Oligopeptides derived from solid-state fermented cottonseed meal showed good antioxidant activity in both in vitro and in vivo studies

Jiancheng Liu
Xinjiang Agricultural University
Fengming Li
Xinjiang Agricultural University
Xiaoyang Zhang
Shihezi University
Cunxi Nie
Shihezi University
Wenju Zhang (zhangwj1022@sina.com)
Shihezi University

Research Article

Keywords: cottonseed meal, oligopeptides, antioxidant activity, free radical, mice

Posted Date: May 9th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1629250/v1

Abstract

To determine the *in vitro* and *in vivo* antioxidant activities of oligopeptides derived from solid-state fermented cottonseed meal. The free radical scavenging *in vitro* as well as the antioxidative function and lipid metabolism in an oxidized stress mice model was applied in the present study and results indicated that the oligopeptides showed good antioxidant activity both *in vitro* and *in vivo*. A positive correlation between the oligopeptides content, free radical scavenging ability and total antioxidant activity was observed when the oligopeptides concentration ranged from 0.5 mg/mL to 8 mg/mL. While the *in vivo* study indicated that feeding oligopeptides increased the activities of T-AOC, T-SOD, CAT, GSH-Px in the liver and serum of mice ($p<0.05$) and decreased the content of MDA ($p<0.01$). Our results indicated that the oligopeptides showed good antioxidant activity and could improve the antioxidant capacity as well as blood lipid metabolism in mice.

Introduction

Redox reactions are indispensable physiological activities in the metabolism of animals with a generation of large numbers of oxygen free radicals. Those free radicals (includes O_2^-, •OH, H_2O_2, and $^1O_2$) are a class of special chemical groups with unpaired electrons. They are extremely unstable and have high reactivity (Obeagu, 2018). It has been reported that excessive accumulation of free radicals in animal cells could directly damage the protein structure, DNA, lipid as well as other biological macromolecules, and eventually lead to cell death and tissue and organ aging (Pouya, 2020), closely associated with coronary heart disease, aging, cancer or other diseases (Valko et al., 2007; Pourahmad et al., 2016; Suleman et al., 2019). In addition to ascorbic acid, vitamin E, β-carotene or other common antioxidants, as non-enzyme free radical scavengers, and antioxidant peptides have also attracted much attention (Nwachukwu and Aluko, 2019). Isolation of antioxidant peptides from plants and animals (e.g. soy, rapeseed, rice, wheat, corn, chickpea, milk, sardines, etc.) has been widely reported (Davalos et al., 2004; Arcan et al., 2007; Bougatef et al., 2010; He et al., 2013; Wang et al., 2014; Wang et al., 2017; Meza-Espinoza et al., 2018; Bisly et al., 2021). Compared with the synthetic antioxidants, antioxidant peptides were more acceptable by consumers for their safety and other advantages (Wen et al., 2020; Salvado et al., 2021; Serajul et al., 2022).

As a by-product of cottonseed during cotton oil manufacture, the cottonseed protein shows a good balance in composition of amino acids and is considered as an important plant protein resource (Nagalakshmi et al., 2007; Nie et al., 2015; Mohammadrezaei et al., 2021). A lot of work has been done to improve the economic value of cottonseed protein. For instance, oligopeptides was one kind of small molecule protein obtained by enzymatic hydrolysis or microbial fermentation of cottonseed protein and has shown much biological activity such as *in vitro* antioxidant activity, and blood pressure reduction (Gao et al., 2010; Wang et al., 2021). However, as far as we know, few studies have focused on the antioxidant activity of cottonseed oligopeptides *in vivo*.

In the present study, the oligopeptides derived from the solid-state fermented cottonseeds meal, of that ability to reduce the free radical of 1, 1-diphenyl-2-trinitrophenylhydrazine (DPPH), hydroxyl (•OH), anti-superoxide anion (O^{2-}), and total antioxidant capacity (T-AOC) was investigated *in vitro*, while its effects on the antioxidant and lipid metabolism in serum and liver were investigated using an oxidative stress mode in mice.

Materials And Methods

Materials

Oligopeptides with a molecular weight less than 3 kD was isolated and purified from the solid-state fermented cottonseed meal used mixture bacterium of *Bacillus subtilis* (CICC 1201) and *Saccharomyces cerevisiae* (CICC 1001) (Liu et al., 2018). 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Shanghai Biological Engineering Co., Ltd. O^{2-}·, •OH, T-AOC, T-SOD, CAT, GSH-Px, GSH, T-CHO, TG, HDL-C, LDL-C, MDA and protein assay kits were available from Jiancheng Ltd., (Nanjing, China). All other chemicals and reagents used in this study were of analytical grade and purchased from Yongsheng Fine Chemical Co., Ltd (Tianjin, China).

Dpph Radical Scavenging Activity

The DPPH free radical scavenging activity was measured according to Zhang et al. (2011) with some modification. Briefly, 100 μL oligopeptides with different concentrations (0.5, 1, 2, 4 and 8 mg/mL) were mixed thoroughly with 2.9 mL DPPH (0.1 mM in methanol solution) and kept in the dark for 30 min. Then, the absorbance of the samples was measured with an ELIASA (EONC, USA). The DPPH free radical scavenging rate was calculated by the following formula:

DPPH radical scavenging activity (%) = $[1-(A_{sample}-A_{blank})/A_{control}] \times 100$

$A_{control}$ was the absorbance after adding the methanol solution, A_{sample} was the absorbance of the oligopeptides and A_{blank} was the absorbance after adding the methanol solution to the oligopeptides solution.

Inhibition Of Hydroxyl Radical Ability

Hydroxyl radical was generated by iron-catalysed Fenton reaction. Briefly, 20 μL of oligopeptides with different concentrations (0.5, 1, 2, 4 and 8 mg/mL) was first combined with 20 μL of ferrous sulfate and 40 μL of 1, 10-phenanthroline. The mixture was incubated at 37°C for 1 min. Then, 200 μL of chromogenic agent was added to the reaction. The mixture was incubated at 25°C for 20 min, and the absorbance at 550 nm was measured by an ELIASA (EONC, USA). The inhibition of hydroxyl radical ability was calculated by the following formula:

Inhibition of hydroxyl radical ability (Unit/mL) = [($A_{control}$ -A_{sample})/($A_{standard}$ -A_{blank})]×Standard concentration (8.824 mmol/L) ×1 mL/0.02 mL

$A_{control}$ was the absorbance of the double distilled water replace of oligopeptides, $A_{standard}$ was the absorbance of the 0.03% H_2O_2 replace of oligopeptides, and A_{blank} was the absorbance of the double distilled water replace of oligopeptides, 1,10-phenanthroline and ferrous sulfate.

Superoxide Anion Radical Scavenging Activity

The superoxide anion radical scavenging activity was measured according to the method described by Ji et al (2018), with some modification. This assay is based on the removal rate of xanthine/xanthine oxidase generated superoxide by measuring the reduction of nitro blue tetrazolium (NBT). The reaction mixture contained 50 µL different concentrations of oligopeptides (0.5, 1, 2, 4 and 8 mg/mL), 1mL of a mixture of xanthine (0.1 mM) and NBT (0.2 mM) in potassium phosphate buffer (50 mM, pH 7.5) containing EDTA(0.05 mM), 0.1 mL xanthine oxidase (0.8 unit/mL diluted in 50mM phosphate buffer, pH 7.5) was incubated at 37°C for 20 min. Addition of 2 mL of 2.5 N HCl to the mixtures terminated the reaction, followed by increase of coloration of NBT, which was measured at 560nm against a blank. The scavenging of superoxide anion in percentage was calculated using the equation described as in the case of DPPH.

Total Antioxidant Capacity

The total antioxidant capacity (T-AOC) of oligopeptides was determined using a commercial kit and the result was calculated by the equation below.

Total antioxidant capacity (Unit/mL) = (A_{sample} – $A_{control}$)/0.3×N×n

Where A_{sample} is the absorbance value of the sample; $A_{control}$ is the absorbance value of the control; N is dilution of the reaction system; n is the dilution of the sample.

The activities of antioxidant enzymes as well as the lipid metabolism of mice were analyzed according to the instructions of the assay kit.

Mouse Grouping And Feeding Management

Male BALB/c mice at 6 weeks of age were purchased from the laboratory animal center of Shihezi University. Mice were maintained under a 12-h dark/light cycle at a temperature of 22 ± 3°C (Napoli et al., 2003). The mice were fed with basal diet or high fat content diet, where the nutritional composition of those diets is listed in supplementary Table 1. For the experiment, forty-five BALB/c mice were divided into 5 groups and each group had 3 replicates. The control group (CK) was fed with the basal diet and

250 μL sterile saline. The high fat diet (HFD) was fed with high fat diet and 250 μL sterile saline. The low (CP-4), medium (CP-20) and high (CP-40) group were fed with high fat diet, and 4 mg/mL, 20 mg/mL and 40 mg/mL oligopeptides in 250 μL sterile saline, respectively. The mice were fed twice a day (10 AM and 6 PM, lasting for 30 days), and during which period, the mice were free access to drinking water.

At the end of the trial period, the mice were fasted for 12 h, and weighed. After the ether anesthesia, blood was collected from the eye socket and the mice were sacrificed by cervical dislocation. Then, 0.1 g liver was placed in 0.9 mL sterile saline to prepare a 10% liver suspension, and stored at -80^{o}C for future analysis. All experimental procedures using laboratory animals were approved by the Animal Care and Use Committee of Shihezi University (A2020-069-01).

Statistical analysis

Statistical analysis was performed using the SPSS software program (Version: 20.0, USA). The data was subjected to one-way analysis of variance (ANOVA) for mean comparison, and the significant differences were detected by the Duncan`s test ($p < 0.05$ indicated the difference was significant, $p < 0.01$ indicated extremely significant).

Results And Discussion

Antioxidant activity of cottonseed meal oligopeptides

In order to better understand the chemical antioxidant activity of oligopeptides derived from solid-fermented cottonseed meal, four different methods were applied in this study, including DPPH free radical scavenging, hydroxyl radical inhibitory, anti-superoxide anion and total antioxidant capacity. As shown in Fig. 1A, the DPPH scavenging effect of cottonseed meal oligopeptides was gradually enhanced with the increase of its concentration, and the DPPH clearance rate reached a maximum of 31.55 ± 1.76% at the concentration of 8 mg/mL. The inhibition of superoxide anion by oligopeptides is depicted in Fig. 1B. The inhibitory capacity of the oligopeptides to hydroxyl radicals increased gradually with the increase of oligopeptides concentration (range of 0.5 to 8 mg/mL). When the oligopeptides concentration was 0.5 mg/mL, the inhibition ability was 1.71 ± 0.26 U/mL. When the concentration of oligopeptides increased to 8 mg/mL, the inhibition ability increased to the maximum of 48.97 ± 0.16 U/mL.

The cottonseed meal oligopeptides inhibited the production of O^{2-} in xanthine and xanthine oxidase reaction system and its ability to inhibit the generation of superoxide anion radicals was positively correlated with the oligopeptides concentration and the maximum of anti-superoxide anion activity was 84.49 ± 10.55 U/L when the concentration of oligopeptides was 8 mg/mL (Fig. 1C). The total antioxidant capacity of cottonseed meal oligopeptides was positively correlated with its concentration. A linear regression equation was obtained ($y = 0.9309x + 0.0562$) with a linear regression coefficient of 0.996 Fig. 1D. When the concentration of cottonseed meal oligopeptides was 8 mg/mL, the total antioxidant capacity reached the maximum of 7.45 ± 0.13 U/μL.

Most of the natural antioxidants were multi-functional and in order to better evaluate their antioxidant ability, we should choose several methods with different oxidation conditions. In other words, for a comprehensive evaluation of natural antioxidants, several methods should be applied (Schlesier et al., 2002). Up to now, many methods were applied to evaluate the antioxidant activity of antioxidants. These methods could be mainly divided into *in vitro* and *in vivo*. The *in vitro* methods, used for convenience and lower cost, have been widely used as the initial exploration of antioxidant mechanisms (Xie et al., 2008). In the present study, our results showed that the oligopeptides derived from solid-state fermented cottonseed meal had DPPH scavenging activity, inhibited the generation of hydroxyl radical and superoxide anion free radicals, and its total antioxidant capacity showed a significant dose-effect relationship with its concentration. Sun et al. (2015) reported a higher hydroxyl free radical scavenging activity in cottonseed meal oligopeptides produced by solid-state fermentation and presumed that the antioxidant activity was closely associated with its amino acid composition. Gao et al. (2010) reported that after the addition of neutral protease, four different cottonseed meal peptides were isolated by Sephadex G-25. All had higher superoxide anion scavenging activity than the raw cottonseed meal and presumed that these peptide fragments might contain certain electron donors and thus could react with free radicals and the reaction was converted to a more stable product to terminate the free radical chain, subsequently, resulting in an antioxidant effect.

Effects Of Cottonseed Meal Oligopeptides On Growth Performance Of Mice Fed With High-fat Diet

The results showed the effect of cottonseed meal oligopeptides on body weight of mice fed with high-fat diet in Table 1. There was no significant difference in mean body weight between the groups at the beginning of the experiment ($p > 0.05$). However after 30 days, the mean weight of mice in HFD group, CP-4 group, CP-20 group, CP-40 group and CK group increased significantly compared with the initial weight ($p > 0.05$). Particularly, the average daily gain of CP-40 group was significantly higher than that of the CK group ($p < 0.01$), HFD group and CP-4 group ($p < 0.05$), while no significant differences were observed in the CP-20 group.

Table 1
Effects of cottonseed meal oligopeptides on growth performance of mice fed with high-fat diet.

Items	CK[1)]	HFD[2)]	CP-4[3)]	CP-20	CP-40
Initial weight (g)	27.40 ± 0.87	27.50 ± 0.92	27.33 ± 0.65	27.50 ± 1.50	27.47 ± 1.53
Final weight (g)	30.57 ± 1.72 a	32.10 ± 1.65 ab	32.27 ± 1.30 ab	32.83 ± 1.43 ab	33.93 ± 1.76 b
Weight gain per day (g)	0.11 ± 0.03 a	0.15 ± 0.03 b	0.16 ± 0.02 b	0.18 ± 0.01 b	0.22 ± 0.03 A
[1)] CK: the control group;					
[2)] HFD: the high fat diet group;					
[3)] CP: the cottonseed meal oligopeptides group;					

Effects of cottonseed meal oligopeptides on activity of antioxidant enzymes in the liver of mice fed with high-fat diet

As shown in Table 2, the activities of antioxidant enzymes T-AOC ($p < 0.05$), T-SOD ($p < 0.05$) and CAT ($p < 0.05$) in the liver of mice were significantly lower as well as the activity of GSH-Px ($p < 0.01$) in the HFD group than in the CK group, while the content of MDA was significantly increased in the HFD group ($p < 0.01$). By adding different concentrations of cottonseed meal oligopeptides to high fat diets, the activities of T-AOC, T-SOD, CAT and GSH-Px in mouse liver increased significantly ($p < 0.05$) and restored to the normal level (CK group). Particularly, with the increase of oligopeptides concentration, the MDA content in mouse liver decreased significantly ($p < 0.01$).

Table 2
Effects of cottonseed meal oligopeptides on antioxidant function in liver of mice fed with high-fat diet.

Items	CK[1)]	HFD[2)]	CP-4[3)]	CP-20	CP-40
T-AOC (U/mgprot)	0.30 ± 0.01[a]	0.25 ± 0.02 [b]	0.26 ± 0.03 [a]	0.30 ± 0.04 [a]	0.31 ± 0.02 [a]
T-SOD (U/mgprot)	58.16 ± 7.95 [a]	45.81 ± 6.02 [b]	47.19 ± 4.29 [b]	51.76 ± 3.66 [a]	58.46 ± 4.93 [a]
CAT (U/mgprot)	1.55 ± 0.16 [a]	1.34 ± 0.16 [b]	1.39 ± 0.07 [a]	1.40 ± 0.05 [a]	1.46 ± 0.09 [a]
GSH-Px (U/mgprot)	185.68 ± 10.90[A]	136.62 ± 15.02 [B]	158.81 ± 17.44 [B]	177.81 ± 14.82 [A]	200.06 ± 6.48 [A]
GSH (μmol/gprot)	0.30 ± 0.06	0.26 ± 0.07	0.28 ± 0.05	0.31 ± 0.03	0.31 ± 0.03
MDA (nmol/mgprot)	3.63 ± 0.09 [A]	4.58 ± 0.24 [B]	3.45 ± 0.10 [A]	2.61 ± 0.08 [C]	2.09 ± 0.06 [D]
[1)] CK: the control group;					
[2)] HFD: the high fat diet group;					
[3)] CP: the cottonseed meal oligopeptides group;					

High fat diets could induce obesity and oxidative stress in animals. The results of this study showed that feeding a high-fat diet could significantly increase the daily gain and final weight of mice. Intake of oligopeptides had a positive effect on the weight gain of mice fed with high-fat diet. Antioxidant enzymes were important antioxidants in the body and studies have demonstrated that oxidative stress was often accompanied by a decrease in the level of antioxidant enzymes (Li et al., 2007). In the present study, we found both a significantly lower activity of antioxidant enzymes such as T-AOC, CAT and GSH-Px and a higher MDA level in the liver and serum of mice fed with high-fat diet compared with the control group (CK). These changes may be attributed to the fact that high-fat diets increased the body's lipid oxidation process and produced excess free radicals (Yang et al., 2006; Qu et al., 2016; Chen et al., 2020).

Effects of cottonseed meal oligopeptides on antioxidant function in serum of mice fed with high-fat diet

The effect of cottonseed meal oligopeptides on serum antioxidative of mice fed with high-fat diet was shown in Table 3. Compared with the CK group, the activities of T-AOC ($p < 0.05$), CAT ($p < 0.05$) and GSH-Px ($p < 0.01$) in the HFD group were decreased significantly, while the MDA content was increased significantly ($p < 0.01$). The activity of T-SOD and the content of GSH were decreased also, but there were no significantly difference compared with the CK group ($p > 0.05$). By adding different concentrations of cottonseed meal oligopeptides (CP-4, CP-20, CP-40 groups) to mice fed with high-fat diets, the activities of T-AOC ($p < 0.05$), CAT ($p < 0.05$) and GSH-Px ($p < 0.01$) in serum of mice were higher than the HFD

group. The content of MDA in serum of mice fed with cottonseed meal oligopeptides was decreased significantly compared with the HFD group ($p < 0.01$).

Table 3
Effects of cottonseed meal oligopeptides on antioxidant function in serum of mice fed with high-fat diet.

Items	CK[1)]	HFD[2)]	CP-4[3)]	CP-20	CP-40
T-AOC (U/mL)	1.95 ± 0.44 [a]	1.23 ± 0.11 [b]	1.66 ± 0.58 [a]	1.83 ± 0.24 [a]	2.04 ± 0.25 [a]
T-SOD (U/mL)	145.76 ± 8.30	136.72 ± 6.38	133.93 ± 6.52	137.18 ± 11.14	144.06 ± 7.44
CAT (U/mL)	6.52 ± 0.09 [a]	5.79 ± 0.35 [b]	5.98 ± 0.56 [a]	6.18 ± 0.44 [a]	6.25 ± 0.30 [a]
GSH-Px (U/mL)	155.05 ± 7.56 [A]	98.14 ± 11.16 [B]	131.13 ± 8.11 [C]	142.27 ± 7.73 [A]	167.84 ± 9.92 [A]
GSH (μmol/L)	3.35 ± 0.37	2.48 ± 0.77	2.97 ± 0.37	3.47 ± 1.31	3.59 ± 0.57
MDA (nmol/mL)	4.69 ± 0.18 [A]	6.06 ± 0.23 [B]	5.64 ± 0.18 [B]	5.43 ± 0.18 [C]	4.73 ± 0.27 [A]
[1)] CK: the control group;					
[2)] HFD: the high fat diet group;					
[3)] CP: the cottonseed meal oligopeptides group;					

Effects of cottonseed meal oligopeptides on lipid metabolism in serum of mice fed with high-fat diet

The effect of cottonseed meal oligopeptides on the lipid metabolism in serum of mice fed with high-fat diet was shown in Table 4. Compared with the CK group, the contents of TC ($p < 0.01$), TG ($p < 0.01$) and LDL-C ($p < 0.05$) were increased in HFD group whereas the HDL-C content was significantly decreased ($p < 0.01$). The TC, TG, HDL-C and LDL-C content in the serum of the CP-4, CP-20, and CP-40 groups showed no significant difference with the CK group. These results indicated that the cottonseed meal oligopeptides could promote the recovery of HDL-C and LDL-C in high-fat diet mice to normal levels.

Table 4
Effects of cottonseed meal oligopeptides on lipid metabolism in serum of mice fed with high-fat diet.

Items	CK[1)]	HFD[2)]	CP-4[3)]	CP-20	CP-40
TC (mmol/L)	2.09 ± 0.16 ^{A}	2.82 ± 0.39 ^{B}	2.36 ± 0.09 ^{A}	2.26 ± 0.11 ^{A}	2.12 ± 0.12 ^{A}
TG (mmol/L)	0.97 ± 0.13 ^{A}	1.73 ± 0.33 ^{B}	1.33 ± 0.05 ^{A}	1.10 ± 0.09 ^{A}	0.94 ± 0.10 ^{A}
HDL-C (mmol/L)	1.20 ± 0.06 ^{A}	0.81 ± 0.02 ^{B}	0.87 ± 0.05 ^{B}	0.97 ± 0.14 ^{B}	1.14 ± 0.08 ^{A}
LDL-C (mmol/L)	0.86 ± 0.08 ^{a}	1.16 ± 0.03^{b}	1.08 ± 0.13 ^{b}	1.04 ± 0.16 ^{ab}	0.93 ± 0.12 ^{a}
[1)] CK: the control group;					
[2)] HFD: the high fat diet group;					
[3)] CP: the cottonseed meal oligopeptides group;					

In addition to decreasing the activity of antioxidant enzymes, the high fat diet also caused the disorder of blood lipid metabolism in mice, and this significantly increased the content of TC, TG and LDL-C, and decreased the content of HDL-C. Khaled et al. (2012) showed that protein hydrolysates could alleviate oxidative stress in the body by increasing the animal's antioxidant enzymes. In our study, we found that the activities of T-AOC, T-SOD, CAT and GSH-Px in the liver and serum could be increased by adding a certain amount of cottonseed meal oligopeptides to high fat diet fed mice, while the TC, TG, LDL-C, HDL-C content in serum could remain at a normal level and the MDA content decreased significantly. As a final product of lipid peroxidation, the MDA content reflects the body`s oxidative stress levels (Niki et al., 2010), and a reduction in MDA was clearly associated with an increase in antioxidant enzymes (Young et al., 2010). Considering the results from the present study, we might speculate that the cottonseed meal oligopeptides could increase the antioxidant function of the body by increasing the activity of antioxidant enzymes and the process of fat metabolism.

Conclusion

In conclusion, the oligopeptides produced by solid-state fermentation of cottonseed meal has a good ability to scavenge DPPH, •OH, and O^{2-} free radicals as well as a good chelating effect on Fe^{2+}. Particularly, oligopeptides can enhance the antioxidant activity by increasing the activity of antioxidant enzymes and affect the process of fat metabolism in oxidative stressed mice fed with high fat diet. Our results indicate that the oligopeptides derived from solid-state fermented cottonseed meal have good antioxidant activity both *in vitro* and *in vivo*.

Statements And Declarations

Ethics approval and consent to participate

The use of experimental mice in this study has been approved by the Animal Care and Use Committee of Shihezi University. All experimental procedures involving mice were performed according to the Guidelines of The Care and Use of Laboratory Animals in China.

Human and animal rights

All animal work in this paper was conducted according to relevant national guidelines. Animal care for the experiment complied with the regulations for the Animal Welfare Committee of Shihezi University (Ethical code: A2020-069-01).

Consent for publication

Not applicable.

Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Funding

This work was supported by the Bingtuan international scientific and technological cooperation plan (grant no. 2017BC002) and the National Natural Science Foundation of China (grant no. 31360564).

Conflicts of interest

The authors declare that they have no conflict of interest.

Acknowledgments

We would like to thank Dr. Sun, H. for excellent technical assistance. We extend our acknowledgment to the management, Shihezi University for providing the facilities.

References

1. Arcan I, Yemenicioglu A (2007) Antioxidant activity of protein extracts from heat-treated or thermally processed chickpeas and white beans. Food Chem 103(2):301–312. https://doi.org/10.1016/j.foodchem.2006.07.050
2. Bisly AA, Hettiarachchy NS, Kumar T, Lay JO (2021) Antioxidant activities of solid-state fermentation derived proteins and peptides from heat-stabilized defatted rice bran. JAOCS https://doi.org/10.1002/aocs.12558
3. Bougatef A, Nedjar-arroume N, Manni L, Ravallec R, Barkia A, Guillochon D, Nasri M (2010) Purification and identification of novel antioxidant peptides from enzymatic hydrolysates of

Sardinellaaurita by-products proteins. Food Chem 118(3):559–565. https://doi.org/10.1016/j.foodchem.2009.05.021

4. Chen Y, Wang F, Zhou J, Niu T, Xuan R, Cheng H, Wu W (2020) In Vivo Antifatigue Activity of Spirulina Peptides Achieved by Their Antioxidant Activity and by Acting on Fat Metabolism Pathway in Mice. Nat Prod Commun 15(8):1934578X2094623. http://doi.org/10.1177/1934578X20946233
5. Davalos A, Miguel M, Bartolome B, Lopez-Fandino R (2004) Antioxidant activity of peptides derived from egg white protein by enzymatic hydrolysis. J Food Protect 67(9):1939–1944. https://doi.org/10.4315/0362-028X-67.9.1939
6. Gao DD, Gao YS, Li HX (2010) Antioxidant activity of peptide fractions derived from cottonseed protein hydrolysate. J Sci Food Agric 90(11):1855–1860. https://doi.org/10.1002/jsfa.4024
7. Harman D (2001) Aging: overview. Ann N Y Acad Sci 928(1):1–21. https://doi.org/10.1111/j.1749-6632.2001.tb05631.x
8. He R, Alashi A, Malomo SA, Girgih AT, Chao DF, Ju XR, Aluko RE (2013) Antihypertensive and free radical scavenging properties of enzymatic rapeseed protein hydrolysates. Food Chem 141(1):153–159. https://doi.org/10.1016/j.foodchem.2013.02.087
9. Ji EJ, Chang BA, Jae YJ (2018) Purification and characterization of antioxidant peptides from enzymatically hydrolyzed ark shell (*Scapharca subcrenata*). Process Biochem 72:170–176. https://doi.org/10.1016/j.procbio.2018.06.001
10. Johansen JS, Harris AK, Rychly DJ, Ergul A (2005) Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical practice. Cardiovasc Diabetol 4(1):5–16. https://doi.org/10.1186/1475-2840-4-5
11. Khaled HB, Ghlissi Z, Chtourou Y (2012) Effect of protein hydrolysates from sardinelle (*Sardinellaaurita*) on the oxidative status and blood lipid profile of cholesterol-fed rats. Food Res Int 45(1):60–68. https://doi.org/10.1016/j.foodres.2011.10.003
12. Li L, Ng TB, Song M, Yuan F, Liu ZK, Wang CL, Liu F (2007) A polysaccharide-peptide complex from abalone mushroom (*Pleurotusabalonus*) fruiting bodies increases activities and gene expression of antioxidant enzymes and reduces lipid peroxidation in senescence-accelerated mice. Appl Microbio Biotech 75(4):863–869. https://doi.org/10.1007/s00253-007-0865-4
13. Liu JC, Sun H, Nie CX, Ge WX, Wang YQ, Zhang WJ (2018) Oligopeptide derived from solid-state fermented cottonseed meal significantly affect the immunomodulatory in BALB/c mice treated with cyclophosphamide. Food Sci Biotechnol 27(6):1791–1799. https://doi.org/10.1007/s10068-018-0414-1
14. Meza-Espinoza L, Sayago-Ayerdi SG, Yahia EM et al (2018) Antioxidant capacity of egg, milk and soy protein hydrolysates and biopeptides produced by Bromelia pinguin and Bromelia karatas-derived proteases. Emirates J Food Agric 30(2):122–130. http://doi.org/10.9755/ejfa.2018.v30.i2.1604
15. Mohammadrezaei M, Navidshad B, Gheisari A, Toghyani M (2021) Cottonseed Meal Bioactive Peptides as an Alternative to Antibiotic Growth Promoters in Broiler Chicks. Int J Pept Res Ther 27(1):329–340. https://doi.org/10.1007/s10989-020-10086-8

16. Nagalakshmi D, Rao SVR, Panda AK, Sastry VRB (2007) Cottonseed meal in poultry diets: A review. The J Poul Sci 44(2):119–134. https://doi.org/10.2141/jpsa.44.119
17. Napoli C, Martin-padura I, De nigris F, Giorgio M, Mansueto G, Somma P, Pelicci PG (2003) Deletion of the $p66^{Shc}$ longevity gene reduces systemic and tissue oxidative stress, vascular cell apoptosis, and early atherogenesis in mice fed a high-fat diet. PNAS 100(4):2112–2116. https://doi.org/10.1073/pnas.0336359100
18. Nie CX, Zhang WJ, Wang YQ, Liu YF, Ge WX, Liu JC (2015) Tissue lipid metabolism and hepatic metabolomic profiling in response to supplementation of fermented cottonseed meal in the diets of broiler chickens. J Zhejiang Univ-Sci B (Biomed & Biotechnol) 16(6):447–455. https://doi.org/10.1631/jzus.B1400255
19. Niki E (2010) Assessment of Antioxidant Capacity *in vitro* and *in vivo*. Free Radical Bio Med 49(4):503–515. https://doi.org/10.1016/j.freeradbiomed.2010.04.016
20. Nwachukwu ID, Aluko RE (2019) Structural and functional properties of food protein-derived antioxidant peptides. J Food Biochem 43(1):1–13. https://doi.org/10.1111/jfbc.12761
21. Obeagu EI (2018) A review on free radicals and antioxidants. Int J Curr Res Med Sci 4(2):123–133. https://doi.org/10.22192/ijcrms.2018.04.02.019
22. Pourahmad J, Salimi A, Seydi E (2016) Role of Oxygen Free Radicals in Cancer Development and Treatment. http://www.intechopen.com/books /free-radicals-and-dise ases
23. Pouya FA (2020) The theory of free radicals and aging: A review article. J Gerontol 4(3):8–12. https://doi.org./10.29252/joge.4.3.8
24. Qu D, Han J (2016) Investigation of the antioxidant activity of chitooligosaccharides on mice with high-fat diet. R Bras Zootec 45(11):661–666. https://doi.org/10.1590/S1806-92902016001100004
25. Salvado A, Leito JH, Fonseca LP (2021) Enzymatic Production of Bioactive Peptides from Whey Proteins: Their Active Role and Potential Health Benefits. https:// doi.org/10.1007/978-3-030-58315-6_15 Accessed 02 December 2020
26. Schlesier K, Harwat M, Bohm V, Bitsch R (2002) Assessment of antioxidant activity by using different *in vitro* methods. Free Radical Res 36(2):177–187. https://dor.org/10.1080/10715760290006411
27. Serajul I, Wang HG, Habtamu A, Abdellatief AS, Wei FA (2022) Health benefits of bioactive peptides produced from muscle proteins: Antioxidant, anti-cancer, and anti-diabetic activities. Process Biochem 116:116–125. https://doi.org/10.1016/j.procbio.2022.03.007
28. Suleman M, Khan A, Baqi A (2019) Antioxidants, its role in preventing free radicals and infectious diseases in human body. Pure Appl Biol 8(1):380–388. https://dor.org/10.19045/bspab.2018.700197
29. Sun H, Yao XH, Wang X, Wu YF, Liu Y, Tang JW, Feng J (2015) Chemical composition and *in vitro* antioxidant property of peptides produced from cottonseed meal by solid-state fermentation. CyTAJ Food 13(2):264–272. https://doi.org/10.1080/19476337.2014.948072

30. Valko M, Leibfrita D, Moncol J, Cronin MTD, Mazur M, Telser J (2007) Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Bio 39(1):44–84. https://doi.org/10.1016/j.biocel.2006.07.001
31. Wang LY, Ma MT, Yu ZP, Du SK (2021) Preparation and identification of antioxidant peptides from cottonseed proteins. Food Chem 352(7):129399. https://doi.org/10.1016/j.foodchem.2021.129399
32. Wang XJ, Zheng XQ, Kopparapu NK, Cong WS, Deng YP, Sun XJ, Liu XL (2014) Purification and evaluation of a novel antioxidant peptide from corn protein hydrolysate. Process Biochem 49(9):1562–1569. https://doi.org/10.1016/j.procbio.2014.05.014
33. Wang XM, Chen HX, Fu XG, Li SQ, Wei J (2017) A novel antioxidant and ACE inhibitory peptide from rice bran protein: Biochemical characterization and molecular docking study. Food Sci Tech 75:93–99. https://doi.org/10.1016/j.lwt.2016.08.047
34. Wen C, Zhang J, Zhang H, Duan Y, Ma H (2020) Plant protein-derived antioxidant peptides: Isolation, identification, mechanism of action and application in food systems: A review. Trends Food Sci Technol 105(1):308–322. https://doi.org/10.1016/j.tifs.2020.09.019
35. Xie ZJ, Huang JR, Xu XM, Jin ZY (2008) Antioxidant activity of peptides isolated from alfalfa leaf protein hydrolysate. Food Chem 111(2):370–376. https://doi.org/10.1016/j.foodchem.2008.03.078
36. Yang RL, Le GW, Li AL, Zheng JL, Shi YH (2006) Effect of antioxidant capacity on blood lipid metabolism and lipoprotein lipase activity of rats fed a high-fat diet. J Nutri 22(11–12):1185–1191. https://doi.org/10.1016/j.nut.2006.08.018
37. Young D, Fan MZ, Mine Y (2010) Egg Yolk peptides up-regulate glutathione synthesis and antioxidant enzyme activities in a porcine model of intestinal oxidative stress. J Agric Food Chem 58(13):7624–7633. https://doi.org/10.1021/jf1011598
38. Zhang YW, Zhang H, Wang L, Guo XN, Qi XG, Qian HF (2011) Influence of the degree of hydrolysis (DH) on antioxidant properties and radical-scavenging activities of peanut peptides prepared from fermented peanut meal. Eur Food Res Tech 232(6):941–950. https://doi.org/10.1007/s00217-011-1466-0

Figures

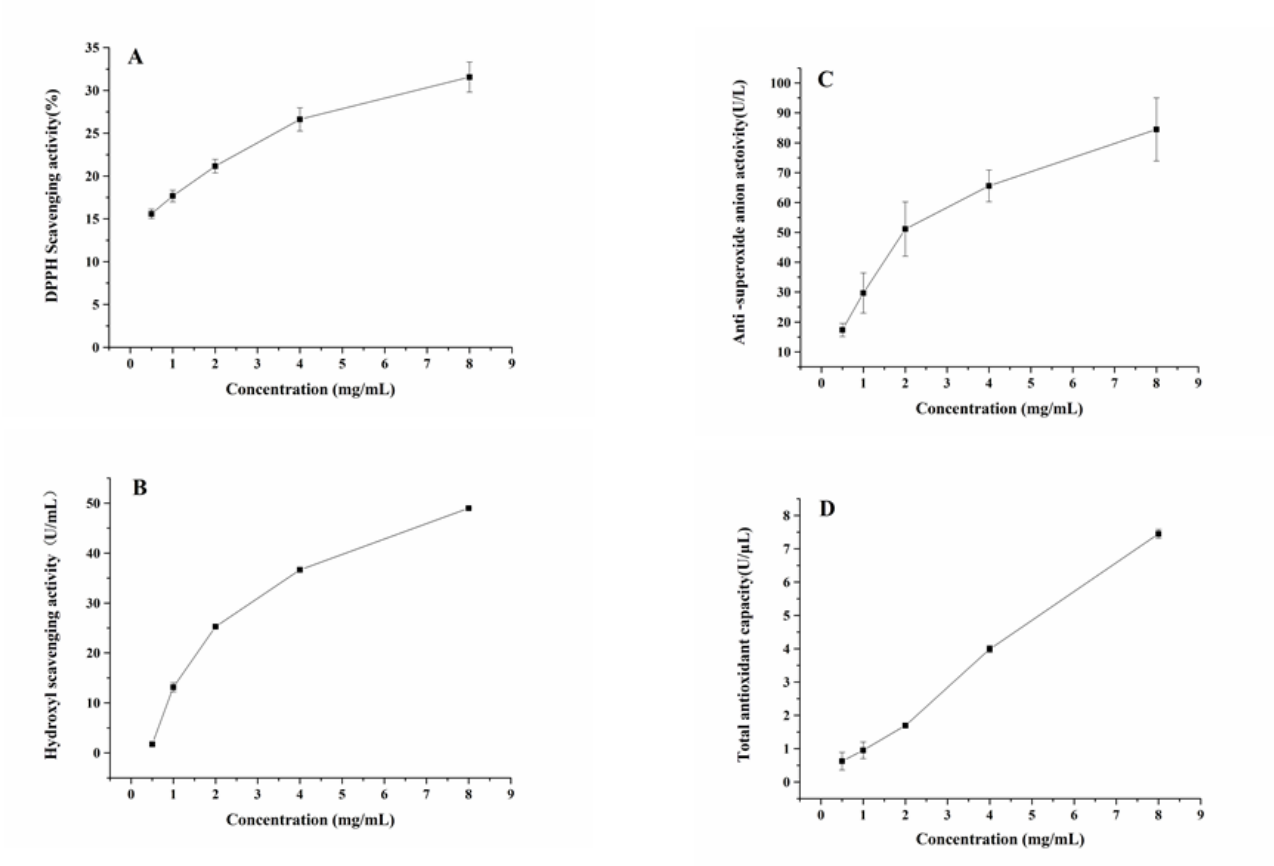

Figure 1

Chemical antioxidant activities of oligopeptides derived from solid fermented cottonseed meal A: DPPH radical scavenging activity; B: Hydroxyl (•OH) radical scavenging activity; C: Superoxide anion ($O_2^{-}\cdot$) radical scavenging activity; D: Total antioxidant capacity (T-AOC)

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryinformation.docx