International Food Research Journal 30(6): 1471 - 1480 (December 2023)
Journal homepage: *http://www.ifrj.upm.edu.my*

Preparation of chickpea peptide-calcium chelates with antioxidant activity

*Shi, W. and Tan, C. Y.

School of Food and Pharmaceutical Engineering, Zhaoqing University, Zhaoqing 526000, China

Article history

Received:
18 November 2022
Received in revised form:
27 April 2023
Accepted:
16 August 2023

Keywords

antioxidant,
chickpea peptides,
peptide-calcium chelate

Abstract

Chelation of antioxidant chickpea peptides with calcium can improve the absorption rate of calcium in human intestinal tract. In the present work, the optimum enzymatic hydrolysis scheme was determined by a single-factor test with an orthogonal experimental design with the following conditions: material-liquid ratio, 1:15; enzymatic hydrolysis time, 10 min; enzyme-substrate ratio, 1:20 (papain protease); and enzymatic hydrolysis temperature, 60°C. The protein hydrolysate obtained under these conditions had high antioxidant activity. The free radical scavenging rates of •OH, O^{2-}•, DPPH•, and H_2O_2 were 49.44, 63.64, 66.57, and 57.64%, respectively. The reducing power was 0.75. The optimum conditions for the preparation of calcium chelate from chickpea peptides were determined by an orthogonal optimisation test: the peptide-calcium ratio, 7:1; the chelation temperature, 50°C; the initial pH of the reaction solution, 7.0; the chelation time, 40 min; and the chelation rate, 39.95%. Antioxidant chickpea peptide-calcium chelates could have the potential to induce synergistic physiological effects.

DOI
https://doi.org/10.47836/ifrj.30.6.10

© All Rights Reserved

Introduction

Chickpea (*Cicer arietinum* L.) is the third most important legume in the world after soybean (*Glycine max* (L.) Merr.) and pea (*Lathyrus oleraceus* Lam.). Chickpea has been used as food and medicine for thousands of years. The protein content of chickpea is 18 - 25%, and this legume is one of the most economical sources of plant protein (Rasul *et al.*, 2022). As an excellent plant protein, chickpea protein has many physiological functions, such as reducing blood lipids, reducing cholesterols, and preventing osteoporosis and menopause syndromes (Milán-Noris *et al.*, 2019). However, protease inhibitors are especially abundant in soybeans and chickpeas, and may be considered anti-nutritional factors because they reduce protein digestibility and hence diet quality (Idate *et al.*, 2021). These protease inhibitors are always proteins or peptides (Idate *et al.*, 2021). Some individuals may even be allergic to chickpea proteins (Misra *et al.*, 2009).

The hydrolysis of chickpea protein can eliminate some anti-nutritional factors and allergens (Rivero-Pino *et al.*, 2021). Peptides with physiological activity can be produced by enzymatic hydrolysis of chickpea proteins. Natural antioxidant peptides prepared from plant proteins have attracted the attention of many researchers due to their environmental friendliness, safety, and other advantages (Mariaelena *et al.*, 2019). Bromelain is a pure natural plant protease with considerable biological activity, and has been widely used in various applications, especially in the food industry (López-Pedrouso *et al.*, 2020). Small-molecular peptides are more easily absorbed and utilised by the human body. They not only have good nutritional properties but also have biological activities such as antioxidative, antibacterial, anti-inflammatory, metal-chelating, blood pressure-lowering, and cholesterol-lowering activities (Shi *et al.*, 2021).

Calcium plays an important role in various physiological activities of the human body. Long-term insufficient or excessive intake will have adverse effects on human health. The average dietary calcium intake level of Chinese population is 366.1 mg/D, which is significantly lower than the intake level of people in Western countries (800 – 1,000 mg/D), and significantly lower than the recommended intake for Chinese population. The dietary calcium intake of approximately 70.2% of

*Corresponding author.
Email: shiwen@zqu.edu.cn

Chinese population does not reach the recommended intake level, thus suggesting that there is a widespread risk of insufficient calcium intake (Curhan *et al*., 1993).

The absorption mode of trace elements in the small intestine reduces their bioavailability to a certain extent. Chelates can improve the bioavailability of inorganic metal ions by means of the absorption mechanism of peptides (Zhang *et al*., 2021b). At present, the preparation of peptide-calcium chelates, such as egg white peptide, soybean peptide, and rapeseed peptide, is a research hotspot (Hu *et al*., 2021). Peptide-calcium chelates can improve the absorption of calcium in the human body (Zhang *et al*., 2021a). In terms of intestinal absorption, calcium-chelated peptides have less energy consumption, faster transport, fewer saturated carriers, and more advantages than peptides before chelation (Jlab *et al*., 2021). Peptide-chelated calcium not only improves the bioavailability of calcium but also improves human health (Liu *et al*., 2021; Sun *et al*., 2022). It can be used as new calcium supplement which adds new vitality to the calcium product market. After trace elements are chelated with short peptides with special biological activities, the chelates retain biological activities such as antioxidative activity, antibacterial activity, immune regulation, blood lipid reduction, and blood glucose reduction, so they have very important research significance and application value.

When compared with short peptide chelates from plants, short peptide chelates from animals have the characteristics of easy absorption and high nutritional value (Xue *et al*., 2021). There are many factors that affect the chelation reaction between peptides and metals, such as the mass ratio of materials, pH value, reaction time and temperature, as well as the degree of hydrolysis in the process of protease hydrolysis (Tian *et al*., 2019). Research on protein peptide chelates from different sources can not only make full use of raw materials and save resources, but also further enhance their application value (Luz *et al*., 2021).

In the present work, chickpea protein was hydrolysed with bromelain to obtain antioxidant chickpea peptides with high activity. Then, the chickpea peptides were chelated with calcium ions to prepare chickpea peptide-calcium chelates with a high chelation rate. The mixture of chickpea peptides and chickpea peptide-calcium chelates is a high-calcium supplement with strong antioxidant activity that can slow human aging and reduce blood pressure, blood lipids, and other physiological properties.

Materials and methods

Preparation of chickpea protein

The experimental method followed that of Xue and Shi (2008). Chickpea was ground into powder by a pulveriser, and then filtered through 80-mesh stainless steel mesh filter. The ratio of chickpea powder to petroleum ether was 1:10 (w/v). The chickpea powder was stirred with a magnetic stirrer for 1 h, and the petroleum ether was recovered. This extraction process was repeated twice. Finally, the chickpea powder was stored at room temperature overnight, and put in a fume hood to volatilise the residual petroleum ether. The treated defatted powder was put into a bag, and refrigerated at 4°C.

Defatted chickpea powder was mixed with water at a ratio of 1:10 (w/v), adjusted to pH 8.0 with 0.5 mol/L NaOH, stirred with a magnetic stirrer for 1 h, centrifuged at 4,000 rpm for 20 min, and all of the supernatant was poured into a beaker. After that, the protein was precipitated with 0.5 mol/L HCl to the isoelectric point (pH 5.5), centrifuged at 4,000 rpm for 10 min, and the supernatant was poured out. The precipitate was placed in a 50°C air blast drying oven, and dried to obtain chickpea protein isolate.

Preparation of chickpea protein peptides

The experimental method followed that of Xue and Shi (2008). An appropriate amount of chickpea protein powder was weighed and dissolved in distilled water so that the material-liquid ratio of protein powder to water was 1:20. The sample was placed in a constant-temperature water bath, and mixed evenly. After preheating at 80°C for 20 min, the mixed solution was quickly adjusted to the optimal temperature (50 - 55°C) and pH (6.8 - 7.0). Papain and neutral protease were added while stirring so that the enzyme complex accounted for 8% of the substrate, and a burette was used to drop 0.5 mol/L NaOH solution to keep the pH of the system constant. The consumption of NaOH was recorded, and hydrolysis was performed for 3 h. The enzyme hydrolysate was placed in a 90 - 95°C water bath to inactivate the enzyme for 10 min, cooled, and centrifuged at 6,000 rpm for 20 min. Finally, the supernatant was taken as the chickpea protein peptide solution and frozen.

Effect of material-liquid ratio on protein hydrolysis of chickpea

The enzyme-substrate ratio, enzyme hydrolysis temperature, and enzyme hydrolysis time were set to 1:20, 55°C, and 10 min, respectively. The material-liquid ratio refers to the ratio of protein mass to water mass, and the value was adjusted to 1:5, 1:10, 1:15, and 1:20 to explore the effect of material-liquid ratio on the preparation of chickpea protein peptides.

Effect of enzyme hydrolysis time on protein hydrolysis of chickpea

The enzyme-substrate ratio, enzyme hydrolysis temperature, and material-liquid ratio were set to 1:20, 1:10, 55°C, and 10 min, respectively, and the enzyme hydrolysis time of the system was adjusted to 5, 10, 15, and 20 min to explore the effect of enzyme hydrolysis time on the preparation of chickpea protein peptides.

Effect of enzyme-substrate ratio on protein hydrolysis of chickpea

The enzyme-substrate ratio, enzyme hydrolysis temperature, and material-liquid ratio were set to 1:10, 55°C, and 10 min, respectively. The enzyme-substrate ratio refers to the ratio of enzyme mass to protein mass, and the enzyme-substrate ratio of the system was adjusted to 1:10, 1:20, 1:30, and 1:40 to explore the effect of the enzyme-substrate ratio on the preparation of chickpea protein peptides.

Effect of enzyme hydrolysis temperature on protein hydrolysis of chickpea

The enzyme-substrate ratio, material-liquid ratio, and enzyme hydrolysis time were set to 1:20, 1:10, and 10 min, respectively, and the enzyme hydrolysis temperature of the system was adjusted to 45, 50, 55, and 60°C to explore the effect of the enzyme hydrolysis temperature on the preparation of chickpea protein peptides.

Preparation of peptide-calcium chelates

The amount of anhydrous calcium chloride was fixed at 0.30 g. A mass of titanium powder corresponding to a fixed peptide-calcium ratio was weighed into a small beaker, and distilled water was added. The mixture was stirred until the peptide powder was completely dissolved, the peptide solution was preheated in a water bath for 10 min, 0.3 g of anhydrous calcium chloride was added, the mixture was stirred until dissolved, and the pH was adjusted to the specified value within 2 min. The peptide-calcium mixed solution was placed in a water bath for a certain period, 1 mL of the mixed solution was transferred into a conical flask, and the total calcium content in the solution was determined by EDTA titration. At the same time, 1 mL of the reaction solution was transferred into a 50 mL centrifuge tube, 15 mL of absolute ethanol was added, and the mixture was shaken for 30 min in a constant-temperature oscillator. The mixture was centrifuged for 10 min at 4,000 rpm, the supernatant was poured out, the volume of the precipitate was adjusted to 25 mL with deionised water, and 2.5 mL of this solution was used for absorption. The content of chelated calcium was determined by EDTA titration.

Effect of peptide-calcium mass ratio on calcium chelation rate

A volume of peptide solution corresponding to the desired peptide-calcium mass ratio was taken, the mass of anhydrous calcium chloride was fixed to 0.10 g, the pH was adjusted to 7.0, and the chelation time and temperature were set to 30 min and 50°C, respectively. The peptide-calcium ratio was controlled to 1:1, 3:1, 5:1, 7:1, and 9:1 to explore the effect of peptide-calcium mass ratio on calcium chelation rate.

Effect of chelation time on calcium chelation rate

The mass of anhydrous calcium chloride was fixed at 0.30 g, the pH was 7.0, the chelation temperature was 50°C, and the peptide-calcium mass ratio was 7:1. The chelation time was set to 10, 20, 30, 40, and 50 min to explore the effect of chelation time on calcium chelation rate.

Effect of chelation temperature on calcium chelation rate

The chelation time, pH, and peptide-calcium ratio were fixed at 30 min, 7.0, and 7:1, respectively, and the chelation temperature was controlled at 30, 40, 50, 60, and 70°C to explore the effect of temperature on calcium chelation rate.

Effect of pH on calcium chelation rate

The peptide-calcium mass ratio, reaction temperature, reaction time, and calcium concentration were set to 7:1, 50°C, 30 min, and 100 mg/mL, respectively, and the pH of the system was adjusted to 5.0, 6.0, 7.0, 8.0, and 9.0 to explore the effect of pH on calcium chelation rate.

Orthogonal experiment

Based on the results of the single-factor experiment, four significant factors influencing the protein hydrolysis of chickpea were selected, and four significant factors influencing the peptide calcium chelation rate were also selected. Three levels were selected for each factor to optimise the preparation process of peptide-calcium chelate. The factor levels are shown in Table 1.

Table 1. Factor levels of the orthogonal test.

		A	B	C	D
Enzymolysis	**Factor level**	**Material-liquid ratio (g/ml)**	**Time (min)**	**Enzyme-substrate ratio (%)**	**Temperature (°C)**
	1	1:5	5	5.0	50
	2	1:10	10	6.0	55
	3	1:15	15	7.0	60
Preparation of calcium-peptide chelate	**Factor level**	**Peptide-calcium mass ratio**	**Time (min)**	**pH**	**Temperature (°C)**
	1	1:5	20	5.0	40
	2	1:7	30	6.0	50
	3	1:9	40	7.0	60

Detection of antioxidants during peptide-calcium chelation

Determination of •OH clearance rate: H_2O_2 was mixed with Fe^{2+} to produce •OH, and then salicylic acid was added to the system to capture •OH, and produce coloured substances with a maximum absorption at 510 nm. The reaction system contained 2 mL of 8.8 mmol/L H_2O_2, 2 mL of 9 mmol/L $FeSO_4$, 2 mL of 9 mmol/L salicylic acid ethanol, and 2 mL of ginger flavonoid solution with different concentrations. Finally, H_2O_2 was added to initiate the reaction, which proceeded at 37°C for 0.5 h. The absorbance of each different concentration solution was measured at 510 nm with distilled water as a reference. Considering the absorbance value of each component, 2 mL of 9 mmol/L $FeSO_4$ and 2 mL of salicylic acid ethanol were used. Next, 2 mL of ginger flavonoid solution with different concentrations provided the background absorption of flavonoids. The calculation formula for clearance rate was as follows: •OH clearance rate = $[A_0 – (A_x A_{x0})] / A_0 \times 100$, where A_0 = absorbance of the blank control solution, A_x = absorbance after adding the flavonoid solution, and A_{x0} = absorbance of the background without adding the H_2O_2 flavonoid solution.

Determination of O^{2-}• clearance rate: the pyrogallol autoxidation method was used; 4.5 mL of pH 8.2, 50 mmol/L Tris HCl buffer solution, and 4.2 mL of distilled water were well mixed and kept warm in a 25°C water bath for 20 min. After the mixture was removed, 0.3 mL of 3 mmol/L pyrogallol preheated at 25°C (prepared with 10 mmol/L HCl; 10 mmol/L HCl was to replace the HCl solution of trihydroxybenzenes in the blank tube) was added, and the mixture was quickly shaken well, and poured into a cuvette. The absorbance was measured every 30 s at 325 nm. The increase in absorbance per minute was calculated within the linear range. Before pyrogallol was added, a certain volume of sample solution was added, and distilled water was added as needed; then, the inhibition rate was calculated using the formula as follows: Inhibition rate (%) = $[(\Delta A_0 – \Delta A) / \Delta A_0] \times 100$, where, ΔA_0 = autoxidation rate of pyrogallol, ΔA = rate of autoxidation of pyrogallol after the addition of flavone solution; and the constant is the increase in absorbance per minute.

Determination of DPPH• clearance rate: in a reaction volume of 2 mL, DPPH was dissolved in a small amount of methanol with 50% ethanol to a concentration of 120 μmol/L. A total of 0.1 mL of sample solution and 1.9 mL of DPPH were added for the reaction. The absorbance change at 525 nm was measured after standing at room temperature for 20 min. The percentage of DPPH removal by the sample = $1 – [(A – B) / A_0] \times 100$, where, A_0 = absorbance of DPPH without sample addition (1.9 mL DPPH + 0.1 mL 50% ethanol), A = absorbance of the sample after reacting with DPPH, and B = absorbance of the blank (0.1 + 1.9 mL 50% ethanol).

Determination of reducing power: to 2.5 mL of sample ethanol solution were added 2.5 mL of 0.2 mol/L, pH 6.6 phosphoric acid buffer solution, and

2.5 mL of 1% potassium ferricyanide; after 20 min of heating in a 50°C water bath, the mixture was cooled rapidly, and 2.5 mL of 10% trichloroacetic acid solution was added. The mixture was centrifuged at 3,000 rpm for 10 min. Next, 5 mL of supernatant was collected and mixed with 4 mL of distilled water and 1 mL of 0.1% $FeCl_3$, and the absorbance value at 700 nm was measured 10 min after mixing. The greater the absorbance value, the stronger the resilience.

Results and discussion

Single-factor experimental results of chickpea proteolysis

The material-liquid ratio refers to the ratio of protein mass to water mass. The material-liquid ratio and enzymatic hydrolysis time of chickpea protein were investigated. The results are shown in Figure 1A. In the range of material-liquid ratios of 1:5 - 1:20, the •OH scavenging rate first increased and then decreased, reaching a maximum at 1:10.

As shown in Figure 1B, the enzymatic hydrolysis time affected the degree of hydrolysis *versus* the •OH scavenging rate. At enzymatic hydrolysis times in the range of 5 - 20 min, the •OH scavenging rate first increased and then decreased. It reached its maximum at 10 min.

Figure 1C shows that the enzymatic hydrolysis temperature affected •OH scavenging. From 45 - 60°C, the •OH scavenging rate first increased and then decreased, reaching the highest value at 55°C.

In Figure 1D, at an enzyme-substrate ratio of 10 - 40%, increases in the amount of bromelain relative to the substrate increased the •OH scavenging rate with first increased and then decreased trend was observed, reaching the highest value at 20%.

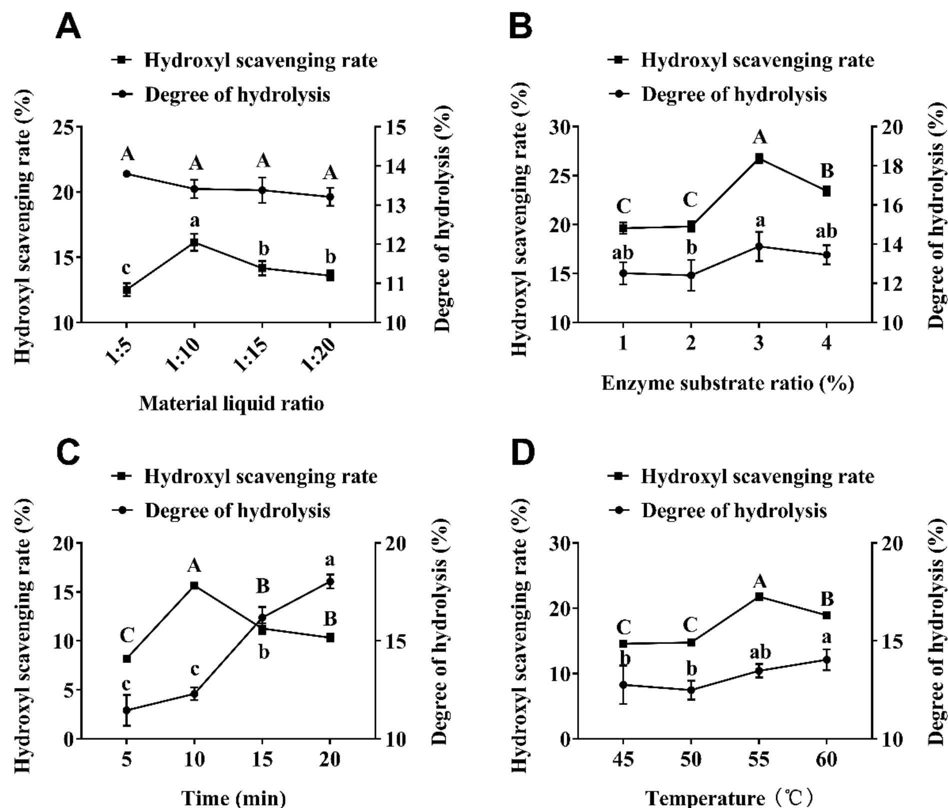

Figure 1. Results of single-factor experiment of chickpea protein hydrolysis.

Orthogonal experiment results of chickpea proteolysis

An L_9 (3^4) orthogonal test was designed, and the protocol and results are presented in Table 2.

As shown in Table 2, the order of the primary factors was C > A > D > B, that is, enzyme-substrate ratio > material-liquid ratio > enzymatic hydrolysis temperature > enzymatic hydrolysis time. The optimal scheme was $A_3B_2C_2D_3$. The optimal conditions for hydroxyl scavenging were as follows: 1:15 feed to liquid ratio, 10 min enzymatic hydrolysis time, 20% enzyme to substrate ratio, and 60°C enzymatic hydrolysis temperature. The hydroxyl scavenging rate of the optimal regime was 49.44%.

Table 2. Results of the orthogonal test of chickpea protein hydrolysis.

Test no.	A	B	C	D	Hydroxyl scavenging rate (%)
1	1	1	1	1	8.33
2	1	2	2	2	11.81
3	1	3	3	3	5.56
4	2	1	2	3	15.28
5	2	2	3	1	8.33
6	2	3	1	2	8.33
7	3	1	3	2	6.94
8	3	2	1	3	14.58
9	3	3	2	1	13.89
K1	25.7	30.55	31.24	30.55	
K2	31.94	34.72	40.98	27.08	
K3	35.41	27.78	20.83	35.42	
k1	8.57	10.18	10.41	10.18	
k2	10.65	11.57	13.66	9.03	
k3	11.80	9.26	6.94	11.81	
R	3.24	2.31	6.72	2.78	
Primary and secondary factors: C > A > D > B					
Optimal scheme: $A_3B_2C_2D_3$					

Results of chickpea peptide antioxidant experiment

The scavenging rate of •OH was 49.44%, the scavenging rate of O^{2-}• was 63.64%, the scavenging rate of DPPH• was 66.57%, the scavenging capacity of hydrogen peroxide was 57.64%, and the reducing power was 0.753. The results showed that the chickpea protein product hydrolysed by bromelain had good free radical scavenging ability and reducing power.

Single-factor experimental results for preparation of peptide-calcium chelates

It can be seen from Figure 2A that pH had a great impact on the calcium chelation rate of chickpea peptides. This could be explain by the fact that when the acidity of the solution is too low, too much H^+ will combine with the amino groups of polypeptides, thus competing with Ca^{2+}; this process affects the formation of calcium peptide chelates, thus leading to a reduction in chelation rate. When the alkalinity of the solution is too high, Ca^{2+} and OH^- will combine to form a precipitate, which will also greatly reduce the chelation rate. The polypeptides and calcium should be chelated in a neutral solution. It can be seen from Figure 2A that when the pH was 7.0, the maximum calcium chelation rate of chickpea peptides was 39.20%.

It can be seen from Figure 2B that the longer the chelation time, the higher the calcium chelation rate of chickpea peptides. At the initial chelation stage, chickpea peptides fully contacted calcium ions, and chelated with the maximum chelation rate. However, after 30 min, the chelation rate did not increase, and remained stable, which indicated that the chickpea peptides had fully chelated with metal calcium ions at a chelation time of 30 min, and the peptide-calcium chelation rate could not be improved with additional time. When the chelation time was 30 min, the maximum calcium chelation rate of chickpea peptides was 33.62%.

When the ratio of chickpea peptides to calcium was in the range of 1:1 - 7:1 (Figure 2C), the chelation rate gradually increased, and the peptide-calcium chelation rate was the highest at 7:1, which was 22.42%. When the mass ratio of peptides to calcium exceeded 7:1, the chelation rate of chickpea peptides with calcium no longer increased.

It can be seen from Figure 2D, between 30 and 50°C, the chelation temperature had little effect on the calcium chelation rate of chickpea peptides. The

maximum chelation rate was 39.89% when the chelation temperature was 50°C. However, when the temperature exceeded 50°C, the chelation rate decreased. The reason could be that the higher the temperature, the stronger the movement of molecular ions, which results in free calcium ions after the polypeptides chelate with metal calcium ions, thus leading to a reduction in chelation rate.

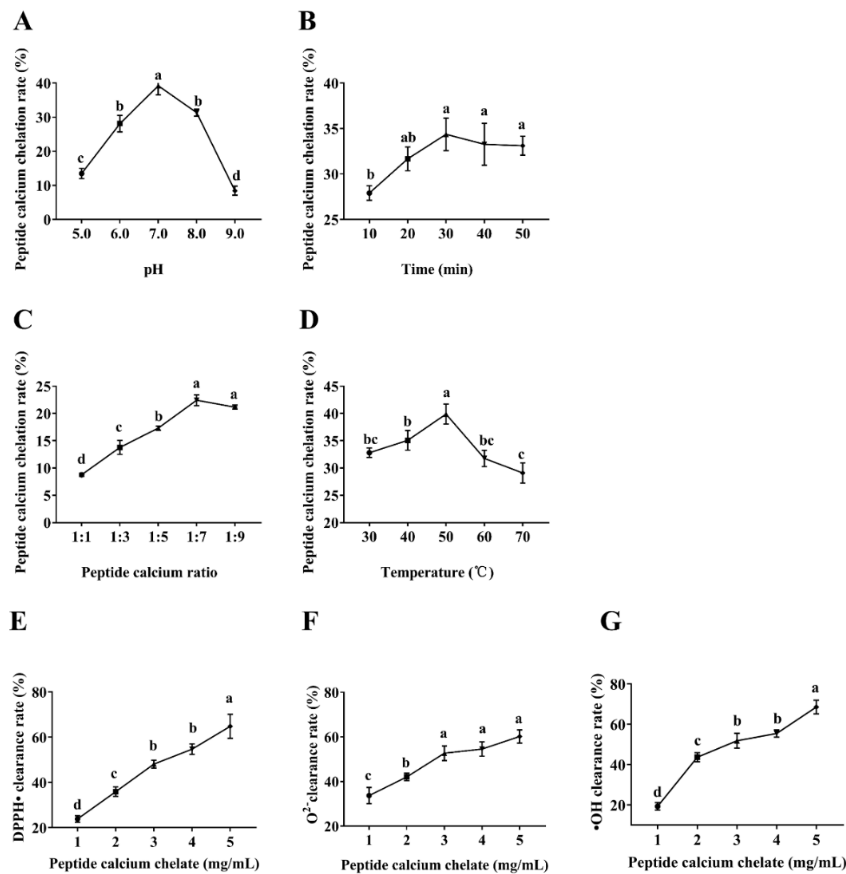

Figure 2. (A), **(B)**, **(C)**, and **(D)**: single-factor experimental results of chickpea peptide-calcium chelate preparation. **(E)**, **(F)**, and **(G)**: results of the antioxidation experiment of chickpea peptide-calcium chelates.

Orthogonal experimental results for preparation of peptide-calcium chelates

Based on the orthogonal test results of the calcium chelation rate of chickpea peptides in Table 3, the influence of each single factor on the chelation rate followed the order of C > A > B > D. The best chelation condition was $C_2A_2B_3D_2$; that is, when the solution pH was 7.0, the peptide calcium mass ratio was 7:1, the chelation time was 40 min, and the chelation temperature was 50°C, the chelation rate between polypeptides and calcium ions was the highest. The validation experiment was conducted under this optimal condition, and the calcium chelation rate of chickpea peptides was 39.95%.

The best chelation condition was $C_2A_2B_3D_2$ based on the results of the orthogonal test. Under these conditions, chickpea peptide-calcium chelates were prepared, and their antioxidant activity was studied.

Experimental results of antioxidant activity of peptide-calcium chelates

Figure 2E shows that in the DPPH radical clearance experiment, when the mass concentration of chickpea peptide-calcium chelates was 1.0 - 5.0 mg/mL, the DPPH radical clearance rate increased with increasing mass concentration. The antioxidation of chickpea peptide-calcium chelates was positively correlated with the mass concentration. When the mass concentration was less than 3.0 mg/mL, the scavenging rate increased rapidly; when the mass concentration was 5.0 mg/mL, the scavenging rate reached 60.24%.

Figure 2F shows that within the measurement range of the O^{2-} free radical scavenging experiment (*i.e.*, mass concentrations of chickpea peptide-calcium chelates of 1.0 - 5.0 mg/mL), the O^{2-} scavenging rate increased with the mass

Table 3. Results of the orthogonal test of the calcium chelation rate of chickpea peptides.

Test no.	A	B	C	D	Chelation rate (%)
1	1	1	1	1	30.23
2	1	2	2	2	36.51
3	1	3	3	3	32.24
4	2	1	2	3	38.01
5	2	2	3	1	34.32
6	2	3	1	2	35.60
7	3	1	3	2	30.62
8	3	2	1	3	30.54
9	3	3	2	1	37.45
K_1	98.98	98.86	96.37	102.00	
K_2	107.93	101.37	111.97	102.73	
K_3	98.61	105.29	97.18	100.79	
k_1	32.99	32.95	32.12	34.00	
k_2	35.98	33.79	37.32	34.24	
k_3	32.87	35.10	32.39	33.60	
R	3.11	2.14	5.20	0.65	
Primary and secondary factors: C > A > B > D					
Optimal scheme: $C_2A_2B_3D_2$					

concentration, and the antioxidation of chickpea peptide-calcium chelates was positively correlated with the mass concentration. When the mass concentration was 5.0 mg/mL, the O^{2-} removal rate reached 64.77%.

Based on Figure 2G, the chickpea peptide-calcium chelates had a strong •OH scavenging rate. Within the determination range of the •OH scavenging experiment (mass concentrations of chickpea peptide-calcium chelates of 1.0 - 5.0 mg/mL), the •OH scavenging rate increased with the mass concentration, and the antioxidation of chickpea peptide-calcium chelates was positively correlated with the mass concentration. When the mass concentration was 5.0 mg/mL, the •OH removal rate reached 68.54%.

Conclusion

Through two orthogonal tests, peptide-calcium chelates with antioxidant activity were prepared in the present work. The two components had synergistic physiological effect in the body to promote human health. The optimum enzymatic hydrolysis scheme was determined by a single factor test and an orthogonal experimental design: material-liquid ratio, 1:15; enzymatic hydrolysis time, 10 min; enzyme-substrate ratio, 20%; and enzymatic hydrolysis temperature, 60°C. The protein hydrolysate obtained under these conditions had high antioxidant activity. The free radical scavenging rates of •OH, O^{2-}, DPPH•, and H_2O_2 were 49.44, 63.64, 66.57, 57.64%, respectively. The reducing power was 0.753. The optimum conditions for the preparation of calcium chelates from chickpea peptides were determined by an orthogonal optimisation test: the peptide-calcium ratio, 7:1; the chelation temperature, 40°C; the initial pH of the reaction solution, 7.0; the chelation time, 20 min; and the chelation rate, 20.86%. The preparation of chickpea peptide-calcium chelates demonstrated in the present work could be a novel method for the comprehensive utilisation and deep processing of chickpea.

Acknowledgement

The authors gratefully acknowledge the financial support received from the Guangdong Provincial Junior Innovative Talents Project for Ordinary Universities (grant no.: 2020KQNCX097).

References

Curhan, G. C., Willett, W. C., Rimm, E. B. and Stampfer, M. J. 1993. A prospective study of dietary calcium and other nutrients and the risk of symptomatic kidney stones. The New England Journal of Medicine 328(12): 833-838.

Hu, S., Lin, S., Wang, D., Zhang, S. and Sun, N. 2021. Antarctic krill-derived peptides with consecutive Glu residues enhanced iron binding, solubility and absorption. Food and Function 12(7): 455-461.

Idate, A., Shah, R., Gaikwad, V., Kumathekar, S. and Temgire, S. 2021. A comprehensive review on antinutritional factors of chickpea (*Cicer arietinum* L.). Pharma Innovation 10(5): 816-823.

Jlab, C., Xy, D., Ops, E., Yza, B. and Yu, F. 2021. Phosphorylation modification of collagen peptides from fish bone enhances their calcium-chelating and antioxidant activity. LWT – Food Science and Technology 72(12): 867-876.

Liu, H., Lv, Y., Xu, J., Chen, C. and Guo, S. 2021. Changes in the secondary structures and zeta potential of soybean peptide and its calcium complexes in different solution environments. Food and Functions 12: 5967-5974.

López-Pedrouso, M., Borrajo, P., Pateiro, M., Lorenzo, J. M. and Franco, D. 2020. Antioxidant activity and peptidomic analysis of porcine liver hydrolysates using alcalase, bromelain, flavourzyme and papain enzymes. Food Research International 137(10): 109389.

Luz, A., Costa, R., Medeiros, G., Piuvezam, G. and Morais, A. 2021. What are the digestion and absorption models used to reproduce gastrointestinal protein processes?: A protocol for systematic review. Medicine 100(30): e26697.

Mariaelena, D., Biase, A. and Rita, B. E. 2019. *Lactobacillus plantarum* ITM21B fermentation product and chickpea flour enhance the nutritional profile of salt reduced bakery products. International Journal of Food Sciences and Nutrition 31(2): 186-194.

Milán-Noris, A., Montoya-Rodríguez, A., Rosa-Millán, J. and Serna-Saldivar, S. O. 2019. Health effects of starch and undigested carbohydrates associated to chickpea-based foods. In Lund, A. T. and Schultz, N. D. (eds). Handbook of Chickpeas: Nutritional Value, Health Benefits and Management, p. 117-124. United States: NOVA Science Publishers.

Misra, A., Prasad, R., Das, M. and Dwivedi, P. D. 2009. Probing novel allergenic proteins of commonly consumed legumes. Immunopharmacology and Immunotoxicology 15(8): 324-331.

Rasul, N. H., Asdagh, A., Pirsa, S., Ghazanfarirad, N. and Sani, I. K. 2022. Development of antimicrobial/antioxidant nanocomposite film based on fish skin gelatin and chickpea protein isolated containing microencapsulated *Nigella sativa* essential oil and copper sulfide nanoparticles for extending minced meat shelf life. Materials Research Express 9(2): 25306.

Rivero-Pino, F., Espejo-Carpio, F. J. and Guadix, E. M. 2021. Unravelling the alpha-glucosidase inhibitory properties of chickpea protein by enzymatic hydrolysis and in silico analysis. Food Bioscience 31(5): 356-364.

Shi, Z., Dun, B., Wei, Z., Liu, C. and Yao, Y. 2021. Peptides released from extruded adzuki bean protein through simulated gastrointestinal digestion exhibit anti-inflammatory activity. Journal of Agricultural and Food Chemistry 53(6): 441-454.

Sun, N., Hu, S., Wang, D., Jiang, P., Zhang, S. and Lin, S. 2022. Calcium delivery systems assembled using Antarctic krill derived heptapeptides: Exploration of the assembly mechanism, *in vitro* digestion profile and calcium absorption behavior. Journal of Agricultural and Food Chemistry 70(6): 358-365.

Tian, T., Li, X.-H. and Wang, Q. 2019. Development of cell penetrating peptides for enhancing drug absorption. Chinese Pharmaceutical Journal 31(2): 186-194.

Xue, H., Han, J., He, B., Yi, M. and Li, J. 2021. Bioactive peptides release and absorption tracking of casein in the gastrointestinal digestion of rats. Food and Function 135(2): 1185-1192.

Xue, Z. and Shi. 2008. Technology optimization for alcalase hydrolysis of chickpea isolated protein. Journal of the Chinese Cereals and Oils Association 25(9): 324-331.

Zhang, H., Zhao, L., Shen, Q., Qi, L. and Richel, A. 2021a. Preparation of cattle bone collagen

peptides-calcium chelate and its structural characterization and stability. LWT - Food Science and Technology 144(12): 111264.

Zhang, X., Jia, Q., Li, M., Liu, H. and Liu, Z. 2021b. Isolation of a novel calcium-binding peptide from phosvitin hydrolysates and the study of its calcium chelation mechanism. Food Research International 141(3): 110169.