

Trends in Gelatin Molecular Weight Research: A Scientometric Approach

Juliana Ramírez Candamil^{1,*}, Valeria Monsalve Castaño², Liliana María Lellesch³, Victor Alonso García Londoño³, Leydi Yuliana Vargas Soto³, Daniela Parra Ramírez³

¹Departamento de Matemáticas, Universidad de Caldas, Carrera, Edificio Orlando Sierra, Manizales, Caldas, COLOMBIA.

²Joven Investigador de Minciencias, Universidad de Caldas, Manizales, Caldas, COLOMBIA.

³Productora de Gelatina S.A.S., Manizales, Caldas, COLOMBIA.

ABSTRACT

Gelatin, a natural polymer derived from collagen, exhibits key physicochemical and rheological properties that are largely dependent on its molecular weight, influencing its functionality and applications in sectors such as food, biomedicine, and pharmaceuticals. Despite numerous studies on molecular weight, measurement methods, and their relationship with these properties, no comprehensive systematic review has been reported that compiles and analyzes the main contributions in this field, facilitating an understanding of the current state of research. To address this issue, a scientometric analysis was conducted using academic databases such as Web of Science (WoS) and Scopus. Search equations were applied to gather relevant information, integrating the records into a single dataset. The methodology consisted of two phases: first, a quantitative mapping analyzed scientific production by country, journal, and author, providing a comprehensive view of the contributions and their geographic scope. In the second phase, the Tree of Science metaphor was used to identify the main theoretical contributions. This historical approach allowed us to examine the evolution of molecular weight studies, measurement methodologies, and their relationship to the properties and applications of gelatin. Key subfields were identified, such as the use of gelatins from alternative sources in biomedical applications, the development of biomaterials, and the influence of proteases on molecular weight modification. These findings offer valuable information for future research and industrial applications.

Keywords: Gelatin, Measurement Methodologies, Molecular Weight, Scientometrics.

Correspondence:

Juliana Ramírez Candamil

Departamento de Matemáticas,
Universidad de Caldas, Carrera 23 #60-63,
Edificio Orlando Sierra, Manizales, Caldas,
C.P. 170004, COLOMBIA.
Email: juliana.ramirez@ucaldas.edu.co

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INTRODUCTION

Gelatin is a soluble natural polymer obtained by partial hydrolysis of collagen, a protein predominant in both humans and animals. Although collagen is found in various body structures, its concentration is particularly high in skin, bones, tendons, and ligaments (Bou-Gharios *et al.*, 2020; Johnston-Banks, 1990). The hydrolysis process, generally carried out through controlled treatments with acids or alkalis, hydrolyzes collagen into smaller peptides, resulting in a substance that, when dissolved in water, form gels, acts as a water-binding agent, stabilizes emulsions and foams, has thickening and binding properties, and improves the texture of products (Yang *et al.*, 2016).

The applicability, functionality, and commercial value of gelatin directly depend on its physical properties, such as viscosity, gel

strength, and thermal stability, as well as its chemical properties, such as moisture and pH. These characteristics are primarily determined by the amino acid composition, molecular weight, and proportion of α -chains (Gómez-Guillén *et al.*, 2011; Gómez-Guillén *et al.*, 2002). In particular, gel strength and viscosity increase with the amount of high-molecular-weight fractions, while the gelation rate and melting point also depend on the proportion of these fractions (Muyonga *et al.*, 2004a).

Several review articles have analyzed different aspects of gelatin. Rohman *et al.* (Gullón *et al.*, 2017) reviewed analytic methods to differentiate porcine and bovine gelatins in halal products, improving authenticity using techniques such as Fourier-Transform Infrared Spectroscopy (FTIR), chromatography, and polymerase chain reaction. Alipal *et al.* (2021) examined properties, extraction methods, and industrial applications. Recently, studies such as Rather *et al.* (2022) investigated the impact of modifications on eco-friendly packaging, while Mikhailov (2023) addressed its use in photographic and pharmaceutical applications (Naharro-Molinero *et al.*, 2024). Analyzed how the formulation of soft gelatin capsules affects their mechanical and thermal properties. By contrast, Zhang *et al.* (2024) reviewed the properties and applications of modified gelatin in the food industry.



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While the 2008 study by Wiegand and Köhler (2008) provides an overview of experimental techniques for determining average molecular weights and their distributions in polymers, a comprehensive systematization of the evolution of these methodologies and their impact on functionality and specific applications has not been performed in recent years. The lack of systematic reviews and scientometric analyses hampers comparisons between previous studies and limits the identification of trends in the state of the art. In this context, this work aims to perform a scientometric analysis of the scientific literature related to molecular weight, considering different measurement methods and their relationship to the properties and applications of gelatin.

To achieve this objective, academic databases such as Web of Science and Scopus are used. Relevant information is collected and analyzed by applying search equations. The records obtained from both databases are integrated into a single data set, to which the 'Tree of Science' (ToS) methodology applied using the TOSR package in R (<https://cran.r-project.org/web/packages/tosr/index.html>, accessed on January 21 2025). A scientometric analysis is then conducted to identify the main trends in global scientific production.

This is a novel article that provides a systematic view of the evolution of gelatin molecular weight measurement methodologies and their impact on properties and applications. The review is divided into three parts. The first describes the methods and key aspects of the "tree of science" approach used in this research. The second presents the methodology used to conduct the analysis, from the identification of references to their evaluation. The third section presents and discusses the results in two dimensions: first, the scientometric analysis of the evolution of scientific production, including countries, journals, and authors; second, the intellectual structure of the field of study using ToS. Finally, the conclusions and cited references are included.

METHODOLOGY

Figure 1 presents the general flow that was used and follows the typical steps that are used in scientometric analysis (Gómez-Ortiz and Vivares-Vergara, 2024; Simantiris and Vardaki, 2025).

Identification of References

The first phase focused on identifying a set of references related to the molecular weight of gelatin, using the parameters summarized in Table 1. In this case, 461 references were identified in Web of Science (WoS), which is more than double the amount of literature found in Scopus. After removing duplicates, 614 references from unique documents were obtained.

Data Extraction and Preprocessing

Unifying data across these databases represents a significant challenge due to the differences in their formats. Therefore,

one of the main contributions of this work is the integration of information from WoS and Scopus to perform a robust scientometric analysis. This integration has been highlighted in previous studies as an approach that enriches and broadens the perspective of scientometric analysis, but it is often a weakness in the literature because few articles achieve this goal (Ariza-Colpas *et al.*, 2024; Gómez-Ortiz and Vivares-Vergara, 2024).

The collected documents contain a wealth of data that allows for various types of analysis. To this end, text mining and data extraction techniques using web scraping were implemented, which facilitated exhaustive preprocessing. This process resulted in an Excel file structured into 22 sheets, from which key inputs were obtained for the development of tables and figures used in the analysis. At this stage, computer tools such as the R, Bibliometrix and TOSR packages were used, which have demonstrated their relevance in previous research (Aria and Cuccurullo, 2017; Robledo *et al.*, 2022).

Scientometric Analysis

Scientometrics, also related to bibliometrics or infometrics in some studies (Hood and Wilson, 2001), is a discipline that quantitatively analyzes different publications, allowing bibliographic data to be transformed into useful information for detecting emerging trends and findings from scientific processes in specific fields of study (Khokhlov, 2020; Leydesdorff and Milojević, 2012). In this sense, Mingers and Leydesdorff (2015) define scientometrics as the study of the quantitative aspects of science, which gives it a crucial role in measuring and evaluating research performance at different levels, from individual researchers to institutions and scientific journals.

The article presented by Khokhlov (2020) highlights the increasingly central role of scientometric indicators in the formal assessment of scientific production. The metrics addressed in this study are the temporal evolution of scientific production, identifying the most cited works, countries, authors, and sources (both journals and other formats) that have been the most prolific. Furthermore, a network-based approach was applied to reveal the interconnections between countries, authors, and sources to identify working groups or thematic clusters, providing insight into the dynamics of collaboration within the scientific community studying the molecular weight of gelatin.

Tree of Science Algorithm (ToS)

ToS is an algorithm created several years ago with the work of Robledo *et al.* (2014) and was first applied to a literature review on networking for small businesses. From there, three works were carried out to mathematically improve the algorithm (Eggers *et al.*, 2022; Hurtado-Marín *et al.*, 2021; Robledo *et al.*, 2020). ToS operates as a metaphor with the parts of a tree to identify roots, trunk and branches of a field of study; these three components make up an intellectual structure for that field. The roots

encompass highly relevant works that provide a foundational basis for the field. The trunk presents references that provide structural support by connecting roots and branches. Finally, the branches correspond to more recent thematic trends.

The tree is obtained from an initial set of references called the seed. In this case, the seed is the references identified in Table 1. From there, the algorithm crawls the cited references and forms a network containing a much larger database of works than the seed. This network can articulate works not in WoS or Scopus, or even those that do not exactly meet the keywords or time window, but that are relevant. In this work, the references identified by ToS were analyzed from a comprehensive perspective to understand the intellectual structure of the field of study and thus make a useful contribution to the scientific community. It is worth highlighting that the algorithm has been successfully used in previous scientometric analyses of various areas, such as those addressed by Guerrero-Molina *et al.* (2024), Ariza-Colpas *et al.* (2024), Urina *et al.* (2024), Gómez and Vivares (2024), Cano and Osorio (2024), among others.

RESULTS AND DISCUSSION

Scientometric Analysis

Scientific production

Annual analysis of scientific output on a specific topic is essential, as it facilitates the identification of key moments over time, revealing trends and advances in the field of study. Figure 2 shows the annual output related to gelatin molecular weight and measurement methodologies over the past 25 years. A higher number of publications are observed in WoS in almost every year (yellow bar). However, Scopus output (green bar) since 2000 has contributed unique articles to the total number of articles (red bar). Overall, the figure shows relatively stable performance over the past 8 years.

The timeline shows that the first peak in citations occurred in 2003, possibly influenced by WoS publications. During this period, relevant articles on the subject were published, such as the one presented by Teo *et al.* (2003), which explores the cloning,

expression, and characterization of a new metalloprotease (Pap6) from *Vibrio harveyi* strain AP6, a key enzyme in bacterial virulence. They analyzed its impact on the degradation of host structural proteins, its independence from zinc, and its role in the pathogenesis of infections in shrimp farming. This finding is relevant for the study of the molecular weight of gelatin, since susceptibility to degradation by metalloproteases such as Pap6 can vary depending on its molecular weight. Pap6's enzymatic preferences for substrates with certain molecular characteristics suggest that the molecular weight of gelatin influences its interaction with this protease, which could have implications for both biotechnology and the understanding of pathogenic processes.

Articles published in 2005 generated the highest number of citations in the dataset (2,120 as of the cutoff date mentioned in the methodology). Two papers were highly influential in this trend. The most cited article was Balakrishnan and Jayakrishnan (2005), which linked hydrogels to tissue regeneration and produced a highly effective injectable hydrogel. To measure molecular weight, they used High-Performance Liquid Chromatography-Gel Exclusion Chromatography to identify properties that control the gelation rate, strength, stability, and degradation of hydrogels. That same year, Balthasar *et al.* (2005) developed a study on modified gelatin nanoparticles for use as a targeted drug delivery method. Size exclusion chromatography was used to determine the molecular weight profile, highlighting the importance of nanoparticles with the characterization and quantification of the chemical reaction during the preparation process for system efficiency.

Some more recent works have started to receive citations quickly, despite not having many years to be cited, such as the article by Liu *et al.* (2020), which analyzes how cross-linking with Transglutaminase (MTG) improves the physical and mechanical properties of Type A and Type B gelatin by increasing their molecular weight. Type A gelatin shows higher cross-linking efficiency and better thermal stability due to its chemical composition, which highlights the potential of MTG to optimize gelatin properties in industrial applications; and the publication

Table 1: Search strategy and general results.

Parameter	Wos	Scopus
Range	2,000-2,024	
Date	January 2025	
Document type	Paper, book, chapter, conference proceedings	
Words	("gelatin" OR "gelatine") AND ("molecular weight" OR "molecular mass" OR "molecular size" OR "molecular weight determination" OR "molar mass") AND ("method" OR "technique" OR "measurement" OR "assay" OR "analysis")	
Results	461	198
Total (Wos + Scopus)	614	

Source: self-prepared.

by Du *et al.* (2021), which is a year more recent, addresses the stabilization of high thermal stability gelatin emulsions by transglutaminase and its application in 3D printing. Highlighting how cross-linking with transglutaminase increases the molecular weight of gelatin, improving the thermal stability and rheological properties of emulsions, allowing them to maintain their structure at high temperatures and optimizing their use in the manufacture of personalized foods. This research focuses on how MTG increases the molecular weight of gelatin and improves its thermal and mechanical stability.

Countries

Table 2 shows the scientific output on molecular weight measurement methodologies in ten countries, along with their impact metrics (measured in citations) and quality (according to Scimago quartiles). China leads in productivity with 15.87%, followed by the United States with 12.27%. An interesting fact is the comparison of impact, since, although China has a higher output, both countries have a similar impact in citations, with the United States reaching 14.3% and China 14.19%, with the U.S. being the country with the highest number of citations. In terms of quality, China leads with a large number of publications in Q1.

Studies on molecular weight measurement methodologies are closely related to countries with strong economies, as the industrial development of these nations generates the need to optimize processes and improve product quality. India ranks third in terms of citations, with 9.76% of the total. It is worth noting that most of the leading countries in scientific production also have significant production of gelatin and collagen, intended for food, pharmaceutical, and cosmetic applications.

One of the most recent studies from China, by Wang *et al.* Looks at how water content and low molecular weight components such as sugars and acids affect phase separation and gelation in gelatin and glucose syrup mixtures. Reducing water content

and acidification enhance gelation, while soluble citrates and sugars can weaken it. In the United States, one of the most recent studies³⁶ investigates how the properties of Methacrylated Gelatin (GelMA) and its flow behavior, influenced by factors such as time and temperature, affect cell viability and the sustained release of minocycline, a high-molecular-weight compound. By contrast, a recent study with authors of Indian affiliation developed hydrogels from tragacanth gum and gelatin through graft copolymerization. These hydrogels were characterized using techniques such as Fourier-Transform Infrared Spectroscopy (FTIR), C-nuclear magnetic resonance, among others, to determine their molecular weight, demonstrating properties suitable for controlled drug release.

Figure 3 presents the global network of cross-country collaborations, with six representative communities. The most notable is the purple community, with nodes in the United Kingdom, the United States, and China, collaborating on the research and application of the physicochemical, functional, and mechanical properties of collagens and gelatins in areas such as biotechnology, food, biomaterials, tissue engineering, and drug delivery. In recent collaborations, the United States and China 2024 (Xu *et al.*, 2024) developed a human neurovascular unit chip that simulates the neurovascular microenvironment using 3D bioprinting technology with GelMA (Methacrylated Gelatin) as one of the main bioinks.

The second community, represented in light green, is led by Germany, the Netherlands, and Belgium. It focuses on studies of the properties, techniques, and applications of biomaterials, especially gelatin, in fields such as drug delivery, biocompatibility, degradation, nanoparticles, molecular analysis, and their interaction in biological systems. Finally, the third community includes countries such as Spain, Denmark, Italy, and Colombia, which focus their collaborations on the characterization, extraction, and applications of biomaterials such as collagen

Table 2: Scientific production and impact by country.

Country	Production		Citation		Q1	Q2	Q3	Q4
	Count	Percentage	Count	Percentage				
China	110	15.87%	3,167	14.19%	72	15	6	6
United States	85	12.27%	3,191	14.3%	45	15	4	0
India	65	9.38%	2179	9.76%	20	19	7	4
Japan	33	4.76%	1,129	5.06%	13	5	1	0
Brazil	32	4.62%	667	2.99%	16	10	1	1
Germany	29	4.18%	1289	5.78%	18	3	1	1
UK	25	3.61%	1,051	4.71%	17	2	1	0
Spain	23	3.32%	705	3.16%	14	1	0	0
Italy	22	3.17%	458	2.05%	17	2	1	0
Korea	18	2.6%	491	2.2%	11	3	0	0

Source. self-prepared.

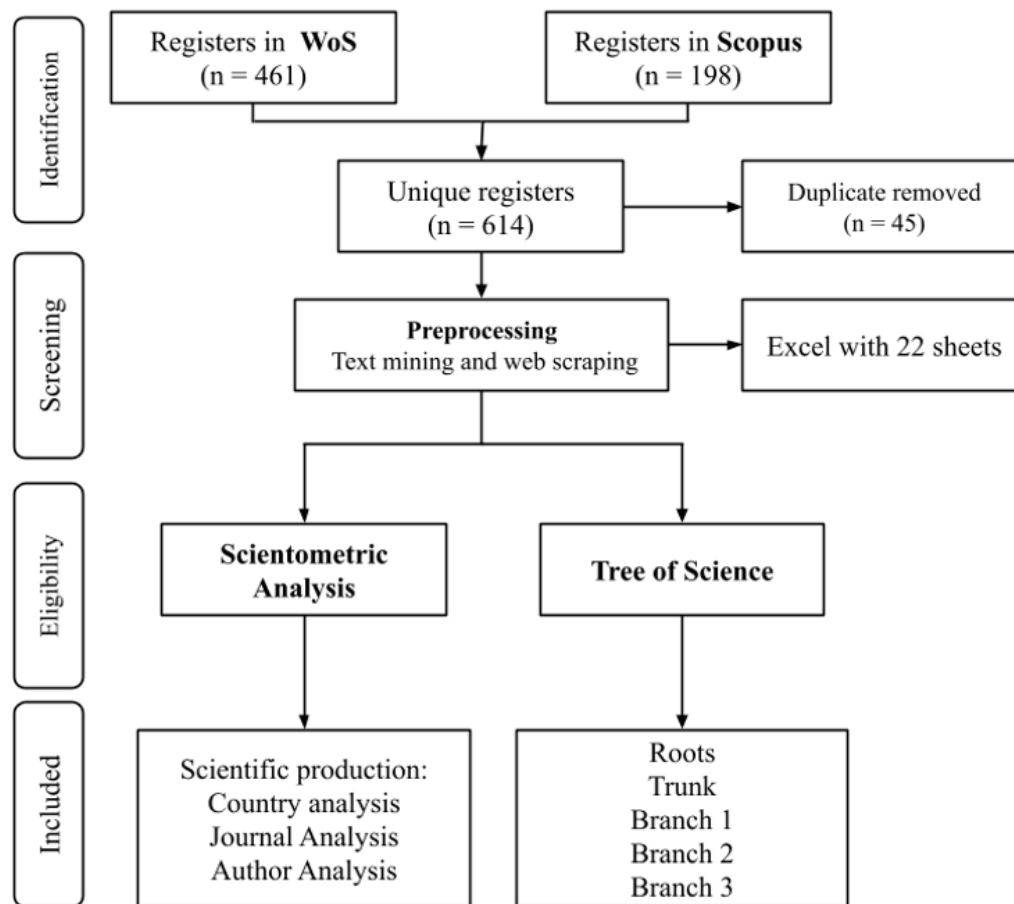


Figure 1: General methodology. Source: Self-prepared.

and gelatin, exploring their functional properties, degradation, regeneration, and uses in biomedicine and biotechnology.

These communities have collaborative networks, such as the one established in 2024 by Belgium and Italy (Greant *et al.*, 2024). This study investigated the adipogenic differentiation of human bone marrow-derived stem cells within photocurable polyester scaffolds with a gelatin hydrogel encapsulating stem cells. Gel permeation chromatography was used to determine the number-average Molecular Weight (Mn) and weight-average molecular weight of the polymer backbone. The results showed successful differentiation of human bone marrow-derived stem cells into mature adipocytes, suggesting that this hybrid strategy is suitable for applications in adipose tissue engineering.

Journals

The journals analyzed in Table 3 shows a predominance in the Q1 quartile, with Food Hydrocolloids standing out with the highest impact factor (2.717) and an H-index of 211, followed by *Journal of Controlled Release* (impact factor: 2.157, H-index: 313) and PLOS one, which leads in overall impact with an outstanding H-index of 435. In terms of coverage, *Food Hydrocolloids* and *International Journal of Biological Macromolecules* stand out in

WoS (26 and 22 records), while biomacromolecules stand out for its balanced presence in WoS and Scopus (5 records in both).

In the highly cited Food Hydrocolloids article, Zunying *et al.* (2012). A found that gelatin-chitosan interactions are stronger when chitosan with higher molecular weight or deacetylation degree is used, which improves the mechanical properties of gelatin films by increasing their tensile strength and glass transition temperature, making them more robust and stable. Ruican *et al.* (2024a) analyzed how phase separation and gelation of gelatin and glucose syrup mixtures are affected by moisture content, sugars, citric acid, and citrates, showing how long-chain polysaccharides in glucose syrups can vary depending on the molecular weight of the gelatin.

Citation networks in leading journals show three communities of scientific journals, classified by size in Figure 4. Their evolution over time and the collaborative relationships between them are also presented. The upper graph indicates that a few communities, such as the first (purple), account for most journals (more than 200), while other smaller communities have fewer than 50 journals. On the timeline, the lower graph reveals a decrease in the percentage of nodes (journals) and links (collaborations) since 2005. However, this apparent decline should not be

interpreted as a reduction in scientific collaboration or field contraction. In longitudinal citation analyses, such patterns are primarily explained by citation latency and temporal truncation (edge effect), since recently published journals and articles have had limited time to accumulate citations. Consequently, lower connectivity in recent years reflects the maturation dynamics of citation networks rather than a genuine decrease in interjournal collaboration.

The collaboration network reveals communities with distinct thematic objectives. The first community (purple) stands out for its research in controlled drug release and pharmacology that influences the formation of sustained-release and biocompatible matrices. The second community (green) focuses on biochemistry and cellular physiology, with applications of gelatin's molecular weight in the creation of tissue engineering scaffolds or drug encapsulation systems. The third community (orange) addresses immunology and infections, investigating how the properties of gelatin, influenced by its molecular weight, improve the stability and functionality of vaccines or immunologic delivery systems.

Thicker connections between nodes, such as those linking Journal of Biological Chemistry and Pharmaceutical Research, reflect stronger collaborative relationships, while the size of the nodes

shows the relevance of certain journals within their respective fields.

Author collaboration network

Table 4 presents the ten most productive researchers in the study of molecular weight measurement in polymers, a key aspect for industrial development based on the use of gelatin. Among them, Samiullah Khan stands out as the author with the highest number of publications in this field, with an h-index of 17. He recently published a study (Khan and Anwar, 2021) on the development of pH-responsive hydrogels based on gelatin and carboxymethylcellulose for the controlled release of anticancer drugs. In this research, various relationships between structure and properties were analyzed, including molecular weight and physical characteristics. For material characterization, techniques such as FTIR, X-ray diffraction, and Differential Scanning Calorimetry were used, in addition to Scanning Electron Microscopy to confirm some structures.

The second most productive researcher is Marta Branquinho, with five publications and an h-index of 30. Her work (De Santa-Izabel *et al.*, 2004) focuses on the analysis of proteinase profiles in different parasite strains, using sodium dodecyl

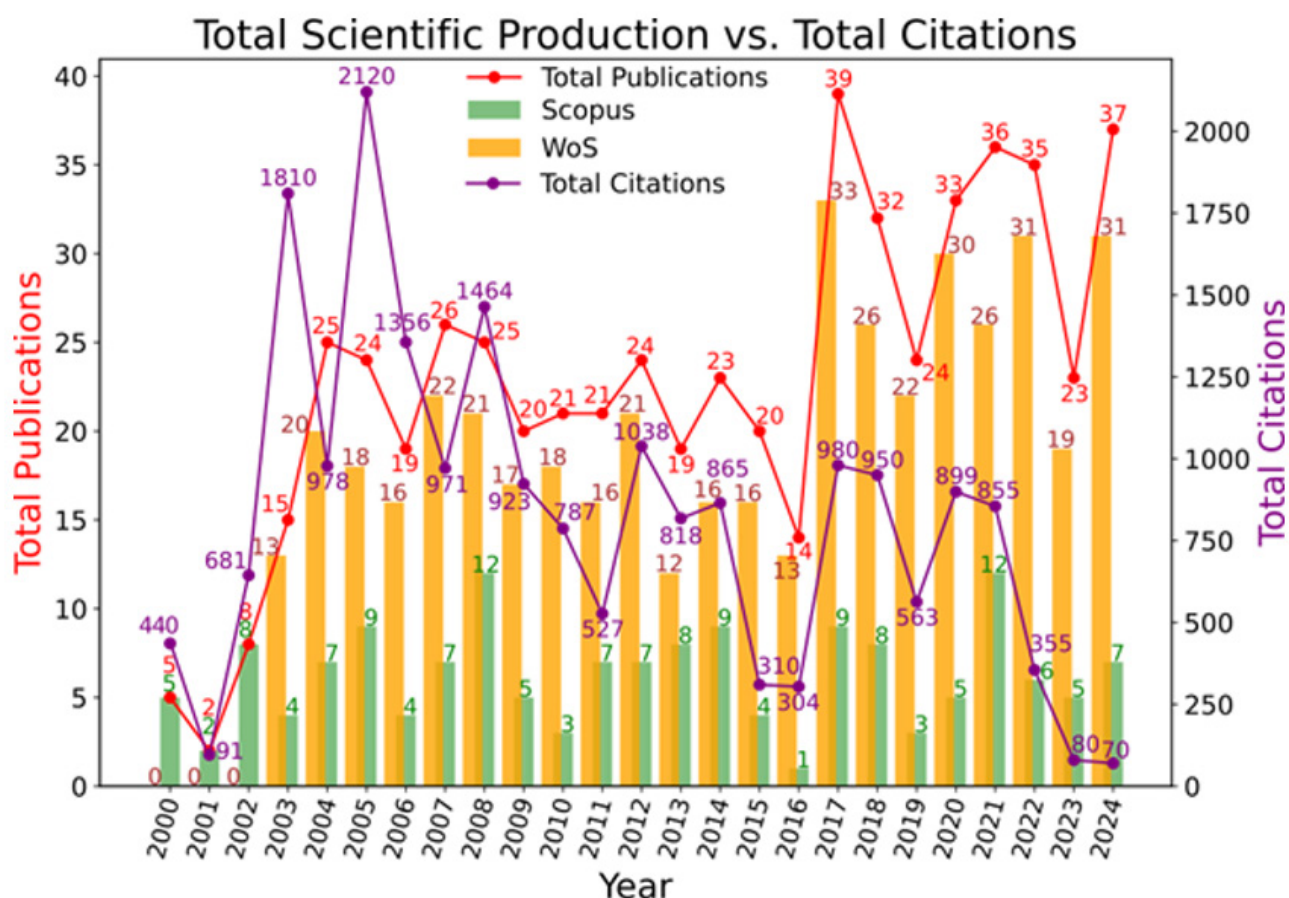


Figure 2: Scientific Production vs. total Citations over the time. Source: Self-prepared.

sulfate-polyacrylamide gel electrophoresis as a substrate to evaluate enzymatic activity.

Meanwhile, Yasuhiko Tabata, who holds the highest h-index of 107, has investigated (Young *et al.*, 2005) the use of gelatin as a polymer in pharmaceutical and medical applications. His study addresses the extraction of gelatin to obtain acidic or basic gelatin, preserving the biological activity of biomolecules for applications in tissue engineering, therapeutic angiogenesis, gene therapy, and drug delivery. To determine the molecular weight profile of gelatin, he used the High-Performance Liquid Chromatography (HPLC) method.

Figure 5 represents the scientific collaboration network among the authors, structured based on personal networks (ego networks). Two major collaboration clusters are visualized: the first, highly cohesive, is made up of leading researchers in the field and, notably, is subdivided into three subgroups with the participation of scientists from China, Europe, Australia, and

other countries. This group forms an influential network in the field of biopolymers, particularly in gelatin research.

Within this group, researcher Zhihua Shan stands out. He has studied the use of a deep eutectic solvent based on sodium acetate trihydrate and urea to dissolve gelatin and prevent its gelation (Cui *et al.*, 2021). His work focuses on harnessing the physical and chemical properties of gelatin to reduce its viscosity and improve its applicability. Using gel permeation chromatography, it was shown that the gelatin did not undergo significant changes in its molecular weight, thus preserving its functional properties for industrial applications.

Another key author in this group is Yasuhiko Tabata, who has collaborated with researchers from different countries on the development of biomaterials (Ratanavaraporn *et al.*, 2010). His work focuses on the use of chitosan combined with gelatin to modulate the activity of bone marrow-derived stem cells in mice. This study highlights the importance of the chemical characteristics of materials, as well as the influence of the molecular

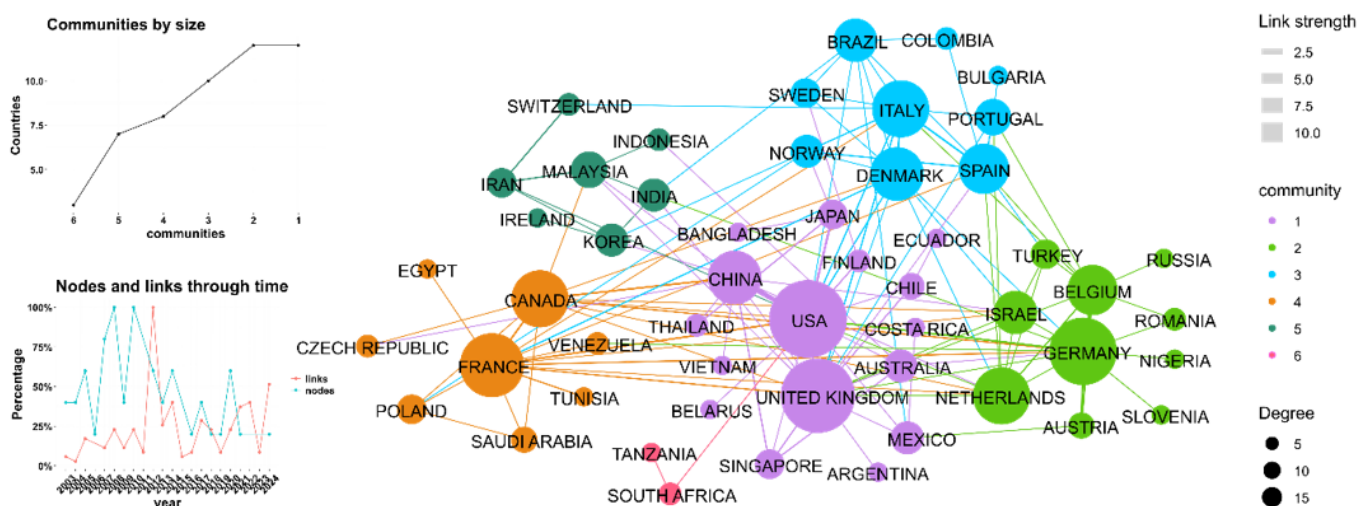


Figure 3: Global collaboration network. Source: Self-prepared.

Table 3: Productivity and impact of leading journals.

Journal	WoS	Scopus	Impact factor	Quartile	H index
Food hydrocolloids	26	5	2.717	Q1	211
International Journal of Biological Macromolecules	22	0	1.245	Q1	191
Food chemistry	6	2	1.745	Q1	324
Applied Biochemistry and Biotechnology	7	0	0.548	Q2	135
Biomacromolecules	5	5	1.232	Q1	252
Journal of Applied Polymer Science	7	0	0.557	Q2	193
Journal of Food Science	7	0	0.783	Q1	179
Marine drugs	5	0	0.88	Q1	159
Plos one	6	0	0.839	Q1	435
Journal of Controlled Release	4	0	2.157	Q1	313

Source. self-prepared. Abbreviations: WoS: Web of Science.

draw on pioneering research that has laid the methodological foundations for measuring molecular weight in molecules, proteins, and polymers. This work has led to the development of increasingly precise techniques, facilitating the understanding of how molecular weight distribution influences the rheological and structural properties of gelatin.

The theoretical foundations of this line of research include key studies that have developed innovative methodologies. A key

work is that of Lawry *et al.* (1951), who proposed a method based on the reaction of proteins with the Folin-Ciocalteu reagent. His research demonstrated how different proteins, including gelatin, exhibit variations in quantification due to their molecular weights. Subsequently, Laemmli (1970) carried out a study on bacteriophage T4 using gel electrophoresis, identifying new proteins and establishing a separation method widely adopted in biochemistry. Complementing these findings, Bradford (1976) developed techniques for the identification and

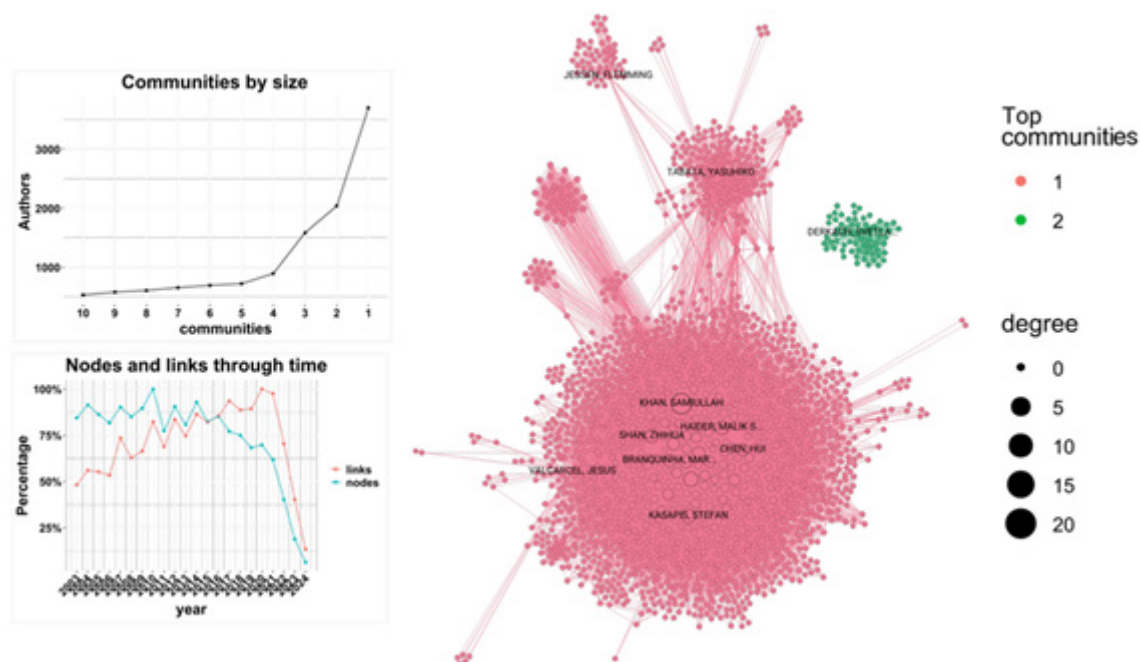


Figure 5: Network collaboration of the most productive authors. Source: Self-prepared.

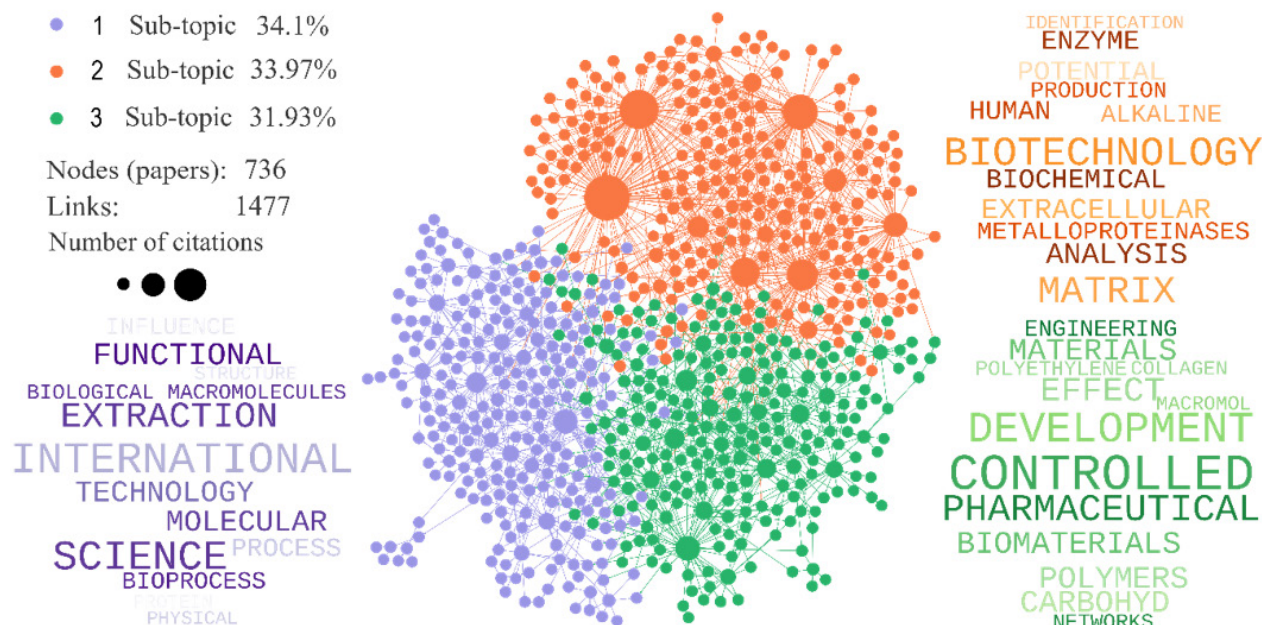


Figure 6: Branches (citation network analysis). Source: Self-prepared.

Table 4: Top production by author.

Sl. No.	Author	Total Publications	h- index	Affiliation
1.	khan, Samiullah	7	17	The Islamia University of Bahawalpur. Punjab, Pakistan.
2.	Branquinha, MARTA H.	5	30	Univ. Federal do Rio de Janeiro. Rio de Janeiro, Brazil.
3.	Haider, malik Salman	5	5	Bahauddin Zakariya University. Multan, Pakistan.
4.	Kasapis, Stefan	5	52	RMIT University. Melbourne, Australia.
5.	shan, Zhihua	5	20	Sichuan University. Chengdu, China
6.	CHEN, hui	4	20	Sichuan University. Chengdu, China.
7.	Tabata, Yasuhiko	4	107	Kyoto University. Kawara-cho Shogoin, Japan.
8.	Derkach, Svetlana R.	3	24	Murmansk State Technical University. Murmansk, Russia.
9.	Valcarcel, JESUS	3	21	Instituto de Investigaciones Marinas (IIM-CSIC). Pontevedra, Vigo, Spain.
10.	Jessen, Flemming	3	34	Technical University of Denmark. Kongens Lyngby, Denmark.

Source. S3.elf-prepared.

quantification of small quantities of proteins through protein-dye interactions, mentioning for the first time mass spectrometry as a tool for molecular weight measurement, although without specifically applying it to gelatin. Further on, we find research that focuses on components of our research. For example, Johnston (Johnston-Banks, 1990) addresses the physical and chemical properties of gelatin, as well as the amino acid compounds that make up both gelatin and collagen. He also describes the various structures of gelatin and details production methods, explaining how it works in industry. Gilsenan and Murphy (2000) also examined the physical and chemical properties of gelatin in various species, applying these findings to industry. In their study, they analyzed both its molecular and gelling characteristics, as well as its molecular weight, highlighting its impact on rheological properties. They also noted that differences in molecular properties and amino acid composition cause mammalian-derived and marine-derived gelatin to vary considerably. Gómez *et al.* (2002) also conducted studies on the composition and structure of gelatin in marine species, exploring the relationship between molecular weight distribution and amino acid composition. They used the polyacrylamide gel electrophoresis technique to identify the molecular weight of gelatin in various species. Later, Muyonga *et al.* (2004b) studied the molecular weight distribution of collagen in gelatin using FTIR spectroscopy and how the amino acid structures of gelatin influence the structural properties of gelation, as in previous studies. In the field of chromatography, Arnesen and Gildberg (2007) were the first to apply HPLC to analyze the molecular weight distribution in gelatin extracts, highlighting its implications for improving industrial quality. Karim and Bhat (2009) investigated fish gelatin as an alternative to mammalian gelatin, comparing amino acid distribution without focusing

on molecular weight measurement methods. Finally, Gómez *et al.* (2011) conducted a review of the functional and bioactive properties of collagen and gelatin, highlighting methods such as High-Performance Liquid Chromatography-Tandem Mass Spectrometry (HPLC-MS/MS) and FTIR-ATR (Attenuated Total Reflection) for molecular weight identification and qualitative differentiation between bovine and porcine gelatins. These studies have been instrumental in establishing precise methodologies and understanding the influence of molecular weight on gelatin properties. Their impact remains significant in the improvement of industrial processes and the characterization of gelatin-based biomaterials.

Trunk

The properties of gelatin are determined by various factors, including its extraction method, amino acid composition, molecular weight distribution, and rheological characteristics. To better understand these aspects, studies have been conducted analyzing how different processing and characterization techniques affect gelatin's structure and functionality. In this context, numerous authors have provided solid theoretical foundations and practical applications using advanced methodologies, such as gel electrophoresis, liquid chromatography, and spectroscopy, to evaluate the relationship between molecular weight, structural stability, and gelatin authenticity. These studies have not only made it possible to differentiate gelatins from different sources but also to establish correlations between their molecular profile and their physicochemical properties, providing relevant information for their application in the food and biomedical industries.

Articles such as that by Giménez *et al.* (2005) investigated how the drying method used to preserve fish skins influences the

gel properties of gelatin. To evaluate the molecular weight distribution, electrophoretic analysis was performed, allowing a detailed characterization of the resulting gelatin. Similarly, Chiou *et al.* (2006) analyzed the rheological and mechanical properties of gelatin derived from *Alaska pollock* and *Alaskan pink salmon*, using gel electrophoresis to determine the molecular weight profile and amino acid composition. They observed that, due to their amino acid composition, fish gelatins exhibit lower gelation and melting temperatures compared to porcine gelatin. In a parallel study, Zhang *et al.* (2006) applied gel electrophoresis to evaluate the molecular weight distribution and amino acid composition of bovine gelatin, establishing their relationship with the isoelectric point, helical conformation, and resistance of collagen to enzymatic hydrolysis. Subsequently, Zhang *et al.* (2009). HPLC-MS/MS to identify marker peptides in gelatin, effectively differentiating bovine and porcine gelatin using this analytic methodology. Additionally, Azira *et al.* (2014) developed a study based on sodium dodecyl sulfate-polyacrylamide gel electrophoresis and principal-component analysis to differentiate porcine and bovine gelatins, finding significant differences in the molecular weight profiles of the polypeptides, which validates the method as an effective tool for the authentication of foods with gelatin. Netter, Goudoulas, and Germann (2020) analyzed the impact of Bloom on the rheological properties of porcine gelatin, concluding that higher Bloom values correlate with higher molecular weights. They also observed an increase in gelation and melting temperatures with increasing Bloom number. To evaluate molecular weight distribution, they employed asymmetrical flow field-flow fractionation, an advanced technique for the separation of macromolecules and nanoparticles based on the size and molecular weight of gelatin. Finally, Rohman *et al.* (2020) conducted a comprehensive review of the analytic methods used to differentiate porcine and bovine gelatins in food products, highlighting their importance in halal authentication. In their study, they described the application of FTIR spectroscopy to identify functional groups, liquid chromatography to analyze amino acid composition, gel electrophoresis to assess the molecular weight of polypeptides, and real-time polymerase chain reaction as a DNA-based method for species identification. The combination of these analytic methods is key to ensuring accuracy in identifying the origin of gelatin. In conclusion, the methodologies used to measure gelatin properties have evolved significantly, allowing for more precise characterization of its authenticity and composition. These advances have strengthened quality control in the food and biomedical industries, ensuring the reliability of gelatin's origin and functionality in various applications.

Branch 1. Gelatin from alternative sources: Properties, optimization, and biomedical applications

Bovine and porcine gelatins are the most widely used in the industry due to their availability and gelling properties. However, gelatins from alternative sources, such as fish and poultry, have gained interest due to their functionality and biomedical applications. A key factor in their performance is molecular weight, which influences viscosity, gelling capacity, and structural stability.

Haryati *et al.* (2025) optimized the extraction of gelatin from *Yellowfin tuna* skin using bromelain hydrolysis and ultrasound, improving its properties. Ruan *et al.* (2023) showed that trypsin pretreatment modifies the composition and molecular weight distribution of gelatins from different fish species. Lamers *et al.* (2024) highlighted the sequential extraction of turkey gelatin as a strategy to improve its performance and functionality. The molecular weight and rheological properties of these gelatins influence their stability and functionality. Derkach *et al.* (2024) compared fish and mammalian gelatin hydrogels, observing differences in viscosity and mechanical strength with impact on biomedical applications. He *et al.* (2024) analyzed how molecular weight distribution affects the gelation of bovine bone gelatin, favoring the formation of more homogeneous gels. Yang *et al.* (2024) explored its use in stabilizing fish oil powders, concluding that its type and concentration determine the product's stability. In the biomedical field, these gelatins have demonstrated promising properties. Islam *et al.* (2024) developed biomedical-grade gelatin from sturgeon skin, ensuring its purity for advanced medical applications. Zhan *et al.* (2024) investigated the anti-inflammatory capacity of squid cartilage gelatin in the treatment of diabetic wounds, showing that the therapeutic effects varied depending on the molecular weight of the biopolymer. Zhang *et al.* (2024) evaluated the effects of low molecular weight E-Jiao peptides on hematopoietic regeneration after chemotherapy, highlighting that their smaller size facilitates absorption and bioavailability in the body. Finally, Sha *et al.* (2023) analyzed the *in vitro* digestion of modified fish gelatins with transglutaminase, observing that the structural strength and enzymatic degradation were directly related to the molecular weight of the gelatin. These studies reinforce the importance of exploring alternative sources of gelatin and optimizing its properties, considering the influence of molecular weight on its functionality and applications. Furthermore, the search for sustainable sources and the implementation of biotechnological strategies can contribute to the production of gelatins with specific properties, tailored to the needs of various industries.

Branch 2. Proteases and gelatin: Influence of molecular weight on biomedical and industrial applications

Proteases play a fundamental role in protein degradation, being essential in multiple biological processes with applications in both biomedicine and industry. Their study allows us to understand how they act in the digestion of structural and functional proteins, facilitating their use in areas such as healthcare, biotechnology, and pharmacology. Several investigations have addressed the activity of these enzymes in different organisms and conditions, analyzing their specificity and stability to optimize their application in different contexts.

In the case of *Candida parapsilosis* (Gandra *et al.*, 2024), analyzed the ability of its secreted proteases, Sapp1 and Sapp2, to degrade proteins such as albumin and gelatin, generating high-molecular-weight polypeptides, demonstrating their ability to process structural proteins relevant to the infection. Similarly, Pandey and Hajela (2023) reported a protease isolated from *Artocarpus heterophyllus* with a molecular weight of 74 kilodaltons and activity on gelatin, determined by zymography, highlighting its potential for industrial and pharmaceutical applications. Likewise, Jana *et al.* (2022) described the activity of *Bacillus cereus* keratinase on proteins such as gelatin and albumin, highlighting its stability over wide pH and temperature ranges, which expands its applicability in biotechnology. The use of gelatin zymography has been key to characterizing enzymatic activity in different organisms. Wei and Kavitha (n.d.) used this technique to identify gingipains from *Porphyromonas gingivalis*, determining that these proteases have molecular weights of approximately 50 and greater than 245 kilodaltons, which allowed a more specific characterization of their activity. Similarly, Ligi *et al.* (2020) optimized gelatin zymography to improve the detection of Metalloproteinases de Matriz MMP-2 and MMP-9, key enzymes in degenerative diseases. Furthermore, it introduces a more stable gelatinase calibrator, allowing the identification of high molecular weight MMP-9 complexes in peripheral blood, which could improve the diagnosis and monitoring of these pathologies. In the dental field, Gou *et al.* (2020) demonstrated that gelatinases can process essential proteins such as dentin sialoprotein in odontoblasts, suggesting a relevant function in tooth remodeling. The molecular weight of biopolymers also influences their functionality, as evidenced by studies on gelatin and its derivatives. Liu *et al.* (2020) analyzed how reducing the molecular weight of hyaluronic acid affects its interaction with gelatin, compromising its lubricating capacity on cartilage surfaces and suggesting implications for the treatment of osteoarthritis. Likewise, Li *et al.* (2020) studied the coacervation of hydrolyzed collagen and observed that its lower molecular weight influences its aggregation and solubility in aqueous media, differentiating it from conventional gelatin. By contrast, Glodowsky *et al.* (2020) evaluated the effect of a *Penicillium chrysogenum* transglutaminase on gelatin gels,

finding that this enzyme increases the gel's mechanical strength, suggesting potential applications in the food and biomedical industries. These studies highlight the importance of proteases and molecular weight in gelatin functionality, demonstrating their impact in various scientific and industrial fields. The characterization of these enzymes and biopolymers not only expands our knowledge of their structural and biochemical properties but also opens up new opportunities for their application in biomedicine, biotechnology, and pharmacology.

Branch 3. Gelatin as a base for biomaterials: Properties, applications and analysis methods

The development of gelatin-based biomaterials has sparked growing interest due to its physical, chemical, and structural properties, which influence its functionality in various applications. In this context, multiple studies have explored methods to optimize its composition and improve its performance in the biomedical and industrial fields.

Song *et al.* (2024) successfully expressed humanlike recombinant gelatin for biomedical applications, highlighting the importance of its chemical composition in its final properties. To characterize its structure, they performed HPLC analysis. Morrish *et al.* studied genipin-cross-linked gelatin networks for the controlled transport of bio actives, evaluating their rheological behavior using FTIR and applying Flory-Rehner theory to analyze their chemical properties and molecular transport capacity. In the industrial field, Ding *et al.* (2022) explored gelatin hydrolysis to obtain peptides of different molecular weights and their impact on gypsum setting. Using FTIR and X-ray diffraction, they determined that the low-molecular-weight peptides effectively inhibited the formation of gypsum dihydrate, suggesting potential applications in construction and advanced materials. These findings reinforce the key role of gelatin and its derivatives in diverse areas, from biomedicine to the materials industry. Optimizing their properties through chemical and enzymatic modifications opens up new opportunities for their application in the development of advanced biomaterials and specific industrial processes.

CONCLUSION

This study demonstrates how systematization and quantitative analysis have made it possible to trace the evolution of the methodologies used to measure the molecular weight of gelatin, highlighting their impact on the characterization of its physical and chemical properties. Key analytic approaches were identified that seek to standardize studies and optimize their application in industrial processes and biomaterial development.

The findings highlight the fundamental role of advanced techniques such as size exclusion chromatography and High-Performance Liquid Chromatography-Gel Exclusion Chromatography in the accuracy of molecular weight profiling,

as well as the contribution of emerging methodologies, such as mass spectrometry and asymmetrical flow fractionation, in the differentiation of gelatins from diverse sources. Furthermore, the influence of factors such as chemical composition and hydration on their functionality was confirmed, expanding their applications in the biomedical, food, and pharmaceutical sectors.

Globally, scientific production in this area has experienced significant growth in recent years, with an increase in international collaborations. A strong concentration of research is observed in the United Kingdom, the United States, and China, along with a growing network of collaborations in areas such as bioprinting, nanotechnology, and tissue engineering. The most influential publications, especially in high-impact journals such as *Food Hydrocolloids*, have driven the consolidation of specialized scientific communities and advancements in analytic techniques. The scientometric analysis developed in this study has allowed us not only to assess the evolution of scientific production but also to identify the authors, journals, and countries with the greatest impact on gelatin research. This knowledge mapping facilitates future collaboration and development initiatives, providing a solid foundation for optimizing the use of innovative analytic tools in the characterization and quantification of gelatin.

Finally, the findings highlight the importance of methodologies and analytic criteria in the effectiveness and applicability of results across different industries. The exploration of new analytic tools and the diversification of their applications will strengthen research in this field. Given the continued growth in biomaterial development, gelatin remains a key component in the improvement of industrial materials, thanks to its functional properties and technological advances that expand its uses in strategic sectors.

ABBREVIATIONS

3D: Three-Dimensional; **AF4:** Asymmetrical Flow Field-Flow Fractionation; **ATR:** Attenuated Total Reflection; **DNA:** Deoxyribonucleic Acid; **FTIR:** Fourier-Transform Infrared Spectroscopy; **FTIR-ATR:** Fourier-Transform Infrared Spectroscopy–Attenuated Total Reflection; **GelMA:** Methacrylated Gelatin; **HPLC:** High-Performance Liquid Chromatography; **HPLC-GEC:** High-Performance Liquid Chromatography–Gel Exclusion Chromatography; **HPLC-MS/MS:** High-Performance Liquid Chromatography–Tandem Mass Spectrometry; **MMP:** Matrix Metalloproteinase; **Mn:** Number-Average Molecular Weight; **MTG:** Transglutaminase; **PCR:** Polymerase Chain Reaction; **Q1:** Quartile 1; **SDS-PAGE:** Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis; **ToS:** Tree of Science; **WoS:** Web of Science; **XRD:** X-ray Diffraction.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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