

# The health benefits, functional properties, modifications, and applications of pea (*Pisum sativum* L.) protein: Current status, challenges, and perspectives

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## Abstract

In recent years, the development and application of plant proteins have drawn increasing scientific and industrial interests. Pea (*Pisum sativum* L.) is an important source of high-quality vegetable protein in the human diet. Its protein components are generally considered hypoallergenic, and many studies have highlighted the health benefits associated with the consumption of pea protein. Pea protein and its hydrolysates (pea protein hydrolysates [PPH]) possess health benefits such as antioxidant, antihypertensive, and modulating intestinal bacteria activities, as well as various functional properties, including solubility, water- and oil-holding capacities, and emulsifying, foaming, and gelling properties. However, the application of pea protein in the food system is limited due to its poor functional performances. Several frequently applied modification methods, including physical, chemical, enzymatic, and combined treatments, have been used for pea protein to improve its functional properties and expand its food applications. To date, different applications of pea protein in the food system have been extensively studied, for example, encapsulation for bioactive ingredients, edible films, extruded products and substitution for cereal flours, fats, and animal proteins. This article reviews the current status of the knowledge regarding pea protein, focusing on its health benefits, functional properties, and structural modifications, and comprehensively summarizes its potential applications in the food industry.

## KEYWORDS

applications, functional properties, health benefits, modifications, pea protein

Abbreviations: AAA, aromatic amino acid; ACE, angiotensin I-converting enzyme; AE/IP, alkaline extraction/isoelectric precipitation; AF-PPH, PPH obtained by alcalase/flavorzyme; ALA,  $\alpha$ -linolenic acid; Alc-PPH, PPH obtained by alcalase; APP, alkaline pH-treated pea protein isolate; BP, blood pressure; CLA, conjugated linoleic acid; DBP, diastolic blood pressure; DDSA, dodecyl succinic anhydride; DH, degree of hydrolysis; DHA, docosahexaenoic acid; EA, emulsifying ability; EAI, emulsifying activity index; EBI, electron beam irradiation; EC, emulsifying capacity; ES, emulsion stability; ESL, emulsifying stability index; FC, foaming

capacity; Fla-PPH, PPH obtained by flavorzyme; FS, foaming stability; GA, gum arabic; GIS, gastrointestinal simulation; GIS-PPH, PPH obtained by in vitro gastrointestinal simulation; GTE, green tea extract; HAA, hydrophobic amino acid; HME, high-moisture extrusion; HP, high pressure; HT, high temperature; LE, licorice extract; LGC, least gelation concentration; LME, low-moisture extrusion; MTG, microbial transglutaminase; MZ, maize zein; OHC, oil-holding capacity; OP, oxygen permeability; OSA, *n*-octenyl succinic anhydride; Pap-PPH, PPH obtained by papain; PCAA, positively charged amino acid; PF, pea dietary fiber; *pI*, isoelectric point; PPC, pea

## 1 | INTRODUCTION

Pea (*Pisum sativum* L.) represents one of the major legumes in the world, with a global annual production estimated at about 13.5 million metric tons and a producer price of 200 USD/ton around, and it is cultivated today in more than 90 countries (FAOSTAT, 2018). Owing to its excellent yields, availability, and low-price production, pea is most widely used as a source of commercial proteins (Sun & Arntfield, 2012). Therefore, the development and application of pea protein have attracted much attention in the food industry.

Both the review by Burger and Zhang (2019) and the chapter by Singhal, Karaca, Tyler, and Nickerson (2016) have described the fractions of pea protein in detail. Overall, pea protein is mainly composed of 7S/11S globulin (salt-soluble, 65% to 80% of total) and albumin 2S (water-soluble, 10% to 20%) protein classes (Karaca, Low, & Nickerson, 2011), and contains high levels of lysine, which can be used to balance its deficiency in cereal-based diets (Iqbal, Khalil, Ateeq, & Khan, 2006). Compared to soybean protein, pea protein is generally recognized as a nonfood allergen with relatively high nutritional value and without genetic modification, offering a clean label for food products (Day, 2013; Krefting, 2017). Many studies suggested that pea protein (in many cases, pea protein hydrolysates [PPHs] and specific peptide fractions) has antioxidant (Ndiaye, Vuong, Duarte, Aluko, & Matar, 2012; Sun & Xiong, 2015), antihypertensive (Aluko et al., 2015; Liao, Fan, Liu, & Wu, 2019), anti-inflammatory (Ndiaye et al., 2012), lowering cholesterol (Sirtori et al., 2012), and modulating intestinal bacteria activities (Swiatecka, Markiewicz, & Wroblewska, 2012; Swiatecka, Narbad, Ridgway, & Kostyra, 2011). In addition, some biological activities of pea protein or PPH can be further enhanced by chemical or combined treatment approaches (Li et al., 2020; Swiatecka et al., 2011; Wang et al., 2017; Zha, Yang, Rao, & Chen, 2019). Hence, the regular dietary intake of foods rich in pea protein may have promising potential to reduce the risk of certain chronic diseases and thus is beneficial for improving human health (Dahl, Foster, & Tyler, 2012; Li et al., 2011).

In addition to its health benefits, the functional properties of pea protein also play a vital role in food processing (Burger & Zhang, 2019; Ladjal-Ettoumi, Boudries, Chibane,

& Romero, 2016; Lam, Karaca, Tyler, & Nickerson, 2018; Lam, Warkentin, Tyler, & Nickerson, 2017; Munialo, van der Linden, Ako, & de Jongh, 2015). Here, the functionality of pea protein refers to all properties contributing to the structure and texture of food products, including its solubility, water-holding capacity (WHC) and oil-holding capacity (OHC), emulsifying properties, foaming properties, and gelling properties. However, the applications of pea protein in food products are still challenging due to its poor functional performances. To overcome these drawbacks, recent studies have explored some modification methods such as physical, chemical, enzymatic, and combined treatments that can be applied to improve functional properties of pea protein by modifying its inherent structure (Burger & Zhang, 2019; Chao & Aluko, 2018; Pillai, Morales-Contreras, Wicker, & Nickerson, 2020; Warnakulasuriya, Pillai, Stone, & Nickerson, 2018; Wei et al., 2020; Zhan, Shi, Wang, Li, & Chen, 2019), and thus expand its application in food formulations.

Nowadays, different applications of pea protein in food-related products have been widely studied, such as encapsulation for bioactive ingredients (Jansen-Alves, Krumreich, et al., 2019; Jansen-Alves, Maia, et al., 2019), edible films (Carvajal-Pinero, Ramos, Jimenez-Rosado, Perez-Puyana, & Romero, 2019; Perez-Puyana, Felix, Romero, & Guerrero, 2017), extruded foods (Osen, Toelstede, Wild, Eisner, & Schweiggert-Weisz, 2014; Philipp, Emin, Buckow, Silcock, & Oey, 2018), substitution for cereal flours, fats and animal proteins (Ben-Harb et al., 2018, 2019; Liu et al., 2020; Muneer et al., 2018; Song & Yoo, 2017), and other products (Feng, Wang, Li, Zhou, & Meng, 2018; Tan, Siow, Peh, & Henry, 2018).

To our knowledge, although there have been a few reviews about certain properties of pea protein (Burger & Zhang, 2019; Lam et al., 2018), this is the first comprehensive review of the current scientific knowledge on pea protein, mainly related to health benefits, functional properties, structural modifications, and potential applications in food-related products (shown in Figure 1). The review paper will give readers a clear understanding of the potential application on pea protein in different food systems and help them think about further research scope in pea protein. Additionally, some products from pea protein in the market are also exhibited in Figure 1.

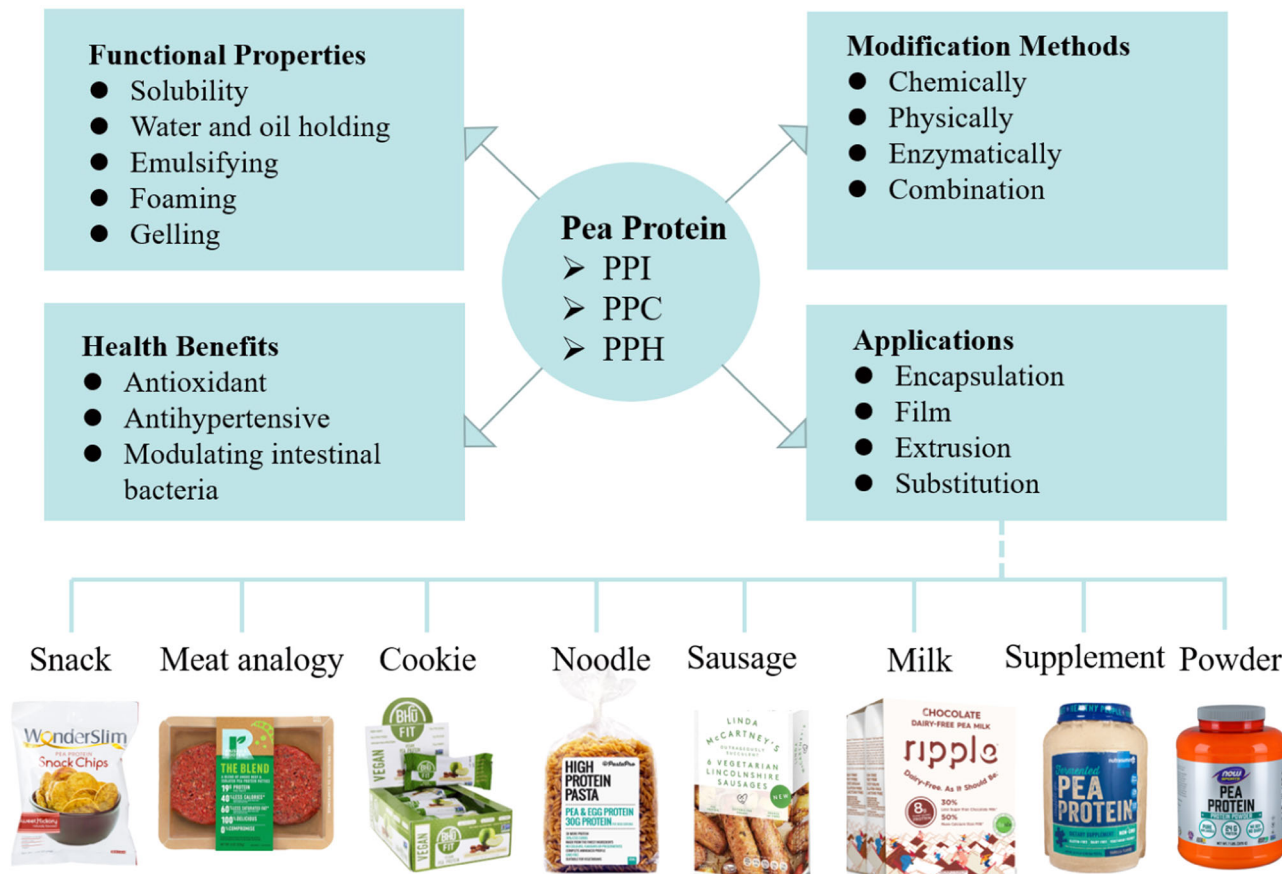
## 2 | HEALTH BENEFITS OF PEA PROTEIN OR PPH AND THE APPROACH TO IMPROVE THEM

### 2.1 | Antioxidant capacity

Antioxidants are bioactive compounds that have the function of inhibiting and/or reducing damages caused by the

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protein concentrate; PPH, pea protein hydrolysate; PPI, pea protein isolate; PPIc, commercial PPI; PPI-MP, PPI obtained by micellar precipitation; PPI-SE, salt-extracted PPI; Pro-PPH, PPH obtained by protamex; RH, relative humidity; RP-HPLC, reverse-phase high-performance liquid chromatography; SA, succinic anhydride; SBP, systolic blood pressure; SE, salt extraction; SHR, spontaneously hypertensive rat; SOD, superoxide dismutase; SPE, solid-phase extraction; SPI, soy protein isolate; SPIc, commercial SPI; TA, tannic acid; The-PPH, PPH obtained by thermolysin; Try-PPH, PPH obtained by trypsin; TyrBm, tyrosinase from *Bacillus megaterium*; UF, ultrafiltration; US, ultrasound; WHC, water-holding capacity; WPI, whey protein isolate; WVP, water vapor permeability.



**FIGURE 1** Research progress of pea protein and its application in market products.

deleterious action of free radicals or nonradical reactive species, playing an important role in various cardiovascular diseases (Roy, Boye, & Simpson, 2010). Despite the existence of some pea protein researches, most investigations on antioxidant property have been focused on PPHs (Tamm, Herbst, Brodkorb, & Drusch, 2016; Zha, Yang, et al., 2019). In this section, PPH could be obtained using enzymatic and physical combined enzymatic treatments. In addition, the effect of several chemical treatments on the antioxidation of pea protein is given. As presented in Table 1, the antioxidant properties of pea protein and PPH prepared by different methods are described in detail, including preparation condition, detection method, and result. Therefore, pea protein and PPH have a potential to substitute traditional/synthetic antioxidants in the food system and make more safe products.

### 2.1.1 | Chemical treatments for improving the antioxidant capacity

Chemical treatments have been reported to improve the antioxidant capacity of pea protein or PPH (Jiang, Zhu, Liu, & Xiong, 2014; Li et al., 2020; Tsai & She, 2006; Zha, Yang, et al., 2019). Tsai and She (2006) investigated that pea protein treated with five phenolics under different heating conditions possessed higher superoxide dismutase (SOD) activity,

DPPH scavenging ability, and reducing power than the control (untreated pea protein). Compared to the control and pea protein samples treated with hydroxybenzoic acids (catechin and gallic acid), pea protein samples treated with hydroxycinnamic acids (ferulic acid, coumaric acid, and caffeic acid) showed higher SOD activity. Among them, coumaric acid was the most effective phenolic compound to enhance the antioxidant capacity of pea protein and had the highest binding capacity with pea protein. Similar results were reported by Li et al. (2020) that pea protein–tannic acid (TA) complexes formed by protein–polyphenol interaction exhibited higher lipid oxidation stability than native pea protein. Jiang et al. (2014) observed that the antioxidant activity of alkaline pH-treated pea protein isolate (APP) is 60% greater than that of pea protein isolate (PPI) in terms of ABTS<sup>•+</sup> scavenging assay. APP is nearly twice as effective as PPI in inhibiting the TBARS formation in the oxidizing liposome model. Compared to PPI, the O/W emulsion prepared with APP is less prone to oxidation (malonaldehyde, peroxide) during storage. According to Zha et al. (2019a), PPH was first explored to conjugate with gum arabic (GA) through Maillard-driven chemistry and studied antioxidant property of conjugate product. These results shown that PPH-GA-1 (PPH-GA synthesized after 1 day of conjugation) exhibited superior capacity

**TABLE 1** The antioxidant properties of pea protein and its hydrolysates and the approaches to improve them

Approach	Preparation condition	Detection method	Result	Reference
<b>Chemical treatment</b> <ul style="list-style-type: none"> <li>Protein–polyphenol interaction</li> </ul>	<ul style="list-style-type: none"> <li>Pea is immersed separately in different phenolic solutions (gallic acid, catechin, ferulic acid, coumaric acid, and caffeic acid) of 1 g/L for 6 hr. After drying pea at different temperatures (30 to 70 °C) for different durations (0 to 8 hr).</li> <li>PPIC-TA complexes are obtained by mixing PPIC and TA (at pH 7.0) solutions together 0.5 hr on stirrer 25 °C; PPIC concentration is fixed (1.0%), whereas a range of TA concentrations are used (0% to 0.5%).</li> </ul>	<ul style="list-style-type: none"> <li>✓ SOD activity assay</li> <li>✓ DPPH scavenging assay</li> <li>✓ Reducing power assay</li> <li>✓ Conjugated dienes assay</li> <li>✓ TBARS analysis</li> <li>✓ O/W emulsion system</li> </ul>	<ul style="list-style-type: none"> <li>Phenols enhanced the antioxidant capacity of pea protein by interacting with protein during heating.</li> <li>Compared with PPIC, PPIC-TA complexes had strong antioxidant activity in O/W emulsion during storage.</li> </ul>	Li et al. (2020); Tsai and She (2006)
<b>Chemical treatment</b> <ul style="list-style-type: none"> <li>Alkaline pH treatment</li> </ul>	<ul style="list-style-type: none"> <li>PPI (20 mg/mL, pH 7.0) solution is adjusted to pH 12 and held at this pH for 1 hr to induce partial unfolding and then titrate back to pH 7.0 to allow refolding.</li> </ul>	<ul style="list-style-type: none"> <li>✓ ABTS scavenging assay</li> <li>✓ POV analysis</li> <li>✓ TBARS analysis</li> </ul>	<ul style="list-style-type: none"> <li>APP had higher antioxidant capacities than PPI in terms of ABTS•<sup>+</sup> scavenging assay, lipid oxidation, and O/W emulsion oxidation.</li> </ul>	Jiang et al. (2014)
<b>Chemical treatment</b> <ul style="list-style-type: none"> <li>Maillard-driven reaction</li> </ul>	<ul style="list-style-type: none"> <li>PPH and GA (1:4, w/w) are mixed and then hydrated (1:2, w/v) for 24 hr on stirrer at 22 °C. The slurry eventually is adjusted to pH 7.0 and lyophilized. PPH-GA products are obtained by exposing the lyophilized powder to 79% relative humidity and 60 °C for variable time (0, 1, 3, and 5 days).</li> </ul>	<ul style="list-style-type: none"> <li>✓ Hydroperoxide analysis</li> <li>✓ Hexanal analysis</li> <li>✓ O/W emulsion system</li> </ul>	<ul style="list-style-type: none"> <li>Compared with PPH and PPH-GA mixture, PPH-GA-1 effectively inhibited lipid oxidation of O/W emulsion during storage.</li> </ul>	Zha et al. (2019a)
<b>Enzymatic treatment</b> <ul style="list-style-type: none"> <li>Alcalase: 50 °C, pH 8.5</li> <li>Flavorzyme: 50 °C, pH 7.0</li> <li>Papain: 40 °C, pH 6.5</li> <li>Trypsin: 37 °C, pH 8.0</li> <li>α-Chymotrypsin: 37 °C, pH 8.0</li> </ul>	<ul style="list-style-type: none"> <li>PPI solution (5%, w/v) is heated at appropriate temperature and pH prior to add different enzymes, and then maintain constant for 4 hr. The slurry eventually is adjusted to pH 4.0 to stop the enzyme reaction.</li> </ul>	<ul style="list-style-type: none"> <li>✓ DPPH scavenging assay</li> </ul>	<ul style="list-style-type: none"> <li>DPPH radical scavenging activity of Fla-PPH was significantly (<math>P &lt; 0.05</math>) the highest, whereas Alc-PPH and Try-PPH were the lowest.</li> </ul>	Humiski and Aluko (2007)

(Continues)

TABLE 1 (Continued)

Approach	Preparation condition	Detection method	Result	Reference
<b>Enzymatic treatment</b> <ul style="list-style-type: none"> <li>Thermolysin: 55 °C, pH 8.0</li> </ul>	<ul style="list-style-type: none"> <li>*PPI solution (6%, w/v) is heated at 55 °C and pH 8.0, and then maintain constant for 3 hr. Enzyme reaction eventually is stopped by heating slurry at 95 °C and holding for 15 min.</li> </ul>	<ul style="list-style-type: none"> <li>✓ DPPH scavenging assay</li> <li>✓ Reducing power assay</li> <li>✓ Superoxide scavenging assay</li> <li>✓ Hydrogen peroxide scavenging assay</li> <li>✓ Metal chelating assay</li> <li>✓ Hydroxyl scavenging assay</li> <li>✓ Inhibition of linoleic acid oxidation</li> </ul>	<ul style="list-style-type: none"> <li>Except for reducing power and linoleic acid oxidation, the contents of HAA, PCAA, and AAA affected most antioxidant capacities of PPH peptide fractions.</li> </ul>	Pownall et al. (2010, 2011)
<b>Enzymatic treatment</b> <ul style="list-style-type: none"> <li>Thermolysin: 55 °C, pH 8.0</li> </ul>	<ul style="list-style-type: none"> <li>PPH is obtained by the above method*. Culture cells for 1 hr, then add PPI or PPH to cells at different final concentrations (25 to 1.56 µg/mL). After 12 hr pretreatment, cells are stimulated with 10 ng/mL LPS and 10 units/mL IFN-γ. The activated cells are further incubated for 24 hr and collect supernatant to determine nitrite concentration.</li> </ul>	<ul style="list-style-type: none"> <li>✓ LPS/IFN-γ-activated RAW 264.7 NO(-) macrophages model</li> <li>✓ Nitric oxide product assay with Griess reaction</li> </ul>	<ul style="list-style-type: none"> <li>PPH exerted potent antioxidant capacity in macrophages model than PPI.</li> </ul>	Ndiaye et al. (2012)
<b>Enzymatic treatment</b> <ul style="list-style-type: none"> <li>Trypsin, chymotrypsin, papain, and pepsin at 37 °C</li> <li>Alcalase, flavorzyme, and protamex at 50 °C</li> </ul>	<ul style="list-style-type: none"> <li>PPI solution (2%, w/v) with or without preheating (90 °C, 5 min) is hydrolyzed for 0.5 hr at appropriate temperature and pH using different enzymes. Enzyme reaction is stopped at 80 °C for 15 min and then solution is neutralized to pH 7.0.</li> </ul>	<ul style="list-style-type: none"> <li>✓ Liposomal model</li> <li>✓ O/W emulsion system</li> <li>✓ POV analysis</li> <li>✓ TBARS analysis</li> </ul>	<ul style="list-style-type: none"> <li>All PPH inhibited lipid oxidation.</li> <li>PPH and LE were applied together; both liposomal model and O/W emulsion showed synergistic inhibition on lipid oxidation.</li> </ul>	Zhang et al. (2013, 2014)
<b>Enzymatic treatment</b> <ul style="list-style-type: none"> <li>Flavorzyme: 50 °C, pH 6.0</li> </ul>	<ul style="list-style-type: none"> <li>PPI solution (5%, w/v) is hydrolyzed for 0.5 hr at 50 °C and pH 6.0. Enzyme reaction is stopped at 80 °C for 15 min and solution is neutralized to pH 7.0.</li> </ul>	<ul style="list-style-type: none"> <li>✓ ABTS scavenging assay</li> <li>✓ Reducing power</li> <li>✓ TBARS analysis</li> <li>✓ Protein carbonyls analysis</li> </ul>	<ul style="list-style-type: none"> <li>Both PPI and PPH had remarkable ABTS•<sup>+</sup> scavenging activity and reducing power, meanwhile effectively inhibited the oxidation of lipid and protein.</li> <li>PPH had better pigment-protection efficacy than PPI.</li> </ul>	Sun and Xiong (2015)
<b>Enzymatic treatment</b> <ul style="list-style-type: none"> <li>Trypsin: pH 8.0</li> <li>Alcalase: pH 8.0</li> </ul>	<ul style="list-style-type: none"> <li>PPI solution (5%, w/v) is hydrolyzed to different degree hydrolysis (DH: 1%, 2%, 4%, 6%, or 8%). Required DH is obtained and enzyme reaction is stopped at 75 °C for 30 min.</li> </ul>	<ul style="list-style-type: none"> <li>✓ Hydroperoxide analysis</li> <li>✓ O/W emulsion system</li> </ul>	<ul style="list-style-type: none"> <li>Compared with PPI and Alc-PPH, Try-PPH effectively inhibited lipid oxidation of rapeseed oil in spray-dried emulsion during storage.</li> </ul>	Tamm et al. (2016)

(Continues)



TABLE 1 (Continued)

Approach	Preparation condition	Detection method	Result	Reference
<b>Enzymatic treatment</b> <ul style="list-style-type: none"> <li>• Pancreatic trypsin: pH 8.0</li> </ul>	<ul style="list-style-type: none"> <li>• PPC solution is hydrolyzed for 25 or 120 min at pH 8.0. Enzyme reaction is stopped using boiling water for 10 min.</li> </ul>	<ul style="list-style-type: none"> <li>✓ DPPH scavenging assay</li> <li>✓ ABTS scavenging assay</li> <li>✓ Folin–Ciocalteu analysis</li> </ul>	<ul style="list-style-type: none"> <li>• PPH exhibited lower antioxidant activities than PPC.</li> </ul>	Felix et al. (2017)
<b>Physical combined enzymatic treatment</b> <ul style="list-style-type: none"> <li>• HP: 200, 400, or 600 MPa, 5 min and alcalase: 50 °C, pH 9.0</li> <li>• HT: 100 °C, 30 min and alcalase: 50 °C, pH 9.0</li> </ul>	<ul style="list-style-type: none"> <li>• PPI solution (5%, w/v) with HP or HT pretreated is hydrolyzed for 4 hr at pH 9.0. Enzyme reaction is stopped using boiling water for 15 min.</li> </ul>	<ul style="list-style-type: none"> <li>✓ ORAC scavenging assay</li> <li>✓ DPPH scavenging assay</li> <li>✓ FRAP scavenging assay</li> <li>✓ Metal chelating assay</li> <li>✓ Superoxide radical-scavenging assay</li> <li>✓ Hydroxyl radical-scavenging assay</li> </ul>	<ul style="list-style-type: none"> <li>• Except for FRAP, HP pretreatment effectively promoted the antioxidant properties of PPH than HT pretreatment.</li> </ul>	Girgih et al. (2015)
<b>Physical combined enzymatic treatment</b> <ul style="list-style-type: none"> <li>• EBI: 5, 10, 30, or 50 kGy and flavorzyme: 50 °C</li> </ul>	<ul style="list-style-type: none"> <li>• PPI solution (5%, w/v) with EBI pretreated is hydrolyzed for 1.5 hr at optimum hydrolysis conditions. Enzyme reaction is stopped using boiling water for 15 min. After cooling, the slurry is adjusted to pH 7.0.</li> </ul>	<ul style="list-style-type: none"> <li>✓ DPPH scavenging assay</li> <li>✓ ABTS scavenging assay</li> </ul>	<ul style="list-style-type: none"> <li>• Compared to nonpretreated PPH, PPH pretreated with EBI showed the higher antioxidant activities.</li> </ul>	Wang et al. (2017)

Abbreviations: DPPH, 2,2-diphenyl-1-picrylhydrazyl; SOD, superoxide dismutase; ABTS, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; POV, peroxides; TBARS, thiobarbituric acid-reactive substances; ORAC, oxygen radical absorbance capacity; FRAP, ferric reducing antioxidant power; PPI, pea protein isolate; PPH, pea protein hydrolysate; TA, tannic acid; GA, gum Arabic; LE, licorice extract; HAA, hydrophobic amino acids; AAA, aromatic amino acids; PCAA, positively charged amino acids; HP, high pressure; HT, high temperature; EBI, electron beam irradiation.

than PPH and PPH-GA mixture against lipid oxidation of corn oil-in-water emulsion during storage at 37 °C. Overall, chemical treatment is an effective approach to improve the antioxidant capacity of pea protein or PPH. In the review paper from Liu, Ma, Gao, and McClements (2017), it is also highlighted that chemical treatment (such as free radical grafting and alkaline treatment) had positive influences on antioxidant activity of proteins. On this research subject, it is worth further doing more work.

### 2.1.2 | Enzymatic treatments for preparing PPH

PPH is of interest for researchers due to its beneficial effects on human health. Enzymatic treatment is a commonly used and relatively safe pathway to prepare PPH with antioxidant capacity. Multiple conditions such as enzyme type, amino acid composition, degree of hydrolysis (DH), and hydrolysis time contribute to the antioxidant property of PPH (Humiski & Aluko, 2007; Pownall, Udenigwe, & Aluko, 2010, 2011; Tamm et al., 2016). Humiski and Aluko (2007) found that the PPH obtained by flavorzyme (Fla-PPH; EC 3.4.15.1) showed higher DPPH radical-scavenging activity than that of papain

(EC 3.4.22.2),  $\alpha$ -chymotrypsin (EC 3.4.21.1), alcalase (Alc-PPH; EC 3.4.21.1), and trypsin (Try-PPH; EC 3.4.21.4) in order. But overall, the DPPH scavenging activity of these PPHs was very poor (about 7% to 11%). Pownall et al. (2010) reported the relationship between amino acid composition and antioxidant activities of PPH obtained by thermolysin (The-PPH; EC 3.4.24.27) peptide fractions (F1 to F5, shown in Table 2), and found that the antioxidant activity of peptides (F1 to F5) from The-PPH depends on the amounts of constituent hydrophobic (HAA) and aromatic amino acids (AAA). The fractions F3 to F5 with high HAA and AAA content exhibited the strongest radical scavenging and metal chelating activities (shown in Table 2). Although The-PPH and its fractionated peptides had very low reducing power, HAA content did not contribute to reducing power of the peptides. In contrast, The-PPH and its peptide fractions were effective at inhibiting linoleic acid oxidation regardless of HAA content. Additionally, Pownall, Udenigwe, and Aluko (2011) further investigated the relationship between positively charged amino acid (PCAA) content and antioxidant activities of The-PPH peptide fractions (F11 to F55, shown in Table 2), which showed that antioxidant properties of

**TABLE 2** Amino acid composition (%) of the <3-kDa pea protein hydrolysate obtained by thermolysin (The-PPH) and its HPLC fractions (F1-F5) and FPLC fractions (F11-F55)

Amino acid	The-PPH	HPLC fractions (F1-F5)					FPLC fractions (F11-F55)				
		F1	F2	F3	F4	F5	F11	F22	F33	F44	F55
ASX	13.79	13.94	10.63	12.59	10.85	11.04	15.09	11.23	9.86	8.12	6.61
THR	3.6	3.89	3.86	3.34	3.11	3.22	4.43	3.42	3.59	2.68	1.74
SER	6.2	6.63	5.71	6.19	4.41	3.82	5.16	3.96	5.73	3.72	4.24
GLX	13.92	17.12	14.78	13.75	12.87	6.64	20.30	22.14	10.81	9.47	9.04
PRO	5.15	2.33	6.47	5.14	5.42	8.05	5.16	5.79	4.56	2.37	2.93
GLY	3.76	3.52	5.00	3.96	4.66	3.26	4.11	3.83	3.47	3.87	2.0
ALA	5.01	5.54	4.30	5.03	3.44	3.62	5.47	4.31	4.62	4.18	2.48
CYS	0.24	0.18	0.39	0.39	0.38	0.29	0.41	0.26	0.13	0.12	0.11
VAL	5.63	5.23	4.45	4.13	5.82	7.68	4.77	4.28	3.64	2.66	2.02
MET	0.91	0.70	1.70	0.87	1.07	0.68	1.07	0.88	0.96	0.51	0.22
ILE	5.43	4.13	4.04	6.71	5.85	9.13	4.99	3.23	3.22	2.25	2.14
LEU	9.91	8.70	6.68	9.95	14.57	19.48	10.16	6.55	8.22	8.92	6.64
TYR	3.87	2.77	5.33	7.15	5.09	2.44	3.41	4.12	3.22	5.61	3.48
PHE	7.41	3.97	7.76	8.73	12.03	16.44	6.81	4.50	6.20	4.86	2.24
HIS	1.61	2.49	3.28	1.90	1.81	0.63	1.05	3.55	3.76	4.22	4.26
LYS	6.1	9.07	7.35	4.26	3.31	1.20	3.40	8.03	16.38	11.79	16.76
ARG	6.83	9.79	8.00	5.15	3.97	1.22	3.12	9.32	10.19	24.50	30.18
TRP	0.68	0.00	0.27	0.74	1.36	1.16	1.06	0.63	1.46	0.14	2.93
HAA	44.24	33.55	41.39	48.84	55.03	68.97	43.31	34.55	36.23	31.62	25.19
PCAA	14.54	21.35	18.63	11.31	9.09	3.05	7.57	20.9	30.33	40.51	51.2
NCAA	27.71	31.06	25.41	26.34	23.72	17.68	35.39	33.37	20.67	17.59	15.65
AAA	11.96	6.74	13.36	16.62	18.48	20.04	11.28	9.25	10.88	10.61	8.65

Note. Combined total of hydrophobic amino acids (HAA): alanine, valine, isoleucine, leucine, tyrosine, phenylalanine, tryptophan, proline, methionine, and cysteine; positively charged amino acids (PCAA): arginine, histidine, and lysine; negatively charged amino acids (NCAA): Asx and Glx; aromatic amino acids (AAA): phenylalanine, tryptophan, and tyrosine. Data are from the studies by Pownall et al. (2010, 2011).

Abbreviations: Asx, aspartic acid and asparagine; Glx, glutamic acid and glutamine.

its peptide fractions could be influenced by PCAA content. Although F11 with the least PCAA content had the highest activity compared to F22 to F55 (shown in Table 2), there is no linear relationship between the PCAA of The-PPH peptide fractions (F11 to F55) and its DPPH radical scavenging activity. The scavenging capacities for  $O_2^{\cdot-}$  and  $H_2O_2$  were negatively related with the PCAA content (shown in Table 2). Similarly, Pownall et al. (2010) reported that The-PPH peptide fractions showed weak reducing power. The-PPH and its peptide fractions all displayed strong inhibition against linoleic acid oxidation during 7-day storage period, although The-PPH peptide fractions did not have hydroxyl radical scavenging and metal chelating activities. Compared to the study by Humiski and Aluko (2007), The-PPH reported by Pownall et al. (2010, 2011) had stronger DPPH radical-scavenging activity due to its higher HAA and AAA content (shown in Table 2). Different from the above studies, Ndiaye et al. (2012) used LPS/IFN- $\lambda$ -activated RAW 264.7 NO(-) macrophages model to research the antioxidant property of PPI and PPH, and indicated that PPI did not show any

significant effect, whereas PPH significantly inhibited NO production of activated macrophages after 12 hr pretreatment in a dose-dependent manner.

Zhang, Xiong, Chen, and Zhou (2013) studied the synergistic inhibition of lipid oxidation by PPH (PPI with or without preheating before enzyme hydrolysis) coupled with licorice extract (LE) in a liposomal model. Almost all PPHs, except those obtained from native PPI with pepsin (EC 3.4.23.15) and alcalase, significantly suppressed lipid oxidation. PPHs obtained from preheated PPI with flavorzyme and protamex (Fla-PPH and Pro-PPH) were the most effective, which found 24.0% and 22.3% TBARS reductions from native PPI ( $P < 0.05$ ), respectively. When PPH and LE were applied together in liposomal model, the most remarkable synergistic effects were observed on both Fla-PPH and Pro-PPH with LE, up to 57.7% and 50.2%, respectively. After that, Zhang, Xiong, Chen, and Zhou (2014) further reported synergy of LE and PPH for lipid oxidation of soybean oil-in-water emulsion system (O/W emulsion). Both Fla-PPH and Pro-PPH significantly retarded oxidation ( $P < 0.05$ ) of the emulsion when

stored at 37 °C for 14 days. Similarly, when PPH and LE were applied together in O/W emulsion, a remarkable synergistic oxidation inhibition was observed with both Fla-PPH and Pro-PPH; among them, Pro-PPH was the most effective up to 30.9% and 40.8%, for peroxides value (POV) and TBARS, respectively. Compared with the study by Zhang et al. (2013), synergistic inhibition by PPH and LE on lipid oxidation in O/W emulsion system was less than that in liposomal model. According to Sun and Xiong (2015), the ABTS<sup>•+</sup> scavenging activity of PPI and PPH at concentration of 2 g/100 g was equivalent to 2.4 and 2.7 mmol/L trolox, respectively. And reducing power of PPI and PPH at concentration of 2 g/100 g was equivalent to 112.9 and 123.3 mmol/L FeSO<sub>4</sub>, respectively. The addition of PPI or PPH significantly decreased the lipid and protein oxidations of cooked cured beef, thus reducing the color of cooked cured beef influenced by oxygen. The study of Tamm et al. (2016) investigated the antioxidant effects of PPI and PPHs (different enzymes hydrolysis and various DH, detailed description shown in Table 1) on microencapsulated rapeseed oil. The O/W emulsion prepared by Alc-PPH was very unstable, not to mention antioxidant capacity of Alc-PPH at the oil/water interface. In contrast, Try-PPH exhibited superior antioxidant properties than PPI for reducing lipid oxidation of rapeseed oil in spray-dried emulsions during storage, meanwhile the inhibition of lipid oxidation increased with increasing DH. Felix, Perez-Puyana, Romero, and Guerrero (2017) looked into the antioxidant activities of gels prepared from pea protein concentrate (PPC) and its various time hydrolysates (PPH25 and PPH120, shown in Table 1) at three pH values (2.0, 6.5, and 8.0). It was found that PPH showed lower antioxidant activities than PPC and the antioxidant activities decreased with increasing hydrolysis time (equivalent to increasing DH), which were contrary to the study by Tamm et al. (2016). This may be the result of the different matrix system; nevertheless, real reasons still need further exploration. In addition, initially antioxidant activities of samples were not pH dependent, whereas excessive DH converted PPC gels into pH dependent.

### 2.1.3 | Physical combined enzymatic treatments for preparing PPH

Recently, various physical combined enzymatic treatments have been reported to produce PPH. These techniques include high pressure (HP), high temperature (HT), or electron beam irradiation (EBI) combined different enzymatic treatments (shown in Table 1). Girgih et al. (2015) studied the antioxidant properties of PPHs from HP- or HT-pretreated PPI and hydrolyzed by alcalase. Results found that PPHs from 400 to 600 MPa-pretreated PPI significantly ( $P < 0.05$ ) exhibited higher ORAC values and metal chelating activity than control (PPH from non-HP-pretreated PPI and hydrolyzed by alcalase). Meanwhile, DPPH, hydroxyl and superoxide

radical-scavenging abilities of PPH from HP-pretreated PPI were also significantly ( $P < 0.05$ ) improved (25%, 20%, and 40%, respectively) in comparison to control, but all PPHs had low reducing power. Different from HP pretreatment, PPHs from HT-pretreated PPI showed lower DPPH radical-scavenging and metal chelating activities than control (PPH from non-HT-pretreated PPI and hydrolyzed by alcalase), and these PPHs had no ORAC, superoxide, or hydroxyl scavenging activities but exhibited significantly ( $P < 0.05$ ) improved (80%) reducing power. In contrast, Zhang et al. (2013) indicated that PPHs prepared from HT-pretreated PPI and hydrolyzed by different enzymes (flavorzyme and protamex) markedly suppressed lipid oxidation compared with the control. Moreover, Wang et al. (2017) used EBI combined flavorzyme treatment to prepare PPHs, and found that antioxidant activities of PPH from EBI-pretreated PPI increased with increasing irradiation dose. Compared to nonpretreated PPH, PPH pre-irradiated at 50 kGy possessed the strongest scavenging effect, namely, the DPPH and ABTS<sup>•+</sup> radical-scavenging activity levels increased by 32.73% and 52.80%, respectively. Generally, PPH obtained by physical combined enzymatic treatment shows the better antioxidant property in comparison to enzymatic treatment; nevertheless, it is the key point to choose adequate physical pretreatment technology. Furthermore, some novel physical techniques, for instance, ultrasound, pulsed electric fields, microwave, and high-pressure homogenization, may also be combined with enzymatic treatment to prepare PPH with strong antioxidant activities, which needs more research to explore in the future.

## 2.2 | Antihypertensive capacity

Hypertension (defined as high systolic blood pressure [SBP] and diastolic blood pressures [DBP]) is directly associated with the development of cardiovascular disease in humans. Many studies have shown a promising potential of PPH to reduce blood pressure (BP). Most antihypertensive peptides from PPH were usually characterized as inhibitors of angiotensin I-converting enzyme (ACE) or renin, given the essential role of the renin-angiotensin system in regulating BP (Barbana & Boye, 2010; Li et al., 2011). In addition, a new study revealed that angiotensin-converting enzyme 2 (ACE2)-upregulating peptides from PPH might be also considered as a strategy for identifying antihypertensive capacity (Liao et al., 2019). Nowadays, enzymatic, fermentation combined with enzymatic and physical combined enzymatic treatments is frequently used to produce PPH with the function of reducing BP.

### 2.2.1 | Enzymatic treatments for preparing PPH

It is well known that enzymatic treatment is a common method for preparing PPH. The study of Vermeirssen, Camp, and



Verstraete (2002) first reported that PPH obtained by digestion with trypsin showed ACE inhibitory activity. Before digestion, the ACE inhibitory activity in PPI was  $35 \pm 7\%$ ; after digestion (from PPI to PPH) this value increased to  $99 \pm 1\%$  and  $IC_{50}$  value for PPH was 1.36 mg/mL. In a follow-up study (Vermeirssen, Camp, & Verstraete, 2005), PPH was obtained through the hydrolysis with in vitro gastrointestinal enzymes (pepsin, trypsin and  $\alpha$ -chymotrypsin), indicating that PPH had high ACE inhibitory activity ( $IC_{50} = 0.070$  mg/mL). It is worth noting that the ACE inhibitory activity of PPH was considerably improved upon purification by ultrafiltration (UF)/centrifugation ( $IC_{50} = 0.055$  mg/mL) and reverse-phase high-performance liquid chromatography (RP-HPLC) ( $IC_{50} = 0.016$  mg/mL). Li and Aluko (2010) investigated the ACE and renin inhibitory activities of Al-PPH. The peptide fractions from PPH were fractionated by cationic solid-phase extraction (SPE) and exhibited different inhibitory capacity against ACE and renin. Meanwhile, these results found that the ACE inhibition was positively correlated with electric charges and the SPE fractions 4 and 5 possessed superior inhibition against ACE. Moreover, the SPE fraction 5 had the highest renin inhibition activity because of its PCAA residues. Further, Li et al. (2011) explored the blood pressure-lowering activity of The-PPH using different hypertensive rat models as well as human subjects. PPH (1 mg/mL) showed weak in vitro renin and ACE inhibitory activities with 17% and 19%, respectively. Compared with PPI, oral administration of PPH to spontaneously hypertensive rats (SHR) at doses of 100 and 200 mg/kg body weight led to a lowering of hourly SBP, with a maximum reduction of 19 mmHg at 4 hr. Han:SPRD-cy rat (a chronic kidney disease model) with oral administration of PPH for 8 weeks resulted in 29 and 25 mmHg reductions in SBP and DBP, respectively. Besides, in a 3-week randomized double blind placebo-controlled crossover human intervention trial (seven volunteers), significant ( $P < 0.05$ ) reductions (over placebo) in SBP of 5 and 6 mmHg were obtained in the 2nd and 3rd weeks, respectively, for the PPH group. According to Barbana and Boye (2010), PPC had no inhibitory effect against ACE, but PPHs (obtained by in vitro using gastrointestinal simulation [GIS-PPH], alcalase/flavorzyme [AF-PPH], and papain [Pap-PPH]) showed inhibitory effect against ACE and increased with increasing concentration. Pap-PPH showed the higher ACE inhibition ( $IC_{50} = 0.128$  mg/mL) compared to AF-PPH ( $IC_{50} = 0.412$  mg/mL) and GIS-PPH ( $IC_{50} = 0.159$  mg/mL), whereas the GIS-PPH in this study possessed the lower ACE inhibition than Vermeirssen et al. (2005) reported ( $IC_{50} = 0.070$  mg/mL). The ACE inhibitory property of PPH obtained by in vitro gastrointestinal enzymic hydrolysis ( $\alpha$ -amylase [EC 3.2.1.1], pepsin, and pancreatin) was also reported by Jakubczyk and Baraniak (2014); here, GIS condition including simulated saliva solution and type of enzymes was different from Vermeirssen et al.'s (2005) study. It was shown

that the PPH exhibited a lower ACE inhibitory ratio ( $IC_{50} = 0.72$  mg/mL) than that of others (Barbana & Boye, 2010; Vermeirssen et al., 2005). Similarly, PPI showed no ACE inhibitory activity. The fraction F8 from PPH separated using ion-exchange chromatography had the highest ACE inhibitory activity ( $IC_{50} = 0.0014$  mg/mL). This fraction (F8) was further separated on Sephadex G10, founding fraction (B) with the highest ACE inhibitory activity ( $IC_{50} = 0.073$  mg/mL). Aluko et al. (2015) examined the antihypertensive activity of The-PPH and its fractions (separated by RP-HPLC), and suggested that fraction 7 possessed the highest dual inhibitions for renin and ACE with 52.16 and 95.17%, respectively. Researchers further found that fraction 7 mainly consisted of five peptides; among them, LTFPG, IFENLQN, and FEGTVFENG exhibited significantly ( $P < 0.05$ ) higher inhibitions for ACE and renin activities. These three peptides were orally administered to SHR at dose of 30 mg/kg body weight, and the results indicated that LTFPG had significantly ( $P < 0.05$ ) the fastest decrease in SBP with a maximum of  $-37$  mmHg after 2 hr. In contrast, the maximum effects of IFENLQN ( $-37$  mmHg) and FEGTVFENG ( $-25$  mmHg) were observed after 4 hr. Nevertheless, the three peptides had significantly ( $P < 0.05$ ) better SBP-reducing effects than PPH, which only gave a maximum of  $-14$  mmHg after 6 hr. It is worth mentioning that Liao et al. (2019) first validated the activity of PPH in upregulating ACE2 expression in A7r5 cells, in which PPH was generated via a combination hydrolysis of thermoase and pepsin. Results showed that only AKSLSDRFSY peptide played a predominant role in PPH to upregulate ACE2 expression, which also provided a new strategy for identification of antihypertensive peptides from food protein sources.

### 2.2.2 | Fermentation combined with enzymatic treatments for preparing PPH

As reviewed in Section 2.2.1, enzymatic hydrolysis is an effective way to release antihypertensive peptides. Meanwhile, fermentation is also a common processing technology for releasing bioactive or functional peptides from food proteins. Several researchers have reported the antihypertensive properties of PPH produced by fermentation combined with enzymatic treatment. Vermeirssen, Camp, Decroos, Wijmelbeke, and Verstraete (2003) first indicated that PPH, which was obtained through in vitro GIS (pepsin, trypsin, and  $\alpha$ -chymotrypsin) hydrolysis after fermented by *Lactobacillus helveticus* (at 28 °C) or *Saccharomyces cerevisiae* (at 37 °C) in monoculture, showed ACE inhibitory activity. However, after hydrolysis, both fermented ( $IC_{50}$ : 0.23 to 0.11 mg/mL) and nonfermented ( $IC_{50} = 0.12$  mg/mL) samples reached maximum ACE inhibitory activity. These results suggested that enzymic hydrolysis was the predominant factor controlling the formation of ACE inhibitory activity, whereas

fermentation processing did not further enhance the ACE inhibitory activity of PPH. The study of Jakubczyk, Karas, Baraniak, and Pietrzak (2013) researched the ACE inhibitory capacity of PPHs, which were obtained through in vitro GIS ( $\alpha$ -amylase, pepsin, and pancreatin) hydrolysis after fermented by *Lactobacillus plantarum* 299v in monoculture under different time (3 hr, 3 days, and 7 days) and temperature (22, 30, and 37 °C) conditions. It shown that PPH after fermentation (after 7-day fermentation at 22 °C) had the lowest IC<sub>50</sub> value (0.19 mg/mL), compared with that of nonfermented PPH (0.37 mg/mL). Different from report by Jakubczyk et al. (2013), Vermeirssen et al. (2003) demonstrated that fermentation processing with *L. plantarum* was beneficial to the improvement in ACE inhibitory activity of PPH. The sequence of peptide (PPH from 7-day fermentation at 22 °C) derived from pea protein was eventually identified as KEDDEEEEQGEEE.

### 2.2.3 | Physical combined enzymatic treatments for preparing PPH

It is an emerging technology to prepare PPH by physical combined enzymatic treatment. Heat and HP treatment are the most common physical processing to change protein structure and thus enhancing the efficiency of protein hydrolysis in the food industry. Nowadays, there is few studies on the antihypertensive capacity of PPH obtained by physical combined enzymatic treatment. Only Chao, He, Jung, and Aluko (2013) studied the ACE- and renin-inhibitory properties of Arc-PPH (1% to 4%, w/w) after HP (200 to 600 MPa, 5 min at 24 °C) or heat (100 °C, 30 min) pretreatment. Compared to the 24 °C PPH, heat pretreatment of PPH at 100 °C led to the production of peptides with significantly ( $P < 0.05$ ) reduced ACE-inhibitory activity, whereas HP pretreatment had no significant influence on ACE-inhibitory activity of PPH in comparison to 0 MPa PPH. PPH produced from HP pretreatment had higher ACE-inhibition capacity than that from heat pretreatment. Overall, heat or HP pretreatment favored to improve ACE- and renin-inhibitory activity of PPH at a lower (1%) alcalase concentration. In general, compared with enzymatic treatment, the antihypertensive capacity of PPH obtained by physical combined enzymatic treatment was not markedly enhanced. More work is warranted to further explore the antihypertensive efficacy of PPH prepared using physical combined enzymatic treatment.

## 2.3 | Modulating intestinal bacteria

The natural intestinal bacteria, intensively colonized ecological niches in the lower parts of the gastrointestinal tract, possesses protective, immunostimulatory metabolic, and detoxication functions (Ridlon, Kang, & Hylemon, 2006). The distribution of intestinal bacteria is not only related to the homeostasis of local intestinal environment, but also related

to human health status. On this topic, the studies mainly focused on the influences of pea protein and PPH produced by enzymatic hydrolysis on the intestinal bacteria ecosystem (Swiatecka et al., 2011, 2012; Swiatecka, Kostyra, & Swiatecki, 2010; Swiatecka, Malgorzata, Aleksander, Henryk, & Elzbieta, 2010; Swiatecka, Swiatecki, Kostyra, Marciniak-Darmochwal, & Kostyra, 2010).

### 2.3.1 | Chemical treatments for improving intestinal bacteria ecosystem

Glycation (called nonenzymatic glycosylation or Maillard reaction), a chemical reaction process, triggers carbohydrate condensation in protein molecules and thereby alters the structure of protein, leading to changes in biological characteristics. Swiatecka, Kostyra, et al. (2010) first studied the impact of glycated pea protein on the activity of free-swimming and immobilized bacteria. Compared with pea protein, glycated pea protein influenced the physiological activity of bacteria by stimulating the proliferation rate and metabolic activity of free-swimming and immobilized bacteria, and thus improved the bacterial intestinal ecosystem. In a follow-up study, Swiatecka, Malgorzata, et al. (2010) explored the effect of glycated pea protein on adhesion of the bacteria from the genera: *Lactobacillus*, *Enterococcus*, and *Escherichia*, which were typical for the human small intestine. These results demonstrated that both pea protein and glycated pea protein might act as modulators of both metabolic activity and adhesive potential of bacteria adhered to the intestinal cells, and modulate the adhesion of beneficial bacteria to the surface of enterocytes, thereby exerting a health-promoting effect on the local environment. Additionally, Swiatecka et al. (2011) further researched the impact of glycated pea protein on the intestinal bacteria from healthy humans. It was shown that glycated pea protein affected the growth of gut commensal bacteria, particularly *Lactobacilli* and *Bifidobacteria*, whose levels increased significantly compared to pea protein, which indicated that glycated pea protein might be a positive modulator of gut environment.

### 2.3.2 | Enzymatic treatments for preparing PPH

The study of Swiatecka, Swiatecki, et al. (2010) first illustrated the influence of PPH (from pepsin hydrolysis) on the physiological activity of bacteria commonly colonizing the human small intestine. They found that PPH could diversely modulate the physiological activity of bacteria existing in different states. And *Lactobacilli* displayed adaptive properties enabling them to utilize PPH regardless of their existing state. Furthermore, Swiatecka et al. (2012) stated the impact of PPH (from pepsin hydrolysis) on modulating the adhesion of bacteria to enterocytes, epithelial proliferation, and cytokine secretion. Obtained results shown that PPH stimulated adhesion of

*Lactobacillus/Enterococcus*, but did not influence the adhesion of *Escherichia coli*. Moreover, pea protein and PPH hindered the mitotic division of Caco-2 cells as well as triggered a significant higher secretion of Interleukin 8. PPH may therefore be considered as a potential modulator for bacterial adhesion and metabolic activity of enterocytes and thus modulated a human health status.

### 3 | FUNCTIONAL PROPERTIES OF PEA PROTEIN

The functional properties of pea protein discussed here refer to the technofunctionality, including solubility, WHC and OHC, emulsifying properties, foaming properties, and gelling properties. These properties will determine the behavior and performance of pea protein in food systems during preparation, processing, storage, and consumption, thereby affecting food texture, stability, and organoleptic characteristics.

#### 3.1 | Solubility

Solubility is a prerequisite to other functional properties of protein, and plays a vital role in food applications. High protein solubility helps in the production of beverages, infant milk powder, imitation milk, and other food products that require instant solubility without residue. However, low-solubility protein has very limited utilization potential in food production. Given to the summary of pea protein solubility shown in Table 3, pea genotype, protein extraction method and protein fraction, pH, and ionic strength all could influence pea protein solubility profile, because differences in these conditions lead to changes in protein conformation and surface properties and in turn affects solubility. Generally, PPI is prepared using alkaline extraction/isoelectric precipitation (AE/IP) and displays minimum solubility in water near pH 4.5 (isoelectric point). Its solubility significantly increases with pH shifting to either more acidic or alkaline conditions and performs a typical “U-shape” in pH-solubility profile (Ladjal-Ettoumi et al., 2016; Shand, Ya, Pietrasik, & Wanasundara, 2007; Wei et al., 2020; Withana-Gamage, Wanasundara, Pietrasik, & Shand, 2011; Zhao, Shen, Wu, Zhang, & Xu, 2020). Compared to native PPI, the lower solubility of commercial pea protein isolate (PPIc) may be resulted from denaturation and aggregation caused by HT during spray-drying processing condition (Shand et al., 2007; Stone, Karalash, Tyler, Warkentin, & Nickerson, 2015; Taherian et al., 2011). According to Shand et al. (2007) and Zhao et al. (2020), PPI generally had a lower solubility profile than soy protein isolate (SPI). For proteins obtained by AE/IP, their solubility (pH 7.0) was lowest at 61.4% for PPI, and was higher than 90% for faba, chickpea, and lentil protein isolates, and was highest at 96.5% for SPI (Karaca et al., 2011), whereas

other studies suggested that PPI had a similar solubility to chickpea and lentil proteins at pH ranging 2.0 to 8.0 (Ladjal-Ettoumi et al., 2016; Withana-Gamage et al., 2011). Although the same extraction method (e.g., AE/IP), PPI obtained from various genotypes or cultivars showed significantly different solubility profile (detailed results shown in Table 3), which attributed to the difference of storage protein content and composition (Barac et al., 2010; Lam et al., 2017; Shevkani, Singh, Kaur, & Rana, 2015; Stone, Karalash, et al., 2015; Stone, Avarmenko, Warkentin, & Nickerson, 2015). Moreover, pea protein can be obtained through different extraction methods, such as precipitation (acid precipitation and heat-acid precipitation, AE/IP, micellar precipitation), UF, and salt extraction (SE) (Boye et al., 2010; Fuhrmeister & Meuser, 2003; Karaca et al., 2011; Stone, Karalash, et al., 2015; Taherian et al., 2011). These results summarized in Table 3 showed that the extraction method influenced the solubility of pea protein in various degree, which was due to the selection of different protein types during extract processing. Some relevant literatures also reported that PPI was made up of different protein fractions, for instance, PPI was classified into vicilin (7S) and legumin (11S) based on their sedimentation coefficient (Kimura et al., 2008; Liang & Tang, 2013), and also was classified into water-soluble, salt-soluble, ethanol-soluble, and alkaline-soluble fractions based on their solubility in different solvents (Adebisi & Aluko, 2011). The different protein fractions had a significant effect on the solubility of PPI, and the detailed comparison results are described in Table 3.

#### 3.2 | WHC and OHC

WHC and OHC of protein are related to the texture, mouthfeel, and flavor retention of food products. For instance, the protein with high WHC is beneficial to reduce moisture loss in packed bakery goods and maintain freshness and moist mouthfeel of baked foods, whereas the protein with high OHC is good for improving mouthfeel and flavor retention of certain food products. Therefore, it is essential to fully understand the factors affecting WHC and OHC in terms of maintaining product quality and meeting consumer acceptability. At present, many researchers have reported WHC and OHC of pea protein, and the results in different conditions are shown in detail in Table 4. In the study of Withana-Gamage et al. (2011), PPI obtained by AE/IP method had WHC and OHC of 2.7 and 2.8 g/g, respectively, which were lower than SPI. Similar result was reported by Zhao et al. (2020): the WHC of commercial SPI (SPIc; 5.168 g/g) was almost 1.5 times PPIc (3.389 g/g), but they had a similar OHC around 1.2 g/g. According to the description in Table 4, it was concluded that different extraction methods could significantly affect WHC and OHC of PPI (Boye et al., 2010; Fuhrmeister & Meuser, 2003; Moreno et al., 2020; Stone, Karalash, et al., 2015).

**TABLE 3** The solubility of pea protein in different conditions

Extraction methods	Conditions	Major findings	References
AE/IP	<ul style="list-style-type: none"> <li>• PPI and PPIc, pH 3.0 to 10.0</li> <li>• PPI and others bean protein isolates, pH 2.0 to 8.0</li> <li>• PPIc and others commercial protein isolates, pH 2.0 to 12.0</li> <li>• PPIc, pH 2.0 to 11.0</li> </ul>	<ul style="list-style-type: none"> <li>✓ All PPI (including PPIc) had minimum solubility near pH 4.5</li> <li>✓ PPI had higher solubility than PPIc at all pH values</li> </ul>	Shand et al. (2007); Ladjal-Ettoumi et al. (2016); Wei et al. (2020); Withana-Gamage et al. (2011); Zhao et al. (2020)
	<ul style="list-style-type: none"> <li>• Six pea genotypes (Maja, Calvedon, Miracle of America, L1, L2, and L3), pH 3.0, 5.0, 7.0, and 8.0</li> <li>• Five pea cultivars (IC 394027, IC 342028, IC 291541, IC 381453, and IC 299013), pH 2.0 to 9.0</li> <li>• Seven pea cultivars (CDC Striker, CDC Golden, Cooper, CDC Dundurn, MFR042, CDC Meadow and Kaspa), pH 7.0</li> <li>• Three pea cultivars (CDC Striker, CDC Meadow, and CDC Dakota), pH 7.0</li> <li>• Six pea cultivars (Agassiz, CDC Golden, CDC Dakota, CDC Striker, CDC Tetris, and Cooper), pH 7.0</li> </ul>	<ul style="list-style-type: none"> <li>✓ All PPI (six genotypes) had significantly better solubility than PPIc at all pH values and showed high solubility at pH 7.0 and 8.0, L1 with the highest (approximately 85%) and L2 with the lowest (70%) solubility at pH 7.0, Maja with the highest solubility at pH 3.0</li> <li>✓ All PPI (five cultivars) had minimum solubility between pH 4.0 and 5.0, PPI solubility ranged between 65.7% and 77.2% (pH 2.0), 2.5% and 3.6% (pH 5.0), 64.2% and 79.9% (pH 7.0), and 69.7% and 95.2% (pH 9.0)</li> <li>✓ PPI (seven cultivars) solubility was significantly different, with 54% to 76% (pH 7.0)</li> <li>✓ All PPI (three cultivars) had similar solubility, with 62.7% to 64.4% (pH 7.0)</li> <li>✓ The mean solubility of PPI (six cultivars) ranged between 62.5% and 75.2% (pH 7.0)</li> </ul>	Barac et al. (2010); Lam et al. (2017); Shevkani, Singh, Kaur, et al. (2015); Stone, Avarmenko, et al. (2015); Stone, Karalash, et al. (2015)
AP and HP; UF	<ul style="list-style-type: none"> <li>• PPI-AP, PPI-HP, and PPI-UF, pH 2.0 to 10.0</li> </ul>	<ul style="list-style-type: none"> <li>✓ Extraction process impacted PPI solubility</li> <li>✓ Solubility (at all pH values): PPI-UF &gt; PPI-AP &gt; PPI-HP</li> </ul>	Fuhrmeister and Meuser (2003)
AE/IP; UF	<ul style="list-style-type: none"> <li>• PPC-AE/IP, and PPC-UF, pH 1.0 to 10.0</li> </ul>	<ul style="list-style-type: none"> <li>✓ Extraction process significantly affected PPC solubility at pH 1.0 and 3.0</li> <li>✓ Solubility: PPC-UF (60%) &lt; PPC-AE/IP (90%) at pH 1.0</li> <li>✓ Solubility: PPC-UF (56%) &gt; PPC-AE/IP (29%) at pH 3.0</li> </ul>	Boye et al. (2010)
AE/IP; SE	<ul style="list-style-type: none"> <li>• PPI-AE/IP and PPI-SE, pH 7.0</li> </ul>	<ul style="list-style-type: none"> <li>✓ Extraction process significantly affected PPI solubility</li> <li>✓ Solubility (at pH 7.0): PPI-AE/IP (61.42%) &gt; PPI-SE (38.12%)</li> </ul>	Karaca et al. (2011)
UF	<ul style="list-style-type: none"> <li>• PPIc and PPI-UF, pH 2.0 to 9.0</li> </ul>	<ul style="list-style-type: none"> <li>✓ PPI-UF had a higher solubility than PPIc at all pH values</li> </ul>	Taherian et al. (2011)
AE/IP; SE; MP	<ul style="list-style-type: none"> <li>• Three pea cultivars (CDC Striker, CDC Meadow, and CDC Dakota)</li> <li>• PPIc, PPI-AE/IP, PPI-SE, and PPI-MP, pH 7.0</li> </ul>	<ul style="list-style-type: none"> <li>✓ Extraction process significantly affected PPI solubility</li> <li>✓ Solubility: PPI-SE (85.7% to 91.1%) &gt; PPI-AE/IP (62.7% to 64.4%) &gt; PPI-MP (42.8% to 49.0%) &gt; PPIc (5.0%)</li> </ul>	Stone, Karalash, et al. (2015)
Refer to reference for details	<ul style="list-style-type: none"> <li>• 7S and 11S globulins</li> <li>• pH 2.2 to 9.5 and pH 3 to 9 at different ionic strengths (0.08 and 0.5)</li> </ul>	<ul style="list-style-type: none"> <li>✓ The solubility of 7S and 11S globulins from pea protein varied significantly at different ionic strengths</li> </ul>	Kimura et al. (2008)

(Continues)



TABLE 3 (Continued)

Extraction methods	Conditions	Major findings	References
	<ul style="list-style-type: none"> <li>• PPIc, WS, SS, AS, and ES fractions from PPIc, pH 3 to 8</li> </ul>	<ul style="list-style-type: none"> <li>✓ Protein fraction solubility was significantly different</li> <li>✓ All protein fractions (except ES fraction, which was insoluble in aqueous solution) had better solubility than PPIc at all pH values</li> <li>✓ WS fraction had the best solubility</li> </ul>	Adebiyi and Aluko (2011)
Refer to reference for details, PPI was obtained by AE/IP	<ul style="list-style-type: none"> <li>• PPI, PV, and PL, pH 2 to 10</li> </ul>	<ul style="list-style-type: none"> <li>✓ The solubility varied with the different fractions</li> <li>✓ Solubility (at pH &lt; 5.0): PL &gt; PV &gt; PPI</li> <li>✓ Solubility (at pH &gt; 5.0): PL ≈ PV &gt; PPI</li> <li>✓ PPI had the lowest solubility at all pH values</li> </ul>	Liang and Tang (2013)

Note. PPI-AP, PPI-HP, PPI-AE/IP, PPI-MP, PPI-UF, and PPI-SE are PPIs obtained from acid precipitation, heat-acid precipitation, alkaline extraction/isoelectric precipitation, micellar precipitation, ultrafiltration, and salt extraction, respectively. WS, SS, AS, and ES are water-soluble, salt-soluble, alkaline-soluble, and ethanol-soluble fractions obtained from PPIc, respectively. PV and PL are purified 7S (vicilin) and 11S (legumin) globulins, respectively. Abbreviations: AE/IP, alkaline extraction/isoelectric precipitation; AP, acid precipitation; HP, heat-acid precipitation; MP, micellar precipitation; UF, ultrafiltration; SE, salt extraction; PPI, pea protein isolate; PPIc, commercial PPI

However, there were no significant differences among cultivars for WHC and OHC of PPI (Lam et al., 2017; Shevkani, Singh, Kaur, et al., 2015; Stone, Avarmenko, et al., 2015; Stone, Karalash, et al., 2015). Therefore, based on the above researches, we could tailor WHC and OHC of PPI according to different extraction methods, thereby expanding the application of PPI in the food industry.

### 3.3 | Emulsifying properties

The emulsifying properties of protein play an important role in its applications as food ingredients. For example, protein with superior emulsifying properties could be used to prepare stable food products, including milk, cream, mayonnaise, ice cream, butter, and so on. At present, emulsifying capacity (EC), emulsifying ability (EA), emulsion stability (ES), emulsifying activity index (EAI), emulsifying stability index (ESI), and creaming stability (CS) are quality indexes commonly used to evaluate the emulsifying properties of protein. Besides, the EA of protein is also sometimes assessed by measuring the droplet size or droplet size distribution of emulsion after homogenization or during storage. For emulsifying properties of pea protein, we found that different researchers used various indexes and units to describe the emulsifying properties, which makes it difficult to compare these results. Therefore, the emulsifying properties of pea protein in different conditions are summarized in detail in Table 5. Overall, the emulsifying properties of pea protein are highly dependent on its extraction method. Fuhrmeister and Meuser (2003) and Taherian et al. (2011) reported that PPI obtained by UF had a higher EAI and ES than those by other methods. The study of Karaca et al. (2011) showed that PPI prepared by AE/IP exhibited a better EAI and ESI than those by SE. In

contrast, Boye et al. (2010) reported that the extraction process had little impact on the emulsifying properties, and PPC obtained by UF and AE/IP possessed similar EAI and ESI. According to Stone, Karalash, et al. (2015), salt-extracted PPI (PPI-SE) possessed a better EC than PPI-AE/IP, whereas PPI obtained by micellar precipitation (PPI-MP) did not exhibit the characteristic emulsifying behavior according to the EC test, and all PPIs had similar ES regardless of extraction methods. Even with the same extraction method (e.g., AE/IP), PPI extracted from different cultivars or genotypes had significantly different emulsifying characteristics (Barac et al., 2010; Lam et al., 2017; Shevkani, Singh, Kaur, et al., 2015; Stone, Avarmenko, et al., 2015; Stone, Karalash, et al., 2015; detailed comparison results are shown in Table 5). In general, PPIc had lower emulsifying properties than PPI obtained by any extraction methods (Stone, Karalash, et al., 2015; Taherian et al., 2011). Withana-Gamage et al. (2011) demonstrated that PPI had a lower EAI and ESI compared with SPI, but Zhao et al. (2020) found that EAI and ESI of PPIc were similar to SPIc. Additionally, different protein fractions also had significant influences on emulsifying properties of PPI (Kimura et al., 2008; Adebiyi & Aluko, 2011; Liang & Tang, 2013). Apart from the above factors, some environmental conditions such as ionic strength, pH, and protein concentration could affect the emulsifying properties of PPI to different degree as shown in Table 5 (Aluko, Mofolasayo, & Watts, 2009; Adebiyi & Aluko, 2011; Kimura et al., 2008; Liang & Tang, 2013; Ladjal-Ettoumi et al., 2016). PPI exhibited better emulsifying properties at pH deviating from isoelectric point (*pI*) than at pH around *pI*. At acidic conditions (pH 2.4 or 3.0), the adsorbed PPI could form more viscoelastic interfacial films at the interface, and the formed emulsions are more stable against creaming than that formed at pH 7.0 (Gharsallaoui, Cases, Chambin, & Saurel, 2009; Liang & Tang, 2014).



**TABLE 4** The water (WHC) and oil holding capacities (OHC) of pea protein in different conditions

Extraction methods	Conditions	Major findings	References
AP and HP; UF	• PPIc, PPI-AP, PPI-HP, and PPI-UF	<ul style="list-style-type: none"> <li>✓ Extraction process affected WHC and OHC of PPI</li> <li>✓ WHC: PPIc (4 g/g) &gt; PPI-AP (2.7 g/g) &gt; PPI-HP (2.2 g/g) &gt; PPI-UF (0 g/g)</li> <li>✓ OHC: PPIc (159%) &gt; PPI-UF (132%) &gt; PPI-AP (90%) &gt; PPI-HP (87%)</li> </ul>	Fuhrmeister and Meuser (2003)
AE/IP; UF	• PPC-AE/IP and PPC-UF	<ul style="list-style-type: none"> <li>✓ Extraction process affected WHC and OHC of PPC</li> <li>✓ WHC: PPC-AE/IP (4.5 mL/g) &gt; PPC-UF (3.9 mL/g)</li> <li>✓ OHC: PPC-UF (177%) &gt; PPC-AE/IP (120%)</li> </ul>	Boye et al. (2010)
AE/IP; SE; MP	<ul style="list-style-type: none"> <li>• Three pea cultivars (CDC Striker, CDC Meadow, and CDC Dakota)</li> <li>• PPI-AE/IP, PPI-SE, and PPI-MP</li> </ul>	<ul style="list-style-type: none"> <li>✓ WHC: PPI-MP (3.2 to 3.6 g/g) <math>\approx</math> PPIc (3.1 g/g) &gt; PPI-AE/IP (2.4 to 2.6 g/g) &gt; PPI-SE (0.34 to 2.6 g/g)</li> <li>✓ OHC: PPI-SE (5.2 to 5.4 g/g) &gt; PPI-AE/IP (3.5 to 3.8 g/g) <math>\approx</math> PPI-MP (3.6 to 3.7 g/g) &gt; PPIc (1.0 g/g)</li> </ul>	Stone, Karalash, et al. (2015)
AE/IP; WE	• PPIc-AE/IP and PPIc-WE	<ul style="list-style-type: none"> <li>✓ Extraction process significantly affected WHC and OHC of PPIc</li> <li>✓ WHC: PPIc-AE/IP (4.19 mL/g) &gt; PPIc-WE (2.30 mL/g)</li> <li>✓ OHC: PPIc-AE/IP (3.10 mL/g) &gt; PPIc-WE (2.85 mL/g)</li> </ul>	Moreno et al. (2020)
AE/IP	<ul style="list-style-type: none"> <li>• Five pea cultivars (IC 394027, IC 342028, IC 291541, IC 381453, and IC 299013)</li> <li>• Seven pea cultivars (CDC Striker, CDC Golden, Cooper, CDC Dundurn, MFR042, CDC Meadow, and Kasper), pH 7.0</li> <li>• Six pea cultivars (Agassiz, CDC Golden, CDC Dakota, CDC Striker, CDC Tetris, and Cooper), pH 7.0</li> <li>• PPI and others bean protein isolates</li> <li>• PPIc and others commercial protein</li> </ul>	<ul style="list-style-type: none"> <li>✓ (Five cultivars) WHC: 3.9 to 4.8 g/g; OHC: 5.5 to 7.2 g/g</li> <li>✓ (Seven cultivars) No significant differences among cultivars for WHC (1.88 to 2.37 g/g) and OHC (1.07 to 1.40 g/g)</li> <li>✓ (Six pea cultivars) No significant differences among cultivars for OHC (3.1 to 3.3 g/g) &gt; PPIc (1.5 g/g)</li> <li>✓ WHC: 2.7 g/g</li> <li>✓ OHC: 2.8 g/g</li> <li>✓ WHC: 3.389 g/g</li> <li>✓ OHC: 1.2 g/g</li> </ul>	Lam et al. (2017); Shevkani, Singh, Kaur, et al. (2015); Stone, Avarmenko, et al. (2015)  Withana-Gamage et al. (2011)  Zhao et al. (2020)

*Note.* PPI-AP, PPI-HP, PPI-AE/IP, PPI-MP, PPI-UF, PPI-SE, and PPI-WE are PPIs obtained from acid precipitation, heat–acid precipitation, alkaline extraction/isoelectric precipitation, micellar precipitation, ultrafiltration, salt extraction, and water extraction, respectively.

Abbreviations: AE/IP, alkaline extraction/isoelectric precipitation; AP, acid precipitation; HP, heat–acid precipitation; MP, micellar precipitation; UF, ultrafiltration; SE, salt extraction; WE, water extraction; PPI, pea protein isolate; PPIc, commercial PPI.

### 3.4 | Foaming properties

In food products such as ice cream, cake, bread, and meringue, foaming formation plays vital textural and structural roles. Foaming capacity (FC) and foaming stability (FS) are the most commonly used indicators to describe the foaming

properties of protein. In terms of pea protein, its foaming properties are affected by factors similar to those that affect its emulsifying properties, including extraction method, cultivar, fraction, pH, protein concentration, and ionic content. The foaming properties of PPI in different conditions are

**TABLE 5** The emulsifying properties of pea protein in different conditions

Extraction methods	Conditions	Major findings	References
AP and HP; UF	<ul style="list-style-type: none"> <li>• PPIc, PPI-AP, PPI-HP, and PPI-UF</li> <li>• pH 5.0 and 7.0</li> </ul>	<ul style="list-style-type: none"> <li>✓ EAI: pH 5.0, PPI-UF (13.5 m<sup>2</sup>/g) &gt; PPIc (7.8 m<sup>2</sup>/g) &gt; PPI-HP (2.7 m<sup>2</sup>/g) &gt; PPI-AP (2.0 m<sup>2</sup>/g)</li> <li>✓ EAI: pH 7.0, PPI-UF (27.4 m<sup>2</sup>/g) &gt; PPI-HP (14 m<sup>2</sup>/g) &gt; PPIc (12.1 m<sup>2</sup>/g) &gt; PPI-AP (10.1 m<sup>2</sup>/g)</li> <li>✓ ES: pH 5.0, PPI-UF (80%) &gt; PPIc (59.7%) &gt; PPI-HP (56.9%) &gt; PPI-AP (55.3%)</li> <li>✓ ES: pH 7.0, PPI-UF (68.4%) &gt; PPI-HP (60.3%) &gt; PPIc (55.5%) &gt; PPI-AP (53.7%)</li> </ul>	Fuhrmeister and Meuser (2003)
UF	<ul style="list-style-type: none"> <li>• PPIc and PPI-UF</li> <li>• pH 3.4 and 6.8</li> </ul>	<ul style="list-style-type: none"> <li>✓ The pH influenced emulsifying properties</li> <li>✓ ES: PPIc and PPI-UF had better ES at pH 6.8 than pH 3.4</li> <li>✓ ES: PPI-UF had better ES than PPIc at both pH values</li> </ul>	Taherian et al. (2011)
AE/IP; SE	<ul style="list-style-type: none"> <li>• PPI-AE/IP and PPI-SE</li> <li>• pH 7.0</li> </ul>	<ul style="list-style-type: none"> <li>✓ EAI: PPI-AE/IP (42.87 m<sup>2</sup>/g) &gt; PPI-SE (42.73 m<sup>2</sup>/g)</li> <li>✓ ESI: PPI-AE/IP (12.40 min) &gt; PPI-SE (10.89 min)</li> <li>✓ CS: PPI-AE/IP &gt; PPI-SE</li> <li>✓ Droplet size (Ds, μm), PPI-AE/IP (1.85 μm) &lt; PPI-SE (42.73 μm)</li> </ul>	Karaca et al. (2011)
AE/IP; UF	<ul style="list-style-type: none"> <li>• PPC-AE/IP and PPC-UF</li> <li>• pH 7.0</li> </ul>	<ul style="list-style-type: none"> <li>✓ Extraction process had little impact on emulsifying properties</li> <li>✓ EAI: PPC-AE/IP (4.8 m<sup>2</sup>/g) &gt; PPC-UF (4.6 m<sup>2</sup>/g)</li> <li>✓ ESI: PPC-AE/IP (18.5 min) &gt; PPC-UF (18 min)</li> </ul>	Boye et al. (2010)
AE/IP; SE; MP	<ul style="list-style-type: none"> <li>• Three pea cultivars (CDC Striker, CDC Meadow, and CDC Dakota)</li> <li>• PPI-AE/IP, PPI-SE, and PPI-MP, pH 7.0</li> </ul>	<ul style="list-style-type: none"> <li>✓ EC: PPI-SE (193.7-243.7%) &gt; PPI-AE/IP (187.5-193.7%) &gt; PPIc (177.1%)</li> <li>✓ ES: PPI-AE/IP (96.7-99.9%) ≈ PPI-SE (97.0-99.6%) ≈ PPI-MP (99.5-99.7%) &gt; PPIc (80.7%)</li> </ul>	Stone, Karalash, et al. (2015)
AE/IP	<ul style="list-style-type: none"> <li>• Six pea genotypes (Maja, Calvedon, Miracle of America, L1, L2, and L3), pH values (3.0, 5.0, 7.0, and 8.0)</li> <li>• Seven pea cultivars (CDC Striker, CDC Golden, Cooper, CDC Dundurn, MFR042, CDC Meadow, and Kaspá), pH 7.0</li> <li>• Three pea cultivars (CDC Striker, CDC Meadow, and CDC Dakota), pH 7.0</li> <li>• Five pea cultivars (IC 394027, IC 342028, IC 291541, IC 381453, and IC 299013), pH 7.0</li> </ul>	<ul style="list-style-type: none"> <li>✓ (Six genotypes) genotype and pH significantly influenced emulsifying properties, EAI: pH 8.0 with highest values (93.92-291.94 m<sup>2</sup>/g) and pH 5.0 with lowest values (9.27-31.63 m<sup>2</sup>/g); ESI: Maja with highest values at all pH values (except pH5.0) and L2 with lowest values at all pH values (except pH5.0)</li> <li>✓ (Seven cultivars) cultivar influenced emulsifying properties, EAI: Cooper (32.57 m<sup>2</sup>/g) and CDC Dundurn (31.09 m<sup>2</sup>/g) had lower values than other cultivars (36.14-39.05 m<sup>2</sup>/g); ESI: all cultivars had similar values (10.97-11.26 min)</li> </ul>	Barac et al. (2010); Lam et al. (2017); Shevkani, Singh, Kaur, et al. (2015); Stone, Avarmenko, et al. (2015); Stone, Karalash, et al. (2015)

(Continues)

TABLE 5 (Continued)

Extraction methods	Conditions	Major findings	References
	<ul style="list-style-type: none"> <li>Six pea cultivars (Agassiz, CDC Golden, CDC Dakota, CDC Striker, CDC Tetris, and Cooper), pH 7.0</li> </ul>	<ul style="list-style-type: none"> <li>(Three cultivars) EC: CDC Meadow (193.7%) &gt; CDC Striker (187.5%) <math>\approx</math> CDC Dakota (187.5%); ES: all cultivars had similar values (96.7-99.9%)</li> <li>(Five cultivars) cultivar influenced emulsifying properties, EAI: 11.8-14.1 m<sup>2</sup>/g; ESI: 52.6-95.4 min</li> <li>(Six pea cultivars) ES: all cultivars had similar values (95.1-96.1%) &gt; PPIc (79.3%)</li> </ul>	
Refer to reference for details	<ul style="list-style-type: none"> <li>7S and 11S globulins</li> <li>Ionic strengths: 0.5 and 0.08</li> <li>PPIc, WS, SS, AS, and ES fractions from PPIc</li> <li>pH 4.0, 7.0, and 9.0</li> </ul>	<ul style="list-style-type: none"> <li>EA: droplet size (D<sub>s</sub>, <math>\mu</math>m), D<sub>s</sub> (11S) &lt; D<sub>s</sub> (7S) at same ionic strength</li> <li>EA: D<sub>s</sub> (0.5) &lt; D<sub>s</sub> (0.08) at same globulin</li> <li>Both fraction and pH influenced emulsifying properties</li> <li>EA: PPIc and AS fraction had better EA at pH 7.0 and 9.0 than 4.0</li> <li>EA: ES fraction with highest EA at all pH values</li> <li>ES: WS, ES and SS fractions had better ES at pH 4.0, whereas PPIc and AS fraction had better ES at pH 7.0 and 9.0</li> </ul>	<p>Kimura et al. (2008)</p> <p>Adebiyi and Aluko (2011)</p>
Refer to reference for details, PPI was obtained by AE/IP	<ul style="list-style-type: none"> <li>PPI, PV, and PL</li> <li>pH 3.0, 5.0, 7.0, and 9.0</li> </ul>	<ul style="list-style-type: none"> <li>Both fraction and pH influenced emulsifying properties</li> <li>EA: PL had better EA than PV or PPI at pH 3.0; PV and PPI had better EA than PL at pH 7.0 and 9.0</li> <li>All proteins had least EA at pH 5.0, and had better EA at pH 3.0 than 7.0 or 9.0</li> <li>CS: PL and PV had better CS than PPI at pH 3.0, 7.0, and 9.0</li> </ul>	Liang and Tang (2013)
AE/IP	<ul style="list-style-type: none"> <li>PPI (10, 25, and 50 mg/mL)</li> <li>pH 3.0, 5.0, and 7.0</li> <li>PPI, pH 3.0, 4.5, 7.0, and 8.0</li> <li>PPI and others bean protein isolates</li> <li>PPIc and others commercial protein, pH 7.0</li> </ul>	<ul style="list-style-type: none"> <li>EA: droplet size (D<sub>s</sub>, <math>\mu</math>m), no significant concentration impact on EA of PPI at pH 5.0 and pH 7.0, but D<sub>s</sub> was reduced with increasing concentration at pH 3.0</li> <li>ES: no significant concentration impact on ES of PPI at pH 5.0 and pH 7.0, but ES was improved with increasing concentration at pH 3.0</li> <li>The pH influenced emulsifying properties</li> <li>EA: the minimum EA at pH 4.5</li> <li>ES: the minimum ES at pH 4.5</li> <li>EAI: 0.9 m<sup>2</sup>/g</li> <li>ESI: 17 min</li> <li>EAI: 118 m<sup>2</sup>/g</li> <li>ESI: 14 min</li> </ul>	<p>Aluko et al. (2009)</p> <p>Ladjal-Ettoumi et al. (2016)</p> <p>Withana-Gamage et al. (2011)</p> <p>Zhao et al. (2020)</p>

Note. PPI-AP, PPI-HP, PPI-AE/IP, PPI-MP, PPI-UF, and PPI-SE are PPIs obtained from acid precipitation, heat-acid precipitation, alkaline extraction/isoelectric precipitation, micellar precipitation, ultrafiltration, and salt extraction, respectively. WS, SS, AS, and ES are water-soluble, salt-soluble, alkaline-soluble, and ethanol-soluble fractions obtained from PPIc, respectively. PV and PL are purified 7S (vicilin) and 11S (legumin) globulins, respectively.

Abbreviations: AE/IP, alkaline extraction/isoelectric precipitation; AP, acid precipitation; HP, heat-acid precipitation; MP, micellar precipitation; UF, ultrafiltration; SE, salt extraction; PPI, pea protein isolate; PPIc, commercial PPI; EC, emulsifying capacity; EA, emulsifying ability; ES, emulsion stability; EAI, emulsifying activity index; ESI, emulsifying stability index; CS, creaming stability.

**TABLE 6** The foaming properties of pea protein in different conditions

Extraction methods	Conditions	Major findings	References
AP and HP; UF	<ul style="list-style-type: none"> <li>• PPIc, PPI-AP, PPI-HP, and PPI-UF</li> <li>• pH 5.0 and 7.0</li> </ul>	<ul style="list-style-type: none"> <li>✓ FC: pH 5.0, PPI-UF (559%) = PPIc (559%) &gt; PPI-AP (446%) &gt; PPI-HP (432%)</li> <li>✓ FC: pH 7.0, PPI-HP (385%) &gt; PPI-UF (377%) &gt; PPI-AP (351%) = PPIc (351%)</li> <li>✓ FS: pH 5.0, PPI-UF (100%) = PPIc (100%) &gt; PPI-AP (63%) &gt; PPI-HP (53%)</li> <li>✓ FS: pH 7.0, PPIc (100%) &gt; PPI-UF (82%) &gt; PPI-HP (69%) &gt; PPI-AP (51%)</li> </ul>	Fuhrmeister and Meuser (2003)
AE/IP; UF	<ul style="list-style-type: none"> <li>• PPC-AE/IP and PPC-UF</li> <li>• pH 7.0</li> </ul>	<ul style="list-style-type: none"> <li>✓ Extraction method had no significant effect on foaming properties</li> <li>✓ FC: PPC-AE/IP (102%) &gt; PPC-UF (99.5%)</li> <li>✓ FS: PPC-AE/IP (42%) &gt; PPC-UF (40%)</li> </ul>	Boye et al. (2010)
AE/IP; SE; MP	<ul style="list-style-type: none"> <li>• Three pea cultivars (CDC Striker, CDC Meadow, and CDC Dakota)</li> <li>• PPI-AE/IP, PPI-SE, and PPI-MP, pH 7.0</li> </ul>	<ul style="list-style-type: none"> <li>✓ Extraction method influenced foaming properties</li> <li>✓ FC: PPI-SE (163.3% to 258.3%) had better FC than PPI-AE/IP (155.0% to 183.3%) and PPI-MP (133.3% to 193.3%)</li> <li>✓ FS: PPI-AE/IP (68.0% to 69.6%) had better FS than PPI-MP (52.8% to 77.8%) and PPI-SE (48.9% to 69.6%)</li> </ul>	Stone, Karalash, et al. (2015)
UF	<ul style="list-style-type: none"> <li>• PPIc and PPI-UF, pH 2.0, 3.5, 5.0, 7.0 and 9.0</li> <li>• NaCl concentration: 0.25%, 0.5%, and 1%</li> <li>• Protein concentration: 2%, 6%, and 10% (w/v)</li> </ul>	<ul style="list-style-type: none"> <li>✓ pH, NaCl and protein concentration all influenced foaming properties</li> <li>✓ FS: PPIc and PPI-UF had better FS at pH 2.0, 3.5, 7.0, and 9.0 than 5.0</li> <li>✓ FS: PPI-UF had better FS than PPIc at all pH values</li> <li>✓ FS: Addition of 0.25% NaCl improved FS of PPIc and PPI-UF</li> <li>✓ FS: Increased protein concentration improved FS of PPI-UF</li> </ul>	Taherian et al. (2011)
AE/IP	<ul style="list-style-type: none"> <li>• Six pea genotypes (Maja, Calvedon, Miracle of America, L1, L2, and L3), pH 3.0, 5.0, 7.0, and 8.0</li> <li>• Three pea cultivars (CDC Striker, CDC Meadow, and CDC Dakota), pH 7.0</li> <li>• Five pea cultivars (IC 394027, IC 342028, IC 291541, IC 381453, and IC 299013), pH 7.0</li> <li>• Six pea cultivars (Agassiz, CDC Golden, CDC Dakota, CDC Striker, CDC Tetris, and Cooper), pH 7.0</li> </ul>	<ul style="list-style-type: none"> <li>✓ (Six genotypes) genotype and pH significantly affected foaming properties, FC: Maja with highest values (293.93% to 439.39%) and pH 5.0 with lowest value for all genotypes; FS: L1 at pH 8.0 with highest value (127.30%)</li> <li>✓ (Three pea cultivars) FC: CDC Striker (183.3%) &gt; CDC Meadow (163.3%) &gt; CDC Dakota (155.0%); FS: the similar FS (68.0% to 69.6%)</li> <li>✓ (Five pea cultivars) FC: 87% to 132%; FS: the similar FS (94% to 96%)</li> <li>✓ (Six pea cultivars) FC: (167.4% to 243.7%) &gt; PPIc (165.6%); FS: the similar FS (73.5% to 75.2%) &gt; PPIc (56.6%)</li> </ul>	Barac et al. (2010); Lam et al. (2017); Stone, Karalash, et al. (2015)

(Continues)

TABLE 6 (Continued)

Extraction methods	Conditions	Major findings	References
Refer to reference for details	<ul style="list-style-type: none"> <li>• PPIc, WS, SS, AS, and ES fractions from PPIc</li> <li>• pH 4.0, 7.0, and 9.0</li> </ul>	<ul style="list-style-type: none"> <li>✓ pH greatly influenced foaming properties</li> <li>✓ FC: WS fraction had significantly higher FC than other fractions at pH 4.0 and 7.0 (except pH 9.0)</li> <li>✓ FS: FS of PPIc and SS fraction increased from pH 4.0 to 9.0</li> </ul>	Adebiyi and Aluko (2011)
AE/IP	<ul style="list-style-type: none"> <li>• PPI (10, 25, 50, and 100 mg/mL)</li> <li>• pH: 3.0, 5.0, and 7.0</li> </ul>	<ul style="list-style-type: none"> <li>✓ FC: Concentration significantly affected FC of PPI, FC at 100 mg/mL was significantly reduced for all pH values</li> <li>✓ FS: FS of PPI was highly dependent on concentration and pH</li> </ul>	Aluko et al. (2009)
	<ul style="list-style-type: none"> <li>• PPIc and others commercial protein, pH 7.0</li> </ul>	<ul style="list-style-type: none"> <li>✓ FC: 20 mL of foam volume</li> <li>✓ FS: 89.74%</li> </ul>	Zhao et al. (2020)

Note. PPI-AP, PPI-HP, PPI-AE/IP, PPI-MP, PPI-UF, and PPI-SE are PPIs obtained from acid precipitation, heat–acid precipitation, alkaline extraction/isoelectric precipitation, micellar precipitation, ultrafiltration, and salt extraction, respectively. WS, SS, AS, and ES are water-soluble, salt-soluble, alkaline-soluble, and ethanol-soluble fractions obtained from PPIc, respectively. AE/IP, alkaline extraction/isoelectric precipitation; AP, acid precipitation; HP, heat–acid precipitation; MP, micellar precipitation; UF, ultrafiltration; SE, salt extraction; PPI, pea protein isolate; PPIc, commercial PPI; FC, foaming capacity; FS, foaming stability.

presented in Table 6. As is shown in Table 6, the studies of Fuhrmeister and Meuser (2003), Stone, Karalash, et al. (2015) and Taherian et al. (2011) all shown that foaming properties of PPI were significantly affected by extraction method (detailed comparison listed in Table 6). However, Boye et al. (2010) reported the different result, and demonstrated that extraction method had no significant effect on the foaming properties of PPC, although PPC prepared by AE/IP had better foaming properties than those by UF. Many studies also explored the foaming properties of PPI from different cultivars or genotypes (Barac et al., 2010; Lam et al., 2017; Stone, Karalash, et al., 2015). Based on the data shown in Table 6, overall, it indicated that cultivars or genotypes had a significant influence on FC of PPI, but no significant effect on its FS. Pea protein could be divided into different protein fractions. Adebiyi and Aluko (2011) illustrated that water-soluble fraction exhibited significantly higher FC at pH 4.0 and 7.0 but not at pH 9.0 compared to PPIc, ethanol-soluble, alkaline-soluble, and salt-soluble fractions. According to Zhao et al. (2020), the FC of PPIc was similar to SPIc, but its FS was higher than SPIc. In addition, some other factors such as pH, protein concentration, and NaCl content also affected the foaming properties of PPI. Among them, pH significantly affected the foaming properties of PPI, and PPI displayed the lowest foaming properties near its *pI* (Adebiyi & Aluko, 2011; Aluko et al., 2009; Barac et al., 2010; Fuhrmeister & Meuser, 2003). However, only under certain conditions might protein concentration and NaCl content significantly influence the foaming properties of PPI. As shown in Table 6, the increased protein concentration and NaCl content had significant effects on the foaming properties of PPI at low protein concentra-

tion and NaCl content (Aluko et al., 2009; Taherian et al., 2011).

### 3.5 | Gelling properties

Gelation is a pretty important property of protein, and it plays a significant role in sensory and textural properties of many different food products. It is reported that gelling formation by globular protein was a complex process, usually involving several steps including denaturation, aggregation, and network formation (Mession, Chihi, Sok, & Saurel, 2015). Generally, protein gel can be divided into heat-induced and cold-set gelation: (a) Heating protein above its denaturation temperature (the protein has higher concentration than its least gelation concentration [LGC]) leads to the protein partial unfolding and exposes its interaction sites, giving rise to intermolecular interactions, eventually resulting in clustering of protein aggregates to form a spatial gel network—the formed gel is called heat-induced gelation; (b) Cold-set gel requires certain preheating treatment for protein, namely, low-concentrated protein suspension at pH far from its *pI* and in the absence of salt ions it is heated to prepare soluble protein aggregates, and then cooling it, the cold-set gelation is carried out by adding salts, acidifying agents, or enzymes, allowing it to assemble into network structure (Mession et al., 2015). For pea protein gelation, heat-induced gels are the main ones, whereas only a few cold-set gels have been reported (details are shown in Table 7). To better visualize the gelling properties of pea protein, rheological characterizations (including the LGC, gelation temperature [T<sub>gel</sub>], storage modulus [*G'*], loss modulus [*G''*], gel reinforcement [Gre], and loss tangent value [tan  $\delta$ ]



**TABLE 7** The gelling properties of pea protein in different conditions

Extraction methods	Conditions	Major findings	References
AE/IP	<ul style="list-style-type: none"> <li>• PPI, 2% to 20% (w/v)</li> <li>• PPI, 12% (w/v), pH 7.0</li> <li>• PPIC and others commercial protein, pH 7.0</li> <li>• PPIc (19.6%, w/w)</li> <li>• Heating temperature (79 to 95 °C)</li> <li>• NaCl (0% to 2.0%, w/w), pH 6.1 to 8.1</li> <li>• Five pea cultivars (Solara, Supra, Classic, Finale, and Espace), 10% to 20% (w/v), pH 7.6; 18% (w/v), pH 7.6, heating and cooling rates: 1.0/1.0, 0.5/1.0, or 1.0/0.2 °C/min</li> <li>• Five pea cultivars (IC 394027, IC 342028, IC 291541, IC 381453. and IC 299013), 15% (w/v)</li> </ul>	<ul style="list-style-type: none"> <li>✓ LGC: 14% (w/v)</li> <li>✓ Tgel: 81.8 °C</li> <li>✓ G' (354.3 Pa) and Tan δ (0.223)</li> <li>✓ LGC: 14% (w/v)</li> <li>✓ The optimal gel conditions for PPIc: 19.6% (w/w), heating temperature (93 °C), NaCl (2.0%, w/w), pH 7.1</li> <li>✓ (Five pea cultivars) LGC: 16% (w/v) for all PPI; heating rate did not impact gelling properties, slower cooling rate increased gel strength; Solara with the highest 11S content had stronger gel</li> <li>✓ (Five pea cultivars) Tgel: 84.0 to 93.1 °C; Gre: 107 to 277 Pa; Tan δ: 0.17 to 0.23</li> </ul>	<p>Withana-Gamage et al. (2011)</p> <p>Zhao et al. (2020)</p> <p>Shand et al. (2007)</p> <p>O'Kane et al. (2005); Shevkani, Singh, Kaur, et al. (2015)</p>
AE/IP; UF	<ul style="list-style-type: none"> <li>• PPC-AE/IP and PPC-UF</li> <li>• 2% to 20% (w/v), pH 7.0</li> </ul>	<ul style="list-style-type: none"> <li>✓ LGC: PPC-UF (12%) &lt; PPC-AE/IP (14%)</li> </ul>	Boye et al. (2010)
AE/IP; WE	<ul style="list-style-type: none"> <li>• PPIc-AE/IP and PPIc-WE</li> </ul>	<ul style="list-style-type: none"> <li>✓ LGC: PPIc-WE (17%) = PPIc-AE/IP (17%)</li> </ul>	Moreno et al. (2020)
SE	<ul style="list-style-type: none"> <li>• At the same conditions (0.3 M NaCl and pH 5.65): PPI-SE (4% to 18%, w/v) and PPIc (8% to 20%, w/v)</li> <li>• PPI-SE (14.5%, w/v), heating and cooling rates (0.5, 1, 2, and 4 °C/min)</li> <li>• PPI-SE and PPIc, 14.5% (w/v), 2 °C/min heating and cooling rates</li> </ul>	<ul style="list-style-type: none"> <li>✓ LGC: PPI-SE (5.5%) &lt; PPIc (14.5%)</li> <li>✓ Tgel: Tgel (83.2 to 85.6 °C) of PPI-SE was concentration independent, but heating and cooling rates significantly impacted Tgel (61.1 to 85.0 °C) of PPI-SE</li> <li>✓ G' and G'' increased with increasing PPI-SE concentration, whereas Tan δ decreased; and final G' and G'' decreased with higher heating and cooling rates</li> <li>✓ Tgel: PPI-SE (85.1 °C) &lt; PPIc (87.85 °C), G': PPI-SE (3212.5 Pa) &gt; PPIc (349.5 Pa), G'': PPI-SE (532.6 Pa) &gt; PPIc (253.5 Pa), Tan δ: PPI-SE (0.17) &lt; PPIc (0.73)</li> </ul>	Sun and Arntfield (2010)
SE	<ul style="list-style-type: none"> <li>• PPI-SE, 14.5% (w/v), 0.3 M NaCl, pH (between 5.65 and 5.70), heating and cooling rates (0.5, 1, 2, and 4 °C/min)</li> <li>• PPIc and PPI-SE, 10.5% (w/v), 0.3 M NaCl, heating and cooling rates at 2 °C/min</li> </ul>	<ul style="list-style-type: none"> <li>✓ Tgel: Tgel of PPI-SE was heating rate dependent, but cooling rate independent</li> <li>✓ G': Cooling rate had greater impact on final G' than heating rate, and final G' increased with decreasing cooling rate</li> <li>✓ G': PPI-SE (291.6 Pa) &gt; PPIc (1.97 Pa), G'': PPI-SE (48.8 Pa) &gt; PPIc (2.55 Pa)</li> <li>✓ Tan δ: PPI-SE (0.167) &lt; PPIc (1.751)</li> </ul>	Sun and Arntfield (2011a)

(Continues)

TABLE 7 (Continued)

Extraction methods	Conditions	Major findings	References
UF	<ul style="list-style-type: none"> <li>• PPIc and PPI-UF, 20 % (w/v), pH 6.5</li> </ul>	<ul style="list-style-type: none"> <li>✓ Tgel: PPI-UF (75.7 to 85.6 °C) &lt; PPIc (above 90°C)</li> </ul>	Taherian et al. (2011)
Refer to reference for details	<ul style="list-style-type: none"> <li>• PPIc, WS, SS, AS, and ES fractions from PPIc</li> <li>• 1% to 20% (w/v)</li> </ul>	<ul style="list-style-type: none"> <li>✓ LGC: AS fraction (10%) &lt; PPIc (20%)</li> <li>✓ WS and SS fractions were unable to form firm gels</li> <li>✓ ES fraction had no LGC due to complete insolubility in water</li> </ul>	Adebiyi and Aluko (2011)
	<ul style="list-style-type: none"> <li>✓ PPI (4% w/v), 7S (4% w/v), and 11S (3.5% w/v) fractions</li> <li>✓ GDL-induced cold-set gel</li> </ul>	<ul style="list-style-type: none"> <li>✓ Final <math>G'</math>: 7S (619 Pa) &gt; PPI (567 Pa) &gt; 11S (200 Pa)</li> <li>✓ Tan <math>\delta</math>: 7S (0.23) &gt; PPI (0.21) &gt; 11S (0.2)</li> </ul>	Mession et al. (2015)
SE	<ul style="list-style-type: none"> <li>• PPI-SE, 14.5% (w/v), pH (3.0 to 11.0), and NaCl (0 to 2.0 M)</li> <li>• NaCl (0 to 2.0 M), pH 5.65</li> </ul>	<ul style="list-style-type: none"> <li>✓ Both pH and NaCl significantly impacted PPI-SE gel properties</li> <li>✓ The strongest gel stiffness (<math>G'</math>: 4516 Pa, <math>G''</math>: 757 Pa, Tan <math>\delta</math>: 0.17) was obtained at 0.3 M NaCl and pH 5.65</li> <li>✓ Both pH and NaCl significantly affected Tgel, the highest Tgel (89.1 °C) was obtained at pH approximately 6.0, and Tgel (60.15 to 93.65 °C) increased with increasing salt concentration at pH 5.65</li> </ul>	Sun and Arntfield, (2011b)
	<ul style="list-style-type: none"> <li>• PPI-SE, 15% (w/v), CaCl<sub>2</sub> (0.01 and 0.1 M) and NaCl (0.3 M), pH (3.0, 5.0, 7.0, and 9.0)</li> <li>• PPI-SE, 10% to 15% (w/v) at pH 3.0, pH (3.0 to 4.2) at 10% (w/v)</li> </ul>	<ul style="list-style-type: none"> <li>✓ Mechanical deformation properties of PPI-SE gel were pH and salt conditions dependent</li> <li>✓ The better PPI-SE gel was obtained at pH away from its <math>pI</math> and at low ionic strength</li> <li>✓ Fracture stress: the value increased with increasing protein concentrations, while decreased with increasing pH values</li> <li>✓ Fracture strain: the similar values at different protein concentrations, the biggest value at pH 4.2</li> <li>✓ Young's modulus: the value increased with increasing protein concentrations, the biggest value at pH 3.4</li> <li>✓ Recoverable energy: the biggest value at 13% (w/v), the similar values at pH 3.0 to 3.8 and the lowest value at 4.2</li> </ul>	Munialo et al. (2014); Munialo et al. (2015)

*Note.* PPI; PPI-AE/IP, PPI-UF, PPI-SE, and PPI-WE are PPIs obtained from alkaline extraction/isoelectric precipitation, ultrafiltration, salt extraction, and water extraction, respectively. WS, SS, AS, and ES are water-soluble, salt-soluble, alkaline-soluble, and ethanol-soluble fractions obtained from PPIc, respectively. Mechanical deformation properties include Young's moduli, elastically stored energy, fracture stress, and fracture strain.

Abbreviations: AE/IP, alkaline extraction/isoelectric precipitation; UF, ultrafiltration; SE, salt extraction; WE, water extraction; PPI, pea protein isolate; PPIc, commercial; 7S, vicilin globulin; 11S, legumin globulin; LGC, the least gelation concentration; Tgel, gelation temperature; Gre, gel reinforcement.

=  $G''/G'$ ) and mechanical deformation properties (including elastically stored [recoverable] energy, Young's moduli [gel stiffness], fracture stress [gel strength], and fracture strain [gel brittleness]) are widely used to evaluate viscoelastic and textural properties of protein gel. Normally, high  $G'$  and Gre values

indicate stronger intermolecular network and increased interactions between proteins, whereas low LGC, Tgel, and tan  $\delta$  values demonstrate better gelling capacity and more elastic network (Sun & Arntfield, 2011a). Similarly, the higher values in mechanical deformation properties usually suggest

that the protein gel possesses the better textural properties (Munialo, van der Linden, & de Jongh, 2014).

It can be seen from Table 7, the cultivar, protein fraction, extraction method, and some environmental conditions including pH, protein concentration, ionic strength, heating temperature, and heating and cooling rates all can affect gel network formation of pea protein. In general, PPI was usually obtained by AE/IP method, and its LGC was 14% to 17% (w/v), forming a weak gel (Boye et al., 2010; Moreno et al., 2020; O’Kane, Vereijken, Gruppen, & van Boekel, 2005; Withana-Gamage et al., 2011; Zhao et al., 2020). Some studies have reported that PPI obtained by different extraction methods (e.g., UF and SE methods) exhibits significantly different gelling properties, including LGC, Tgel,  $G'$ ,  $G''$ , and  $\tan \delta$  (Boye et al., 2010; Sun & Arntfield, 2010; Taherian et al., 2011; Sun & Arntfield, 2011a; detailed results are shown in Table 7). However, Moreno et al. (2020) indicated that the two PPIc obtained by AE/IP and water extraction had the same LGC. According to O’Kane et al. (2005) and Shevkani, Singh, Kaur, et al. (2015), even though PPI was obtained by the same extraction method, its gel and rheological properties from different pea cultivars/lines had some variation due to their differences in physicochemical and structural composition. Pea protein is a mixed protein, which can be divided into different fractions according to different classification methods, such as vicilin (7S) and legumin (11S) fractions or water-soluble, salt-soluble, ethanol-soluble, and alkaline-soluble fractions. Some researchers have suggested that different protein fractions have significantly different gelling performance (Adebiyi & Aluko, 2011; Messin et al., 2015), for example, LGC of the alkaline-soluble fraction was 10% (w/v), but the ethanol-soluble fraction had no LGC (Adebiyi & Aluko, 2011). Compared to PPI, PPIc usually exhibited poor gelling properties, such as higher LGC (about 20%, w/v), Tgel, and  $\tan \delta$  and lower  $G'$  and  $G''$ , because it was extensively denatured during its large-scale production (Shand et al., 2007; Adebiyi & Aluko, 2011; Sun & Arntfield, 2011a; Taherian et al., 2011). However, the study of Sun and Arntfield (2010) showed that the LGC of PPIc was 14.5%, much lower than other reports. It might be that NaCl favored gel formation through enhancing intermolecular hydrophobic interactions, and reducing electrostatic repulsion and altering water structure around PPIc, thereby enhancing the hydration of protein molecules and reducing the LGC. Zhao et al. (2020) also reported the similar result, for example, the LGC of PPIc was 14% (w/v). Additionally, several environmental factors, such as pH, protein concentration, ionic strength, heating temperature, and heating and cooling rates, have been reported to affect the gelling properties of PPI. Based on the summary in Table 7, it is found that pH, ionic strength, and cooling rate had significant effects on the gelling properties of PPI (Munialo et al., 2014, 2015; O’Kane et al., 2005; Sun et al., 2010, 2011a, 2011b).

## 4 | MODIFICATION METHODS TO IMPROVE THE FUNCTIONALITY OF PEA PROTEIN

As described above, the application of pea protein in food systems is limited by its low water solubility (e.g., low acidic solubility) and some poor functional performance. To overcome these drawbacks, the following modification methods could be performed to improve its functionality: (a) chemical modification, (b) physical modification, (c) enzymatic modification, and (d) combined modification, including chemical combined physical modification and physical combined enzymatic modification. In this section, the specific methods, materials, characterization techniques, and major findings of pea protein modification are presented in Table 8. Furthermore, Table 9 summarizes the main advantages and disadvantages of different modified techniques, which can provide some useful information for the food processing industry.

### 4.1 | Chemical modification

In terms of improving the functional properties of pea protein, chemical modification is one of the most commonly used methods. Among all chemical treatments shown in Table 8, the complex coacervation and conjugation between pea protein and polysaccharide are two traditional and simple methods. The fabrication of complex coacervate is based on pH-dependent associative phase behavior when protein and polysaccharide are oppositely charge, and this complexation is generally considered to follow nucleation and growth-type mechanism (Sanchez, Mekhloufi, & Renard, 2006). Generally, the level of complexation between PPI and polysaccharide depends on pH, biopolymer mixing ratio, and the polysaccharide’s molecular characteristics such as degree of esterification, molecular weight, and charge density, besides, turbidimetric and phase diagram analysis are well-established methods to identify complexes formation. Nowadays, numerous studies have explored the functional properties of PPI–polysaccharide complexes (see Table 8), such as PPI–GA (Liu, Elmer, Low, & Nickerson, 2010), PPI–high methoxyl pectin (HMP) (Lan, Chen, & Rao, 2018; Wei et al., 2020), PPI–high methoxy citrus pectin (P90), PPI–apple pectin (P78), PPI–sugar beet pectin (P62), PPI–low methoxy citrus pectin (P29) (Warnakulasuriya et al., 2018), PPI–propylene glycol alginate (PGA) (Guo, Su, Yuan, Mao, & Gao, 2019), PPI–native high methoxy citrus pectin (NP), PPI–modified pectins (MP42, MP37, and MP33) (Pillai et al., 2020), PPI–corn fiber gum, PPI–carboxymethyl cellulose, and PPI–konjac glucomannan (Wei et al., 2020). The studies of Liu et al. (2010), Lan et al. (2018), and Guo et al. (2019) all indicated that the presence of polysaccharides shifted the pH of PPI minimum solubility toward more acidic value. Compared with unmodified PPI and others complexes, the

**TABLE 8** Modification methods to improve the functionalities of pea protein

Modification method	Modification material	Characterization techniques	Major findings	References
Chemical modification	Complex coacervation <ul style="list-style-type: none"> <li>• PPI-GA</li> <li>• PPI-HMP</li> <li>• PPI-P90, PPI-P78, PPI-P62, and PPI-P29</li> <li>• PPI-PGA</li> <li>• PPI-NP, PPI-MP42, PPI-MP37, and PPI-MP33</li> <li>• PPI-corn fiber gum, PPI-HMP, PPI-carboxymethyl cellulose, and PPI-konjac glucomannan</li> </ul>	<ul style="list-style-type: none"> <li>✓ UV/Vis spectrophotometer</li> <li>✓ Zeta potential measurement</li> <li>✓ Phase diagram</li> </ul>	<ul style="list-style-type: none"> <li>• Improved solubility, ES and FS</li> <li>• Improved solubility</li> <li>• Improved solubility (PPI-P90, PPI-P78, and PPI-P62)</li> <li>• Improved solubility, EAI and ESI, and physical stability</li> <li>• Improved solubility (PPI-NP and PPI-MP42)</li> <li>• Improved physical stability</li> </ul>	Guo et al. (2019); Lan et al. (2018); Liu et al. (2010); Pillai et al. (2020); Warnakulasuriya et al. (2018); Wei et al. (2020)
	Conjugation <ul style="list-style-type: none"> <li>• PPI-pectin</li> <li>• PPI-GA, PPC-GA, and PPH-GA</li> </ul>	<ul style="list-style-type: none"> <li>✓ SDS-PAGE</li> <li>✓ SEM</li> <li>✓ FT-IR</li> <li>✓ UV/Vis spectrophotometer</li> <li>✓ Chroma Meter</li> </ul>	<ul style="list-style-type: none"> <li>• PPI-pectin conjugates blend ratio (1:3, incubated 48 hr, 60 °C), enhanced solubility, improved EA and ES of emulsion during storage</li> <li>• PPI-GA, PPC-GA, or PPH-GA conjugates blend ratio (1:4, incubated 1, 3, or 1 day, 60 °C), enhanced solubility, improved EA and physical stability of emulsion against environment stresses</li> </ul>	Tamnak, Mirhosseini, Tan, Ghazali, et al. (2016); Zha et al. (2019a, 2019b); Zha, Yang, et al. (2019)
	pH-shifting treatment <ul style="list-style-type: none"> <li>• PPI, alkaline pH treatment</li> </ul>	<ul style="list-style-type: none"> <li>✓ TEM</li> </ul>	<ul style="list-style-type: none"> <li>• Improved physical stability (lower values in Ps and SLH) of emulsion during storage</li> </ul>	Jiang et al. (2014)
	Acylation treatment <ul style="list-style-type: none"> <li>• PPI-SA, PPI-OSA, and PPI-DDSA</li> </ul>	<ul style="list-style-type: none"> <li>✓ SDS-PAGE</li> <li>✓ FT-IR</li> </ul>	<ul style="list-style-type: none"> <li>• Enhanced solubility, ESI, FC, FS, and WHC</li> </ul>	Shah et al. (2019)
	Phosphorylation treatment <ul style="list-style-type: none"> <li>• PPI-sodium tripolyphosphate</li> </ul>	<ul style="list-style-type: none"> <li>✓ SEM</li> <li>✓ FT-IR</li> </ul>	<ul style="list-style-type: none"> <li>• Improved solubility, EAI, ESI, FC, FS, and OHC</li> </ul>	Liu et al. (2020)
	Protein-polyphenol interaction <ul style="list-style-type: none"> <li>• PPI-TA</li> <li>• Oxidized PPI</li> </ul>	<ul style="list-style-type: none"> <li>✓ UV/vis spectrophotometer</li> <li>✓ ITC</li> <li>✓ Zeta potential measurement</li> </ul>	<ul style="list-style-type: none"> <li>• PPI-TA complexes (TA concentration: 0.01% and 0.1%), improved physical stability (lower Ps) of emulsions during storage at 25, 37, and 55 °C</li> </ul>	Li et al. (2020)
		<ul style="list-style-type: none"> <li>✓ SDS-PAGE</li> <li>✓ SEC</li> <li>✓ Spectrofluorimeter</li> </ul>	<ul style="list-style-type: none"> <li>• (24-hr oxidized PPI) Improved coalescence stability of emulsion</li> <li>• Reduced solubility</li> </ul>	Hinderink et al. (2020)

(Continues)

TABLE 8 (Continued)

Modification method	Modification material	Characterization techniques	Major findings	References
	<ul style="list-style-type: none"> <li>• PPC-WPI, thermal co-gel</li> <li>• PPC-starch, pressure-induced gel</li> </ul>	<ul style="list-style-type: none"> <li>✓ Dynamic rheology</li> <li>✓ TPA</li> <li>✓ SEM</li> </ul>	<ul style="list-style-type: none"> <li>• PPC-WPI blend ratios in NaCl solution (2:8, pH 6.0), enhanced gel properties (higher values in G' and gel hardness, lower LGC)</li> <li>• Enhanced gel properties (higher G', lower tan δ and LGC)</li> </ul>	Sim et al. (2020); Wong et al. (2013)
Physical modification	Heat treatment: PPI <ul style="list-style-type: none"> <li>• 95 °C/30 min</li> <li>• 50 to 100 °C/30 min</li> <li>• 140 °C/4 s and 121 °C/2.8 min</li> <li>• 130 °C/0.5 to 20 min</li> </ul>	<ul style="list-style-type: none"> <li>✓ SDS-PAGE</li> <li>✓ Spectrofluorimeter</li> <li>✓ FT-IR</li> <li>✓ HP-SEC</li> </ul>	<ul style="list-style-type: none"> <li>• Improved creaming stability of emulsion during storage, reduced solubility</li> <li>• Improved EA at pH 7.0, similar solubility, reduced FC and FS at all pH values (3.0, 5.0 and 7.0)</li> <li>• Reduced solubility, (121 °C/2.8 min) &gt; (140 °C/4 s)</li> <li>• Reduced solubility, (20 min) &gt; (5 min) &gt; (0.5 min)</li> </ul>	Beck, Knoerzer, Sellahewa, et al. (2017); Bogahawaththa et al. (2019a); Chao and Aluko (2018); Peng et al. (2016)
	Heat with shear treatment: PPI <ul style="list-style-type: none"> <li>• Shear (250 to 3,000/s) treatment during heating at 130 °C for 0.5, 5, and 20 min</li> <li>• Shear (400 or 700/min) treatment during heating at 130 to 170 °C</li> <li>• Shear (100 or 1,500/s) treatment during heating at 90 °C for 5 min</li> </ul>	<ul style="list-style-type: none"> <li>✓ FT-IR</li> <li>✓ SDS-PAGE</li> </ul>	<ul style="list-style-type: none"> <li>• Reduced solubility</li> <li>• Solubility: (Heat with shear treatment) &gt; (Pure heat treatment)</li> </ul>	Beck, Knoerzer, Sellahewa, et al. (2017); , Beck, Knoerzer, and Arcot (2017); Bogahawaththa et al. (2019b)
	Ultrasonic treatment: PPI <ul style="list-style-type: none"> <li>• 20 kHz, 1 to 40 min</li> <li>• 20 kHz, amplitude: 30% to 90%, 30 min</li> </ul>	<ul style="list-style-type: none"> <li>✓ SDS-PAGE</li> <li>✓ CD</li> <li>✓ Spectrofluorimeter</li> </ul>	<ul style="list-style-type: none"> <li>• Improved solubility</li> <li>• Improved FC and FS</li> </ul>	Xiong et al. (2018); Ye et al. (2016)
	<ul style="list-style-type: none"> <li>• PPI, high-pressure supercritical CO<sub>2</sub> treatment</li> </ul>	<ul style="list-style-type: none"> <li>✓ SEM</li> <li>✓ FT-IR</li> </ul>	<ul style="list-style-type: none"> <li>• Improved FS</li> </ul>	do Carmo et al. (2016)
	<ul style="list-style-type: none"> <li>• PPI, high pressure treatment</li> </ul>	<ul style="list-style-type: none"> <li>✓ SDS-PAGE</li> <li>✓ Spectrofluorimeter</li> </ul>	<ul style="list-style-type: none"> <li>• Significantly improved EA and FC at pH 3.0 and ES at pH 7.0</li> <li>• Similar solubility</li> </ul>	Chao, Jung, et al. (2018)
	<ul style="list-style-type: none"> <li>• PPI, cold atmospheric plasma treatment</li> </ul>	<ul style="list-style-type: none"> <li>✓ Spectrofluorimeter</li> </ul>	<ul style="list-style-type: none"> <li>• Improved solubility</li> </ul>	Bussler et al. (2015)
	<ul style="list-style-type: none"> <li>• PPI with GA or MD, solid dispersion-based spray-drying treatment</li> </ul>	<ul style="list-style-type: none"> <li>✓ FT-IR</li> <li>✓ SEM</li> <li>✓ XRD</li> </ul>	<ul style="list-style-type: none"> <li>• Both PPI/GA and PPI/MD improved solubility at pH 7.0</li> <li>• PPI/GA improved solubility at pH 4.5</li> </ul>	Lan et al. (2019)

(Continues)



TABLE 8 (Continued)

Modification method	Modification material	Characterization techniques	Major findings	References
	<ul style="list-style-type: none"> <li>• PPI mixed MD, modulate viscosity treatment</li> </ul>	<ul style="list-style-type: none"> <li>✓ Rotational rheometer</li> </ul>	<ul style="list-style-type: none"> <li>• Improve FS</li> </ul>	Moll et al. (2019)
Enzymatic modification	MTG treatment <ul style="list-style-type: none"> <li>• PPIc, heat-induced gel</li> <li>• PPI-SE, heat-induced gel</li> </ul>	<ul style="list-style-type: none"> <li>✓ Dynamic rheometer</li> <li>✓ SDS-PAGE</li> </ul>	<ul style="list-style-type: none"> <li>• Enhanced gel properties (higher shear strain and elasticity)</li> <li>• Enhanced gel properties (higher strength and elasticity, lower LGC)</li> </ul>	Shand et al. (2008); Sun and Arntfield, (2011c)
	TyrBm treatment <ul style="list-style-type: none"> <li>• PPC or PPC-Zein</li> </ul>	<ul style="list-style-type: none"> <li>✓ SDS-PAGE</li> </ul>	<ul style="list-style-type: none"> <li>• Improved physicochemical stability of emulsions (TyrBm-treated PPC-Zein &gt; TyrBm-treated PPC) during storage</li> </ul>	Glusac et al. (2019)
	Enzymatic hydrolysis treatment <ul style="list-style-type: none"> <li>• PPI, chymosin or papain</li> <li>• PPI, trypsin</li> <li>• PPC, trypsin</li> <li>• PPC, pancreatic trypsin</li> <li>• PPI, alcalase, flavorzyme, neutrase, alcalase-flavorzyme, neutrase-flavorzyme</li> </ul>	<ul style="list-style-type: none"> <li>✓ SDS-PAGE</li> <li>✓ SEC</li> <li>✓ Small amplitude oscillatory rheometer</li> </ul>	<ul style="list-style-type: none"> <li>• Improved solubility, EAI, ESI and FC</li> <li>• Improved physicochemical stability of emulsion during storage</li> <li>• Improved solubility, enhanced stability of emulsion</li> <li>• Improved solubility, similar gel properties</li> <li>• Improved solubility and FC</li> </ul>	Bajaj et al. (2017); Barac et al. (2011, 2012); Felix et al. (2017); Klost and Drusch (2019a); Tamm et al. (2016)
Chemical combined physical modification	<ul style="list-style-type: none"> <li>• PPI, pH-shifting combined ultrasound (US) treatment</li> </ul>	<ul style="list-style-type: none"> <li>✓ SDS-PAGE</li> </ul>	<ul style="list-style-type: none"> <li>• Improved solubility</li> <li>• Solubility: (pH-shifting combined US treatment) &gt; (pH-shifting treatment)</li> </ul>	Jiang et al. (2017)
	<ul style="list-style-type: none"> <li>• PPI-RPI complexes combined direct steam injection treatment</li> </ul>	<ul style="list-style-type: none"> <li>✓ SDS-PAGE</li> </ul>	<ul style="list-style-type: none"> <li>• Improved solubility, EAI, FS, and OHC</li> <li>• Similar FC</li> <li>• Reduced ESI</li> </ul>	Pietrysiak et al. (2018)
	<ul style="list-style-type: none"> <li>• PPI-SSPS complexes combined freeze-drying treatment</li> </ul>	<ul style="list-style-type: none"> <li>✓ FT-IR, AFM</li> <li>✓ Zeta potential measurement</li> <li>✓ Spectrofluorimeter</li> </ul>	<ul style="list-style-type: none"> <li>• Improved FC, FS and EAI</li> <li>• Similar ESI</li> </ul>	Zhan et al. (2019)
Physical combined enzymatic modification	<ul style="list-style-type: none"> <li>• PPI, electron beam irradiation combined flavorzyme hydrolysis treatment</li> </ul>	<ul style="list-style-type: none"> <li>• Gel permeation chromatography</li> </ul>	<ul style="list-style-type: none"> <li>• Improved FC, FS and EAI</li> <li>• Reduced ESI</li> </ul>	Wang et al. (2017)

Abbreviations: PPI, pea protein isolate; PPIc, commercial PPI; PPC, pea protein concentration; Ps, particle size; ZP, zeta-potential; SLH, serum layer height; EA, emulsifying activity; FA, foaming activity; RPI, rice protein isolate; SSPS, soluble soybean polysaccharide; SEC, size exclusion chromatography; AFM, atomic force microscope; TEM, transmission electron microscope; SEM, scanning electron microscopy; CD, circular dichroism; XRD, X-ray diffraction; FT-IR, Fourier transform infrared spectroscopy; ITC, isothermal titration calorimetry; TPA, texture profile analysis; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; MD, maltodextrins; MTG, microbial transglutaminase.

**TABLE 9** Comparison of different modification techniques of pea protein

Modification techniques	Advantages	Disadvantages	Using status
Chemically	<ul style="list-style-type: none"> <li>• High reaction rate</li> <li>• High efficiency in the functional improvement</li> <li>• Low cost</li> <li>• High yield</li> </ul>	<ul style="list-style-type: none"> <li>• Undesirable or toxic side product</li> <li>• Pollute the environment</li> </ul>	Industry application phase ✓ Complex coacervation ✓ Conjugation Research phase ✓ Other modification ways
Physically	<ul style="list-style-type: none"> <li>• Clean production</li> <li>• High yield</li> </ul>	<ul style="list-style-type: none"> <li>• High equipment requirement</li> <li>• High cost</li> <li>• Low efficiency in the functional improvement</li> </ul>	Industry application phase ✓ Non-thermal processing Research phase ✓ Other modification ways
Enzymatically	<ul style="list-style-type: none"> <li>• Reaction specificity</li> <li>• Clean production</li> <li>• High efficiency in the functional improvement</li> <li>• Mild reaction condition</li> </ul>	<ul style="list-style-type: none"> <li>• Low reaction rate</li> <li>• High cost</li> <li>• Low yield.</li> </ul>	Industry application phase ✓ Microbial transglutaminase-catalyzed cross-linking ✓ Commercial enzyme-hydrolysis

PPI–HMP complex with a mass ration of 1:1 and the PPI–PG complex with a mass ration of 3:1 had the highest protein solubility at pH 4.5 and 4.0, reaching 93% and 74.18%, respectively (Guo et al., 2020; Lan et al., 2018). In addition, compared to PPI (approximately 4%), the formation of PPI–P90, PPI–P78, and PPI–P62 complexes significantly increased the PPI solubility at pH 4.5 to 13%, 45%, and 29%, respectively; however, PPI–P29 complexes did not change the PPI solubility (Warnakulasuriya et al., 2018). Similar results were also reported by Pillai et al. (2020) who found that PPI–NP and PPI–MP42 complexes had significantly higher the PPI solubility values of 16.5% and 19.9% at pH 4.5, whereas the protein solubility of PPI–MP33 and PPI–MP37 was similar to PPI (2.8%). Therefore, in terms of improving PPI’s solubility at pH 4.5, the complexes formed by PPI and higher degree of esterification pectin might be a much better choice. Furthermore, the emulsifying properties, FS, and physical stability of PPI under acidic conditions were also significantly enhanced by this modification method (Guo et al., 2019; Liu et al., 2010; Wei et al., 2020). For coacervation modification, it is generally believed that during an acid titration polysaccharide- and pH-induced changes to the protein’s conformation could lead to different surface properties of the formed complexes, thus improving the functional performance of PPI. Overall, the formation of PPI and polysaccharides soluble complexes improves solubility and physical stability of protein at acidic environment, which contributes to the application and development of pea protein-based acidic beverage formulas.

Conjugation is an important chemical modification technique different from coacervation, namely, conjugation of protein with polysaccharide through Maillard-driven reaction, which has become a promising green chemical technique to improve functionality of protein (Tamnak, Mirhosseini, Tan, Ghazali, & Muhammad, 2016). In general, conjugation of protein with polysaccharide is affected by the following factors: (a) the nature and ration of each polymer, (b) relative humidity (RH), and (c) incubation time and temperature. Fourier transform infrared spectroscopy and sodium dodecyl sulfate-polyacrylamide gel electrophoresis are effective methods to characterize and analyze conjugated product formation. Until now, some researchers have studied the functionality of pea protein–polysaccharide conjugates formed at 60 °C and 79% RH with various incubation times, such as PPI–pectin (Tamnak, Mirhosseini, Tan, Ghazali, et al., 2016), PPI–GA, PPC–GA, and PPH–GA (Zha, Dong, Rao, & Chen, 2019a, 2019b; Zha, Yang, et al. 2019). Findings from these studies suggested that the solubility of pea protein or PPH was remarkably improved after conjugating with polysaccharide at appropriate incubation time (shown in Table 8), but longer incubation time had an adverse impact on the solubility of pea protein–polysaccharide conjugates. Additionally, the O/W emulsions stabilized by pea protein–polysaccharide or PPH–polysaccharide conjugates also showed smaller particle size, higher surface charge, and great physical stabilities against pH (2 to 8), thermal processing (25, 37, and 72 °C), and ionic strength (0, 100, 300, and 500 mM), as compared

to pea protein or PPH. Therefore, pea protein–polysaccharide conjugation through controlling Maillard reaction could be an effective strategy to enhance the solubility and emulsifying property of pea protein or PPH.

The pH-shifting treatment, namely, subjecting protein solution to an extreme acidic or alkaline pH condition and then to a neutral pH environment, allows protein to undergo partial unfolding and then refolds to assume a molten globule conformation that has unique surface properties (Wang & Xiong, 2019). This approach has been applied to improve the functional properties of proteins (Wang & Xiong, 2019). However, only Jiang et al. (2014) reported that the O/W emulsion prepared with alkaline pH-modified PPI was more effective against oil droplets coalescence during storage, as compared with native PPI, suggesting alkaline pH-modified PPI exhibited superior emulsifying property. Furthermore, both acylation and phosphorylation modifications are cheap and efficient chemical methods to improve the functional properties of pea protein. The study of Shah, Umesh, and Singhal (2019) showed that the functional properties (solubility, FC, FS, ESI, and WHC) of PPI were improved substantially through hydrophobically modified by N-substitutions with succinic anhydride (SA), *n*-octenyl succinic anhydride (OSA), and dodecyl succinic anhydride (DDSA) to synthesize PPI–SA, PPI–OSA, and PPI–DDSA, respectively. Similar results were reported by Liu et al. (2020), finding that the functional properties (solubility, EAI, ESI, FC, FS, and OHC) of PPI were also remarkably enhanced using modified by sodium tripolyphosphate. Both acylation and phosphorylation modification introduced negative charge into pea protein structure and enhanced the electrostatic repulsion between protein molecules, and thus improving the functionalities of pea protein.

The studies of Li et al. (2020) and Hinderink, Kaade, Sagis, Schroen, and Berton-Carabin (2020), respectively, indicated that the O/W emulsions prepared by PPI–TA complexes and oxidized PPI exhibited better ES than native PPI. In addition, pea protein has inferior gelling properties than soybean protein. Both studies of Wong, Vasanthan, and Ozimek (2013) and Sim and Moraru (2020) demonstrated that adding whey protein isolate (WPI) or starch to PPC forming thermal co-gel or pressure-induced gel (600 MPa for 4 min) significantly enhanced the gelation properties of PPC.

## 4.2 | Physical modification

Some physical technologies to modify pea protein for functionality improvement are gradually becoming available (see Table 8). Many studies have shown that compared with unmodified PPI, the solubility of heat-treated PPI with different parameters did not improve but might decrease significantly (Beck, Knoerzer, Sellahewa, Emin, & Arcot,

2017; Chao & Aluko, 2018; Bogahawaththa, Chau, Trivedi, Dissanayake, & Vasiljevic, 2019a; Peng et al., 2016). The elevated temperature typically caused interprotein interactions (hydrophobic and covalent), and thus resulting in reduced solubility due to protein aggregation and precipitation. Although heat treatment was not conducive to the improvement of protein solubility, Peng et al. (2016) found that emulsion stabilized by heat-treated PPI exhibited a better CS than those formed by unmodified PPI. The study of Chao and Aluko (2018) also suggested that heat treatment enhanced the EA of PPI at pH 7.0, but reduced its foaming properties. In addition, Beck, Knoerzer, Sellahewa, et al. (2017), Beck, Knoerzer, and Arcot (2017), and Bogahawaththa, Chau, Trivedi, Dissanayake, and Vasiljevic (2019b) investigated the effect of combined heat and shear treatment technology on PPI solubility. Compared to native PPI, the solubility of the protein was obviously reduced by the combined heat and shear treatment, but this modification process resulted in higher protein solubility compared to the pure heat treatment. Apart from heat treatment, ultrasound (US) treatment can also modify protein conformation and structure, resulting in the increased hydrophilicity of protein molecules. Ye et al. (2016) indicated that US treatment significantly improved PPI solubility, and its solubility further increased with increasing sonication time. Moreover, Xiong et al. (2018) showed that high-intensity US processing effectively improved foaming properties of PPI, in which the foaming ability and FS of PPI increased from 145.6% and 58.0% to 200.0% and 73.3%, respectively. HP is a nonthermal treatment and can significantly affect noncovalent bonds, thereby changing the protein conformation. The FS of PPI was effectively improved through HP supercritical CO<sub>2</sub> treatment (do Carmo et al., 2016). HP treatment had positive effects on emulsifying and foaming properties of PPI at a certain pH, whereas its solubility was not improved significantly (Chao, Jung, & Aluko, 2018). According to Moll, Grossmann, Kutzli, and Weiss (2019), the addition of nonsurface-active maltodextrin was able to considerably improve FS of PPI by modulating the viscosity of the continuous phase, compared to the same concentration PPI. In addition, Bussler, Steins, Ehlbeck, and Schluter (2015) illustrated that cold atmospheric pressure plasma treatment significantly enhanced the solubility of PPI. Solid dispersion-based spray-drying technique is used to disperse poor water-soluble ingredients in an amorphous matrix carrier and obtain improved solubility ingredients through spray drying (Lan, Xu, Ohm, Chen, & Rao, 2019). Lan et al. (2019) first reported that this technique remarkably enhanced the solubility of PPI at pH 4.5 and 7.0, using GA and maltodextrins as amorphous matrix carrier. This modification method is considered as a clean and efficient technique to improve solubility of PPI, which is worthy of further research.

### 4.3 | Enzymatic modification

Enzyme-modified approach for the improvement in functionality of protein is considered to be cleaner and more efficient than chemical and physical modifications, and is also easily favored by consumers. For pea protein, enzyme-catalyzed cross-linking shown in Table 8 can significantly enhance its gelling and emulsifying performances, whereas enzymatic hydrolysis can improve its solubility and emulsifying and foaming properties.

Microbial transglutaminase (MTG) is an enzyme commonly used for the cross-linking of protein, which can modify protein by catalyzing the acyl transfer between a  $\lambda$ -carboxamide of a peptide/protein bound glutamine and lysine forming an  $\epsilon$ -( $\lambda$ -glutamyl) lysine [ $\epsilon$ -( $\lambda$ -Glu) Lys] cross-link (Kuraishi, Yamazaki, & Susa, 2001), thus effecting gel performance of protein. Shand, Ya, Pietrasik, and Wanasundara (2008) and Sun and Arntfield (2011c), respectively, reported that the gelation properties of PPIc and PPI-SE were dramatically improved by MTG-catalyzed cross-linking, meanwhile showed that gel strength of MTG-treated PPI was stronger than that of SPI with and without MTG treatment. Moreover, the study of Glusac, Davidesko-Vardi, Isaschar-Ovdat, Kukavica, and Fishman (2019) explored the effect of tyrosinase-crosslinking (tyrosinase from *Bacillus megaterium* [TyrBm]) of PPI and PPI–zein complexes on the properties of O/W emulsion, and the emulsion stabilized by TyrBm-crosslinked PPI–zein had the best physicochemical stability, followed by TyrBm-crosslinked PPI, compared to PPI alone stabilized emulsion. This study is meaningful because it provides a novel way to improve the stability of PPI stabilized emulsion.

Enzymatic hydrolysis usually decreases molecular weight, increases the number of ionizable groups, and exposes the hydrophobic group buried in the protein core, which has a potential to improve hydrophobicity, solubility, and emulsifying and foaming properties of protein. Many researchers have applied this structure-modifying technique to effectively improve the solubility of pea protein at different pH values (Bajaj et al., 2017; Barac et al., 2011, 2012; Felix et al., 2017; Klost & Drusch, 2019a). Commercial proteases, such as chymosin (EC 3.4.23.4), papain, trypsin, alcalase, flavorzyme, neutrase (EC 3.4.24.28), mixed alcalase-flavorzyme, and neutrase-flavorzyme, are generally used. Barac et al. (2011, 2012) used chymosin and papain to hydrolyze PPI, and found an overall tendency toward improving its emulsifying and foaming properties. Bajaj et al. (2017) indicated that different enzymatic treatment significantly increased the foaming ability of PPI, among which FC of alcalase was the highest, followed by neutrase-flavorzyme, neutrase, and alcalase-flavorzyme. In addition, the FC for all PPI was highest at 30 min of hydrolysis and decreased with increasing hydrolysis time. According to Tamm et al. (2016)

and Klost and Drusch (2019a), trypsin-treated PPI-stabilized emulsions exhibited smaller oil droplet and higher surface charge than PPI-stabilized emulsion; moreover, the emulsifying properties of trypsin-hydrolyzed PPI increased significantly with increasing DH. However, enzyme hydrolysis treatment had no positive effect on the gelling property of PPC (Felix et al., 2017). Overall, both type of enzyme and DH influence the extent of improvement in pea protein functionalities, and limited enzyme hydrolysis treatment is an appropriate way to obtain desirable functional properties.

### 4.4 | Combined modification

Combined modification (shown in Table 8), for example, different modified methods work together, is an emerging technology that can effectively improve the functional properties of pea protein. Jiang et al. (2017) studied the effect of pH-shifting (at pH 2, 4, 10, and 12) and US combined process on the functionality of PPI, showing that pH-shifting at pH 12 and US combined treatment (pH12-US) significantly improved PPI solubility, whereas the solubility of PPI treated by acidic conditions combined US treatment had no obvious enhancement. And the pH12-US-modified PPI had a solubility seven times higher than native PPI. The study of Pietrysiak, Smith, Smith, and Ganjyal (2018) indicated that pea–rice protein isolate complexes combined direct steam injection treatment enhanced the functionality of protein, for instance, solubility (from 3% to 41%, at pH 7), EAI (from 5.9 to 52.5 m<sup>2</sup>/g), FS (from 68.2% to 82.8%), and OHC (from 1.8 to 4.9 g/g) values drastically increased compared to the untreated protein. Furthermore, the emulsifying and foaming properties of PPI were improved obviously by protein–polysaccharide complexes combined freeze-drying treatment (Zhan et al., 2019). Wang et al. (2017) modified PPI by electron beam irradiation combined flavorzyme hydrolysis treatment, and the results were similar to Zhan et al. (2019), among which FC and FS of PPI increased with increasing irradiation dose and reached a maximum at 10 kGy.

## 5 | APPLICATIONS OF PEA PROTEIN IN THE FOOD PRODUCTS

### 5.1 | Pea protein-based encapsulation systems for bioactive ingredients

In food system, the application of some bioactive ingredients (e.g., hydrophobic and hydrophilic compounds, probiotics, and minerals) is limited by their instability, low bioavailability, and unfavorable flavors. Encapsulation is a promising technique to solve the above problems of bioactive ingredients. In recent years, there is an increasing research interest in pea protein as encapsulating materials, due to its health

**TABLE 10** The summary of pea protein-based encapsulation systems for bioactive ingredients

Composition	Bioactive ingredients	Technique	Result	References
<ul style="list-style-type: none"> <li>• PPC</li> <li>• PPC/M mixture</li> </ul>	$\alpha$ -tocopherol	<ul style="list-style-type: none"> <li>• Spray drying</li> </ul>	<ul style="list-style-type: none"> <li>✓ Improved stability</li> </ul>	Pierucci et al. (2006, 2007)
<ul style="list-style-type: none"> <li>• PPC</li> <li>• PPC/M mixture</li> </ul>	CLA	<ul style="list-style-type: none"> <li>• Spray drying</li> </ul>	<ul style="list-style-type: none"> <li>✓ Similar stability in both wall materials</li> </ul>	Costa et al. (2015)
<ul style="list-style-type: none"> <li>• PPI</li> <li>• PPI/HMP mixture</li> </ul>	PUFA-rich oil (rich in omega-3)	<ul style="list-style-type: none"> <li>• Spray drying</li> </ul>	<ul style="list-style-type: none"> <li>✓ Improved oxidative stability</li> </ul>	Aberkane et al. (2014)
<ul style="list-style-type: none"> <li>• PPI</li> </ul>	Flaxseed oil (rich in ALA)	<ul style="list-style-type: none"> <li>• Spray drying</li> </ul>	<ul style="list-style-type: none"> <li>✓ Highest encapsulation efficiency at 1:5 core-to-wall ratio</li> </ul>	Bajaj et al. (2015)
<ul style="list-style-type: none"> <li>• PPI/M mixtures</li> </ul>	Black pepper seed oil	<ul style="list-style-type: none"> <li>• Spray drying</li> </ul>	<ul style="list-style-type: none"> <li>✓ Highest encapsulation efficiency (1% PPI + 39% M)</li> </ul>	Karaca (2019)
<ul style="list-style-type: none"> <li>• PPC</li> </ul>	Iron	<ul style="list-style-type: none"> <li>• Spray drying</li> </ul>	<ul style="list-style-type: none"> <li>✓ Masked undesirable taste</li> <li>✓ Controlled release</li> <li>✓ Preserved bioaccessibility</li> </ul>	Bittencourt et al. (2013)
<ul style="list-style-type: none"> <li>• PPC</li> </ul>	Propolis extract	<ul style="list-style-type: none"> <li>• Spray drying</li> </ul>	<ul style="list-style-type: none"> <li>✓ Improved thermal stability</li> <li>✓ Reduced antioxidant activity</li> <li>✓ Kept antimicrobial activity</li> </ul>	Jansen-Alves, Krumreich, et al. (2019); Jansen-Alves, Maia, et al. (2019)
<ul style="list-style-type: none"> <li>• PPI/SA mixtures</li> <li>• PPI/I-CGN mixtures</li> <li>• PPI/GG mixtures</li> </ul>	Acid-sensitive probiotic: <i>Bifidobacterium adolescentis</i>	<ul style="list-style-type: none"> <li>• Freeze drying</li> </ul>	<ul style="list-style-type: none"> <li>✓ Improved acid survivability</li> <li>✓ Effective bioactivity in rat (PPI/SA capsule)</li> </ul>	Varankovich et al. (2015)
<ul style="list-style-type: none"> <li>• PPI/AL mixture</li> </ul>	Acid-sensitive probiotic: <i>Bifidobacterium adolescentis</i>	<ul style="list-style-type: none"> <li>• Extrusion</li> </ul>	<ul style="list-style-type: none"> <li>✓ Improved acid survivability</li> </ul>	Khan et al. (2013)
<ul style="list-style-type: none"> <li>• PPI</li> <li>• PPI-MS complexes</li> </ul>	Canola oil (rich in DHA)	<ul style="list-style-type: none"> <li>• Freeze drying</li> </ul>	<ul style="list-style-type: none"> <li>✓ Improved oxidative stability</li> </ul>	Yildiz et al. (2018)
<ul style="list-style-type: none"> <li>• PPI at pH 3.0</li> </ul>	$\beta$ -carotene	<ul style="list-style-type: none"> <li>• Pickering emulsion</li> </ul>	<ul style="list-style-type: none"> <li>✓ Sustained release</li> <li>✓ Improved stability during the digestion</li> </ul>	Shao and Tang, (2016)
<ul style="list-style-type: none"> <li>• PPI-pectin conjugate</li> </ul>	Tartrazine	<ul style="list-style-type: none"> <li>• W/O/W emulsion</li> </ul>	<ul style="list-style-type: none"> <li>✓ Higher encapsulation stability</li> </ul>	Tamnak, Mirhosseini, Tan, Amid, et al. (2016)
<ul style="list-style-type: none"> <li>• PPI and others protein isolates</li> </ul>	Omega-3 oil Fish oil	<ul style="list-style-type: none"> <li>• O/W emulsion</li> </ul>	<ul style="list-style-type: none"> <li>✓ Similar lipid digestion</li> <li>✓ Similar FFA release</li> <li>✓ Encapsulated 10 % omega-3 oil</li> <li>✓ Better oxidative stability</li> </ul>	Gumus et al. (2017a, 2017b, 2017c)
<ul style="list-style-type: none"> <li>• PPI</li> <li>• PPI/SPI mixtures</li> <li>• PPI/WPI mixtures</li> </ul>	Lycopene	<ul style="list-style-type: none"> <li>• O/W emulsion</li> </ul>	<ul style="list-style-type: none"> <li>✓ PPI/WPI mixtures had best lycopene retention</li> </ul>	Ho et al. (2018)
<ul style="list-style-type: none"> <li>• PPI</li> <li>• PPI/SC mixtures</li> <li>• PPI/WPI mixtures</li> </ul>	Curcumin Canola oil Sunflower oil	<ul style="list-style-type: none"> <li>• O/W emulsion</li> </ul>	<ul style="list-style-type: none"> <li>✓ Improved encapsulation stability</li> </ul>	Hinderink et al. (2019); Yerramilli et al. (2017; 2018)
<ul style="list-style-type: none"> <li>• PPI</li> <li>• PPI-TA complexes</li> </ul>	Flaxseed oil (rich in ALA)	<ul style="list-style-type: none"> <li>• O/W emulsion</li> </ul>	<ul style="list-style-type: none"> <li>✓ PPI-TA complexes had better oxidative stability</li> <li>✓ Similar lipid digestion</li> </ul>	Li et al. (2020)

(Continues)



TABLE 10 (Continued)

Composition	Bioactive ingredients	Technique	Result	References
• PPC	Vitamin D	• O/W emulsion	✓ Improved cellular uptake efficiency	Walia and Chen (2020)
• Modified-PPI	Vitamin D	• O/W emulsion	✓ Improved bioefficacy in rat	Almajwal et al. (2019)
• Modified-PPI	Vitamin D	• O/W emulsion	✓ Improved UV light stability ✓ Improved bioavailability	Jiang et al. (2019)
• PPI-MG complexes, pH 5.0	Quercetin	• Complexes	✓ Improved UV light stability ✓ Improved oxidative stability	Cuevas-Bernardino et al. (2018)
• PPI-HMP complexes	Curcumin	• Complexes	✓ Improved stability against UV light and thermal	Guo et al. (2020)

Abbreviations: PPI, pea protein isolate; PPC, pea protein concentration; SPI, soybean protein isolate; SC, sodium caseinate; WPI, whey protein isolate; TA, tannic acid; M, maltodextrin; HMP, high methoxyl pectin; AL, alginate; SA, sodium alginate; I-CGN, iota-carrageenan; GG, gellan gum; MS, modified starch; CLA, conjugated linoleic acid; PUFA, polyunsaturated fatty acids; FFA, free fatty acids; MG, mesquite gum.

benefits, hypoallergenic issues, and no issues of genetic modification (Amagliani & Schmitt, 2017). Spray drying, emulsions, and complexes are now the main technologies to involve the utilization of pea protein-based as encapsulation materials. The stability and function of bioactive ingredients can be improved through using pea protein-based encapsulation systems, as shown in Table 10. Although these are still at exploring research stage, it could provide useful technical support for industrial production in the future.

In pea protein-based encapsulation systems, spray drying is a widely used encapsulation technology for the production of microparticles. Many lipophilic bioactive components, for instance,  $\alpha$ -tocopherol (Pierucci, Andrade, Baptista, Volpato, & Rocha-Leao, 2006, 2007), omega-3 fatty acids (Aberkane, Roudaut, & Saurel, 2014),  $\alpha$ -linolenic acid (ALA) (Bajaj, Tang, & Sablani, 2015), conjugated linoleic acid (CLA) (Costa et al., 2015), and black pepper oil (Karaca, 2019), first been encapsulated in feed emulsions stabilized by pea protein and then obtained microparticles through spray drying. Both PPC- and PPC/maltodextrin-encapsulated microparticles effectively improved the stability of  $\alpha$ -tocopherol during 90-day storage, and PPC-encapsulated microparticle exhibited higher  $\alpha$ -tocopherol retention (Pierucci et al., 2006, 2007). PPC and PPC/maltodextrin as wall materials also used to encapsulate CLA; there was no difference between them on the oxidative stability of CLA (Costa et al., 2015). Aberkane et al. (2014) reported that PPI-HMP as encapsulation material performed better than PPI in protecting omega-3 against oxidation during storage. Bittencourt, Pedrosa, de Sousa, Pierucci, and Citelli (2013), Jansen-Alves, Krumreich, et al. (2019), and Jansen-Alves, Maia, et al. (2019) used PPC to encapsulate iron and propolis extract, and the results suggested that the stability and function of bioactive substances were improved or preserved. Compared to spray drying, freeze-drying encapsulation system is more suitable

for thermosensitive bioactive ingredients. The capsules of docosahexaenoic acid (DHA) with different wall materials were prepared by freeze-drying; both PPI and PPI-modified starch complexes-based microencapsulation provided a good protection of DHA against oxidation during 30 days storage (Yildiz et al., 2018). Acid-sensitive probiotic, *Bifidobacterium adolescentis*, was encapsulated in PPI-based microparticles using freeze-drying and extrusion techniques, and its survivability was obviously improved in synthetic stomach juice (pH 1.8, 37 °C) and a commercial yogurt, respectively (Khan, Korber, Low, & Nickerson, 2013; Varankovich, Khan, Nickerson, Kalmokoff, & Korber, 2015). Moreover, a study in rats suggested that, among all PPI-based coated microparticles, PPI/sodium alginate (PPI/SA mixtures) microparticle effectively protected probiotic cells from highly acidic condition of the stomach with subsequent release of probiotic from the microparticle into the intestine (Varankovich et al., 2015).

In addition, some lipophilic bioactive components, for example,  $\beta$ -carotene (Shao & Tang, 2016), omega-3 oil (Gumus, Decker, & McClements, 2017a, 2017c), lycopene (Ho, Schroen, San Martin-Gonzalez, & Berton-Carabin, 2018), curcumin (Yerramilli, Longmore, & Ghosh, 2018), canola oil (Yerramilli, Longmore, & Ghosh, 2017), flaxseed oil (rich in ALA) (Gumus, Decker, & McClements, 2017b; Li et al., 2020), and vitamin D (Almajwal et al., 2019; Jiang et al., 2019; Walia & Chen, 2020), can also be encapsulated into pea protein-based stabilized emulsions. For example, pea protein-based stabilized O/W emulsions exhibited significantly higher vitamin D bioavailability than free vitamin D suspension (Almajwal et al., 2019; Jiang et al., 2019; Walia & Chen, 2020). Jiang et al. (2019) also showed that modified PPI-prepared emulsion had good protection of vitamin D against UV radiation. The studies of Gumus et al. (2017a, 2017b, 2017c) compared the gastrointestinal fate, encapsulation characteristic, and oxidation stability of bioactive



components in encapsulated emulsion systems stabilized by lentil, pea, and faba bean proteins. There were no significant differences between them in free fatty acids release, lipid digestion, and encapsulation capacity, but in terms of the encapsulation stability, lentil protein-encapsulated oil droplet was the most stable at environmental stresses such as pH, ionic strength, and temperature changes, whereas pea and faba bean proteins-coated oils showed better oxidative stability. Moreover, two encapsulation materials' blend or protein–polyphenol complexes contributed to increase the encapsulation stability. Compared with emulsions prepared by the individual proteins (PPI, sodium caseinate, or WPI), the emulsions stabilized by 1:1 mixtures of PPI and sodium caseinate or WPI not only did not display any creaming or aggregation, but it remained stable for long time (Hinderink, Munch, Sagis, Schroen, & Berton-Carabin, 2019; Yerramilli et al., 2017). The study of Ho et al. (2018) illustrated that emulsions stabilized by PPI-based blends exhibited higher lycopene retention than emulsion stabilized by PPI alone. In addition, emulsions stabilized with PPI–TA complexes more effectively delayed flaxseed oil oxidation, but had similar lipid digestion, compared to emulsion stabilized by PPI alone (Li et al., 2020). According to Shao and Tang (2016), Pickering emulsion stabilized by PPI nanoparticle at pH 3.0 exhibited a great potential to act as intestine-targeted and sustained release delivery system for  $\beta$ -carotene. Water-in-oil-in-water (W/O/W) is a type of the double emulsion; it can be used to encapsulate the hydrophilic bioactive compounds. Compared with nonencapsulated tartrazine, tartrazine loaded in W/O/W emulsion stabilized by PPI–pectin conjugate had desirable release behavior and high encapsulation stability after 1-month storage (Tammak, Mirhosseini, Tan, Amid, et al., 2016).

The complexes formed between protein and polysaccharide by electrostatic interaction can serve as a novel delivery system for functional components. The quercetin loaded in PPI–mesquite gum complexes exhibited higher chemical and oxidative stability during exposure to UV light for 4 hr, in comparison to free quercetin (Cuevas-Bernardino et al., 2018). Similar results were reported by Guo et al. (2020), suggesting curcumin in the PPI–HMP complexes had better capacity against UV light and thermal stress; moreover, the complexes also delayed the release of curcumin in the simulated gastrointestinal tract.

## 5.2 | Use of pea protein-based in films

More and more researchers are recognizing the importance of using natural polymers for the preparation of biodegradable packaging. Pea protein, as a biocompatible and biodegradable natural polymer, has been extensively studied to produce edible/biodegradable films, which will provide a promising possibility for the application of pea protein-based film in food industrial-scale production. Pea protein-based films, includ-

ing processing condition and parameter, reference object, and major result, are listed in Table 11. In general, the desirable film needs to have excellent barrier properties for gas and water (e.g., low oxygen permeability [OP] and low water vapor permeability [WVP]), superior mechanical properties (e.g., tensile strength, elastic modulus, puncture strength, Young's modulus, elongation at break, stress, and strain), and good appearance properties (e.g., transparency). Table 11 shows that solvent casting, injection molding, and electrospinning are now main technologies used to prepare pea protein-based films. The film-forming properties of PPI alone are influenced by plasticizer type, protein/plasticizer ratio, pH, heat treatment, and injection parameters (Carvajal-Pinero et al., 2019; Kowalczyk & Baraniak, 2011; Kowalczyk, Gustaw, Swieca, & Baraniak, 2013; Perez, Felix, Romero, & Guerrero, 2016; Perez-Puyana, Felix, Romero, & Guerrero, 2016; Shevkani & Singh, 2015). All PPI films showed excellent barrier properties to UV light, which could contribute to prevent the degradation of UV-sensitive food ingredients (Kowalczyk et al., 2011, 2013). The type of plasticizer had a great influence on WVP and mechanical characteristics of PPI films, but the effect of pH and heat treatment was not significant, and the films plasticized with sorbitol exhibited superior water barrier and mechanical properties, in comparison with glycerol-plasticized films (Kowalczyk et al., 2011, 2013). In most cases, glycerol is the more commonly used plasticizer because of its safety. Many studies have demonstrated that glycerol-plasticized PPI film with around 40% plasticizer possessed the good processability; further, glycerol-plasticized PPI film with more excellent mechanical properties could be obtained by controlling mixing parameters (mixing speed and time) or injection parameters (molding time and injection pressure) (Carvajal-Pinero et al., 2019; Felix, Perez-Puyana, Romero, & Guerrero, 2016; Perez et al., 2016; Perez-Puyana et al., 2016). According to Shevkani and Singh (2015) and Felix et al. (2016), film forming of PPI showed the most desirable properties compared to proteins from soybean, kidney bean, amaranth, rice, albumen, and crayfish. Pea protein is a good film former and has excellent water vapor and UV light barriers at low RH; however, due to its hydrophilic nature, it is poor moisture barrier compared to synthetic film. The study of Kowalczyk et al. (2016) revealed that the combination of PPI with different lipid materials to form emulsion films was an effective way to enhance functionality of PPI film, among which candelilla wax was most effective in enhancing the water vapor barrier property and simultaneously provoked the lowest increase in OP and the lowest decrease in the mechanical strength of the films. In addition, the mixed films formed by PPI and other polymers (e.g., hydrophobic protein or polysaccharide) had better moisture barrier and mechanical properties than that of any individual polymer. For example, incorporation of maize zein (MZ) into PPI film-forming solution improved flexibility, surface hydrophilicity,

**TABLE 11** The summary of properties of films formed by pea protein and other polymers

Compositions	Conditions	Results	Contrast	References
Protein-alone PPI	Plasticizers: glycerol (3% to 7% w/w) or sorbitol (4% to 8% w/w), pH (7.0, 9.0, and 11.0), nonheating or heating (90 °C, 20 min), casting	<ul style="list-style-type: none"> <li>✓ All firms had better barrier properties to UV light</li> <li>✓ Films plasticized with sorbitol had lower WVP</li> <li>✓ pH and heating had no effect on WVP</li> <li>✓ Heating enhanced film transparency</li> </ul>	PPI films prepared at different conditions	Kowalczyk and Baraniak (2011)
PPI		<ul style="list-style-type: none"> <li>✓ Films plasticized with sorbitol had higher TS, EM, PS and lower EBA</li> <li>✓ Higher plasticizer content reduced TS, EM, and PS, but did not affect EBA</li> <li>✓ Higher pH improved EBA, PS and TS of only glycerol-plasticized films</li> <li>✓ Heating improved mechanical strength</li> </ul>		Kowalczyk et al. (2013)
PPI	PPI/glycerol ratios (80/20, 70/30, 60/40, and 50/50), injection molding	<ul style="list-style-type: none"> <li>✓ PPI/glycerol (70/30 and 60/40) films had better processability</li> </ul>	PPI/glycerol films at different ratios	Perez et al. (2016)
PPI	PPI/glycerol (60/40) film, parameters: moulding time (100, 200, and 300 s) and injection pressure (100, 300, 500, and 900 bar), injection moulding	<ul style="list-style-type: none"> <li>✓ Higher moulding time increased breaking strain, reduced transparency and YM</li> <li>✓ Higher injection pressure increased breaking strain</li> </ul>	Films at different injection parameters	Perez-Puyana et al. (2016)
PPI	PPI/glycerol (60/40) film, parameters: mixing speed (10, 30, 50, 70, and 90 rpm) and mixing time (1 to 180 min), injection molding	<ul style="list-style-type: none"> <li>✓ The film had better processability at mixing speeds (30 rpm) and mixing times (1 and 10 min)</li> </ul>	Films at different mixing parameters	Carvajal-Pinero et al. (2019)
PPI	pH (7.0, 8.0, and 9.0), nonheating or heating (90 °C, 20 min), casting	<ul style="list-style-type: none"> <li>✓ PPI film had the most properties (highest TS and transparency)</li> <li>✓ Higher pH improved transparency</li> <li>✓ Heating improved TS and decreased WVP</li> </ul>	KBI film and API film	Shevkani and Singh (2015)
PPI-based	Lipid (AMF, CNW, LEC and OLA) and content (0.5%, 1.0%, 1.5%, and 2.0%), different lipid was added to sorbitol-plasticized PPI film-forming, casting	<ul style="list-style-type: none"> <li>✓ All firms had barrier properties to UV light</li> <li>✓ Lipid-added increased OP, reduced mechanical strength (TS, EM, PS and EBA)</li> <li>✓ CNW-added films had the most properties (lower WVP, higher OP and transparency)</li> </ul>	PPI film and various lipid-added films	Kowalczyk et al. (2016)

(Continues)

TABLE 11 (Continued)

Compositions	Conditions	Results	Contrast	References
Protein based-active ingredient PPI- $\alpha$ -tocopherol	60 or 100 mg $\alpha$ -tocopherol g/protein was added to PPI film-forming solution, RH: 33%, 53%, and 75 %, homogenization conditions: rotor–stator or microfluidizer, casting.	<ul style="list-style-type: none"> <li>✓ <math>\alpha</math>-tocopherol addition provided antioxidant properties of films</li> <li>✓ At 33% and 53% RH, <math>\alpha</math>-tocopherol addition decreased WVP and OP, but did not affect transparency</li> <li>✓ At 53% and 75% RH, <math>\alpha</math>-tocopherol in microfluidized films increased resistance to break and extensibility</li> </ul>	PPI film	Fabra, Jimenez, et al. (2014)
PPI-lysozyme	50, 75, or 100 mg lysozyme g/protein was added to PPI film-forming solution, casting.	<ul style="list-style-type: none"> <li>✓ At 10 °C, two types of films had effective antimicrobial activity</li> <li>✓ At 25 °C, antimicrobial effectiveness significantly decreased and PPI-lysozyme films showed the best antimicrobial activity</li> </ul>	Corn starch-lysozyme film at the same preparation conditions	Fabra, Sanchez-Gonzalez, et al. (2014)
PPI-nisin	2% or 4% nisin was added to PPI film-forming solution, injection molding.	<ul style="list-style-type: none"> <li>✓ Nisin addition inhibited growth of Gram-positive bacteria, improved YM and reduced breaking strain</li> </ul>	PPI film	Perez-Puyana et al. (2017)
PPI/polyvinyl alcohol (PVA)-cinnamaldehyde (CA)	<ul style="list-style-type: none"> <li>• PPI/PVA ratios (80/20, 70/30, 60/40, 50/50, 40/60, 30/70, and 20/80), pH (2.0, 7.0, and 9.0)</li> <li>• CA (0.25, 0.5, 1, and 1.5 wt %) was added to PPI/PVA (50/50), electrospinning.</li> </ul>	<ul style="list-style-type: none"> <li>✓ PPI/PVA (50/50, 40/60, 30/70, and 20/80) at pH 9.0 formed homogeneous nanofibers</li> <li>✓ CA addition inhibited growth of Gram negative (at 1 and 1.5 wt %) and Gram positive (at 0.5, 1, and 1.5 wt %) bacteria</li> </ul>	No	Maftoonazad et al. (2019)
Protein-polymer PPI-Maize zein (MZ)	PPI/MZ ratios (0/100, 10/90, 20/80, 30/70, 40/60, 50/50, 60/40, 70/30, 80/20, 90/10, and 100/0), extrusion and hot press processing.	<ul style="list-style-type: none"> <li>✓ PPI into MZ enhanced the flexibility and surface hydrophilicity of MZ film</li> <li>✓ PPI-MZ (10/90, 20/80, and 30/70) films had better flexibility, surface hydrophilicity, and cytocompatibility</li> </ul>	MZ film	Liu et al. (2010)
PPI-pullulan (PUL)	PPI/PUL ratios (20/80, 50/50, and 80/20) containing Tween 80 (10 and 20 wt %), pH 12, electrospinning.	<ul style="list-style-type: none"> <li>✓ PUL addition increased YM, maximum stress, and breaking strain</li> </ul>	PPI alone did not form electrospun film	Aguilar-Vazquez et al. (2018)

Abbreviations: PPI, pea protein isolate; AMF, anhydrous milk fat; CNW, candelilla wax; LEC, lecithin; OLA, oleic acid; WVP, water vapor permeability; OP, oxygen permeability; TS, tensile strength; EM, elastic modulus; PS, puncture strength; YM, Young's modulus; EBA, elongation at break; KBI, kidney bean isolate; API, amaranth protein isolate; RH, relative humidity.

and cytocompatibility of MZ film (Liu et al., 2010). PPI did not form electrospun film until pullulan was added to protein solution (Aguilar-Vazquez, Loarca-Pina, Figueroa-Cardenas, & Mendoza, 2018). Some bioactive compounds, such as  $\alpha$ -tocopherol (Fabra, Jimenez, Talens, & Chiralt, 2014), nisin (Perez-Puyana et al., 2017), cinnamaldehyde (Maftoonazad, Shahmirian, John, & Ramaswamy, 2019), and lysozyme (Fabra, Sanchez-Gonzalez, & Chiralt, 2014), have been added into PPI-based film-forming solution to produce antioxidative and antimicrobial films, thus extending the shelf life and safety of food. Moreover, the addition of functional ingredients might have some effects on WVP, OP, and mechanical properties of the film, as shown in Table 11.

### 5.3 | Use of pea protein in extruded products

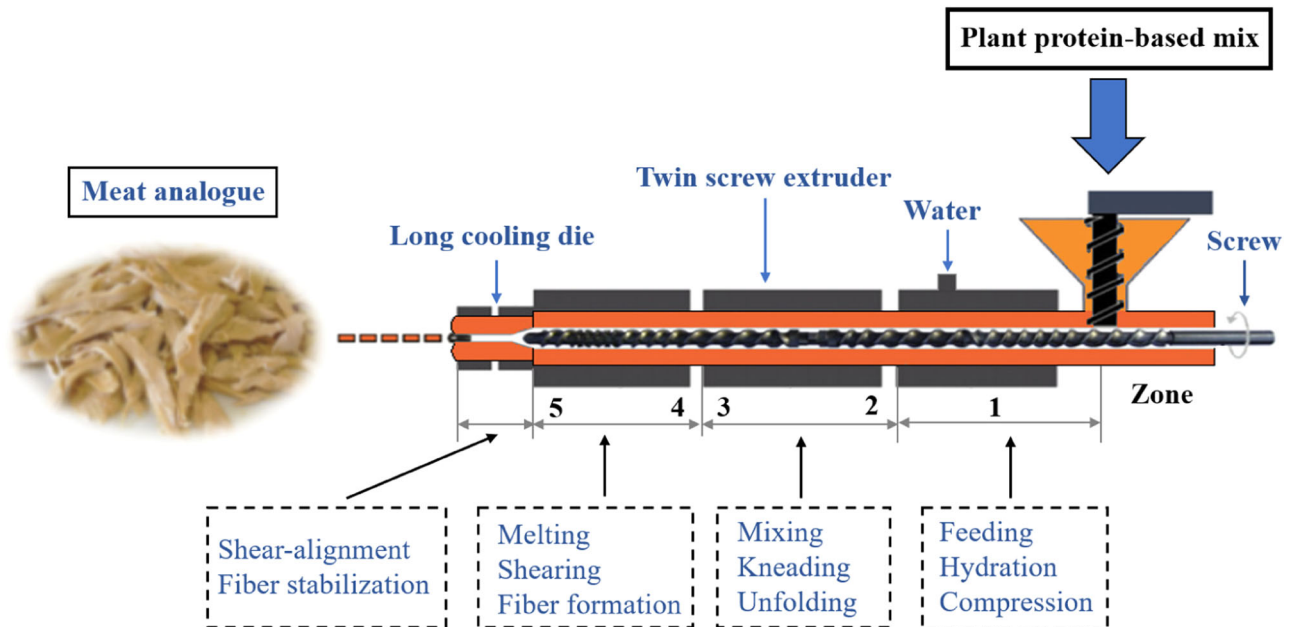
Extrusion techniques include low-moisture extrusion (LME; <35%) and high-moisture extrusion (HME; >40%), both of which are widely used in commercial food production. Generally, LME is used for extruded snacks preparation, whereas HME is used for meat analogue preparation. Nowadays, the research of extruded products based on pea protein has been extensively reported. Many studies reported that PPI was added to different starches such as rice starch (Beck et al., 2018; Philipp, Buckow, Silcock, & Oey, 2017; Philipp, Oey, Silcock, Beck, & Buckow, 2017; Philipp et al., 2018), wheat starch (Lopez-Baron et al., 2018), and corn grits (Garcia-Segovia, Igual, Noguero, & Martinez-Monzo, 2020) to prepare protein-fortified extruded snacks by LME, and the results demonstrated that PPI-fortified extruded products exhibited high protein content and balanced amino acid profile compared to pure starch extrudates. Furthermore, PPI-fortified extrudates with rich nutrition and desirable physicochemical characteristics could be prepared by controlling the protein content and extrusion process parameters. Philipp, Oey, et al. (2017) and Beck et al. (2018) indicated that adding intermediate PPI amount (10% and 20%, w/w) significantly improved the expansion behavior and microstructure of the pure rice starch extrudates, whereas the extrudate containing up to 50% PPI exhibited the poorest expansion, highest bulk density, and the biggest hardness structure. The study of Garcia-Segovia et al. (2020) revealed that the expansion behavior of corn grits-based extrudate was not strongly affected by the addition of 5% PPI. An increase of extruder screw speed from 400 to 600 rpm resulted in remarkably improved expansion of rice starch-pea protein snacks, whereas increasing die temperature (from 130 to 150 °C) or moisture content (from 23% to 26%) did not significantly change expansion of different extrudates (Philipp, Buckow, et al., 2017; Philipp, Oey, et al., 2017). In general, extruded product with crisp, expanded, and light texture is considered a promising snack in market. Philipp, Buckow, et al. (2017) showed the texture of highly expanded extrudates as crisp, whereas less expanded extrudates were

perceived as hard, crunchy, and noncrispy. In addition, Lopez-Baron et al. (2018) also studied the effect of PPI on the digestibility of starch in protein-fortified extrudate, and found that protease-hydrolyzed PPI (not native PPI) suppressed the *in vitro* digestibility rate of wheat starch in extruded protein-fortified extrudate and thus delayed the release of soluble glucans and glucose. Overall, under the appropriate preparation conditions, pea protein-fortified starch-extruded products not only have higher nutritional benefits, but also have superior texture characteristics, which provide a direct basis for market production.

In recent years, researchers and entrepreneurs have been exploring the preparation of pea protein-based meat analogue products using high-moisture extrusion technique (Osen et al., 2014; Osen, Toelstede, Eisner, & Schweiggert-Weisz, 2015; Samard & Ryu, 2019; Schreuders et al., 2019). Pea protein-based meat analogue products manufactured by Beyond Meat was available in 2019. The flow chart of plant proteins-formed mimic meat by twin-screw extruder at high moisture is shown in Figure 2 (Chen, Wei, & Zhang, 2011; Liu & Hsieh, 2008). Several studies have reported that pea protein can form fibrous meat-like structure using twin-screw extruder at high moisture, but the fibrous structure of meat analogue was significantly affected by extrusion conditions (Osen et al., 2014; Osen et al., 2015; Samard & Ryu, 2019). For example, Osen et al. (2014) showed that cooking temperature significantly affected the cutting strength and degree of texturization of PPI-prepared meat analogue, and extrudate only exhibited a dough-like soft texture without any fibrous structures below 120 °C. Unlike twin-screw extruder, Schreuders et al. (2019) prepared meat analogues using PPI/wheat gluten blends through a high-temperature conical shear cell device, and they also suggested that shearing temperatures greatly affect fibrous morphology of meat analogue. PPI/wheat gluten blends formed distinct fibrous morphology when sheared and heated at 120 °C; however, there was a weak product without fibers at low temperature. In addition to the advantages of hypoallergenic and non-GMO, the texture characteristics of pea protein-prepared bionic meat were also comparable to that of soybean protein (Samard & Ryu, 2019; Schreuders et al., 2019). Till date, researchers mainly focus on the study to mimic the appearance and texture of fibrous whole-muscle meat; there is no information about sensory and digestibility analysis of PPI-formed meat analogue. Therefore, future research should pay more attention to these areas and do more meaning works.

### 5.4 | Use of pea protein in flour products

Studies have shown that addition of pea protein to cereal products improved the nutritional values of these products by providing the essential amino acid profile, meanwhile, the texture and structure were also affected at various degrees



**FIGURE 2** The flow chart of plant protein-based material prepared meat analogue through high-moisture extrusion. Information from Liu and Hsieh (2008) and Chen, Wei, and Zhang (2011)

(Bustillos, Jonchere, Garnier, Reguerre, & Valle, 2020; Morales-Polanco, Campos-Vega, Gaytan-Martinez, Enriquez, & Loarca-Pina, 2017; Narciso, & Brennan, 2018; Song & Yoo, 2017; Wee, Loud, Tan, & Forde, 2019). Compared to commercial crackers using wheat flour as a principal ingredient, the cracker prepared by dehulled oat and pea protein exhibited lower hardness (19.04 N) and gumminess (4.07 N) and higher values of cohesiveness (0.35), springiness (0.45 mm), and chewiness (0.35) (Morales-Polanco et al., 2017). The pasta-like food made from PPI and pea dietary fiber (PF) at 100 PPI and 90/10 PPI–PF blends showed highest strength and extensibility, whereas the E-modulus was similar for all the blends (around 38 MPa) (Muneer et al., 2018). The incorporation of PPI remarkably reduced the viscosity parameters of rice noodle, and further addition of green tea extract (GTE) significantly recovered these values. In addition, the rice-substituted noodle with added PPI and GTE not only exhibited better cooking and viscoelasticity properties, but also had higher antioxidant activity (Song & Yoo, 2017). The study of Narciso and Brennan (2018) indicated that in PPI-fortified rice starch products, pea protein could be effectively digested without causing a significant increase in blood glucose levels. Although addition of native or denatured PPI did not affect obviously product texture and sensory perceptual characteristics of wheat noodles, the noodle with added denatured PPI had a reduction in in vitro glucose release (Wee et al., 2019). PPI with various contents (0%, 10%, 20%, 30%, and 40%) was added in substitution to wheat flour to form five batters: the batter air volume fraction decreased with increasing PPI content, thereby increasing the density and apparent Young modulus of cake (Bustillos et al., 2020). Gluten-free bread

is known for its poor nutritional quality, crumbly texture, and light color. The incorporation of PPI not only increased nutritional quality of starch-based gluten-free breads, but also improved the color of their crust (Pico, Reguilon, Bernal, & Gomez, 2019; Shevkani & Singh, 2014).

### 5.5 | Use of pea protein in substitution for fat or animal protein products

Plant protein could be used as a substitution for fat or animal protein to meet the need of lacto-vegetarians and make food healthier. Several studies have explored partly or fully substituting dairy proteins by pea protein in emulsion or gel products and the impact on structure and taste of these products (Ben-Harb et al., 2020; Ben-Harb et al., 2018, 2019; Klost & Drusch, 2019b; Youssef, Lafarge, Valentin, Lubbers, & Husson, 2016). The partial substitution of milk protein with PPI did not improve the texture and flavor of the yoghurt gel, and 10% PPI was most similar to conventional dairy products (Youssef et al., 2016). The study of Klost and Drusch (2019b) showed the addition of rapeseed oil and/or oat fiber could strongly increase the shear modulus and the maximum structuring velocity of yoghurt-style gels containing 10% PPI, but the taste of PPI yoghurt alternative still needs to improve. In contrast, Ben-Harb et al. (2019) revealed that 100% PPI emulsion after fermenting generated a roasted/grilled aroma, whereas the fermented emulsion containing 50% PPI and 50% milk protein released a fruity, lactic aroma. Further, Ben-Harb et al. (2020) indicated that aromatic components from 100% PPI formed gel were mainly smoked/onion/garlic, whereas those in the mixed gel (50% PPI and 50% milk protein) were



perceived as dairy/cheese/fruit. According to Lin, Tay, Yang, Yang, and Li (2017), the eggless cake containing PPI, 0.1% xanthan gum, and 1% soy lecithin as egg substitutes had similar physical properties to traditional cakes in terms of specific gravity, crumb color, and crumb pore characteristics. The study of Liu et al. (2020) reported that phosphorylated PPI mixed with 0.4% (w/v) xanthan gum was used as fat mimics to substitute the light cream in mango mousse cake, and showed that up to 20% of light cream could be replaced without affecting its taste. Compared to chicken nugget, the chicken nugget with added PPI as a meat substitute displayed high protein content and WHC, low cooking loss, and acceptable sensory score (Shoaib, Sahar, Sameen, Saleem, & Tahir, 2018).

## 5.6 | Use of pea protein in other products

At present, although there are few studies on pea protein in three-dimensional printing product and beverage, it is also necessary to summarize it, thus providing some references for researchers. Feng et al. (2018) studied the effect of PPI on the properties of potato starch-based three-dimensional printing materials, and indicated that the structural and physicochemical properties of printing products changed regularly with increasing PPI content and the best printing quality was obtained at 1% PPI. Tan et al. (2018) showed that the sugar-sweetened beverages with added PPI reduce postprandial glycemic responses, compared to the carbohydrate-matched control beverage with negligible amount of protein.

## 6 | CONCLUSIONS AND FUTURE DIRECTIONS

As being a clean label plant protein, pea protein plays a key role in healthy diet and food industry. Pea protein, especially its hydrolysates obtained by enzymatic treatment or combined treatment, has better antioxidant, antihypertensive, and modulating intestinal bacteria activities, which help manage disease symptoms and promotes human health. However, it is still necessary to perform new investigations on pea protein and its bioactive peptides to demonstrate specifically the mechanisms of action that contribute to the health benefits. Additionally, pea protein provides comparable WHC and OHC and emulsifying, foaming, and gelling properties that allow it to meet current consumer demands to substitute plant-based protein sources. These functional properties of pea protein are dependent upon the cultivar, protein fraction, extraction method, and surrounding environmental conditions. Overall, chemical, physical, enzymatic, and combined modification methods can improve the functionality of pea protein, among which chemical and enzymatic modifications are the most effective ways to enhance the solubility and emulsifying prop-

erties of pea protein, so as to broaden its market potential in the food industry.

At present, the studies about the application of pea protein in food system are extensive and mainly focused on encapsulation for bioactive ingredients, edible films, extruded foods, and substitution for cereal flours, fats, and animal proteins. Although meat analogue obtained by high moisture extrusion has been researched, the sensory, nutritional, and digestibility properties of pea protein-based meat analogue products are also topics worthy of future study. Meanwhile, how to expand the commercial application of pea protein in flour and dairy products without affecting the texture and flavor of food still needs to further explore. Additional research is also needed to investigate the application of pea protein in three-dimensional printing product and beverage.

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## AUTHOR CONTRIBUTIONS

Jiao Ge searched and analyzed the literature and wrote the initial manuscript. Cui-Xia Sun, Harold Corke, Khalid Gul, Ren-You Gan, and Yapeng Fang critically revised the article. Ren-You Gan and Yapeng Fang conceived the idea and scientific guidance through the process and edited the manuscript. The final version was approved by all authors.

## CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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