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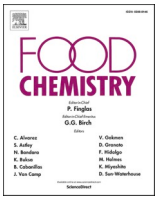

Improving functional properties of pea protein through "green" modifications using enzymes and polysaccharides

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ABSTRACT

Pea proteins have gained significant interest in recent years. The objective of this study was to enhance pea protein functional properties through enzymatic and/or conjugation modifications and understand the physicochemical properties of the modified proteins. Molecular changes of the proteins were characterized, and protein functionality, *in vitro* digestibility, and sensory properties were analyzed. The proteins crosslinked with transglutaminase showed significantly improved water holding capacity (5.2–5.6 g/g protein) compared with the control pea protein isolate (2.8 g/g). The pea proteins conjugated with guar gum showed exceptional emulsifying capacity (EC) and stability (ES) of up to 100% compared with the control protein (EC of 58% and ES of 48%). Some sequentially modified pea proteins, such as transglutaminase crosslinking followed by guar gum conjugation had multiple functional enhancement (water holding, oil holding, emulsifying, and gelation). The functionally enhanced pea proteins had comparable sensory scores as the control protein.

1. Introduction

The demand for food proteins is continually increasing worldwide, due to the rapid growth of global population and needs for healthy and nutritious diets. Proteins are the essential building blocks and dietary macronutrients for human body. In addition to the nutritional value, protein ingredients deliver crucial techno-functional properties that contribute to food quality and sensory characteristics (Chen et al., 2021). In recent years, plant proteins have attracted more attention from consumers because of their lower cost, energy efficiency, and environmental sustainability compared with animal proteins (Li, 2020).

Pea protein is one of the most used plant proteins, after wheat gluten and soy proteins. It contains high levels of lysine, threonine, and tryptophan and has good digestibility, non-transgenicity, and low allergenicity (Fang, Xiang, Sun-Waterhouse, Cui, & Lin, 2020; Xiong et al., 2018). However, the commercial utilization of pea protein is still relatively limited, owing to its less desirable functional characteristics in some applications and beany flavor (Zha, Dong, Rao, & Chen, 2019b), which may be improved through physical, chemical, or enzymatic modifications. When pea protein suspension with higher concentration was served, people could feel the gritty texture, and lumps could get adhere to throat during swallowing (Fang et al., 2020).

Enzymatic deamidation using protein glutaminase was reported to modify pea proteins, which converts some amide groups (glutamine or asparagine) to carboxyl groups (glutamic acid or aspartic acid) (Chen et al., 2021; Fang et al., 2020). The deamidation modification increased the concentration of negatively charged carboxyl group and exposed some hydrophobic side chains of the protein, which shifted the isoelectric point to the acidic side (Fang et al., 2020; Jiang et al., 2015). Some protein functional properties, such as solubility, foaming capacity, and emulsifying stability were improved through the enzymatic deamidation under appropriate conditions (Kunarayakul, Thaiphanit, Anprung, & Suppavorasatit, 2018). Previous studies reported that the enzymatic deamidation enhanced protein solubility of wheat gluten (Yiehui Yong, Yamaguchi, & Matsumura, 2006), zein (Yong, Yamaguchi, Gu, Mori, & Matsumura, 2004), and oat proteins (Jiang et al., 2015). Sensory profiles affected included enhanced umami and reduced bitter flavor in deamidated wheat gluten, and reduced beany taste and lumpiness in deamidated pea protein (Fang et al., 2020; Liu, Zhu, Guo, Peng, & Zhou, 2017). Transglutaminase is another enzyme commonly used to modify food proteins, and it catalyzes the covalent crosslinking between amino group on lysine residues and carboxyamide group on glutamine residues in protein (Marco, Pérez, Ribotta, & Rosell, 2007). This modification can convert some soluble proteins to insoluble higher

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molecular weight polymers through inter- and intra-molecular interactions (Sun & Arntfield, 2011). In addition, many studies reported that pea protein modified by transglutaminase had enhanced gelation property (Shand, Ya, Pietrasik, & Wanasundara, 2008; Sun & Arntfield, 2011).

Protein-polysaccharide conjugation is another green approach to modify the protein through glycosylation reaction between the carbonyl groups of polysaccharide and amine groups of protein. The conjugation modification enhances protein hydrophilicity and affects the balance of protein hydrophilicity and hydrophobicity. The modified protein may have more favored protein-water interaction, resulting in some improved functional properties, for example, emulsification property (Zha, Dong, Rao, & Chen, 2019a). Pea proteins conjugated with pectin, gum arabic, and soybean polysaccharide showed improved emulsifying, foaming properties, solubility, and thermal stability (Lan, Chen, & Rao, 2018; Zha, Yang, Rao, & Chen, 2019).

Previously, we investigated the effect of acylation or/and conjugation on pea protein functionalities, and we found that the sequential acylation and conjugation modifications had exceptional synergistic and positive effects on protein emulsification, oil holding capacity, and gelation properties (Shen & Li, 2021). Because of the concerns of using synthetic chemicals such as acetic anhydride or succinic anhydride (21CFR172.892) during acylation modification, the aim of this study was to develop greener approaches based on enzymes and natural polysaccharides for protein functional enhancement. Although some previous studies have reported the functional improvement of plant proteins through enzymatic or conjugation modification alone with different enzymes or polysaccharides, combining both modifications may deliver some synergistic effects and produce more functional protein ingredients. Therefore, the objective of this study was to enhance the functional properties of pea protein through sequential enzymatic modification and polysaccharides conjugation, in comparison with enzymatic modification or polysaccharide conjugation alone, and understand the physicochemical and sensory properties of the modified proteins. The new modification methods have many advantageous natures, such as clean-label, mild reaction, safety, and efficiency. The newly modified and functionally enhanced pea proteins will further expand the uses of plant proteins in broader food applications and better meet the increasing protein demands.

2. Materials and methods

2.1. Materials

Yellow pea flour was provided by ADM (Chicago, IL, USA). Guar gum (Judee's, Plain City, OH, USA), gum arabic (Fisher Scientific, Hampton, NH, USA), protein-glutaminase (Amano Enzyme Inc, Nagoya, Japan), and transglutaminase (Modernist pantry, Eliot, ME, USA) were used as received. Other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Preparation of pea protein isolate

The yellow pea flour was first defatted with hexane. The defatted yellow pea flour was dispersed in distilled water at a 10% solid concentration. The pH was adjusted to 8.5 using 1.0 M NaOH, and the slurry was mixed at 500 rpm for 1 h at room temperature. Then, the slurry was centrifuged at 8000 × g for 20 min at 4 °C. The supernatant was collected, and pH was adjusted to 4.5 using 1.0 M HCl, which was then allowed to precipitate the protein at 4 °C for 2 h. After that, the protein was recovered by centrifugation (8000 × g, 20 min), washed twice using distilled water, and re-adjusted to pH 7.0. Finally, the protein suspension was lyophilized and stored at 4 °C for further study.

2.3. Preparation of modified pea proteins

Enzymatically modified pea proteins were prepared by reacting the protein (10% concentration in water) with 1% (protein basis) transglutaminase at 40 °C or 1% (protein basis) protein-glutaminase (pH 6.5) at 55 °C for 3 h, respectively. At the end of the reaction, the protein slurry was heated to 100 °C to inactivate the enzyme. Conjugated pea proteins were prepared by incubating the protein (10% concentration in water) with 5% guar gum or gum arabic (protein basis) at 60 °C for 24 h. Enzyme treated/polysaccharide conjugated proteins were also prepared to investigate their synergistic effects, where after the deactivation of the enzyme, the protein slurry was added with guar gum/gum arabic (5%, protein basis) at 60 °C for 24 h. The slurries of modified proteins were lyophilized and stored at 4 °C till further analysis.

2.4. Functional properties

Protein functional properties, including solubility, emulsifying properties, water and oil holding capacities, and least gelation concentration were determined using our previous methods without modification (Shen & Li, 2021).

2.5. Physicochemical properties and in vitro gastrointestinal digestibility

Protein physicochemical properties, including free sulfhydryl group content, free amino group content, protein secondary structures, surface hydrophobicity, and *in vitro* gastrointestinal digestibility were determined following previous methods without any modification (Shen & Li, 2021).

Size exclusion chromatography (*SEC*-HPLC) was conducted to estimate molecular size changes of pea proteins with different modifications. The protein sample (1 mg/mL) was dispersed in sodium phosphate buffer (pH 6.8). The suspension was vortexed and vigorously mixed for 1 hr to dissolve the protein, followed by centrifugation at 4000 × g for 5 min. The supernatant was collected and filtered through a 0.45 μm filter (Biomed Scientific, Forest, VA, USA). The protein separation was achieved using a Phenomenex *SEC*-4000 column (7.8 × 300 mm) at 30 °C with Agilent 1100 HPLC system (Santa Clara, CA, USA). The mobile phase included phase A (water with 0.1% trifluoroacetic acid) and phase B (acetonitrile), with gradient elution of 20% phase B at 0 – 20 min, 30% phase B at 20 – 25 min, 35% phase B at 25 – 40 min, and 20% phase B again at 40 min to elute all the residues. Flow rate was set at 0.7 mL/min. Proteins were detected at 214 nm using a diode array detector (Agilent, Santa Clara, CA, USA). Standard proteins with known molecular sizes, including thyroglobulin bovine (670 kDa), γ-globulins from bovine blood (150 kDa), bovine serum albumin (60 kDa), and chicken egg grade VI albumin (44 kDa) (Sigma-Aldrich, St. Louis, MO, USA) were analyzed under the same chromatography conditions to estimate the molecular weight of the pea proteins.

2.6. Sensory analysis

Descriptive sensory analysis of pea and the modified pea proteins was conducted by six well-trained panelists to determine the flavor characteristics, including beany, starchy, grain, green, powdery mouthfeel, umami, sweet, astringent, bitter, and metallic flavors. The descriptive analysis was conducted using an intensity scale with 0.5 increments (0 = none; 15 = extremely intense). For each protein sample, 1.2 g protein was dispersed in 30 mL distilled water to obtain an aqueous dispersion of 4% (Cosson et al., 2020). The protein dispersion was placed in a transparent cup with a lid labeled with a randomly selected three-digit code. Before being served, the panelists manually remixed the suspension to achieve a homogenous dispersion. Pure water, unsalted crackers, and mozzarella cheese were used for mouth rinsing between samples to avoid any carry-over effect. The panelists completed one 1 h orientation session in order to align on the attributes and

Table 1
Physicochemical properties including free sulfhydryl group content, free amino group content, secondary structures of pea and modified pea proteins. *Means with different letters in each column indicate significant differences ($p < 0.05$). ** ND: not detected.

Samples	Free SH (μmol/g)	Free NH_2 (mmol/g)	α-Helix (%)	β-Sheet (%)	β-Turn (%)	Random coil (%)	Hydrophobicity (H0)
Control	13.52 ± 0.09^{a}	8.44 ± 0.06^{a}	18.64 ± 0.09 ^{cd}	27.52 ± 4.37^{bc}	11.48 ± 2.27^{bc}	42.37 ± 6.55^{a}	202,096 ± 12,306^{b}
PG	9.91 ± 0.06^{c}	7.53 ± 0.13^{b}	53.72 ± 0.48^{a}	26.67 ± 2.96^{bc}	19.61 ± 2.48^{a}	ND	161,826 ± 1,274^{d}
TG	11.86 ± 0.08^{b}	5.30 ± 0.06^{c}	21.97 ± 1.60^{c}	60.69 ± 3.30^{a}	17.33 ± 1.70^{ab}	ND	73,910 ± 1,500^{f}
Guar	7.68 ± 0.02^{d}	7.31 ± 0.16^{b}	37.62 ± 1.56^{b}	52.54 ± 0.78^{a}	9.84 ± 0.77 ^{cd}	ND	93,342 ± 1,099^{e}
Arabic	6.46 ± 0.03^{ef}	7.56 ± 0.22^{b}	41.20 ± 10.39^{ab}	48.08 ± 9.75^{ab}	10.71 ± 0.64 ^{cd}	ND	105,724 ± 1,995^{e}
PG-Guar	5.61 ± 0.00 ^{g}	7.34 ± 0.29^{b}	9.66 ± 0.38 ^{cd}	47.96 ± 1.33^{ab}	6.88 ± 0.10 ^{cd}	35.51 ± 1.05^{a}	186,742 ± 3,243^{c}
PG-Arabic	4.89 ± 0.04 ^{h}	7.41 ± 0.22^{b}	7.39 ± 1.02^{d}	58.92 ± 1.72^{a}	4.85 ± 0.28^{d}	28.84 ± 2.46^{a}	230,281 ± 1,223^{a}
TG-Guar	6.68 ± 0.04^{e}	5.39 ± 0.06^{c}	10.29 ± 2.14 ^{cd}	56.58 ± 12.13^{a}	6.05 ± 0.80 ^{cd}	27.08 ± 15.06^{a}	28,158 ± 1,846 ^{g}
TG-Arabic	6.28 ± 0.15^{f}	5.19 ± 0.10^{c}	20.90 ± 0.54 ^{cd}	22.33 ± 4.19^{c}	8.86 ± 2.34 ^{cd}	47.91 ± 5.99^{a}	22,563 ± 1,098 ^{g}

reference materials and three 1 h evaluation sessions. The evaluation was completed based on a modified flavor profile approach using consensus (Koppel & Koppel, 2018). The references and definitions of flavor attributes used for this study were provided in the Supplementary Document. The sensory analysis was approved by the KSU Institutional Review Board committee, IRB-5930.

2.7. Statistical analysis

All the tests were conducted in at least duplicates, and the results were presented as mean ± standard deviation (SD). All the results were evaluated by one-way ANOVA, and Tukey's post-hoc test was conducted using SAS University Edition software (SAS Institute, Cary, NC, USA) to assess the significant differences ($p < 0.05$) among different treatments.

3. Results and discussion

3.1. Free sulfhydryl group and free amino group

The free sulfhydryl (SH) content of the control and modified pea proteins is summarized in Table 1. The enzymatically modified and/or conjugated pea proteins showed significantly reduced free SH content than the control pea protein (13.5 μmol/g). The pea protein deamidated by PG, crosslinked by TG, and conjugated with guar gum or gum arabic all had decreased free SH group, which was attributed to the fact that the mechanical mixing in air condition during the modification processes favored the oxidation reaction by converting some free SH groups to disulfide bonds (Netto et al., 2007). The conjugated proteins exhibited significantly lower free SH group content than the enzymatically modified proteins, which was ascribed to the higher reaction temperature during the conjugation than the deamidation and crosslinking reactions; thus, more disulfide linkages were formed. The sequentially modified proteins exhibited even lower free SH group content than the proteins from deamidation or crosslinking reaction alone, which is because the former proteins underwent heat treatments twice during the combined modifications.

Free amino group content indicates the degree of enzymatic and conjugated modifications in the modified pea proteins, as the amino group was a major reaction site during the modifications. Overall, all the modified pea proteins showed significantly ($p < 0.05$) lower content of free amino group compared with the control protein (8.44 mmol/g) (Table 1). The pea protein crosslinked by transglutaminase and/or conjugated with guar gum or gum arabic exhibited the lowest free amino group content, which was attributed to formation of ε-(γ-Glu)-Lys polymers with the free aminos (Sun & Arntfield, 2011). The decreased free amino group in deamidated proteins occurred because the conversion of amide groups to carboxyl groups in the presence of protein glutaminase, as ammonia was formed, and free amino group content was reduced. The reduced free amino group in the proteins conjugated with gums was due to the Maillard reaction that consumed some amino groups (Zha et al., 2019a).

3.2. Protein secondary structures

The control pea protein consisted of 18.64% α-helix, 27.52% β-sheet, 11.48% β-turn, and 42.37% random coil (Table 1). With different modifications, the secondary structure composition was greatly changed. For example, the proteins modified by PG, TG, guar gum, and gum arabic did not have any random coils, while the proteins modified by TG, guar gum, and gum arabic had greatly increased α-helix and β-sheet, and the protein modified by PG and TG had increased β-turn, compared with the control. However, the sequential enzymatic and conjugated modifications increased the random coil, reduced β-turn, and slightly reduced α-helix contents (in PG-Guar and PG-Arabic) compared with the enzymatic or conjugated protein alone. These results demonstrated that the enzymatic or conjugated modifications enabled the protein to be unfolded, and some random structures could be converted to more regular and ordered structures. Jiang et al. (2015) reported that α-helix content was increased in deamidated oat protein compared with the control because of increased flexibility protein molecules. Further, they observed that β-sheet was decreased with higher degree of protein deamidation. Mattice and Marangoni (2021) reported that both β-sheet and random coil were increased in TG crosslinked zein. Therefore, it can be concluded that secondary structure composition of modified proteins was affected by the nature of the modification, degree of modification, enzyme and protein types, and extent of non-covalent interactions.

3.3. Surface hydrophobicity

Protein surface hydrophobicity was measured to estimate the availability of nonpolar amino acid residues exposed to the surface of the protein (Cabra, Arreguin, Azquez-Duhalt, & Farres, 2007). Overall, the enzyme modified and/or conjugated pea proteins showed significantly decreased surface hydrophobicity compared with the control, except for the PG-Arabic (Table 1). The decreased surface hydrophobicity for the protein deamidated by PG might be because the deamidation modification increased carboxylic acid residues and favored hydrophobic interactions of the protein (Chen et al., 2021). Our result agreed with that reported by Miwa et al. (2013), who showed that deamidated whey protein by protein glutaminase had decreased surface hydrophobicity. However, some other studies reported increased surface hydrophobicity for deamidated proteins, such as barley hordein (Zhao, Tian, & Chen, 2010), wheat gluten (Qiu, Sun, Cui, & Zhao, 2013), and zein (Cabra et al., 2007). Surface hydrophobicity of deamidated proteins are affected by many factors, such as protein type and original hydrophobicity/hydrophilicity, enzyme concentration, and other reaction parameters (Chen et al., 2021). The proteins crosslinked by transglutaminase (e.g., TG, TG-Guar, TG-Arabic) showed dramatically decreased surface hydrophobicity compared with the control and other modified proteins, which was attributed to the aggregated proteins formed during crosslinking and partial burial of the hydrophobic cavities in the protein core (Agyare & Damodaran, 2010), thus reducing protein surface hydrophobicity. Shen et al. (2021) indicated that freeze-dried quinoa protein

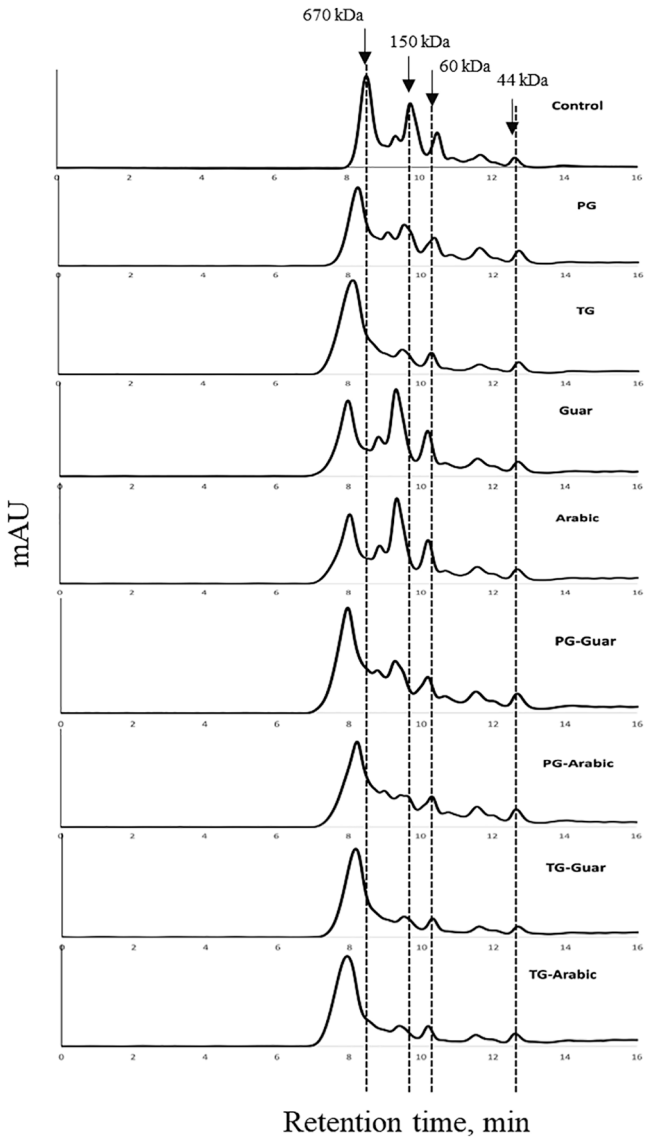

Fig. 1. *SEC*-HPLC chromatograms of the control and modified pea proteins.

had higher surface hydrophobicity than spray-dried protein, which was attributed to the extent of protein denaturation during the different drying processes.

3.4. Sec-HPLC

Four proteins with known molecular sizes, including thyroglobulin bovine (670 kDa), γ-globulins from bovine blood (150 kDa), bovine serum albumin (60 kDa), and chicken egg grade VI albumin (44 kDa), were separated with the same chromatography conditions and marked on the chromatogram as molecular weight references (Fig. 1). With enzymatic modification and/or conjugation with polysaccharides, some proteins with larger molecular sizes were formed compared to those in the control pea protein, as indicated by the left shift of the first peak (670 kDa) on the chromatograms. The modified pea proteins from conjugation alone (e.g., Guar, Arabic) had similar peak patterns as the control one, except that the peak size between 150 and 670 kDa was increased, while the peak around 670 kDa was relatively decreased, which was caused by the alteration of the sizes of medium molecule proteins during conjugation. For all the modified proteins involving enzymatic treatment, there was a dramatic decrease of peak sizes in the range of 60 to 150 kDa, which was caused by the formation of larger

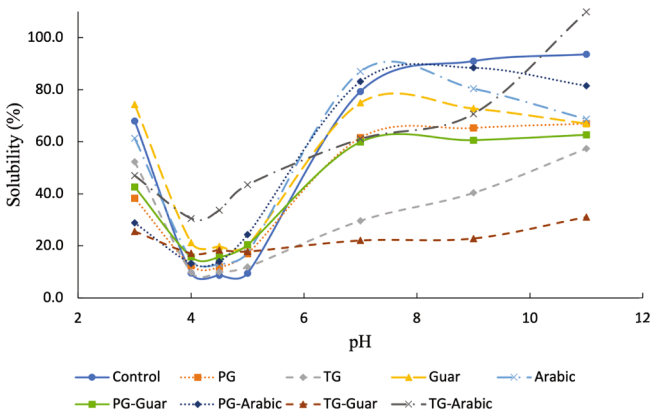

Fig. 2. Solubility of pea and modified pea proteins.

proteins (670 kDa) through various crosslinking mechanisms. The mechanical mixing during the enzymatic and conjugation modifications along with increased temperature favored the oxidation reaction to induce protein crosslinking. The PG and TG protein samples underwent enzyme deactivation (i.e., boiling the protein slurries at 100 °C for 10 min) after protein deamidation and crosslinking reactions, which also favored protein crosslinking, in addition to the enzymatically induced crosslinking reactions. Furthermore, the sequential enzymatic and conjugated proteins exhibited even larger molecular size, especially for the TG-Guar and TG-Arabic samples. Several peaks disappeared, and some small peaks were merged into one prominent peak, similar to the sample TG. This *SEC*-HPLC result can be associated with the free sulfhydryl content (Table 1) and confirmed that the modified pea protein had exhibited a larger molecular size partially due to the protein crosslinking reaction.

3.5. Solubility

The control pea protein, which was extracted from pea flour in the lab and lyophilized, exhibited great solubility when the pH was away from the isoelectric point (PI, pH 4–5). The solubility was also much better than commercial pea protein (Shen and Li (2021)), which implied that the commercial processing conditions of the proteins might cause more intensive structural denaturation that impaired the solubility (Zha, Dong, et al., 2019a). With the enzymatic and/or conjugation modifications, most of the modified pea protein had similar or decreased solubility than the control pea protein when the pH was away from the PI, while the modified pea proteins had slightly increased solubility at the PI (Fig. 2). Some of the pea proteins crosslinked with transglutaminase (e.g., TG, TG-Guar) were the least soluble at pH above the PI compared with the other modified protein samples. Pea protein contains high amount of lysine, and it favors the crosslinking reaction catalyzed by transglutaminase (Marco et al., 2007). This reaction enabled the formation of larger protein polymers, which became less soluble (Marco et al., 2007). Notably, the protein sample treated with TG and gum arabic had much greater solubility at PI and pH 11 compared with the control and TG and TG-Guar proteins, which may be attributed to the synergistic effects of transglutaminase and gum arabic modifications. Zha et al. (2019a) reported that commercial pea protein conjugated with gum arabic showed significantly improved solubility, because the less soluble 11S and 7S subunits of pea protein and hydrophilic gum arabic were involved in forming conjugates, which improved the overall solubility. Shen and Li (2021) also reported a similar finding showing improved solubility for commercial pea protein isolate conjugated with guar gum. Even for the lab extracted protein, our results showed that pea protein conjugated with gum arabic or treated with PG-Arabic had slightly increased solubility at pH 4.5–7 compared with the control and other treatments. Previous studies reported that enzymatic deamidation

Table 2
Functional properties including water holding capacity (WHC), oil holding capacity (OHC), emulsion capacity (EC), emulsion stability (ES), and least gelation concentration (LGC) of pea and modified pea proteins. *Means with different letters for each column indicate significant differences ($p < 0.05$).

Samples	WHC (g/g)	OHC (g/g)	EC (%)	ES (%)	LGC (%)
Control	2.66 ± 0.06^{f}	2.76 ± 0.05^{c}	58.58 ± 2.21^{c}	48.14 ± 1.77^{d}	11^{d}
PG	3.62 ± 0.04^{d}	2.68 ± 0.08^{c}	63.46 ± 4.95^{bc}	51.91 ± 0.95 ^{cd}	15^{a}
TG	5.31 ± 0.08^{b}	3.08 ± 0.03^{b}	94.51 ± 0.33^{a}	57.69 ± 1.39^{b}	11^{d}
Guar	3.62 ± 0.04^{d}	2.62 ± 0.04 ^{cd}	97.94 ± 0.34^{a}	96.31 ± 0.95^{a}	9^{e}
Arabic	2.66 ± 0.01^{f}	2.50 ± 0.06^{d}	57.79 ± 4.05^{c}	52.11 ± 2.81^{c}	13^{b}
PG-Guar	5.06 ± 0.02^{c}	3.36 ± 0.05^{a}	100.00 ± 0.00^{a}	97.74 ± 0.08^{a}	12^{c}
PG-Arabic	3.27 ± 0.03^{e}	2.75 ± 0.04^{c}	67.57 ± 1.48^{b}	56.71 ± 2.15^{b}	15^{a}
TG-Guar	5.62 ± 0.04^{a}	2.98 ± 0.07^{b}	100.00 ± 0.00^{a}	100.00 ± 0.00^{a}	9^{e}
TG-Arabic	5.21 ± 0.06^{b}	2.70 ± 0.02^{c}	66.51 ± 4.65^{b}	54.62 ± 1.97^{bc}	9^{e}

improved the solubility of gluten proteins (Yong et al., 2006) and zein proteins (Yong et al., 2004), because the induction of additional carboxyl groups to the protein molecules provided a newly balanced amphiphilicity that favored protein interaction with water. As for some of our modified pea proteins from deamidation and/or conjugation, the solubility was not improved, which was because the native structure of the control pea protein was more favorable to solubility, compared to the denatured and modified structures.

3.6. Water and oil holding capacity

Overall, the proteins treated by transglutaminase, for example, TG, TG-Guar, and TG-Arabic, had significantly higher water holding capacities of 5.31, 5.62, and 5.21 g water /g protein, respectively, compared with the control pea protein (2.66 g/g) (Table 2). The PG-Guar also exhibited a significantly higher water holding capacity of 5.06 g/g. Transglutaminase catalyzed covalent crosslinking between lysine and glutamine residues in forming inter- or intra- molecular ε-(γ-Glu)-Lys polymers, which resulted in larger protein molecules and more intensive protein aggregation, favoring water holding capacity (Sun & Arntfield, 2011). Further, the newly formed crosslinking structures may enhance protein gel formation with better water holding capability due to the stronger hydrogen-bonded water shown in Raman bands (Kang et al. (2016)). The pea proteins modified by protein glutaminase or guar gum alone also had improved water holding capacity up to 3.62 g/g compared with the control. With sequential modification using both protein glutaminase and guar gum, the water holding capacity was further improved to 5.06 g/g, implying synergistic effects from multiple modification approaches.

The control pea protein had an oil holding capacity of 2.76 g oil/g protein, which was more than twice of that reported for commercial pea protein (1.03 g/g) (Shen & Li, 2021). Among all the modified pea proteins, the PG-Guar protein exhibited significantly higher oil holding capacity than the control and other treatments (Table 2). However, the oil holding capacity of the protein deamidated by protein glutaminase or conjugated with guar gum alone did not significantly differ from the control protein, which may be attributed to their lower surface hydrophobicity as compared to the control or PG-Guar (Table 1). The PG-Guar treatment showed synergistic effect benefiting oil holding capacity. The oil holding capacity of pea protein conjugated with guar gum was similar to the control protein in this study, all around 2.6–2.7 g/g. Shen and Li (2021) reported that the commercial pea protein conjugated with guar gum had significantly increased oil holding capacity (2.02 g/g) than the control protein (1.03 g/g). This was because the heat treatment during the conjugation had altered and unfolded protein structures, and more hydrophobic amino acid residues were exposed, resulting in improved oil holding capacity.

3.7. Emulsifying properties

The emulsifying characteristics of proteins, including emulsion capacity (EC) and emulsion stability (ES), are affected by the rate of protein adsorption and the ability to reorganize at the oil/water interface during emulsifying. The protein molecules act as barrier against the droplet coalescence and provide steric and electrostatic repulsions against flocculation in forming stable interfacial layer (Ma, Forssell, Partanen, Buchert, & Boer, 2011). As shown in Table 2, some of the modified pea proteins possessed greatly ($p < 0.05$) improved emulsifying properties than the control pea protein (EC: 58%, ES: 48%), especially for the treatments involving guar gum, such as Guar, PG-Guar, and TG-Guar with emulsion capacity of 97–100% and emulsion stability of 96–100%. Additionally, the pea proteins that conjugated with gum arabic (i.e., Arabic, PG-Arabic, TG-Arabic) had similar emulsifying properties as the control. Gum arabic has a very different structure compared with guar gum, and it is a complex mixture of glycoproteins and polysaccharides predominantly consisting of arabinose and galactose. After conjugating with pea protein, the proteins with guar gum seem to have a more balanced hydrophilicity and hydrophobicity that favored their surface activities at oil/water interface compared to the proteins with gum arabic. Gum arabic had a relatively low hydration radius and effective volume (Bai, Huan, Li, & McClements, 2017), and it is less viscous than guar gum when applied at the same concentration in water. The conjugated proteins with gum arabic might be insufficient to span the surface of oil droplet when used at the same concentration as the protein conjugates with guar gum, resulting in the destabilization or flocculation of protein emulsions (Liu, Elmer, Low, & Nickerson, 2010).

The emulsifying properties of the protein deamidated by PG were not significantly different from the control, while the protein crosslinked by TG had significantly increased emulsion capacity and stability, although the stability was still much lower than those conjugated with guar gum. The interfacial film formed by the crosslinked protein by transglutaminase had higher resistance to destabilization, and relatively lower solubility of the crosslinked protein enabled a thicker interface with better steric stability, thus improved emulsion capacity (Nivala, Nordlund, Kruus, & Ercili-Cura, 2021). However, the absorption of the crosslinked proteins at the oil and water interface was not able to sustain the environmental stress (e.g., high temperature and shearing) during stability tests due to the larger molecular sizes and lack of molecule flexibility, which led to lower surface coverage and decreased emulsion stability (Færgemand, Otte, & Qvist, 1998). The pea protein deamidated by protein glutaminase had no significant differences with the control protein, because the protein deamidation had increased carboxylic acid residues and improved electrostatic repulsion, but it might weaken the hydrophobic interaction and hydrogen bonds, which resulted in structures that were less surface active (Chen et al., 2021). In summary, the sequential enzymatic modification and conjugation (PG-Guar and TG-Guar) had synergistic effects on the emulsifying properties, implying that protein functionalities could be better enhanced by combining different modifications approaches.

3.8. Protein gelation property

Heat-induced gelation is one of the most important functional properties of protein, as it is associated with the texture, quality, and sensory aspects of the foods. When pea protein slurry was heated above the denaturation temperature, the globulins were unfolded and rearranged to form soluble aggregates; while when the protein solution was cooled, the electrostatic repulsions were reduced between the

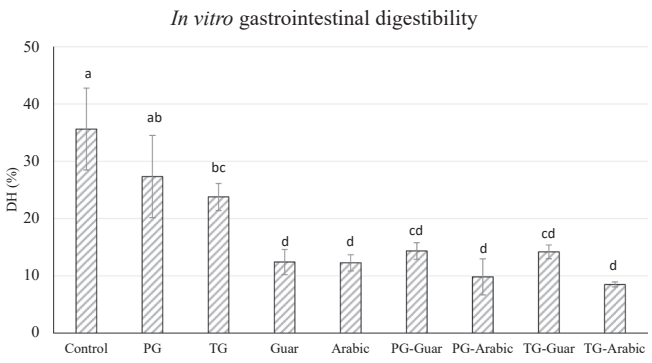

Fig. 3. *In vitro* gastrointestinal digestibility (DH%) of pea and modified pea proteins. *Means with different letters indicate significant differences ($p < 0.05$).

aggregated proteins, and the proteins were assembled to form the structured get network entrapping water molecules (Mession, Sok, Assifaoui, 2013). The control pea protein had a good gelation potential, with a least gelation concentration (LGC) of 11%, which was much lower than that of commercial pea protein (LGC of 18%). The modified pea proteins from guar gum conjugation (i.e., Guar) or transglutaminase crosslinking plus conjugation (i.e., TG-Guar, TG-Arabic) had further significantly improved gelation property with LGC of 9%, compared with the control protein (Table 2). The inclusion of guar gum during the protein conjugation can unfold the protein structure and enhance the hydrophobic interaction to create more stable and firm gel networks (Shen & Li, 2021). The addition of transglutaminase in the protein promoted the crosslinking among protein molecules and improved gelation ability (Sun & Arntfield, 2011). The proteins deamidated by glutaminase (i.e., PG, PG-Arabic, PG-Guar) had significantly decreased gelling property than the control, which might be partially attributed to the increased electrostatic repulsion between carboxylic acid groups (Miwa et al., 2013). The pea protein conjugated with gum arabic alone did not show gelation improvement, as contract to that with guar gum. This was probably related to the lower viscosity of gum arabic in water than guar gum (Saha & Bhattacharya, 2010). In addition, Alam et al. (2021) reported that the taro starch with guar gum had lower swelling power due to the fact that the tightening of starch granules restricted the exudation process, and improved gelation property. However, the gum arabic effectively facilitates the water penetration and eventually increases the swelling power due to the increased interactions between gelatinized starch granules; thus, the taro starch with gum arabic exhibited poorer gelation. Some of the polysaccharide properties may be carried over to the conjugated proteins and affect protein functional properties. The protein crosslinked by transglutaminase and followed by conjugation showed synergistic advantage in improving gelation property. These combined modification approaches could be used in many food applications that rely on protein gelation, such as condiments, meat patties, dairy, and cake batter products.

3.9. *In vitro gastrointestinal digestibility*

The digestibility of the pea and modified pea proteins was determined and presented as degree of hydrolysis of the proteins after the *in vitro* gastrointestinal digestion (Fig. 3). Overall, the modified pea proteins showed significantly decreased digestibility ($p < 0.05$) compared with the control pea protein, except for the sample PG, which was also reduced but not significantly different from the control ($p > 0.05$). The conjugated proteins and the proteins modified by a combination of enzymatic crosslinking and conjugation had increased molecular weight and were more potent to aggregate; thus, they became less accessible to the digestible enzymes as compared with the control. Gan et al. (2009) and Glusac et al. (2020) reported that soy and chickpea proteins crosslinked with transglutaminase also had decreased digestibility. The treatment of pea protein with protein glutaminase increased protein electrostatic repulsion, which may favor enzyme accessibility during digestion. Qiu et al. (2013) reported that the deamidated gluten had decreased pepsin digestibility, which was attributed to the acidic shift of the protein's isoelectric point after deamidation and resulted in more protein aggregates under pepsin digestion condition (pH = 2). However, the digestibility of the deamidated gluten was increased during pancreatin digestion due to increased solubility and loss of protein structures.

3.10. *Descriptive sensory analysis*

The sensory scores from descriptive analysis are summarized in Table 3. Overall, the modified pea proteins had comparable sensory scores for most attributes as the control pea protein, and all the modification treatments did not obviously decrease most sensory scores (Table 3). One interesting observation is that the proteins crosslinked with transglutaminase (e.g., TG, TG-Guar, TG-Arabic) had obviously increased pulpy mouthfeel (scores 3 – 5) compared with the control (score 0), which was attributed to the increased protein molecular sizes and aggregation because of crosslinking. The umami taste of several modified proteins (PG, PG-Guar, TG-Guar) was reduced to zero compared with the control (score 2). All the modified proteins had similar scores for beany related attributes (beany, green, astringent, bitter, and metallic) as the control.

4. Conclusions

Enzymatic modification and/or conjugation with polysaccharides altered pea protein secondary structure compositions, molecular sizes, surface hydrophobicity, and contents of free sulfhydryl and amino groups, thus resulting in different functional characteristics. The pea proteins conjugated with guar gum (i.e., Guar, PG-Guar, TG-Guar) had greatly enhanced emulsifying properties compared with the control pea protein. The pea proteins crosslinked by transglutaminase (i.e., TG, TG-Guar, TG-Arabic) had water holding capacity twice of that of the control. Sequential modification of pea protein with transglutaminase and guar

Table 3
Sensory descriptive analysis score of pea and the modified pea proteins.

Sample	Beany	Starchy	Grain	Green	Pulpy	Powdery mouthfeel	Umami	Astringent	Bitter	Metallic
Control	6	6	5	3	0	5.5	2	2.5	2.5	1.5
PG	5.5	5	6	3	0	5.5	0	2.5	2	1.5
TG	6	6.5	5	2.5	5	5	2	2	3	0
Guar	6.5	7	4.5	2.5	0	5.5	2	2.5	2.5	1.5
Arabic	6	4	6	3	0	5	2	2.5	2	1.5
PG-Guar	6	6	4.5	2.5	0	5	0	2	2	1.5
PG-Arabic	5	5	5	3	0	5	2	2.5	2.5	1.5
TG-Guar	6	6	5.5	2.5	3	5.5	0	2.5	3	1.5
TG-Arabic	6	5	5	3	3	6	2	2.5	2.5	1.5

Note: The descriptive analysis was conducted using intensity scale with 0.5 increments (0 = none; 15 = extremely intense). References and definition of the sensory attributes are available in the Supplementary Document.

gum (TG-Guar) led to multiple functional enhancement of pea protein, including increased water holding capacity, oil holding capacity, emulsion capacity, emulsion stability, and gelation, and decreased protein solubility. The modified pea proteins had comparable sensory scores as the control pea protein, and these modifications overall did not negatively affect protein sensory properties. However, the modified pea proteins showed decreased *in vitro* gastrointestinal digestibility compared with the control protein. The newly developed pea proteins through green modifications may expand their uses in various food applications and better meet the increasing demand for more functional plant proteins.

CRediT authorship contribution statement

Yanting Shen: Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Shan Hong:** Investigation, Writing – review & editing. **Gaganpreet Singh:** Investigation, Writing – review & editing. **Kadri Koppel:** Methodology, Investigation, Resources, Writing – review & editing, Supervision. **Yonghui Li:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.132687.

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