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Review

Preparations, characterizations, assembly behaviors, applications, and perspectives of whey protein amyloid fibrils: A review

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ABSTRACT

Whey protein, nutritionally complete with all essential amino acids, is the main component of milk protein. Although the nutritional potential of whey protein meets the needs of some food formulations, a limited range of functional properties hinders its wider application. Whey protein fibrillization refers to the protein aggregation behavior wherein whey protein self-assembles under specific conditions, forming highly ordered fibril aggregates known as whey protein amyloid fibrils (WPAFs). Whey protein fibrillization is considered to be an innovative modification method to improve and enhance the structure and function of whey protein. This paper reviewed the various strategies, influencing factors, and characterization techniques of WPAFs. The assembly pathway of WPAFs and its application in various scientific fields were highlighted and introduced. Finally, the limitations of current research on WPAFs were discussed, and the future research direction and application prospects of WPAFs were proposed.

1. Introduction

Recently, there has been a significant interest and notable advancement in the development of protein amyloid fibrils (Meng et al., 2022; Zhang et al., 2024). Protein fibrillization refers to aggregation behavior in which protein self-assembles under specific conditions to form highly ordered fibril aggregates known as protein amyloid fibrils (Meisl et al., 2016). Initially, amyloid fibrils were believed to be associated with debilitating neurodegenerative diseases such as Alzheimer's, Parkinson's, Huntington's, and Creutzfeldt–Jakob diseases (Frey et al., 2024). Later, it was indicated that the formation of protein amyloid fibrils was a common characteristic of most proteins (Mohammadian & Madadlou, 2018). Protein fibrillization can be deliberately induced to obtain protein amyloid fibrils with specific structural and functional features in vitro conditions (An et al., 2022). Food-grade proteins have been proven to be capable of converting into amyloid fibrils under appropriate fibrillization conditions, displaying unique structural and functional features (Cao & Mezzenga, 2019; Xu et al., 2023). Based on both in vitro and in vivo assessments, food protein amyloid fibrils are considered to be safe nutritional ingredients (Jansens et al., 2018; Xu et al., 2023). At present, food-grade protein fibrillization is considered to be an innovative modification method to improve and enhance the structure and function of protein, and is expected to become a new raw material for the production of future food formulations.

Plant proteins are regarded as renewable and sustainable, with abundant availability, easy accessibility, and environmental friendliness (Liang et al., 2024; Nasrabadi et al., 2021). Several plant proteins, such as soybean protein (Wang et al., 2025), rice protein (Qi et al., 2023), pea protein (Wu et al., 2022), peanut protein (Xu et al., 2025), oat protein (Xu, Tang, et al., 2024), and wheat protein (Lambrecht et al., 2021), have begun to be developed for the preparation of protein amyloid fibrils. It is worth noting that most of the developed applications of amyloid fibrils have primarily relied on animal proteins (Lambrecht et al., 2019). Compared to plant proteins, animal proteins have a more comprehensive amino acid profile, higher digestibility, and superior bioavailability (Zeng et al., 2024). In the practice of seeking sustainable and innovative development of the food industry, the high-value utilization of by-products has become a strategic imperative (Ouyang et al., 2024). Whey protein is the main animal protein source for the preparation and application of amyloid fibrils (Lendel & Solin, 2021). Whey

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protein is derived from whey, a byproduct of cheese or casein production, through a series of processes including clarification to remove impurities, ultrafiltration and nanofiltration for protein concentrate, and drying to produce protein powder (Tang, 2021). Whey protein is a collective term referring to the diverse proteins present in whey, including β-lactoglobulin (β-LG, 65%), α-lactalbumin (α-LA, 25%), bovine serum albumin (BSA, 8%), and minor proportions of other proteins (Fig. 1) (Boscaini et al., 2023; Falsafi et al., 2022). The basic properties of the three main proteins in whey protein are summarized in Table 1. Whey protein is considered a high-quality protein source with significant nutritional value, containing all essential amino acids (Chen et al., 2023). It has been incorporated into various food formulations due to its small molecular weight and high solubility (Zhu, 2024). Whey protein fibrillization has become a current research hotspot as a promising strategy to improve and expand the functional properties of whey protein. There is a large amount of research devoted to understanding the aggregation behavior of whey protein amyloid fibrils (WPAFs) (Hoppenreijs et al., 2022; Leal et al., 2024; Wang et al., 2023; Yang, Guan, et al., 2023).

Based on data from the Web of Science database, there has been a substantial increase in the number of publications related to WPAFs in relevant fields (e.g., "Biochemistry Molecular Biology", "Food Science Technology", "Chemistry Applied") over the past five years. The primary emphasis of these studies has been focused on the formation, mechanisms, structure, and characterization of WPAFs, as depicted in Fig. 2. At present, numerous studies have been published on the formation conditions, assembly mechanisms, and structural characteristics of WPAFs. How to efficiently and scientifically optimize the functional properties and applications of WPAFs is the primary challenge for future research. In this review, the diverse fibrillization factors, characterization technologies, and assembly behaviors of whey protein fibrillization were introduced. The application designs and future perspectives of WPAFs were discussed. This review aims to provide a comprehensive understanding of whey protein fibrillization, serving as a reference for future research and applications of WPAFs.

2. Various strategies of whey protein amyloid fibrils formation

The formation of WPAFs is usually conducted under extreme and controlled environmental conditions. Different fibrillization conditions can affect the formation, structure and function of WPAFs (Zhang et al., 2024). Acidic thermal treatment is one of the main strategies to fabricate WPAFs (Mohammadian & Madadlou, 2018). The exact factors influencing whey protein fibrillization include protein type, protein concentration (Xu, Zhang, et al., 2024), heating temperature (Ouyang et al., 2023), heating time (Lin et al., 2025), pH value (Heyn et al., 2023), and ionic strength (Guan et al., 2022). The formation of WPAFs is not only related to the conventional fibrillization factors, but also closely related to external conditions. Besides the acidic thermal treatment, a variety of specialized strategies can be employed to influence whey protein fibrillization. These strategies encompass physical processing conditions (Leal et al., 2024), chemical environmental factors (Liu et al., 2021), as well as biological auxiliary treatments (Yang et al., 2023). Table 2 illustrates various preparation conditions for WPAFs.

Table 1
Physicochemical properties of three main proteins in whey protein.

Parameters	β-LG	α-LA	BSA
Molecular weight (kDa)	18.3	14.2	66.4
Amino acid residues	162	123	583
Isoelectric point (pI)	5.3-5.5	4.5-4.8	4.7-5.1
Denaturation temperature (°C)	83	68	70
Content in whey protein (%)	60-65	20-25	8
Secondary structure	Nine antiparallel β-strands and one α-helix segment	Major α-subdomain and minor β-subdomain	Largely α-helix in conformation comprising of three domains and six sub-domains
Tertiary structure	5 cysteine residues, that forms 2 intramolecular disulfide bonds and 1 free sulfhydryl group	8 cysteine residues, that forms 4 intramolecular disulfide bonds	35 cysteine residues, that forms 17 intramolecular disulfide bonds and one free sulfhydryl group

2.1. Key conditions

WPAFs are formed through the formation of building blocks from the denatured whey protein and the subsequent assembly of these building blocks (Hoppenreijs et al., 2022). The secondary structure of whey protein is mainly composed of α-helix, β-sheet, β-turn and random coil, while the tertiary structure of whey protein is mainly maintained by intramolecular disulfide bonds and free sulfhydryl groups. The secondary and tertiary structures of whey protein are highly susceptible to changes in pH value and temperature. Heating whey protein solution above its denaturation temperature at an acidic environment far from the isoelectric point is regarded as the standard strategy for preparing WPAFs. Heat treatment (≥ 70°C) induces denaturation and partial unfolding of whey protein, leading to the exposure of hydrophobic domains and free sulfhydryl groups. Extreme pH (≤ 3.5) induces the acid hydrolysis of whey protein and the aggregation of peptides, leading to the formation of WPAFs with extreme aspect ratio. The partial unfolding and acid hydrolysis are indispensable steps for the formation of WPAFs. Electrostatic interaction and hydrophobic interaction drive the transition of disordered building blocks into ordered fibril aggregates, forming WPAFs. Notably, the structure and morphology of WPAFs are dependent on protein type, pH value, ionic strength, temperature, protein

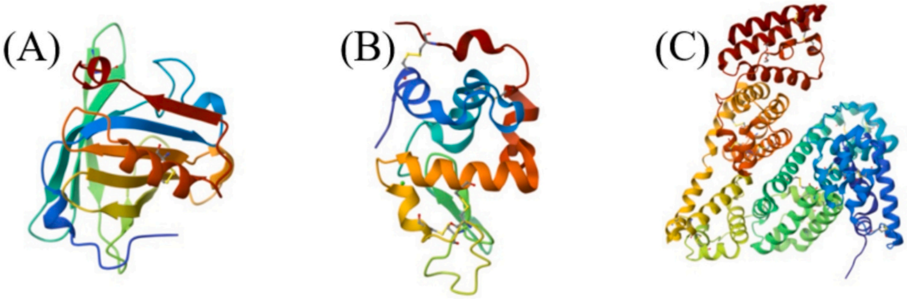

Fig. 1. Structure of β-LG (A), α-LA (B), and BSA (C).

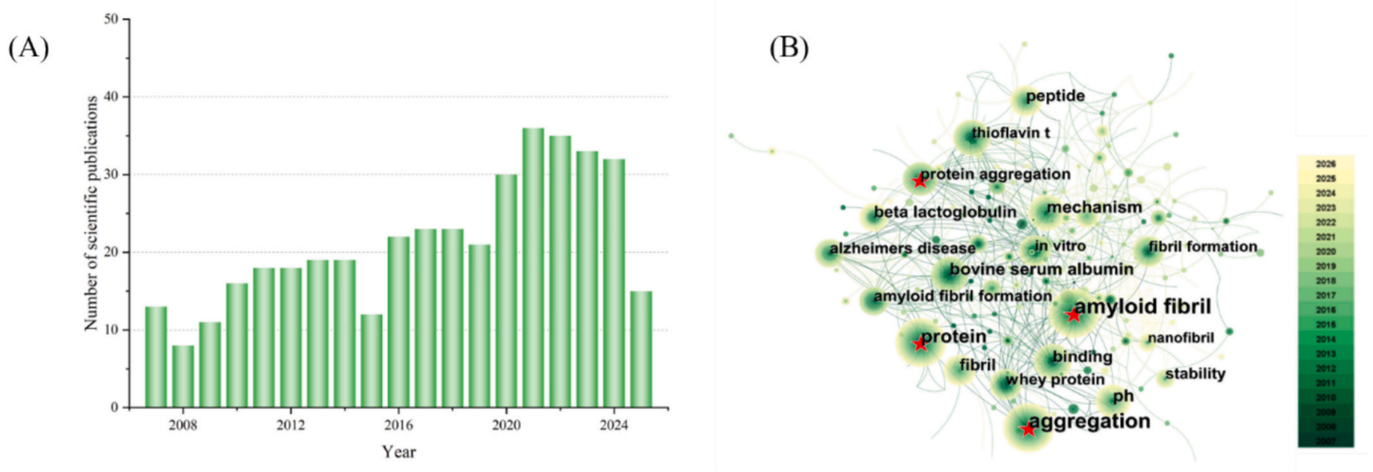

Fig. 2. Number of scientific publications in the Web of Science database containing the term WPAFs in the topic between the years 2007 and 2025 (A). Co-occurrence diagram of keywords WPAFs based on the Web of Science database (B).

concentration and so on. Whey protein is a mixture of various proteins such as β-LG, α-LA, and BSA. The ability of these proteins to form amyloid fibrils is different. β-LG is the major protein in whey protein and plays a dominant role in the formation of WPAFs. It has become an important model protein for studying amyloid fibrils. β-LG contains two specific peptide sequences, including N-terminal regions (residues 1-53) and C-terminal regions (residues 138-162), which are the key building blocks of the formation of amyloid fibrils. α-LA (Goers et al., 2002) and BSA (Yang et al., 2024) can also produce amyloid fibrils, but different conditions are required (Kroes-Nijboer et al., 2012). Kang et al. (2024) and Oboroceanu et al. (2014) reported the formation of α-LA and BSA amyloid fibrils through long-term acidic heating mediation and ultrasound assistance.

pH can regulate the degree of acid hydrolysis and the strength of electrostatic interactions, resulting in WPAFs with different morphologies (Farrokhi et al., 2019). In an acidic environment, the continuously increasing number of positive charges on the surface of whey protein induces changes in its spatial conformation to produce building blocks. Acid hydrolysis of whey protein induces peptide bond dissociation and increases electrostatic repulsion, resulting in the aggregation of building blocks and facilitating the fibrillization process (Bolder et al., 2007). In general, elongated WPAFs are usually formed at pH $\leq$ 2.0, while worm-like WPAFs are formed at pH $\leq$ 3.5 (Heyn et al., 2023). The differences of elongated and worm-like WPAFs are attributed to differences in the building blocks (Ye et al., 2018). Heyn et al. (2019) pointed out that whey protein undergoes specific acid hydrolysis at pH 2.0 to generate whey protein peptides and non-specific hydrolysis at pH 3.5 to produce non-hydrolyzed whey protein.

Temperature is confirmed as an important factor in the formation of WPAFs (Heyn et al., 2020). WPAFs are usually formed by heating whey protein solution above their thermal denaturation temperature (typically 70-95°C). When the whey protein solution is heated above its denaturation temperature, the peptide bonds of whey protein are break and hydrophobic residues are exposed. Increasing temperature can accelerate the fibrillization process and induce the formation of WPAFs (Farrokhi et al., 2019). However, prolonged high temperature heating can cause the depolymerization of preformed WPAFs, which may be due to hydrophobic driven aggregation and the dissolution of hydrogen bonds (Jones & Mezzenga, 2012). Excessively high temperature ($\geq$ 100°C) induced local gelation of whey protein, accompanied by a significant increase in turbidity and the destruction of the cross-β structure (Loveday, Wang, et al., 2012). The optimal temperature for the formation of WPAFs is usually 90°C (Ouyang et al., 2023).

Whey protein concentration is a key factor affecting the number of fibril-building blocks and the conversion efficiency of WPAFs. The optimal fibrillization concentration is an important prerequisite for the formation of WPAFs with good morphology. Whey protein fibrillization needs to occur at a specific critical aggregation concentration (Kroes-Nijboer et al., 2009). Kroes-Nijboer et al. (2011) pointed out that the assembly of peptides into amyloid fibrils is the rate-limiting step of fibrillization in low whey protein concentrations, while the degree of hydrolysis is the limiting factor of fibrillization in high whey protein concentrations. Xu et al. (2024) reported the formation kinetics of WPAFs at a concentration of 1-5%. Long semi-flexible WPAFs can be formed at a concentration of 2%, and WPAFs were entangled with each other at a concentration of 4%. Excessively high fibrillization concentration ($\geq$ 5% (w/w)) can cause an increase in the viscosity of whey protein solution and even induce whey protein gelation, thereby reducing the WPAFs conversion rate (Oboroceanu et al., 2014).

Ionic strength has the potential to significantly impact the fibrillization kinetics and the ultimate morphology of WPAFs due to the charge shielding effect (Yang et al., 2022). In environments of low ionic strength, relatively strong electrostatic repulsion promotes the formation of long and semiflexible fibrils using fibril-building blocks in ordered arrangement. Conversely, high ionic strength induced the formation of short and worm-like fibrils aggregates using fibril-building blocks in disordered arrangement. Electrostatic repulsion of WPAFs increases with the enhancement of ionic strength. The presence of different ion types and ionic strength can influence the formation of WPAFs by altering the electrostatic interaction and hydrophobic interaction (Loveday et al., 2010). Adding $CaCl_2$ and NaCl on the whey protein solution showed that Ca^{2+} and Na^{+} caused the disordered aggregation to form short and curly WPAFs by shielding negatively charged regions (Loveday, Wang, et al., 2011). The morphology of the WPAFs changes from long and semi-flexible to short and tightly curled when NaCl $\geq$ 60 mM or CaCl2 $\geq$ 33 mM (Loveday et al., 2010). In addition, the addition of salt ions can also induce the gelation of the WPAFs solution. Mohammadian and Madadlou (2016b) reported that Zn^{2+} induced cold-set hydrogels with higher water holding capacity and a denser microstructure compared to Mn^{2+} and Ca^{2+}.

2.2. *Physical processing conditions*

Several common physical techniques including mechanical stress, ultrasound, dielectric heating, and microfluidization-assisted pretreatment, have been employed to optimize the functional characteristics and structural morphology of WPAFs. Mechanical stress (shear flow or magnetic stirring) impacts the formation kinetics of WPAFs through additional mechanical energy input (Dunstan et al., 2009). Appropriate shear and stirring increase the collision and alignment of hydrolyzed

Table 2
Summary of the preparation conditions for WPAFs.

Types	Concentration (%)	pH	Temperature (°C)	Time (h)	Addition pretreatment	Morphology	References
Key factors	5	2	85	5	-	WPAFs with nanometric diameter (less than 15 nm) and micrometric length.	(Mohammadian et al., 2019)
	4	2	85	5	-	WPAFs with nano-thick diameters less than 10 nm and heterogenous in length.	(Mohammadian & Madadlou, 2016a)
	3	2	85	48	-	α-LA fibrils with a width of 10–15 nm and a length of 0.5–2.0 μm.	(Kang et al., 2024)
	5	2	85	24	-	BSA fibrils with nanometric diameter (less than 15 nm) and micrometric length (1-1.5 μm).	(Fan et al., 2024)
	1	2.0-4.0	65	0-12	-	Short-bent fibrils at pH 2.0; Micron-length fibrils and nanometer-scale filaments at pH 2.5; Regular spherical aggregates at pH 3.0; Worm-like fibrous aggregates at pH 3.5; amorphous aggregates.	(Yang et al., 2024)
	5-10	2.0, 7.0	85	12; 0.5	-	WPAFs aggregates at pH 2.0 and 12 h; Granular aggregates at pH 7.0 and 0.5 h.	(Wang et al., 2024)
	2.5	2.0, 3.5	90	5	-	Straight fibrils at pH 2.0 and worm-like aggregates at pH 3.5.	(Heyn et al., 2019)
	2	2.0-6.0	80, 90	5	-	Long with linear structure at pH2.0; fibril aggregations at pH 4.0-6.0.	(Farrokhi et al., 2019)
	6.5	2.0, 8.0	85	0.25, 5	-	Nano-thick fibrils at pH 2.0 and 5h; Wormlike aggregates at pH 8.0 and 0.25 h.	(Mohammadian et al., 2018)
	0.5-4	1.5-3.0	80-110	0-24	-	Semiflexible fibrils presented with a slightly curved and unbranched filament at 6h; Multistranded-helical semiflexible fibrils and single-filament flexible fibrils (about 5 nm in diameter) at 24h.	(Ouyang et al., 2023)
	2	2	85	5-20	-	Self-assembled into fibrils at 10 h; Entangled with each other at 15 h; The collapse of the fibrils and dense clumps formation at 20 h.	(Meng et al., 2024)
	2.5	2	95	0-24	-	Short fibrils with a length of around 87 nm at 2h; Longer fibrils with a contour length of around 205 nm at 6h; Longer and more branched fibrils with a length of about 240 nm at 8 h; Large fibrillar aggregates with a contour length of about 770 nm at 12h; Clusters with a contour length of around 1080 nm at 24h.	(Liu et al., 2024)
	2	2	90	4-24	-	A small number of fibrils with small length at 4 h; Height reached 3–4 nm at 8 h; The mature fibrils begin to break, and entangle to form aggregates at 12h, 16h, and 24h.	(Wang et al., 2024)
	1-5%	2	90	16	-	Several fibrils at 1%; Long fibrils at 2%; Entangled fibrils at 3-4%; Few fibrils at 5%	
	0.5-5%	2	80	0-34	-	Low fibrils conversion at 0.5%; Long semi-flexible fibrils at 2%.	(Bolder, Vasbinder, et al., 2007)
Ionic strength	0.7	2	90	1-10	$CaCl_2$ (0-50 mM)	Ca^{2+} induced disordered aggregation.	(Yang et al., 2022)
	6	2	85	5	$CaCl_2$, $MnCl_2$ and $ZnCl_2$	Cations induced fibrils gelatin.	(Mohammadian & Madadlou, 2016b)
	2	2	80	0-24	$CaCl_2$ (0-120 mM)	Ca^{2+} and Na^{+} induced increased the proportion of curly, twisted fibrils.	(Loveday, Su, et al., 2011)
	1	1.6-2.4	80	0-24	NaCl, $CaCl_2$ (0-100 mm)	Ca^{2+} and Na^{+} induced worm-like fibrils when $\geq$60 mm NaCl or $\geq$33 mm $CaCl_2$.	
Physical techniques	2.5	2, 3.5	80	0.5-4	Shear flow	Shearing promotes the formation of semiflexible fibrils at pH 2.0; worm-like fibrils at pH 3.5.	(Heyn et al., 2023)
	2	2	80	20	Magnetic stirring	Stirring promotes the formation of uniform and homogeneous fibrils.	(Rathod & Amamcharla, 2024)
	1	2	90	6	Ohmic heating	Ohmic heating promotes fibrils with length ranging (0.67-1 μm), and a diameter (14-20 nm).	(Leal et al., 2024)
	3	2	-	2	Ultraviolet B radiation	Ultraviolet B radiation accelerated worm-like aggregation at pH 3.5; Decelerated fibrils formation at pH 2.0.	(Fitzner et al., 2023)
	3.5	2	-	5, 7	Radio frequency heating	Radio frequency heating promotes the formation of longer and more flexible fibrils at 7 h.	(Wang et al., 2023)
	3	2	80	4	Microwave heating	Microwave heating promotes the formation of entangled long fibrils with many branches, clear shapes and clear network structures.	(Jiao et al., 2021)
	5-9	2	80	-	High pressure microfluidization	High pressure microfluidization promotes the formation of uniformly and shorter fibrils.	(Koo et al., 2018)
Chemical solvents	0.5%	2	85	24	Ethanol	Ethanol induced the formation of short worm-like nanofibrils.	(Liu et al., 2021)
	2.5	2	30-50	5	Ethanol	Ethanol induced the formation of worm-like aggregates together with spherical aggregates within 1–2 h.	(Kayser et al., 2020)
	-	7	37	-	Urea	Urea induced the formation of unbranched, twisted fibrils with diameters of 8–10 nm.	(Hamada & Dobson, 2002)
	1	2	80	1	Glycerol and sorbitol	Glycerol and sorbitol induced the formation of straight and unbranched fibrils with uniform width.	(Dave et al., 2014)

(continued on next page)

Table 2 (*continued*)

Types	Concentration (%)	pH	Temperature (°C)	Time (h)	Addition pretreatment	Morphology	References
Limited enzymatic hydrolysis	2	2	25	24	Trifluoroethanol	Trifluoroethanol induced the formation of worm-like fibrils.	(Chen et al., 2025)
	2	2	90	10	Alkaline protease	Fibrils with loosen the cross-β structure packing.	(Yang et al., 2023)
	3	2	37	24	Endoproteinase GluC	Linear shape and shorter fibrils with the length of 0.4–0.6 μm.	(Feng et al., 2019)
	4	2	85	5	Bacterial endo-peptidase	Nano-thick fibrils and the diameter less than 10 nm.	(Mohammadian & Madadlou, 2016a)
	3	2	90	0-10	Trypsin	More fibrils with longer and more semi-flexibility with massive unbranched fibrils at 5 h.	(Gao et al., 2013)

peptides, thereby accelerating the self-assembly of WPAFs and increasing the number of WPAFs (Heyn et al., 2023; Hill et al., 2006). Excessive high mechanical stress leads to breakage of long semiflexible WPAFs into short rigid WPAFs (Akkermans, van der Goot, Venema, van der Linden, & Boom, 2008). Ultrasound treatment has been reported to induce protein conformation changes by disrupting non-covalent bonds (Zhao et al., 2018). Cavitation bubbles produced by ultrasound treatment can lead to a series of physicochemical effects, including high shear, instantaneous high temperatures and high pressures within the solution (Ashokkumar, 2011). These physicochemical effects can effectively accelerate the spontaneous nucleation of whey protein monomers, thereby improving the efficiency of fibrillization and producing uniform WPAFs (Yang et al., 2024). It was reported that ultrasound pretreatment promotes unfolding of β-LG and exposure of hydrophobic residues, and accelerates amyloid-like aggregation of β-LG (Chandrapala et al., 2012). Conventional heat conduction and convection heating caused the whey protein solution to be heated unevenly, and the heat transfer efficiency was affected by the volume and viscosity of the whey protein solution (Jaeger et al., 2016).

Dielectric heating refers to the conversion of electrical energy into thermal energy to generate heat under the action of high-frequency electric field, which can effectively solve the problem of low heating efficiency of protein dissolution (Wang et al., 2023). At present, the commonly used dielectric heating methods include microwave heating and radio frequency heating, which are based on volume heating (Jiao et al., 2018). Microwave heating is based on electromagnetic waves in the wavelength and frequency range of 1 mm to 1 m and 300 MHz to 300 GHz, respectively, with uniform heating and high heating efficiency (Nasrabadi et al., 2021). Since the microwave energy is lower than the energy of the chemical bonds, microwave radiation can directly induce torsional vibration of the whey protein skeleton without destroying the primary structure of whey protein so that the secondary and tertiary structure of whey protein can be changed through denaturation and unfolding. Jiao et al. (2021) used microwave heating instead of conventional heating to promote the formation of WPAFs, which can significantly shorten the formation time of WPAFs. Compared to microwave heating, radio frequency has longer wavelength and occupies the region between 1 and 300 MHz. The action of free radicals generated by dipole and ion motion under the effect of the radio frequency field plays a role in the structure of proteins. Radio frequency heating also enables a more uniform and rapid heat treatment. Wang et al. (2023) successfully formed WPAFs featuring a micrometric length and proper aspect ratio via radio frequency heating. The fiber conversion and fluorescence intensity of radio frequency heating were significantly higher than those of conventional heating, and the fibrillization time and browning degree of whey protein were effectively reduced.

Ohmic heating is a heat treatment method that applies alternating electric currents directly to whey protein solution medium (Varghese et al., 2014). According to Joule's law, passing a moderate and alternating electric current through whey protein solution provides fast and uniform heating as well as electrical effects. Leal et al. (2024) reported that ohmic heating induced β-LG unfolding, denaturation and formation of uniform sized protein aggregates. It showed that ohmic heating can be used as an alternative to conventional heating to prepare fibril networks with distinctive morphologies.

Ultraviolet (UV) light with the wavelength of 200-280 nm specifically targets aromatic amino acids such as tryptophan, tyrosine, and phenylalanine. UV radiation affects the conformation of whey protein and promotes protein unfolding and the formation of aggregates. Unfolding exposes charged amino acids, promotes hydrophobic interactions and increases the solubility of the protein. UV radiation can be used as a conformational induction treatment to promote temperature-induced protein unfolding. Fitzner et al. (2023) investigated the effect of unfolding on the formation of amyloid fibrils and confirmed that UV radiation induces unfolding of β-LG. Microfluidization, a dynamic high-pressure homogenization technique, generates highly turbulent flow and shear forces with a larger energy density. Microfluidization treatment can change the secondary structure of whey protein. Microfluidization process generates highly turbulent flow under high pressure and high speed, inducing the denaturation of whey protein and the structural changes of amyloid fibrils (Oboroceanu et al., 2011). Koo et al. (2018) reported extensive protein aggregation caused by reduced electrostatic repulsion between protein molecules by combined thermal-microfluidization treatment, resulting in a reduction in the average length of WPAFs (from about 310 to 97 nm). Oboroceanu et al. (2011) reported that the effect of dynamic high pressure microfluidization on β-LG and whey protein solution before and after fibril formation. Fibril length distribution of β-LG and whey protein before and after microfluidization was measured by shear birefringence. These results indicate that pressure (≥50 MPa) micro fluidization treatment resulted in protein breakup into more uniformly sized and much shorter fibrils, while pressure (170 MPa) micro fluidization treatment resulted in slightly shorter fibrils but did not completely dissociate them.

2.3. *Chemical environment conditions*

Chemical solvents induction can also be used to prepare WPAFs. Chemical denaturants can be an alternative to heat denaturation for preparing WPAFs. Previous studies have reported that WPAFs can be formed under several different chemical conditions including the presence of trifluoroethanol, guanidine hydrochloride, polyethylene glycol, urea as well as alcohol (Hamada & Dobson, 2002; Ma et al., 2013; Yoshida et al., 2012). Ethanol has a relatively low dielectric constants and molecular polarity, which can induce the denaturation of whey protein and weaken hydrophobic interaction (Cao & Mezzenga, 2019). Urea can solvate the peptide skeleton, exposing polar and aromatic residues and leading to the further unfolding of whey protein (Hoppenreijs et al., 2022). Ethanol has been proven to induce the formation of WPAFs by altering the polarity of the whey protein solution and promoting hydrophobic interactions of building blocks (Jordens et al., 2011). It has been reported that trifluoroethanol can induce the unfolding of WP into small oligomers with predominant β-sheet conformations at 25°C, resulting in the formation of worm-like WPAFs (Chen et al., 2025).

2.4. Biological auxiliary conditions

In addition to the above-mentioned influencing factors, biological auxiliary conditions can also affect the formation of WPAFs. The partial unfolding and acid hydrolysis of whey protein play a significant role in the whey protein fibrillization and can regulate the structure and morphology of WPAFs. Enzymatic hydrolysis can be applied as an alternative to acid hydrolysis to produce peptide fragments with increased aggregation propensity (Mohammadian & Madadlou, 2016a). Whey protein is hydrolyzed into polypeptides by enzymes under appropriate temperature and pH conditions, which leads to the formation of random aggregates. Subsequently, the pH of the solution was adjusted to 2.0 to promote the formation of WPAFs with micrometer length and nanometer diameter (Akkermans, Venema, van der Goot, Boom, & van der Linden, 2008). The combination of heating and enzymatic treatment of the whey protein solution has successfully induced the formation of WPAFs. Limited enzymatic hydrolysis (4 h) could promote the formation rate and the yield of WPAFs (Yang et al., 2023). Different enzymes could hydrolyze whey protein to form WPAFs. Gao et al. (2013) reported the influence of trypsin, pepsin, protease M, and protease A on the formation of WPAFs. Trypsin is a specialized peptide chain endonuclease that specifically targets peptide bonds formed by the amino acid arginine or lysine carboxyl groups (Pi et al., 2023). It was found that the hydrolysis products of trypsin had the highest ability to form WPAFs because the whey protein sequences prone to self-assembly remain intact under the hydrolysis of trypsin.

3. Structure and characterization of whey protein amyloid fibrils

Fibrillization provides an attractive strategy for the modification of whey protein (Zhu, 2024). The molecular arrangement of WPAFs depends on the different fibrillization conditions. The availability of additional functions of WPAFs mainly depends on the structure and morphology changes of WPAFs caused by fibrillization conditions. Therefore, it is crucial to correctly understand the morphology and structure changes of whey protein during fibrillization. The rational application of various characterization techniques is of great significance for exploring the morphology and structure changes of whey protein during fibrillization.

3.1. Structural features of whey protein amyloid fibrils

WPAFs are denatured whey protein aggregates composed of β-sheets and β-strands, which are parallel and perpendicular to the amyloid fibril axis, respectively (Venturi et al., 2025). Whey protein undergoes conformational changes under specific denaturation conditions, generating a large number of protomers. Conformational changes promote whey protein fibrillization by intermolecular interactions such as hydrophobic interactions, electrostatic interactions, van der Waals forces, disulfide bonds, and hydrogen bonds (Mohammadian & Madadlou, 2018). Protomer is the smallest fibril structure and also the basic building block of amyloid fibrils. A large number of protomers are assembled and transformed to form protofilaments. Two or more protofilaments are arranged in parallel and close to each other, further polymerizing and twisting to form various forms of amyloid fibrils (Lendel & Solin, 2021). WPAFs with various structures can be obtained by adjusting the fibrillization methods and conditions, leading to the formation of WPAFs with tunable height, length, flexibility, and molecular packing patterns (Hoppenreijs et al., 2022). The typical feature of WPAFs is fibril structures with micrometer length and nanometer thickness. Generally, WPAFs have structural similarities and polymorphisms. WPAFs share a remarkable similarity at the atomistic length scale, namely ordered β-sheet (antiparallel or parallel arrangement) and cross-β structures. The β-strands are stacked perpendicular to the fibril axis to form β-sheet. Adjacent β-sheets are arranged parallel or antiparallel to the fibril axis, which assemble into a cross-β structure. The β-strands distance is about 4.8 Å, whereas the β-sheets distance is about 6-12 Å (Cao & Mezzenga, 2019). Cross-β structures are extended along the longitudinal direction to form protofilaments instead of stacking in the latitude direction, further polymerizing into mature amyloid fibrils. In addition to the common structural similarity, WPAFs share structural polymorphism and molecular polymorphism. Polymorphism is defined as different amyloid structures formed by the same polypeptide, including the number of protofilaments, the relative arrangement of the protofilaments, and the configuration of protofilaments (Ostermeier et al., 2021). The interdigitating side chains in cross-β structures appear like the teeth of a zipper, thousands of cross-β structures are closely arranged to form a "steric zipper" structure when looking down the fibril axis (Nelson et al., 2005). The extreme stability of WPAFs is due to the existence of “steric zipper” structure. The “steric zipper” structure is not unique. Based on the differences in the sources of protomers, the registration and combination between protomers, a homo-steric or a hetero-steric zipper structure can be formed in the protofilament.

3.2. Characterization technologies of whey protein amyloid fibrils

The identification and characterization of WPAFs are based on the unique cross-β structure and extreme high aspect ratio structure. Rational identification and characterization methods contribute to a deeper understanding of the formation mechanism, structural composition, and functional characteristics of WPAFs. Currently, various technologies have been developed for the qualitative and quantitative study of whey protein fibrillization. Several complementary methods are usually used to improve the accuracy of the identification and characterization of WPAFs. The formation of WPAFs is accompanied by the appearance of cross-β structure. Congo red staining and Thioflavin T (ThT) fluorescence are sensitive techniques used to identify WPAFs, as they can specifically bind to the cross-β structure in WPAFs through electrostatic interaction and hydrophobic interaction. Congo red staining shows green, yellow, or orange birefringents under a polarized light microscope (Howie et al., 2008). However, dyeing operation of Congo red is rather complex, with background interference and low reproducibility (Biancalana & Koide, 2010). The binding of ThT to WPAFs results in a marked increase in fluorescence intensity. Correspondingly, the enhancement of ThT fluorescence intensity indicates the increase of cross-β-structure content. ThT fluorescence does not affect whey protein fibrillization process, which can monitor the growth kinetics of WPAFs (Sebastiao et al., 2017). Therefore, ThT fluorescence is an effective gold standard for detecting WPAFs. X-ray diffraction (XRD) is a more convenient method for identifying WPAFs (Sunde et al., 1997). The cross-β-structure in amyloid fibrils has distinctive reflection in the XRD pattern, which can be used to determine the β-strands and β-sheets distances of cross-β structures (Langkilde et al., 2015). XRD is the first method used to characterize the cross-β structures of amyloid fibrils. Fourier transform infrared spectroscopy (FTIR), and circular dichroism (CD) have also been used to analyze the β-sheet content in WPAFs. FTIR spectroscopy is a vibrational spectroscopy technique that quantitatively calculated the relative percentages of α-helix, β-sheet, β-turn, and random coil using PeakFit software (Zandomeneghi et al., 2004). CD spectroscopy is a spectral method based on the absorption of left and right circularly polarized light, which can quantitatively analyze the secondary structure changes of proteins using CDNN software (Micsonai et al., 2015). Atomic force microscopy (AFM), transmission electron microscopy (TEM), and scanning electron microscopy (SEM) are used to observe and analyze the macroscopic morphology and mesoscopic structure of WPAFs based on microscopic techniques, which can obtain high-precision structural information for further analysis and interpretation of the formation of WPAFs (Adamcik et al., 2010; Adamcik & Mezzenga, 2012). Mass spectrometry (MS) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) are used to monitor the

Table 3
Overview of structural characterizations of WPAFs.

Method	Principle	Comments	References
Congo red stain	Different affinities of amyloid fibrils and non-amyloid fibrils for Congo red	Amyloid fibrils exhibit typical apple green birefringence under polarized light microscopy.	(Howie et al., 2008)
Congo red absorption	Congo red binds cross-β-sheet structure of amyloid fibrils with relatively high affinity.	An increase in absorbance and a red shift in the maximum absorption wavelength when Congo red binds to amyloid fibrils; The high absorbance indicates the formation of more ordered amyloid fibrils.	(Qi et al., 2023)
Thioflavin T (ThT) fluorescence	ThT binds cross-β-sheet structure of amyloid fibrils with relatively high specificity.	An increase in ThT fluorescence intensity when ThT binds to amyloid fibrils; ThT fluorescence intensity is positively correlated with the number of amyloid fibrils; ThT fluorescence intensity can be used to evaluate the growth kinetics of amyloid fibrils.	(Sebastiao et al., 2017)
X-ray diffraction (XRD)	Cross-β-sheet structure of amyloid fibrils has distinctive reflections in the XRD pattern	Specific diffraction pattern of amyloid fibrils can be used to identify β-strands and β-sheets distances of cross-β structures; The distance between β-strands is 4.8 Å; The distance between β-sheets is 6–12 Å	(Langkilde et al., 2015)
Fourier transform infrared spectroscopy	Secondary structures of amyloid fibrils have different absorption peak intensities in the FTIR spectroscopy.	A blue shift of the amide I band signifies the transition of α-helix and random coils into β-sheet during fibrillization process; Amide I band in FTIR spectroscopy can be used to identify secondary structures of amyloid fibrils.	(Yang et al., 2022)
Circular dichroism (CD)	β-sheet structure of amyloid fibrils has different ellipticity signal in the CD spectroscopy.	Strong peak at 218 nm represents the β-sheet structure; An increase in ellipticity signal at 218 nm indicates an increased proportion of β-sheet structure.	(Wang et al., 2023)
Atomic force microscopy (AFM)	Based on interaction changes between sharp probes and fibrils surfaces to obtain AFM images of amyloid fibrils.	AFM can be used to observe surface structural features and monitor dynamic growth process of amyloid fibrils; AFM images can confirm fibrils formation and depict mesoscopic morphological parameters of amyloid fibrils including height profiles, contour length, persistence length, and curvature.	(Yang et al., 2023)
Transmission electron microscopy (TEM)	Focused electron beam projected onto amyloid fibrils surfaces to obtain TEM images of amyloid fibrils.	TEM images can confirm fibrils formation and observe the mesoscopic morphology of amyloid fibrils including length, diameter, branching, and aggregates	(Liu et al., 2024)
Dynamic light scattering (DLS)	Based on light dispersion phenomena to measure of size distribution within the nanometer to micrometer range during whey protein fibrillization.	DLS can be used to monitor the formation and growth of amyloid fibrils.	(Mukhopadhyay et al., 2022)
Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)	SDS-PAGE is used to monitor the molecular weight change during fibrillization process	SDS-PAGE indicates that whey protein is unfolded and hydrolyzed to be peptides; The density of main whey protein bands continues to decrease and the appearance of new band during whey	

(*continued on next page*)

Table 3 (*continued*)

Method	Principle	Comments	References
Rheology	Used to measure extensional flow and shear flow of amyloid fibril solutions.	protein fibrillization. Extensional flow was more sensitive to amyloid fibrils dispersions alteration than shear flow.	(Zhao et al., 2022)

molecular weight change during whey protein fibrillization (Bleiholder et al., 2011). Moreover, the advancements in techniques for studying WPAFs, such as dynamic light scattering (DLS), rheology (Loveday, Su, et al., 2012), quartz crystal microbalance (QCM) (Kotarek et al., 2008), intrinsic fluorescence, and extrinsic fluorescence have also been introduced to analyze the assembly behavior and growth kinetics of WPAFs. Table 3 describes detailed information about the application and results of these technologies to characterize the structure and function of WPAFs.

4. Assembly behaviors of whey protein amyloid fibrils

A comprehensive understanding of the growth process and formation mechanism of WPAFs is essential, as this is of great significance for the precise regulation of WPAF structure and function (Hoppenreijs et al., 2022). At present, pathways, mechanisms, and models used to describe the formation of WPAFs mainly include the self-assembly pathway and the nuclei-induced pathway. A schematic diagram of the possible assembly behaviors of WPAFs is depicted in Fig. 3.

4.1. *Self-assembly pathway*

WPAFs are usually considered to be denatured whey protein aggregates under specific denaturation conditions (Hoppenreijs et al., 2022). The self-assembly pathway is widely recognized mechanism used to describe the aggregation of denatured whey protein into amyloid fibrils (Tomadoni et al., 2020). Monomer model, polypeptide model, and nucleation-growth model are contributing to understanding the formation of WPAFs involved self-assembly pathway. The monomer model was first proposed to explain the whey protein fibrillization, including activation, nucleation, polymerization, growth, and termination steps (Morris et al., 2009). In this model, whey protein was unfolded to form active monomers under denaturation conditions, which suggested that active monomers are the basic building blocks of WPAFs. Activated monomers undergo nucleation, polymerization, and growth, ultimately self-assembling into amyloid fibrils. Activation and nucleation are the rate-limiting steps in monomer model. Monomer model suggested that the limited conversion of globular whey protein into WPAFs was attributed to insufficient active monomer as a result of whey protein hydrolysis (Bolder et al., 2007). Subsequently, polypeptide model was developed to further explain the whey protein fibrillization (Kroes-Nijboer et al., 2011). Polypeptide model mainly emphasizes the key role of whey protein hydrolysis during fibrillization process. Polypeptide model suggested that peptides are the basic building blocks of WPAFs instead of active monomers (Akkermans et al., 2008). With the deepening of the research on whey protein fibrillization, active monomers and polypeptides are both considered to be the basic building blocks of WPAFs. In addition to the above-mentioned monomer model and polypeptide model, nucleation-growth model is currently more accepted model to explain whey protein fibrillization. Nucleation growth model mainly emphasizes the crucial role of nucleation during fibrillization process (Kumar et al., 2017). Whey protein fibrillization follows an "S"-shaped growth curve, which can be characterized by a lag stage (monomer → nucleus), a growth or elongation stage (nucleus → protofibril), and a steady or maturation stage (protofibril → mature fibril) (Kroes-Nijboer et al., 2011). Lag stage is a slow spontaneous nucleation process to prepare the further formation of new ordered structures called "nuclei". The unfolding and hydrolysis of whey protein promote the formation and growth of nuclei. Growth or elongation stage corresponds to the formation and elongation of protofilament. The fibril conversion rate is the highest in this stage. When whey protein is exhausted and WPAFs stop growing, reaching a balanced state is called steady or maturation stage.

4.2. *Nuclei induction pathway*

Based on the nucleation growth model, nuclei induction pathway is another effective pathway to prepare WPAFs. Nuclei induction pathway

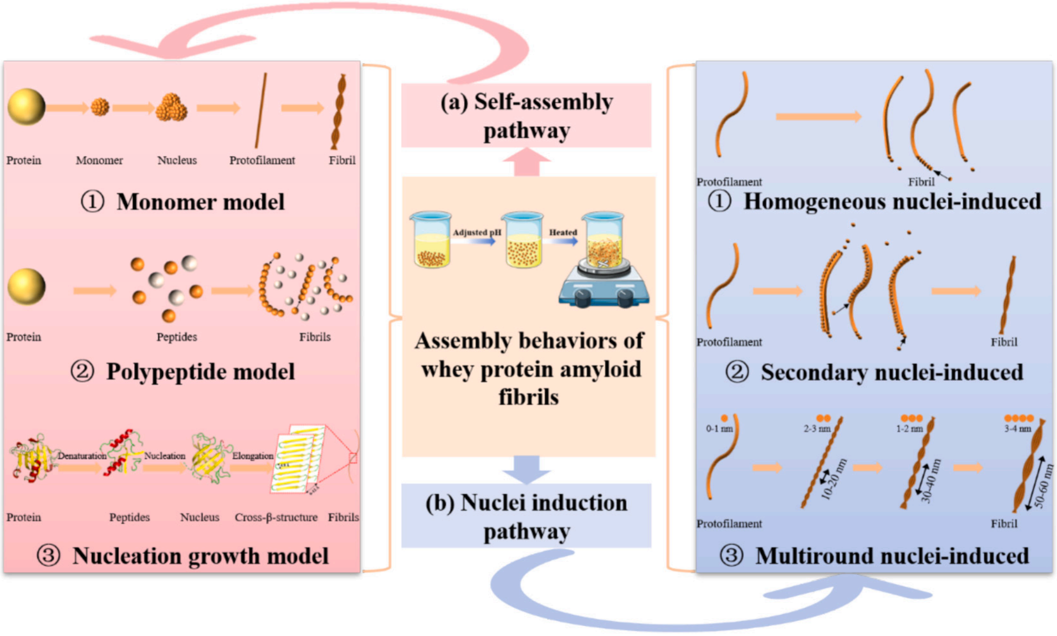

Fig. 3. Schematic diagram of the possible assembly behaviors of WPAFs.

refers to adding nuclei as seeds to induce the polymerization of protofilament and the formation of amyloid fibrils (Bolder et al., 2007). The incorporation of nuclei has an inducing effect, immediately eliminating the lag stage and accelerating the formation of amyloid fibril (Arosio et al., 2015). The growth of amyloid fibrils is an autocatalytic process, adding nuclei into whey protein solution to form “critical nuclei” with high free energy. whey protein monomers attach to the surface of the “critical nuclei”, and then grow and elongate to form WPAFs (Guan et al., 2022). Nuclei induction pathway can be divided into homogeneous induction, secondary induction, and multiple rounds induction. In the nucleation growth model, the high energy nuclei formed in the lag stage are referred to as homogeneous nuclei. The high energy nuclei further aggregate to form protofilament during the growth or elongation are termed secondary nuclei (Dovidchenko et al., 2014). Correspondingly, the process of accelerating whey protein fibrillization by adding preformed homogeneous nuclei or secondary nuclei as seeds to the whey protein solution is termed homogeneous induction and secondary induction, respectively. The addition of whey protein molecules on the basis of the presence of homogeneous and secondary nuclei can directly induce intermolecular polymerization, which can accelerate fibril formation by shortening or bypassing the lag phase and increasing the quantity of fibrils. Yang et al. (2022) found that WPAFs could not only be formed by self-assembly pathway spontaneously, but could also be formed by nuclei induction pathway. Previous studies suggested that homogeneous induction and secondary induction can accelerate whey protein fibrillization, thereby shortening the lag time and increasing the number of fibrils (Tan et al., 2018). Multiround induction pathway, a novel fibrillization approach, uses “nuclei” as seeds to make amyloid fibrils through repeated seeding reactions. Briefly, homogeneous nuclei or secondary nuclei are used as seeds to prepare amyloid protofibrils. Amyloid protofibrils are used as new seeds to prepare new amyloid protofibrils. By repeating the above procedure, a series of protofibrils with different seeding cycle numbers is produced (Wang et al., 2021). Researchers have used secondary nuclei as seeds to induce the polymerization of whey protein monomers and have successfully prepared amyloid fibrils (Guan et al., 2022). Previous studies have suggested that multiround induction can change the structure of WPAFs with greater height and periodicity, which made the amyloid fibril more flexible and hydrophobic (Yang, Song, et al., 2024).

5. Application design of whey protein amyloid fibrils

Whey protein fibrillization is an economical and feasible strategy that can improve structural characteristics and expand the functional properties of native whey protein, which makes WPAFs have an important place in application design. The structural characteristics and functional properties of WPAFs determined their applications and effects in different fields. In order to explore the feasibility of WPAFs in

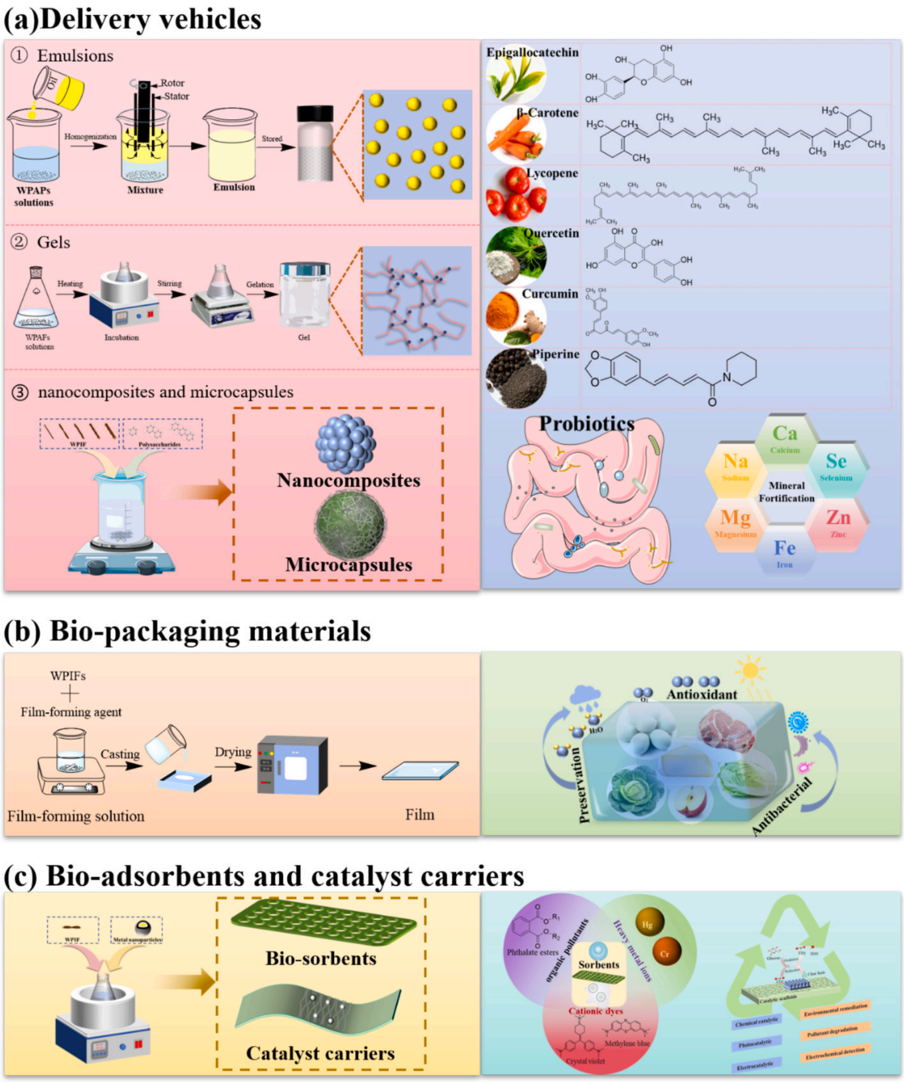

Fig. 4. Summary of the application designs of WPAFs.

various application designs, a large amount of research work has been performed. Nowadays, previous studies have successfully reported WPAFs as functional materials and structural building blocks in various fields such as food science, materials science, environmental science, biomedicine, tissue engineering, and nanotechnology (Su et al., 2024). Fig. 4 summarizes the diverse applications of WPAFs in various aspects, including delivery vehicles, bio-based packaging materials, bio-absorbent, and catalyst carriers.

5.1. Delivery vehicles

Bioactive ingredients mainly promote beneficial human health by regulating physiological or cellular activities. Common bioactive ingredients include polyphenols, essential oils, carotenoids, vitamins, minerals, phytosterols, bioactive peptides, probiotics, and other nutritional factors, all of which have been proven to exert positive biological functions. However, many bioactive ingredients have poor solubility, low physicochemical stability, and decreased bioavailability, which limit their applications in health fields. Therefore, there is an urgent need for a method to improve the bioavailability of bioactive ingredients, protecting and delivering them to targeted sites, thereby promoting absorption and metabolism of bioactive ingredients. WPAFs possess a number of outstanding attributes, such as micro-nanoscale size, high aspect ratio, ordered structures, superior mechanical strength, and nutritional benefits. These unique features endow WPAFs with considerable potential for the encapsulation, protection, and delivery of bioactive components, making them attractive candidates for applications in the food, pharmaceutical, and nutraceutical industries. Currently, emulsions, gels, nanocomposites, and microcapsules are important vehicles for delivering bioactive substances.

Emulsions are defined as a homogeneous dispersion composed of two immiscible phases, wherein, the dispersed phase is dispersed in the form of droplets in the continuous phase. Better interfacial activity, superior emulsifying property, and excellent deformation resistance of WPAFs can be used as stabilizer or emulsifiers to fabricate different types emulsions such as emulsions (Liu et al., 2024), double emulsions (Cui et al., 2023), high internal phase emulsions (Yang et al., 2022). WPAFs can increase surface activity and reduce interfacial tension by forming high interfacial moduli within the emulsion matrix. Recently, WPAFs-stabilized emulsion as an ideal delivery system for bioactive ingredients has attracted widespread attention. Previous studies have successfully used WPAFs emulsions to deliver bioactive components. Chen et al. (2025) studied the delivery capacity of β-carotene by elongated and worm-like WPAFs, found that elongated fibrils had higher release of β-carotene in the gastrointestinal digestion. Zhu et al. (2024) reported that WPAFs stabilized Pickering emulsion can protect lycopene from UV and heat degradation.

Gels are solid three-dimensional networks that trap and immobilize the solvent. Food gels are promising carriers for the delivery system for bioactive ingredients due to their advantages such as renewability, low cost, biocompatibility, and biodegradability (Cao & Mezzenga, 2020). WPAFs, with high stiffness, extreme aspect ratio, ordered structures, accelerated gelation rate, and superior viscoelastic properties hold significant promise as an ideal matrix material for food gels. Previous studies have successfully used WPAFs as gelling agents to fabricate various types of gel-based delivery systems, such as hydrogels (Mohammadian & Madadlou, 2016b), aerogels (Peydayesh et al., 2020), oleogels (Mykolenko et al., 2025), and mixed gels (Chen et al., 2020). WPAFs have micro-nanoscale size and high stiffness. These structural advantages allow them to significantly enhance the mechanical strength and water-holding capacity by forming rigid networks within the gel matrix. (How et al., 2021) successfully prepared the β-LG amyloid fibril hydrogels to deliver riboflavin, found that the release rate of riboflavin was correlated with the number of amyloid fibrils. Zhu et al. (2022) reported that WPAFs and gliadin nanoparticles can assemble into hydrogels for curcumin loading. Moreover, the hydrogel could effectively load and protect curcumin from thermal degradation and photodegradation. Emulsion gels are complex colloidal materials composed of emulsion droplets embedded in gel structures. Compared with emulsions and gels, three-dimensional gel network effectively captures emulsion droplets, thereby improving stability and preventing phase separation. As a novel type of delivery system, emulsion gel has been widely used in the development of delivery system for delivery of bioactive ingredients. Wang et al. (2024) prepared WPAFs emulsion gels to deliver curcumin, noting that these emulsion gels exhibited a high curcumin retention ratio and enhancement of bioaccessibility during simulated gastrointestinal digestion.

In addition to emulsion and gel-based delivery systems, WPAFs can also be developed into nanocomposites and microcapsules as delivery systems for bioactive ingredients. WPAFs have good interfacial and surface activity due to multiple functional groups, which can spontaneously bind hydrophobic compounds to form nanocomposites through non-covalent interactions, thus allowing for the co-encapsulation and co-delivery (Meng et al., 2024). Also, positively charged WPAFs can co-assemble with negatively charged polysaccharides to form composites through electrostatic interaction. These complexes have high surface activity and charged adsorption layers, which can be utilized to construct microcapsule shells of the microcapsule by the layer-by-layer assembly approach to protect, control, or target the release of bioactive ingredients (Humblet-Hua et al., 2012). Numerous studies have successfully developed nanocomposites and microcapsules into delivery vehicles for the loading and encapsulation of bioactive compounds (Kang et al., 2024; Li et al., 2023). Zhang et al. (2021) reported that WPAFs and β-carotene form nanocomposites through van der Waals force, hydrogen bonds, and hydrophobic interactions for the protection and delivery of β-carotene. The results showed that worm-like WPAFs nanocomplexes have a high encapsulation rate and loading capacity of β-carotene, while elongated WPAFs nanocomplexes have a higher release of β-carotene during gastrointestinal digestion (Chen et al., 2025). Hu et al. (2020) prepared microcapsules with chitosan and WPAFs, which have been proven to have the potential as effective carriers for curcumin and showed improved aqueous dispersion stability and antioxidant activity. Shen et al. (2017) and Zhou et al. (2025) have demonstrated that WPAFs can serve as both anti-oxidizing nanocarriers and colloidal stabilizers, thus playing a crucial role in iron fortification for both animals and humans.

5.2. Bio-based packaging materials

Whey protein is nutritionally complete with the presence of all the essential amino acids (Boscaini et al., 2023). The antioxidant activity of whey protein is enhanced by hydrolyzing or unfolding the native conformation to improve the solvent accessibility of active amino acid residues (Mohammadian & Madadlou, 2016a). The antioxidant activity and antibacterial property of WPAFs are improved, which can be attributed to the exposed amino acids with sulfur groups or aromatic side chains during fibrillization treatment (Zhao et al., 2021). WPAFs with film-forming property are expected to be applied in bio-based packaging materials to achieve better packaging performance because of antioxidant activity, antibacterial property, and food-grade biological properties (Zheng et al., 2024). Most petroleum-based polymers and plastics packaging materials are non-biodegradable and nonrenewable, which can cause environmental pollution and affect human health. Global plastic pollution has a catastrophic impact on the earth ecosystem. The development of green bio-based packaging materials is an effective way to solve the environmental pollution caused by petroleum-based polymers and plastics packages (Yuan et al., 2021). Edible film and coating have been considered as potential approaches to reduce traditional packages, which can help protect food products from multiple factors such as physical, chemical, and biological deterioration (Kumar et al., 2022). Previous reports showed that WPAFs have been incorporated into edible films and edible coatings to design bio-based packaging materials, which can improve the mechanical and functional properties of packaging materials (Feng et al., 2018; Yang et al., 2024). Edible film constitutes a preformed thin layer made of edible components that, once formed, can be placed on or between food components. Edible coating refers to a thin layer of coating formed on the surface of food, usually in liquid form, applied to the surface of food by means of brushing, spraying, or dipping (Hasan et al., 2023). Usually, edible films and coatings can be eaten as part of food. The incorporation of WPAFs in the film or coating has been reported as a more suitable approach for application in food packaging. WPAFs were reported to be incorporated into starch coating, which showed superior antioxidant properties and inhibited the browning of fresh-cut apples during storage (Zheng et al., 2024). Additionally, the addition of polyphenols to WPAF-based films resulted in synergistic antibacterial effects the addition of polyphenols to WPAF-based films (Liu et al., 2023). Peydayesh et al. (2021) noted that sustainable bioplastic films produced by combining WPAFs with biodegradable polymers exhibited highly uniform surfaces, acceptable water stability, good barrier properties, and enhanced antioxidant activity, improving their suitability for efficient food packaging. Furthermore, incorporating anthocyanins into bioplastic films to prepare sustainable smart packaging materials demonstrated significant performance in monitoring food freshness and extending shelf life. These materials exhibited strong pH-responsivity, which allowed for precise and sensitive colorimetric detection of seafood spoilage in pH-sensitive smart sensors (Peydayesh et al., 2024).

5.3. *Bio-adsorbent*

Environmental contaminants are currently a major threat to human health and ecosystem sustainability. Organic pollutants, metal pollutants, dyes, bacteria, and viruses all cause serious environmental problems, seriously affecting ecosystems and human health. Therefore, there is an urgent need to develop efficient, safe, sustainable, and environmentally friendly porous adsorption materials to remove contaminants. WPAFs have high specific surface area, increased surface activity, and diverse functional groups, which make them suitable as functional materials to produce bio-absorbents for the removal of contaminants (Jia et al., 2022). WPAFs have increasingly been employed as scaffolds and building blocks in the fabrication of various porous adsorption materials. These include aerogels, biopolymer foams, and adsorbent membranes, all of which exhibit remarkable adsorbent characteristics in diverse fields. Aerogels are an emerging category of ultralight solid and porous materials with high porosity and low density, which can be obtained by removing solvents from the gel. Notably, aerogels prepared using WPAFs exhibit more available adsorption sites, thereby demonstrating superior adsorption performance (Wang et al., 2024). WPAFs-based aerogels as a sustainable, efficient, and inexpensive solutions, exhibit excellent adsorption capacity for organic pollutants, dyes, heavy metals, bacteria, and viruses, which minimize the impact of pollutants on the ecological environment and human health (Peydayesh et al., 2022). Researchers have successfully utilized the adsorption properties of WPAFs aerogels to remove organic pollutants from water (Peydayesh et al., 2020) and recover gold from electronic waste (Peydayesh, Boschi, Donat, & Mezzenga, 2024). Furthermore, given the biocompatibility, biodegradability, and environmental friendliness of WPAFs, the composite biopolymer foams formed by WPAFs have performed exceptionally well in the applications of adsorbing dibutyl phthalate and removing heavy metals, with removal efficiency exceeding 98% (Wang et al., 2025). Membrane filtration is a promising technique for removing heavy metals and purifying water (Ramírez-Rodríguez et al., 2020). WPAFs and activated porous carbon were employed to fabricate hybrid membranes capable of selectively adsorbing heavy metal ions and radioactive waste in solutions (Bolisetty & Mezzenga, 2016). These hybrid membranes have demonstrated considerable potential for environmental remediation and wastewater treatment.

5.4. *Catalyst carriers*

Metal nanoparticles have gained significant importance in industrial applications as catalysts due to their exceptional catalytic activity and stability. However, a major challenge arises from their high surface energy, which tends to cause nanoparticle aggregation. This phenomenon notably hinders the catalytic performance of metal nanoparticles, highlighting the need for strategies to mitigate aggregation and enhance their functionality (Lai, Wang, & Lin, 2024). WPAFs serve as the immobilization substrates for metal nanoparticles due to unique structural stability, high mechanical strength, and superior biocompatibility (Lai, Huang, et al., 2024). In the presence of WPAFs, metal nanoparticles can effectively reduce aggregation, improving the catalytic activity and thermodynamic stability. Recent studies have demonstrated that metal nanoparticles formed with the participation of WPAFs have been successfully applied in the fields of chemical catalysis and electrocatalysis (Lai et al., 2024). WPAFs were chosen as the support of titanium dioxide nanoparticles (Lai et al., 2024) and silver nanoparticles (Lai et al., 2022) and utilized for the catalytic reduction of methylene blue. WPAFs participate in the modification of electrochemical sensors, which demonstrate enhanced electron transfer capabilities and increased electrocatalytic activity (Lai et al., 2024). In addition, the application of metal nanoparticles developed from WPAFs in biological and environmental fields is currently a subject of ongoing exploration (Peydayesh et al., 2024; Wang et al., 2024). These nanoparticles exhibit considerable potential as carriers and substrates for catalysts and enzymatic reactions, positioning them as promising materials for future advancements.

6. Conclusion and future perspectives

Whey protein is renowned for its wide availability, biocompatibility, and biodegradability, making it a primary source of high-quality protein for the fabrication of amyloid fibrils. Fibrillization is a promising strategy to improve and broaden the structural and functional properties of whey protein. In this paper, we reviewed the preparation conditions, structural characterizations, assembly behaviors, and application designs of WPAFs. The fibrillization conditions of pH 2.0 and 90°C has been regarded as the standard condition for whey protein fibrillization, the integration of physical and chemical techniques is employed to optimize and regulate the structure and function of the WPAFs. WPAFs are characterized by cross-β structure, which is mainly composed of β-sheets parallel to the fibril axis and β-strands perpendicular to the fibril axis forming highly anisotropic aggregates. WPAFs exhibit significant potential for implementation in various industries, including as components in delivery vehicles, bio-based packaging materials, bio-absorbent products, and as carriers for catalysts. WPAFs not only provide the essential amino acids needed for comprehensive nutrition but also offer exceptional digestibility. Investigating these diverse applications of WPAFs across different sectors holds considerable promise. However, there are various limitations in present studies that need to be addressed in future studies. From our perspective, when exploring the future research directions of WPAFs, the following aspects should be taken into consideration: (1) Food-derived amyloid fibrils have been validated as safe nutrients, with extensive evidence from both animal models and human clinical trials supporting their biocompatibility and safety profile. Whey proteins serve as the primary sources in the formation of amyloid fibrils. Most studies have focused on the exact structural characteristics and functional properties of WPAFs. In fact, the reproducibility and scalability of WPAFs still need to be improved to promote the sustainable application in multiple fields due to their structural polymorphism and functional diversity. (2) WPAFs usually can be formed under controlled conditions with heating treatment and acidic environment. Extreme acidic conditions promote the formation of amyloid fibrils, while also posing challenges for food processing applications. Acidic environment exceeds the acceptable range for food processing, as the pH range of most foods is usually between 4.0 and 7.0 to ensure safety, taste and overall quality. Therefore, it is necessary to consider the impact of post-fibrillization pH adjustment on the morphology, stability, and functionality of food processing under different storage conditions. (3) WPAFs are employed as a matrix material for constructing composite systems with other functional materials. Significant consideration should be given to the addition reactions, interfacial interactions, and structural adaptability between individual components, which serve as the critical prerequisite for ensuring the structural stability and functional synergy of the composite systems.

CRediT authorship contribution statement

Caiyun Cheng: Writing – original draft, Software, Investigation. **Qian Xu:** Validation. **Yang Li:** Methodology, Investigation. **Eric Haubruge:** Methodology, Investigation. **Ning Liao:** Supervision, Data curation. **Guangsu Zhu:** Supervision, Investigation. **Kunlun Liu:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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