INTRODUCTION

Curcumin is a natural low molecular weight phenolic substance obtained from the dried rhizome of perennial herb Curcuma longa (Xiang, Sun-waterhouse, Cui, Wang, & Dong, 2018). This bioactive molecule has a variety of beneficial biological functions, such as antioxidant, anticancer, anti-inflammatory, and antibacterial properties (Tapal & Tiku, 2012; Medina-Torres et al., 2019). It was also reported that curcumin can prevent Alzheimer’s disease, diabetes, and atherosclerosis (Akl et al., 2016). However, the applications of curcumin as a biologically active compound in different functional foods and beverages have been limited due to its extremely low water solubility, low chemical stability, poor oral bioavailability, and rapid metabolism (Tapal & Tiku, 2012). Curcumin is poorly soluble under both neutral and acidic conditions, it degrades in alkaline solutions, and it is sensitive to UV light (Zheng, Zhang, Chen, Luo, & McClements, 2017). Therefore, to overcome these challenges, several strategies and approaches have been proposed to improve the water solubility, chemical stability, and bioavailability of curcumin such as encapsulation in hydrogel beads (Zheng et al., 2017), cold-set hydrogels (Alavi et al., 2018), and zein–curcumin particles (Patel, Hu, Tiwari, & Velikov, 2010). Despite enhancing the bioavailability and water...
disperability of curcumin, these common approaches have specific disadvantages such as the usage of surfactants and organic solvents which may have toxic effect on the finished product (Chen et al., 2014; Pan, Luo, Gan, Baek, & Zhong, 2014). Therefore, inexpensive and organic solvent-free approaches should be employed to improve the solubility and bioavailability of curcumin, which are more suitable for the fabrication of functional food products and beverages (Wang et al., 2019).

The complexation of proteins and curcumin has recently attracted a great deal of interest for improving the curcumin aqueous solubility and physico-chemical stability. Various proteins have been used as carriers for curcumin delivery such as whey protein nanofibrils (Mohammadian et al., 2019a), zein protein (Patel et al., 2010), and soy protein isolate (Chen, Li, & Tang, 2015a, 2015b).

Whey proteins as a valuable by-product of cheese industry are widely used in food products, because of their high nutritional value and excellent technological functionalities including good emulsifying, foaming, and gelling properties (Wichchukit, Oztop, McCarthy, & McCarthy, 2013). Whey proteins also were used to produce carriers for the encapsulation and protection of bioactive molecules (Wang et al., 2016) due to their ability to bind with various hydrophobic molecules such as curcumin in aqueous solution (Liu et al., 2017). It was also investigated that the solubility and photo-stability of bioactive molecules were boosted through the binding with whey proteins (Liu, Chen, Cheng, & Selomulya, 2016).

The previous studies suggested that the alkaline pH shifting method can be effectively used to modify the proteins through unfolding and refolding of their structure. With the controlling of dissociation and re-association by changing the pH, proteins can effectively encapsulate the hydrophobic polyphenols (Wang et al., 2019). It was investigated that curcumin has an appropriate solubility at high alkaline pH values owing to the deprotonation of its hydroxyl groups. Therefore, it seems that the curcumin can dissolve better in the presence of alkaline-dissociated proteins and form soluble complexes during the acidification process improving its water dispersibility, chemical stability, and antioxidant properties (Pan et al., 2014).

Accordingly, this study aimed to fabricate whey protein isolate (WPI)-curcumin complexes by a pH shifting method without using any solvents such as ethanol as well as any heating step. After that, the characteristics of the resulting curcumin-WPI complexes were studied at pH values of 7.0 and 3.0 by various experiments.

2 | MATERIALS AND METHODS

2.1 | Materials

WPI with >90% protein content was supplied by Hilmar Ingredients (CA, USA). Curcumin, pancreatin, and pepsin were supplied by Bio Basic (Bio Basic Inc., Canada). Hydrochloric acid (HCl), ethanol, sodium hydroxide (NaOH), and other chemicals used at this study were obtained from Sigma-Aldrich (St. Louis, Mo, USA).

2.2 | Complexation of curcumin with WPI

Curcumin-WPI complexes were prepared by pH shifting method according to Pan, Chen, Baek, and Zhong (2018) with slight modifications. WPI powder was dispersed at a concentration of 20 mg/ml in distilled water and were continuously stirred for 2 hr at room temperature and then they were fully hydrated overnight at 4°C. WPI solution was adjusted to pH 12.0 using 4.0 M NaOH. Then the curcumin crystals were added to the WPI dispersion at a final concentration of 0.4 mg/ml (curcumin to WPI ratio of 1:50). After stirring on a magnetic stirrer for 30 min, the mixture was acidified to pH values of 7.0 and 3.0 using 4 M HCl. For comparison, WPI-curtcumin samples were also prepared at pH values of 7.0 and 3.0 without using the pH shifting method. In these cases, curcumin was directly added to the WPI solutions with the pH values of 3.0 and 7.0.

2.3 | Curcumin solubility and loading amount (LA)

Solubility of curcumin was measured according to Pan et al. (2018) with some modifications. All the samples containing curcumin which were prepared with or without pH shifting method were centrifuged at 6,000 rpm for 15 min to remove the free (unloaded) curcumin and big particulates. The supernatant was transferred into a dark bottle and mixed with ethanol for extracting the curcumin. The amount of soluble curcumin was determined spectrophotometrically at a wavelength of 420 nm using an ethanolic curcumin standard curve (0.1–10 μg/ml, R² = 99.99%). The solubility of curcumin and its loading amount in different samples were determined by following equations:

\[
\text{curcumin solubility (\%) = } \frac{\text{amount of curcumin in the supernatant}}{\text{total amount of added curcumin}} \times 100
\]

\[
\text{LA } (\text{mg}) = \frac{\text{loaded amount of curcumin}}{\text{total amount of protein}}
\]

The curcumin sedimentation was also investigated during 10 days of storage by monitoring the visual appearances of different samples.

2.4 | Characterization of curcumin-WPI complexes

The curcumin-WPI complexes formed without pH shifting method showed very low curcumin solubility. Therefore, only the samples (WPI and C-WPI) which were produced by the pH shifting method (i.e., from pH 12 to pH values of 7.0 and 3.0) were employed for the following experiments.

2.4.1 | Fluorescence spectroscopy

To investigate the binding of curcumin to WPI, the protein and curcumin fluorescence properties were studied with a
spectrofluorometer (Cary Eclipse, Palo Alto, CA). Samples were diluted to protein and curcumin concentration of 0.2 mg/ml and 4 μg/ml, respectively. The emission spectra of curcumin were obtained from 450 to 700 nm with an excitation wavelength of 420 nm. The protein emission fluorescence was also recorded from a range of 300 to 450 nm with the excitation wavelength of 280 nm. The aqueous solutions of curcumin and WPI with the same protein and curcumin concentration at the pH values of 7.0 and 3.0 were also used as controls for the fluorescence measurement.

2.4.2 Zeta-potential measurements

The zeta-potential of WPI and C-WPI solutions was measured at pH values of 7.0 and 3.0 using a dynamic light scattering (DLS) apparatus (Brookhaven Instruments Corp., USA) in the zeta-potential mode. Distilled water with pH values of 7.0 and 3.0 was employed to dilute the samples prior to this test.

2.4.3 Fourier transform infra-red (FT-IR) spectroscopy

FT-IR spectra of freeze-dried samples were recorded using a Bruker FT-IR spectrophotometer (Billerica, Massachusetts, United States) at the wavenumber range from 4,000 to 600 1/cm for investigating their molecular attributes. For this purpose, the freeze-dried samples were pressed within a KBr disk and scanned.

2.4.4 Circular dichroism (CD) spectroscopy

The CD spectroscopy was used to evaluate the secondary structural changes of WPI resulted from the complexation with curcumin. The CD spectra of samples (with the protein concentration of 0.2 mg/ml) were obtained at the far-UV (190–260 nm) region with a spectropolarimeter (model/J810, Jasco, Japan).

2.4.5 X-ray diffraction analysis (XRD)

The XRD patterns of powdered samples were recorded on a Philips PW1730 X-ray diffractometer (PANalytical, Netherlands). The scanned angle was set from 2θ of 10° to 50°. The accelerating voltage and emission current were 40 kV and 30 mA, respectively.

2.4.6 Curcumin heat- and photo-stability

The heat- and photo-stability experiments of the curcumin-WPI complexes at the pH values of 7.0 and 3.0 were studied by monitoring the absorption spectrum at 420 nm. The curcumin-WPI complexes were heated in a water bath at 85°C for 60 min (by sampling at 0, 15, 60, 45, and 60 min). For the photo-stability experiment, the samples were placed uncovered into the transparent glass vials. Then samples were exposed to a fluorescent lamp of 40 W at a distance of 15 cm at room temperature for up to 10 days and sampling was carried out at the designed time points (1, 3, 6, and 10 days). Finally, the curcumin content in the samples during the above-mentioned tests was determined spectrophotometrically at 420 nm.

2.4.7 Antioxidant activity

The antioxidant activity of WPI (pH values of 7.0 and 3.0) and C-WPI (pH values of 7.0 and 3.0) was determined by reducing power assay according to the method of Tapal and Tiku (2012). About 0.5 ml of samples (WPI and curcumin concentrations of 5 and 0.1 mg/ml, respectively) was charged with 1.25 ml of 0.2 M phosphate buffer at pH 6.6 and 1.25 ml of K3[Fe(CN)6] (10 g/L). The resulting mixtures were heated at 50°C for 20 min. Then an aliquot (1.25 ml) of 100 mg/ml TCA was added. Subsequently, the samples were centrifuged at 1,500 × g for 10 min. After that, 1.25 ml of supernatants was charged with 1.25 ml of distilled water and 250 μl of FeCl3 (1.0 mg/ml). Finally, the absorbance of the samples was measured spectrophotometrically at 700 nm after 10 min incubation at room temperature.

2.4.8 In vitro release behavior

The in vitro curcumin release behavior under simulated gastrointestinal conditions was evaluated with a dialysis sac method (Akl et al., 2016). For this purpose, the pH of curcumin-WPI complex which was produced by pH shifting method was adjusted to 1.2. After that, 3 ml of the resulting mixture with the pH value of 1.2 was filled into a dialysis bag (with 12 kDa molecular cut-off) and then was mixed with 3 ml of simulated gastric fluid (SGF, consisted of NaCl, HCl, and 3.2 g/L pepsin with a final pH value of 1.2). The bag was immersed in a mixture of SGF without pepsin (75 ml) and ethanol (75 ml) as the release medium. After 2 hr of shaking at 37°C and 100 rpm, the mixture was charged with 6 ml of simulated intestinal fluid (SIF, consisted of KH2PO4, NaOH, and 10 g/L of pancreatin with the final pH value of 7.5) and the dialysis bag was moved to a beaker comprising 150 ml of SIF (without enzyme)-ethanol mixture and was kept for 4 hr at 37°C under shaking condition of 100 rpm. Then aliquots of the dialysate were taken out at the predetermined time intervals (0, 1, 2, 3, 4, 5, and 6 hr) and the amount of curcumin in the dialysate was quantified using a UV/visible spectrophotometer.

2.5 Statistical analysis

Statistical analysis of the data was done by one-way analysis of variance (ANOVA) using IBM SPSS software version 23. The comparisons between samples were assessed by Duncan test (p = .05). All of the experiments were carried out in triplicate.

3 RESULTS AND DISCUSSION

3.1 Curcumin solubility and loading amount

The results of curcumin solubility and loading amount for different samples (curcumin-WPI complexes prepared without or with pH shifting method) under different pH values (7.0 and 3.0) are shown...
in Table 1. The solubility and LA of curcumin for complexes prepared by pH shifting method were significantly higher compared to the samples formed without pH shifting. The solubility and loading amount of curcumin for CWPI samples at pH 3.0 were slightly lesser than those prepared at pH 7.0, which can be related to the lower protein hydrophobicity due to its more compacted structure at acidic pH (Wang, He, Labuza, & Ismail, 2013) as well as lower curcumin solubility at acidic condition compared to the neutral and alkaline pH values (Yang, Wu, Li, Zhou, & Wang, 2013). According to Wang et al. (2019), the solubility of curcumin was significantly improved by the pH shifting method with porcine protein plasma (PPP) as a carrier and they reported that the curcumin-PPP complex reached the maximum solubility of 65.4%, whereas in our study, the maximum curcumin solubility was about 80%, suggesting that the WPI has a high capacity for being the carrier for curcumin in the pH shifting method. The curcumin sedimentation in samples was also observed during 10 days of storage (Figure 1). The solutions of WPI-curcumin complexes formed without pH shifting at pH values of 7.0 and 3.0 became colorless after storage as a result of more curcumin precipitation. Moreover, it should be noted that in the case of WPI-curcumin complexes which were produced without pH shifting, curcumin sediment was observed immediately after the preparation. Whereas no obvious sediment was observed in the curcumin-WPI complexes produced by pH shifting method. Therefore, the binding of curcumin to whey proteins through a solvent-free approach can be useful to improve the water dispersibility of curcumin which expands its uses in different fields such as food science and drug delivery.

### 3.2 Fluorescence spectroscopy

Fluorescence measurement was used to investigate the interactions between WPI and curcumin as well as the micro-environmental changes of curcumin with respect to this fact that curcumin fluorescence in aqueous solution is very low due to its insolubility. The fluorescence spectra of free curcumin in distilled water, WPI, and curcumin-WPI complexes with pH values of 7.0 and 3.0 at the excitation wavelength of 420 nm are displayed in Figure 2a. The free curcumin at both pH values of 7.0 and 3.0 exhibited a low-intensity broad peak at 493 nm. However, when curcumin was bounded to WPI, its fluorescence intensity was drastically increased. This observation proposed that curcumin was transferred from a hydrophilic environment to a more hydrophobic environment as a result of binding to the hydrophobic domains of WPI (Liu et al., 2016). This was in accordance with the previous reports of Mohammadian et al. (2019a) investigating the interaction between whey protein nanofibrils and curcumin. They observed that the fluorescence intensity of curcumin increased upon binding to whey protein-originated nanofibrils through the generation of hydrophobic interactions. Furthermore, the higher fluorescence intensity and blue shift of the emission peak of curcumin at complexes formed at pH 7.0 compared to the curcumin-WPI complexes at pH 3.0 suggested that the binding sites of WPI for curcumin are higher in pH 7.0 which was also confirmed by the results of the solubility test and was also in harmony with those of Li, Ma, and Ngadi (2013). Our finding was also in accordance with previous reports on the binding of curcumin with proteins such as porcine plasma protein (Wang et al., 2019) and soy protein isolate (Chen, Zhang, & Tang, 2016).

Fluorescence quenching measurement of protein has been widely used to investigate the binding properties of small ligands such as curcumin at the excitation wavelength of 280 nm to evaluate the accessibility of bioactive molecules to the fluorophore groups of protein (Mohammadi, Bordbar, Divsalar, Mohammadi, & Saboury, 2009). The fluorescence spectra of samples at an excitation wavelength of 280 nm are displayed in Figure 2b. The intrinsic fluorescence emission of proteins comes from their tryptophan and tyrosine residues. The WPI samples at pH values of 7 and 3.0 showed strong fluorescence peaks at wavelengths around 343 and 336 nm.

### Table 1 Curcumin solubility and loading amount in different samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Curcumin solubility (%)</th>
<th>Loading amount (μg/mg)</th>
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<tr>
<td>WPI (pH 3.0)</td>
<td>17.83 ± 0.99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.56 ± 0.19&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>WPI (pH 7.0)</td>
<td>21.74 ± 0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.34 ± 0.04&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>WPI (pH 12 to 3.0)</td>
<td>76.59 ± 1.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.31 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>WPI (pH 12 to 7.0)</td>
<td>79.80 ± 3.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.96 ± 0.70&lt;sup&gt;a&lt;/sup&gt;</td>
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Note: Means with different letters in the same column are significantly different (p < .05).
respectively. After the complexation with curcumin, the fluorescence intensity of WPI at both pH values of 7.0 and 3.0 decreased. This decreased fluorescence intensity suggested that the polarity of the microenvironment surrounding the hydrophobic amino acids of WPI was changed by binding to curcumin. Therefore, it is likely that curcumin molecules can bind to the tyrosine and tryptophan residues of whey proteins. Mohammadian et al. (2019a) and Sahu, Kasoju, and Bora (2008) also reported that the intrinsic fluorescence of whey protein nanofibrils and casein micelles was reduced by the addition of curcumin.

3.3 | Zeta-potential measurement

The zeta-potential of samples was determined to evaluate the stability of the curcumin-WPI complexes (Figure 3). As expected, the WPI at pH 7.0 was negatively charged, while at pH 3.0 it was positively charged. The results of the zeta-potential measurements proved that the complexation with curcumin significantly lowered the magnitude of negative surface charge of WPI at the pH value of 7.0, whereas, after complexation with curcumin at pH 3.0, its positive charge significantly increased. These observations were consistent with the results of Chen et al. (2015a) who reported that the magnitude of zeta-potential of soy proteins decreased at pH 7.0 after the complexation with curcumin. These results were also in agreement with those of Patel et al. (2010) who reported that the incorporation of curcumin into the zein colloidal particles at pH 3.0 increased their zeta-potential.

3.4 | FT-IR and CD analysis

The FT-IR and CD spectroscopy were used to study the structural properties (especially the secondary structure of protein) of different samples. The FT-IR spectra of different samples are shown in Figure 4. WPI at pH values of 7.0 and 3.0, exhibited peaks at 2,941 and 2,958 cm⁻¹, respectively, ascribing to the C-H stretching vibrations of WPI functional groups (Alavi et al., 2018), which were shifted to 2,932 and 2,933 cm⁻¹, respectively, after the complexation with curcumin. These changes could be associated with the formation of hydrogen bonds between proteins and phenolic hydroxyl groups in curcumin, reducing the position of stretching vibrations (Mohammadian, Salami, Momen, Alavi, & Emam-Djomeh, 2019b). The spectrum of free curcumin also showed different sharp peaks that were disappeared after complexation with WPI at pH values of 7.0 and 3.0 which may be due to limitation of curcumin stretching and bending vibrations after complexation with WPI (Mohammadian et al., 2019b). These results suggested that the binding of curcumin to the proteins such as whey proteins occurs mainly through hydrophobic interactions and hydrogen bonds. Moreover, WPI at pH values of 7.0 and 3.0, showed sharp peaks at 1635 and 1636 cm⁻¹, respectively, attributed to the C=O groups stretching vibrations of amide I band. Moreover, characteristic peaks at 1528 and 1525 cm⁻¹ were observed for WPI samples at pH values of 7.0 and 3.0, respectively, attributed to the C=O groups stretching vibrations of amide II region (Bagheri, Madadlou, Yarmand, & Mousavi, 2013). The results showed that the complexation of WPI with curcumin had no significant effect on the positions of the specific peaks related to the secondary structures of proteins in neutral and
acidic pH. In addition, the far-UV CD spectra of WPI in the absence and presence of curcumin were also investigated to determine the influence of complexation with curcumin and pH on the secondary structures of whey proteins (Figure 5). The main absorbing groups in far-UV (190–260 nm) region for proteins are peptide bonds (Mohammadi et al., 2009). The CD spectrum of WPI exhibited a broad negative peak around 205 and 206 nm at pH values of 7.0 and 3.0, respectively, indicating $\alpha$-helix conformation (Dai et al., 2016). The results displayed that the complexation of WPI with curcumin did not considerably affect its secondary structures and the CD spectra of C-WPI samples with pH values of 7.0 and 3.0 were almost similar to those of curcumin-free counterparts. Liu et al. (2017) also stated that the interaction of curcumin with $\beta$-lactoglobulin had no significant effect on the protein secondary structures likely due to its high structural flexibility allowing it to form complexes with curcumin without needing significant structural alterations. Generally, the results of FT-IR and CD experiments suggested that the secondary structures of WPI did not change significantly due to the binding of curcumin.

3.5 | XRD analysis

The XRD was performed to assess the effect of complexation with curcumin through the pH shifting method on the crystalline and amorphous structures of WPI (Figure 6). The XRD pattern of curcumin displayed several characteristic peaks associating with its high crystalline structure (Pu, Tang, Li, Li, & Sun, 2019). Native WPI at pH values of 7.0 and 3.0 showed a few characteristic peaks that can be related to the presence of NaCl formed as a result of using NaOH and HCl for adjusting the pH. After complexation with curcumin, the peaks in the diffraction patterns were similar to that of curcumin-free WPI. These findings suggested that the XRD patterns of WPI at pH values of 3.0 and 7.0 were not significantly changed by binding to curcumin. Moreover, it should be noted that the observation of no peak related to the curcumin in the XRD patterns of the curcumin-WPI complexes can be due to this fact that the amount of loaded curcumin in these samples was very low. Similar results have been reported after loading of curcumin in soy protein nanoparticles (Teng, Luo, & Wang, 2012) and whey protein microgels (Mohammadian et al., 2019b), which the XRD patterns of proteins were not significantly influenced by binding to curcumin.

3.6 | Thermal and photo-stability of curcumin

Figure 7a represents the degradation kinetics of curcumin-WPI complexes with pH values of 7.0 and 3.0 when heated at 85°C up to 60 min. The heat-induced degradation of curcumin at pH 3.0 was much lower than pH 7.0. In fact, complexes with pH 3.0 showed a higher thermal
stability compared to the curcumin-WPI complex with the pH value of 7.0. In this regard, approximately 44% of curcumin was degraded at pH 7.0 after heating for 60 min, whereas the curcumin degradation was 11% at the pH value of 3.0. Also, it is well known that curcumin is photosensitive and is rapidly decomposed by light (Chen et al., 2014). Thus, the photo-stability of complexes was also evaluated by a fluorescent lamp with a higher intensity than normal sunlight for a long-time experiment in 10 days. The results showed that the curcumin was degraded during the continuous visible light irradiation (Figure 7b). Moreover, the results indicated that the curcumin-WPI complex with pH 3.0 had a higher photo-stability compared to the complex with pH value of 7.0. In the case of C-WPI with pH 3.0, 43% of curcumin was remained after 10 days of visible light irradiation, whereas the remained curcumin in the case of C-WPI with pH 7.0 was about 13%. This observation indicated that curcumin light-induced degradation is faster at pH 7.0 than at acidic pH values (Wang, Lu, Lv, & Bie, 2009). Generally, the results indicated that the binding of curcumin to WPI through the pH shifting method significantly improved its thermal and photo-stability in an aqueous media. However, the stability of curcumin in acidic pH was significantly higher than neutral pH. These results suggested that the curcumin-WPI complexes can be appropriately used to produce functional beverages (especially acidic drinks) that need thermal treatment with respect to their high stability to heat and light.

3.7 | Antioxidant activity

The reducing power assay was used to determine the antioxidant activity of WPI and C-WPI samples at pH values of 7.0 and 3.0 (Figure 8). The results demonstrated that the C-WPI complexes had higher antioxidant activity compared to the curcumin-free samples due to the presence of curcumin. Moreover, the WPI and C-WPI samples exhibited slightly higher antioxidant activity at pH 7.0 than those at pH 3.0, likely due to the protein structural deformation (Chen et al., 2016). Moreover, as mentioned earlier, the solubility and loading amount were higher for C-WPI complexes at pH 7.0 compared to pH 3.0 which can be accounted for the higher reducing power. This was in accordance with Wang et al. (2019) who reported a higher antioxidant activity for the curcumin-PPP complex in comparison with PPP, and also indicated that the antioxidant activity increased with increasing of pH. In addition, it was reported that the antioxidant activity of curcumin improves in the presence of proteins probably due to this fact that the proteins such as WPI can promote the electron transfer from curcumin to Fe^{3+} as a consequence improves the rate of sequential proton loss electron transfer (SPLET) process as the main mechanism for curcumin antioxidant property (Li et al., 2013). Therefore, the curcumin-loaded WPI can be used as promising antioxidant components in the formulation of functional foods and beverages which can enhance their health-promoting properties.

3.8 | In vitro release behavior

The in vitro release of curcumin from curcumin-WPI complex prepared by pH shifting method was determined under simulated gastrointestinal conditions, which was defined as the percentage of curcumin
which was transferred to the release medium (Figure 9). The release of curcumin in the simulated stomach was slightly burst and after 2 hr reached to about 31.5% which can be due to the degradation of the protein molecules in the gastric condition (Teng, Li, & Wang, 2014) and the liberation of curcumin which was entrapped near the surface of protein particles. When the samples were transferred from SGF to SIF, a relatively sustained release behavior was observed in which the percentage of released curcumin increased slowly with time, and at the end of 6 hr, the total percentage of released‐curcumin reached to 59.31%. A similar result was reported by Peng et al. (2017) studying the release of curcumin from nanoparticles of rice bran albumin and chitosan. They observed a burst and sustained release profile for curcumin under the simulated gastric and intestinal condition, respectively. These observations suggested that the sustained release at the intestinal environment may help maintain the effective concentration of curcumin in plasma for an extended time period.

4 | CONCLUSIONS

In conclusion, WPI can be used as a promising protein-based carrier for encapsulation of curcumin through the solvent-free pH shifting method. The solubility, chemical stability, and antioxidant activity of curcumin are drastically improved by this approach at pH values of 7.0 and 3.0. Hydrophobic interactions and hydrogen bonds were the main interactions involved in the formation of curcumin-WPI complexes as evaluated by fluorescence and FT-IR spectroscopy. The ultimate pH of complexes was effective on the heat- and photo-stability of curcumin; higher protection was observed at pH 3.0 compared to the pH 7.0. The reducing power assay confirmed that curcumin still possessed strong antioxidant activity after binding to WPI. Moreover, a controlled-release profile was investigated for curcumin under simulated gastrointestinal condition. Therefore, the pH shifting approach can be used as a safe, low-cost, convenient, and organic solvent-free encapsulation method for curcumin and has considerable potential for development of novel functional polyphenol-enriched beverages. However, further works are necessary to investigate the bioavailability and safety of WPI-curcumin complexes formed by pH shifting method in the biological systems using in vivo digestion models.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

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