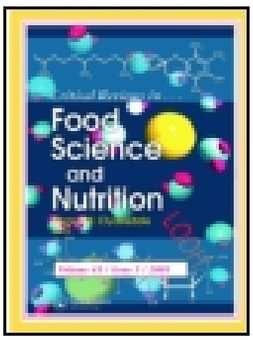

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REVIEW

Latest developments in the applications of microfluidization to modify the structure of macromolecules leading to improved physicochemical and functional properties

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ABSTRACT

Microfluidization is a unique high-pressure homogenization technique combining various forces such as high-velocity impact, high-frequency vibration, instantaneous pressure drop, intense shear rate, and hydrodynamic cavitation. Even though it is mainly used on emulsion-based systems and known for its effects on particle size and surface area, it also significantly alters physicochemical and functional properties of macromolecules including hydration properties, solubility, viscosity, cation-exchange capacity, rheological properties, and bioavailability. Besides, the transformation of structure and conformation due to the combined effects of microfluidization modifies the material characteristics that can be a base for new innovative food formulations. Therefore, microfluidization is being commonly used in the food industry for various purposes including the formation of micro- and nano-sized emulsions, encapsulation of easily degradable bioactive compounds, and improvement in functional properties of proteins, polysaccharides, and dietary fibers. Although the extent of modification through microfluidization depends on processing conditions (e.g., pressure, number of passes, solvent), the nature of the material to be processed also changes the outcomes significantly. Therefore, it is important to understand the effects of microfluidization on each food component. Overall, this review paper provides an overview of microfluidization treatment, summarizes the applications on macromolecules with specific examples, and presents the existing problems.

KEYWORDS

Dietary fibers; dynamic high-pressure microfluidization; high-pressure microfluidization; microfluidization; polysaccharides; proteins; starches

Introduction

From the standpoint of food science, scientists have been trying to understand the effects of processing techniques on size, structure, conformation, and material characteristics that have certain influences on physicochemical and functional properties. Specifically, in the search of specific physicochemical and functional properties, some applications on foods aim to modify the structure and conformation to enhance rheological, mechanical, and emulsifying properties (e.g., consistency, viscosity, stability, strength), to improve antimicrobial properties by extending the availability of certain compounds (e.g., essential oils, phenolics), to contribute to sensory properties (e.g., color, flavor, taste), and to produce micro- and nano-structures to improve material characteristics for specific purposes (e.g., higher surface area, better solubility) (Chaudhry et al. 2008).

The conformation of molecules within a material has great impacts on specific behaviors such that different constructional frameworks such as sheets, helices, and spirals result in numerous bonding possibilities inter- and intramolecularly, which leads to changes in functional properties (Villay et al. 2012). In addition to conformation, physical (e.g., size, surface area) and rheological (e.g., viscosity) properties play a significant role in incorporating food components into new formulations to have better functional properties. These are some examples of why researchers have proposed a great number of applications and treatments to reveal the best characteristics of a material or to modify and improve these characteristics. In this perspective, chemical (e.g., pH treatment, crosslinking), enzymatic (e.g., hydrolysis, catalysis), and physical (e.g., thermal treatments, ultrasonication, microwave) methods have been used to obtain greater functionalities compared to the native version of the food components (Dabbour et al. 2019; Juodeikiene et al. 2020; Wang et al. 2021). Recently, non-thermal physical processing techniques have been highly popular in the food industry and among high-pressure treatments, even though its applicability on large scale is still limited, microfluidization has had an important place (Ganesan et al. 2018). Specifically, it has been commonly utilized for the modification of biological macromolecules, formation of nanoemulsions and nanoparticles (Páez-Hernández, Mondragón-Cortez, and Espinosa-Andrews 2019), and enhancement of rheological (He et al. 2020), emulsifying properties like stability (Santos et al. 2019), and functional properties like water holding and binding capacity (Ozturk

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and Mert 2019), and solubility (Ge et al. 2021). Its advantageous aspects including low treatment temperature, short processing time, little nutritional loss, and no exogenous chemical formation (Hu et al. 2011) give it an edge compared to other high-pressure systems as well.

The applications of microfluidization in the food industry began with dairy products in the late 1980s (Cook and Lagace 1985; Paquin and Giasson 1989) and the positive results leading to better physical properties in dairy products have attracted the attention of researchers in other fields. The increasing interest in this technique resulted in the spread of its use on different products and sectors including beverages, cereals, proteins, and polysaccharides (e.g., gums, dietary fibers, starches). Even though, in the early years, it was commonly preferred because of its effects on size reduction, later, researchers found that this technique also contributes to many other improvements in food properties such as enhancement of hydration properties (Ozturk and Mert 2019), viscosity (He et al. 2020; Li et al. 2018), and cation-exchange capacity (Wang et al. 2012), increase in bioavailability of phenolic compounds (Cikrikci, Demirkesen, and Mert 2016; Morales-Medina et al. 2020), and functionalization of fibrous structures (Mert et al. 2014), which make way for the enhanced material to be used in other food applications. Even the major application area of this technique is still related to emulsion-based products, especially dairy and beverage industries (Kumar et al. 2019; Martin-Piñero, Muñoz, and Alfaro-Rodriguez 2020; Wang et al. 2019), the shift in the research explained above has led to new research topics using microfluidization. Therefore, in this review, emulsion-based systems were excluded and the main focus was given to the direct effect of this innovative technology on macromolecules. In this perspective, the primary objective of this review is to serve as a solid reference for microfluidization by presenting an outline about its working principle and major applications in modifying food macromolecules. Specifically, the focus is kept on reviewing the improvements on the physicochemical and functional properties of food macromolecules and discussing the findings of researchers using this emerging technology.

General mechanism

Although microfluidization is known as a type of high-pressure processing technique, it is quite different from high-pressure homogenization in terms of its working principle. In high-pressure homogenization, the shear forces are due to the sudden restriction of flow leading to the fluctuations in pressure. The change in pressure during the treatment results in products with wider size distributions. In microfluidization, on the other hand, the fixed geometry of the instrument leads to the delivery of constant pressure profiles, which are provided with compression and suction strokes. The pressure provided by the pump is constant during the compression strokes, meaning constant feeding, while zero pressure is applied during suction strokes, indicating no feeding. Due to the continuous constant and zero pressure cycles, it produces particles with smaller size and narrower size distribution (Mert 2020).

In addition to its high-pressure effect, microfluidization combines many other features such as high-velocity impact, high-frequency vibration, instantaneous pressure drop, intense shear rate, and hydrodynamic cavitation (Liu et al. 2009; Mert 2020). In microfluidization, inertial forces in turbulent flow, cavitation, and laminar elongation flow are the dominant ones responsible for the disruption of structures (Galooyak and Dabir 2015; Jafari, He, and Bhandari 2007). Figure 1 illustrates the schematic representation of the microfluidization process (Figure 1A) and the reaction chamber of the equipment (Figure 1B). As illustrated, the fluid is pumped with a high-pressure into the interaction chamber, where it is first divided into two or more microstreams possessing high velocity. This high velocity is due to the high-pressure inlet that flows through the narrow microchannels. Then, the microstreams are collided with each other at the impingement area, generating intense disruptive forces such as high impact energy (Alkanawati et al. 2018; Ganesan et al. 2018). This collision results in deformation and changes in the material structure and formation of very fine particles (Bai and McClements 2016). Also, the decrease of dimensions in microtubes, in regions just before the collision region, creates high shear rates, which enhance the effects further. Besides, due to the instantaneous pressure drop at the exit of the interaction chamber, the product expands, leading to irreversible changes such as the formation of pores, tissues, and cavities. At the end of the process, the temperature of the product is cooled down and the product is collected in the outlet reservoir (Mert 2020; Ozturk and Mert 2018a).

Depending on the type of treated dispersion, the design of the interaction chamber varies as shown in Figure 2. Type Y is generally used for liquid-liquid dispersions such as the formation of emulsions and liposomes, while type Z is more efficient for processing solid-liquid dispersions such as disruption of solid structures and formation of nano-dispersions (Nekkanti, Vabalaboina, and Pillai 2012). In microfluidizers having type Y interaction chamber, the fluids divided into two streams are forced to pass through smaller tubes, resulting in a higher velocity and a consequent higher shear rate. Then, these microstreams collide with each other in the impact zone with a very high velocity, which causes further changes in structure leading to the formation of finer emulsions (Mert 2012). On the other hand, in microfluidizers with a Z-type interaction chamber, the fluid under high-pressure is forced to pass through zigzag microtubes, resulting in a collision at the walls leading to disruption of solid particles and formation of homogenous dispersions.

Improvements on physicochemical and functional properties

It is generally speculated that microfluidization should be considered as a pretreatment and the effective modification of samples are due to other processing techniques used along with microfluidization. However, as introduced in the

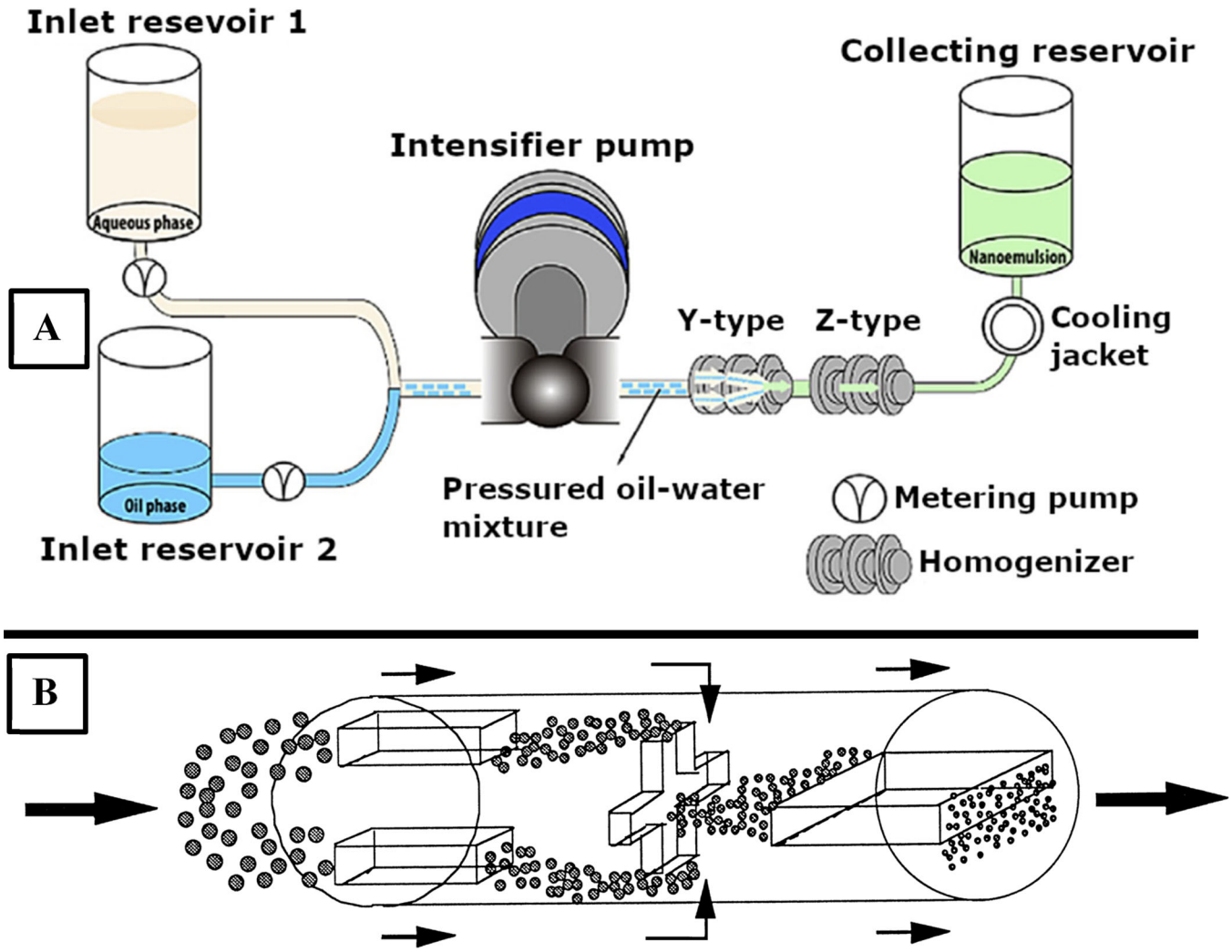

Figure 1. Schematic representation of (A) production using one-step dual-channel microfluidization (B) the reaction chamber of the equipment. Reproduced from Bai et al. (2016) and Lagoueyte and Paquin (1998) with the permission from the publisher.

first section, there is a growing interest in the use of microfluidization as the primary processing method rather than as a pretreatment. Therefore, it should be clarified that even though microfluidization is commonly used as a pretreatment in food science, especially in emulsion-based systems, the aim of this review is to gather the research using microfluidization as the primary and mostly the sole processing method to modify the physicochemical and functional properties of food components and to address the question of whether the modifications of samples are due to microfluidization or other methods.

Macromolecules such as proteins and polysaccharides are the major components in a food matrix and highly influence the functional and textural properties of food. However, the desired properties for each component differ from each other (e.g., high dispersibility and solubility for most proteins, slow retrogradation properties for starches, minimized gas production for dietary fibers). Therefore, there is a need to modify the food components to reveal these desired properties. Nowadays, there is a tendency for the use of environmentally friendly physical methods among consumers and microfluidization can be thought as a physical modification method leading to changes in food components. Mostly, it has a superior effect on the particle size of materials leading to micro- or nano-size particles, which leads to numerous other changes in the properties of the material such as increased surface area, solubility, and diffusion rate, the formation of new structures through the matrix, and changes in conformational structure. However, these changes can be in many different ways depending on the treated material. Therefore, in this section, we systematically investigated the effects of this innovative technique on macromolecules separately. The main topics reviewed in this paper have been outlined in Figure 3.

Proteins/enzymes

In the food industry, there are several proteins and byproducts with a high protein content that have very limited application area or only utilized as animal feed due to lack of their functionality and desired properties to be incorporated into human food products. There has been a growing interest to improve the functional properties of these protein-rich byproducts or proteins directly to utilize these materials in new applications. Since microfluidization makes changes in conformational structure of the proteins (e.g.,

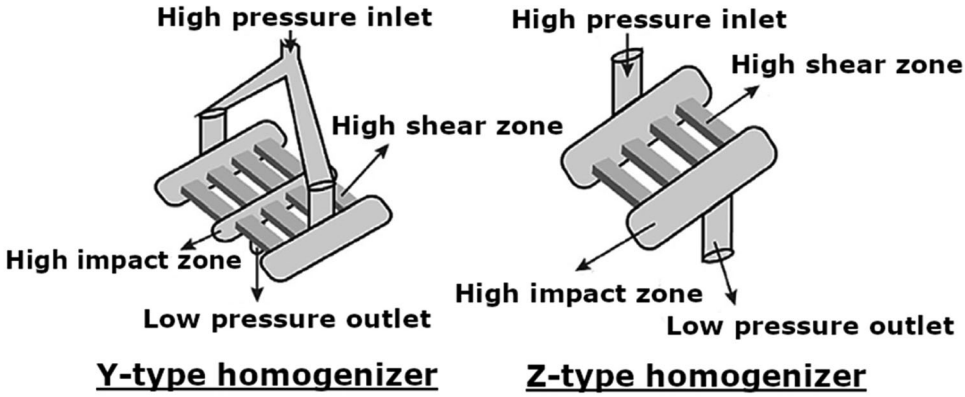

Figure 2. Schematic drawing of Y- and Z-type homogenizer chambers. Reproduced from Bai et al. (2016) with the permission from the publisher.

secondary, ternary), it contributes to the formation of new structures and provides interactions between different groups in protein structure, which leads to improvements in functional properties of proteins (Iordache and Jelen 2003). In this section, its applications on different proteins (Table 1) were discussed in detail to present how proteins can be modified to improve specific properties. For this purpose, first its impacts on chemistry and conformation were discussed, then changes in functional properties as a result of these chemical changes were detailed with specific food examples.

Chemical changes

Secondary structure. Hu et al. (2011) used microfluidization on peanut protein isolate (PPI) and observed that microfluidization effectively changed the physicochemical and functional properties by unfolding the structure of PPI due to changes in its secondary structure. They showed that the β-sheet and random coil structures increased at the expense of α-helices and β-turns, leading to a looser structure. Similarly, Gong et al. (2019) worked on PPI in aqueous dispersion to investigate the driving forces and mechanism behind disintegration and reaggregation of this protein when treated with microfluidization. They proposed the mechanism depicted in Figure 4 as a response to the structural changes that happened with the change in treatment pressure. They observed an increase in β-sheet content when the treatment pressure was as high as 120 MPa, similar to the findings of Hu et al. (2011) who also reported an increase in β-sheet content at the same pressure; however, Gong et al. (2019) reported a lower β-sheet content when the pressure exceeded 120 MPa. They concluded that, as shown with the mechanism in Figure 4, the treatment at 120 MPa led to the highest disintegration in the aggregated structure and increasing pressure further caused reaggregation.

FT-IR results of Liu et al. (2017) on ovalbumin showed that although there was conformational changes for the samples treated at 80 and 160 MPa, interestingly, no change was observed for the sample treated at 120 MPa. However, for this analysis, they only considered the peak position of the Amide I without determining the secondary structure content. On the other hand, with hydrogen/deuterium exchange-mass spectroscopy analysis (HDX-MS), they showed that the treatment was specifically effective on α-helix and small β-sheets whereas big six strands of β-sheets resisted to impacts of microfluidization. Therefore, although a treatment at 80 or 120 MPa led to a more flexible and open structure for ovalbumin, only partial unfolding could be mentioned (Liu et al. 2017).

Liu et al. (2009, 2010) reported a decrease in α-helix content of mushroom polyphenoloxidase (PPO) and trypsin as shown with FTIR results. A partially unfolded and more open structure was obtained as a result of the changes in conformation, which significantly benefited the enzyme activity. They also observed some conformational changes in the tertiary and quaternary structures as a result of the exposure of tryptophan and tyrosine residues due to unfolding.

Fluorescence spectroscopy, circular dichroism, and thermal analyses on β-lactoglobulin (Chen et al. 2016) showed that there were changes in the tertiary and secondary structure of the protein due to protein unfolding; a decrease in β-sheets and an increase in α-helix structures. These changes in conformation resulted in significant improvements in binding capacity leading to reduced allergenicity. Similarly, Zhong et al. (2019)'s work on investigation of antigenicity and conformational changes on β-lactoglobulin with kestose glycation and microfluidization presented that the use of microfluidization resulted in unfolding of protein structure and the formation of two conjugates with different molecular weights. However, they only reported a significant increase in α-helix content and did not observe any change in other secondary structures (β-sheets, β-turns, and unordered). The authors hypothesized that this conformational change was the basis for the decrease in antigenicity due to masked or disrupted epitopes in the structure of the protein.

Fan et al. (2020) applied microfluidization on a very interesting natural product, water-insoluble fraction of edible birds' nest (EBN) or cubilose that is produced from saliva of some bird species. They observed conformational changes in protein structure such that the percentage of α-helix and β-turn structures increased at the expense of random coils mostly. They also noted that tryptophan residues were less exposed to solvent, denoting more hydrophobic nature.

Table 1. An overview of research articles using microfluidization on proteins.

Protein/Enzyme	Modification/Change in properties	References
Peanut protein isolate	Unfolding of protein, changes in secondary structure (increased β-sheet and random coils at the expense of α-helices and β-turns), exposure of hydrophobic groups • An increase in protein dispersibility and solubility, and a decrease in surface hydrophobicity	Hu et al. (2011)
Peanut protein isolate	Disintegration (up to 120 MPa) and reaggregation (>120 MPa) of protein • Favored surface hydrophobicity, increased the number of disulfide bonds, and decreased free sulfhydryl groups, but the extent of these changes was dependent on pressure level	Gong et al. (2019)
Pea albumin aggregate	Reduction in particle size, unfolding • Foaming and emulsifying properties of pea albumin aggregates were favorably influenced	Djemaoune, Cases, and Saurel (2019)
Ovalbumin	Unfolding of protein • A lower deuterium integration for β-sheets	Liu et al. (2017)
Fish gelatin	Polyporous microstructure formation • Enhanced emulsifying and foaming properties, higher to pepsin digestibility, lower melting point	Sha et al. (2018)
β-lactoglobulin	Changes in the tertiary and secondary structure of the protein due to protein unfolding • Decreased binding capacity of immunoglobulin E (IgE) with glycation	Chen et al. (2019, 2016, 2017)
β-lactoglobulin	Emergence of smaller peptide pieces, a decrease in particle size • Enhanced the gastrointestinal digestibility of β-lactoglobulin, an increase in hydrophobicity	Chen et al. (2019)
β-lactoglobulin	Unfolding of protein structure and change in the secondary structures • Decrease in antigenicity due to masked or disrupted epitopes	Zhong et al. (2019)
Whey protein fibrils	Fragmentation and partial disruption of biopolymer aggregates • Phase separation (transparent at the top and turbid at the bottom) due to the thermodynamic incompatibility between the components, decrease in apparent viscosity	Koo et al. (2018)
Whey protein	limited the sedimentation of whey protein polymers (especially at lower pH level) • Enhancement in the degree of solubility	Iordache and Jelen (2003)
Corn gluten meal	Disintegration of hydrophobic structures into micro particles, formation of tissues, cavities, and micropores • A substantial decrease in particle size with narrower particle size distribution, enhanced hydration properties due to increase in the number of available water binding sites	Ozturk and Mert (2018a, 2018b, 2019)
Soy protein isolate	Conformational changes observed as unfolding of protein and rearrangement of structure, interchanges between free sulfhydryl group (SH) and disulfide bond (SS) contents • Higher protein solubility, surface hydrophobicity, disulfide bond content, and emulsifying ability	Shen and Tang (2012)
Polyphenoloxidase	Changes in the conformation (decrease in α-helix content) • Increased enzyme activity	Liu et al. (2009, 2010)
Trypsin	Exposure of tryptophan and tyrosine residues, and an increase in exposed sulfhydryl groups (-SH) due to newly formed unfolded structure • Improved pH and thermal stability in spite of no impact on enzyme activity	Liu et al. (2010)
Edible birds' nest (EBN) or cubilose	Conformational changes in protein structure (α-helix and β-turn structures increased at the expense of random coils) Higher solubilization, more functional protein	Fan et al. (2020)
Eucommia ulmoides Oliver seed meal proteins	Exposure of hydrophilic groups possessing more waster binding sites Enhanced water holding capacity and solubility, higher emulsifying capabilities	Ge et al. (2021)
Potato protein isolate	Disruption of secondary and tertiary structures Enhanced functional properties	Hu et al. (2020a, 2020b)
Pea globulin	Structural rearrangements within the globulin aggregates Better stability such as limited flocculation or coalescence, lower hydrophobicity, better emulsifying properties	Oliete et al. (2018, 2019)

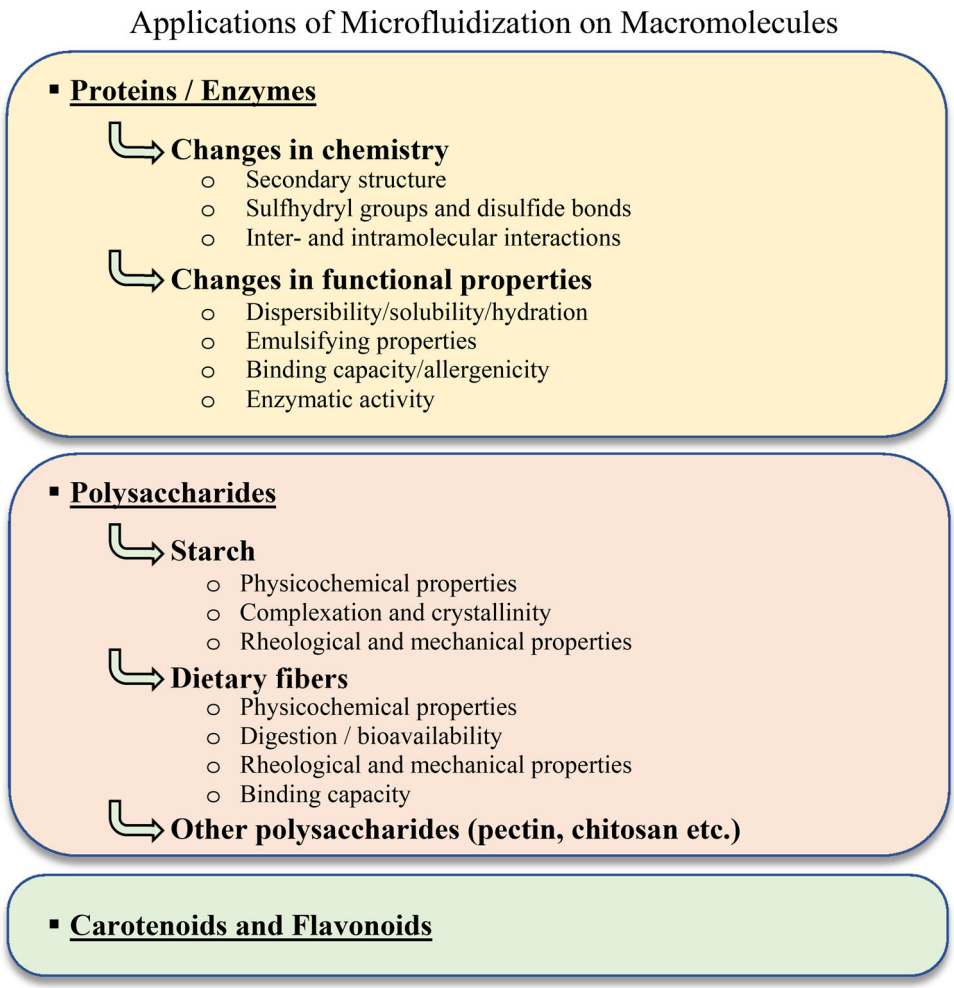

Figure 3. Applications of microfluidization on macromolecules.

The modification of *Eucommia ulmoides* Oliver seed meal proteins with microfluidization treatment (Ge et al. 2021) resulted in conformational changes in protein structure; α-helix to β-sheet ratio increased upon the treatment due to mostly the conversion of β-sheets to β-turns. They speculated that the increase in this ratio correlates with degradation of protein, which can be read as enhanced bioavailability in vivo.

Sulfhydryl groups (-SH) and disulfide bonds (-SS). Liu et al. (2009, 2010) reported an increase in exposed sulfhydryl groups (-SH) on the surface of mushroom polyphenoloxidase and trypsin due to newly formed unfolded structure. Similarly, Gong et al. (2019) showed that the modified structure of PPI with microfluidization (due to unfolding of the protein), as proposed in Figure 4, altered the polarity of the environment, resulting in favored surface hydrophobicity, increased the number of disulfide bonds, and decreased free sulfhydryl groups, but the extent of these changes was dependent on the pressure level. Li et al. (2019) also speculated that the emergence of new glycation sites upon microfluidization might be associated with the disruption of disulfide bonds and changes in tertiary structure in α-lactalbumin.

Shen and Tang (2012) showed that in addition to the conformational changes observed as unfolding of soy protein isolate with microfluidization, interchanges between free sulfhydryl group (-SH) and disulfide bond (-SS) contents also provided a significant contribution to improvements in physicochemical and emulsifying properties, such that a higher protein solubility, surface hydrophobicity, and emulsifying ability index were determined for the microfluidized samples.

Intermolecular and intramolecular bonds/interactions. Gong et al. (2019) concluded that a combination of various forces (hydrophobic interactions, hydrogen bonds, disulfide/free sulfhydryl group reactions, and electrostatic interactions) are responsible for changes in PPI structure rather than the individual effects of these forces. They claimed that disintegration in protein structure was favored at pressures lower than 120 MPa since electrostatic repulsion was dominant over intermolecular interactions in this range. On the other hand, they speculated that, above this level, the smaller particles produced with the treatment started to collide with each other, causing the formation of bonds and interactions between fragments leading to reaggregation and embedding of some functional groups in this reaggregated structure. Therefore, they concluded that the samples treated at 120 MPa had the most disintegrated structure and the degree of protein unfolding reached its peak, which was achieved

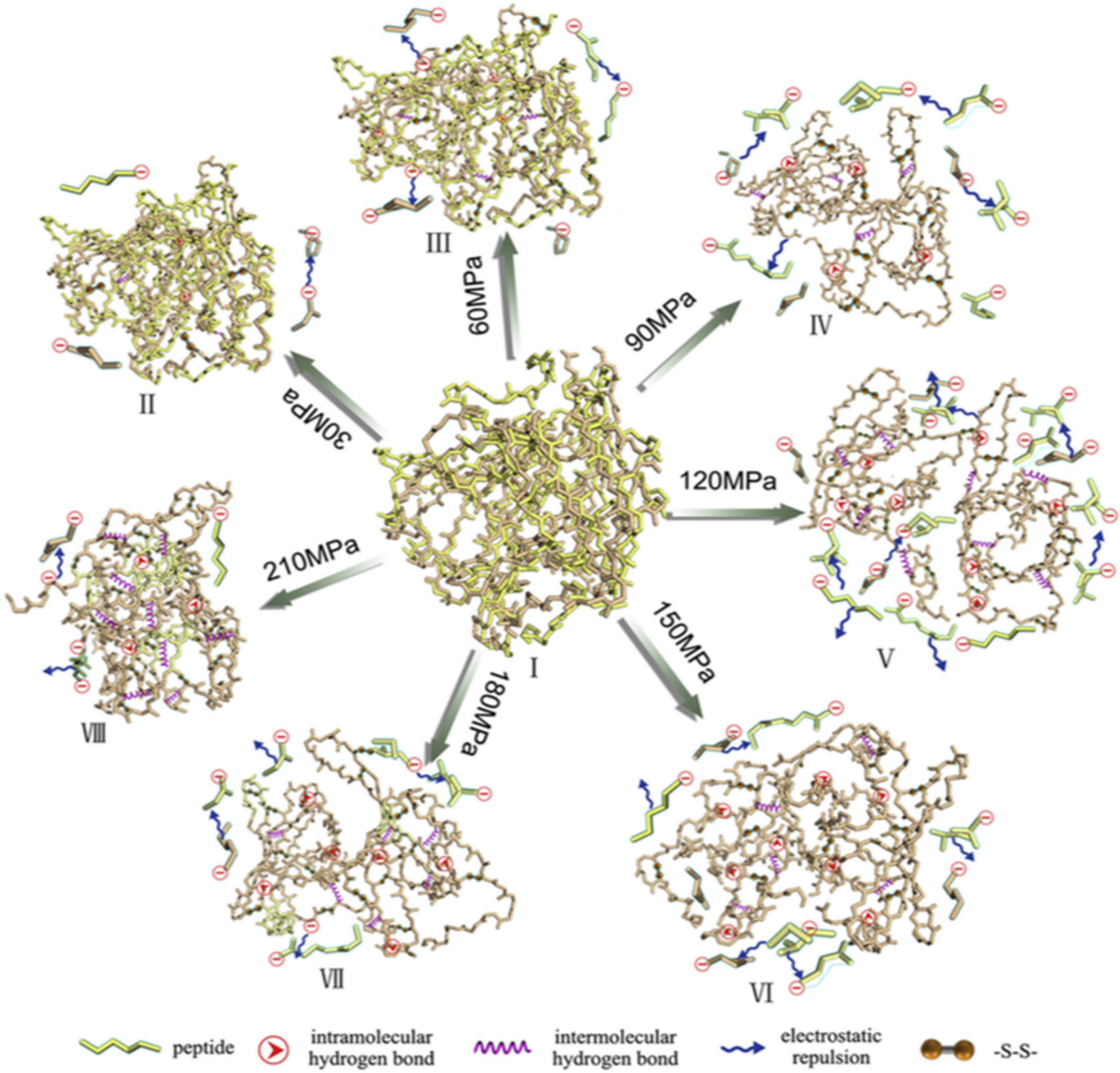

Figure 4. Schematic diagram depicting the proposed mechanism of disaggregation and aggregation of PPI in aqueous solution when subjected to HPM. I, natural state of PPI; II–V, disaggregation of PPI; VI–VII, aggregation of PPI. From II to VII, with the pressure increasing, -S-S- and surface hydrophobicity continued to increase. For peptide, intramolecular hydrogen bond and electrostatic repulsion first increased and then decreased with the boundary pressure of 120 MPa. Reproduced from Gong et al. (2019) with the permission from the publisher.

with maximum repulsion and optimum fragment size leading to less interaction between particles. This finding suggests that the changes in the protein structure and properties due to microfluidization are dependent on processing pressure, and this dependence is not always linear. Especially, a specific processing pressure is needed for each material to observe the greater influence on functional properties, which was found to be 120 MPa for peanut protein isolate.

Hu et al. (2020a, 2020b) found that secondary and tertiary structures of potato protein isolate were disrupted by microfluidization treatment, whereas chitosan could serve as a protective mechanism against the disruption of molecular structure of the protein by forming new hydrogen bonds in the complex. This finding suggests that in addition to enhanced functional properties on proteins as a result of microfluidization treatment, the addition of chitosan to protein systems that is microfluidized can limit the changes on chemical conformation of protein to maintain some desired functionalities.

Changes in functional properties

It should be noted that almost all changes in functional properties upon microfluidization process are a result of changes in protein conformation due to particle size

reduction. Therefore, the particle size effect of microfluidization was taken as a constant throughout this review and was not mentioned separately in a subsection. Instead, the focus was given to improvements in other functional properties.

Dispersibility and solubility. Hu et al. (2011) reported that the conformational changes in secondary structure of PPI with microfluidization led to an increase in protein dispersibility and solubility, and a decrease in surface hydrophobicity, due to the exposure of hydrophobic groups that were initially hidden inside the structure with structural changes. They speculated that microfluidization followed by transglutaminase crosslinking might even lead to more drastic changes in the folded structure of PPI, leading to better adsorption at the oil-water interface and thereby greater emulsion stability. Shen and Tang (2012) presented that this process led to the formation of a soluble structure by transforming the insoluble aggregates of soy protein isolate, which was attributed to conformational changes and interchanges between free sulfhydryl group (SH) and disulfide bond (SS) contents. Ge et al. (2021) measured that microfluidization noticeably improved the solubility of *Eucommia ulmoides* Oliver seed meal proteins, which was found to be at its highest upon treatment at 80 MPa and showed a lower solubility value at higher pressure treatments. This decreasing trend was attributed to the exposure of more hydrophobic groups at higher pressure diminishing interaction between protein and water. Fan et al. (2020)'s study on edible birds' nest showed that almost insoluble fraction (1–2%) of protein was solubilized up to some extent (26–27%) upon microfluidization treatment (at 120 MPa), which could be attributed to changes in secondary structure of the protein in addition to particle size reduction. The authors claimed that the findings of this study can serve as a reference to functionalize the water-insoluble proteins to be used in the industry upon microfluidization treatment.

Many proteins in aqueous solutions tend to either precipitate due to the formation of insoluble aggregates after heat treatments or form viscoelastic gel-like structures if the protein concentration is high. Iordache and Jelen (2003) used microfluidization to improve the functionality of heat-denatured whey proteins and claimed that this process limited the sedimentation of whey protein polymers and even made the structure completely resistant against sedimentation at low pH values (3.8), indicating an enhancement in the degree of solubility. These findings show that since heat treatment affects the solubility of a material, a treatment like microfluidization might be a desirable step after heat treatment to obtain a structure with limited sedimentation behavior and increased solubility.

Emulsifying properties. Djemaoune, Cases, and Saurel (2019) reported that the foaming and emulsifying properties of pea albumin aggregates were favorably influenced by the microfluidization process, specifically at neutral pH levels, due to changes in aggregation characteristics. Similarly, Sha et al. (2018) showed that the treatment of fish gelatin with microfluidization significantly enhanced the emulsifying and foaming properties in addition to pepsin digestibility. Interestingly, while microfluidization did not have significant effects on molecular weight distribution and gel strength, it decreased the melting temperature, especially at high-pressure levels. These modified protein structures could be used in food formulations since emulsifying and foaming properties are considered to maintain the quality and stability of foods.

The treatment of *Eucommia ulmoides* Oliver seed meal proteins (ESMPs) (Ge et al. 2021) and potato protein isolate (PPI)-chitosan complex (Hu et al. 2020a) with microfluidization resulted in higher emulsifying capabilities. For both proteins, emulsifying activity of the proteins significantly increased; however, the extent of this increase was dependent on the applied pressure. It was noted that the exposure of more hydrophobic groups hidden inside the structure upon microfluidization enhanced the adsorption capacity of proteins around oil-water interfaces, leading to a higher activity for both proteins. However, while emulsion stability of ESMPs' improved with the treatment, that of PPI-chitosan complex's deteriorated. This discrepancy between studies might be associated with the protein structure as well as the flocculation effect seen in PPI-chitosan complex. These behaviors suggest that the nature of protein and also its interaction with other components, if any in the system, are crucial for emulsifying properties.

Thermal treatment of proteins generally provides aggregates, which makes them good candidates to be used in oil in water interfaces. However, even though the formation of disulfide bonds and the increase in surface hydrophobicity during the process stabilizes the structure up to some extent, coalescence and flocculation still occurs in such emulsion systems by time. Oliete et al. (2018, 2019) processed soluble pea globulin using a microfluidizer to eliminate these drawbacks and to investigate its impacts on the emulsifying properties and structural characteristics. They observed structural rearrangements within the globulin aggregates, inducing better characteristics to maintain the stability, such as smaller particle size and lower hydrophobicity. The effect of microfluidization on stability was found to be dependent on the treatment pressure; more stable at higher pressure levels. At high levels, inter-droplet interactions limited the flocculation or coalescence, and gel-like characteristics were dominant in this stable structure. They concluded that negative aspects of thermal aggregation of pea globulin on stability can be overcome using microfluidization, especially at high-pressures, to form new characteristics within the protein structure.

Binding capacity and allergenicity. Chen et al. (2016) proposed the use of microfluidization as a physical treatment along with glycation of β-lactoglobulin with galactose to improve its functional properties and reduce allergenicity. They found that microfluidization of glycated β-lactoglobulin decreased the binding capacity of immunoglobulin E (IgE) for cow's milk allergy. The order of the treatments affected IgE binding differently; it seems that higher microfluidization pressures had a greater effect on the binding when it was performed as a pretreatment before glycation.

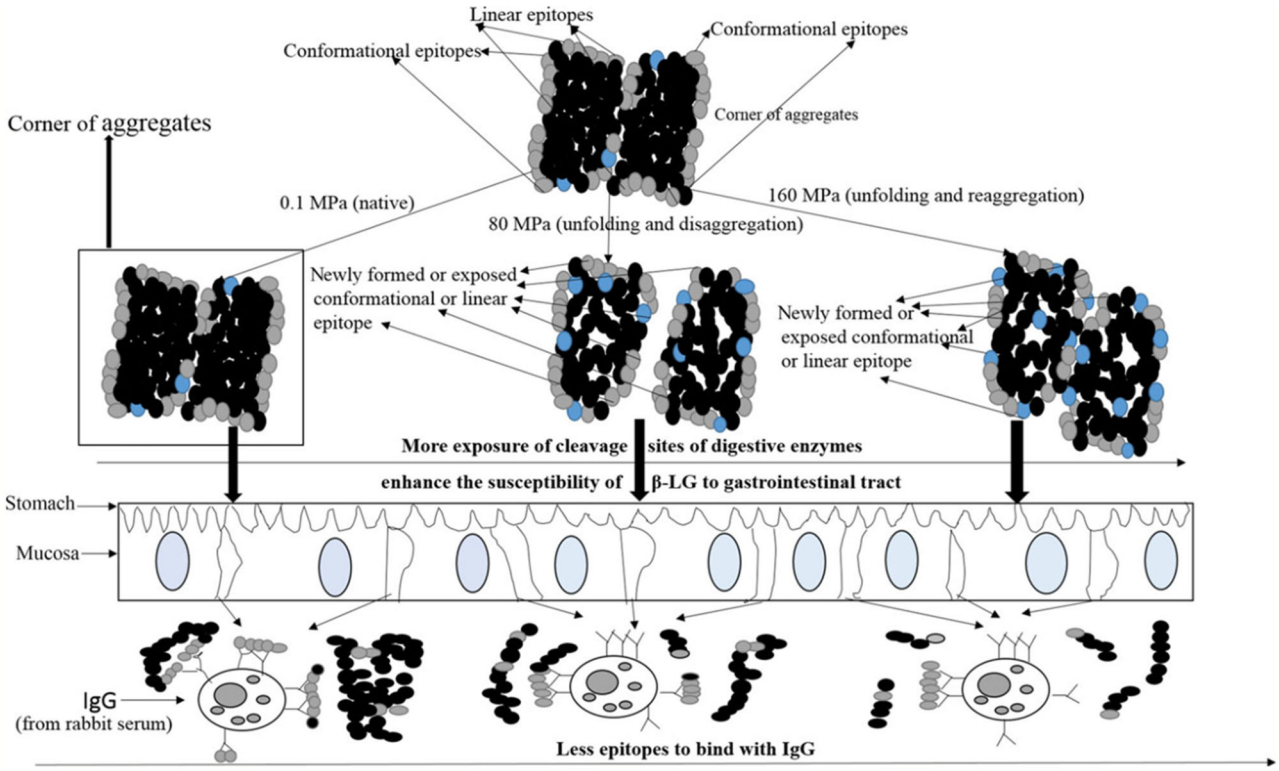

Figure 5. The schematic diagram of mechanism of antigenicity changes of DM-b-LG-VD. Blue circle represents cleavage site of enzymes; Gray circle represents epitopes. Reproduced from Chen et al. (2019) with the permission from the publisher.

Later, the same group (Chen et al. 2017) revealed that the number of glycation sites in β-lactoglobulin increased from 11 to 12 after microfluidization treatment. The emergence of a new site (Lys60) suggests that the treatment altered conformation of the protein, explaining higher binding capacity and the decrease in allergenicity. Chen et al. (2019) built a follow-up study on their study relating microfluidization effects on β-lactoglobulin to allergenicity (Chen et al. 2016) and proposed a mechanism explaining the reduction in antigenicity of β-lactoglobulin with microfluidization (Figure 5). Another study from the same group (Zhong et al. 2019) showed that glycation could be made through some other sugar units, specifically 1-kestose in this study. A decreasing trend in antigenicity was observed with increasing pressure level, such that treatment at 80 MPa led to a decrease up to 58%.

Likewise, Li et al. (2019) modified α-lactalbumin using microfluidization and glycation with lactose to decrease the binding capacity of immunoglobulin E and G (IgE/IgG). The authors found that dynamic high-pressure microfluidization promoted a reduction in binding capacities even further in addition to the glycation effect and 110 MPa was found as the optimum treatment pressure. In addition to native glycation sites of α-lactalbumin (K62, K94, K98), microfluidization pretreatment revealed two more active glycation sites (K108 and K114) as well as improving the extent of glycation for native K94 and K98 sites. These studies clearly show that the characteristics and the extent of glycation sites, and also changes in conformation, disulfide bonds, and aggregation level are likely the reasons behind lower IgE/IgG binding capacities of various proteins. These studies demonstrate that microfluidization combined with glycation would be a promising method to modify the binding capacity and decrease the allergenicity of proteins similar to what has been done on β-lactoglobulin and α-lactalbumin.

Hydration properties. Ozturk and Mert (2018a, 2018b) investigated the potential of microfluidization as a value-adding process on corn gluten meal to improve the water-holding ability of this underutilized corn protein-rich byproduct. They reported that microfluidization disintegrated the large hydrophobic structures, formed micro and nanoparticles, and even modified the smooth surfaces into a branchy network by forming tissues, cavities, and micropores through the structure. The changes in structure also led to a substantial decrease in particle size and formation of homogenous suspension by narrowing the particle size distribution (Ozturk and Mert 2018b). They indicated that the modification of hydrophobic structures increased the number of available water binding sites, leading to enhanced hydration properties (Ozturk and Mert 2019). They were able to obtain a compatible structure to be used in gluten-free bread formulations, which is not possible for corn gluten meal due to its high hydrophobicity and insolubility in water (Ozturk and Mert 2018a, 2018b). The measurements on dough and pieces of bread from microfluidized corn gluten meal showed that microfluidization was not only a potential method to improve the hydration properties of corn gluten meal, but it also helped the formation of a stronger dough network, leading to improved textural attributes such as hardness, springiness, and cohesiveness.

Table 2. An overview of research articles using microfluidization on starches.

Starch	Modification/Change in properties	References
Corn amylose	Alteration in surface appearance and partial gelatinization • Decrease in gelatinization temperature and enthalpy, increased swelling power, and decreased solubility	Tu et al. (2013)
Lotus seed starch	Partial destruction of the crystalline region led to a disorder in starch structure • Promoted formation of double helix structure, obtainment of various crystalline structures depending on treatment pressure	Zheng et al. (2020)
Chickpea and lentil starches	Significant changes on morphology, destruction of the crystalline structure • Higher water absorption capacity, higher swelling power	Bitik, Sumnu, and Oztop (2019)
Potato starch	Disintegration of large granules into a block-like structures with irregular shapes, disruption of crystalline and short-range ordered structures • Improvements in pasting properties, such as viscosity and setback value, enhanced mechanical properties related to rigidity	He et al. (2020)
Rice starch	Changes in morphology and crystallinity of rice starch (degree of crystallinity decreased) • Improvements in rheological properties – lower pasting temperature and higher peak viscosity	Li et al. (2018)
Rice starch-bamboo shoot dietary fiber composite	Changes in crystalline structure and rheological properties • Increased relative crystallinity, resistant starch fraction, lower pasting viscosity and viscoelasticity	Wang et al. (2020)
Rice amylose	• Improved the physicochemical and rheological properties	Duan et al. (2017)

Modification of *Eucommia ulmoides* Oliver seed meal proteins with microfluidization at acidic conditions (pH < 6) enhanced the water holding capacity of the protein (Ge et al. 2021). Acidic conditions promoted the impacts of microfluidization treatment and led to the exposure of more hydrophilic groups possessing more water binding sites. Therefore, microfluidization was found to be a promising method to treat low-acid foods requiring high water retention.

Enzymatic activity. This innovative technique was also employed on enzymes to test the hypothesis that whether this technique can completely inactivate or limit the enzyme activity. Liu et al. (2009, 2010), for instance, explored the use of microfluidization as a potential modification method to observe the effects on enzyme activity of mushroom polyphenoloxidase (PPO) and trypsin, respectively, due to changes in the conformation. They reported that the activity of these enzymes increased at each pressure level for PPO and remained at a similar level for trypsin, concluding that microfluidization cannot be used to inactivate these enzymes. Contrarily, it can be a new method for enzyme modification leading to higher activity levels. This suggests that the effect of microfluidization may differ depending on the type of material, which necessitates the evaluation of its applicability on specific materials.

Other physicochemical properties. Microfluidization was also found to affect the surface tension and flexibility of materials as exemplified on pea albumin aggregates (Djemaoune, Cases, and Saurel 2019) and ovalbumin (Liu et al. 2017) as a result of the reduction in particle size and the changes in protein structure leading to unfolding. Microfluidization has also been utilized for designing and producing protein-based foods and beverages with desirable sensory and physical properties. Koo et al. (2018), for example, examined the effects of different treatments (heat, microfluidization, and pH) on the physicochemical properties of solutions containing whey protein fibrils as the main constituent and chitosan as a fiber supplementary in a beverage formulation. The authors clearly showed that the transparent appearance was lost (turbidity increased) with heat treatment, which was associated with the formation and aggregation of protein-rich structure favored by the presence of chitosan. On the other hand, microfluidization of the heated system resulted in phase separation; transparent at the top and turbid at the bottom, which was attributed to the thermodynamic incompatibility between the components due to fragmentation of whey protein particles by microfluidization. Subsequent microfluidization treatment led to an excessive decrease in apparent viscosity compared to the heated system, indicating the reduction in effective volume fraction by partial disruption of biopolymer aggregates formed with heating prior to microfluidization, which can be a base point to modulate and obtain the desired properties for beverages with whey protein. This study showed that microfluidization has a promising modification technique along with a thermal and pH adjustment to improve beverages' physicochemical and sensory properties.

In addition, Liu et al. (2010) found that the pH and thermal stability of trypsin could be improved with dynamic high-pressure microfluidization. While the activity of trypsin without a treatment decreased 86% of its original activity after incubation at 45 °C, microfluidized trypsin was able to present almost the same activity level. These studies show the potential of this technique to be utilized on enzymes to affect their activities that can be useful in designing new food formulations. In the follow-up study of Chen et al. (2019), it was shown that microfluidization process also enhanced the gastrointestinal digestibility of β-lactoglobulin, which can be attributed to the emergence of smaller peptide pieces, the increase in hydrophobicity, and the decrease in particle size.

Table 3. An overview of research articles using microfluidization on dietary fibers.

Dietary fiber	Modification/Change in properties	References
Insoluble peach and oat fiber	Reduction in fiber particle size, increase in the composition of soluble fibers • Improved physicochemical properties to some extent after microfluidization, lowered postprandial serum ability and increased the inhibition activity of pancreatic lipase	Chen et al. (2013)
Soybean residues	Decrease in particle size, puffed morphology • Increase in the water holding capacity, increased porosity	Liu, Liang et al. (2016)
Hazelnut skin	Formation of strong and elastic web-like fibrous structures • higher viscoelastic moduli, yield stress, and consistency index values	Cikrikci, Demirkesen, and Mert (2016)
Hazelnut skin	Changes in morphology • Release of some phenolic compounds trapped within cellulosic structures, retarded retrogradation when incorporated into cookie formulation	Yildiz, Demirkesen, and Mert (2016)
Wheat bran	Formation of a separated fibrous structure • Higher water holding capacity, greater surface area, and more available methanol soluble-free phenolic content • Higher yield stress, consistency index, and viscoelastic moduli values of batter samples with microfluidized wheat bran fibers	Mert et al. (2014)
Soybean residue and rice bran	Alteration of surface structures • Increased lead (II) ion adsorption, increased the binding capacity	Wang et al. (2016); Wang, Wu et al. (2018)
Pea hull fiber	Defibrillation of aggregated cellulosic macrostructure into microfibrils • Formation of a thermally stable and viscoelastic behavior with greater elastic modulus	Morales-Medina et al. (2020)
Wheat and corn bran	Separation of structural components, expanded porous structure • Substantially decrease in particle size and bulk density, increased surface area • Enhancements in water-holding, swelling, cation-exchange, and oil-holding capacities	Wang et al. (2012); He et al. (2016); Wang, Sun et al. (2013)
Wheat bran	Reduction in fiber particle size, changes in morphology • Liberation of phenolic compounds that are bound to polysaccharides and are embedded in the fiber matrix • Increase in acid hydrolyzable, alkaline, and surface-reactive phenolics	Wang, Raddatz, and Chen (2013)
Wheat bran	Changes in appearance, but not reduction in the particle size • Formation of stable and viscous dispersions	Rosa-Sibakov et al. (2015)
Inulin	Formation of a web-like network consisting of agglomerates • Transformation form less viscous to a more viscous state, reduction in solid inulin particle size, and formation of smoother texture	Ronkart et al. (2010)

Polysaccharides

Starches

Polysaccharides, especially starch, have been commonly used as stabilizers, gelling and thickening agents in the food industry. However, they present many drawbacks including low solubility, high tendency to retrogradation, and low heat resistance, that need to be improved to be used in food product formulations (Ozturk and Takhar 2018). Microfluidization presents great potential with its modification effects on the structure and rheology of starches. The research using microfluidization to modify the structure of starches to add new properties or to improve the existing ones is listed in Table 2.

Physicochemical properties. Tu et al. (2013), for example, explored the changes in morphology and physicochemical properties of corn amylose after a single-pass dynamic high-pressure microfluidization treatment at various pressures. The process altered the surface appearance, led to partial gelatinization due to a decrease in gelatinization temperature and enthalpy, increased swelling power, and decreased solubility whereas no significant changes were observed in freeze-thaw stability. Interestingly, the mean diameter of granule size increased when the treatment pressure was higher than 120 MPa, which might be attributed to the aggregation or partial gelatinization of starch amylose. Even though it was not mentioned in this study, this increase in granule size above this pressure level could be associated with a higher probability of collision and re-coalescence at higher energy input (Jafari et al. 2008). It was shown that the timescale of collision becomes shorter at a high energy level, causing re-coalescence when the droplet encounters another (Lobo and Svereika 2003). This phenomenon was also named as "over-processing" by some other researchers (Mert 2012; Olson, White, and Richter 2004).

Starches are commonly modified with octenyl succinic anhydrides to obtain a starch derivative that is widely used in a range of applications in the food industry, such as a thickener, additive, emulsifier, or wall material for microencapsulation, given the superior emulsifying properties of amphiphilic polymer structure formed after the transformation due to esterification between starch and octenyl succinic anhydride. Li et al. (2018) hypothesized that microfluidization as a pretreatment to assist octenyl succinic anhydride for the modification of rice starch might provide

Table 4. An overview of research articles using microfluidization on non-starch and non-dietary fiber polysaccharides.

Polysaccharide	Modification/Change in properties	References
High-methoxyl pectin	Extended conformation and disruption of chains while preserving the primary structure of pectin, formation of small porous structures • Decrease in molecular weight, apparent viscosity, and particle size while increase in the amount of reducing sugars	Chen et al. (2012)
Pectin extracted from black cherry tomato waste	Formation of foamed filiform parts • Better fluidity and higher consistency for the solutions, decrease in apparent viscosity and consistency index	Zhang et al. (2018)
Pectin	Disintegration of aggregated structure in water-ethanol systems	Liu et al. (2018)
Lentinan	Assistance to extract lentinan • Higher scavenging capacity for many free radicals, enhancements in extraction yield and antioxidant activity.	Huang et al. (2012)
Chitosan	Fragmentation and chain scission with mechanical action • Narrower molecular weight distribution than that of the original polymer	Kasaai et al. (2003)
Cellulose	Disintegration of microcrystalline cellulose and exposed surface groups from residual hemicellulose • Decreased particle aggregation tendency and improved dispersion of cellulosic fibers within the matrix • Formation of a stronger polymer structure with enhanced filler-matrix interaction, improved homogeneity of composite films	Otoni et al. (2018)
Whole cowpea flours	Efficient disruption of cotyledon and seed coat, liberation of embedded protein and starch granules in the cotyledon, and the fragmentation of fibers in the seed coat, generation of fluffy and amorphous microstructures • Decrease in bulk density and particle size, increase in total water-extractable protein amount, water-holding capacity, swelling capacity, and oil-holding capacity	Adjei-Fremah et al. (2019)
Polysaccharides from *Mesona chinensis* Benth	Changes in morphology (formation of curly structures) • Alteration in monosaccharide composition, enhanced the antioxidant activities, increase in uronic acid and total sugar content while a decrease in protein content	Huang et al. (2018)
Oat β-glucan	More spherical and denser appearance • Narrowed molecular weight distribution, stabilized storage-related viscosity, decrease in both the viscosity and the molar mass	Kivela et al. (2010)
Xanthan gum	Conformational transformation from an ordered structure to a disordered structure and in a degradation in polymer • Decrease in both the viscosity and the molar mass, thereby a reduction in stabilizing properties	Lagoueyte and Paquin (1998)
Xanthan gum	Reduction in aggregated state by degradation, separation of the double-helical structure of xanthan into a single chain and even attack to molecule backbone after a few process cycle • Continual increase in reducing ends and decrease in molecular weight	Laneuville, Turgeon, and Paquin (2013)
Guar gum, sodium carboxy-methylcellulose, gum arabic, hydroxyethylcellulose, and sodium alginate	Mechanical degradation of polysaccharides • Decrease in apparent and intrinsic viscosities and reduction of the molar mass of polymer chains	Villay et al. (2012)
Saccharomyces cerevisiae β-D-glucan	Disruption of strong intra- and inter-molecular H-bonds Lower crystallinity and high solubility	Liu et al. (2020); Liu, Li et al. 2016)
Citrus pectin	Significant changes in morphology and structure, formation of compact interfacial layer Decrease in particle size, molecular weight, and polydispersity, decreased degree of branching, high thermal and centrifugal stability, better encapsulation	Wang et al. (2021)
Honey	Evaluation of microfluidization instead of thermal processes Higher phenolic and antioxidant activity	Leyva-Daniel et al. (2020)

an opportunity to alter the physicochemical properties, which would make the structure more suitable for further chemical modifications.

Wang, Zhu et al. (2018), on the other hand, processed a suspension of rice amylose at various microfluidization pressure levels (60, 100, 140, and 180 MPa). They reported the

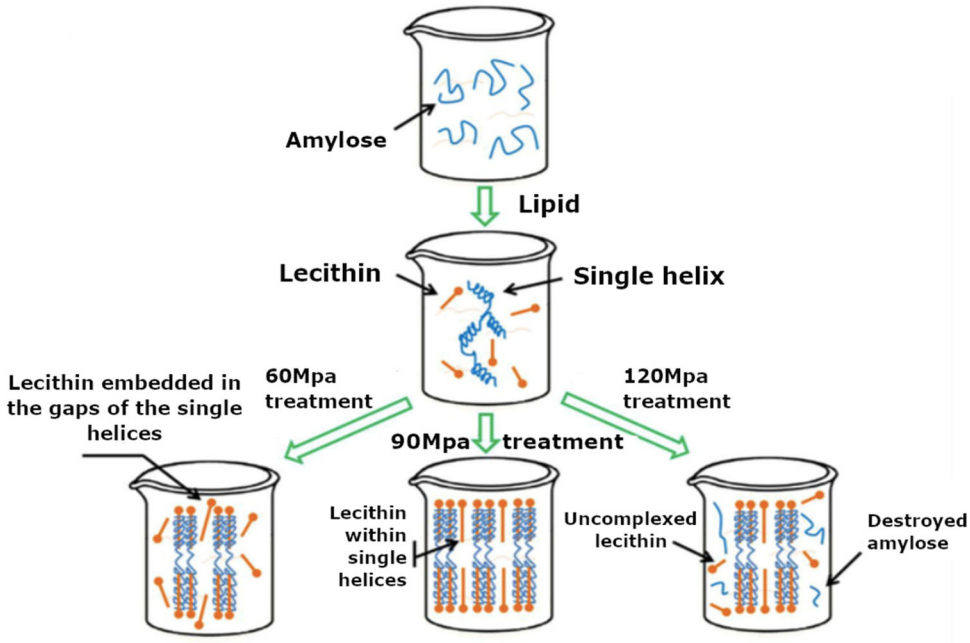

Figure 6. The diagram of the formation mechanism of lotus seed starch-lecithin complexes. Reproduced from Zheng et al. (2020) with the permission from the publisher.

formation of hollows and grooves on the surface leading to higher specific surface area for rice amylose, while smaller uniform particles were obtained as the pressure increased. FT-IR results indicated that even though microfluidization treatment altered the structure, the primary structure of rice amylose remained intact. A new peak emerged at 1636 cm-1 and also the peaks at 1660, 1643 cm^{-1}, assigned to the vibrations of H-O-H bending, and the peak at 1363 cm^{-1} shifted to lower wavelengths, which might be attributed to a better interaction between amylose and water, possibly due to the enhanced surface properties and crosslinking reactions because of reduction of free molecular ends.

Bitik, Sumnu, and Oztop (2019) modified chickpea and lentil starches using microfluidization and ultrasonication to improve both functional and rheological-thermal properties. It was reported that the morphology of starches was significantly affected by both treatments leading to changes in swelling power, solubility, apparent viscosity, and volume mean diameter. Time-domain NMR analysis showed that modification of starch lengthened the T_2 relaxation time, which might be associated with the higher water absorption capacity of microfluidized samples, meaning higher free water content after the treatment. This finding also supported the swelling power increase such that the higher interaction between starch and water due to more available free water resulted in a higher swelling power.

Complexation and crystallinity. Zheng et al. (2020) studied the formation mechanism behind the lotus seed starch-lecithin complexes formed with dynamic high-pressure microfluidization to understand the effects of processing conditions on structural properties during complexation. It was found that the complexes formed with 5% lecithin under 90 MPa pressure preserved their integrity, which was also displayed with the highest complex index for these samples. Retrogradation of amylose and degradation of amylopectin were inhibited when the complexes were created, such that they presented different A-, V_{6I}-, and V_{6II}-type crystalline patterns. When the homogenization pressure increased, partial destruction of the crystalline region led to a disorder in starch structure leading to a decrease in proportions of the microcrystalline region and subcrystalline region of lotus seed starch. While the increase in lecithin concentration resulted in complexes with single-helix, the double helix degree (995/1022 peak ratio in FT-IR) significantly increased as the pressure increased, indicating the promoted formation of double helix structure as supported by morphological properties (FESEM) and crystalline pattern (XRD). In the light of obtained results, the authors came up with a formation mechanism (Figure 6) showing the effect of homogenization pressure. At the low pressure (60 MPa), only a small fraction of amylose contributed to the complex formation with lecithin, resulting in low-density V_{6II}-complexes due to the embedded lecithin in the gaps of single helices of amylose. The medium processing pressure (90 MPa) led to the formation of stable V_{6I}-complexes by improving the dissolution of single helix amylose, providing an enhanced interaction between the single helix amylose and lecithin. However, a further increase in the pressure (120 MPa) destroyed the single helix structure of amylose and retarded the interaction between components, leading to A- or V_{6II}-type structures, not stable V_{6I}. Bitik, Sumnu, and Oztop (2019) studied chickpea and lentil starches and observed that the structural changes are also associated with the destruction of the crystalline structure due to the high-pressure effect of the treatment, which was verified with FTIR analysis showing changes in peak intensity at 1000 cm^{-1}. Li et al. (2018) showed that microfluidization altered the morphology and crystallinity of rice starch, such that the original starch morphology was lost and the degree

of crystallinity decreased from 29.4% to 25.7% as the treatment pressure increased. Wang, Zhu et al. (2018)'s study on rice amylose showed that the crystalline structure of amylose could be completely destructed when the pressure level was above 100 MPa as shown with the shift of peak (XRD) at around 17° peak to 18°, and the decrease in intensity of XRD reflections. These studies particularly show that microfluidization can be used to obtain desired properties for formulations including starch with its modification effects on the crystalline structure and complexation mechanism.

Wang et al. (2020) observed a significant increase in gelatinization enthalpy (17%) and relative crystallinity (by 63%) for microfluidized (100 MPa) bamboo shoot dietary fiber (BSDF)-rice starch composites compared to untreated composite samples. They also determined that the replacement of BSDF with rice starch at 10% increased the resistant starch fraction, leading to a reduced starch hydrolysis extent and rate. This impact could be attributed to the formation of a protective layer around rice starch granules by flaky BSDF. This strategy, the coating ability of BSDF, holds promise for new products aiming the delivery of starches to distal part of small intestine to activate gut-brain axis via L-cells in this region.

Rheological and mechanical properties. Although the application of microfluidization on the industrial scale is very limited, He et al. (2020) recently designed and used an industry-scale microfluidizer to treat potato starch. They observed remarkable destruction of starch granules at 120 MPa leading to the disintegration of large granules into block-like structures with irregular shapes, and the disruption of crystalline and short-range ordered structures, which was positively correlated with pressure level. In addition to the improvements in pasting properties, such as viscosity and setback value, mechanical properties related to rigidity were enhanced. Li et al. (2018) reported similar rheological improvements for rice starch (lower pasting temperature and higher peak viscosity) after microfluidization treatment in addition to enhancements in emulsifying activity and the emulsion stability. Duan et al. (2017) reported that the modification of rice amylose by dynamic high-pressure microfluidization significantly improved the physicochemical and rheological properties, which can serve as an example for the starch industry to use this technique in developing modified starches. In the light of results in these studies, microfluidization would be a practical, safe, and simple industrial application to modify materials with large granule sizes, and the application of this technology at the industrial level may be used for processing bigger materials like whole grain flours. Even though these findings are promising, it should be noted that its use in the industry is still limited due to many problems as discussed in the last section.

Wang et al. (2020) tested the use of microfluidization at various pressure levels on bamboo shoot dietary fiber (BSDF)-rice starch composites and reported that the combination treatment (the replacement of 10% rice starch with BSDF and microfluidization) resulted in a decrease in rheological indexes, such that lower pasting viscosity and viscoelasticity were recorded when microfluidization was introduced at the lowest pressure level tested. However, changes in pressure led to fluctuations in these rheological indexes.

Although the use of microfluidization on starches has gained popularity in recent years, the research generally focused on how this treatment alters swelling, solubility, and gelation properties. However, a deeper investigation is required to shed light on the effect of this innovative treatment on the hierarchical structure in starch granules. For example, to our knowledge, there is no study investigating how amylose/amylopectin ratio in granules is modified during the process or how the modified structures affect the digestion profile, which would lead to new application areas. Also, most research has only studied the changes in physicochemical properties such as particle size, surface area, solubility, etc. on a specific starch concentration. A detailed chemical investigation is needed to completely comprehend the effects on the hierarchical structure of starch.

Dietary fibers

One of the hot topics among public and food manufacturers recently is to find a way to increase the consumption of dietary fibers in the daily diet because many studies have reported that fiber consumption decreases the risk of heart diseases, obesity, diabetes, and even cancers in addition to their regulatory effects on human gastrointestinal and neurological systems (Mert 2020; Zhang and Hamaker 2017). It has also been reported that dietary fibers can bind toxic heavy metals and reduce or eliminate the risk of heavy metal poisoning (Wang, He, and Chen 2014). On the other hand, some studies have documented that the high amount of dietary fiber consumption in diet might lead to discomfort due to gas production and delays in intestinal gas transit (Mert 2020). Also, the physicochemical and functional properties of most dietary fibers, such as water and oil holding, and swelling capacities are lacking to incorporate them into industrial products. Research using microfluidization has been conducted to improve these properties of dietary fibers as presented in Table 3.

Digestion profile. Chen et al. (2013), for example, investigated the effects of microfluidization on insoluble dietary fibers from peach and oat. They found that, in addition to a reduction in fiber particle size, the composition of soluble fibers increased and physicochemical properties were improved to some extent after microfluidization. They also showed that fibers treated with this process lowered postprandial serum ability and increased the inhibition activity of pancreatic lipase. However, it should be noted that the transition from insoluble to soluble fiber would affect the digestion profile of these materials in the gastrointestinal tract such that soluble fibers are mostly absorbed in the small intestine whereas insoluble fibers reach the colon. This difference in digestion profile would also influence the beneficiaries from these materials; the former can be sensed with L-cells and activate the gut-brain axis while the latter can be

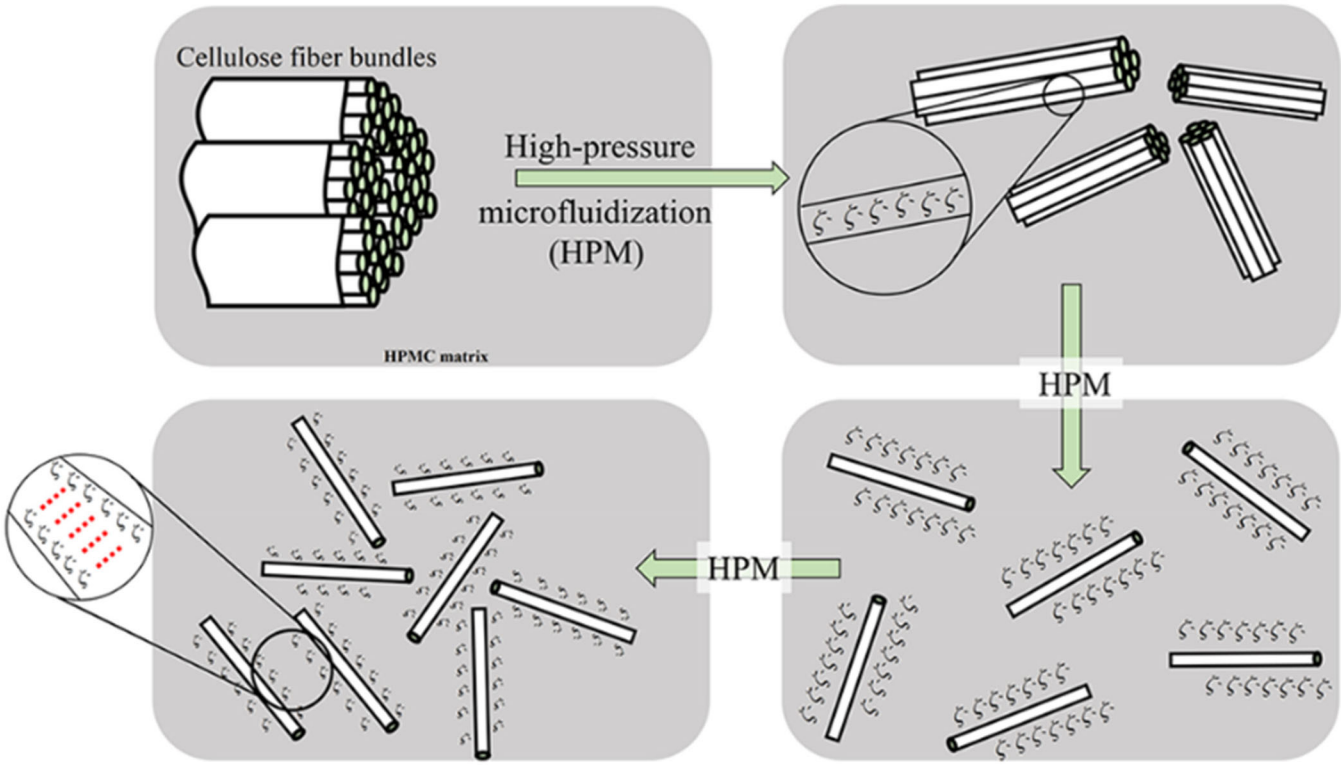

Figure 7. Schematic representation of the effects of high-pressure microfluidization on cellulose fiber bundles. Reproduced from Otoni et al. (2018) with the permission from the publisher.

utilized by the gut microbiome in the colon (Zhang and Hamaker 2009). Therefore, there is a necessity to test how this transition in fiber solubility affects the utilization of these fibers in human physiology.

Physicochemical properties. Liu, Liang et al. (2016) modified insoluble dietary fiber from soybean residues with microfluidization and they found that modification with microfluidization led to an increase in the water holding capacity, and a decrease in particle size, and puffed morphology. The investigation of Mert et al. (2014) on the use of microfluidization as a milling process for wheat bran revealed that this process formed a separated fibrous structure, leading to a higher water holding capacity, and greater surface area.

Wang et al. (2012), He et al. (2016), and Wang, Sun et al. (2013) studied the potential of microfluidization on the treatment of wheat bran and corn bran since bran from cereals has a high potential to be used in fiber-enriched products in the food industry. They demonstrated that this process could effectively increase the surface area of the material by separating the structural components, substantially decreasing the particle size and bulk density. They indicated that unlike traditional methods that cause a collapse during the breakdown process, microfluidization created an expanded structure with increased porosity. This expanded structure along with other changes in material properties led to enhancements in physicochemical properties of wheat and corn bran, such as water-holding, swelling, cation-exchange, and oil-holding capacities increased up to 90-140% for corn bran.

Rheological properties. Microfluidized insoluble dietary fiber from soybean residues were incorporated into rice starch to investigate the rheology and gelatinization characteristics to evaluate the use of this mixture in food applications for designing fiber-rich products. It was found that the addition of modified insoluble soybean fiber resulted in a decrease in breakdown and setback values, pointing out increased stability of paste and restriction of short-term retrogradation of gels (Liu, Liang et al. 2016). Cikrikci, Demirkesen, and Mert (2016) and Yildiz, Demirkesen, and Mert (2016)'s studies on hazelnut skin compared the effectiveness of microfluidization with conventional milling processes and showed that microfluidization led to improvements in rheological properties (resulted in much higher viscoelastic moduli, yield stress, and consistency index values), due to the formation of strong and elastic web-like fibrous structures similar to gluten systems (Cikrikci, Demirkesen, and Mert 2016). Similar rheological improvements (higher yield stress, consistency index, and viscoelastic moduli values of batter samples prepared with microfluidized wheat bran) were presented by Mert et al. (2014). These improvements indicated that the microfluidized wheat bran fibers could form a strong fibrous matrix, a desirable characteristic to use in batter formulations for bakery products. Therefore, the microfluidized wheat bran was used to produce a model cake system and it was shown that microfluidization on wheat bran resulted in an enhancement of water holding capacity and retardation on staling. Even though very promising results were reported in these studies, the formulated products were just some prototypes and the incorporation of modified dietary fibers into real food formulations is still

very limited. Especially, given the limitations of the microfluidization process, the focus should be given to the usability of microfluidization to create more functional fiber structures on the industrial scale.

Morales-Medina et al. (2020) evaluated the effects of microfluidization on particle size, and subsequently on functional and microstructural properties of suspensions prepared with pea hull fiber. The decrease in particle size led to the formation of a thermally stable and a viscoelastic behavior with greater elastic modulus. Besides, the structure had interfibrillar gaps between the particles which might increase the flexibility and provide an opportunity to use this material in functional products.

Rosa-Sibakov et al. (2015) also investigated the potential of different disintegration methods such as dry and wet grinding, enzymatic treatment, and microfluidization to stabilize the enriched liquid food matrix with wheat bran at a high moisture content. Among these treatments, microfluidization provided the most stable and viscous dispersions, which might be associated with better homogenization due to the modifications on the bran structure. They did not observe a reduction in the particle size of wheat bran after the microfluidization process unlike the study of Wang, Raddatz, and Chen (2013). This discrepancy is probably due to the application of microfluidization after wet milling by Rosa-Sibakov et al. (2015), so that wet milling probably reduced the particle size of wheat bran to its limit, and microfluidization did not affect the size further.

All studies discussed above investigated the degradation of polysaccharide structures leading to new or modified functional properties, which can be hypothesized as the primary effect of this treatment. On the other hand, Ronkart et al. (2010) showed that the effect of microfluidization is dependent on the material. For example, they clearly observed that, although the chemical composition of inulin was not changed as a result of the process, microfluidization resulted in a transformation of the inulin-water system from a less viscous to a more viscous state depending on the concentration of inulin (2, 7, and 15% (w/w)) and the number of microfluidization passes. Optical and electron microscopy showed that a web-like network consisting of agglomerates was formed, leading to a textural modification due to the increase in interactions between the system and the solution. Also, microfluidization led to a reduction in solid inulin particle size, providing a smoother texture.

Bioavailability. The bioavailability of phenolics is not high since they appear as bound compounds in the cereal kernels, which limits the accessibility of them by enzymes. Therefore, recent studies have investigated the alternative methods that can accompany the traditional methods to increase the bioavailability of these compounds and enhance health-related properties. Cikrikci, Demirkesen, and Mert (2016) and Yildiz, Demirkesen, and Mert (2016), for example, employed microfluidization for the production of fibrous structures from hazelnut skin containing high amounts of fibers and natural phenolic antioxidants, and the microfluidized material was used in cake and cookies as model bakery products, respectively. Yildiz, Demirkesen, and Mert (2016) also indicated that microfluidization led to the release of some phenolic compounds trapped within cellulosic structures and the incorporation of microfluidized hazelnut skin into cookie formulation enhanced the nutritional value. Similarly, Mert et al. (2014) reported more available methanol soluble-free phenolic content for wheat bran after microfluidization treatment. Aggregated cellulosic macrostructure of pea hull fiber was defibrillated into microfibrils, resulting in more interaction between the particles due to friction and electrostatic forces (Morales-Medina et al. 2020). Even though there were no changes in the amount of alcohol-soluble substances for suspensions with higher a particle size ($D_{90} > 60\,\mu m$), a further decrease in the particle size ($D_{90} = 60\,\mu m$) resulted in the release of these substances from the pectic and hemicellulosic structures.

Wang, Raddatz, and Chen (2013) showed that the phenolic compounds that are bound to polysaccharides and are embedded in the fiber matrix in wheat bran could be partially liberated when microfluidization was employed as a milling process. While the solvent extractable phenolic contents decreased, since they dispersed in water during the process, the contents of acid hydrolyzable, alkaline, and surface-reactive phenolics were increased by 20, 60, and 280%, respectively.

Binding capacity. Wang et al. (2016) and Wang, Wu et al. (2018) introduced the utilization of microfluidized insoluble dietary fiber from soybean residue and rice bran, respectively, to be used in the lead (II) ion adsorption. The primary structure remained intact while microfluidization altered the surface structure of the fiber, which had more contribution to the adsorption ability depending on the physical properties as well. It was found that even though microfluidization increased the binding capacity more than 13% and 36% for fiber materials from soybean residue and rice bran, respectively, when the treatment pressure increased to 80 MPa and 150 MPa, in vitro studies showed that the net adsorption value of fibers from soybean was significantly lower in the intestine (~9 μmol/g) compared to that in the stomach (~48 μmol/g). This decreasing trend in net adsorption value through the gastrointestinal tract could be attributed to competitive adsorption due to enzymes like pancreatin and cholate in the small intestines. Among physicochemical properties, total negative charges exhibited a strong relationship with the adsorption capacity and affinity as presented by a high Person's coefficient correlation (0.923 and −0.951, respectively, for fiber from soybean residue).

Microfluidization provides an opportunity to improve functional properties, thereby the fibers treated with this process can be utilized as functional ingredients in the food industry to enrich the fiber content of products. Also, the use of microfluidized and modified dietary fibers can be used in food applications prohibiting lipid absorption, requiring oil and moisture retention, and controlling glucose levels.

Table 5. An overview of research articles using microfluidization on carotenoids and flavonoids.

Sample	Modification/Change in properties	References
Total phenolic content of Ottoman Strawberry juice	Enhancements on physical and chemical properties, such as antioxidant activity, phenolic content, and color	Karacam, Sahin, and Oztop (2015)
Lycopene from ketchup mixes	Disintegration of cellular material, formation of more fibrils and stronger network of aggregates • Decrease in size of tomato solids, increase in detectable lycopene content of samples	Mert (2012)
Flavonoids from *Cyperus esculentus* L. leaves	Efficient extraction of flavonoids in terms of yield and boosted their activity • Pronounced antioxidant activity elevated superoxide dismutase	Jing et al. (2016)

Other polysaccharides

Other than the main categories like starches and dietary fibers under polysaccharides, other polysaccharides treated with microfluidization process were discussed in this section and an overview of research articles using microfluidization on other non-starch and non-dietary fiber polysaccharides is given in Table 4.

Physicochemical properties. Although pectin has been successfully used in pharmaceutical and food industries as a thickener, emulsifier, gelling agent, and stabilizer, the optimal utilization of this material has been always a problem. Chen et al. (2012) used dynamic high-pressure microfluidization to degrade high-methoxyl pectin, which led to a decrease in molecular weight, apparent viscosity, and particle size while an increase in the amount of reducing sugars was observed. Although degradation of pectin in the natural state is very limited, the proposed model in this study shows that mechanical treatments, especially microfluidization, might open the chains and extend the conformation, which was also supported with morphological observations, such that big chunk-like flakes turned into small porous structures with increasing pressure. Also, the correlation between the increase in the amount of reducing sugars and the decrease in molecular weight suggested that microfluidization induced the disruption of glycosidic bonds, leading to fragmentation into smaller segments while preserving the primary structure of pectin. Wang et al. (2021)'s study on citrus pectin showed that the combination of microfluidization and ultrasonication could lead to a further decrease in particle size, molecular weight, and polydispersity of the samples compared to only ultrasonicated samples. They also indicated that the original thick and branched structure of pectin was significantly modified to thinner fibrils with decreased degree of branching.

Zhang et al. (2018) argued that dynamic high-pressure microfluidization increased the average particle size of pectin extracted from black cherry tomato waste with the formation of foamed filiform parts. It was shown that pectin after microfluidization treatment exhibited two different structures: 1-fragmented and fluffy filiform parts and 2-flake-like parts, which were also supported with two major peaks at particle size analysis. They also indicated that the process pressure did not change galacturonic acid with esterified structures although the degree of esterification increased when the pressure was higher than 40 MPa.

Kasaai et al. (2003) studied the fragmentation of chitosan by microfluidization and observed that exposure time, pressure or intensity of turbulence, and hydrodynamic parameters (polymer concentration and molecular weight) had great effects on the fragmentation of chitosan, which was also modeled to relate the chain scission with mechanical action. The extent of chain scission increased with increasing pressure and exposure time, indicating the susceptibility of chitosan to mechanical degradation. Continuous microfluidization process was found to be more effective than volume pass mode for fragmentation and it produced narrower molecular weight distribution than that of the original polymer, showing microfluidization favorably fragmented larger macromolecules. However, it is difficult to evaluate whether this decrease in molecular weight distribution was really associated with the breakage of polymer chains or the separation of aggregates. Therefore, there is a need for further investigation to verify the reason behind this decrease.

Otoni et al. (2018) proposed microfluidization as a green tool to process biodegradable materials, such as cellulose, to produce particles before biofilm formation from particles formed. Microfluidization did not have any effect on the crystallinity of cellulose whereas it broke down microcrystalline cellulose and exposed surface groups from residual hemicellulose, which decreased particle aggregation tendency and improved dispersion of cellulosic fibers within the matrix.

Similar to other materials discussed in this section, Adjei-Fremah et al. (2019) reported some improvements in functional properties of whole cowpea flours; decrease in bulk density and particle size by up to 68.7% and 92.3%, respectively, increase in water-holding capacity, swelling capacity, and oil-holding capacity by up to 16.1%, 107.7%, and 162.1%, respectively.

Huang et al. (2018) suggested dynamic high-pressure microfluidization as an alternative method to modify the physical properties and functional characteristics of polysaccharides from *Mesona chinensis* Benth. They reported an increase in uronic acid and total sugar content while a decrease in protein content after the treatment of this polysaccharide was observed. High-pressure treatment significantly altered the monosaccharide composition such that the molar ratio of galactose: xylose: and galacturonic acid was increased from 2.8: 5.5: 2.4 to 3.8: 7.4: 3.0. The treatment had no significant effect on the molecular weight and the chemical interactions as observed by FT-IR, while it turned

the morphology into a curly structure and enhanced the antioxidant activities.

Liu et al. (2020) and Liu, Li et al. 2016) treated *Saccharomyces cerevisiae* β-D-glucan with the combination of ionic liquids and microfluidization. Specifically, the combination of 1-ethyl-3-methylimidazolium acetate (EmimAc) and microfluidization significantly enhanced the solubility (85.01% with 10 recycles), resulted in two fractions in terms of molecular weight, and lowered thermal stability. Even though new H-bonds were formed between (EmimAc) and β-D-glucan, the authors also determined that strong intra- and inter-molecular H-bonds in β-D-glucan were disrupted during the treatments, leading to changes in structure with lower crystallinity and high solubility.

Rheological and mechanical properties. Zhang et al. (2018) investigated the flow behavior of pectins extracted from black cherry tomato waste and the power-law model was well-fitted to explain this behavior at low shear rate and sweep frequency. They observed that microfluidization resulted in a decrease in apparent viscosity and consistency index. Pectin treated with microfluidization had better fluidity and higher consistency for the solutions. Different than the previous studies on pectin, Liu et al. (2018) proposed the treatment of pectin in water-ethanol systems rather than in aqueous systems. While pectin was found to exhibit aggregation in the absence of treatment, microfluidization highly disintegrated the aggregated structure of pectin and even degradation was observed when the ethanol concentration was low. This study might help the researchers optimize the environmental and processing conditions while incorporating pectin in food formulations.

Otoni et al. (2018) studied the optimization of the mechanical performance of cellulosic composites from cellulose particles formed by microfluidization. These particles led to the formation of a stronger polymer structure as filler-matrix (microfluidized cellulose-hydroxypropyl methylcellulose) interaction was enhanced due to the improved homogeneity of composite films, given the effects of microfluidization on the dispersion of cellulose as shown in Figure 7. The authors found that the most effective operating condition for the stiffest and strongest structure can be achieved with seven microfluidization cycles.

Kivela et al. (2010) investigated the influence of different homogenizers on flow and molecular properties of oat β-glucan during fragmentation. Although each homogenizer had clear and irreversible impacts on viscosity and molar mass, an increase in energy input during microfluidization treatment (called high-pressure homogenizer in the study) led to a linear decrease in both the viscosity and the molar mass, resulting in a more spherical and denser appearance, a narrower molecular weight distribution, and a stabilized storage-related viscosity.

The study of Lagoueyte and Paquin (1998) on xanthan gum showed similar results to the former study, such that an increase in pressure and number of passes decreased the viscosity, hydration rate, and molecular weight. However, contrary to the former study, they argued that the decrease in these properties led to a reduction in stabilizing properties. They indicated that microfluidization resulted in a conformational transformation from an ordered structure to a disordered structure and degradation in polymer due to opening the structure with its combined forces such as high shear, cavitation, and turbulence, producing less stable functional properties. Laneuville, Turgeon, and Paquin (2013), similarly, investigated the effects of microfluidization on xanthan gum; however, they mainly focused on the correlation between the degradation level and aggregate content by proposing a degradation mechanism. They argued that the aggregated state of xanthan gum was reduced in the first pass through the microfluidization process, leading to a xanthan solution without aggregates; however, they observed viscosity recovery after one-pass treatment, pointing out partial recovery. On the other hand, they hypothesized that the increase in the number of passes might lead to a separation of the double-helical structure of xanthan into a single chain and even attack to molecule backbone, resulting in irreversible changes as determined by a continual increase in reducing ends and a decrease in molecular weight. Similarly, Villay et al. (2012) investigated the mechanical degradation of polysaccharides such as guar gum, sodium carboxy-methylcellulose, gum arabic, hydroxyethylcellulose, and sodium alginate with microfluidization. They observed that apparent and intrinsic viscosities of polysaccharides in solution decrease in addition to the reduction of the molar mass of polymer chains, while the extent of these changes was dependent on treatment pressure and the number of cycles.

Extraction and bioavailability. Huang et al. (2012) used dynamic high-pressure microfluidization to assist and increase the yield of lentinan extraction. They optimized the extraction of lentinan using response surface methodology (RSM) considering three main independent variables (microfluidization pressure, extraction temperature, and water-raw material ratio) and showed that the extraction of lentinan through microfluidization assistance, compared to traditional hot water extraction, resulted in a higher scavenging capacity for many free radicals such as superoxide anion free, hydroxyl, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, leading to an enhancement in extraction yield and antioxidant activity. Huang et al. (2018) showed that 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl radical scavenging activities of the polysaccharide from *Mesona chinensis* Benth ascended from 65.4% to 72.1% and from 61.8% to 87.1%, respectively.

Adjei-Fremah et al. (2019) recently used microfluidization with a Z-type interaction chamber to process the whole cowpea flours to explore its effects on microstructure, protein profile, and physicochemical properties. Both of two major parts of cowpea seed, cotyledon and seed coat, were efficiently disrupted, leading to the liberation of embedded protein and starch granules in the cotyledon, and the fragmentation of fibers in the seed coat. The disruption increased the total water-extractable protein amount by up to 39.7%, which was also supported by SDS-Page analysis.

The confocal micrographs revealed that the generated fiber fragments had fluffy and amorphous microstructures.

Wang et al. (2021) reported that the combination treatment (microfluidization and ultrasonication) on citrus pectin provided the highest thermal and centrifugal stability due to the formation of more compact interfacial layer, leading to improvement in encapsulation of cholecalciferol (Vit D_3). They also indicated that DPPH radical scavenging capacity of modified pectin with the combination treatment was significantly higher than both unmodified and ultrasonicated samples.

Leyva-Daniel et al. (2020) evaluated the use of microfluidization instead of thermal processes for industrial honey processing. They determined that compared to control, the honey treated with microfluidization had higher phenolic (9.8–53.9%) and antioxidant activity (7.5–37.2%) depending on process conditions such as pressure, the number of cycles, and the type of interaction chamber. While Z-type interaction chamber was found to have a greater release profile for both (phenolics and antioxidants), the optimum pressure and number of cycles differed, such that two cycles at 52 MPa was the best condition for total phenolic content whereas five cycles at 69 MPa gave the highest value for antioxidant activity.

All these studies have shown that high-pressure and high shear stress during microfluidization leads to structural changes in food structures, the extent of which depends on material characteristics in addition to process conditions. While the major effect of microfluidization on proteins was the improvement of their solubility and hydrophobicity, its major impact on polysaccharides is seen as functionalization through fragmentation.

Carotenoids and flavonoids

Carotenoids and flavonoids are another group of food components, whose functionalities were tested upon exposure to microfluidization. There are only a few research articles that have been conducted on carotenoids and flavonoids as presented in Table 5. A more detailed discussion was given below for these studies.

Karacam, Sahin, and Oztop (2015), for example, conducted experiments to investigate the effects of microfluidization on Ottoman Strawberry juice and reported that, depending on the working pressure and the number of passes, microfluidization can be used to enhance the physical and chemical properties, such as antioxidant activity, phenolic content, and color.

Mert (2012) evaluated the effects of high-pressure microfluidization on the lycopene content of ketchup mixes. Reducing the size of tomato solids by changing their native structure through microfluidization was reported to increase the detectable lycopene content of samples, almost doubled compared to the high-pressure valve homogenization, which was also supported with color parameter a^* indicating redness of the sample. They showed that the intense impact of microfluidization formed more disintegrated cellular material, leading to the formation of more fibrils and a stronger network of aggregates compared to the untreated samples, although the treatment at high-pressure levels (above 1600 MPa) caused some unexpected and undesired consequences due to over-processing effect.

Flavonoids from *Cyperus esculentus* L. leaves were extracted using dynamic high-pressure microfluidization by Jing et al. (2016). This study indicated that microfluidization treatment was not only effective in the efficient extraction of flavonoids in terms of yield, but also boosted the activity of them. In vivo study on mice showed that pronounced antioxidant activity elevated superoxide dismutase, an enzyme that helps eliminate harmful oxygen molecules in the cells, without changing the level of malondialdehyde causing oxidative stress. It was also found that even though there was no observed effect on fungi such as *Aspergillus* and *Penicillium*, these flavonoids could inhibit the growth of both Gram-positive and negative bacteria. These and other studies have shown that microfluidization can be an effective method to extract and also enhance the antioxidant activity of substances that can be used in the pharmaceutical and food industry.

Disadvantages and problems

Although microfluidization has been reported as one of the promising technologies to enhance the structural and physicochemical properties of materials, its handling and operation are not convenient to use. Even in the lab-scale, it presents some disadvantages such as difficulties for cleaning and operation. For example, it is not easy to determine whether the interaction chamber is contaminated or blocked since the size of this part is quite small (10-100 µm), which can affect the quality and safety of the product. Although there are a few production scale microfluidizers available to be used in the pharmaceutical, cosmetic, and chemical industries, compared to other high-pressure technologies such as high-pressure homogenization, its practical applications in the field are not as common and, as exemplified throughout the text, this technique has been used for the formation of prototypes rather than in real food formulations. Moreover, current studies are mainly based on the use of microfluidization for one or a few similar components, but real food formulations are very complex systems with many food components including biological macromolecules altogether. Therefore, it is essential to investigate whether this technology can be applied to more complex systems to be tested in the food industry.

One of the biggest problems with this technology is the limitation of scale-up to be used in the industry. In addition to the expensiveness of the technology, especially at the industrial scale, the design of the technology in terms of the requirement for small interaction chamber and microchannels, necessitates additional equipment, such as homogenizers and millers, prior to the use of microfluidization. Also, this design limits the production capacity of the microfluidizer to 1-10 L/min depending on the viscosity and the concentration of the material being processed. To overcome these drawbacks, a new design for an industrial scale

microfluidizer that has a larger production capacity is required. However, even though an ideal design is achieved, the operating cost will be extremely high as opposed to simple milling systems. Lastly, although it has been presented as a non-thermal processing technology, high-velocity collision of microstreams and intense shear rate in the interaction chamber might result in a temperature increase of the product, which might influence the material properties further. However, the studies summarized in this review have not considered this temperature increase, which might be due to their smaller scales.

Conclusions and future prospects

The studies reviewed here demonstrate that microfluidization has a great influence on materials' structure even though the final outcome is dependent on different factors such as process conditions (e.g., number of cycles, pH) and solvent type, but mostly the type of the material and its concentration. Moreover, additional treatments like pre-heating and pH adjustment might favor the effect of microfluidization even though the extent of these changes is dependent on the structural characteristics of the material treated. The only common aspect of the treated materials is the size reduction due to physical impacts during the process. In addition to the size reduction aspect, conformational changes happening in the molecular structure might lead to different functionalities in a desired or undesired way, meaning microfluidization has unique effects on each material. Therefore, it is necessary to conduct extensive studies to completely understand the mechanistic effect of microfluidization.

In this review paper, the general working mechanism and various applications of microfluidization in food science are reviewed to serve as a solid reference. More specifically, the studies using microfluidization treatment for improvements on the physicochemical and functional properties of food macromolecules such as proteins and polysaccharides are summarized. The studies gathered here clearly showed that microfluidization presents promising results to alter/modify the physicochemical and emulsifying properties of materials, which may result in the use of treated materials in different applications in the food industry to formulate new products. However, there are still some drawbacks that need to be addressed. Especially, its use is very limited on the industrial scale due to problems in design that needs to be improved. Despite these drawbacks, the research summarized in this review presents the potential of the microfluidization process to obtain value-added novel ingredients with improved functionalities.

Based on the scientific literature on the use of microfluidization in food science, this review shows that this emerging technology is about to become a crucial tool, not only for the research community but also for the industry to develop new food products. While microfluidization is very commonly used in the dairy industry and for some basic emulsion preparation, it has become a major technique to modify macromolecules in the last decade. Even though these are not the focused topics in this review, some new application areas have been emerging for microfluidization, such as production of liposomes, encapsulation of biodegradable compounds and molecules, and formation of nanoparticles. Also, as mentioned in this review, researchers started to scale up the microfluidizer designs to fit them into industrial production lines for continuous processes. Even though it is still a costly process for the industry similar to other innovative techniques like ultrasound and freeze-drying, the use of microfluidization in the industry should lead to a decrease in cost in time, the same way it happened to extrusion. We believe the most important aspect is to show its superiority to modify the food structures leading to new or improved functionalities, as summarized here. If this continuous development is allowed to bloom and if the necessary commercial support takes place, we can soon expect a range of new areas of use for microfluidization as well as more common utilization of it in the industry. However, we acknowledge that the opinion and attitude of the industry toward microfluidization process is extremely important for the development of this innovative technique. Given the outcomes of many studies, our hope is that the industry will invest on this promising technique and use this process in their production lines in the near future.

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Author contributions

Oguz Kaan Ozturk planned and wrote the manuscript. Hazal Turasan contributed to writing and edited the manuscript.

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