive population in future osteoarthritis trials evaluating structural modification; however, a larger sample size, longer study duration and/or improved methods of measurement will be required, as the rate of joint space width loss seen on plain radiographs is much slower than previously appreciated (Sawitzke et al., 2008). When compared to glucosamine-chondroitin sulfate and placebo, the combination of glucosamine–chondroitin sulfate–methylsufonylmethane (GCM) supplements was more effective in lowering pain and improving function in individuals with Kellgren Lawrence I-II knee osteoarthritis, while the glucosamine-chondroitin sulfate supplement was shown to be no better than a placebo in terms of clinical effectiveness (Siagian, 2014). In the symptomatic treatment of knee

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Although glucosamine and chondroitin as osteoarthritis therapy show results vary from patient to patient; simple fixes can be used in clinical settings. These medications can be used safely as an initial treatment for some osteoarthritis patients before beginning treatment with NSAIDs, acetaminophen, and other conventional medications (Narvy and Vangsness, 2010). Under mixed-mode lubrication conditions, chondroitin sulfate demonstrated to be an excellent cartilage lubricant. Supplemental chondroitin sulfate that diffused into the specimens, on the other hand, had no effect on cartilage fluid load support (Katta et al., 2009). Oral chondroitin in the prescribed dosage is more effective than placebo for pain relief and physical function. Glucosamine had a substantial influence on the result of stiffness when compared to placebo (Zhu et al., 2018).

Chondroitin sulfate patches are developed as an alternative to oral chondroitin sulfate which is more difficult to administer. With a partition value of 2.22, 150 mg of chondroitin sulfate in a transdermal patch provides continuous penetration in 19 hours (Sopyan et al., 2017). A topical cream combining glucosamine sulfate, chondroitin sulfate, and camphor is more effective than a placebo in reducing joint discomfort in individuals with knee osteoarthritis (Cohen et al., 2003).

It can be concluded that clinically, chondroitin sulfate can result in reduced pain and increased joint mobility in osteoarthritis patients and slows down joint damage (Zaki, 2013).

10.6.1.2 Gastric Ulcer

Gastric ulcer is one type of inflammatory disease that is often found both in adults and adolescents as a result of a decrease or damage to the resistance of the gastric mucosa. Treatment of gastric ulcers has shifted to cytoprotective drugs, namely drugs that can increase protection and resistance of the gastrointestinal mucosa, especially the stomach. Oral chondroitin sulfate showed a protective effect on the gastrointestinal mucosa of rats. The higher the chondroitin sulfate dosage, the greater the protective effect. A dosage of 4 g/kg body weight of chondroitin sulfate had the same excellent protective effect as sucralfate as a positive control at a level of 500 mg/kg body weight. Because chondroitin sulfate had a pH of 7.4 in the stomach of rats, it was an antacid or neutralized gastric acid (Wikanta et al., 2000).

10.6.1.3 Atherosclerosis

Atherosclerosis is one of the most prevalent illnesses in the elderly, generating fatty liver, renal failure, myocardial infarction, coronary heart disease and other cardiovascular disorders, and excessive cholesterol is one of the primary

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10.6.1.4 Cancer

Muscle-invasive bladder cancer (MIBC) was studied for treatment using chondroitin sulfate. When cisplatin-resistant MIBC overexpresses certain sugar chains compared to chemotherapy-naive MIBC, enhanced cellular expression of chondroitin sulfate was discovered in a study using a mouse model. This fact shows that targeting chondroitin sulfate with VDC886 drug conjugate is a promising strategy, especially in cisplatin-resistant MIBC. This discovery might pave the way for a new therapy paradigm for human MIBC patients who aren't responding to cisplatin (Seiler et al., 2017).

Chondroitin sulfate and/or glucosamine has been explored as a colorectal cancer (CRC) chemoprevention agent, but clear evidence of an independent preventive effect of chondroitin sulfate and/or glucosamine on CRC has not been obtained, as the observed effect could be associated with concomitant use of non-steroidal anti-inflammatory drugs (NSAIDs). Their low toxicity, however, warrants additional investigation into their impact and prospective function as chemopreventive drugs (Ibáñez-Sanz et al., 2018).

10.6.1.5 Inhibition of Mast Cells

Chondroitin sulfate appears to be a powerful mast cell inhibitor of both allergic and non-immune activation, with therapeutic consequences. Mast cells are responsible for the development of allergies and potential inflammatory responses and originate in the bone marrow. Chondroitin sulfate inhibited mast cell secretagogue compound 48/80 (48/80)–induced histamine production in rat peritoneal mast cells in a dose-dependent manner. Inhibition by chondroitin sulfate increased with pre-incubation time and remained after the medication was washed off, but cromolyn's impact was restricted by rapid tachyphylaxis. Histamine production from rat connective tissue mast cells (CTMCs) was also suppressed immunologically. Ultrastructural autoradiography reveals that chondroitin sulfate is mostly linked with the plasma and perigranular membrane (Theoharides et al., 2000).

10.6.1.6 Reconstructed Cornea

A porous collagen/glycosaminoglycan-based scaffold seeded with stromal keratocytes and then epithelial and endothelial cells was used to imitate the corneal extracellular matrix. The collagen-chondroitin sulfate scaffold is an excellent substrate for the creation of artificial corneas due to its capacity to sustain long-term culture, lifespan and

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10.6.1.7 Coating Composite Dental Implant

The chewing function of dental implants is based on osseointegration. Nerve growth factor (NGF) can stimulate bone healing as a neurotrophic agent. The impact of an NGF-chondroitin sulfate/hydroxyapatite (CS/HA)–coated composite implant on osseointegration and innervation is an essential factor to consider when using it. NGF-CS/HA coating has been demonstrated to significantly accelerate implant osseointegration and enhance peri-implant nerve regeneration in the mandible of beagle dogs. This might provide a scientific basis for using NGF-CS/HA–coated implants in oral implants (Ye et al., 2021).

10.6.2 Potential Application for Food Supplements

The European Medicines Agency regulates chondroitin sulfate as an anti-osteoarthritis prescription medication, whereas the U.S. Food and Drug Administration (FDA) classifies it as a dietary supplement, allowing U.S. customers more freedom to purchase and use chondroitin. Chondroitin sulfate is one of the most popular joint health supplements in the United States (You, 2021). A review based on available data prefers to classify glucosamine and chondroitin sulfate as food supplements rather than drugs (Šimánek et al., 2005).

In cases of symptomatic spinal disc degeneration, daily intake of glucosamine and chondroitin sulfate-based capsules as a food supplement for two years for disc recovery, where long-term intake of this supplement can counteract the symptoms of spinal disc degeneration, especially in the early stages. The disc degeneration/regeneration process was objectively observed using magnetic resonance imaging (MRI) in clinical studies of these food supplements. Why these medicines may have a cartilage structure and symptom modifying effect has been biochemically explained, demonstrating their therapeutic effectiveness against osteoarthritis in general (van Blitterswijk et al., 2003).

There were no histopathological alterations in intestinal tissue in rabbit tests, indicating that the liposomal formulation employed was safe. As a result, liposomes can be regarded as a viable oral permeation enhancer system for glucosamine sulfate and chondroitin sulfate, even though bioavailability studies are still warranted (Agiba et al., 2018). In comparison to placebo, glucosamine and chondroitin sulfate offered statistically significant pain alleviation (Barrow, 2010). A study revealed that a single dosage of up to four capsules containing 500 mg chondroitin sulfate and 400 mg glucosamine sulfate was well tolerated, with a profile that was consistent with 12-hour treatment (Toffoletto et al., 2005). Preventive therapy, larger dosages and multimodality methods with certain combination treatments were

Administration (FDA) classifies it as a dietary supplement, allowing U.S. customers more freedom to purchase and use chondroitin. Chondroitin sulfate is one of the most popular joint health supplements in the United States (You, 2021). A review based on available data prefers to classify glucosamine and chondroitin sulfate as food supplements rather than drugs (Šimánek et al., 2005).

In cases of symptomatic spinal disc degeneration, daily intake of glucosamine and chondroitin sulfate-based capsules as a food supplement for two years for disc recovery, where long-term intake of this supplement can counteract the symptoms of spinal disc degeneration, especially in the early stages. The disc degeneration/regeneration process was objectively observed using magnetic resonance imaging (MRI) in clinical studies of these food supplements. Why these medicines may have a cartilage structure and symptom modifying effect has been biochemically explained, demonstrating their therapeutic effectiveness against osteoarthritis in general (van Blitterswijk et al., 2003).

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The dosage form quality control carried out for products containing glucosamine and chondroitin sulfate does not all reflect the claims stated on the label on the tablet or capsule. Weight variation tests for nutritional supplements proved insufficient to evaluate dosage forms because they did not truly reflect the amount of active ingredient present. Therefore, there needs to be regulation to protect consumers of products containing glucosamine or chondroitin sulfate (Adebowale et al., 2000). Using a supplement based on results from another product does not guarantee effectiveness. The quality and content of nutritional supplements varies. It is in the best interests of patients to carefully examine items and provide informed recommendations (Schenck, 2000).

10.6.3 Potential Application for Nutraceutical Products

Nutraceuticals dominated the market in 2020, accounting for more than 36.0% of worldwide sales. In the nutraceutical sector, sodium chondroitin sulfate is commonly used as a dietary supplement for the treatment of osteoarthritis and joint discomfort. They are consumed to relieve arthritic pain and to strengthen joints, cartilages and bones. Chondroitin sulfate is employed in a variety of foods, including drinks, chewing gums and dairy products (Grand View Research. 2021).

New probiotic drink product development was carried out by combining the healthy properties of milk-based products with the properties of non-dairy probiotic drinks, namely the presence of chondroitin sulfate as a prebiotic and supplement that modulates the immune system and the number and diversity of probiotic strains that have health benefits (Pacini and Ruggiero, 2017). Joint-protecting beverages containing one or more active substances that enhance cartilage and synovial fluid, or a combination of these chemicals with bone-building calcium and phosphorus additions are invented. The active substances in these drinks are consumed in liquid form, which allows the human body to absorb and utilize them more effectively. Glucosamine sulfate, glucosamine hydrochloride, chondroitin sulfate, methyl sulfonylmethane, and hyaluronic acid may be included in the active component combination. High fructose corn syrup, citric acid, ascorbic acid, arabic gel, sodium benzoate and potassium sorbate may all be used to make various concentration drinks (Li and Xu, 2004).

A nutritional supplement in the form of a beverage containing cartilage added with cetyl myristolate was developed. The beverage is a combination of juice drinks manufactured from water-based fruit-flavored juice pasteurized at a relatively high temperature and a cartilage supplement solution created from cartilage supplements prepared at a relatively low temperature. Carbonated drinks, non-carbonated drinks, and concentrated drinks are all options. Chondroitin, glucosamine, and hyaluronic acid are examples of cartilage supplements (Stone, 2010).

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Deep processing in the blueberry beverage industry requires stable blueberry anthocyanins through the development of a nanocomplex embedded hydrogel system using anthocyanin and chondroitin sulfate co-pigmentation and then incorporation into a kappa-carrageenan hydrogel. The system can improve the stability of blueberry anthocyanins by providing effective protection, mainly due to low pH exposure during processing in the blueberry beverage industries. The chondroitin sulfate group on the nanocomposite generates a hydrophilic surface and offers good colloidal stability. Because of its high charge density, chondroitin sulfate can preserve the stacking structure of anthocyanins as a stabilizer (Xie et al., 2020).

Snack bars enriched with chondroitin sulfate are made by melting confectioner's peanut butter ingredients mixed with high fructose corn syrup and molasses. The liquid mixture was then supplemented with salt, chondroitin sulfate, glucosamine sulfate and hyaluronic acid. The sugar, whey protein, rice flour and soy protein are then added until homogeneous. The mixture is extruded at room temperature, cut into individual bars and then coated with melted chocolate confectionery before cooling. The final bar weighs around 70 grams and has roughly 300 calories (Stone, 2010).

10.6.4 Potential Application for Personal Care and Cosmetics

Chondroitin sulfate is commonly used in skin conditioning agents or hair treatments because of its capacity to moisturize, hea, and soothe skin, as well as its anti-inflammatory characteristics and ability to rebuild the intercellular matrix. Chondroitin sulfate is a substance found in skin care and cosmetics. Chondroitin sulfate is generated from muco-polysaccharides and has the ability to produce collagen and elastin, which can be used directly or incorporated into skin care products externally (Newseed, 2015). Treatment with chondroitin sulfate promotes the proliferation of keratinocytes and fibroblasts, as well as the migration and synthesis of fibroblast extracellular matrix components. Chondroitin sulfate also promotes the production of type I procollagen via activating extracellular signal-regulated kinase pathways. The chondroitin sulfate therapy improves skin wound healing and regeneration using a full-thickness skin wound model and an aging skin model. As a result, chondroitin sulfate can be used in therapeutic settings to improve skin aging (Min et al., 2020).

Chondroitin sulfate is used in hair care products like shampoo and conditioner to keep hair moist and silky and prevent it from drying out. Chondroitin sulfate is commonly used in rejuvenating serums, sun lotions, lip balms, pain relief creams and a variety of other products. In addition to skin and hair treatments, different grades of sodium chondroitin sulfate are used in beauty and personal care products, with 20%, 40%, and 80% being the most common (Newseed, 2015).

10.7 CONCLUSIONS

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10.7 CONCLUSIONS

Marine chondroitin sulfate has good prospects to be used for various purposes because it is safe for consumption and there are no objections to its use and consumption for cultural, religious and harmful disease reasons. Marine biota that have been explored for the extraction and isolation of chondroitin sulfate by various methods are still limited, and many of them need upscaling and commercialization studies. The health-related efficacy of chondroitin sulfate has been widely revealed, not only for osteoarthritis which has been widely known to the public, so it is likely to encourage an increase in the need for chondroitin sulfate. Chondroitin sulfate has the potential to be applied for the production of food supplements, nutraceuticals and personal care products by taking advantage of its biocompatible, anti-inflammatory, biodegradable, non-immunogenic and non-toxic properties, in addition to the health benefits that have been demonstrated.

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