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nano encapsulation of curcumin in mung protein -maltodextrin conjugate, estimation of physicochemical and release properties

Somayeh Aziznia[a], Gholamreza Askari*[a], Zahra EmamDjomeh[a], Maryam salami[a]

a: Department of Food Science and Technology, Faculty of Agriculture, University of Tehran, Karaj, Iran

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*Corresponding Author E-iraskari@ut.ac.ir

ABSTRACT

A delivery system was developed according to maillard reaction using mung bean protein and maltodextrin for encapsulation and sustain release of curcumin. The ultrasound assisted (150 W, 80 °C, 10 min) and classic wet heating (80 °C, 60 min), were used to prepare conjugates at three different ratios of Mung bean protein isolate to maltodextrin. Degree of conjugation was measured using OPA method Uv-visible spectroscopy was used to estimate the final products of maillard reaction. Different amounts of curcumin (0, 0.2, 0.4 and 0.6 mg. mL^{-1} was loaded using ethanol and change in pH. Primary analysis showed that the optimized samples were obtained when 0. 4 mg. mL^{-1} of curcumin was encapsulated using pH change method. FTIR spectra confirmed the conjugation of the MPI and MD and showed the electrostatic and hydrophobic interactions as well as hydrogen bonding are the main reasons of conjugates stability and curcumin encapsulation. The prepared curcumin containing conjugates under optimized method showed the higher DPPH radical scavenging activity. Our results showed that the release rate of encapsulated curcumin under simulated condition of gastrointestinal tract (GIT) was controlled and lower than that which encapsulated in mung bean protein.

1- Introduction

Curcumin (di-feruloylmethane) is a phenolic and lipophilic compound with low molecular weight that is present in turmeric (dried rhizome of Curcuma longa plant) [1 and 2]. Turmeric is usually used as a spice and food color, but this plant has gained a lot of attention in the medicine and food industry in recent years. Extensive biological activities such as antioxidant, anti-inflammatory, anti-cancer, anti-tumor, anti-allergy, antimicrobial, anti-diabetic, etc. properties have been reported for curcumin [1 and 3]. Curcumin is almost insoluble in water, sensitive to alkaline conditions and photodegradation. It also has little resistance to heat and oxidation. Due to rapid metabolism and low intestinal absorption, it has low bioavailability and is quickly broken down and excreted. Therefore, although curcumin has unique and significant medicinal and nutritional properties, regrettably, due to its short half-life and rapid elimination, the body is not able to use the health benefits of it entirely. Therefore, it is absolutely necessary to develop suitable delivery system to overcome these limitations [2 and 4]. encapsulation can be a suitable option to minimize these limitations. In recent years, extensive research has been conducted on the effect of encapsulation and controlled release on various food and pharmaceutical compounds, including curcumin. Since in the process of encapsulation and delivery in food products, food-grade raw materials and green methods must be used, therefore, the use of biological materials derived from animal and plant sources such as proteins, saccharides and lipids, alone or in combination with each other, is used [5]. Mung bean with the scientific name (*Vigna radiata* (L.)) is a legume that is more than 2000 years old. The ability to detoxify the human body, reduce blood cholesterol, anti-tumor and anti-inflammatory properties are among the biological functions of this seed [6]. It has 25-28% protein and except for methionine, cystine and tryptophan, it has all essential amino acids including lysine in amounts close to egg amino acids [7]. Physicochemical and functional properties of mung bean protein have shown that this protein has a high potential for use in delivery systems [6 and 8]. On the other hand, mung bean is a low-input, drought-resistant crop with a short growth cycle, and it can be cultivated in loamy to sandy soils and in areas with irregular rainfall [7, 9]. The cultivated area of mung bean in the world is about 6 million hectares and its production is 4 million tons with an average global yield of 667 kg/ha. The cultivated area of mung bean in Iran is 16167 ha with a production rate of 11661 tons [10]. Therefore, considering the biological and nutritional value and the abundance of this product in Iran and the guarantee of the supply market, mung bean protein has been considered in the current study. In recent years, researches have shown that the stability of proteins and colloids covered with proteins has been significantly improved by conjugation with saccharides. Conjugation improves the functional properties of food proteins, and it is done in different ways, such as physical mixing, creating cross-links by enzymatic or chemical methods, and the maillard reaction [11]. To use conjugates as a food ingredient, the maillard reaction must be conducted properly to avoid the advanced stages and formation of harmful nitrogenous polymers and co-polymers of brown coloration [12]. On the other hand, micro and nano capsules prepared by conjugation by maillard method have been developed to protect bioactive compounds, better stability against environmental stresses (such as high temperature, acidic conditions and ionic shifts). In various researches, maltodextrin has been used in combination with gums, pectin, alginate and serum proteins for encapsulating bioactive compounds and improving emulsifying properties, reducing oxygen permeability from the capsule wall and controlling the release profile [13]. Therefore, in the current study, in addition to mung bean protein isolate, maltodextrin was used to fabricate curcumin conjugate and encapsulation.

2 -Materials and Methods

2-1 Materials

Mung bean seeds were purchased from local farm in Darehshahr city, Ilam province. Maltodextrin (DE = 13 to 16) was kindly provided by Ingredion (Germany, GmbH). All other chemicals and solvents were at analytical grade and were purchased from Sigma-Aldrich (MA, USA), Merk (Darmstadt, Germany) and Dr. Mojallali Lab (Iran).

2-1-1 Preparation of mung bean protein isolate (MPI)

Mung bean protein isolation was prepared by alkaline extraction and acid precipitation method according to Du's method [13].

2-1-2 Preparation of Mung bean protein isolate – Maltodextrin conjugates (MPI-MD)

Mung bean protein isolate powder (1% w/v) and maltodextrin (1, 1.5, 2 % w/v) were dissolved in phosphate buffer solution (0.2 mol L^{-1}, pH 7.2) separately. Sodium azide (0.02 % w/w) was added to prevent bacterial growth during storage. The solutions were mixed with a magnetic stirrer for 2 hours at a temperature of 25 °C and kept overnight at a temperature of 4 °C to ensure complete hydration. The hydrated and completely solubilized samples were filtered to remove insoluble residues and then mixtures of MPI and MD at the distinctive mass ratio (1:1, 1:1.5 and 1:2) were prepared and stirred 1h at room temperature separately. The first group of conjugates was prepared using ultrasonic waves and based on the method of Wang et al (2016) with some modifications [14]. the temperature of solutions set at 80 °C in water bath, then treated with ultrasound power 150 W, for 10 min (with a sequence of 5s of sonication and 5s of rest). After that, the samples were subsequently cooled in ice water to prevent further reactions. For these samples, the U code was considered and to show the ratios of 1:1, 1:1:5, and 1:2 of MPI: MD, the codes U11, U1 1.5, and U1 2 were included, respectively. In second group, the ratios prepared from mung bean protein isolate and maltodextrin mentioned above were placed at 80 °C for one hour and after that, the samples were cooled. For these samples, the W code was considered, and to show the ratios of 1:1, 1:1:5, and 1:2 of MPI:MD, the codes W11, W1 1.5, and W1 2 were included, respectively.

2-1-3 Preparation of curcumin- conjugate nanoparticles

In order to encapsulate curcumin, the pH shifting method (without heat and organic solvent) according to Pan et al.'s report (2018) and the conventional method (simple dissolving of curcumin in organic solvent) according to He et al (2021) was used [15, 16]. A mixture of mung bean protein isolate - maltodextrin conjugate solution and different concentrations of curcumin (0.6, 0.4, 0.2 and 0 mg/ ml) and a mixture of mung bean protein isolate solution and different concentrations of curcumin were formed by pH shifting method. Each of the samples was separately adjusted to pH 12 and mixed with curcumin for 20 minutes and then adjusted to pH 7.2 and then in order to remove the unloaded curcumin, the samples were centrifuged at 4000 ×g for 10 minutes. The samples were called PCC (pH-shifted curcumin- conjugated Mung bean isolate-Maltodextrin) and PCM (pH-shifted curcumin-Mung bean isolate). A complex of mung bean protein isolate-maltodextrin conjugate solution and different concentrations of curcumin and a complex of mung bean protein isolate solution and different concentrations of curcumin were also prepared in a conventional method and called CCC (Conventional curcumin-conjugated Mung bean isolate- Maltodextrin) and CCM (Conventional curcumin- Mung bean isolate). In order to remove the unloaded curcumin, the samples were centrifuged at 4000 ×g for 10 minutes. In all the samples, the procedures were carried out in dark containers to prevent curcumin degradation.

2-2 Methods

2-2-1 Determination of proximate composition and protein recovery

The chemical composition (moisture, ash, protein and fat) of flour and mung bean protein isolate was determined according to the method of Brishti et al (2017) [17]. A 105 °C oven was used for moisture, a Soxhlet method was used to determine the fat, a Kjeldahl method (6.25 × N) was used to determine protein, and a 550 °C oven was used to determine ash. Carbohydrate was calculated by subtracting the total amount of protein, fat, moisture and ash from 100.

Total yield percentage and percentage protein recovery were calculated based on the following equations [18]:

(1) $\text{Yield} = P/S\ (\%)$

(2) $\text{Recovery} = P1/P2\ (\%)$

Where P is the weight of mung bean protein isolate powder, S is the weight of whole grain, P1 is the protein content of mung bean protein isolate powder, P2: protein content of whole grain.

2-2-2 Amino acid analysis

The amino acid content was analyzed during three stages according to the method of Brishti et al (2017) with some modifications [17]. In briefly 1- acid hydrolysis stage, to release amino acids with 6 M hydrochloric acid containing 0.1% phenylphthalein, for at least 24 hours at 110 °C; 2- derivatization by phenylisothiocyanate, drying and redissolving the sample in a suitable solvent of phosphate buffer and acetonitrile and 3- Separation, identification and quantification of the derivative obtained from the preparation stage using HPLC (Elmer series 200, Perkin, USA).

2-2-3 Determination of degree of graft (DG)

The degree of glycosylation was estimated by measuring the free amino groups via the OPA assay according to the method of Zhuo et al (2013) with some modifications [19].

2-2-4 Estimation of the final maillard reaction products

to estimate the progress of the maillard reaction and the production of melanoidin compounds, the absorbance of samples (diluted with 0.1 % SDS) was measured at 420 nm. The mixture of distilled water and SDS was used as control [20].

2-2-5 Determination of encapsulation efficiency and loading capacity

The encapsulation efficiency (EE) and loading capacity (LC) of curcumin were evaluated according to the method of Peng et al (2020), with some modifications [21]. free (unloaded) curcumin in the solutions was removed by centrifugation at 4000 × g for 10 minutes. Then, by diluting the supernatant with ethanol and centrifuging again under the previous conditions, soluble curcumin was extracted and its absorbance at 420 nm was measured by a spectrophotometer. The standard curve of curcumin concentration in ethanol was drawn and its standard equation was determined. For this purpose, curcumin solution was first prepared at a concentration of 10 ppm and then by diluting it from 10 to 1 ppm, other solutions were prepared. The absorbance of each solution at 420 nm was determined. Finally, the standard curve of concentration and absorption was prepared and the standard equation was obtained. The encapsulation parameters were calculated by using following equations:

(3) EE (%) = m1/ m2 ×100

(4) LC (%) = m1/ m3

Where EE is the encapsulation efficiency, LC is the amount of loading, m1 is the amount of encapsulated curcumin, m2 is the amount of total curcumin, and m3 is the amount of total protein.

2-2-6 Determination of particle size distribution

The particle size distribution of diluted samples was measured by dynamic light scattering (DLS) at pH 7.2. The average diameter of the particles, the polydispersity of dispersion and the zeta potential were studied in measurements at a laser wavelength of 659 nm, a scattering angle of 90 degrees and a temperature of 25 °C using a particle analyzer (Brookhaven Instruments Corp., Holtsville,201 NY, USA).

2-2-7 Fourier transform infrared (FTIR) spectroscopy

In order to evaluate the chemical structure of the samples and investigate alterations occurred in functional groups, Fourier transform infrared spectroscopy was used. The samples were dried by a freeze dryer, mixed with potassium bromide (KBr) at a ratio of 1 to 10, and then compressed to form pellets. The FTIR spectrum were recorded on an infrared spectrometer (Spectrum two; Perkin Elmer, MA, USA) over a wavelength range of 500- 4000 cm^{-1} [22].

2-2-8 Evaluation of DPPH radical scavenging activity

The DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical inhibitory activity of the samples was performed according to the method of Yi et al (2016), with minor modifications [23]. 2 ml of the sample solution (with protein concentration of 5 mg/ mL and curcumin concentration of 0.2 mg/ ml) was added to 2 ml of 0.12 mM DPPH ethanol solution. after mixing, the samples were kept in a dark place for 30 minutes at room temperature and then centrifuged at 750 × g for 10 minutes. Then, the absorbance of the supernatant solution of the samples was read at a wavelength of 517 nm using a spectrophotometer. 2 ml of sample solution and 2 ml of ethanol were used as blank and 2 ml of distilled water and 2 ml of DPPH solution were used as control samples.

DPPH radical scavenging activity was calculated using the following equation:

(5) Radical scavenging activity(%) = $\frac{Ac-(As-Ab)}{Ac} \times 100$

Where, As is the absorbance of samples, Ab is the absorbance of blank and Ac is the

absorbance of control.

2-2-9 In vitro curcumin release

The amount of curcumin release in the in vitro simulated condition of gastrointestinal tract (GIT) was investigated based on the method of Maltais et al. (2009) with some modifications [24]. Briefly, 3 mL of each of the samples were poured separately into a dialysis bag (12 kDa) and mixed with 3 mL of simulated gastric fluid (SGF) containing HCl, NaCl, and pepsin enzyme (with a concentration of 3.2 mg/ml) at a pH 1.2. Then, each dialysis bag was immersed in 150 ml of release medium containing SGF and ethanol (without enzymes) and stirred for 2 hours at 37 °C. Then, the content of each bag was mixed with 6 ml of simulated intestinal medium (SIF) including sodium hydroxide, monobasic potassium phosphate and pancreatin enzyme (with a concentration of 10 mg/ mL) at pH 7.5. Each dialysis bag was immersed in 150 ml of fresh release medium containing SIF and ethanol (without enzymes) and stirred at 37°C for 4 hours. To determine the amount of release, every hour, a portion of the release medium in which the bag was removed and after determining its absorbance at 420 nm, it was returned to the release medium in order to prevent the volume of the release medium from changing. Finally, the curcumin standard curve was used to calculate the released curcumin concentration.

2-2-10 Statistical analysis

The results were analyzed by analysis of variance (ANOVA) using SPSS (version 16 USA). Duncan's multiple range tests was carried out to compare 95% confidence level.

3-Results and Discussion

3-1 proximate composition and protein recovery

The chemical composition of a food has an important role in estimating its nutritional quality and its uses in the food industry [17]. The chemical composition of mung bean flour and mung bean protein isolate are shown in Table 1. The amount of moisture, protein, fat, ash and carbohydrates content in mung bean flour were 8.96%, 20.07%, 1.02%, 3.39% and 66.56%, respectively and in mung bean protein isolate, 5.81%, 81.13%, 0.19%, 4.02% and 8.85% were obtained respectively. The lower fat content in the isolate was due to the defatting of the flour before the protein extraction process. The difference in the amount of humidity can be caused by the isolation production process or the difference in the relative humidity of the environment. Li et al. (2010) examined 60 varieties of mung beans and for their flour moisture 7.49-8.45%, protein content 24.26-28.50%, fat content 1.86-0.57%, ash content 4.24-3.64%, and carbohydrates 54.25-58.69% were reported. Also, also for protein isolate obtained from different varieties, moisture, protein, fat and ash were measured in the ranges of 8. 20-9.28%, 69.22-74.85%, 0.36-0.64%, and 2.19- 3.04% respectively. These researchers attributed the high amount of mung bean ash compared to many legumes to the presence of high salts in this grain.

Table1. Proximate composition of mung bean flour and mung bean isolate

	Mung bean flour	Mung bean protein isolate
Moisture (%)	8.96±0.11	5.81±0.64
Protein (%)	20.07±1.29	81.13±1.17
Fat (%)	1.02±0.35	0.19±0.34
Ash (%)	3.39±0.56	4.02±0.52
Carbohydrate (%)	66.56±2.34	8.85±1.52

Each value in the table represents the mean± SD of triplicate determinations.

The higher amount of ash in the isolate compared to flour may be due to the formation of salt due to the addition of acid and alkali during the process of preparing the isolate [25].

Total yield and protein yield were 18% and 72.76%, respectively. The efficiency of protein extraction by alkaline extraction - isoelectric precipitation and extraction with salt was 66.5% and 40.9% respectively by Rahma et al (2000) And for these two methods and their combination with each other, 30.4% to 67.5% has been reported by Wintersohle et al (2023) [26, 27].

3-2 Amino acid analysis of mung bean protein isolate

Since the behavior of proteins depends to a large extent on the amino acids composition, therefore, in this research, the amino acid profile of isolated mung bean protein was determined. As shown in Table 2, the total free amino acids content was 625.77 mg/g, of which 312.38 mg/g was hydrophilic amino acids and 313.39 mg/g was hydrophobic amino acids.

Table 2. Amino acid profile of mung bean flour and mung bean isolate

	Amino acids	mg/g of protein
Hydrophilic	Aspartic acid	68.57±1.23
	Glutamic acid	126.34±0.36
	Histidine	21.1±3.01
	Arginine	37.83±1.67
	Threonine	29.2±2.03
	Tyrosine	29.34±3.12
	Total	312.38
Hydrophobic	Glycine	25.32±1.63
	Alanine	28.31±0.12
	Proline	36.84±1.04
	Valine	25.16±2.11
	Methionine	10.27±2.26
	Cysteine	4.14±1.19
	Isoleucine	19.13±3.42
	Leucine	31.84±1.32
	Phenylalanine	33.68±1.89
	Total	313.39

Data are expressed as the mean of tow replicate± SD

Kudre et al. (2013) investigated the compounds of mung bean protein isolate. In this research, the amount of total protein was 87.8%, the amount of total amino acid was 800.2 mg /g, the essential amino acids were 43.5%, and the content of sulfur-containing amino acids was about 1.6% of the total amino acids [28]. Brishti et al. (2017) compared the amino acid profile of mung bean protein isolate with soy protein isolate. They reported the amount of hydrophilic (653.68 mg/g) and hydrophobic (493.4 mg/g) amino acids of mung bean protein were significantly higher than hydrophilic (568.16 mg/g) and hydrophobic (429/47 mg/g) amino acids of soy protein. However, the ratio of hydrophilic to hydrophobic amino acids was almost similar in both isolates [17]. Dahiya et al. (2015) reviewed the nutritional and technological potentials of mung bean in research and pointed out that different values for amino acids of mung bean have been reported in researches due to the difference in the tested variety and the methods of determining amino acids [29]. On the other hand, the results of the report of researchers that mentioned above, showed that the highest percentage of amino acids in mung bean were glutamic acid and aspartic acid, which was in accordance with the results of our research. These two amino acids are known as monosodium glutamate-like amino acids, which create a pleasant and appetizing smell during food preparation [30].

3-3 Effect of MPI- MD ratio on the graft reaction

The degree of graft (glycosylation) is often used to estimate the extend of the Maillard reaction

and is measured according to the changes of the free amino groups of the protein [19]. The degree of glycosylation of different treatments are shown in Table 3. Conjugates showed higher DG than mung bean protein isolate. The low level of DG in the mung bean protein isolate was probably due to the presence of carbohydrate residues during the preparation of the isolate. With the increase in the ratio of maltodextrin, the amount of DG increased, which indicated that with the increase in the ratio of carbohydrates, more reducing carbonyl groups were provided to interact with the amines of mung bean protein, and as a result, there was a decrease in free amine groups and an increase in DG [31]. Xue et al. (2013) prepared the conjugate of soy protein isolate with maltodextrin and acacia gum separately and after determining the degree of graft of each of the conjugates showed that the protein-maltodextrin reaction rate is significantly higher than that of protein-acacia gum. These researchers involved the flexibility and higher solubility of maltodextrin compared to acacia gum in this difference [32]. In the same ratio of protein to maltodextrin, the higher DG value in the treatments prepared with ultrasound compared to the treatments prepared by classical wet heating. This suggested that ultrasound could improve the graft reaction of MPI and MD [14]. It might be related to the more energy provided by ultrasound waves and available free amino groups for the graft process.

Table 3. Influence of different glycation methods (U and W) on the degree of graft (DG) in controls (U-MPI and W-MPI) and various of MP: MD ratios.

Treatments	U-MPI	U11	U1 1.5	U12	W-MPI	W11	W1 1.5	W12
DG values	2.24±0.03^{e}	33.02±1.58^{c}	35.61±0.71^{b}	38.73±1.57^{a}	2.20±0.02^{e}	29.06±0.02^{d}	29.75±0.36^{d}	33.64±0.77^{c}

Results having different letters are significantly different ($p<0.05$)
Each value in the table represents the mean± SD of triplicate determinations

Peanut protein isolate-glucomannan [33], mung bean protein isolate-glucose [14], beta-conglycinin-maltodextrin [34], black bean protein isolate-glucose [35] and chitosan-fructose [36] are examples of pairs of reactants in the maillard reaction which indicated the improving effect of ultrasound compared to the wet heating treatments to promote the glycosylation. In all these researches, the cavitation is considered as the most important factor in the development of millard reaction. cavitation by opening the interwoven structure of the protein, exposes the internal groups buried inside the structure of the protein chain and thus providing more amino groups and improving the maillard reaction.

3-4 Estimation of the final millard reaction products

Maillard reaction is a complex reaction that occur due to covalent bonds between the amino groups and carbonyl compounds. The final stage of the reaction leads to the formation of brown nitrogenous polymers and copolymers, which are called melanoidins [12]. The progress of the final products of the Maillard reaction was determined by measuring the absorbance of the samples at a wavelength of 420 nm (A420) [37, 38 and 39]. In both groups, the absorption of conjugated increased compared to the control sample (Table 4). At the same ratio of protein to maltodextrin, the intensity of browning in the treatments prepared with ultrasound waves was lower than the treatments prepared by the wet heating treatment.

Table 4. Influence of different glycation methods (U and W) on the absorbance at 420 nm as indicator of advanced stage of maillard reaction in controls (U-MPI and W-MPI) and various of MP: MD ratios.

Treatments	**U-MPI**	**U11**	**U1 1.5**	**U12**	**W-MPI**	**W11**	**W1 1.5**	**W12**
A 420	0.02±0.001^{e}	0.20±0.003^{d}	0.23±0.002^{c}	0.23±0.005^{c}	0.02±0.001^{e}	0.23±0.003^{c}	0.26±0.002^{b}	0.28±0.002^{a}

Results having different letters are significantly different ($p<0.05$)
Each value in the table represents the mean± SD of triplicate determinations

These results are in accordance with the results of Chen et al. (2019) and Zhang et al. (2014) and are probably due to the reduction of side reactions during graft process with ultrasound. These waves have affected the speed and amount of browning by reducing the polymerization of intermediate products and interfering with the formation of final Millard

products [31 and 34]. These waves have affected the rate of browning by reducing the polymerization of intermediate products and interfering with the formation of final millard products [31 and 34]. Contrary to these results, Wang et al (2016) showed that the intensity of browning of the treatments prepared with ultrasound waves was higher than the treatments prepared by the wet heating and suggested the increase in the local temperature of the solutions caused the increase in the value of melanoidin after the use of ultrasound [14].

3-5 encapsulation efficiency and loading capacity results

According to the results obtained from the degree of glycosylation and the estimation of millard's final products, the mung bean protein isolate-maltodextrin treatment at a ratio of 1 to 2 (U12) was selected as the optimal treatment and used to load curcumin. In order to evaluate mung bean protein and mung bean protein-maltodextrin conjugate for curcumin loading, the encapsulation efficiency and loading capacity of the samples were determined. As shown in Table 5, the pH shift method significantly increased the encapsulation efficiency and loading capacity of the treatments compared to the conventional method.

Table 5. Effect of curcumin concentration and loading method on the encapsulation efficiency (EE) and loading capacity (LC)

Curcumin concentration (mg mL^{-1})	EE (%)				LC (%)			
	PCC	PCM	CCC	CCM	PCC	PCM	CCC	**CCM**
0.2	0.23^{a} ·± 57.46	52.51±1.07^{a}	17.25±0.39^{a}	8.32±0.13^{b}	1.15±0.03^{a}	1.05±0.02^{a}	0.34±0.01^{a}	**0.16±0.01^{a}**
0.4	66.18 ±1.15^{c}	63.03±1.12^{c}	20.27± 0.12^{c}	11.78±0.06^{c}	2.64±0.12^{b}	2.52±0.11^{b}	0.81±0.07^{b}	**0.47±0.02^{c}**
0.6	59.23±2.46^{b}	59.18±0.98^{b}	19.25±0.84^{b}	7.07±0.21^{a}	3.55±0.31^{c}	3.55±0.07^{c}	1.15±0.30^{c}	**0.42±0.02^{b}**

Means with different superscripts in the same column differ significantly ($p < 0.05$).

Peng et al. (2020) investigated the effect of the pH shift method to produce nanoparticles with four kinds of biopolymers including: caseinate, milk whey proteins, soy protein and gum arabic loaded with curcumin and pointed this method contributed to the improving encapsulation efficiency and high loading rate [21]. Jiang et al. (2017) considered increasing the ionic strength in the medium in extremely alkaline or acidic pH conditions can be effective in improving solubility, reducing some interactions of side chains and increasing the flexibility of the dissociated protein molecules, and for such proteins have used the term “molten globule” (MG) [40]. In alkaline pH (pH>8), curcumin is dissolved and its solubility increases significantly with the deprotonation of curcumin hydroxyl groups and progressively increase of negative charge density. In the presence of dissociated proteins that have hydrophobic interiors, the curcumin molecules move into hydrophobic parts to reduce their contact with water and protein and curcumin molecules could be co-dissolved. When the pH is then adjusted back to neutral, the protein structure is refolded and the curcumin molecules are enclosed inside the self-assembled protein particles [15,21 and 40]. Based on the results of Table 5, in addition to the loading method, conjugation also had a significant effect on the encapsulation parameters, and the mung bean protein - maltodextrin conjugate treatments prepared by the pH-change method, showed a higher encapsulation efficiency and loading capacity than the other treatment. He et al. (2021) reported higher EE and LC for soy protein isolate-dextran conjugate than soy protein isolates in curcumin loading [15]. The role of ultrasound waves on the changes in the protein structure and possibly the effect on the amount of enclosed curcumin should not be overlooked.

In the present study, three different concentrations of curcumin were considered and for each of the treatments, the percentage of EE and LC was calculated separately. The highest encapsulation efficiency was related to the concentration of 0.4 mg/ml of curcumin. Therefore, to perform other tests, PCC treatment (mung bean isolate protein-maltodextrin conjugate nanoparticles prepared by pH shift method) and 0.4 mg/ml curcumin concentration were considered. In order to make a better comparison, the treatment of PCM (mung bean protein nanoparticles prepared by the pH shift method) was also investigated.

3-6 Evaluation of DPPH radical scavenging activity

In order to investigate the antioxidant activity of the samples, the DPPH radical scavenging ability test was used. As shown in Figure 1, the highest radical inhibitory activity was related to the curcumin-conjugate compound prepared by pH shift method (PCC).

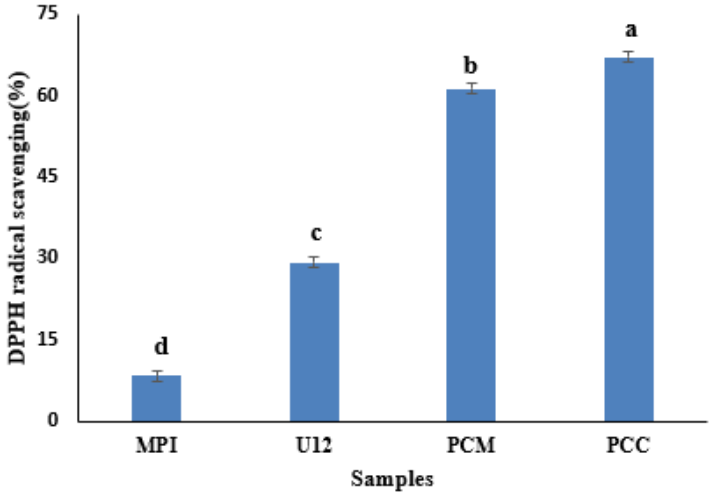

Fig. 1. DPPH radical scavenging activity of different samples including mung bean protein isolate (MPI), ultrasound-assisted maillard reaction with the ratio 1 to 2 of MPI to MD (U12), pH-shifted curcumin- Mung bean isolate (PCM) and pH-shifted curcumin- conjugated Mung bean isolate- Maltodextrin (PCC)

The higher antioxidant activity of PCC compared to PCM (curcumin-mung bean protein isolate) can be due to the higher encapsulation efficiency and the loading capacity of it. Fan et al. (2018) reported an increase in the antioxidant activity of bovine serum albumin-dextran conjugate after loading with curcumin. These researchers attributed it to the presence of curcumin as an inhibitor of free radicals, reducing metal ions, and preventing lipid peroxidation [41]. Mung bean protein isolate (MPI) showed few antioxidant properties. Proteins have antioxidant properties that the amount of it, is corelated with the source, amount and composition of amino acids [23]. The antioxidant properties of hydrophobic amino acids in proteins have also been mentioned in some researches [42]. The conjugate of mung bean protein isolate-maltodextrin (U12) showed higher radical scavenging activity compared to mung bean protein isolate. In accordance with the results of previous research, Gu et al. (2010) showed that casein-glucose conjugate had higher antioxidant properties than casein [43]. The higher inhibitory activity of the conjugate in compare with the protein can be attributed to the formation of some antioxidant products in the maillard reaction [44].

3-7 Fourier transform infrared (FTIR) spectroscopy

In order to study the interactions between curcumin and mung bean protein isolate (PCM) and curcumin with mung bean protein isolate-maltodextrin conjugate (PCC) prepared by pH shift method, fourier transform infrared spectroscopic analysis was used.

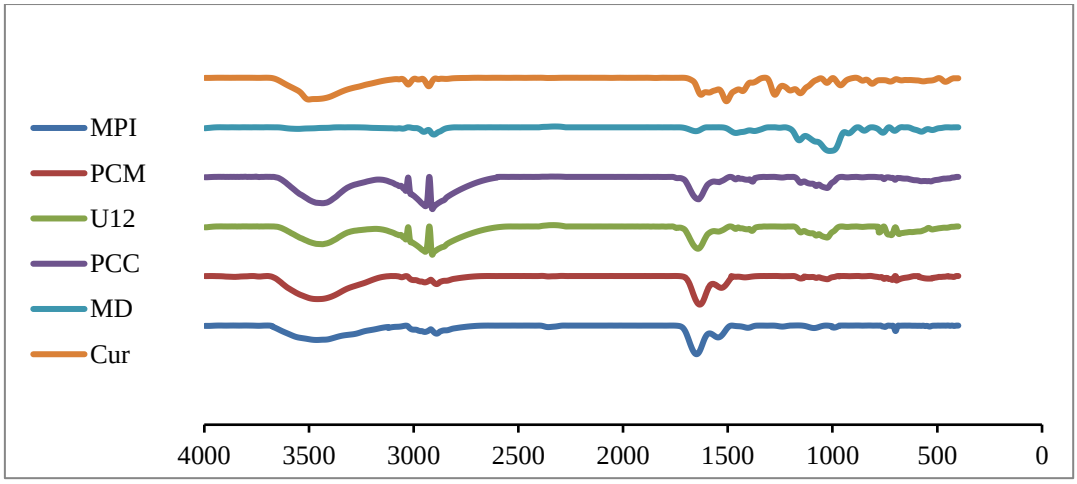

Fig. 2. FT-IR spectra of different samples including mung bean protein isolate (MPI), ultrasound- assisted maillard reaction with the ratio 1 to 2 of MPI to MD (U12), pH-shifted curcumin- Mung bean isolate (PCM) and pH-shifted curcumin-conjugated Mung bean isolate- Maltodextrin (PCC), free curcumin (Cur) and maltodextrin (MD)

As shown in Figure 2, the comparison between the main peaks in MPI and U12 shows a shift of the 3464 cm^{-1} peak and an increase in the absorption intensity in a broad region of ~3670-3030 cm^{-1} and a change in the intensity of the peaks in the 2947-2889 cm^{-1} region that refers to the role of hydrogen bonds in MD-MPI conjugate complex. Also, the shift of amide I and II peaks from 1649 cm^{-1} and 1546 cm^{-1} to 1643 cm^{-1} and 1544 cm^{-1} respectively indicates the role of hydrophobic interactions and electrostatic attraction in the formation of the conjugated complex. In Figure 2, different functional groups of free curcumin show various specific peaks that disappeared after binding to MPI and MD-MPI conjugate. When curcumin was coated inside the mung bean protein structure, there was a shift in the peaks from 1649 cm^{-1}, 1546 cm^{-1}, 1404 cm^{-1} and 3464 cm^{-1} in MPI to 1635 cm^{-1}, 1531 cm^{-1}, 1417cm^{-1} and 3460 cm^{-1} respectively were observed in PCM. The mung bean protein-maltodextrin conjugate with a ratio of 1 to 2 (U12) underwent slight changes after loading with curcumin by pH shift method (PCC). Peaks from 1643 cm^{-1}, 1544 cm^{-1}, 1384 cm^{-1} and 3435 cm^{-1} in U12 shifted in the regions around 1639 cm^{-1}, 1541 cm^{-1}, 1381 cm^{-1} and 3439 cm^{-1} in PCC, respectively. The above changes showed that curcumin was able to be encapsulated in the structure of nanoparticles through hydrophobic interactions, electrostatic attraction and hydrogen bonds. Also, the results showed that in PCM, the role of hydrophobic interactions and electrostatic attraction was more dominant than hydrogen bonds, but in PCC, almost all of the above binding forces played an equal role in the formation of nanoparticles loaded with curcumin. In accordance with the obtained results, Liu et al. (2017) reported in the curcumin-ovalbumin complex, molecular hydrophobic and hydrogen bonding interactions were the most possible interactions in the process of binding curcumin to proteins [45]. Guo et al. (2020) showed that hydrogen bonds, hydrophobic interactions, and electrostatic attraction involved in the encapsulation of curcumin in pea protein-pectin conjugate, and the first two mentioned binding forces were more dominant [46]. Guo et al. (2021) prepared a pea protein isolate-high methoxyl pectin-rhamnolipid complexes for microencapsulation of curcumin and resveratrol and reported that hydrogen bonds, hydrophobic interactions, and electrostatic attractions were main driving forces in the formation of the complexes [47].

3-9 Determination of particle size and size distribution

Table 6 has shown that the mean particle size of PCC was larger than PCM. This difference was due to the presence of maltodextrin as a secondary coating on the protein particles. The particle size in U12 and MPI was about 167.0 and 127.33 nm, respectively (DLS results not shown), and after loading with curcumin, the larger particle size of the PCC nanoparticles than PCM nanoparticles was not unexpected.

Table 6. Z-average, polydispersity index and ζ – potential of pH-shifted curcumin- Mung bean isolate (PCM) and pH-shifted curcumin- conjugated Mung bean isolate- Maltodextrin (PCC)

Particles	Z-average(nm)	PDI	ζ – potential
PCC	195.7±1.64	0.31±0.01	-12.8±0.8
PCM	165.2±2.11	0.29 ±0.01	-13.8 ± 0.2

Zhang et al. (2014) also showed that β conglycinin -dextran particles had a larger size than β conglycinin particles [34]. The zeta potential in PCC was lower than that of PCM, and this difference could be due to the presence of maltodextrin around the proteins and its role as a steric barrier may screen the positively charged surface from the continuous phase [48]. This probably contributed to the higher poly dispersity index (PDI) of this treatment compared to PCM. Fan et al. (2018) used bovine serum albumin-dextran conjugate prepared by dry maillard method to load curcumin. These researchers showed that the particle size increased after loading with curcumin [41]. Ha et al. (2012) showed that the size of nanoparticles prepared from natural polymers after loading of curcumin, was smaller than the size of curcumin and attributed this to the role of hydrophilic polymers in preventing the aggregation of hydrophobic curcumin [49]. In the research conducted by Guo et al. (2020), the size of pea protein isolate-dextran nanoparticles loading curcumin was 559.20 nm and the zeta potential was determined -14.80 [46].

3-10 In vitro release

The in vitro release of curcumin-loaded samples (PCM and PCC) was investigated under simulated condition of gastrointestinal tract. According to Figure 3, after 2 hours of digestion in gastric conditions and with the presence of pepsin, the cumulative release percentage of curcumin in PCC and PCM samples was 24.30% and 34.51%, respectively. After 6 hours of digestion (2 hours in SGF and 4 hours in SIF), the amount of released curcumin for PCC and PCM was determined as 93.93% and 93.31%, respectively.

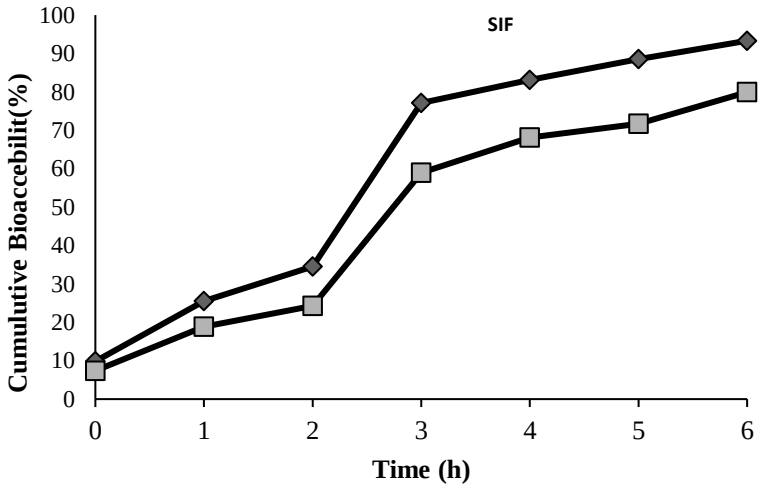

Fig. 3. Profiles of curcumin *in vitro* release from, pH-shifted curcumin- Mung bean isolate (PCM) and pH-shifted curcumin-conjugated Mung bean isolate- Maltodextrin (PCC) under simulated gastrointestinal condition

The results showed that curcumin encapsulated in PCC had a slower release than the PCM sample in both SGF and SIF conditions during the release. Encapsulation of curcumin in mung bean protein-maltodextrin conjugate prepared by pH shift method, due to the steric hindrance created by maltodextrin molecules, reduced the access of enzymes to the reactive site and thus led to differences in digestibility and made it resistant to pepsin and pancreatin enzymes and digestion conditions. The proteins that refolded in the cycle of pH change and surrounded curcumin, had a compact structure that was able to keep curcumin inside its structure for a longer time. As well as, the presence of a second shell of maltodextrin around the nanoparticles, caused PCC to show a more controlled release behavior for curcumin during gastrointestinal digestion compared to PCM. Generally, the results showed that curcumin encapsulation in mung bean protein-maltodextrin conjugate with pH change method may be suitable method to control the release of such lipophilic compounds in digestion

conditions. The results are in accordance with the results of Paşcalău et al. (2016) who used multilayer microcapsules of bovine serum albumin/polysaccharides as a curcumin delivery system and pointed out the good effect of curcumin encapsulation using multilayer membranes in controlled release [50]. Guo et al. (2020) pointed out the controlled release of curcumin in the conjugate of pea protein isolate-pectin during the simulated digestion condition and showed that during 180 minutes of the presence of the complex in digestion condition, the release of curcumin occurred slowly and controlled [46]. Mina et al. (2021) loaded curcumin in whey protein-maltodextrin concentrate conjugate and showed that after two hours of digestion in SGF condition, a small amount of curcumin (12.01%) was released. These researchers attributed the existence of a thick layer of maltodextrin around the carrier particles as the reason for their resistance against disintegration in acidic conditions of the SGF [51].

4- Conclusions

The ratio of protein to maltodextrin and the method of the maillard reaction affect the degree of conjugation and the amount of the final products of maillard, and here the highest degree of conjugation and the lowest amount of melanoidin were obtained in the treatment prepared with ultrasound waves and in a ratio of 1 to 2 from mung bean protein isolate to maltodextrin. Encapsulation efficiency and loading capacity of curcumin were higher in mung bean protein isolate-maltodextrin conjugate than in mung bean protein isolate. Encapsulation of curcumin in conjugated complex showed higher antioxidant activity and slower release in the GIT compared to protein isolate.

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سمیه عزیزنیا[a]، غلامرضا عسکری [a*]، زهرا امام جمعه[a]، مریم سلامی[a]

a: به ترتیب دانشجو، دانشیار، استاد و دانشیار گروه علوم و صنایع غذایی دانشکده کشاورزی دانشگاه تهران

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کورکومین،

پروتئین ماش

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* مسئول مکاتبات:

iraskari@ut.ac.ir

چکیده

در این پژوهش با بهره‌گیری از واکنش میلارد، یک سیستم تحویل برگرفته از منابع گیاهی(ایزوله پروتئین ماش و مالتودکسترین) برای ریزپوشانی و رهایش کنترل شده کورکومین طراحی و ساخته شد. واکنش میلارد به دو روش استفاده از امواج فراصوت (۱۵۰ وات، ۸۰ درجه سلسیوس، ۱۰ دقیقه) و روش مرطوب(۸۰ درجه سلسیوس، ٦٠ دقیقه) برای سه نسبت مختلف ایزوله پروتئین ماش به مالتودکسترین انجام شد. درصد مزدوج سازی به روش OPA و تخمین محصولات نهایی میلارد توسط اسپکتروفوتومتر انجام شد و تیمار بهینه برای بارگذاری کورکومین انتخاب گردید. غلظت‌های مختلف کورکومین (۰/٦ mg mL^{-1}، ۰/٤، ۰/۲، ۰) به دو روش متداول(انحلال در اتانول) و روش تغییر pH (روشی بدون نیاز به حلال‌های آلی) بارگذاری شدند. پس از تعیین کارایی درونپوشانی و میزان بارگذاری کورکومین، تیمار مزدوج ایزوله پروتئین ماش- مالتودکسترین تهیه شده به روش تغییر pH و غلظت ۰/٤ میلی گرم بر میلی لیتر کورکومین به عنوان تیمار بهینه انتخاب و برای مقایسه بهتر نتایج و بررسی تاثیر مزدوج سازی، از تیمار ایزوله پروتئین ماش حامل کورکومین نیز به عنوان کنترل استفاده شد. نتایج تعیین اندازه ذرات(DLS) نشان داد که ذرات در ابعاد نانو بوده و نتایج FTIR نشان داد که کورکومین توانسته از طریق برهمکنش‌های آبگریز، جاذبه الکترواستاتیکی و پیوندهای هیدروژنی در ساختار نانو ذرات کپسوله شود. بررسی فعالیت آنتی اکسیدانی نشان داد که بیشترین قدرت مهارکنندگی رادیکال DPPH مربوط به ترکیب مزدوج- کورکومین تهیه شده به روش تغییر پی اچ بوده است. این امر می‌تواند به دلیل بالاتر بودن کارایی درونپوشانی باشد. رفتار رهایش کورکومین در شرایط هضم متوالی شبیه سازی شده گوارشی(معده و روده) نشان داد که درونپوشانی کورکومین با روش انجام گرفته در این پژوهش باعث رهایش آهسته کورکومین شده و در تیمار تهیه شده با مزدوج ایزوله پروتئین ماش- مالتودکسترین رهایش کورکومین آهسته‌تر از تیمار ایزوله پروتئین ماش بود.