LAPORAN PENELITIAN

ENHANCING FISH GELATIN INTO NANO PARTICLES: FROM WASTE TO PRODUCT OF INDONESIAN PANGASIUS CATFISH Sp.

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ABSTRACT

Fish gelatin, derived from processing by-products, has captured substantial attention due to its sustainability and exceptional attributes. This comprehensive review explores its multifaceted applications, with a special emphasis on the potential of Indonesian Pangasius catfish as a source, showcasing its superior properties and innovative pre-treatment techniques. However, the exploration of fish gelatin at the nano scale remains a largely uncharted territory. In general, interdisciplinary approaches and diverse synthesis methods, such as desolvation and electrospinning, have recently unveiled the vast potential of fish gelatin nanoparticles (NPs). These nanoparticles (NPs) serve as versatile carrier agents, biomaterials, and crucial components in antimicrobial packaging, drawing from various fish species. Furthermore, fish gelatin NPs have seamlessly integrated into nanocomposite (NC) f ilms alongside substances like organic and inorganic NPs, promising significant strides in food safety and preservation strategies. Remarkably, fish gelatin NPs exhibit unique antimicrobial properties, positioning them as promising candidates for applications in both the food packaging industry and biomedicine. Moreover, the inclusion of NPs enhances mechanical performance, augmenting their suitability for a multitude of applications. This review highlights transforming and elevating of Pangasius catfish gelatin-based NPs, drawing inspiration from techniques applied to other fish species. It underscores the imperative need for systematic optimization encompassing gelatin extraction, synthesis, and characterization. Additionally, it addresses the formidable challenges and promising opportunities associated with unlocking the full industrial potential of gelatin-based NPs, thereby pointing towards a sustainable and innovative future in the field of materials science. Readers are cordially invited to embark on this promising avenue of research, where a world of possibilities awaits exploration and the promise of environmentally friendly innovation beckon.

Keywords: Biophysical characterization, fish gelatin, fish processing by-products, nanocomposites, and manoparticles

I. INTRODUCTION

Gelatin has been widely used in food, pharmaceutical, cosmetic, and photographic industries. In food industry, gelatin is used as emulsifier, stabilizer, thickener, biodegradable film, foaming, gelling as well as encapsulation agents and it is estimated around 52% from total gelatin production worldwide. According to market share value, gelatin is the highest among most important hydrocolloid material for food application reaching 21.2% [1] (GME, 2021). Indonesia has been imported gelatin to supply the national demand reaching 4,250,933 kg per year or with import worth of US$ 30,136,022 per year in average (2014-2018) (UN Comtrade, 2020). Most of gelatin is imported from Brazil, India, China, United States, and Australia. In addition, majority of gelatin derived from pig skin (44%), bovine hides (28%), and bovine bones (27%) [2](Zilhadia et al. 2018). However, this mammalian sources have some obstacles related with religion, socio-cultural, health aspects, and virus outbreaks. Therefore, it is urgently needed an alternative gelatin replacing mammalian traditional gelatin. Gelatin from fish processing by-products especially skin and bone are most potential alternative gelatin [3]–[6] (Nitsuwat et al. 2021; Boran & Regenstein, 2010; Gomez-Guillen et al. 2009; Karim & Bhat, 2008) which accepted universally with respect to those the aspects mentioned.

The by-products from fish filleting process are approximately 70% [7] (Olsen et al. 2014) from total of fish weight and around 30% fish weight could be derived from skin and bone [8], [9] (Zamorano-Apodaca, 2020; Sotelo et al. 2016). The others convey that the fish waste portion from fishery commodities are more than 21% [10](Karayannakidis & Zotos, 2016). Ministry of Marine Affairs and Fisheries outlined that fish production in Indonesia in 2015-2017 was 11,042 billion tons per year which divided into marine capture fish, freshwater fish, and farmed fish [11], [12] (BPS, 2021; Statistik-kkp; KKP 2022). So that, the fish processing by-products particularly skin and bone is estimated around 3,512 billion tons per year. If we assumed that the gelatin yield from fish waste adopted from previous research was 2.4% (the lowest yield) [13], [14] (Atma & Ramdhani, 2017; Muyoga et al. 2004), means that it would produce as many as 87,675,177 kg gelatin or would be more than 20-folds higher than the national (Indonesia) need. Furthermore, the fish gelatin from warm-water fish have superiority in term of physical properties compared to cold-water fish [15]–[17] (Chandra & Shamasundar, 2015; Liu et al. 2017; Jellouli et al. 2011). Thus, it will be potential to developed in the future including to export goal.

The Pangasius catfish or *Pangasius sutchi* (*P. sutchi*), *P. hypophthalmus* syn. *P. g*igas, or also known as Patin, holds a prominent position within Indonesia's flourishing fisheries sector, making a substantial contribution to an annual production exceeding 300,000 tons [18], [19]. With 380,000 tons being produced annually by 2022, the Pangasius catfish farming industry also presents a chance for significant financial gain [20]. This indigenous piscine species undergoes diverse processing, resulting in an assortment of products encompassing patin fish fillets, traditional culinary offerings, and a variety of derived edibles. This extensive utilization inevitably leads to the generation of associated by-products, among which fish bones assume significant proportions, accounting for no less than 23-27% of the fish's total weight [21] (Jasmadi et al. 2022). Given the scale of its annual production, this implies a potential accumulation of over 69,000 until 81,000 tons of Patin bones as residual waste on a yearly basis. Considering mounting environmental concerns, the imperative to develop technology aimed at managing and mitigating the disposal of Patin bone waste becomes paramount.

Until the 20th century, gelatin's historical role, predominantly revolved around its essential application in the realm of food products, a role of undeniable historical significance. However, this role, though foremost, is merely the tip of the iceberg [22] (Mikhailov et al., 2023). Gelatin's versatility extends well beyond the culinary domain, finding its utility in addressing a myriad of challenges posed by human activities. It has become a key player in diverse fields, including its crucial involvement in adhesive formulations for artistic creations using materials like wood, leather, textiles, and publications. Additionally, gelatin plays a pivotal role in the formulation of water-soluble coatings for washing and dishwasher tablets and serves as a critical component in ballistic testing, famously recognized as ballistic gelatin [23]–[25] (Ding et al., 2023; Hes et al., 2023; Mauri & Scialla, 2023).

While these applications span various sectors and possess distinct characteristics, they share a common thread anchored in the mechanical and physical attributes of macro-level gelatin structures. However, limited attention has been paid to exploring the potential of fish gelatin at the micro- and nanoscale levels, specifically focusing on its molecular properties and structural organization. The nanoscale dimension unveils exciting opportunities for leveraging gelatin's unique characteristics, leading to a diverse range of applications:

1. *Photographic*: Gelatin's nanostructural organization assumes a pivotal role in the creation of photographic gelatin, a critical component in the production of silver halide

photographic materials used for data storage[26], [27] (Hodgson, 2023; Huang et al., 2004).

2. *Nanoparticle Production and Immobilization*: Gelatin plays a crucial role in the synthesis and immobilization of nanoparticles containing a wide array of chemical compounds [23], [28], [29] (Ding et al., 2023; Kour et al., 2023; Oh et al., 2023).

3. *Medical Applications*: Gelatin is central to the development of medical materials, encompassing capsules, components of nutrient mixtures, culture media, and essential elements in plasma-substituting and diagnostic tools [29]–[32] (Oh et al., 2023; Nassar & Kasapis, 2023; Milano et al., 2023; Huang et al., 2023).

4. *Nanomaterials*: Gelatin-based nanomaterials find extensive utility across various domains, particularly within the realm of protein-based nanotechnologies [33]–[35] (Lou et al., 2023; Sadegi et al., 2023; He et al., 2023).

Definitely, gelatin nanoparticles have garnered prominence in food applications, marking a significant stride in integrating nanotechnology into culinary endeavors [36]–[38] (Wu et al., 2023; Tan et al., 2023; Tang et al., 2023). Given gelatin's historical lineage, rooted in its chemical properties, this discourse primarily delves into the first application area: photographic gelatin. The second application area emerges as a logical extension, as silver halide photographic materials frequently constitute the foundational elements of gelatin-immobilized systems. Notably, these systems often exhibit nanostructural organization, thereby augmenting their significance. The third application, centered on medical purposes, assumes pivotal importance in contemporary practice, particularly within the medical domain, warranting substantial attention, as emphasized earlier. The fourth facet relates to the utilization of gelatin in nanoparticle form, pertinent to diverse nanotechnological pursuits.

The utilization of fish by-products for nano gelatin production presents a diverse spectrum of possibilities [39]–[41] (Labbani et al. 2023; Hussain et al. 2023; Sharma et al. 2023). These resources, comprising elements from warm and cold aquatic realms, have demonstrated distinct variations in gelatin attributes. Gelatin sourced from warm-water fish, exemplified by the Pangasius catfish bone gelatin, exhibits remarkable gel strength akin to mammalian-derived gelatin. This advancement in the knowledge of fish gelatin properties offers substantial insights for various applications within the gelatin industry and beyond.

II. FISH GELATIN AND *PANGASIUS CATFISH* GELATIN

The published work related to fish gelatin is mainly focused on their extraction process or optimization of extraction, and characterization of obtained fish gelatin. The extraction process normally comprises two main stages: pre-treatment and main extraction. The pre-treatment commonly involves soaking the raw material (fish bones, skin, or fish waste) under certain conditions for example in the acid [42]–[45] (Santiz-Gomez et al. 2019; Venupriya et al. 2023; Mahmoodani et al. 2014; Sanei et al. 2013), alkaline [46], [47](Jeya Shakila et al. 2012; Taheri et al. 2009), or weak acid solvent ([48] Pertiwi et al. 2018; [49] Monsur et al. 2014). Subsequently, the main extraction is carried out using a combination of acid, alkaline [50], [51](See et al. 2015; Koli et al. 2012), water [13], [47], [52](Atma et al. 2018; Shyni et al. 2014; Taheri et al. 2009) and ultrasound assisted [53](Mirzapour-Kouhdash et al. 2019). In terms of characterization, a significant portion of the research analyzes the yielded gelatin [54], [55] (Atma & Taufik, 2021; Fan et al. 2017), examines its chemical composition (with a primary focus on proximate analysis and amino acid composition) [56], [57] (Derkach et al. 2020; Atma et al. 2017), and assesses its physical characteristics such as texture profile (particularly gel strength) and viscosity [46], [47], [58] (Mahmoodani et al. 2014; Jeya Shakila et al. 2012; Taheri et al. 2019).

Various types of fish parts, such as their skin, bones, and another by-product, have been harnessed as sources of gelatin. Broadly, they can be categorized into marine and freshwater fish, sourced from warm and cold aquatic regions [59]–[62] (Yang et al. 2022; Alves et al. 2022; Renuka et al. 2019; Ratnasari et al. 2013). Research findings indicate that gelatin derived from warm-water fish exhibits enhanced physical attributes, such as superior on gel strength, thermostability, and and viscosity, when compared to gelatin sourced from cold-water counterparts [5], [63]–[65] (da Trindade Alfaro et al. 2015; Zhang et al. 2011; Gomez-Guillen et al. 2009; Wang & Regenstein, 2009). Among warm-water fish, the studies for gelatin production were covering Tilapia [14] (Muyoga et al. 2004; Niu et al. 2013), certain parts of shark cartilage (*Isurus oxyrinchus*) [66](Cho et al. 2004), cod head [67](Arnesen & Gildberg 2006), lizardfish scale (*Saurida tumbil*) (Wangtueai & Noomhorm, 2009), king weakfish (*Macrodon ancylodon*) [63] (da Trindade Alfaro et al., 2009), catfish (*Clarias gariepinus*) [45] (Sanaei et al. 2013), Tuna [52] (Shyni et al. 2014), and Pangasius catfish (*Pangasius sutchi*) [13], [58] (Atma & Ramdhani 2017; Mahmoodani et al. 2014). Notably, gelatin extracted from the bones of the Pangasius catfish stands out with its elevated yield among these warm-water sources. Mahmoodani's (2014) [44] research underscores that the gel strength of Pangasius

catfish bone gelatin is on par with gelatin from mammalian sources. Indeed, gel strength is one of essential physical characteristic of gelatin. Furthermore, ash content of gelatin from this fish was 2.6 which is commercial gelatin have ash content ≤ 2.6% (Atma, 2017) [57].

Research on gelatin extracted from Pangasius catfish has been conducted by several teams. Mahmoodani et al. (2014) [44] performed gelatin extraction from Pangasius catfish bones using strong acid in the pre-treatment stage, followed by main extraction with distilled water. The extraction process was optimized using the statistical response surface methodology (RSM). Ratnasari et al. (2013) [62] extracted gelatin from Pangasius catfish skin using citric acid for pre-treatment and distilled water for the main extraction. A study conducted by See et al. (2010) [50] focused on gelatin extraction from the skin of Pangasius catfish, utilizing acetic acid for pre-treatment and distilled water for the main extraction.

In Indonesia, studies on gelatin from Pangasius catfish have also been carried out. For instance, a study reported that the bone enables to produce gelatin. Pangasius sutchi's bone was separated from other waste materials like the head, fin, scales, and viscera. The attached flesh was scraped off the bones with a knife and then easily removed by soaking them in warm water (80–90 °C) for 30 minutes. Fish bones were subsequently washed with tap water and kept in the freezer (-20 °C) for a month. In the pretreatment stage, cleaned bones were minced in a meat grinder before being soaked in four different citrus fruit extracts with a bone solvent ratio of 1:5 (w/v). With varying times (24, 36, 48, and 56 hours) to allow for demineralisation, pretreatment was done at room temperature. The leached bone (ossein) was then separated from the citrus fruits (pretreatment solvent) by centrifugation for 10 min at 10.000 xg 4 °C. The ossein was washed in distilled water until the pH level reached seven, neutralising the protein. Following that, for the main extraction stage, the neutralised ossein was diluted 1:5 with water. Various temperatures (45, 55, 65, and 75 °C) were used during the main extraction, lasting 5 hours [54] (Atma & Taufik 2021). Gelatin extraction from Pangasius catfish bones also involved pre-treatment using citric acid [48] (Pratiwi et al. 2018), acetic acid [68] (Pradarameswari et al. 2018), and pineapple waste [13], [69] (Atma et al. 2018; Atma & Ramdhani 2017). During the pre-treatment stage using pineapple waste, liquid waste was pasteurized to prevent gelatin hydrolysis due to the presence of the bromelain. In these studies, the presence of gelatin had identified based on molecular weight (Mw) using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and specific amino acid percentage, the hydroxyproline. In addition, beside the yield analysis, the study also measured the physical

properties of the obtained gelatin, such as texture profile and gel strength [54], [69] (Atma & Taufik 2021; Atma et al. 2018).

Skin is another Pangasius waste product that can be converted into gelatin. Reported by Azizah et al., (2020) [70], the Pangasius (*Hypophthalmus*) skins were kindly provided by a pangasius fillet company in Karawang, Jawa Barat, Indonesia, and transported to the lab while chilled on ice. The skin preparation was followed based on modification from Zhang et al. (2016) [71]. The pangasius skin was squared off (1 cm x 1 cm) and then washed with tap water. For 12 hours, the skin was submerged in a 0.25% citric acid solution at a skin-to-solution ratio of 1:4 (w/v). Deionised water (DI) was used to wash the skin, and it was then extracted at 65 °C for 7 hours using a 1:1 (w/v) solution ratio and the skin. The product of the extraction process was filtered through two layers of cheesecloth to create a gelatin solution. To turn the gelatin solution into gelatin powder, it was dried in an oven at 50 °C for 15 hours. The yield of gelatin from the extracted pangasius skin was 15.07±1.45% in comparison to the starting material, a figure that is comparable to the (6-19%) reported by Karim and Bhat (2009) [6]. According to See et al. (2010) [50], the yield was 10.78% when the skin was removed over 18 hours at 45 ^{o}C. The type of raw material, the pretreatment procedure, and the extraction temperature are key variables that can affect the yield values.

However, study on Pangasius catfish gelatin or fish gelatin in Indonesia remains quite limited. To our knowledge, no published work has progressed to the stage of fish gelatin application, let alone its development as nanoparticles. Table 1 presents gelatin from Pangasius sp., the extraction techniques, and the gelatin properties.

Table 1. Extraction method of fish gelatin, the yield, and gel strength of gelatin

Part of Pangasius sp. Waste	Pre-treatment	Main extraction	Yield (%)	Gel strength (g Bloom)	References
Skin	0.8 N sodium chloride (NaCl) (1:6 w/v) at 5°C for 10 min.	Distilled water at 45°C for 18 hours	10.78	324.53b ± 7.98	See et al. (2010)[50]
Skin	1% (1:3 b/v) citric acid (pH 3) for 12 h	distilled water at 60°C for 6 h	22	273.58±3.54	Ratnasari et al., (2013)[62]
Skin	0.2 N NaOH and 0.1 N acetic acid	Distilled water at 63.7°C for 2.41 h	23.61	438.34 ± 5.55	Mahmoodani, Ardekani, Fern, et al. (2014)[58]
Skin	0.05 M NaOH followed 0.05 M acetic acid	Distilled water at 50°C.	24.65±2.84	105.30±1.08	Pradarameswari et al. (2018)[68]
Skin	4°C in distilled water, lime followed by tamarind.	Warm distilled water (50°C) for 12 hours	19.72 ± 1.26	282.29± 5.33	Ismail & Wan A Latiff (2019)[72]
Skin	Papain 4% soaking for 25 minutes.	Distilled water at 80°C for 3 hours	4.97	115.17	Suparmi et al., (2021)[73]
Skin	0.05 M NaOH solution for 1 h, 0.3% citric acid for 12 h.	Distilled water at 65 °C for 8 h.	18	220.93	Nurilmala et al., (2023)[74]
Bone	2.74% HCl for ˜ 21 h.	Distilled water at 74.7°C for 5.26 h	13.86	254.7 ± 8.65	Mahmoodani, Ardekani, See, et al. (2014)[44]
Bone	1% citric acid for 48 h.	Distilled water at 75 °C for 5 h	6,14	364,19±0,04	Pertiwi et al., (2018)[48]
Bone	5% HCl for 48 hours	Distilled water at 70°C for 5 h	147.74±0.83	147.74±0.83	Asih et al. (2019)[75]
Bone	The Citrus fruits	Water at 55° for 5 h.	6.26	451	Atma & Taufik, (2021)[54]

III. FISH GELATIN NANOPARTICLES: THE STATE OF THE ART

3.1 Study of Fish Gelatin NPs

In the current era, globally, fish gelatin has been tried to be studied for its development at the nano scale, both as a raw material in the form of gelatin nanoparticles and in composite form with other substances, for the synthesis of carrier or delivery agents, biomaterials, metal catalysts, and antimicrobial film packaging (Figure 1). Some fish species have been explored for the extraction of gelatin as a precursor to nano-products, encompassing Tilapia [76]–[80] (Akbar et al. 2020; Subara et al. 2017; Zhang et al. 2017; Hani et al. 2016; Arfat et al. 2015), Mirror carp [81] (Nuerjiang et al. 2023), Tuna [82] (Dara et al. 2021) and Walleye pollock [16] (Liu et al.2016). However, certain investigations employing gelatin from cold-water fish fail to specify the exact fish species. Studies undertaken by Subara et al. (2017) [77] and Khramtsov et al. (2021) [83] have ventured into developing fish gelatin nanoparticles from Tilapia skin and cold-water fish, respectively.

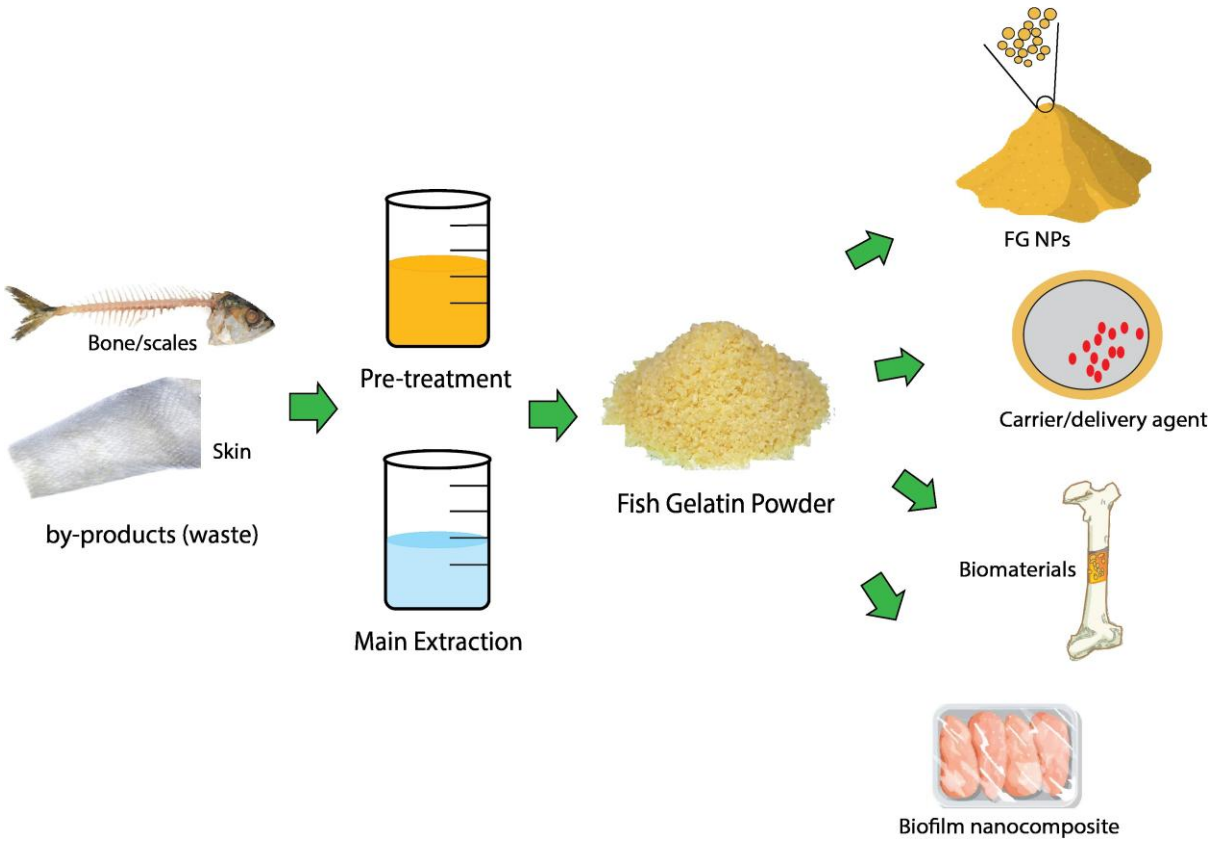

Figure 1. Fish Gelatin Nanoparticles (FGNPs) for different applications

Furthermore, studies conducted by Hani et al. (2016) [79] and Akbar et al. (2020) [76] have respectively delved into gelatin nanofibers and gelatin nanoparticles sourced from Tilapia

skin, employed as carrier agents for *Moringa oleifera* bioactive extract and sunflower-derived biopeptide, respectively. Fish gelatin-based nanoparticles as carrier agents have also been investigated to enhance the bioavailability of polyphenols, with the latter the study employing hybridization of fish gelatin with chitooligosaccharide [84] (Chen et al. 2023).

The evolution of fish gelatin into the nano realm represents a significant stride in contemporary scientific exploration. This trend has witnessed the utilization of diverse fish species, notably Tilapia and cold-water fish [83], [85], [86] (Khramtsov et al. 2021; Kwak et al. 2017; Hosseini et al. 2016), as potential sources for the extraction of gelatin nanoparticles. Furthermore, these nanoparticles are being harnessed for various applications, including their role as carrier agents for bioactive substances [76], [79], [84](Chen et al. 2023; Akbar et al. 2020; Hani et al. 2016), thereby accentuating their relevance in fields spanning biomedicine to sustainable packaging solutions. The ongoing research in this domain underscores the interdisciplinary nature of such studies, bridging the realms of biology, materials science, and nanotechnology to unlock innovative frontiers in sustainable materials and advanced functional systems.

The development of bionanomaterials based on fish gelatin has also been explored. For example, a study on bionanocomposite membranes composed of gelatin from tuna fish skin crosslinked with chitosan and tripolyphosphate (TPP) for applications in biomedicine and tissue engineering was conducted by Dara et al. (2021)[82]. Similarly, research performed by Nerjiang (2023) [81] focused on gelatin nanoparticles derived from the skin of Mirror carp (*Cyprinus carpio*) fish to produce composite films with mechanical properties resembling collagen films. Furthermore, investigations into fish gelatin nanofibrous webs sourced from cold-water fish were undertaken to compare their adhesion capabilities, compatibility, and proliferation in relation to both bulk fish gelatin and mammalian gelatin [85](Kwak et al. 2017). Notably, Wu et al. (2015) [87] synthesized colloidal complex nanoparticles from hydrolyzed gelatin extracted from fish bones, which were self-assembled with tannic acid. This effort aimed at developing materials with the potential to coat intestinal epithelial cells, particularly targeting the Tight Junction (TJ) regions.

The realm of bionanomaterials derived from fish gelatin represents a dynamic frontier in concurrent research. The utilization of fish-derived gelatin in the creation of composite membranes and nanoparticles for diverse applications showcases the adaptability and versatility of this biomaterial. Moreover, the exploration of mechanical properties in composite

films and the assessment of adhesion and compatibility underscore the pivotal role of fish gelatin in constructing materials that closely emulate natural counterparts. The synthesis of complex nanoparticles for cellular interaction signifies a significant stride toward functional coatings with potential implications in biotechnological and medical contexts. This interdisciplinary approach intertwines materials science, biology, and nanotechnology, propelling the development of innovative solutions with broad-reaching applications. Table 2 presenting fish gelatin as NPs, carrier agent and biomaterial.

Table 2. Fish gelatin as NPs, carrier agent and biomaterial

Purposes	Form of Gelatin	Composite	Source of Fish Gelatin	Technique for production	Size of NPs	Size Measurement Principle	Other Characterization	Author(s)
As Raw Material	FGNPs (Single-component particles)	-	Tilapia fish skin	Two-step Desolvation	254±11 nm	Dynamic light scattering (DLS)	Scanning-Electron microscopy (SEM)	Subara et al. (2017) [88]
	FGNPs (Single-component particles)	-	Cold water fish	One-step Desolvation	151±3 until 164±7 nm	DLS, TEM	SEM	Khramtsov et al. (2021)[83]
Carrier	Gelatin nanofiber	-	Black Tilapia skin	Electrospinning+crosslinking	40±5 nm until 120±20 nm	TEM, SEM (diameter distribution)		Hani et al. (2016)[79]
	GNPs		Tilapia	Two step dissolvation	228 to 1305 nm	DLS	SEM	Akbar et al. (2020)[76]
	FG/Chitooligo-NPs		Not reported detail	Maillard reaction	65.2 ± 0.4 until 158.0 ± 1.2 nm	DLS	SEM	Chen et al. (2023)[84]
Biomaterial	Fish gelatin hydrolyste Hybdrid NPs	Tannic acid	Not reported detail	Coacervation	260.8 ± 3.6 nm	DLS, TEM		Wu et al. (2015)[87]
	FG nanofibrous	-	Cold water fish	Electrospinning+crosslinking	Diameter: 91.42 nm until 200.48 nm	SEM		Kwak et al. (2017)[85]
	FG nanocomposite membrane	Chitosan+TPP	Tuna skin	Solvent Casting and Freeze-Drying Method	83 - 193 nm	DLS	SEM	Dara et al. (2021)[82]
	FGNPs	Glutaraldehyde	Mirror carp skin (Cyprinus carpio)	Two-step desolvation	~155–410 nm	DLS	SEM	Nuerjiang et al. (2023)[81]

By thinking out of the box, the study conducted by Sinha et al. (2021) [89] capitalizes on the utilization of gelatin sourced from Labeo rohita scales as a reduction and stabilization agent in the synthesis of silver nanoparticles (Ag NPs). Although, the gelatin was not used as source of nanomaterial, but their role had surprised us as readers. In this study, the synthesis procedure strategically hones in on the optimization of critical reaction parameters, encompassing temperature, pH, and extract concentration, to exert precise control over the synthesis of Ag NPs. The resultant Ag NPs are subjected to meticulous investigation, particularly concerning their catalytic efficacy in the reduction of aromatic nitro compounds in both aqueous and micellar environments. Remarkably, the involvement of fish-derived gelatin introduces a complex interplay within this synthesis process. This intricate dynamic manifests as the gelatin from Labeo rohita scales engages in a multifaceted interaction with the silver ions, thereby acting as a dual role player. Primarily, it serves as a reductive agent, facilitating the conversion of silver ions into their metallic nanoparticulate form. Concurrently, the gelatin imparts an invaluable protective facet by enveloping the Ag NPs, effectively establishing a biomolecular corona. This biomolecular corona furnishes a dual-functionality shield; it augments the generation of Ag NPs by providing the requisite electrons for their reduction while simultaneously functioning as a protective layer, forestalling the premature aggregation of the resultant metallic nanoparticles.

The synthesis of nanocomposites for film applications, particularly in the realm of antimicrobial packaging, based on fish gelatin has garnered significant research attention. A substantial portion of these investigations involves the composite integration of fish gelatin with Zinc Oxide (ZnO) or ZnO nanoparticles (NPs) [16], [78], [80], [90] (Zhang et al. 2017; Liu et al. 2016; Arfat et al. 2015; Rouhi et al. 2013). Additionally, some composites incorporate the biopolymer chitosan, while others feature a combination of Silver-Copper NPs (AgNPs and CuNPs) [91] (Arfat et al. 2017). In assessing the potential of these nanofilms as antimicrobial agents, evaluations are conducted to ascertain their capacity to inhibit the growth of both gram-positive and gram-negative spoilage microorganisms.

Noteworthy studies undertaken by Arfat et al. (2015)[80], Arfat et al. (2016)[92], and Zhang et al. (2017) [78] have respectively synthesized nanofilms from Tilapia fish gelatin, incorporating zinc nanoparticles (ZnO) in tandem with fish protein isolate (FPI), and ZnO NPs+ginger oil respectively. Additionally, Hosseini et al. (2015) [93] and (2016) [86] have

contributed to the field by fabricating nanocomposite films using cold-water fish gelatin reinforced with chitosan NPs. Moreover, Chen et al. (2022) [94] have investigated antibacterial nanofilm composites composed of gelatin from cold-water fish, chitosan, and ZnO. Notably, Arfat et al. (2017) [91] have meticulously prepared and examined the characteristics of antibacterial nanocomposite films originating from gelatin sourced from the bones of cold-water fish, combined with Silver-Copper NPs (AgNPs and CuNPs). The efficacy of these nanocomposite films is evaluated against *L. monocytogenes, E. coli, and S. thyphimurium* and etc [16], [78], [91], [92] (Zhang et al. 2017; Arfat et al. 2017; Arfat et al. 2016; Liu et al. 2016).

This evolving domain epitomizes the merging of innovative materials and advanced functionality. The amalgamation of fish gelatin with diverse additives to engender nanocomposites with antimicrobial properties underscores the potential for these materials to revolutionize packaging and preservation techniques. The exploration of a wide array of composite formulations, the examination of their efficacy against specific bacterial strains, and the comprehensive assessment of their attributes collectively advance our comprehension of how biomaterial-based nanocomposites can pave the way for enhanced food safety and preservation strategies. Table 3 presents nanofilm composite based on fish gelatin.

Table 3. Nanofilm composite based on fish gelatin as antimicrobial film.

Purposes	Form of Gelatin	Composite	Source of Fish Gelatin	Technique for production	Size of NPs	Size Measurement Principle	Other characterization	Inhibited microbe	Author(s)
Antimicrobial film/Packaging	Nanocomposite film (composite)	ZnO nanorods	Not mentioned detail	Solution casting method	-	TEM			Rouhi et al. (2013)[90]
	Nanocomposite film (composite)	Chitosan+TPP	Cold water fish skin	Solution casting method	40-80 nm	DLS for suspension	SEM		Hosseini et al. (2015)[93]
	Nanocomposite film (composite)	ZnO	Tilapia Skin	Solution casting method				*Pseudomonas, Enterobacteriaceae Listeria monocytogenes,*	Arfat et al. (2015)[80]
	Nanocomposite film (composite)	Fish Protein Isolate+ZnO	Tilapia Skin	Solution casting method				*Pseudomonas aeruginosa*	Arfat et al. (2016)[92]
	Nanocomposite film (composite)	ZnO							Liu et al. (2016)[16]
	Nanocomposite film (composite)	ZnO/Chitosan	Walleye pollock	Solution casting method				*L. monocytogenes, S. aureus, E. coli, V. parahemolyticus Staphylococcus aureus, Listeria monocytogenes,*	Liu et al. (2016)[16]
	Nanocomposite film (composite)	Chitosan+TPP	Cold water fish	Solution casting method			FTIR: interaction, SEM	*Salmonella enteritidis and Escherichia coli*	Hosseini et al. (2016)[86]
	Nanofilm (composite)	ZnONPs+Ginger Oil	Tilapia skin	Emulsion casting method		Size of emulsion with mastersizer	SEM	*E. coli & L. monocytogenes*	Zhang et al. (2017)[78]

	Nanocomposite film (composite)	Silver-Copper NPs (AgNPs and CuNPs)	Cold water fish skin	Solution casting method	Particles size each <100 nm (mentioned on the raw materaial charac.	SEM	*L. monocytogenes, E. coli, S. thyphimurium*	Arfat et al. (2017)[91]
	Nanocomposite film (composite)	CM-Chitosan+ZnO	Cold water fish skin	Solution casting method		FTIR: interaction, SEM	*E. coli and S. aureus*	Chen et al. (2022)[94]

3.1 Fish Gelatin Nanoparticle and Nanocomposite Preparation

Several methods have been employed in the synthesis of fish gelatin nanoparticles (NPs) or composite NPs. The methodologies encompass desolvation [76], [77], [81], [83](Nuerjiang et al. 2023; Kramtsov et al. 2021; Akbar et al. 2020; Subara et al. 2017), coacervation [87] (Wu et al. 2015), electrospinning and crosslinking [79], [85] (Kwak et al. 2017; Hani et al. 2016), as well as the Maillard reaction [84] (Chen et al. 2023). Each of these techniques offers distinct advantages and mechanisms for the creation of tailored nanoparticles with specific attributes. All of these methods summarized in an illustration presented on figure 2.

Desolvation serves as a versatile approach, involving the precipitation of dissolved components through the controlled addition of nonsolvent agents. Desolvation is a method frequently employed for generating nanoparticles and microparticles from dissolved materials. This technique operates on the principle of precipitating or aggregating soluble particles within a solution through the removal of the original solvent via the addition of a non-solvent or antisolvent. The desolvation process encompasses several stages, including solution preparation, solvent removal through antisolvent addition, agitation or mixing, particle formation, and eventual separation. Two distinct approaches have been undertaken in the production of fish gelatin nanoparticles using the desolvation method: two-step desolvation [76], [77], [81] (Subara et al. 2017; Akbar et al., 2020; Nuerjiang et al., 2023) and one-step desolvation [83] (Khramtsov et al. 2021). In the two-step desolvation, antisolvent is added twice, sequentially. The antisolvent may comprise acetone, ethanol, or isopropyl alcohol. Within the desolvation technique, a crosslinker agent such as glutaraldehyde is added during the particle formation stage prior to separation via centrifugation.

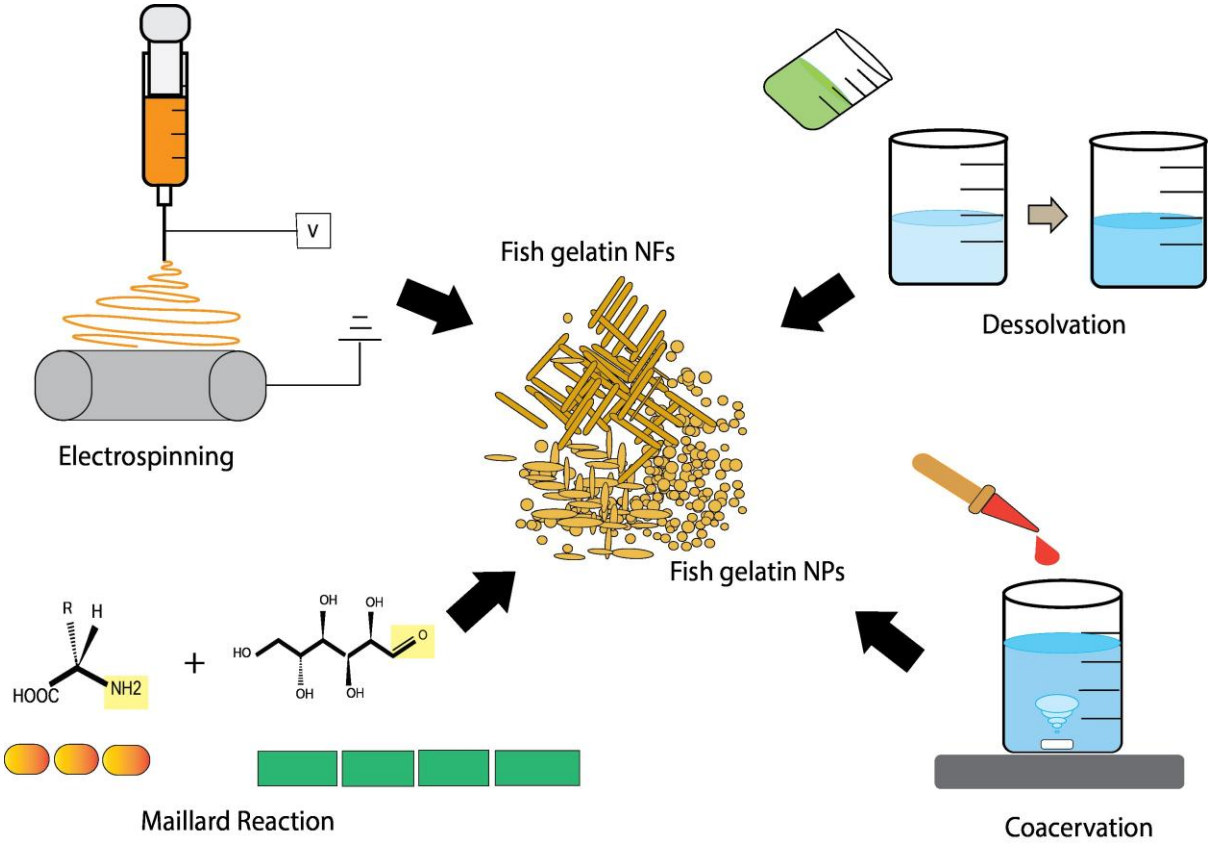

Figure 3. Methods for synthesizing fish gelatin nanoparticles (NPs) or nanofibers (NFs)

Coacervation involves the segregation of colloidal particles from a solution by inducing the formation of an additional liquid phase. The coacervation technique has also been explored in the production of fish gelatin hydrolysate hybrid nanoparticles for use as biomaterials, as studied by Wu et al. (2015) [87]. In this investigation, coacervates were spontaneously generated upon the mixing of fish gelatin solution with tannic acid. Electrostatic interactions and hydrogen bonding were pivotal driving forces behind the creation of the colloidal nanoparticle suspension. This study exemplifies how the coacervation approach can be harnessed to engineer innovative biomaterials through careful manipulation of molecular interactions and phase separation dynamics.

The combination of electrospinning and crosslinking processes facilitates the creation of fiber structures or interconnected networks of nanoparticles by harnessing electrostatic forces, followed by subsequent crosslinking to bolster stability. The underlying principle of electrospinning involves the utilization of an electric field to draw and elongate a charged polymer solution into delicate fibers, overcoming the inherent surface tension of the material.

The application of the electric field induces the polymer solution to form a fine jet, which undergoes stretching and solidification as it advances toward a grounded collector, culminating in the production of nanofibers. Hani et al. (2016) [79] embarked on the production of nanofibers from gelatin sourced from the black Tilapia fish skin, with the intention of developing these nanofibers as delivery agents. The process entailed electrospinning fish gelatin nanofibers using varying concentrations, employing an experimental setup involving high voltage, a syringe pump, and a collector. Similarly, Kwak et al. (2017)[85] focused on generating gelatin nanofibers from cold-water fish for potential biomaterial applications. In their study, fish gelatin nanofibers were produced through the electrospinning method, employing distinct solvent systems—namely, acetic acid/water and trifluoroethanol (TFE).

Conversely, the Maillard reaction exploits the intricate interplay between amino acids and reducing sugars, culminating in the creation of nanoparticles (NPs) endowed with multifaceted functional attributes. In the context of fish gelatin-chitooligosaccharide NPs, the synthesis process involves the preparation of a composite solution comprising gelatin and chitooligosaccharide at pH 6.0. This solution is subjected to incubation at a high temperature of 90°C for a duration of 4 hours, resulting in the formation of a composite product via the Maillard reaction. Subsequently, solutions containing apple polyphenols of varying concentrations are prepared independently. These solutions are then blended in diverse proportions, harnessing the potential of the Maillard reaction and composite formation. Through this methodological approach, the study delves into the characteristics of the resultant particles and investigates how these attributes evolve over time (Chen et al. 2023)[84].

This investigative pursuit underscores the potential of the Maillard reaction to orchestrate intricate nanoscale interactions (Chen et al. 2023)[84], thereby affording a mechanism to engineer nanoparticles with tailored properties. The utilization of composite solutions and the manipulation of reaction parameters further emphasize the intricate control achievable within this methodology. By exploring the transformative journey of these particles and their dynamic attributes, this study extends our comprehension of the complex interplay between molecular constituents and reaction dynamics in the realm of nanoscale materials synthesis.

3.2 Fish gelatin nanofilm composite preparation

The synthesis of composite nanofilms based on fish gelatin has been conducted using two methods: the solution casting method [16], [86], [91], [94] (Chen et al. 2022; Arfat et al.

2017; Hosseini et al. 2016; Liu et al. 2016) and the emulsion casting method [78] (Zhang et al. 2017). In the solution casting method, fish gelatin is dissolved and heated to a specific temperature (45-70 °C), followed by the addition of a plasticizer such as glycerol. This mixture is then combined with a composite solution containing gelatin and other components like ZnO, Chitosan, Ag NPs, or Cu NPs, and homogenized. This stage yields a solution known as the film-forming suspension. Some studies incorporate a degasification step, which involves using a vacuum pump. The resulting film-forming suspension is cast into a silicon resin plate or a rectangular plastic dish and allowed to dry at room temperature. The dried film is subsequently peeled from the mold or plate under controlled temperature and humidity conditions.

On the other hand, the emulsion casting method shares similarities with the solution casting method, but it involves the addition of an emulsified organic solution after mixing fish gelatin with the composite solution. This organic solution has been previously emulsified and is introduced at a specific stage (Zhang et al. 2017)[78].

Both methods exemplify the multifaceted approach to creating composite nanofilms from fish gelatin. The solution casting method showcases the sequential process of preparation, mixing, and casting, culminating in the formation of films with desired properties [16], [80], [86], [91]–[94] (Chen et al. 2022; Arfat et al. 2017; Hosseini et al. 2016; Liu et al. 2016; Arfat et al. 2016; Arfat et al. 2015; Hosseini et al. 2015). In comparison, the emulsion casting method introduces an additional layer of complexity by involving the incorporation of emulsified organic solutions into the process [78] (Zhang et al. 2017). These methodologies contribute to the expanding repertoire of techniques for engineering nanoscale materials, leveraging the inherent properties of fish gelatin and other components to craft advanced composite films with tailored functionalities.

3.3 Characteristics of fish gelatin nanoparticle, nanofiber, or nanocomposite

3.3.1 Biophysical characteristics

Some characteristics need to be analyzed in the production of fish gelatin nanoparticles (NPs). In this review, our focus centers on key attributes, namely size, polydispersity, surface charge, shape (morphology), and, notably, the interactions between fish gelatin and other constituents in the formation of composite nanofilms. Figure 3 depicted approaches that have been used for characterization fish gelatin NPs, NCs or NFs.

The produced fish particles exhibit sizes within the nano and submicron range. The diameter ranges from approximately 40 nm to 200 nm for fish gelatin nanofibers [79], [85]

(Hani et al. 2016; Kwak et al. 2017), while fish gelatin nanoparticles vary between approximately 65 nm and 410 nm [77], [81], [83], [84], [87] (Nuerjiang et al. 2023; Chen et al. 2023; Kramtsov et al. 2021; Subara et al. 2017; Wu et al.2015;). Based on the conducted studies, we conclude that the incorporation of composites does not significantly influence the resulting nanoparticles compared to single fish gelatin. Nonetheless, a more detailed analysis of this aspect is warranted. It is noteworthy that fish gelatin nanofibers from black Tilapia fish skin possess the smallest diameter [79] (Hani et al. 2016). The determination of particle diameters, both for fish gelatin nanoparticles and nanofibers, is primarily achieved through dynamic light scattering (DLS) approach.

DLS, also known as Photon Correlation Spectroscopy (PCS), is a technique employed to measure the size distribution of particles, typically on the nano or micro scale, within a suspension or solution (O'Connell et al. 2023). This method quantifies the movement of particles within a solution, often referred to as Brownian motion, and their interaction with light. The fluctuations of the scattered light resulting from particle motion are captured by a photodetector. Smaller particles exhibit faster fluctuations and consequently contribute to quicker signal fluctuations compared to larger particles [95], [96] (Kazemi et al. 2023; Pravinata et al. 2016). The intensity of these fluctuations is then analyzed using a mathematical concept called the correlation function. The correlation function calculates the correlation between scattered light intensities at different time intervals, enabling the analysis of particle distribution and movement within a solution. Leveraging the Stokes-Einstein Equation and attributing particle movement within a solution to the diffusion coefficient, the hydrodynamic diameter can be determined. Furthermore, the width of the correlation function, referred to as "correlation decay," provides valuable insights into the distribution of particle sizes within the samples, denoted by the polydispersity index (PDI) [97] (Stetefeld et al. 2016). A broader correlation decay signifies a higher PDI, indicative of a wider span of particle sizes [98] (Arnroth 2020). A study undertaken by Khramtsov et al. (2021) [83] illustrated the PDI of fish gelatin nanoparticles synthesized via one-step desolvation ranging from 0.014 to 0.127. Fish gelatin nanoparticles synthesized using the two-step desolvation approach exhibited a PDI approximately ≤ 0.4 [76], [77], [81] (Subara et al. 2017; Akbar et al. 2020; Nuerjiang et al. 2023). Meanwhile, the PDI of fish gelatin/chitooligo-NPs produced through the Maillard reaction ranged from 0.165 to 0.496 (Chen et al. 2023) [84]. Additionally, fish gelatin nanocomposite membranes derived from Tuna skin gelatin displayed pronounced polydispersity (PDI value was 0.535 to 0.890) (Dara et al. 2021)[82].

Particularly, particles with a PDI of 0-0.1 are classified as monodisperse, signifying a high degree of uniformity (Raval et al. 2019) [99]. Particles with a PDI falling within the range of 0.1 to 0.3 denote moderate polydispersity, indicating a relatively uniform distribution of sizes, albeit with a slightly broader size range compared to lower PDIs (Gazzillo et al. 2005). Within the moderate PDI range, most particles exhibit a similarity in size, although a small fraction may deviate in size either larger or smaller. Notably, a PDI range of 0.3-1.0 or >0.7 suggests a wide array of particle sizes within the samples, signifying a mixture containing significantly divergent particle sizes [100] (Mudalige et al. 2019).

Stokes Einstein Equation:

$$D = \frac{KB\,T}{6\,\pi\,\eta\,R} \quad , \quad dH = \frac{KB\,T}{6\,\pi\,\eta\,D}$$

Where K_B is Boltzman's constant, T is temperature, η is viscosity and D is diffusion coefficient, dH is hydrodynamic radius [101] (Nobbmann et al. 2007). And,

Correlation function (c) is defined by:

$C(\Delta t) = (Is\,(t)\;x\;Is\,(t - \Delta t))$, where I_s is light intensity, and t is time (Weiss, 2022)[102]

As per the Stokes-Einstein Equation, it is widely acknowledged that maintaining controlled viscosity and temperature is of first importance in DLS-based size measurements [96] (Pravinata et al. 2016). Consequently, in the context of studies on fish gelatin nanoparticles, the absence of detailed information regarding solution concentrations and viscosity levels results in size measurements being confined to estimations. Moreover, the nanoparticle diameter measurement technique using DLS presents several inherent limitations. For instance, accuracy diminishes when particles within a solution are excessively large, and as the method assumes the particles to be in a spherical form within the solution. It is also sensitive to the presence of particle aggregates in a solution, while occasionally failing to reflect the particle size in dry solid state [97] (Stetefeld et al. 2016). Therefore, alternative techniques are employed to mitigate these shortcomings, such as scanning electron microscopy (SEM) and transmission electron microscopy (TEM), which will be elaborated upon subsequently.

The stability of fish gelatin nanoparticles (NPs) is assessed based on their zeta potential or electrokinetic potential values. The zeta potential values for fish gelatin NPs derived from Tilapia range from 21 ± 2.2 to 29 ± 1.6 mV [76] (Akbar et al. 2020), while those from Mirror

carp fish skin measure between 18.1 to 19.8 mV [81] (Nuerjiang et al. 2023). In the study conducted by Wu et al. (2015) [87], fish gelatin hydrolyste hybrid NPs exhibited a zeta potential of 17.5 ± 0.5 to 22.7 ± 0.9 mV. Furthermore, fish gelatin/chitooligo-NPs display a zeta potential of 21 ± 2.2 to 29 ± 1.6 mV (Chen et al. 2023)[84]. The zeta potential is a crucial parameter for predicting colloidal stability, particle-particle interactions, and the charge of particles or colloids in a solution. A zeta potential of 0 mV is categorized as slight to no stability, ≤15 mV signifies less stability with slight settling, while ≥30 mV indicates moderate stability [103] (Liu et al., 2020). While zeta potential measurements are typically performed using the same instrument as the size distribution analysis, a dynamic light scattering (DLS) with a zetasizer, zeta potential measures the surface charge of colloidal particles using a separate technique known as "electrophoretic mobility" or "phase analysis light scattering (PALS)." In this method, an electric field is applied to the sample, and the velocity of charged particle movement in response to the electric field is measured (Thomas et al. 2002; McNeil-Watson et al. 1998). Utilizing a folded cuvette with two poles, it is possible to determine how quickly particles in a solution move toward positive or negative poles. Zeta potential values are calculated based on electrophoretic mobility at 25°C using the Smoluchowski equation (Sze et al. 2003)[104].

$\zeta = \frac{4\pi\eta\mu}{\varepsilon}$, in which $\mu = \frac{\nu}{E}$

Where ζ is zetapotential, η is viscosity buffer, μ is electrophoretic mobility, ε is the electric constant of the solvent, ν is velocity, and E is electrical field [105] (Doroszkowski 1999)[106].

Transmission electron microscopy (TEM) is a powerful imaging technique that enables scientists to observe the morphology (shape), size, and aggregation of nanomaterials at an exceptionally high resolution. In principle, when an electron beam strikes a sample in TEM, it undergoes interactions with the atoms within the sample. Those electrons that do not interact with the atoms are transmitted through the sample. These transmitted electrons then encounter an array of electromagnetic lenses collectively known as the objective lens system. These lenses serve to magnify the transmitted electrons and concentrate them into a focused, coherent beam. This focused electron beam subsequently impinges upon a fluorescent screen or digital camera, where the interactions between the electrons and the specimen lead to variations in electron intensity. These variations combine to create an image characterized by exceptional

spatial resolution [107]–[110] (Hattar et al. 2021; Bridson et al. 2014; Malkoch et al. 2012; Pennycook 2005).

Research conducted by Nuerjiang et al. (2023) [81] employed TEM to investigate fish gelatin nanoparticles, revealing their spherical shape, monodispersity, and sizes corresponding to those measured using dynamic light scattering (DLS). This study concluded that an increase in glutaraldehyde concentration resulted in reduced nanoparticle aggregation. Conversely, fish gelatin nanoparticles without glutaraldehyde or with fish gelatin-to-glutaraldehyde ratios of 12:1 or 12:2 led to the formation of nanoparticle aggregates. Similarly, Wu et al. (2015) [87] observed spherical fish gelatin nanoparticles with sizes consistent with those determined by DLS analysis. An increase in tannic acid concentration, however, induced nanoparticle aggregations.

Scanning electron microscopy (SEM) has been used in the characterization of fish gelatin nanoparticles, nanofibers, and nanocomposite films. Although SEM images may not precisely depict the actual size of nanoparticles, this technique can excel in visually representing fish gelatin micro and nano-scale features, such as particles [83] (Khramtsov, 2021) or fibers [79], [85] (Hani et al., 2016; Kwak et al., 2017). Recent applications of SEM on fish gelatin nanocomposite have proven particularly valuable in assessing complexation between fish gelatin and its composites. For instance, in a study by Chen et al. (2022)[85], SEM analysis was employed to evaluate the incorporation of ZnO into the gel carboxymethyl cellulose (CMC) and fish gelatin matrix for composite nanofilms used in food packaging. The results demonstrate the compatibility between ZnO and CMC/fish gelatin. Furthermore, SEM has been utilized to analyze cross-sectional thickness and surface morphology, specifically smoothness and roughness, of composite nanofilms. For example, the CMC/fish gelatin/ZnO nanocomposite film exhibited uneven roughness and a fine pore structure (Chen et al., 2022)[94]. The incorporation of fish gelatin nanoparticles into collagen fibers resulted in a homogeneous, compact, flat, and smooth nanocomposite film [81] (Nuerjiang et al., 2023). Similarly, studies have shown that pristine fish gelatin possesses a smooth and homogeneous microstructure, with minimal changes observed when a small quantity of chitosan nanoparticles is added. However, structural alterations become apparent with increased CsNP concentrations [86] (Hosseini et al., 2016). Arfat et al. (2017) [92] observed a similar phenomenon, where SEM images indicated that the smooth surface of neat fish gelatin film became rougher when combined with 2% Ag-Cu.

3.3.2 Functional Properties of Nanocomposites based on Fish Gelatin

Characterization has been conducted on fish gelatin-based nanocomposite films, encompassing mechanical strength assessment as a pivotal component. The introduction of nanoparticles in biopolymer film fabrication has been demonstrated to enhance tensile strength (TS) and Young's modulus (YM) [90], [94] (Chen et al., 2022; Rouhi et al., 2013). This augmentation in the mechanical properties of composite films can be attributed to the robust intermolecular forces operating between carboxymethyl chitosan (CMCS) and gelatin. Notably, Rouhi et al. (2013) [90] observed that the incorporation of ZnO nanorods into gelatin films also led to a reduction in elongation at break (EAB). This phenomenon is intricately linked to moisture content and interfacial interactions between ZnO nanorods and the biopolymer matrix. Moreover, a reduced plasticizer content has been found to elevate TS and YM while diminishing EAB, owing to the pronounced mechanical interplay between the filler materials and the film matrix.

The incorporation of ZnO nanoparticles into CMCS and gelatin composite films derived from cold fish skin has been instrumental in enhancing film plasticity and mechanical strength [94] (Chen et al., 2022). This improvement is underpinned by strong interactions between CMCS, gelatin, and ZnO nanoparticles, closely related to their micron-scale structural characteristics, concurrently contributing to increased crystallinity [111](Gomes-Estaca et al., 2011).

Conversely, the utilization of Ag-Cu nanoparticles at a concentration of 2% in gelatin films sourced from cold fish skin has been found to bolster tensile strength (TS) while diminishing it at nanoparticle concentrations exceeding 2% [91] (Arfat et al., 2017). The excessive loading of nanoparticles beyond 2% impairs the film matrix's capacity to accommodate them, consequently undermining mechanical properties. EAB exhibited a declining trend with increasing nanoparticle concentration, attributed to heightened film rigidity. Notably, Ag-Cu nanoparticles exhibit hydrophobic characteristics and tend to aggregate at specific concentrations, impeding protein domain organization and the amorphous network of gelatin films, thereby diminishing their mechanical attributes [91] (Arfat et al., 2017).

In summary, the characterization of fish gelatin-based nanocomposite films involves a comprehensive evaluation of their mechanical properties. The incorporation of nanoparticles, such as ZnO and Ag-Cu, yields notable enhancements in tensile strength and Young's modulus

while influencing elongation at break. These improvements are ascribed to intricate intermolecular interactions, micron-scale structural features, and nanoparticle concentration effects, which collectively contribute to the overall mechanical performance of the films.

The introduction of chitosan nanoparticles (CSNPs) at an 8% concentration results in a noteworthy improvement in the tensile strength of gelatin films derived from cold-water fish skin, enhancing it to 1.28 ± 1.02 MPa, compared to pure gelatin films devoid of chitosan nanoparticles, which exhibited a tensile strength of 7.44 ± 0.17 MPa [93] (Hosseini et al., 2015). The inclusion of CSNPs leads to the formation of more rigid films due to the reinforcing influence of these nanoparticles within the polymer matrix. However, an inverse correlation is observed in the elongation at break (EAB), showing a diminishing trend with increasing CSNP content, declining from 102.04 ± 28.38% to 32.73 ± 7.38%. This decline signifies an escalation in the brittleness of the fish gelatin film upon chitosan supplementation, primarily attributable to the reduction in free volume among polymer chains owing to heightened intermolecular attractive forces. Consequently, the polymer network becomes densely packed, rendering it less permeable. Furthermore, the elastic modulus (EM) of the gelatin film experiences a substantial increase of 60% with the augmentation of CSNP content, elevating it from 287.03 ± 14.25 to 467.2 ± 49.63 MPa. The EM value of the nanocomposite film from cold-water fish skin surpasses that of chitosan-containing gelatin films derived from fish bone, as reported by Jeya Shakila et al. in 2012, which stood at 378 MPa. This notable enhancement in elastic modulus holds considerable promise, particularly in the context of food packaging applications.

In addition, films fabricated from gelatin extracted from fish skin and fish protein isolates exhibit distinct characteristics under varying pH conditions. Alkaline pH conditions facilitate greater protein solubility, resulting in more substantial molecular stretching. Partial denaturation of myofibrillar proteins and the elongation of gelatin molecules (alpha 1 and alpha 2 chains) contribute to the formation of robust film networks characterized by high cross-linking densities. Films produced from gelatin extracted from fish skin and fish protein isolates, both with and without the incorporation of NP-ZnO, exhibit higher tensile strength (TS) and lower EAB values when formulated under pH 11 conditions, in contrast to those produced at pH 3 [92] (Arfat et al., 2016). The incorporation of NP-ZnO into films at pH 11 enhances the formation of salt bridges between Zn^{2+} ions and negatively charged residues, particularly COO^{-} groups located on protein side chains or C-termini. This phenomenon contributes to an increase in TS in the FPI/FSG-ZnO nanocomposite films. Notably, nanoparticles possess high

surface energy and a substantial specific surface area, fostering robust interfacial interactions between the polymer and nanoparticles, thereby resulting in significant improvements in polymer properties [112][90] (Kovacevic et al., 2008; Rouhi et al., 2013).

3.3.3 Antimicrobial Properties

The incorporation of essential oils has been shown to enhance antimicrobial activity in bio-composite films. Gelatin-based composite films derived from cold-water fish skin, combined with chitosan nanoparticles, initially exhibit no significant antimicrobial activity against bacteria such as S. aureus, L. monocytogenes, S. enteritidis, and E. coli. However, upon the addition of oregano oil, these films manifest remarkable antimicrobial efficacy against all four bacterial strains [86] (Hosseini et al., 2016). A concentration of 1.2% (w/v) oregano oil effectively inhibits bacterial growth, yielding inhibition zones ranging from 26.3 to 33 mm. This antimicrobial activity is attributed to the presence of compounds such as carvacrol, thymol, and p-cymene [113] (Corrales et al., 2014). Carvacrol and thymol can disrupt the outer membrane of gram-negative bacteria, leading to the release of vital cellular components (Burt, 2004), while p-cymene, a biological precursor of carvacrol, exhibits hydrophobic properties, causing the cytoplasmic membrane to expand more than carvacrol [114] (Ultee, Bennik, & Moezelaar, 2002).

Furthermore, when meat is packaged using gelatin films derived from Tilapia fish skin, augmented with 3% nanoparticle ZnO (w/w, gelatine) and 80% ginger essential oil (GEO) (w/w, gelatine), a lower total bacterial count is observed compared to control films and films with varying concentrations (10%, 20%, and 40%) of ginger essential oil [78] (Zhang et al., 2017). The total bacterial counts recorded are 4.6, 4.7, and 2.8 log CFU g-1 for psychrotrophic, mesophilic, and LAB bacterial groups, respectively. These counts are significantly lower ($P < 0.05$) than those found in control films and meat not packaged with the film. All tested samples exhibit bacterial counts below the threshold, except for films containing 10% GEO. The antimicrobial capability of essential oils, including ginger essential oil, is attributed to their ability to enhance cell membrane permeability and induce cytoplasmic leakage. Hydrophobic compounds within these oils can penetrate the double layers of phospholipids and interact with enzymes situated on the membrane [115] (Lee et al., 2016). Notably, at the same GEO concentration, gram-positive bacteria, like *L. monocytogenes*, are more susceptible than gram-negative bacteria such as *E. coli*. This discrepancy arises from the complex structure of gram-negative bacterial cell walls, which consist of a thin peptidoglycan layer, as opposed to the

thick and multi-layered peptidoglycan structure of gram-positive bacteria [116] (Arfat et al., 2014). Gram-negative bacteria are less sensitive due to the presence of an outer lipopolysaccharide membrane, which restricts the diffusion of hydrophobic agents in antibacterial substances. The outer membrane of gram-negative bacteria does not readily absorb Zn^{2+} ions released from ZnONPs and hydrocarbon cyclic hydroxyl groups in GEO compared to gram-positive bacteria [117] (Shahmohammadi & Almasi, 2016). Consequently, the inhibition of foodborne pathogenic microbes in FSG-ZnONP films is enhanced through the incorporation of GEO, especially against gram-positive bacteria.

The combination of gelatin and chitosan demonstrates the potential to inhibit the growth of microorganisms, thereby slowing down the spoilage process and extending shelf life, particularly at low temperatures. The addition of zinc oxide nanoparticles (ZnO NPs) at a 0.6% concentration to nanocomposite films derived from gelatin extracted from walleye pollock (Theragra chalcogramma) skin and chitosan significantly enhances inhibition zones against bacteria including S. aureus, L. monocytogenes, E. coli, and V. parahemolyticus, measuring 8.50, 5.58, 7.91, and 6.97 mm, respectively. Gelatin/chitosan films containing ZnO exhibit substantially elevated antimicrobial activity, primarily due to photocatalysis processes and metal ion release [118] (Yang et al., 2008). The release of Zn^{2+} ions from ZnO NPs penetrates the microbial cell walls, impacting bacterial cell viability. ZnO is also known to mediate the production of hydrogen peroxide (H2O2), leading to membrane damage in bacterial cells [119] (Tayel et al., 2011). This is further corroborated by research conducted by Arfat et al. (2016), demonstrating that ZnO nanoparticles incorporated into FPI/FSG films exhibit higher antibacterial activity against *L. monocytogenes* and *P. aeruginosa* compared to films lacking ZnO nanoparticles.

Furthermore, composite films containing CMCS/Gelatin derived from cold-water fish skin and nano ZnO display greater antibacterial activity against E. coli compared to S. aureus (with antibacterial rates of 99.20% against Escherichia coli and 84.70% against Staphylococcus aureus) [94] (Chen et al., 2022). The variance in antibacterial efficacy is attributed to structural differences in the cell walls of these two bacterial strains. While the cell wall of gram-negative bacteria is complex, it consists of only a thin peptidoglycan layer. In contrast, gram-positive bacteria possess thick peptidoglycan layers with multiple levels (Anitha et al., 2012). When zinc ions released by nano zinc oxide traverse the cell layers, various reactive oxygen species are generated within the cell, resulting in cell death [120] (Pal et al., 2007).

Lastly, composite films made from gelatin extracted from cold fish skin and incorporating Ag-Cu nanoparticles at concentrations ranging from 1% to 4% demonstrate antibacterial activity against L. monocytogenes and S. typhimurium. The inhibitory mechanisms of Ag-Cu NPs against these bacteria involve the release of silver or copper ions, which can penetrate bacterial membrane pores and gaps, interact with disulfide or sulfhydryl enzyme groups, or adhere to negatively charged bacterial cell walls, disrupting metabolic processes, or causing bacterial cell wall rupture, ultimately leading to cell death [121] (Dizaj et al., 2014).

IV. FISH GELATIN NPS AND NCS FROM PANGASIUS CATFISH V.

As far as our current knowledge extends, there have been no published research findings specifically concerning fish gelatin nanoparticles, nanofibers, or composites derived from Pangasius catfish/Patin. However, if gelatin from other fish, especially those from warm-water species like Tilapia [76], [77], [79] (Akbar et al. 2020; Subara et al. 2017; Hani et al. 2016) and Tuna [82] (Dara et al. 2021), can be developed into nanomaterials, it is reasonable to assume that Pangasius catfish gelatin can be developed as well. The process of developing Pangasius catfish gelatin into nanoparticles can follow similar techniques used in the development of gelatin nanoparticles from Tilapia fish skin, such as the two-step desolvation method [76], [77], [81] (Nuerjiang et al. 2023; Akbar et al. 2020; Subara et al. 2017), or into nanofibers using electrospinning [79], [85] (Kwak et al. 2017; Hani et al. 2016). Furthermore, Pangasius catfish gelatin can also be employed as a constituent in nanofilm composites alongside ZnO [16], [80], [94] (Chen et al. 2022; Liu et al. 2016; Arfat et al. 2015). Nevertheless, optimization of the fabrication process and characterization of the final products are essential to compare them with nanoparticles from other fish sources or from commercial products.

Actually, more than a decade ago, techniques for producing gelatin nanoparticles from mammalian gelatin were already studied, but these techniques have not yet been applied to fish gelatin. These techniques include emulsification-solvent evaporation [122], [123] (Bajpai & Choubey 2006; Choubey & Bajpai, 2010), reverse phase microemulsion [124] (Gupta et al. 2004), nanoprecipitation [125], [126] (Khan et al. 2013; Lee et al. 2012), and self-assembly [127], [128] (Li & Gu, 2011; Chen et al. 2010). Figure 4 shows other techniques that potentially can be adopted to produce fish gelatin NPs.

The emulsification-solvent evaporation technique is based on a single water-in-oil (W/O) emulsion, where the aqueous phase containing gelatin is added dropwise into the organic phase while stirring. This process forms an emulsion, which is then cooled to around 5°C to promote nanoparticle formation, followed by crosslinking. In this technique, if the nanoparticles are used as a delivery vehicle, the bioactive compound is initially dissolved depending on its solubility. If the bioactive compound is soluble in water, it is dissolved along with the gelatin solution in the aqueous phase. However, if it is soluble in organic solvents, it

is mixed and dissolved in the organic phase first [122], [123], [129], [130] (Yasmin et al. 2017; Sahoo et al. 2015; Choubey & Bajpai, 2010; Bajpai & Choubey 2006).

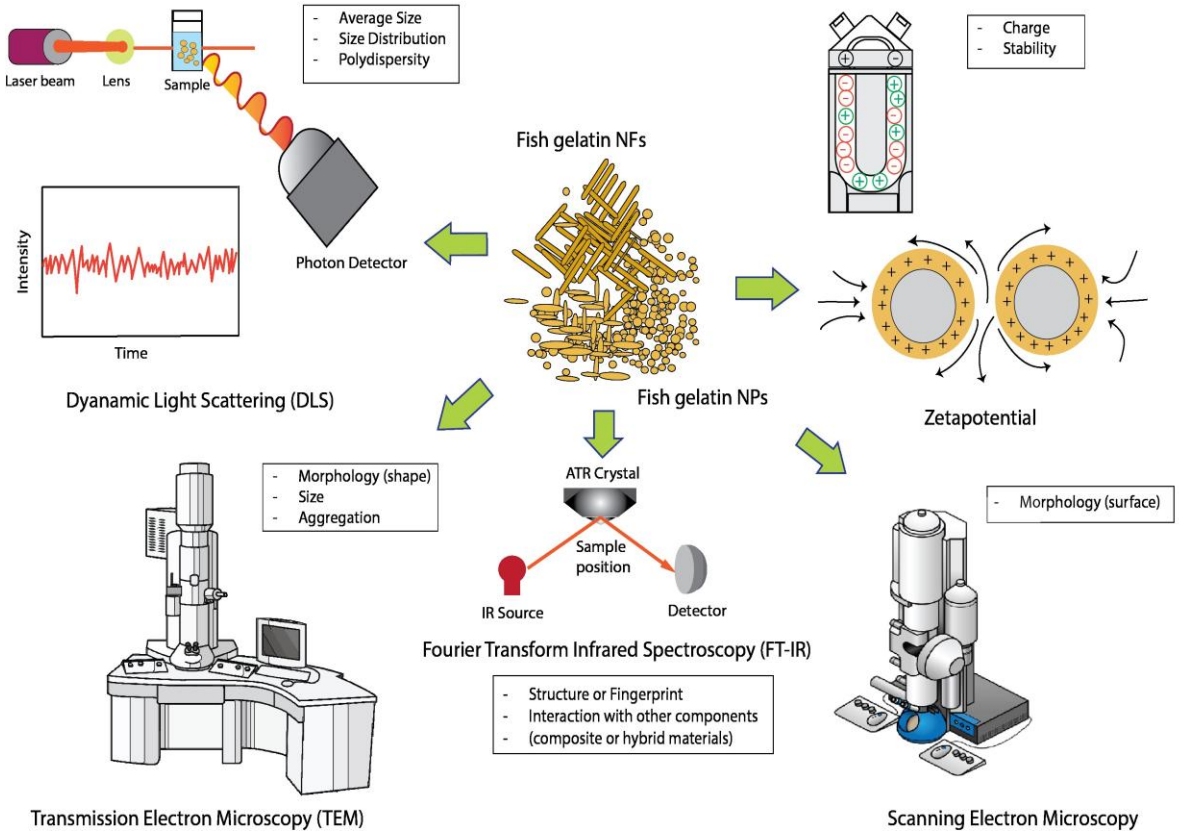

Figure 4. Characterization of fish gelatin nanoparticles and nanocomposites

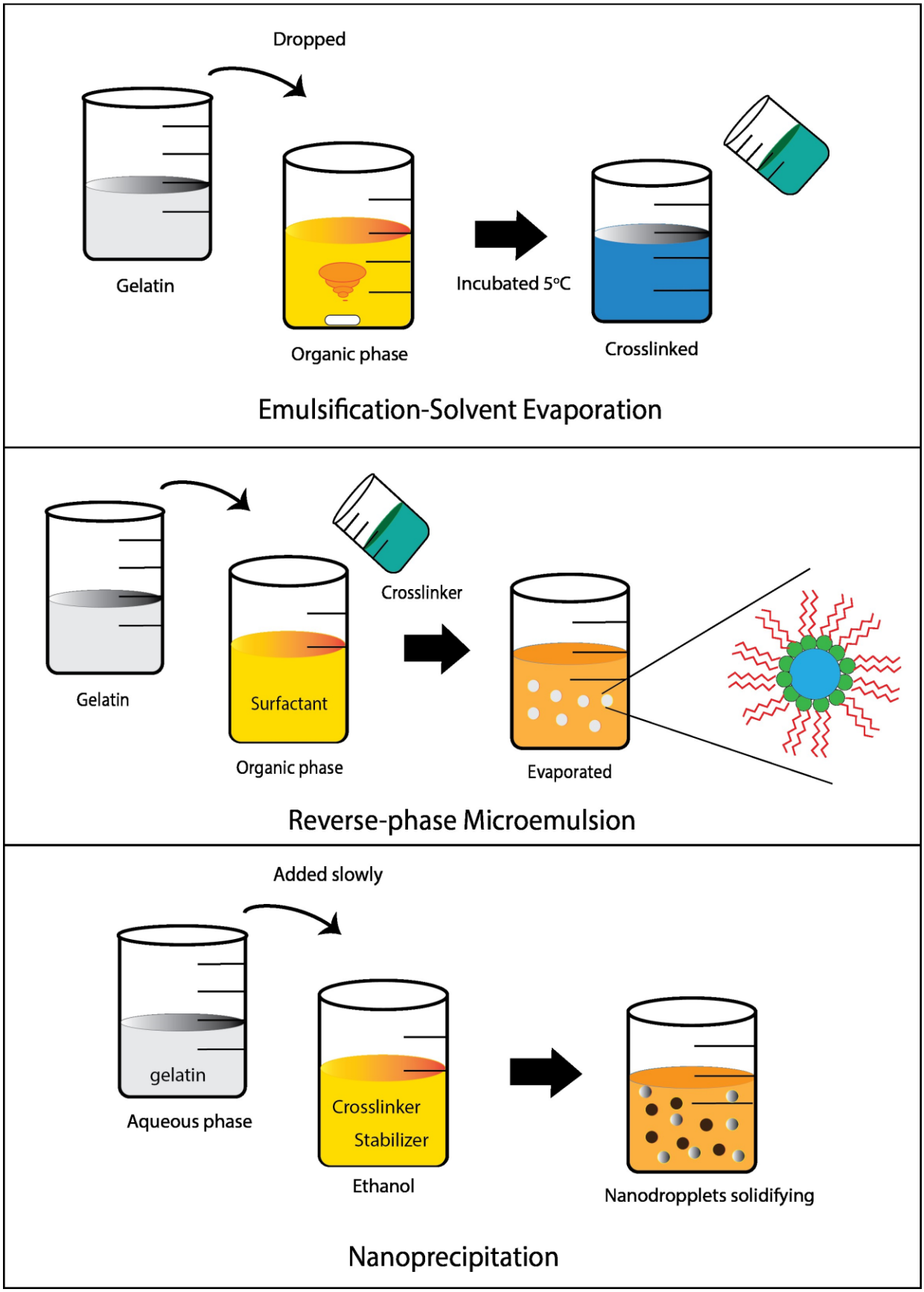

Figure 4. Another option techniques for synthesis FG NPs

Gelatin nanoparticles can also be synthesized using the reverse phase microemulsion technique. In this method, the gelatin solution is poured into the organic phase containing a surfactant, followed by the addition of a crosslinker. The crosslinker acts within the core or internal emulsion (W/O emulsion). The surfactant stabilizes the emulsion by providing its hydrophobic tail to the external part, interacting with the organic phase, while its hydrophilic head interacts with the gelatin in the internal/aqueous phase. Subsequently, evaporation is carried out to remove the organic solvent phase [124], [129]–[131] (Hathout & Metwally, 2019; Yasmin et al. 2017; Sahoo et al. 2015; Gupta et al. 2004).

Another technique is nanoprecipitation, where the gelatin in the aqueous phase is added slowly into ethanol, which serves as the non-solvent phase containing a crosslinker and stabilizer. The mixing process induces interface turbulence due to limited mixing ability, and as turbulent mixing continues, tiny droplets are formed on the nanoscale, called "nanodroplets." These nanodroplets are inherently unstable due to the immiscibility of the two liquids, but the stabilizer prevents them from separating. Over time, the solvent gradually diffuses and spreads into the surrounding liquid, leaving solid particles suspended in a solution [125], [126], [129]–[131] (Hathout & Metwally, 2019; Yasmin et al. 2017; Sahoo et al. 2015; Khan et al. 2013; Lee et al. 2012).

The last technique that can be used to produce fish gelatin nanoparticles is self-assembly. Self-assembly can be utilized to create gelatin nanoparticles for encapsulating bioactive compounds without external process intervention. Essentially, nanoparticles are formed due to hydrogen bonding and hydrophobic interactions either among gelatin structures or with bioactive compounds. Gelatin is a protein with a relatively high content of hydrophobic amino acids such as proline, hydroxyproline, leucine, isoleucine and phenylalanine. The protein structure can be loose and extended in a random coil conformation, and importantly, it has numerous sites on its surface for interacting with other molecules [127], [128], [130] (Sahoo et al. 2015; Li & Gu, 2011; Chen et al. 2010).

In light of the extensive studies conducted on gelatin and fish gelatin nanoparticles (NPs), it is evident that a meticulous optimization process is essential to establish a robust protocol for the production and advancement of fish nanoparticles derived from Patin fish gelatin. This optimization entails a comprehensive series of key stages. Firstly, the selection of an appropriate gelatin extraction methodology from Patin fish waste forms the foundational

step. Subsequently, the characterization of the extracted liquid gelatin is imperative for understanding its properties and quality. Following this, the synthesis of gelatin NPs demands the application of techniques tailored to the desired product specifications. These may include the development of pure gelatin-based particles, fibers, or the creation of hybrid and composite fish nanogelatin structures. The fourth stage necessitates the comprehensive characterization of the produced gelatin NPs, nanofibers, or other nanoproducts to ensure their conformity with predetermined criteria. The ensuing stages encompass the isolation, thorough washing, and purification of these products, culminating in the sixth stage, which involves the drying process. The dried product is then subjected to a detailed characterization in the seventh stage. Finally, the protocol must encompass stability testing throughout storage, accompanied by a rigorous specification analysis. This multifaceted approach ensures the systematic development of Patin fish gelatin-based nanoparticles, fostering a robust foundation for future research and industrial applications. Figure 5 presenting steps that potentially enable to follow in order to develop fish gelatin NPs.

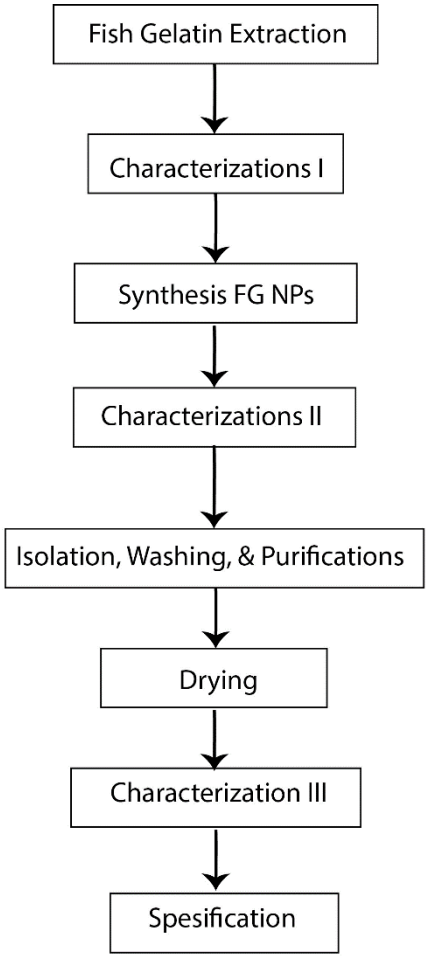

Figure 5. Flow chart steps for FG NPs development from down stream to up stream

V. CHALLENGE AND OPPORTUNITIES ON NPS AND NANOCOMPOSITE FROM PANGASIUS SP. GELATIN

The endeavor to develop fish gelatin and fish gelatin nanoparticles (NPs) from Pangasius catfish into nanomaterials presents an intriguing avenue of research, accompanied by a host of formidable challenges. As of now, no definitive formula has emerged to address these multifaceted issues. These challenges encompass the low extraction yield of gelatin from raw materials [13], [48], [54], [69] (Atma & Taufik 2021; Atma et al. 2018; Pratiwi et al. 2018; Atma & Ramdhani et al. 2017), the inherent weakness of fish gelatin in terms of gel strength [68] (Pradarameswari et al. 2018), prevalent usage of organic solvents [45], [58] (Mahmoodani et al. 2014; Sanaei et al. 2013) and crosslinking agents in the synthesis processes [86], [93] (Hosseini et al. 2016; Hosseini et al. 2015), and the complex regulatory landscape surrounding the commercialization of nanomaterials worldwide like in the European which also implicate to Indonesia [132], [133] (Pavlicek et al. 2021; Lugani et al. 2021). In this section, each of these challenges is elucidated in detail, with an exploration of potential opportunities and solutions that merit pursuit.

*Low extraction yield of gelatin from raw materials***.** The primary challenge encountered in this pursuit is the modest extraction yield of gelatin from raw materials (Pratiwi et al. 2018; Atma & Ramdhani 2017). Specifically, the process of converting raw materials into gelatin yields less than optimal results. While data related to the yield of nanoparticles from fish gelatin remains scarce, it is reasonable to infer that the yield is even lower. To address this, it is imperative to secure a consistent and ample supply of raw Pangasius catfish gelatin material. This necessitates a meticulous approach to optimizing the production of Pangasius catfish gelatin, leveraging statistical methodologies such as response surface methodology (RSM) to ascertain the ideal conditions for pre-treatment and main extraction [58], [74], [134] (Nurilmala et al. 2023; Jakhar et al. 2014; Mahmoodani et al. 2014). Notably, a shift from traditional batch extraction methods to continuous extraction techniques could hold the potential for yield enhancement [135] (Nasrallah et al. 1993). Additionally, under the conventional batch extraction approach, ossein byproduct, which constitutes a substantial portion of the initial raw material weight, is often underutilized. Implementing strategies to re-extract ossein could bolster gelatin yield significantly.

*Weak gel strength of fish gelatin***.** Another critical challenge lies in the inferior gel strength of fish gelatin relative to its mammalian counterparts, thereby limiting its suitability

for applications requiring robust film-forming capabilities. This limitation can be overcome through judicious blending of fish gelatin with compatible materials, as demonstrated in previous studies on fish gelatin nanocomposites. Furthermore, the augmentation of fish gelatin with other polymer substances presents a viable strategy to bolster its gel strength, particularly in contexts such as biomaterials or coatings. Promising candidates for such blending include alginate [136], [137] (Maihemuti et al. 2023; Sow et al. 2019), chitosan [138], [139] (Lin et al. 2020; Uranga et al. 2019), or other proteinaceous materials [140], [141] (Aksun Tümerkan et al. 2023; Demir et al. 2022). Moreover, the illustrative examples are in the review conducted by Atma (2022)[142], which present synthesized a hybrid fish gelatin-composite, resulting in improved gel strength while concurrently embracing principles of sustainability and safety.

Utilization of organic solvents and crosslinker agents. The third critical challenge revolves around the prevalent utilization of organic solvents and crosslinking agents in the process of synthesizing fish gelatin nanoparticles. This practice, while effective, introduces concerns related to biocompatibility, given the inherent toxicity of such agents. To circumvent this challenge, a pivotal strategy is the adoption of more environmentally sustainable nanogelatin production techniques that circumvent the need for organic solvents. Such techniques may involve coacervation [87], [130] (Sahoo et al. 2015; Wu et al. 2015) or self-assembly processes [127], [128], [130] (Sahoo et al. 2015; Li & Gu, 2011; Chen et al. 2010) devoid of crosslinking agents. Alternatively, crosslinkers may be replaced with naturally occurring polymers possessing counteractive charge properties relative to fish gelatin. In practice, crosslinkers primarily serve to facilitate interactions between gelatin and composite materials or gelatin clusters. Recent studies have successfully forged polyelectrolyte complexes by harnessing ionic interactions between gelatin and naturally charged biopolymers. These interactions, synergizing with hydrogen bonding and hydrophobic forces, impart robust binding capabilities between gelatin and its biopolymeric counterparts [143], [144] (de Oliveira et al. 2019; da Silva et al. 2015). This approach also absolutely categorized as green nanotechnology for sustainable environment [95], [145] (Ehtesabi et al. 2023; Kazemi et al. 2023).

*Regulatory complexities***.** The final challenge pertains to the intricate regulatory landscape governing the commercialization of nanomaterials in Indonesia. These regulations present a formidable hurdle to the development and application of nanomaterials. For instance, nano-sized components within products, including proteins such as gelatin, are subject to stringent non-food materials legislation in certain regions, posing substantial compliance and

investment challenges for both laboratory research and industrial-scale production ([146], [147] Talebian et al. 2021; Laux et al. 2018). In light of these regulatory nuances, customizing or formulating fish gelatin to achieve specific size ranges, such as the submicron (100-1000 nm) scale, emerges as a pragmatic approach [148] (Guerra-Garces et al. 2022). Within this submicron range, fish gelatin can be managed akin to conventional components or ingredients, aligning seamlessly with established food regulations and circumventing potential regulatory bottlenecks.

In conclusion, the development of fish gelatin and fish gelatin nanoparticles from Pangasius catfish into nanomaterials is an intricate pursuit marked by multifaceted challenges. These challenges, ranging from suboptimal extraction yields to gel strength limitations, reliance on toxic synthesis processes, and intricate regulatory frameworks, necessitate a comprehensive and innovative approach. Addressing these challenges holds the potential to unlock the vast possibilities of fish gelatin and its nanoparticles in various scientific and industrial applications, ultimately enriching both the scientific discourse and industrial landscape.

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