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Effect of Microwave Irradiation Nonuniformity on the Digestion and Allergenicity of the Glycated Ovalbumin

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Abstract The glycated OVA by microwave irradiation was digested by different methods (gastric digestion, intestinal digestion, and two-step digestion). Digestibility properties change of glycated OVA after digestion were evaluated in this work. The results showed that the samples were mainly digested in the stomach and they were hardly hydrolyzed by trypsin. Glycated OVA digested in stomach or in intestines produced more free amino groups than that by two-step digestion. Calcium ion chelating ability of glycated OVA was much higher than OVA when digested in stomach and intestines; glycated OVA had stronger ferrous ion chelating abilities by intestinal digestion. Glycated OVA after being digested hold the better antioxidant activity than the digested OVA, especially the Sample 3. IgG and IgE binding of all samples after intestinal digestion and two-step digestion were lower than that by gastric digestion. The IgG binding of glycated OVAs were lower than OVA, the result of the IgE binding was the opposite.

Keywords: *ovalbumin, glycation, digestion, microwave*

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1. Introduction

Egg proteins are high-quality proteins [1], Egg white protein is widely used in food processing. It not only contains the essential amino acids of human body, but also shows better processing characteristics during food processing, such as foaming, gel and other characteristics. However, due to the antigenicity of egg white protein, some of its functional characteristics are not significant, improving its processing characteristics and reducing the antigenicity by glycation modification are the focus and hotspot of research at home and abroad. Zhigao Wang ect. find that glycation can improve the gelation property of rapeseed protein isolates [2]. It can also increase the emulsifying property of some proteins [3] and decrease allergicity [4].

However, under normal conditions, glycation proceeds very slowly. Different methods have been found to improve the functional properties of OVA, such as pulsed electric field [5,6], dynamic high pressure micro fluidization [7], and ultrasonic [8]. At present, the research methods of protein glycation include dry process [9,10], wet process [11,12], synergistic treatment [4,13] and microwave heating [14], most of these methods are time-consuming. Microwave heating is efficient and fast and has a good effect on glycation It was found that microwave-assisted glycated OVA had antioxidant activity and hypersensitivity. However, amino acid loss also occurred. At the same time, K and R amino acids on protein were screened to reduce its digestive characteristics in gastrointestinal tract. At present, there is no report on the study of its gastrointestinal digestion.

As a model protein, OVA has been widely used in the study of the effect of glycation on the structural properties of proteins. It has been found that glycation between OVA and carboxymethyl cellulose can improve its functionality [15]. Other reports have studied that glycation can reduce allergenicity of OVA [16]. However, there are few studies on the digestive characteristics and allergenicity of glycated products in gastrointestinal tract.

In this paper, glycated products of OVA were obtained by microwave heating. Digestion characteristics, antioxidant activity and antigenicity of the products were studied in gastric, intestinal, and two-step digestion, so as to provide theoretical guidance for the nutritional safety of glycated proteins.

2. Materials and Method

2.1. Chemicals and Material

OVA (Grade V, A-5530) and D-glucose were purchased from Sigma-Aldrich (St. Louis, MO, USA). Pepsin (1:10000) and trypsin (1:250) were from Solarbio (Beijing solarbio science & technology co., Ltd, Beijing, China).

Goat Anti-rabbit IgG/HRP and Goat Anti-human IgE/HRP were purchased from Bioss antibodies (Beijing Bioss Biotechnology Co., Ltd, Beijing, China). Human serum was obtained from Chongqing Manuik Technology Co. Ltd (Chongqing China). Rabbit serum was made in laboratory. All other chemicals used were of analytical grade.

2.2. Preparation of Samples

2.2.1. Glycation of Samples

Make appropriate modifications according to Duan's method [17]. OVA and D-glucose were dissolved in deionized water severally by the proportion of 1:10 (w/v). Then the OVA solution and D-glucose solution were mixed in the proportion of 1:1 (v/v) by vortex oscillators (WH-8666, Shanghai Jihui Scientific Instrment Co. Ltd, China). The mixture was lyophilized at -80°C for 48 hours by freeze dryer (Yataikerong Scientific; Beijing, China). Afterwards, the lyophilized mixtures were heated by microwave irradiation (Galanz, Guangdong, China) at 560W for 5 min in five different positions. The five samples were named Sample 1 to Sample 5, respectively. The Sample 6 is the mixture of OVA and D-glucose without microwave heating and Sample 7 is the native OVA.

2.2.2. In Vitro Digestion

Before digestion, gastric juice was prepared by dissolving 2 g NaCl and 7 mL 36.5% HCl, followed by adding 3.2 g pepsin before adjusting the pH to 1.2, the volume was finally adjusted to 1000 mL with distilled water [18,19]. Intestinal juice was prepared by dissolving 6.8 g KH_2PO_4 and 190 mL 0.2 M NaOH, adding 2 g trypsin after regulating the pH to 7.5; finally, adjusted volume to 1000 mL [20]. The samples were dissolved with 600 μL distilled water, affiliated 200 μL gastric juice or 200 μL intestinal juice to create a needed digestive environment, after that, the samples were incubated at 37°C for 1.0 h; then inactivated enzyme at 100°C for 10 min. Two step enzyme digestion groups were added 400 μL intestinal juice to react at 37°C for 1.0 h after incubating with gastric juice, the other operations were the same as above.

2.3. Sodium Dodecyl Sulphate-polyacrylamide Gel Electrophoresis (SDS-PAGE)

The digestive juice was added to sample buffer and boiled for 10 min. The treated samples were added to 5% of the concentrated rubber and 12% of the separation glue. The voltage was set to 110 V, and the current was 400 mA. The gel was taken out 2 hours later, and then stained for an hour. Finally, the background color was removed with the destaining solution [21].

2.4. Free Amino Group

Content of free amino groups of samples was obtained in accordance with reference [22,23] with modifications. 10 μL sample to be tested was mixed with 200 μL O-phthalaldehyde reagent (1.905 g sodium tetraborate and 0.05 g sodium dodecyl suifonate were dissolved in distilled water, 0.04 g OPA was dissolved in 1 mL ethanol, 0.044 g dithiothreotol were dissolved in distilled water, and all reagents were mixed and fixed to 50 mL). Put it in dark at room temperature for 2 min and then detected at 340 nm. Content of free amino groups in the sample was calculated according to the standard curve.

2.5. Ion Chelating Capacity

2.5.1. Calcium Chelating Ability

According to the method of Jiménez et al. [24] with a few modifications, 100 μL 10 mg/mL $CaCl_2$ solution was added during digestion process. After digestion, removed the protein and took 200 μL supernatant in a conical bottle, added 50 mL of distilled water, dripped 3 drips HCl solution of 1:1(v/v) and made it homogeneous by mixing, heated and boiled for 30 s, cooled down to 50°C, adjusted the pH by adding 5 mL 20% KOH solution, added about 80 mg calcein-phenolphthalein indicator [25] (0.2 g calcein, 0.07 g phenolphthalein and 20 g KCl were mixed and ground to a powder.), then titrated by using 0.01 mol/L EDTA when fluorescence yellowish green was vanishing and red was appearing [26]. Recorded the volume of the consumption of EDTA. The calculation formula was as follows:

$$Calcium\ chelation\ rate = \frac{Vc - Vs}{Vc} \times 100\% \quad (1)$$

where Vc is the volum of EDTA consumed in the control group; Vs is the volume of EDTA consumed in sample.

2.5.2. Chelating Ability of Ferrous ions

Three phenazines and one divalent iron ion can react to form a purple complex, which can be detected at 562 nm. The reaction is as follows:

$$3\text{Ferrozine} + Fe^{2+} = \left[Fe\left(\text{Ferrozine}\right)_3 \right]^{2+}$$

Modified with reference to the method of [27], digested samples were prepared with different concentration gradients. Took 25 μL diluted samples to mix with 125 μL 1 mmol/L of Fe^{2+} solution of for 2 min and then added with 100 μL 500 μmol/L of phenazine. The absorbance was measured at 562 nm after 10 min. 10 μmol/L EDTA was used as positive control and water as negative control. The results were calculated by the chelating rate of ferrous ions, and the formula was as follows:

$$\text{chelating rate of ferrous ions\%} = \left[1 - \frac{a - b}{c}\right] \times 100 \quad (2)$$

where *a* is the absorbance value of the sample, *b* is the absorbance value of the positive control of EDTA, *c* is the absorbance value of the negative control of water.

2.6. Determination of Intrinsic Fluorescence

Diluted the protein digestive fluid to 1 mg/mL, then the intrinsic fluorescence of the solution was measured by using fluorescence spectrometer (F-4500; Hitachi, Tokyo,

Japan) in accordance with the technique described by Tang, Sun and Foegeding [28]. The excitation wavelength was 286 nm. The emission spectrum was recorded from 300 nm to 500 nm, with excitation and emission slits of 2.5 nm, and a scan speed at 1200 nm/min.

2.7. Determination of Antioxidant Activity

2.7.1. Determination of Reducing Force

Transferred 100 μL sample solution into the colorimetric tube, then added 100 μL phosphate buffer (pH6.6, 0.2 M) and 100 μL 1% $K_3Fe(CN)_6$, rapidly cooled after 20min reaction in a water bath of 50°C. 100 μL 10% (w/v) acetocaustin were needed to join to the solution above, then the mixed liquid was centrifuged at 8000 r/min for 5 min after fully mixed. Absorbed 100 μL supernatant into tube and appended 100 μL distilled water and 20 μL 0.1% (w/v) $FeCl_3$, after that, put it into water bath at 50°C for 10 min, and measured the absorption value at 700 nm [29].

2.7.2. Determination of Free Radical Scavenging Rate of DPPH

Electron-donating ability of samples was determined by employing DPPH radical scavenging assay [30]. 1 mL 100 μmol/L DPPH (Dissolved in anhydrous ethanol) was added into 1 mL sample solution. The mixture was vortexed and left to stand at ambient temperature for 30 min. A reaction mixture containing 1 mL ethanol and 1 mL 100 μmol/L DPPH was served as the control, and the mixture of 1 mL ethanol and 1 mL sample solution was regarded as sample blank. The absorbance of the solution was measured spectrophotometrically at 517 nm. The percentage of DPPH scavenging was calculated from the equation:

$$Clearance\ (\%) = \frac{A0 - (Ai - Aj)}{A0} \times 100 \quad (3)$$

Where A_0 is the absorbance of control, A_i is the absorbance of sample and A_j is the absorbance of sample blank.

2.7.3. Determination of Free Radical Scavenging Rate of $ABTS^+$

Refer to Corzo-Martínez's method [31] and make appropriate modifications. Transferred 80 μL sample solution mixed with 3.92 mL $ABTS^+$ reagents to react 10 min at room temperature. A mixture containing 80 μL deionized water and 3.92 mL $ABTS^+$ reagents was served as the control. The absorbance of the solution was measured at 734 nm. The scavenging rate was calculated according to the following formula:

$$Clearance\ (\%) = \frac{A0 - As}{A0} \times 100 \quad (4)$$

where A0 is the absorbance of control and As is the absorbance of sample.

2.8. Enzyme-linked Immunosorbent Assay (ELISA)

IgG-binding capacity of samples was estimated by an indirect competive ELISA [32] with modifications. 96-well micro-plates were coated with 100 μL/well 16 μg/mL of OVA and then were incubated 1 h at 37°C. Residual free-binding sites were blocked with 2% BSA in phosphate buffer solution Tween solution (PBST) for 30 min at 37°C. The wells of the plates were washed 3 times with PBST. The wells were added 50 μL samples diluted into different concentration in carbonate buffer solution (CBS) and 50 μL rabbit serum diluted 1:100000 in PBST, then the plate was incubated at 37°C for 30 min. After removing the solutions, the wells were washed thrice with PBST. 100 μL of purified Goat Anti-rabbit IgG-HRP conjugate diluted 1:100000 in PBST was added, and the plate was incubated at 37°C for one hour. After washing, 100 μL of tetramethylbenzidine solution was immediately added to each well. The reaction was stopped by adding 100 μL of 2 M sulfuric acid. The absorbance was measured at 450 nm by using Synergy-H1 multimode reader (Bio Tek, USA). Inhibition rate was measured by using the following equation :

$$\text{Inhibition}(\%) = \frac{Ao - A}{Ao} \times 100 \quad (5)$$

Where A and Ao are the absorbance values of the well with and without the inhibitor, respectively.

The IgE-binding capacity of samples was estimated by an indirect competive ELISA with modification. 96-well micro-plates were coated 100 μL/well 2 μg/mL of OVA and were incubated 1h at 37°C. Residual free-binding sites were blocked with 2% BSA in PBST for 1h at 37°C. The wells of the plates were washed 3 times with PBST. The wells were added 50 μL samples diluted into different concentration in CBS and 50 μL human serum diluted 1:50000 in PBST, then the plate was incubated at 37°C for 30min. After removing the solutions, the wells were washed thrice with PBST. 100 μL of purified Goat Anti-human IgG-HRP conjugate diluted 1:100000 in PBST was added, and the plate was incubated at 37 °C for 1 h. After washing, 100 μL of tetramethylbenzidine solution was immediately added to each well. The reaction was stopped by adding 100 μL of 2 M sulfuric acid. The absorbance was measured at 450 nm by using Synergy-H1 multimode reader (Bio Tek, USA).

2.9. Statistical Analysis

All glycation samples and control samples were analysis in triplicate. Statistical analysis was established by ANOVA.

3. Results and Discussion

3.1. SDS-PAGE

After being digested by stomach, intestine and two-step in vitro, respectively, distribution of molecular weight of digestive products was performed by SDS-PAGE. The SDS-PAGE under reducing conditions profiled of glycted OVA digested by different ways was shown in Figure 1. The molecular weight of OVA is between 43 kDa and 45 kDa. Pepsin, which has a molecular weight of 35 kDa, mainly acts on the peptide bond containing phenylalanine

or tyrosine in the protein. The main decomposition product is peptone. Figure 1A shows that the bands were accumulated below 14.4 kDa after experiencing gastric digestion. The difference among them could hardly be observed. But the band at 43 kDa of Sample 3 has vanished and the other bands of the other samples had deeper impressions than the Sample 7. It indicated that the nature one held the lower protein concentration, and also meant that the native OVA was a little more vulnerable to be enzymed by pepsin. The glycation led to the increase of molecular weight so that the pepsin could not hydrolyzed. Therefore, the glycated samples had the higher protein concentration. Trypsin is a serine proteolytic enzyme with a molecular weight of 24 kDa. It is an endonuclease that can cut the carboxyl side of lysine and arginine residues in the polypeptide chain. The bands after being digested by trypsin were totally different from the results of that treated by pepsin. Compared to the sample 7, the band near 43 kDa of other samples were little higher. This proved that the glycated OVA have a bigger molecular weight and Sample 3 was the most prominent. In addition, the band of Sample 3 near 43 kDa is in light color. It was probably that part of the protein in Sample 3 was lost during the process of glycation, resulting in the low protein concentration. Overall, the trypsin did little devastation to the OVA and glycated OVA. The D-glucose tends to bind to the free amino groups on lysine and arginine [33], which prevents trypsin from acting on lysine and arginine because of the space barrier caused by D-glucose.

In the Figure 1C, the major band is near 31 kDa and it exhibited a similar variation tendency with the Figure 1A. A small difference was observed between the bands of 31 and 22.0 kDa. These bands probably belonged to pepsin and trypsin. There were also some bands above 97.4 kDa. It might be some protein polymer. In this section, we can infer that OVA and glycated OVA could be hydrolyzed from 43 kDa to 31 kDa after digestion.

In a word, the digestion of OVA occurred mainly in the stomach, not in the intestine. Digestion band of sample 3 was always different from other samples, which showed that it was the focus in microwave field, and it was caused by the inhomogeneity of microwave heating.

3.2. Free Amino Group

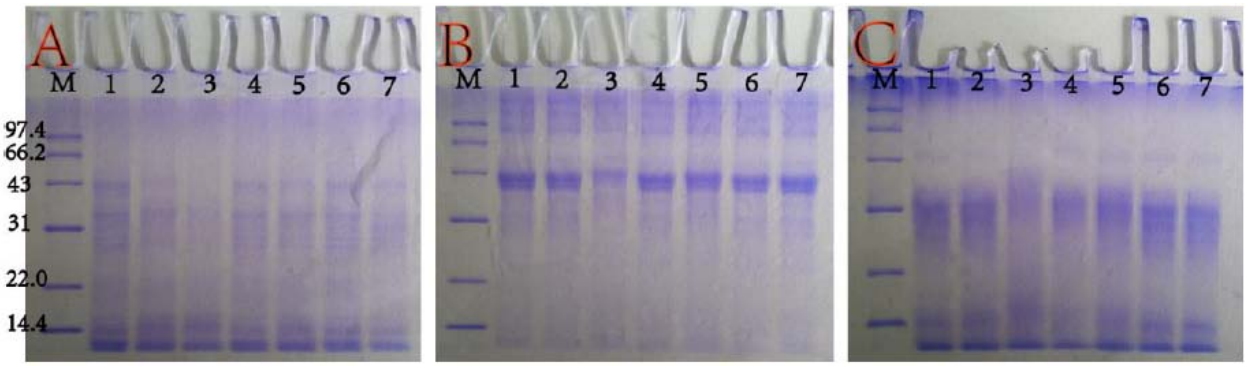
Changes in the free amino groups can reflect the degree of protein digestion to a certain extent. As shown in Figure 2, when it was digested by pepsin, the glycated OVA held slightly more free amino groups than the native OVA. It was speculated that D-glucose could induce the partial expansion of OVA and the microwave irradiation could also make the structure of samples unfold. Results of free amino group showed that free amino content of samples digested in gastric juice was more than that in intestinal juice, which was more than that by two-step digestion. It was because that after two-step digestion, more free amino groups generated, but they would react with redundant D-glucose. Thus, the free amino groups diminished after two-step digestion.

Briefly, microwave treatment combined with glycation exerted a certain influence on the digestion of OVA. In the gastric digestion, Sample 4 and 6 had the most of free amino groups, and Sample 1 held the least. In the intestinal digestion, Sample 2 had the most of free amino groups, Sample 5 had the least, and no difference was found among other samples. In the two-step digestion, Sample 7 had more free amino groups, Sample 3 had the least. These also showed that the microwave radiation was indeed uneven, and the impact on each position was not certain.

3.3. Intrinsic Fluorescence

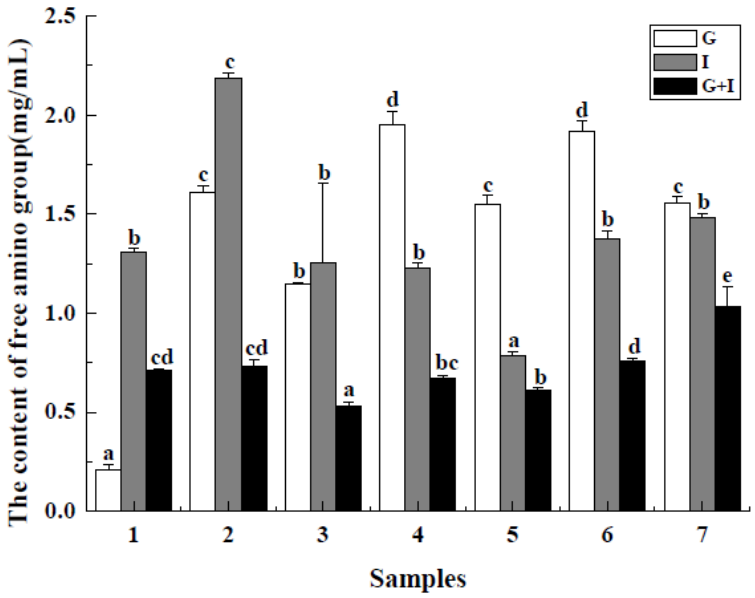
Intrinsic fluorescence can reflect the microenvironment of aromatic amino acids. Pepsin can act on phenylalanine or tyrosine in proteins. As shown in Figure 3A, the fluorescence intensity of Sample 7 was lower than the other samples expect Sample 3. It was that microwave heating can expand the protein's structure and promote the exposure of fluorescent amino acids. Sample 3 might have been destroyed parts of the protein's structure so that it has the lowest fluorescence intensity after gastric digestion.

Trypsin can cut the carboxyl side of lysine and arginine residues in the polypeptide chain. After glycation, the protein was more difficult to be hydrolyzed. As shown in Figure 3B, trypsin has better activity in alkaline environment and the structure of protein can spread cushily, thereby resulting in the highest fluorescence intensity of Sample 7 compared with the other samples. The disturbance of D-glucose reduced the fluorescence intensity; as the D-glucose attached to the protein at lysine and arginine, which impacted the fluorescence intensity of glycated OVA. Sample 3 still had the lowest fluorescence intensity.



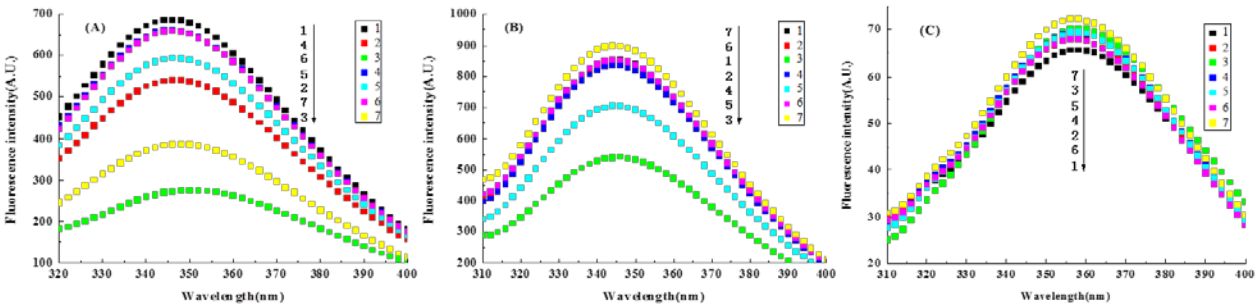
A: digested by simulated gastric juice; B: digested by simulated intestinal fluid; C: digested by simulated gastric juice and intestinal fluid. M: marker; 1-5: position 1-5 in microwave field, respectively; 6: ovalbumin mixed with D-glucose then lyophilized for 48h; 7: ovalbumin without any treatment.

Figure 1. SDS-polyacrylamide gel electrophoresis of samples after digestion



1-5: position 1-5 in microwave field, respectively; 6: ovalbumin mixed with D-glucose then lyophilized for 48h; 7: ovalbumin without any treatment.
G: digestion and decomposition in simulated gastric juice; I: digestion and decomposition of simulated intestinal fluid; G+I: digestion and decomposition in simulated gastric juice and intestinal fluid.
Different letters (a-e) in the picture means significant difference ($p<0.05$).

Figure 2. The content of free amino groups after digestion



(A): digested by simulated gastric juice; (B): digested by simulated intestinal fluid; (C): digested by simulated gastric juice and intestinal fluid.
1-5: position 1-5 in microwave field, respectively; 6: ovalbumin mixed with D-glucose then lyophilized for 48h; 7: ovalbumin without any treatment.

Figure 3. Intrinsic fluorescence of digested samples

Figure 3C showed the change in fluorescence of samples by two-step digestion. The fluorescence intensity decreased sharply compared with that by one-step digestion. Although protein structures were opened after hydrolysis by pepsinate and tyrosinase, Brown movement between many small molecules reduced the fluorescence intensity. Therefore, there was no significant difference among these samples. The fluorescence of digested samples could not fully reveal the digestion degree of glycated OVA.

3.4. Ion Chelation Capacity

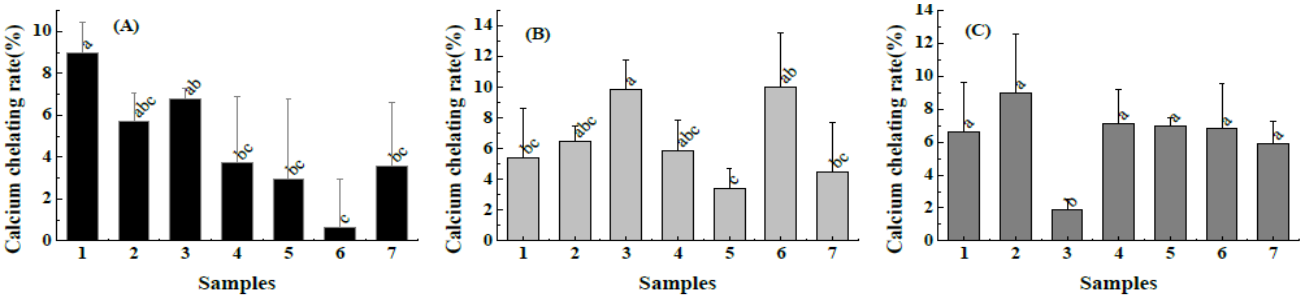
3.4.1. Calcium Chelation Ability

Calcium ions are absorbed slowly in the human body, by combining them with peptides, their absorption into the human body can be promoted [34]. To evaluate the calcium chelation ability of OVA after glycated, the calcium chelation ability had been detected and the results has been shown in Figure 4. Glycation had improved OVA's calcium chelating rate.

At the stage of gastric digestion (Figure 4A), the calcium chelating rate of glycated OVA except Sample 6, were higher than Sample 7. As the structure of protein was expanded by microwave irradiation, more free carboxyl exposed. Besides, in acid condition, amino group was in protonation state and it was easily attracted by positively charged calcium ions.

At the stage of intestinal digestion (Figure 4B), the calcium chelating rate of most glycated samples were higher than Sample 7, Sample 3 and 6 held the highest calcium chelation rate. It was in an alkaline environment; the glycation can be promoted, and many other ionic substances would generate to combine with calcium ions.

However, after two-step digestion, the calcium chelating rate of all samples was much the same except Sample 3. It indicated that previous chelates with protonated amino groups were unstable and could be broken in an alkaline environment. Consequently, during the gastric digestion, the calcium chelating rate could be improved after glycation. After two-step digestion, the calcium chelating rate had no difference to these samples and glycation might affected the chelating if it is overreaction.



(A): digested by simulated gastric juice; (B): digested by simulated intestinal fluid; (C): digested by simulated gastric juice and intestinal fluid.
1-5: position 1-5 in microwave field, respectively; 6: ovalbumin mixed with D-glucose then lyophilized for 48h; 7: ovalbumin without any treatment.
Different markers on the top of the bars denote significant difference ($p < 0.05$).

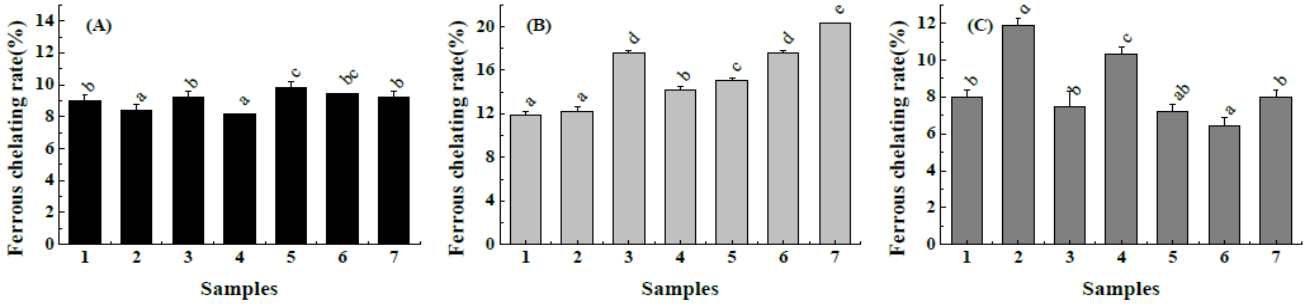
Figure 4. The calcium chelation rate of digested samples

From the above results, it could be seen that reaction degree of microwave radiation to each sample was different, and Sample 3 seemed to be in a special position in the microwave field.

3.4.2. Ferrous Ion Chelating Ability

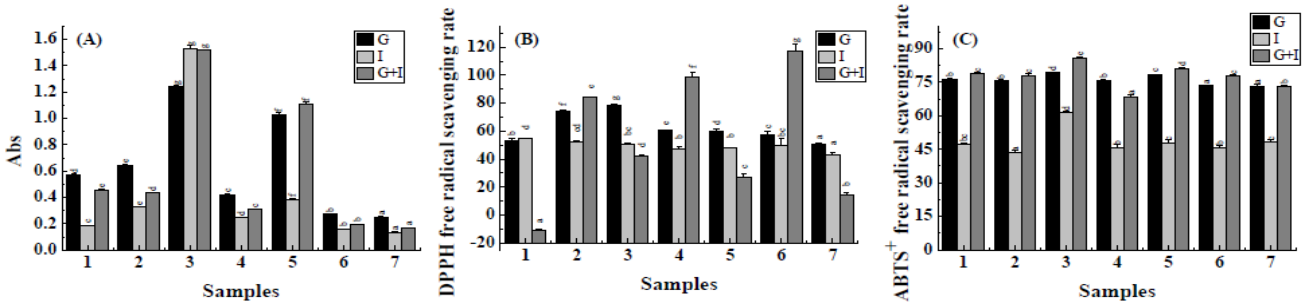
Iron is an essential trace element in the human body and an indispensable component of hemoglobin. There are two forms of iron in food: inorganic iron and organic iron [35]. Although the content of inorganic iron is high, it is not conducive to human absorption. Organic iron, such as ferrous chelating polypeptide, can effectively promote human absorption of iron and has high physiological activity. After enzymatic hydrolysis, proteins may demonstrate better chelation with iron ions. The results are revealed in Figure 5.

At the stage of gastric digestion, the ferrous ion chelating rate of samples had little difference to each other. The ferrous ion of hemoglobin is present in its hydrophobic pocket. It was suspected that ferrous ions were more likely to bind to hydrophobic amino acid. Pepsin destroyed the structure of protein so that there showed no markable changes among samples. In the intestinal digestion, the glycation led to the increase of the hydrophilicity of OVA, so that Sample 7 had the highest ferrous ion chelating rate and Sample 3 had formed hydrophobic products. After two-step digestion, the production of hydrolysates and the change of pH made ferrous chelation more complicated. In general, the ferrous ions chelating rate of glycated samples was higher than Sample 7.



(A): digested by simulated gastric juice; (B): digested by simulated intestinal fluid; (C): digested by simulated gastric juice and intestinal fluid.
1-5: position 1-5 in microwave field, respectively; 6: ovalbumin mixed with D-glucose then lyophilized for 48h; 7: ovalbumin without any treatment.
Different markers on the top of the bars denote significant difference ($p < 0.05$).

Figure 5. The ferrous ion chelating rate of digested samples



(A): Reducing power; (B): DPPH free radical scavenging capacity; (C): ABTS+ free radical scavenging ability.
1-5: position 1-5 in microwave field, respectively; 6: ovalbumin mixed with D-glucose then lyophilized for 48h; 7: ovalbumin without any treatment.
G: digestion and decomposition in simulated gastric juice; I: digestion and decomposition of simulated intestinal fluid; G+I: digestion and decomposition in simulated gastric juice and intestinal fluid.
Different letters (a-e) in the picture means significant difference ($p<0.05$).

Figure 6. Antioxidant capacity of digested samples

3.5. Antioxidant Activity

As shown in the Figure 6A, the reducing power was determined by the following method: potassium ferricyanide was reduced by samples to potassium ferrocyanide, which was then combined with ferric ion, resulting in a Prussian blue color. The reducing power of glycated OVA were considerably stronger than Sample 7., which is attributed to the antioxidants produced during glycation. D-glucose did not contribute to the reducing power, because it reacts with potassium ferricyanide only in strong alkaline environment. It could be seen clearly that in the intestinal stage, the reducing power was lower than the other stage. It might be that the samples did not hydrolyzed by trypsin and small molecular peptides have better reducing power.

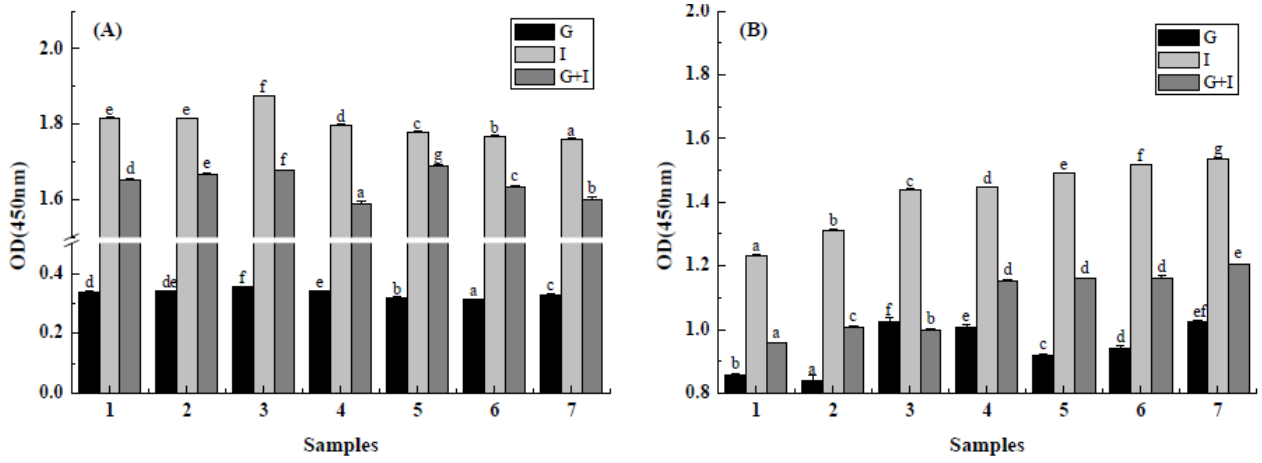
DPPH is a very stable nitrogen-centered radical. Its stability is mainly attributed to the resonance stabilization of three benzene rings and the space barrier, so that the paired electrons trapped in the middle nitrogen atom cannot play their role as paired electrons. As a stable free radical, DPPH can capture other free radicals. In the stomach (Figure 6B), the DPPH free-radical scavenging rates of glycated samples were slightly higher than that of Sample 7 on the score of the free electrons in D-glucose. Glucose also has DPPH free radical scavenging capacity because it has some unpaired electrons that can be captured by DPPH free radicals, thus increasing DPPH free-radical scavenging capacity. In the intestines, little divergence was shown in the outcome. The two-step digestion method exhibited unpredictable test results. Glycated OVA exhibited the highest DPPH free radical scavenging capacity, which might be attributed to the pH value. The DPPH suggested that there were more unpaired electrons in glycated OVA so that they had the ability to scavenge other free radicals.

ABTS is oxidized to green $ABTS^+$ free radical under the action of appropriate oxidants, and the production of $ABTS^+$ free radical is inhibited in the presence of antioxidants. This outcome (Figure 5C) was consistent with the results of electrophoretic separation. We suspected that $ABTS^+$ free radicals could easily capture free radicals in small molecular peptides. Furthermore, the $ABTS^+$ free radical scavenging capacity of all samples reached approximately 80% by pepsin digestion and two-step digestion, and approximately 50% by trypsin digestion. The glycated OVA were still a little bit higher than Sample 7. Sample 3 held the hightest $ABTS^+$ free radical scavenging capacity because it had produced the most antioxidants.

3.6. IgG- and IgE-binding Capacity Analysis

Glycation can reduce the allergenicity of protein [4]. Because the allergen epitopes will be masked after glycation. Nevertheless, allergenicity of glycated samples during digestion remains unknown. Binding capacity of IgG from rabbits having OVA allergy on samples was studied by indirect competitive ELISA. The IgG-binding was determined to evaluate their allergies. As shown in Figure 6A, optical density value of digestion in intestinal and two-step digestion were higher than that in gastric digestion. This might be that pepsin enzymogen the protein into short peptides that more allergenic epitope exposed; trypsin did not make great changes to the protein, and the allergen epitopes were still hidden; after two-step digestion, the protein were hydrolyzed into too small peptides whose allergenic epitope were ruined. Besides, glycated samples had a little lower allergenicity than Sample 7. Allergenicity of Sample 3 was the lowest. It might be that over glycation destroyed its allergic sites.

The binding capacity of IgE from patients having OVA allergy, samples were studied by indirect competitive ELISA. As shown in Figure 7B, samples at the gastric digestion had higher allergenicity than that of intestinal digestion and two-step digestion. Samples digested by intestinal digestion had the lowest allergenicity. It is supposed that trypsin destroyed the allergy sites, while pepsin exposed allergy sites. It was the same as the results of IgG binding. However, the glycated samples had higher allergenicity than Sample 7. It means that glycation could not reduce the IgE binding of OVA, but increase it instead.



(A): IgG binding; (B):IgE binding.
1-5: position 1-5 in microwave field, respectively; 6: ovalbumin mixed with D-glucose then lyophilized for 48h; 7: ovalbumin without any treatment.
G: digestion and decomposition in simulated gastric juice; I: digestion and decomposition of simulated intestinal fluid; G+I: digestion and decomposition in simulated gastric juice and intestinal fluid.
Different letters (a-e) in the picture means significant difference ($p<0.05$).

Figure 7. Allergenicity of digested samples

4. Conclusion

In this study, microwave treatment combined with glycation was used to investigate the digestibility of OVA. The results showed that microwave can unfold the structure of OVA and promote glycation. The OVA was digested more easily in gastric fluid than intestinal fluid. More calcium and ferrous ions attached to the glycated samples and the glycated samples had better antioxidant abilities. As for the allergenicity, glycation could reduce the IgG binding while increasing the IgE binding.

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Abbreviation

ovalbumin, OVA
immunoglobulin G, IgG
immunoglobulin E, IgE
sodium dodecyl sulphate-polyacrylamide gel electrophoresis, SDS-PAGE
enzyme-linked immunosorbent assay, ELISA
phosphate buffer solution Tween solution, PBST
carbonate buffer solution, CBS

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