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Original research

# Improving the water dispersibility and antioxidant activity of curcumin as a hydrophobic bioactive compound by binding to egg white proteins

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A B S T R A C T

In the present study, the fresh egg white was employed as a carrier for enhancing the aqueous solubility and antioxidant activity of the curcumin as a bioactive hydrophobic ingredient. The curcumin-egg white protein complexes were prepared at pH values of 7.0 and 3.0. The results indicated that the binding of curcumin to egg white proteins at pH values of 7.0 and 3.0 drastically improved its water solubility. The fluorescence measurements showed that the hydrophobic interactions were generated between the curcumin and proteins. The curcumin-loaded egg white prepared at pH 7.0 and 3.0 also had a good antioxidant activity that was measured by radical (ABTS and DPPH) scavenging activity and reducing power test. In general, the findings of this study suggested that the egg white can be considered as an efficient system for increasing the aqueous solubility and antioxidant activity of curcumin which enhances its applications in different fields including food, cosmetic, and pharmaceutical industries.

Keywords: Curcumin, Egg white proteins, Fluorescence spectroscopy, Radical scavenging activity, Reducing power

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## 1. Introduction

Curcumin as a widely-used food coloring agent is a phenolic compound derived from the rhizomes of turmeric (Xiang et al., 2018). A wide range of biological and pharmacological activities such antioxidant, anti-inflammatory, and anticancer properties have been reported for the curcumin (Yi et al., 2016; Wang et al., 2018). However, the low water solubility, bioavailability, and chemical stability of curcumin limited its applications in the food and pharmaceutical formulations (Xiang et al., 2018). Different methods such as encapsulation in various delivery systems have been used to overcome these challenges. In this regards, fabrication of food protein-based carriers for improving the solubility and biological activity of curcumin has attracted notable interest for various research are owing to the outstanding of food proteins such as high nutritional value, excellent techno-functional attributes, amphiphilic nature, biocompatibility, and biodegradability (Yi et al., 2016; Abaee et al., 2017; Abbasi Rad & Askari, 2018). Different food proteins such as whey proteins (Liu et al., 2016; Alavi et al., 2018; Mohammadian et al., 2019), soy proteins (Tapal & Tiku, 2012), and rice bran proteins (Liu et al., 2018) have been employed to form curcumin-protein complexes with high water dispersibility and antioxidant activity. Curcumin binds to the protein molecules through the hydrophobic interactions and hydrogen bonds resulting in the formation of soluble complexes (Mohammadian et al., 2019).

Fresh egg white (albumen) is one of the best known sources of proteins (about 9.7-10.6 % w/w) and is extensively widely used in food industry applications such as bakery products, meringues, and meat products (Nasabi et al., 2017). Egg white includes different globular proteins like ovalbumin (54%), ovotransferrin (12%), and lysozyme (3.5%) which play important roles in the formation of egg white-base structures such as hydrogels, nanofibrils, and particles (Babaei et al., 2019). The egg white protein-based structures were used as carriers for different biologically active compounds due to their versatile properties such as remarkable nutritional value, superb technological functionalities, digestibility, self-assembly, and amphiphilic properties (Chang et al., 2019). Therefore, in this study egg white was introduced as new delivery system for a hydrophobic bioactive molecule (i.e. curcumin).

The aim of the present study was to use egg white proteins as novel systems for improving the dispersibility and antioxidant properties of curcumin as a bioactive compound. In this regard, egg white protein-curcumin complexes were produced at pH 3.0 and 7.0 and their properties were studied by different methods.

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## 2. Material and Methods

### 2.1. Materials

Whole eggs were obtained from a local store in Karaj, Iran. Egg whites were separated carefully from the egg yolks and chalaza and then were homogenized by an ultrasonic homogenizer (20 kHz, 100 W) for 3 min to mix the liquid and the thick white. The protein content of egg white was measured by the Kjeldahl method (N×6.25, 10%). Curcumin (with purity more than 95%) was supplied by Bio Basic (Bio Basic Inc., Canada). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis-(3-ethylbanzthiazoline-6-sulfonate) (ABTS) were procured from Sigma-Aldrich (Sa. Louis, MO, USA). All of the other chemicals used in this research also were of analytical grade and obtained from Sigma-Aldrich and Merck.

### 2.2. Complexation of curcumin with egg white

For the production of curcumin-enriched egg white solution, the pH of egg white with protein content of 10% was adjusted to 7.0 and 3.0 using NaOH 5 M and then was charged with curcumin ethanolic dispersion to a curcumin final concentration of 1.0 mg/mL (curcumin to protein ratio of 1:100). The resulting mixtures were then stirred for 5 h at room temperature in a dark place. The final concentration of ethanol in the mixtures never exceeded 0.5% (v/v). Moreover, the aqueous solutions of curcumin at pH 3.0 and 7.0 also were produced by the same conditions.

### 2.3. Solubility measurement

The solubility of curcumin was determined according to Tapal and Tiku (2012). For this purpose, the curcumin-egg white samples and aqueous curcumin dispersions with pH values of 7.0 and 3.0 were centrifuged at 1500 × g for 10 min to remove the undissolved curcumin. Supernatants were diluted with ethanol to extract the curcumin and its absorbance was read spectrophotometrically at 420 nm. Finally, the concentration of curcumin in the supernatant was determined with an ethanolic standard curve of curcumin (0.1-10 µg/mL, R2= 99.99%) and the curcumin solubility in different medium (i.e. distilled water and egg white) was calculated as following:

$$\text{Curcumin solubility (\%)} = \frac{\text{amount of curcumin in supernatant}}{\text{total amount of added curcumin}} \times 100 \quad (1)$$

### 2.4. Fluorescence spectroscopy

The fluorescence properties of samples were studied by a Varian fluorescence spectrofluorometer (Cary Eclipse, Palo Alto, CA). The emission spectra of samples with protein concentration of 0.2 mg/mL and curcumin concentration of 2 µg/mL were recorded from 300 to 400 nm at the excitation wavelength of 280 nm for assessing of the protein intrinsic fluorescence and also were collected from 450 to 700 nm at an excitation wavelength of 420 nm for studying the fluorescence property of curcumin. The emission and excitation slit widths were set at 5 nm.

### 2.5. Antioxidant activity

The antioxidant activity of different samples was evaluated by DPPH and ABTS radical scavenging activity tests and reducing power assay. Before the measurements, samples including egg white, curcumin-egg white and aqueous dispersions of curcumin with pH values of 7.0 and 3.0 were diluted (20-folds) with distilled water of same pH.

For the DPPH radical scavenging (Yi et al., 2016; Mirzakhani et al., 2018), 200 µL of different samples (AS) was charged with 1.0 mL of the ethanolic solution of DPPH (0.1 mM). 1.0 mL of DPPH solution also was added to 200 µL of distilled water as a control (AC). The resulting solutions were mixed and kept for 30 min at room temperature in a dark condition. The absorbance of the resulting mixtures was determined spectrophotometrically at 517 nm and the DPPH radical scavenging activity was calculated using the below equation:

$$\text{DPPH radical scavenging activity (\%)} = \frac{A_C - A_S}{A_C} \times 100 \quad (2)$$

For $ABTS^+$ radical scavenging activity (Chang et al., 2019), the $ABTS^+$ was produced by reacting 7.4 mM ABTS in phosphate buffered saline (pH 7.4) with 2.6 mM potassium persulfate and storing for 18 h at room temperature. The ABTS+ was diluted with distilled water to an absorbance of 0.7 at 734 nm. After that, 200 µL of the samples ($A_{sample}$) or distilled water ($A_{control}$) was mixed with 1.0 mL of the ABTS+ solution and their absorbance was determined after 10 min at 734 nm. Finally, the ABTS radical scavenging activity was calculated with the following equation:

$$\text{ABTS radical scavenging activity (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \quad (3)$$

For reducing power, 1.0 mL of diluted sample solutions was mixed with 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%). The resulting solutions were incubated for 20 min at 50°C. Then, aliquot (2.5 mL) of trichloroacetic acid (10%) was added to the samples. This was followed by 10 min centrifuging at 1500 × g. Subsequently, 2.5 mL of the he supernatants was mixed with 2.5 mL distilled water and 0.5 mL FeCl3 (0.1%) and the absorbance was measured at 700 nm after 10 min incubating at room temperature with a spectrophotometer. A higher absorbance was revealing of a stronger antioxidant capacity (Li et al., 2013).

### 2.6. Statistical analysis

The obtained data were analyzed using Duncan test and on-way analysis of variance (ONOVA) by SPSS 16.0 software package. Difference was considered statistically significant if $P < 0.05$. All the experiments were conducted at least in triplicate.

## 3. Results and Discussion

### 3.1. Curcumin solubility

In the present study, egg white was enriched with curcumin at pH values of 3.0 and 7.0 (Fig. 1). The results of the solubility test (Fig. 2) showed a higher solubility for curcumin in its complex state with egg white proteins compared to its aqueous free form. In fact, the results indicated that the aqueous solubility of curcumin was greatly improved through the complexation with egg white proteins. It was attributed to the formation of soluble complexes

between proteins and curcumin through the hydrophobic interaction resulted in the improvement of the curcumin water dispersibility (Mohammadian et al., 2019). In agreement with our findings, Liu et al. (2016) and Xiang et al. (2018) also respectively reported that the solubility of curcumin was significantly increased by binding to whey proteins and sly protein isolate. Moreover, the results showed a higher solubility for curcumin-egg white complexes prepared at pH 7.0 compared to those formed at pH value of 3.0 which can be due to the higher solubility of curcumin at alkaline and neutral conditions compared to acidic ones. In accordance, Chen et al. (2016) also reported a higher loading amount for curcumin at pH 7.0 compared to pH 3.0 in the case of soy protein isolate attributing to its more compacted structure at pH 3.0 in comparison with pH 7.0.

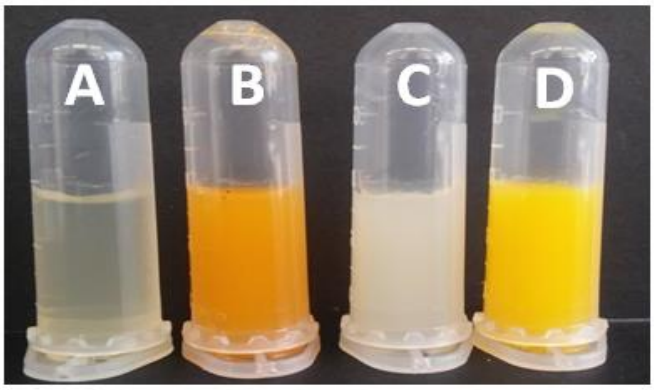


Fig. 1. Visual observations of egg white at pH 7.0 (A) and 3.0 (C) and its complexes with curcumin at pH 7.0 (B) and 3.0 (D).

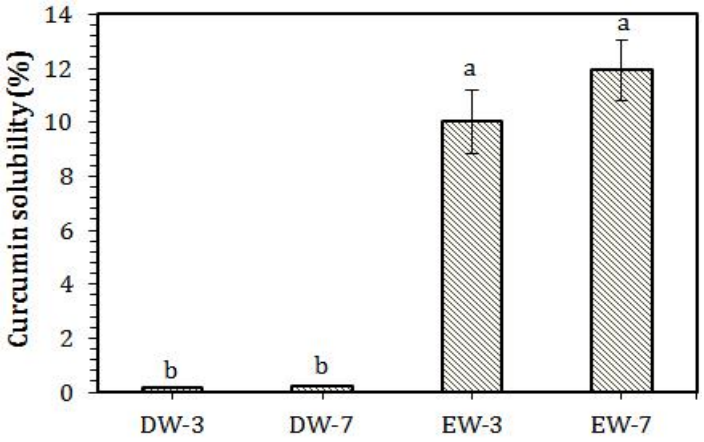


Fig. 2. Solubility of curcumin in distilled water (DW) and egg white (EW) at pH 7.0 and 3.0. Means followed by different letters are significantly different ($p < 0.05$).

### 3.2. *Fluorescence spectroscopy*

The fluorescence spectroscopy was employed to study the interactions of curcumin and egg white proteins. The intrinsic fluorescence of protein was evaluated at an excitation wavelength of 280 nm (Fig. 3A). The results showed that the fluorescence intensity of egg white samples was decreased at both pHs of 7.0 and 3.0 after binding to the curcumin as a ligand. This observation indicated that the binding of curcumin to egg white proteins happened around the fluorophore groups of proteins such as tryptophan and tyrosine residues (Wang et al., 2018). In harmony with our results, it was reported that the fluorescence intensity of soy protein isolate was quenched after binding to the curcumin (Tapal & Tiku, 2012). Liu et al. (2016) also investigated that the intrinsic fluorescence of the whey proteins was decreased after the complexation with the curcumin suggesting that the curcumin was able to form bind the tryptophan or tyrosine residues in the protein. Similar results also were reported for the complexes of the proso millet protein and curcumin by Wang et al. (2018).

The fluorescence spectra of different samples at the 420 nm as the excitation wavelength of curcumin also were recorded (Fig.

3B). The results showed that the fluorescence intensity of curcumin was higher in the complex state compared to its aqueous solution. These results showed that the curcumin was moved from a hydrophilic to a more hydrophobic environment after the complexation with egg white proteins suggesting the formation of hydrophobic interactions between the curcumin and proteins (Li et al., 2013). Our observations are in agreement with those reported by Pan et al. (2013) in the case of curcumin-casein complexes as well as those reported for soy proteins-curcumin complexes by Tapal and Tuku (2012).

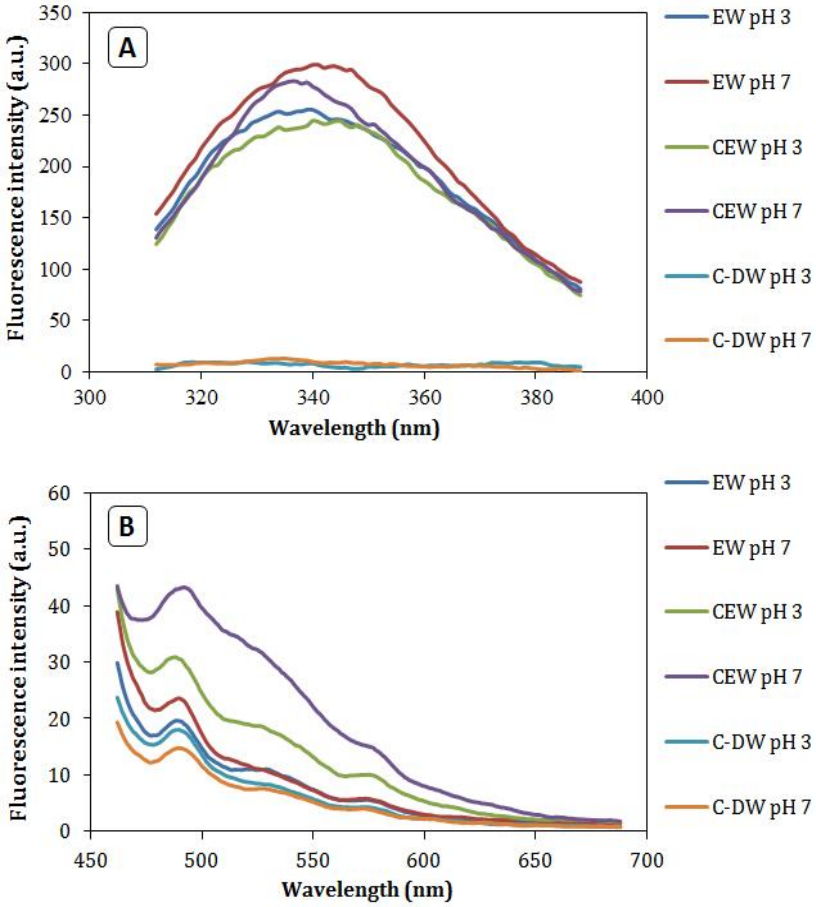


Fig. 3. Fluorescence emission spectra of different samples including free curcumin in distilled water (C-DW), egg white (EW), and curcumin-egg white complex (CEW) with pH values of 3.0 and 7.0 at excitation wavelengths of (A) 280 nm and (B) 420 nm.

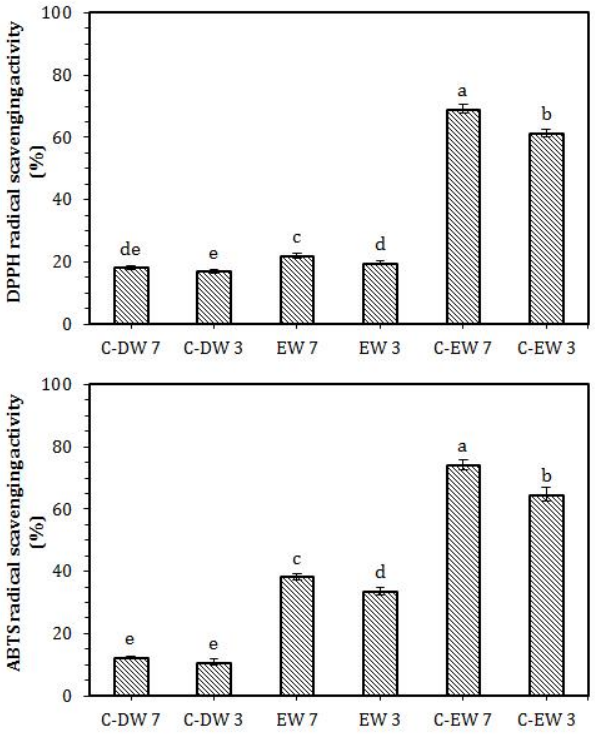


Fig. 4. DPPH and ABTS radical scavenging activity of free curcumin in distilled water (C-DW), egg white (EW), and curcumin-egg white complex (CEW) with pH values of 3.0 and 7.0. Means followed by different letters are significantly different ($p < 0.05$).

### *3.3. Antioxidant activity*

Different methods including ABTS radical scavenging activity (Fig. 4), DPPH radical scavenging activity (Fig. 4), and reducing power (Fig. 5) were used to study the antioxidant activity of the samples. The results showed that in all of the methods, the binding of curcumin to the egg white proteins, significantly improved its antioxidant activity and the curcumin-protein complexes at both pHs of 3.0 and 7.0 had a higher antioxidant activity compared to the curcumin-free counterparts. In fact, the complexation of curcumin with proteins improves its water solubility which results in an increase in the available amount of curcumin for the interaction with free radicals such as DPPH and ABTS radicals (Yi et al., 2016). Moreover, in the case of reducing power assay, the improvement of reducing activity of curcumin after binding to food protein was attributed to the promoting effect of protein for transferring of electron from curcumin to $Fe^{3+}$ which improves the rate of sequential proton loss electron transfer process as the main mechanism for antioxidant activity of curcumin (Li et al., 2013). In a good agreement with our observations, Liu et al. (2017) reported that the free radical scavenging activity and reducing power of curcumin was drastically enhanced after binding to ovalbumin as a major protein of egg white. Mohammadian et al. (2019) also observed that the antioxidant activity of curcumin was significantly improved after the complexation with the whey protein nanofibrils as a nanocarrier. Moreover, our results indicated that the curcumin-egg white complexes with a pH value of 7.0 were more antioxidant that the samples with the pH value of 3.0. This can be due to the higher solubility of the complexes at pH 7.0 compared to pH 30 which was mentioned earlier. Generally, the results of the antioxidant activity measurements suggested that the egg white protein can be efficiently employed as a carrier to increase the antioxidant capacity of curcumin which enhances its uses in the food applications.

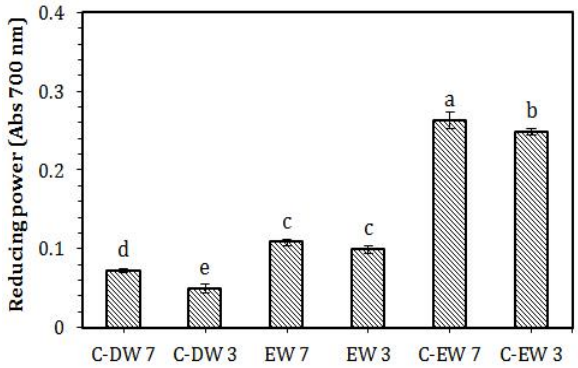


Fig. 5. Reducing power of free curcumin in distilled water (C-DW), egg white (EW), and curcumin-egg white complex (CEW) with pH values of 3.0 and 7.0. Means followed by different letters are significantly different ($p < 0.05$).

## 4. Conclusion

In the current research, the egg white was used as a carrier for improving the aqueous solubility and antioxidant activity of curcumin. The results showed that the complexation of curcumin with egg white proteins at pH values of 7.0 and 3.0 significantly increased its water dispersibility. Fluorescence spectroscopy indicated that the hydrophobic interactions were formed between the curcumin and egg white proteins. The curcumin-loaded egg white prepared at pH 7.0 and 3.0 also showed a good antioxidant activity that was measured by different methods including radical (ABTS and DPPH) scavenging activity and reducing power assay. Generally, the results of the present study suggested that the egg white can be used as an efficient carrier for improving the solubility and antioxidant properties of curcumin as a bioactive ingredient with various health-promoting properties. This study also suggested that the egg white protein-curcumin complexes can be effectively used to produce functional foods such as beverages and drinks. However, further studies are required to investigate the in vivo bioavailability and gastrointestinal fate of the curcumin-loaded egg white proteins.

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