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Investigation of the effects of high hydrostatic pressure on the functional properties of pea protein isolate

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Abstract

This study investigates the effects of high hydrostatic pressure (HHP) on some functional properties of pea protein isolate (PPI). HHP was combined with various temperature and pH conditions to investigate the combined effects of HHP-based food processing conditions on the functional properties of PPI. Herein, PPI solutions prepared at different pH conditions (3.0, 5.0, and 7.0) were subjected to 300, 400, and 500 MPa HHP treatment at 25 and 50°C for 5 min. Water-holding capacity (WHC), solubility, and emulsification activity of PPI samples were determined. Additionally, nuclear magnetic resonance (NMR) relaxometry and Fourier transform infrared spectroscopy experiments were performed for further analysis. Maximum WHC ($p < 0.05$) was observed for the samples treated at 500 MPa-pH 5.0-50°C whereas maximum solubility ($p < 0.05$) belonged to the samples subjected to 300 MPa-pH 7.0-50°C treatment conditions. Better emulsification activity was achieved at pH 3.0 regardless of the pressure level applied. The novelty of this study is that NMR relaxometry was introduced as a fast/nondestructive technique to investigate the changes in the functional properties of PPI samples and one of the functional parameters was correlated with NMR relaxation data. Herewith, the longest transverse relaxation time (T_2) ($p < 0.05$) belonged to the samples with maximum WHC. The results showed that HHP is able to modify the functional properties of PPI at specific temperature-pH combinations, and NMR relaxometry technique has a high potential for such studies.

Practical Applications

High hydrostatic pressure (HHP) is an emerging technology which is used for its diverse range of applications in food science and technology. Modification of physicochemical and functional properties of food ingredients is one of the latest applications of HHP treatment. This study demonstrated that HHP treatment was able to modify some functional properties of pea protein isolate (PPI) samples such as water-holding capacity (WHC), solubility, and emulsification activity. In addition to pressure level, pH and temperature were also effective on modifying the functional properties of PPI samples. For instance, a high pressure (500 MPa) was required to improve WHC whereas lower pressures (300 MPa) improved the solubility of the samples at high pH and temperature. The results of this study could be used in model HHP studies to improve some functional properties of PPI for different purposes.

KEYWORDS
high hydrostatic pressure, pea protein isolate, pH, solubility, temperature, water-holding capacity

1 | INTRODUCTION

Pea proteins attract a great attention in food science and industry since they have an enormous potential for the food industry to meet emerging consumers' needs. Some of the main features of pea proteins that make them a good substitute for animal-based proteins are sustainability, high nutritional value, low cost, and allergenicity (Shevkani et al., 2019). Thus, pea proteins are used in a wide range of food processing and applications including gluten-free goods (cereal and bakery), pasta, baby foods, snack bars, and vegan/vegetarian products (Reinkensmeier et al., 2015). Pea proteins have also been reported to be suitable for the quality enhancement of gluten-free muffins and the preparation of edible films as pea flour (Shevkani et al., 2019).

Pea protein isolate (PPI), pea protein concentrate, and pea flour are the three main distinct forms of commercial pea protein ingredients. It is crucial that PPI has good functional properties. In addition, it is of great importance to find new methods for the improvement of new PPI-based products and to improve their functionality such as water-holding capacity (WHC), solubility, and emulsification activity (Chang et al., 2015). These functional properties could be modified by some processing techniques such as high hydrostatic pressure (HHP) as well as processing conditions including pH and temperature.

HHP has many applications in food industry. Inactivation of unwanted food enzymes and foodborne pathogens, production of high-quality food products, and reduction of the microbial population of spoilage microorganisms are among the wide-range applications of HHP in food industry (Estrada-Girón et al., 2005). Other widespread applications of HHP include the changes in the structures of food-grade biopolymers, that is, gelatinization of starch and protein modification (Yamamoto, 2017). One example for such modifications by HHP is the volume change within the protein molecule structures in solution. Thus, proteins show changes in their native structure due to compression of the protein cavities by HHP and this may alter their functional properties (Akharume et al., 2021). When applied at different pressure, temperature, and time levels, HHP processing could influence quaternary and secondary structures of proteins, which irreversibly affects their unfolding process. Recent studies suggested that HHP promoted conformational changes in globular proteins like walnut, amaranth, and sweet potato protein and caused formation of insoluble aggregates or breaking of ordered secondary structure (Chen et al., 2019). In addition, application of HHP around 300–400 MPa was shown to modify soy and lupin proteins, improving their functionality in food systems (Khan et al., 2015). However, still only limited studies have been reported regarding the effects of HHP on the structural and functional properties of legume proteins, especially pea proteins.

The primary objective of this study was to analyze the effects of HHP processing, temperature, and pH conditions on the functional properties of PPI solutions (45%, w/v). For this purpose, different HHP (300, 400, 500 MPa), pH (3.0, 5.0, 7.0) and temperature (25, 50°C) levels were applied to the samples in this study. WHC, solubility, and emulsification activity experiments were performed to analyze the changes in the functional properties of PPI samples. Additionally, Fourier transform infrared (FTIR) spectroscopy and nuclear magnetic resonance (NMR) relaxometry experiments were also performed to investigate the effects of processing conditions on functional properties of PPI.

2 | MATERIALS AND METHODS

2.1 | Materials

PPI was purchased from Elite Naturel Organik Gida San. ve Tic. A.S. (Ankara, Turkey). Kjeldahl analysis was performed as given in AOAC Official Method (AOAC, 2007), to determine the protein content (85.05%, dry basis) of the PPI samples. All the chemicals, including sodium potassium tartrate tetrahydrate ($KNaC_4H_4O_6{\cdot}4H_2O$), sodium carbonate (Na_2CO_3), copper (II) sulfate pentahydrate ($CuSO_4{\cdot}5H_2O$), sodium hydroxide (NaOH), bovine serum albumin (BSA), Folin-Ciocalteau's phenol reagent, boric acid (H_3BO_3), sulfuric acid (H_2SO_4), hydrochloric acid (HCl), phenolphthalein ($C_{20}H_{14}O$), and methyl red were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Corn oil was purchased from the local market (Evin Yag, Ankara, Turkey) for the emulsification activity experiments.

2.2 | Sample preparation

Pea protein samples of 45% g/ml (w/v) were prepared by 1 M NaOH and 1 M HCl solutions. In this way, pH is set to the required levels (3.0, 5.0, and 7.0). Then, these samples were treated by HHP. PPI concentration was selected high in order to speed up the following freeze-drying process.

2.3 | HHP treatment

A pressure equipment (760.0118, SITEC-Sieber Engineering AG, Zurich, Switzerland) was used for HHP treatment. The internal diameter, length, and capacity of the pressurization chamber were 24 mm, 153 mm, and 100 ml, respectively. The internal temperature of the system was kept constant by a built-in cooling system (Huber Circulation Thermostat, Offenburg, Germany). The pressure transmitting medium was distilled water. Pressure increase–release times were excluded from the pressurization time given in the study. Samples

were placed into 25 ml polyethylene cryotubes (LP Italiana SPA) for HHP application. All samples (pH of 3.0, 5.0, and 7.0) were treated at 300, 400, and 500 MPa (25 and 50°C) for 5 min. The experimental setup was determined for the investigation of the effects of different pH, temperature, and pressure conditions, and their combinations on the functional properties of PPI. 25°C was chosen for its proximity to room temperature in order to investigate the HHP effect alone whereas 50°C was used to understand the effects of mild temperature increase on pressure-applied PPI samples. Higher temperatures were avoided since HHP also induces intense conformational changes on proteins even at low temperatures. Before the analyses, HHP-treated samples were firstly freeze-dried and then stored at −20°C.

2.4 | WHC analysis

The method shown by Bajaj et al. (2015) was used to determine WHC. Firstly, 5% (w/v) PPI solution was prepared and then blended (Ultra Turrax T-18, IKA, Corp., Staufen, Germany) at 6000 rpm for 5 min. Subsequently, these samples (PPI solutions) were placed into 25 ml centrifuge tubes. The overall weight of the tubes containing the samples was measured and recorded. Afterwards, a centrifugation at 2862 × g for 30 min was performed with a Hanil MF80 benchtop centrifuge (Hanil Science Industrial Co., Ltd., Incheon, Korea) for all the samples. Following centrifugation, the supernatant was removed from the tubes and the weight of the residual part was determined. Finally, WHC was determined according to the formula of (Bajaj et al., 2015):

$$\mathrm{WHC}(\mathrm{g\,H_2O\ held\ by\ the\ sample/g\ dry\ protein\ sample}) = \frac{w_t - w_{ct} - w_s - w_{ppi}}{w_{ppi}}, \quad (1)$$

where w_t, w_{ct}, w_s, and w_{ppi} represent the total weight of the PPI solution and the centrifuge tube (PPI solution + centrifuge tube), weight of the centrifuge tube, weight of the supernatant liquid, and weight of the dry PPI sample used, respectively.

2.5 | Solubility analysis

The Lowry method (Lowry & Randall, 1951) was used to detect the soluble protein content of the samples (aqueous solubility). First, the prepared PPI samples (1%, w/v) were blended at 5000 rpm, 5 min (Ultra Turrax T-18 IKA, Corp., Staufen, Germany) for complete dissolution and then centrifuged at 1118 × g (Hanil MF80 Benchtop Centrifuge, Hanil Science Industrial Co., Ltd., Incheon, Korea) for about 15 min. Then, distilled water was used to dilute the supernatant (1:4). Later on, 2.5 ml Lowry reagent was mixed with 0.5 ml sample. These mixtures were kept at room temperature for 10 min. Subsequently, mixtures were mixed with 0.25 ml Folin reagent and kept in a dark place for 30 min. Finally, absorbance of the samples was measured by an UV spectrophotometer (Optizen Pop Nano-Bio, Mecasys Co., Ltd., Daejeon, Korea) at 750 nm.

2.6 | Emulsification activity analysis

A modified form of the method given in the study of Hoang (2012) was used to determine the emulsification activity of the samples. Subsequent to preparation of 1% (w/v) PPI solution, mixing with an Ultra Turrax T-18 (IKA, Corp., Staufen, Germany) at 5000 rpm for 5 min was performed. Then, 0.5 ml corn oil and 1 ml protein solution were mixed by the same equipment but at different conditions (15,000 rpm, 1.5 min). Afterwards, initial height of the samples was measured and then centrifuged at 1118 × g (Hanil MF80 Benchtop Centrifuge, Hanil Science Industrial Co., Ltd., Incheon, Korea) for 1 min. Finally, the emulsification activity of each sample was determined by dividing the emulsified fractions by the initial height of the emulsions. The results were reported in %.

2.7 | FTIR spectroscopy analysis

FTIR measurements of the powdered samples were conducted by IR-Spirit Spectrometer (Shimadzu Corporation, Kyoto, Japan) with an attenuated total reflectance attachment. The resolution was set at 4 cm^{-1}. The total number of scans was 32 and the measurement region was between 600 and 4000 cm^{-1}.

2.8 | NMR relaxometry analysis

PPI samples prepared at 1:3 pea protein to distilled water ratio were used for NMR relaxometry experiments. A 0.5 T (20.34 MHz) benchtop NMR instrument (Spin Track, Resonance Systems GmbH, Kirchheim/Teck, Germany) was used to measure T_2 of the samples. CPMG (Carr-Purcell-Meiboom-Gill) pulse sequence with 800 µs echo time, 512 echo-number, 16 scans, and 1 s relaxation period was used to determine T_2. MATLAB (R2019b, The MathWorks Inc., Natick, MA, USA) was used for the relaxation data analysis. Mono-exponential fit was the appropriate approach for the relaxation analysis of the samples.

2.9 | Statistical analysis

Samples were analyzed by MINITAB (Minitab Inc., Coventry, UK). The effects of the process conditions (variables) were investigated by analysis of variance (ANOVA). Tukey's test was performed with 95% confidence level for the multiple comparison tests. In order to show the differences between the different treatment conditions, small letters were used. At least three replicates were used for each measurement.

3 | RESULTS AND DISCUSSION

3.1 | Water-holding capacity

The maximum level of WHC (5.48 g/g) was observed for the sample treated at 500 MPa (pH 5.0 and 50°C) among all other pressure-pH-

temperature combined treatments (Figure 1). This could be due to the conformational changes of pea protein molecules induced by 500 MPa pressure and pH 5.0 conditions. Since such a high WHC trend was not induced by 500 MPa pressure at pH 3.0 and 7.0, pH could be the main factor here. It was previously demonstrated that 600 MPa pressure treatment produced the most denaturation in yellow field PPI structure compared to the pressures applied at 200 and 400 MPa (Chao et al., 2018). Such high pressures promote a more unfolded protein structure and increase the intensity of interactions between the aromatic groups of proteins and the aqueous environment (Schmidt, 1989). However, besides HHP, pH also has an effect on WHC. pH 5.0 is near the isoelectric point (pI, 4.5) of pea protein (Lam et al., 2018), which would be expected to induce lower WHC of PPI. Nevertheless, this was not the case due to the distinct effects of 500 MPa HHP on conformational behaviors of PPI molecules at their isoelectric point. Close to their pI, pea protein molecules experience maximal protein–protein interactions and water-binding sites are buried in the depths of the molecular conformation (Zayas, 1997). Application of 500 MPa may have exposed these buried water-binding sites to surface and contributed to the high WHC observed at pH 5.0 (Akharume et al., 2021). Since a more proportion of such protein fractions are readily exposed on the surface of pea protein molecular conformations at pH 3.0 and especially at pH 7.0, application of 500 MPa could have produced an opposite effect on these samples. For instance, high pressure may have induced pea protein denaturation at pH 3.0 and 7.0 by exposing the hydrophobic sites in the depths of the protein molecular conformations (Messens et al., 1997). In this way, the water-binding sites may have been buried into the molecular conformation under high pressures at pH 3.0 and 7.0, thereby decreasing the WHC of the samples (Figure 1). The increase in temperature from 25 to 50°C also contributed to the low WHC of the samples at pH 3.0 and 7.0 (Figure 1). Although thermal denaturation of PPI is achieved at temperatures above 75°C (Shand et al., 2007), HHP is able to induce nonthermal denaturation of proteins at much lower temperatures (Messens et al., 1997). Therefore, 500 MPa-50°C samples achieved lower WHC values ($p < 0.05$) compared to 500 MPa-25°C samples at pH 3.0 and 7.0 as shown in Figure 1. This was mostly due to the accelerating effect of high pressure (500 MPa)–high temperature (50°C) treatment on protein denaturation at pH levels far from the pI of the proteins (Akharume et al., 2021). However, the increase in temperature did not induce such an effect on WHC at pH 5.0 since the effects of pressure and temperature on conformations of pea proteins were different at the pI of pea proteins.

3.2 | Solubility

Soluble protein contents of PPI samples did not show any significant difference between pH 3.0 and 5.0, but the increase in pH to 7.0 substantially increased ($p < 0.05$) the aqueous solubility of PPI (Figure 2). This was in agreement with the findings of Barac et al. (2010), where solubility of different pea varieties was highly pH-dependent. All the samples in that study attained higher solubility at pH 7.0 and 8.0. Another study, where solubility of palm kernel cake protein was studied, indicated that pH 7.0 resulted in higher solubility (87.65%). Presence of fewer hydrophobic residues on protein surfaces around pH 7.0 was claimed to be the main reason behind this finding (Chee & Ayob, 2013). At pH 5.0, which is around pI of PPI, approximately 60% lower solubility was observed for all samples compared to that of pH 7.0. Since pH 5.0 is close to the pI of PPI (4.5), proteins did not have a significant net charge, thus they displayed minimum solubility in water (Zayas, 1997), regardless of the applied pressure level. However, their WHC values were not coherent with their solubility results, mostly due to the distinct effects of HHP on PPI molecular conformations at pH 5.0 (close to pI of PPI) as previously explained in Section 3.1.

Generally, HHP did not change the solubility levels, especially at pH 3.0 and 5.0. This result was consistent with the findings of Chao et al. (2018), who reported that HHP treatment at 200, 400, and 600 MPa (5 min, up to pH 7.0) induced soluble isolated pea protein aggregate formation. They also reported that solubility values did not demonstrate any significant difference at different pressure levels. Similar results were also reported for soy proteins by Li et al. (2012), where pressure treatments in the range of 200–400 MPa at pH 3.0 induced no significant difference in solubility values. The impacts of HHP application on solubility were only detectable at pH 7.0. The highest solubility value at pH 7.0 was observed for the samples treated at 300 MPa-50°C (88.59%) (Figure 2). This indicates that solubility

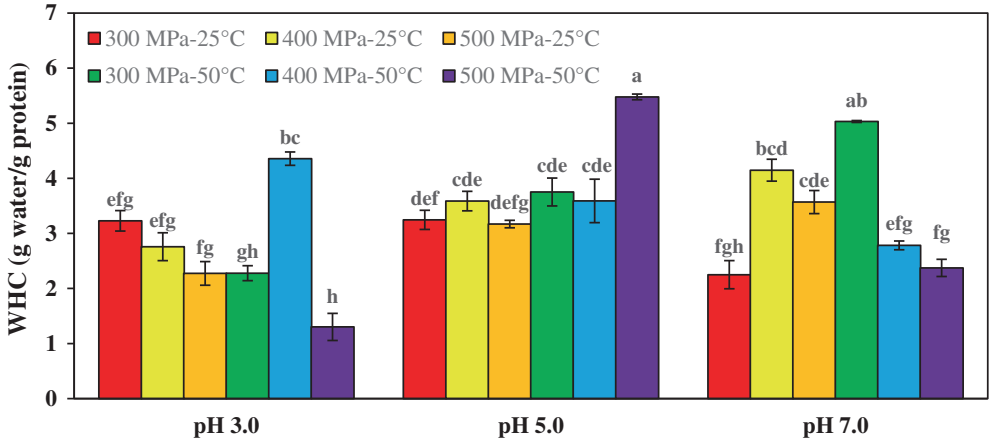

FIGURE 1 Water-holding capacity of HHP-treated PPI samples (300, 400, and 500 MPa) at different pH (3.0, 5.0, and 7.0) and temperature (25 and 50°C) conditions. Different small letters indicate the significant difference between different samples for all given results ($p < 0.05$). Errors are represented as standard errors. HHP, high hydrostatic pressure; PPI, pea protein isolate

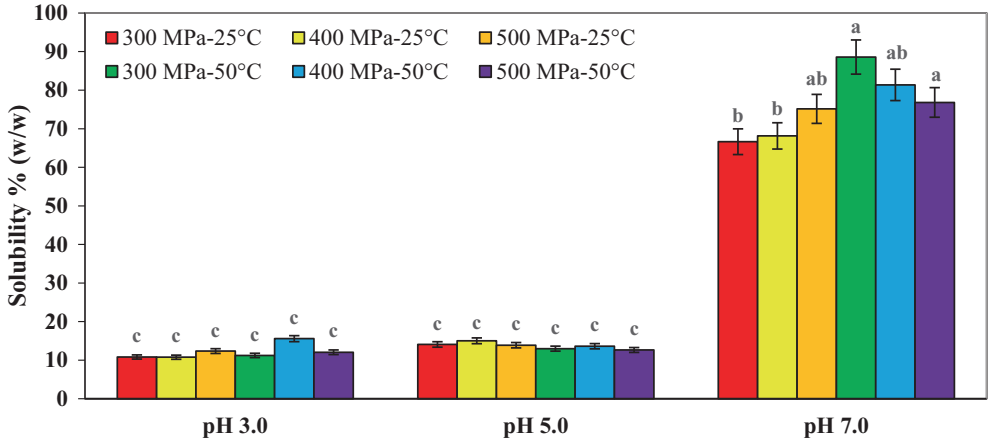

FIGURE 2 Solubility of HHP-treated PPI samples (300, 400, and 500 MPa) at different pH (3.0, 5.0, and 7.0) and temperature (25 and 50°C) conditions. Results are shown separately for each pH value studied. Each pH value contains the results of all pressure–temperature combinations. Different small letters indicate the significant difference between different samples for all given results ($p < 0.05$). Errors are represented as standard errors. HHP, high hydrostatic pressure; PPI, pea protein isolate

of PPI enhanced under milder HHP and higher temperature conditions at pH 7.0. Similarly, another study also stated that the two different kind of PPI investigated demonstrated an increase in solubility with increasing temperature. Moreover, poor solubility values (less than 50%) were recorded below 50°C in the same study (Chen et al., 2019). However, treating the PPI samples at 79–95°C for 25 min reduced the solubility significantly showing that extreme temperatures have an adverse effect on pea protein solubility. Therefore, mild temperature–pressure combinations should be applied at pH 7.0 in order to increase the solubility of PPI. In contrast, high pressures at pH 7.0 and above may increase the surface hydrophobicity of proteins which would result in reduced solubility (Chao et al., 2018). Accordingly, rise of temperature at low pressures and neutral pH (pH 7.0) may have triggered the electrostatic interactions, which is the driving force for the protein–solvent interactions promoting dissolution, thereby increasing the PPI solubility. Barac et al. (2010) also concluded that solubility of seed proteins from various pea genotypes increased around pH 7.0 due to the electrostatic environment created by the high pH condition favorable for protein dissolution. At pH 7.0, which is away from pI of PPI, PPI molecules have net negative charge increasing the repulsion between the protein molecules. Thus, proteins do not form intense interactions with each other and instead interact with water (Aluko et al., 2009). Similarly, temperatures around 50–60°C were reported to facilitate the solubilization of PPI (Shanthakumar et al., 2022). Therefore, the highest solubility value of the samples treated at 300 MPa-pH 7.0-50°C was in agreement with the literature findings.

3.3 | Emulsification activity

Emulsification activity was highly influenced by the change in pH as demonstrated in Figure 3. At pH 3.0, emulsification activities of PPI are significantly higher ($p < 0.05$) than those of pH 5.0 and 7.0 except for 300 MPa-25°C and 500 MPa-50°C samples. In these samples pH 3.0 also produced higher emulsification activity ($p < 0.05$) than pH 7.0. The reason for such high emulsification activities at pH 3.0 may be the formation of stronger and denser viscoelastic networks at the interface induced by pea proteins in acidic conditions, owing to the higher surface viscoelasticity modulus (Gharsallaoui et al., 2009). This claim was in agreement with the study of Liang and Tang (2013), where partially purified globulins and PPI from dry pea seeds showed better emulsifying abilities at pH 3.0 than at pH 7.0 and 9.0. Similarly, Gharsallaoui et al. (2009) claimed that pea protein-stabilized emulsions had better emulsifying properties at pH 2.4 than at pH 7.0. Samples showed more homogeneous particle size distribution and better creaming stability at pH 2.4.

Pressure level of HHP treatment did not show any significant difference in any pressure-temperature-pH combination. The minimum level of HHP treatment (300 MPa) provided similar emulsification activities to the higher (400 and 500 MPa) pressures applied in this study. Pressure levels as low as 200 MPa were previously demonstrated to be sufficient to change the ratio of hydrophilicity to hydrophobicity of pulse proteins in favor of better emulsification activity (Hall & Moraru, 2021). Therefore, 300 MPa was probably sufficient to alter the conformation of pea proteins for a better emulsification activity. A similar result was also reported by Zhao et al. (2015). This study reported the highest emulsification activity index for a major peanut protein (arachin) after a 300 MPa HHP treatment for 10 min. Moreover, Wang et al. (2008) stated that 200 MPa HHP treatment produced a significant increase in soy protein isolate emulsification activity index. However, higher pressures (400–600 MPa) were not effective on emulsion activity. All these studies are in agreement with the results of this study and it can be concluded that the major factor that determined the emulsification activity of PPI emulsions was pH rather than applied pressure levels and temperature.

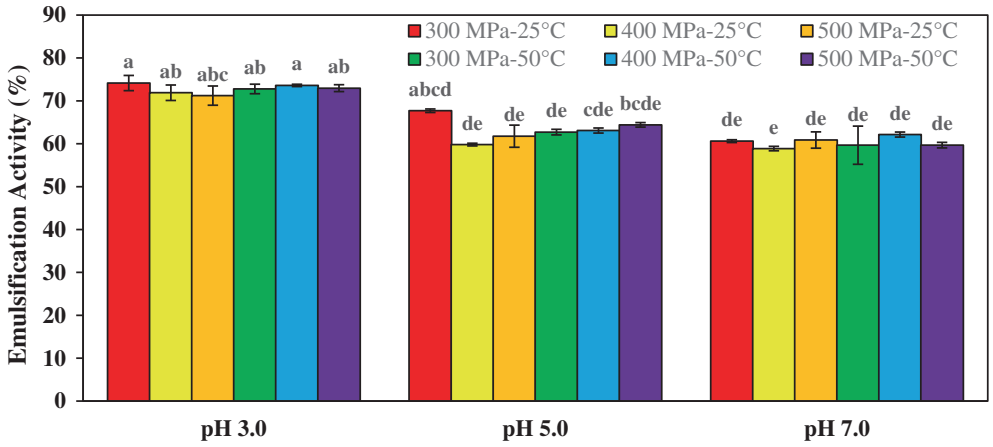

FIGURE 3 Emulsification activity of HHP-treated PPI samples (300, 400, and 500 MPa) at different pH (3.0, 5.0, and 7.0) and temperature (25 and 50°C) conditions. Different small letters indicate the significant difference between different samples for all given results ($p < 0.05$). Errors are represented as standard errors. HHP, high hydrostatic pressure; PPI, pea protein isolate

3.4 | FTIR spectroscopy analysis

FTIR measurements were used to analyze the changes in the molecular conformations of the PPI samples. Peak positions of the bands at each pH studied (3.0, 5.0, and 7.0) are shown in Figure 4. Amide I band (1600–1700 cm^{-1}) demonstrates the C═O stretching vibration of the protein backbone (Moreno et al., 2020). Thus, amide I band is uniquely useful to analyze pea protein secondary structural and conformational changes under different pressurization levels. For instance, amide I peak of the sample treated at 400 MPa-pH 3.0-50°C showed a broader band than the rest of the samples as shown in Figure 4a. Since amide I region is susceptible to conformational changes in protein secondary structures, it can be concluded that this treatment combination induced some changes in the β-sheet structures of the aggregated PPI molecules (Moreno et al., 2020). Changes in the hydrogen bond strength and geometry of the protein secondary structures are some of the examples leading to a band enlargement in amide I region (Carbonaro et al., 2012). Another change in the shape of a band was observed for the hydroxyl group vibrations (3000–3500 cm^{-1}) of the samples treated at 400 MPa-pH 3.0-50°C. These hydroxyl group vibrations include the total stretching of free and bonded O—H and N—H groups. Therefore, an alteration in the frequency and/or broadening of the band in this region could be associated with formation of more hydrogen bonding between the analyzed sample constituents and the surrounding aqueous media (Ebrahimi et al., 2016). In accordance with the higher relative area under their hydroxyl group vibration band compared to other samples, PPI samples treated at 400 MPa-pH 3.0-50°C also had the highest WHC ($p < 0.05$) with respect to the other samples treated at pH 3.0. Additionally, the same samples also attained slightly higher solubility at pH 3.0 but the increase was not statistically significant. Accordingly, FTIR spectrum of the samples treated at 400 MPa-pH 3.0-50°C was in agreement with WHC and solubility results. Other samples (pH 3.0) demonstrated very similar FTIR spectra and this was also compatible with their similar WHC and solubility results. Herein, it could be claimed that 400 MPa pressure was able to induce sufficient changes on the molecular conformations of PPI at pH 3.0 and 50°C so that these samples were also able to show some differences related to WHC and solubility with respect to other samples.

FTIR spectra of the PPI samples at pH 5.0 were more similar and closer to each other with respect to those observed at pH 3.0 (Figure 4b). Samples treated at 500 MPa-pH 5.0-50°C demonstrated one of the largest peaks for amide I and hydroxyl group bands. This was actually in agreement with their highest WHC within the all analyzed samples. The large peaks observed at amide I region could be related to the changes in the molecular conformations of pea proteins (500 MPa-pH 5.0-50°C) which also affected the water absorbing behavior of the same samples (Moreno et al., 2020).

Increasing the pH to 7.0 changed the amide I band shape of some samples, especially the one treated at 500 MPa-50°C (Figure 4c). These samples experienced narrowing in their bands when pH was increased from 5.0 to 7.0. This may be due to the distinct high pressure-induced changes on protein conformation at pH 5.0. Another FTIR spectrum pattern of the samples at pH 7.0 was the broadening of the peaks responsible for hydroxyl group vibrations. These peaks lost their sharp structures at pH 7.0 and formed smoother peaks. Such broadened smoother peaks are associated with a rise in the number of —OH groups involved in hydrogen bonding compared to the number of free —OH groups. These samples also had substantially higher solubility levels at pH 7.0 than at pH 3.0 and 5.0. This also demonstrated that there was an increase in the hydrogen bonding between the PPI molecules and the surrounding aqueous medium at pH 7.0.

3.5 | NMR relaxometry analysis

Mono-exponential T_2 values of the samples showed similar profiles except for one sample as demonstrated in Figure 5. A drastic increase in T_2 ($p < 0.05$) (108 ms) was observed at 500 MPa-pH 5.0-50°C with respect to other HHP-treated samples at different conditions. The main reason for this result could be the presence of a higher amount of free water under these treatment conditions. T_2 represents the efficiency of energy transfer between neighboring spins. When the proximity between the molecules are closer, a shorter T_2 is obtained due to the higher efficiency of energy transfer between the spins. Accordingly, shortest and longest T_2 values are attained for solid and liquid materials, respectively (Kirtil & Oztop, 2016). Therefore, the long T_2

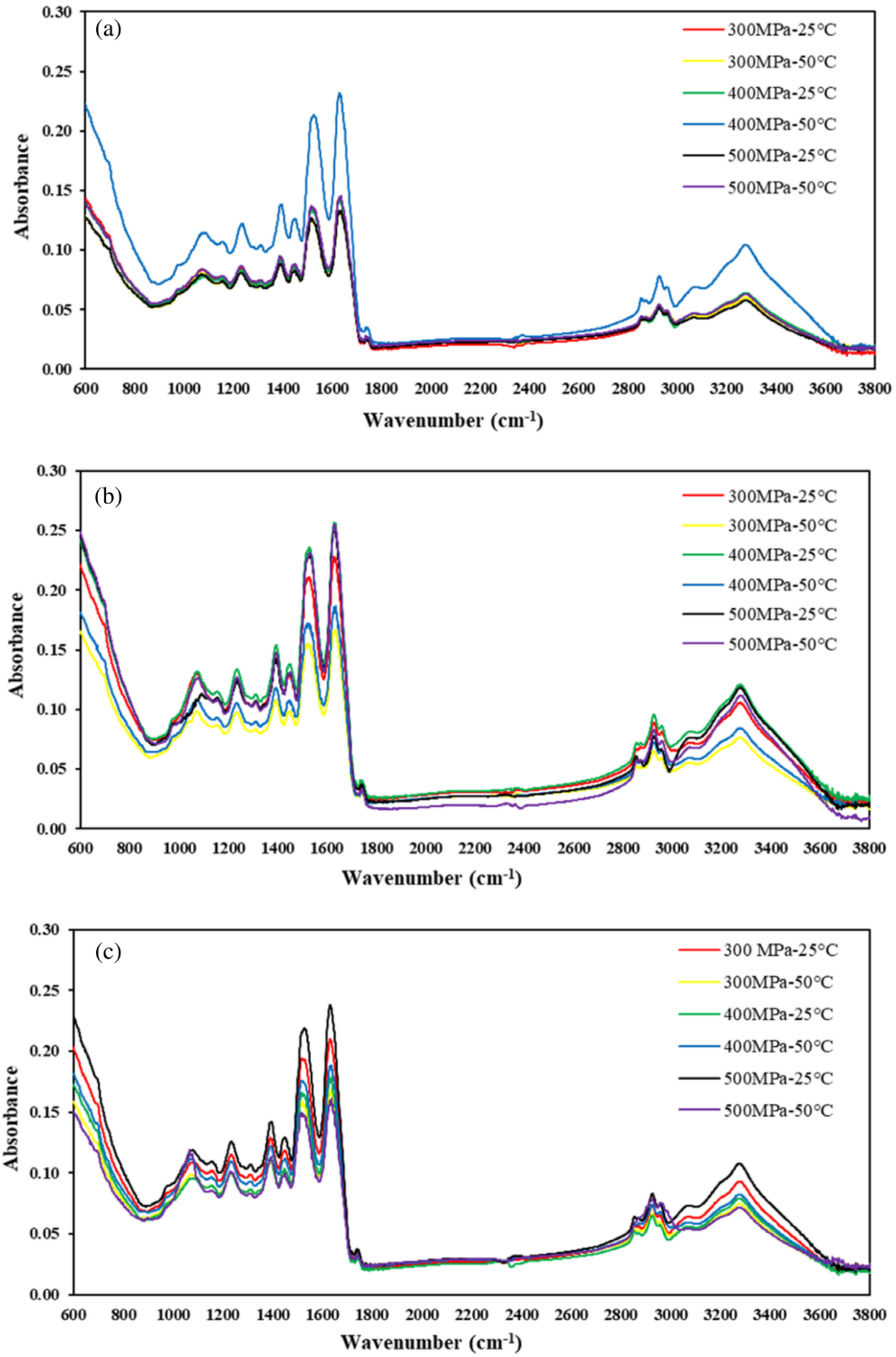

FIGURE 4 FTIR spectra of HHP-treated PPI samples (300, 400, and 500 MPa): (a) at pH 3.0 and 25–50°C; (b) at pH 5.0 and 25–50°C; (c) at pH 7.0 and 25–50°C. HHP, high hydrostatic pressure; PPI, pea protein isolate

trend of 500 MPa-pH 5.0-50°C samples were also consistent with their highest WHC among the other samples since such a longer T_2 shows a higher amount of absorbed water that does not interact heavily with other surrounding molecules.

It is known that the interactions between exchangeable biopolymer protons and solvation water depend on the conformational changes of a protein (Oztop et al., 2010). However, T_2 results suggested that pressure level did not alone have a significant effect on transverse relaxation behavior of the PPI samples. Nonetheless, combination of high pressure (500 MPa) at a specific temperature (50°C) and pH (5.0) resulted in a drastic increase in T_2. Under these experimental conditions, distinct conformational changes in PPI induced by HHP enabled the entrapment of a higher amount of water in PPI structures. Another important point was that temperature increase did not induce any significant difference in T_2 for almost all treatment combinations. This result was consistent with the findings of Coelho

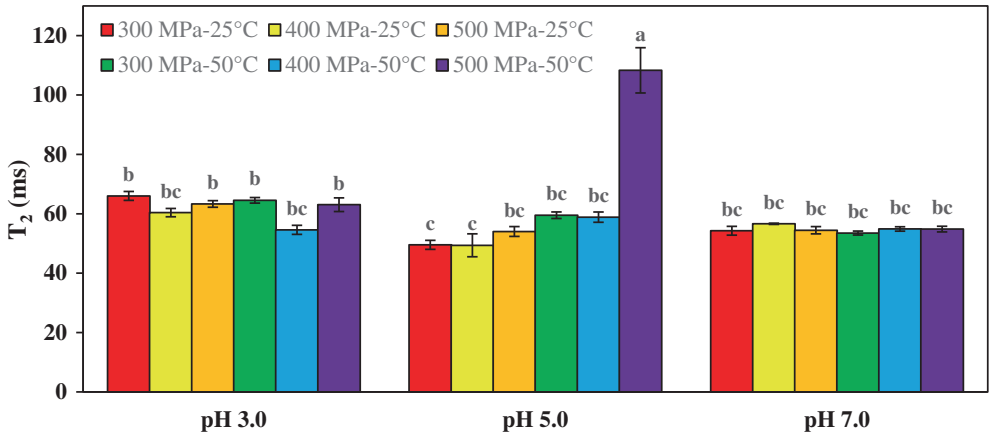

FIGURE 5 Transverse relaxation times of HHP-treated PPI samples (300, 400, and 500 MPa) at different pH (3.0, 5.0, and 7.0) and temperature (25 and 50°C) conditions. Different small letters indicate the significant difference between different samples for all given results ($p < 0.05$). Errors are represented as standard errors. HHP, high hydrostatic pressure; PPI, pea protein isolate

et al. (2007), where heat treatment of β-lactoglobulin as a globular protein showed no change in T_2 at temperatures between 21 and 90°C.

4 | CONCLUSIONS

The results of this study indicated that HHP was effective on functional properties of PPI in combination with pH and temperature. WHC of the PPI samples reached a maximum at 500 MPa-pH 5.0-50°C treatment condition. This suggests that specific HHP, pH, and temperature conditions should be met to increase the WHC of pea proteins. Solubility results showed that the maximum solubility of PPI samples could be achieved at mild pressure (300 MPa), high pH (7.0), and temperature (50°C) conditions. Moreover, pH was the dominant factor on emulsification activity results. A low pH value (3.0) provided better emulsification activity in PPI-based emulsions at all pressure levels. FTIR spectra analysis of the samples demonstrated changes mainly at amide I and hydroxyl group bands. These changes in FTIR spectra were related to the functional changes of the pea proteins. For instance, samples treated at 400 MPa-pH 3.0-50°C produced broader bands in both the amide I and hydroxyl group regions. The same samples also had higher WHC. Finally, T_2 was also introduced for further analysis. T_2 was susceptible to the state of water in pea protein structures and revealed that a longer T_2 was associated with a higher WHC. All in all, this study showed that HHP-induced PPI modifications could be achieved at certain temperature and pH conditions. Moreover, the results of this study can be used in model industrial applications to improve the desired functional properties of various PPI samples. Time domain NMR was also introduced as a novel, nondestructive, and easy characterization technique for such applications.

AUTHOR CONTRIBUTIONS

Asuhan Kalayci: Investigation, Formal analysis, Writing – original draft. **Baris Ozel:** Visualization, Writing – original draft, Writing – review & editing. **Mecit Halil Oztop:** Conceptualization, Methodology, Supervision. **Hami Alpas:** Conceptualization, Methodology, Supervision, Writing – review & editing.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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