Ambidextrous: Journal of Innovation, Efficiency and Technology in Organizations

https://journal.takaza.id/index.php/ambidextrous

Vol. 5, No. 01, 2026, pp. 160-170
E-ISSN: 3031-7002

E-mail:ambidex@takaza.id

The Effect of Non-Thermal Energy Exposure on the Unfolding and Gelation of Local Fish Protein: A Biochemical Approach: A Systematic Literature Review

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Article History:
Received : 31-10-2026
Revised : 10-11-2026
Accepted : 12-11-2026

Keywords: ***Ultrasound; High Pressure Processing; Unfolding; Fish Protein; Gelation***

Abstract: *Myofibrillar proteins play an essential role in determining the texture and gel properties of fish-based products. Non-thermal technologies, particularly ultrasound and High Pressure Processing (HPP), offer alternatives for modifying protein structures without intensive heating. This study aims to analyze the effects of these technologies on protein unfolding, conformational changes, sulfhydryl group exposure, and gelation properties. A Systematic Literature Review (SLR) was conducted using ScienceDirect, Scopus, and Google Scholar, focusing on empirical studies published within the last ten years. The selected literature was analyzed based on treatment mechanisms, structural changes, and functional properties. The findings indicate that ultrasound induces protein unfolding through acoustic cavitation, generating shear forces and shock waves, whereas HPP promotes conformational changes through hydrostatic pressure. Both treatments can expose hydrophobic and sulfhydryl groups, facilitating intermolecular interactions and disulfide bond formation that support gel network development. Controlled structural modification has the potential to improve gel strength, water-holding capacity (WHC), and emulsifying properties. However, excessive treatment may cause protein aggregation and reduce functional quality. Therefore, optimizing treatment intensity and duration is essential to achieving desirable protein functionality.*

How to Cite: . Rizky Tristan H., Vincentius Aurel M., Khanza Putri Salma, Andritta Putri Burnama, Fashya Eliza Rosadian, Syifa Fairuz Haliza, Yunita Satya Pratiwi. (2026). *The Effect of Non-Thermal Energy Exposure on the Unfolding and Gelation of Local Fish Protein: A Biochemical Approach: A Systematic Literature Review*. 5(01). Pp. 160-170. https://doi.org/10.61536/ambidextrous.v5i01.994

https://doi.org/10.61536/ambidextrous.v5i01.994

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Introduction

Protein is a crucial component of food ingredients, determining various functional characteristics and product quality. In fish-based products, myofibrillar proteins play a crucial role in the formation of structure, texture, and gel characteristics during processing. The actomyosin complex, composed of actin and myosin, is a key component of myofibrillar proteins responsible for gel formation in fish products. The structure and interactions of these proteins can change when subjected to certain processing treatments. These changes in protein structure can then affect the intermolecular interactions and functional properties of proteins in food products (Jiang et al., 2020).

Conventional fish protein processing generally involves extensive heating. Heating can provide energy that causes changes in protein structure and affects intermolecular interactions. If the treatment is not controlled, these structural changes can occur excessively, leading to protein aggregation or the formation of undesirable structures. This is crucial because protein structure is closely related to the textural and functional properties of food products. The use of technologies that can modify protein structure more controlledly has become an alternative in the development of food processing technologies (Zhang et al., 2018).

Non-thermal energy technology is one approach that can be used to modify protein structure by reducing reliance on heating. Two technologies widely studied in the context of protein modification are ultrasound and High Pressure Processing (HPP). Both technologies have different physical principles, but can produce effects such as protein unfolding. Ultrasound produces acoustic cavitation that generates shear forces and shock waves, while HPP applies uniform hydrostatic pressure to the material (Ko et al., 2003).

Unfolding is a change in protein conformation when the initially folded protein structure is opened. This process can cause protein groups or parts previously contained within the structure to become more exposed to the environment. In fish proteins, these changes can affect intermolecular interactions and determine the protein's ability to form gel structures. Controlled unfolding of the structure can provide conditions that support intermolecular interactions, while excessive changes can lead to uncontrolled aggregation. Therefore, the degree of unfolding is a critical factor in determining the success of protein modification using non-thermal energy (Cando et al., 2015).

Protein conformational changes are also related to the dynamics of sulfhydryl or –SH groups. When a protein is folded, some –SH groups remain hidden within the protein structure. Exposure to ultrasound or HPP can cause the protein structure to unfold, exposing these groups to the protein surface. The exposed –SH groups can then undergo oxidation, forming disulfide or –S–S– bonds. The formation of new disulfide bonds can strengthen intermolecular interactions and support the formation of protein gel networks (Gao et al., 2021).

Gel formation is an important property of fish protein in the processing of products such as surimi and fish cakes. Gel quality can be assessed using several parameters, including gel strength and water holding capacity (WHC). Gel strength indicates the strength and elasticity of the protein gel, while WHC indicates the gel's ability to retain water and prevent it from easily escaping from the network. The better the gel network formation, the better the product's

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ability to retain texture and water. These two parameters are important indicators in determining the quality of fish protein-based products (Wang et al., 2015).

In addition to gelation, changes in protein structure due to non-thermal energy can also affect other functional properties, one of which is emulsification properties. High-intensity ultrasound can cause cavitation and changes in protein structure, thereby exposing hydrophobic or amphipathic groups. This condition can enhance the protein's ability to adsorb at the oil-water interface and increase the Emulsifying Activity Index (EAI) under optimal treatment conditions. However, treatment that is too intense or too prolonged can cause protein aggregation, thus preventing a sustained increase in emulsification properties (Jiang et al., 2020).

Although ultrasound and HPP have been widely studied as protein modification technologies, an integrated understanding of the relationships between protein structure changes, unfolding, active group exposure, disulfide bond formation, and gelation is crucial. This study is crucial for explaining how non-thermal energy can affect fish protein from the molecular structure level to the functional properties of the product. Understanding these relationships is also necessary for determining treatment conditions that optimally modify protein without causing excessive aggregation (Zhang et al., 2018).

Based on these conditions, this article uses a Systematic Literature Review approach to examine the effects of non-thermal energy exposure on the structure and functional properties of fish protein. The study focuses on the mechanisms of ultrasound and HPP in causing unfolding, changes in secondary structure, sulfhydryl group dynamics, disulfide bond formation, gelation, gel strength, WHC, and protein emulsification properties. The purpose of this study is to analyze the relationship between changes in protein structure due to non-thermal energy with gel formation and improvements in the functional properties of fish protein.

Research Methods

This study used the Systematic Literature Review (SLR) method to identify and synthesize research findings related to the effects of non-thermal energy on fish protein modification. The literature search was conducted through several scientific databases, namely ScienceDirect, Scopus, and Google Scholar. The search was directed at studies discussing the mechanisms of fish protein modification using ultrasound and High Pressure Processing (HPP) technology. The search focus included changes in protein structure, unfolding, conformational changes, sulfhydryl groups, disulfide bonds, gelation, gel strength, WHC, and emulsification properties (Bao et al., 2026).

The keywords used in the literature search included ultrasound, high-pressure processing, fish protein, myofibrillar protein, protein unfolding, protein gelation, sul)hydryl group, disulfide bond, gel strength, water holding capacity, and emulsifying activity. The combination of these keywords was used to obtain research directly related to the mechanisms of changes in protein structure and functional properties due to non-thermal energy treatments. The obtained literature was then selected based on its suitability to the study focus (Chen et al., 2024).

Literature selection focused on empirical research articles within the last ten years that specifically addressed protein changes resulting from ultrasound or HPP treatment. The literature used should provide information on the mechanisms of protein structural changes or

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their impact on protein functional properties. Sources not directly related to non-thermal energy modification of fish proteins were not used as the primary basis for synthesis (Dash et al., 2022).

Literature analysis was conducted systematically by grouping findings based on treatment mechanisms, protein structure changes, and functional impacts. The first stage aimed to identify the working principles of ultrasound and HPP on proteins. The second stage analyzed conformational and secondary structure changes resulting from unfolding. The third stage examined sulfhydryl group exposure and disulfide bond formation. The next stage correlated these structural changes with gel formation, gel strength, WHC, and emulsification properties. Findings from various literatures were then compared to produce a synthesis regarding favorable protein modification conditions and those that can cause structural damage (Jiang et al., 2025).

Results and Discussion

Non-Thermal Energy Principles in Fish Protein Modification

Non-thermal energy technologies can be used to modify proteins through physical mechanisms distinct from conventional heating. The two technologies focused on in this study are ultrasound and HPP. Both are capable of delivering energy to protein systems, resulting in changes in the protein's three-dimensional structure. Although the physical mechanisms differ, both technologies can produce effects such as protein unfolding. These changes form the basis for structural modification and alter the protein's functional properties (Jiang et al., 2020).

Ultrasound works through the phenomenon of acoustic cavitation. High-intensity sound waves can cause microbubbles to form within a medium, which then rapidly grow and collapse. This phenomenon generates shear forces and shock waves that can exert mechanical stress on protein structures. These mechanical forces can disrupt the interactions that maintain the protein's folded structure, causing the structure to become more open (Zhang et al., 2018).



Figure 1. Illustration of Ultrasound Mechanism

HPP has a distinct mechanism because it applies high hydrostatic pressure evenly to the material. At pressures ranging from 200–600 MPa, pressure can affect protein structure through changes in intermolecular interactions. Water molecules can enter the hydrophobic cavities of proteins and exert pressure on the protein structure, causing changes in protein folding. Thus, HPP can induce conformational changes without using heating as the primary mechanism (Ko et al.,

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2003).

These differences in mechanisms indicate that ultrasound and HPP provide different physical stimuli to proteins. However, both can lead to similar conformational changes, namely the unfolding of the protein structure. These changes can then affect the exposure of active groups and intermolecular interactions.

The final effect of the treatment is highly dependent on the intensity, pressure, time, and condition of the protein during the process (Cando et al., 2015).

Protein Unfolding and Secondary Structure Changes

Unfolding is one of the major changes that occurs in proteins due to exposure to non-thermal energy. A folded protein has a three-dimensional structure that retains various functional groups within the molecule. When this structure unfolds, previously hidden groups become more exposed. These changes can increase the likelihood of interactions between proteins and their environment (Gao et al., 2021).

One indicator of changes in protein structure can be observed through FTIR spectroscopy, particularly in the Amide I region around 1600–1700 cm^{-1}. Analysis of this region can provide information about changes in the protein's secondary structure. A decrease in the α-helix structure indicates a change in the native protein structure, while an increase in the random coil structure indicates that the protein chain is experiencing a more open and less ordered state (Wang et al., 2015).

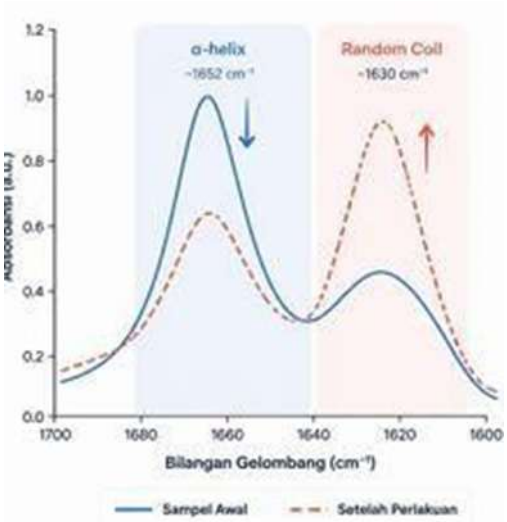


Figure 2. FTIR Spectrum/Secondary Structure Change Diagram

These changes in secondary structure demonstrate that non-thermal energy not only causes physical changes in materials but can also affect the molecular structure of proteins. The α-helix structure is maintained by various interactions, including hydrogen bonds. When these interactions are disrupted, the protein structure can unfold. This condition forms the basis for changes in the functional properties of proteins after treatment (Bao et al., 2026).

Controlled conformational changes can be advantageous by exposing previously unavailable protein regions to interaction. However, if unfolding is too intense, the protein structure can lose stability. This can cause the protein to aggregate and form an irregular structure. Therefore, the success of protein modification is determined not only by the occurrence of unfolding but also by the degree of unfolding (Chen et al., 2024).

Relationship between Unfolding and Gelation Potential

Gelation is a significant change in fish protein during processing. Proteins undergoing conformational changes can interact with each other, forming a three-dimensional network. This network then plays a role in determining the strength and characteristics of the resulting gel. Therefore, changes in protein structure due to non-thermal energy are directly related to gel formation potential (Dash et al., 2022).

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Controlled unfolding can open up a protein's structure, exposing previously hidden regions. Certain hydrophobic and reactive groups can then interact with other protein molecules. These interactions can help form a more organized protein network. This suggests that unfolding a protein's structure may be a crucial initial step in gel formation (Jiang et al., 2025).

Conversely, excessive unfolding can result in random protein aggregation. When large amounts of hydrophobic groups are exposed and proteins interact uncontrollably, they can clump before a regular three-dimensional network is formed. This condition can lead to decreased gel quality. Thus, there is an optimum between a protein structure that is too closed and one that is too open (Bao et al., 2026).

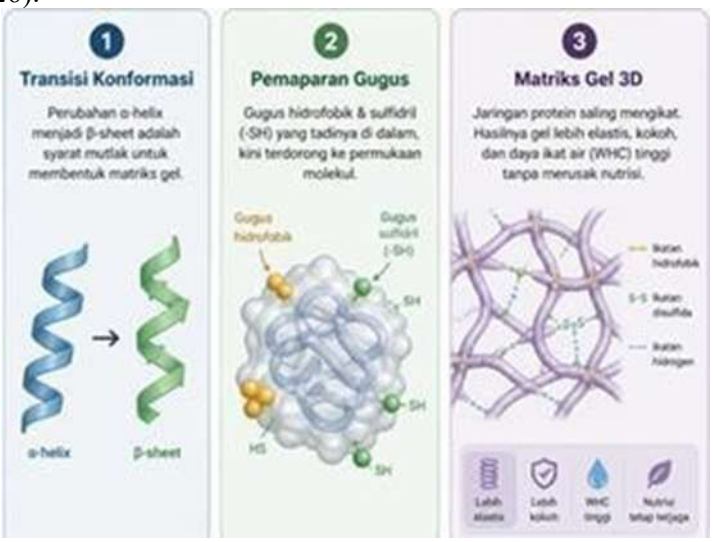


Figure 3. Relationship Diagram

The relationship between unfolding and gelation suggests that protein structural modifications must be controlled. Proteins that do not unfold may retain active groups internally, thus limiting gel-forming interactions. Conversely, excessive unfolding can lead to uncontrolled denaturation and aggregation. Optimal conditions are required for the protein structure to unfold sufficiently to form a network without losing its ability to form a good gel structure (Chen et al., 2024).

Dynamics of Sulfhydryl Groups and Disulfide Bond Formation

The sulfhydryl or –SH group is a functional group that plays a role in protein structural changes after non-thermal energy treatment. In proteins that are still folded, some –SH groups can be hidden within the molecular structure. When the protein unfolds, these groups can become more exposed on the protein surface. This condition increases the opportunity for interactions and oxidation reactions between sulfhydryl groups (Dash et al., 2022).

Ultrasound or HPP exposure can cause protein structures to open up, allowing the –SH groups to interact more easily. The exposed groups can then undergo oxidation, forming disulfide bonds, or –S–S–. These intermolecular disulfide bonds can help connect protein molecules to each other. These interactions can then contribute to the formation of stronger protein networks (Jiang et al., 2025).

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Figure 4. Protein Folding Scheme

Non-thermal energy does not necessarily break existing disulfide bonds. A more important mechanism is a change in protein conformation that causes reactive groups to become more exposed. The exposed –SH groups can then interact and form new disulfide bonds. This bond formation can strengthen the protein gel network and contribute to the resulting gel's characteristics (Bao et al., 2026).

This relationship suggests that changes in protein structure at the molecular level can impact the macroscopic properties of the product. Changing –SH groups to disulfide bonds can strengthen intermolecular interactions, resulting in a stronger gel network. However, network formation remains dependent on treatment conditions, as excessively intense interactions can result in uncontrolled aggregation (Chen et al., 2024).

Gel Strength and Water Holding Capacity

The quality of fish protein gel can be assessed through gel strength and Water Holding Capacity (WHC). Gel strength indicates the gel network's ability to maintain its strength and elasticity. Meanwhile, WHC indicates the gel network's ability to retain water, preventing it from easily escaping from the gel structure. These two parameters are important indicators in determining the quality of fish protein-based products, including surimi and fish cakes (Chen et al., 2024).

The formation of a good gel network requires sufficiently strong and organized intermolecular interactions. Partial unfolding can help expose the active groups, which then interact and form a three-dimensional network. The formation of disulfide bonds can also strengthen the network. When the network is able to retain water well, the WHC value is higher, thus maintaining the product's texture (Jiang et al., 2025).

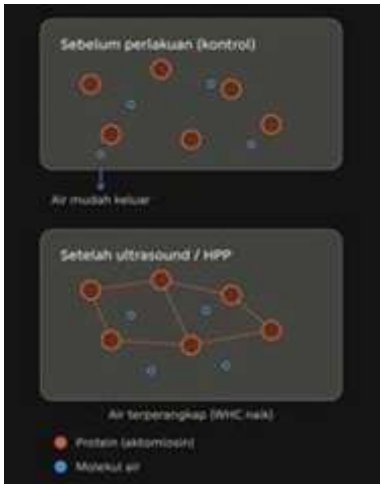


Figure 5. Graph/Data of Gel Strength and WHC Research Results

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Research data presented in the material indicates that changes in protein structure due to non-thermal energy treatment can be associated with changes in gel properties. These results demonstrate the importance of treatment conditions in determining the quality of the final product. Appropriate treatment conditions can help produce a strong gel structure that is able to retain water. Conversely, excessive treatment can cause excessive changes in protein structure and potentially degrade gel quality (Bao et al., 2026).

The Effect of Ultrasound on Protein Emulsification Properties

In addition to its role in gelation, protein modification through ultrasound can also affect emulsification properties. The Emulsifying Activity Index (EAI) indicates a protein's ability to aid emulsion formation. Proteins with amphipathic properties can interact with both the oil and water phases, helping stabilize the interface between the two phases (Chen et al., 2024).

High-intensity ultrasound can produce cavitation, which changes protein structure. This process can induce unfolding, exposing hydrophobic and amphipathic groups previously located within the protein. This can enhance the protein's ability to adsorb at the oil-water interface. Under optimal ultrasound conditions, these changes can increase the EAI value (Dash et al., 2022).



Figure 6. Graph of the Effect of Ultrasound on EAI

The increase in EAI indicates that protein structural modifications not only affect gelation but also other functional properties. Alterations in protein structure can make amphipathic groups more likely to interact with the oil–water interface. However, this improvement has certain limitations. Ultrasound treatment at too high or too long a rate can cause protein aggregation, thus decreasing emulsification ability (Jiang et al., 2025).

These conditions indicate that treatment intensity is a critical factor in determining the success of protein modification. Too low a treatment intensity may not produce sufficient structural changes, while too high a treatment intensity can cause aggregation. Therefore, optimum conditions are needed so that unfolding can increase functional group exposure without causing excessive structural damage (Bao et al., 2026).

Synthesis of the Relationship between Unfolding, Gelation, and Functional Properties of Proteins

Based on a literature synthesis, ultrasound and HPP can induce changes in protein structure through different physical mechanisms. Ultrasound produces cavitation, which exerts mechanical force on proteins, while HPP generates hydrostatic pressure that can affect protein structure from within. Both treatments can cause protein unfolding and conformational changes. These changes can then expose previously hidden active groups (Chen et al., 2024).

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Literatur	Perlakuan	Fokus	Temuan
Jiang et al. (2020)	Ultrasound	Struktur & fungsi	Struktur protein berubah
Zhang et al. (2018)	Ultrasound intensitas tinggi	Struktur & fisik	Sifat fisik berubah
Gao et al. (2021)	Ultrasound intensitas tinggi	Emulsifikasi	**EAI meningkat pada kondisi tertentu**
Ko et al. (2003)	HPP	Myosin	Konformasi berubah
Cando et al. (2015)	HPP	Surimi gel	Pembentukan gel dipengaruhi

Figure 7. Final Synthesis

Controlled unfolding can expose hydrophobic, amphipathic, and sulfhydryl groups, increasing the potential for intermolecular interactions. Exposed sulfhydryl groups can undergo oxidation and form new disulfide bonds. These interactions can support gel network formation and increase gel strength and WHC. At the same time, exposure of amphipathic groups can enhance the protein's ability to interact at the oil-water interface, thus enhancing emulsification properties (Dash et al., 2022).

However, protein modification using non-thermal energy does not always result in improved functional properties. Treatments that are too high or too long can cause excessive unfolding and protein aggregation. Uncontrolled aggregation can inhibit the formation of an ordered gel network and degrade the protein's functional properties. Therefore, the success of non-thermal energy technologies depends heavily on the ability to control the rate of protein structural changes (Jiang et al., 2025).

This synthesis demonstrates that non-thermal energy can be viewed as a protein modification technology that operates at the molecular structural level. Alterations in the protein's three-dimensional structure are the initial step, which then influences the exposure of active groups, intermolecular interactions, gel network formation, and emulsification properties. This relationship suggests that process optimization requires a balance between necessary unfolding and avoidable aggregation (Bao et al., 2026).

Implications and Limitations

1. Implications

This study implies that non-thermal energy technologies have the potential to be used for more targeted modification of fish proteins. Ultrasound and HPP can induce changes in protein structure without relying on heating as the primary mechanism. These changes can open up the protein structure and expose active groups that play a role in intermolecular interactions. This provides an opportunity to enhance the functional properties of proteins in food processing.

Biochemically, protein conformational changes are a key mechanism in determining the final product properties. Controlled unfolding can increase the exposure of sulfhydryl groups and enable the formation of disulfide bonds. These interactions can support gel network formation and increase gel strength and WHC. Furthermore, exposure of amphipathic groups can enhance the protein's ability to form emulsions.

Practically, the application of non-thermal energy technology can be directed towards the development of fish protein-based products with improved textural characteristics and functional properties. This technology has the potential to be used in the processing of products such as surimi and fish cakes. However, its application requires consideration of the protein type, treatment intensity, treatment duration, and processing conditions to ensure that the resulting structural changes remain favorable.

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2. Limitations

This study has limitations because the analyzed results come from previous studies with varying treatment conditions. Differences in ultrasound intensity, HPP pressure, treatment duration, and protein characteristics can result in varying degrees of structural change. This condition means that the results of one study cannot always be directly applied to other processing conditions.

Furthermore, the study material focuses more on the mechanisms of protein structural changes and functional properties related to unfolding and gelation. Not all studies reviewed used the same testing parameters, thus limiting quantitative comparisons between publications. Therefore, further experimental research is needed to determine the optimum non-thermal energy conditions for specific fish protein types and food products.

Conclusion

Non-thermal energy technologies such as ultrasound and High Pressure Processing (HPP) have the ability to modify the structure of fish proteins through conformational changes and unfolding processes. Ultrasound works through acoustic cavitation that produces shear forces and shock waves, while HPP provides hydrostatic pressure that can change the protein structure. Both treatments can open the three-dimensional structure of proteins and expose active groups without directly breaking the main peptide bonds. These changes allow sulfhydryl groups to become more exposed and undergo oxidation to form disulfide bonds that can strengthen the gel network. Controlled unfolding has the potential to improve gel strength, WHC, and emulsification properties through changes in intermolecular interactions and exposure of amphipathic groups. Conversely, too high or too long a treatment can cause uncontrolled protein aggregation, thereby reducing gel quality and functional properties. Thus, the success of protein modification using non-thermal energy depends heavily on controlling the treatment conditions to achieve an optimal unfolding rate without causing excessive aggregation.

Thank-you note

The author would like to express his gratitude to all parties who have provided support in the process of compiling this article, both in literature searches, information processing, and completion of the manuscript.

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