CARPATHIAN JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

journal homepage: http://chimie-biologie.ubm.ro/carpathian_journal/index.html

# EFFECT OF SOYBEAN POLYPEPTIDE FOOD ON PHYSICAL RECOVERY

**Yu Ding***

*Sports Department of Northwest University of Politics and Law, No.88, Chang'an South Road, Xi'an, Shan'xi, 710063, China; * dingyu5669@163.com*

**Article history:**
Received:
5 January 2016
Accepted in revised form:
19 February 2016

**Keywords:**
*Soybean polypeptide;*
*Basketball player;*
*Recovery of physical fitness;*
*Antioxidant;*

**ABSTRACT**

Objective: By analyzing the effect of soybean polypeptide food on physical recovery of basketball players, we explored the influence of soybean peptide on immune system and its antioxidant effect, thus to provide a theoretical basis for the development of soybean peptide.

Methods: By combining single factor experiment with response surface analysis, we did an optimization research on enzymolysis technology of soybean antioxidant peptides; gel filtration chromatography was adopted to separate bean pulp powder hydrolysate and 3 components of polypeptides were obtained; furthermore, in vitro and in vivo antioxidant experiments were performed.

Results: Soybean peptides could eliminate hydroxyl radicals (produced by Fenton reaction) and superoxide anion radical (produced by pyrogallol system); they could significantly inhibit H2O2-induced hemolysis of red blood cells; in addition, they had an inhibitory effect on lipid peroxidation to some degree.

Conclusion: Soybean peptides have health care effects such as enhancement of immunity, oxidation resistance and delaying senescence.

## 1. Introduction

In recent years, with the development of molecular biology, there have been new discoveries in function and nutrition of polypeptides which not only cover all kinds of amino acids, but also have regulatory effect on the physiological function of organisms (Kohsokabe and Kaneko, 2016; Qiang, et al., 2008). Soybean polypeptides are small peptides obtained by protease hydrolysis of soy proteins, featured with high absorptivity and fast absorption rate (Bamba, et al., 2015). Since soybean polypeptides are a kind of bioactive peptides, it is of great value to develop soybean polypeptides as food and health products, which has an important effect on the recovery of basketball players' physical fitness (Hoffman and John-Arne, 2012).

According to a recent investigation, the overall nutrition status of basketball players in China is not optimistic; consequently, basketball players' physical fitness is in need of targeted nutritional supplement (Altendorfer, et al., 2015). Nutrition intervention is effective in preventing basketball players from the decrease of blood sugar and serum iron during basketball training. In other words, nutritional intervention can effectively prevent fatigue, enhance immunity, increase energy, restore physical fitness and improve endocrine function (Rafiee, et al., 2014; Monnier, et al., 2012). The opinion that soybean polypeptide food has an effective effect on the recovery of basketball players'

physical fitness has been approved by a lot of experts and scholars. In 2014, Nikolaidis et al. found that increase of serum creatine kinase after exercise could be relieved by supplementing soybean polypeptides, which indicated that soybean polypeptides could promote the repair of tissue damage and reduce intracellular creatine kinase leakage effect (Nikolaidis, et al., 2014). In 2012, Cherif et al. analyzed basketball players' physical consumption characteristics and energy supply methods; furthermore, they analyzed the relationship between three major energy supply materials (sugar, fat, protein) and basketball training as well as competition, aiming to explore the importance of rational nutrition and balanced diet to the elimination of fatigue and physical recovery of basketball athletes (Cherif, et al., 2012).

Therefore, by increasing the specific immune function of organisms, soybean polypeptides can enhance body immune system so as to promote physical recovery.

## 2. Materials and methods

### 2.1. Function of soybean peptides

*2.1.1. Good solubility and low viscosity at high concentration (Cwalina and Wagner, 2016)*

After hydrolysis, reticular structure of soybean protein is destroyed, which leads to decrease of expansion and viscosity (Zhou, et al., 2015). When the concentration of soybean peptide in solution is 30%, its viscosity is only equivalent to the viscosity of 10% soybean protein solution. Soybean peptide is characteristic of low viscosity at high concentration, which makes it particularly applicable in high protein fluid food.

*2.1.2. Good stability in resistance to acid and heat*

Refined soybean peptide can remain in good dissolved state around isoelectric point pH 4.5 of soybean protein, which means the solution is clear and transparent, without being influenced by pH change or heating (Koji et al., 2011). With a high nitrogen solubility index (NSI) value (more than 90%) and good instant solubility, soybean peptide is applicable in the production of instant drinks, which provides favorable conditions for the development of acidic soybean beverages and protein-rich acidic food.

*2.1.3. Absorption and retention of moisture* (Liping, et al., 2006)

Moisture absorption and retention ability of soybean peptide is better than that of collagen polypeptide and silk protein peptide, which meets the psychological need of pure natural plant cosmetics and low cost.

*2.1.4. Softening gel* (Ueki, et al., 2016)

Soybean peptide can be applied in the production of ham, sausage, fish cake and other high protein food to soften the food, adjust the hardness and improve the taste of food.

*2.1.5. Promoting fermentation* (Palani, et al., 2016)

Soybean peptide can promote microbial growth and activate metabolism; moreover, it can promote the proliferation of lactic acid bacteria, bifidobacteria, yeast, mold and other fungi as well as secretion of useful metabolites. Therefore, soybean peptide can be applied in the production of fermented food, such as lactic acid beverage, cheese, vinegar, soy sauce and fermented ham (Quezada, et al., 2006).

### 2.2. Nutritional value of soybean peptide

Nutritional value of soybean peptide can be concluded as follows: firstly, advantageous in high absorbency, it can promote fat metabolism; secondly, it can enhance the physical fitness of athletes; thirdly, it can increase bone density and prevent osteoporosis (Black, et al., 2007); fourthly, it can lower blood pressure and blood sugar; fifthly, it can inhibit cholesterol; at last, it has functions such as oxidation resistance, low antigen and immune enhancement.

### 2.3. Experimental materials and instruments

Materials included defatted soy pulp, 2709 alkaline protease, neutral protease, efficient hydrolysis protease, flavor protease and papaya protease. Instruments included digital display thermostatic bath, PHS-25 precision pH meter, 722 spectrophotometer, centrifuge and micro Kjeldahl apparatus (Corral, et al., 2016).

### 2.4. Experimental methods

*2.4.1. Enzymatic preparation*

Table 1 shows the most suitable pH and reaction temperature for hydrolysis of defatted soybean pulp by 5 kinds of proteases. Under the condition of same substrate mass fraction and the same amount of enzyme addition, hydrolysis effect and hydroxyl radical clearance rates of the 5 kinds of proteases were compared.

**Table 1.** The most suitable pH and reaction temperature for protease hydrolysis

| Protease types | Neutral protease | Alkaline protease | Efficient hydrolysis protease | Flavor protease | Papaya protease |
|---|---|---|---|---|---|
| pH | 7 | 11 | 7 | 7 | 7.5 |
| Temperature (°C) | 50 | 55 | 50 | 50 | 50 |

*2.4.2. Enzymolysis technology of defatted soybean pulp*

Defatted soybean pulp was added into the hydrolysis reactor, and some distilled water was added; then, it was stirred mildly so that the soybean pulp was evenly dispersed in the water. It was heated at 90 °C for ten minutes; then, as it cooled off till its temperature was right for hydrolysis reaction, NaOH (1.0 mol/L) was used to adjust the potential of hydrogen (pH). And an accurate amount of protease was weighed and added into the hydrolysis reactor, and it was stirred slowly. During the reaction process, NaOH (1.0 mol/L) was added timely to adjust the pH value. When the scheduled reaction time was up, heating and stirring stopped; then, HCL (1.0 mol/L) was used to adjust the pH to 4.5, and the temperature rose up to 80 °C; it was heated for 10 minutes for passivation of proteases.

### 2.5. Determination method of antioxidant activity

Precisely, we mixed the following reagents in test tubes for sample group: 2.0 ml of PBS (150 mmol/L, pH 7.4), 0.2 ml of 1,10-Phenanthroline (7.5 mmol/L), 0.2 ml of FeSO4 (7.5 mmol/L), 0.4 ml of samples (of different concentrations), 0.8 ml of distilled water and 0.4 ml of H2O2 (1%). Each test tube was placed in 37 °C thermostatic water bath for an hour; absorbance value was measured at 536 nm (wavelength) and denoted as Asample. In the blank group, distilled water was used as sample, and the remaining steps were the same as in sample tubes; the measured absorbance was denoted as A0. In the blank control group, distilled water was used instead of sample and H2O2, and the remaining steps were the same as in the sample tubes; the measured absorbance was denoted as Ablank. Calculation formula for clearance rate is shown as follows:

$$E_{\cdot OH^-}(\%) = (A_{\text{sample}} - A_0)/(A_{\text{blankcontrol}} - A_0) \times 100\% \quad (1)$$

### 2.6. Protein supplement for basketball players

Due to the characteristics of basketball sports in strength, endurance and speed, protein synthesis and catabolism of basketball players have distinct features. On the one hand, synthetic metabolism needs to meet the need of physical exercise; on the other hand, endurance and speed training period determines muscle cell damage and intense protein decomposition which require renewal or reestablishment after training or during intermittence. Accordingly, basketball players' protein supplement should coordinate with the supplement of carbohydrate or saccharides (Rhodes, 2006) in recovery phase of exercise.

During high intensity exercise, timely supplement of high biological activity and high-quality protein and amino acids is not only beneficial to exercise training, but also has an important effect on the recovery of basketball players' physical fitness.

## 3. Results and discussions

### 3.1. Effect contrast of single enzyme enzymolysis

As can be seen from Figure 1, hydrolysis ability of neutral protease and efficient protease was similar; flavor protease was the least capable of hydrolyzing soybean protein, while hydrolysis ability of alkaline protease was the greatest with low cost (Alaskar, et al., 2015).

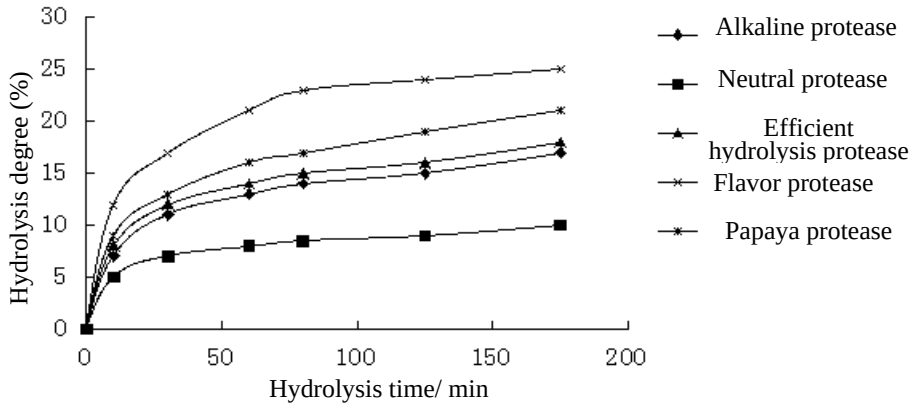


**Figure 1.** Graph of protease hydrolysis process

### 3.2. Clearance effect of different protease hydrolysates on hydroxyl radical ·OH⁻

As can be seen from Table 2, all the five kinds of proteases had antioxidant effect; however, their clearance effects were obviously different. Enzymatic hydrolysate of alkaline protease and papaya protease had better antioxidant effects.

**Table 2.** In vitro antioxidant activity of different protease hydrolysates

| Protease hydrolysates | Clearance rate of hydroxyl radical·OH⁻ (%) |
|---|---|
| Flavor protease | 17.3±0.8 |
| Papaya protease | 59.7±1.9 |
| Efficient hydrolysis protease | 51.4±1.6 |
| Alkaline protease | 66.7±1.0 |
| Neutral protease | 31.3±2.0 |

### 3.3. Effect of enzyme addition on antioxidant activity and hydrolysis degree

As can be seen from Figure 2 and Figure 3, with the increase of enzyme addition, hydrolysis degree improved continuously. Hydrolysis degree of substrate depended on protease concentration. Only when enzyme molecules tended to be saturated and part of them could not contact the substrate, increase of hydrolysis degree would slow down.

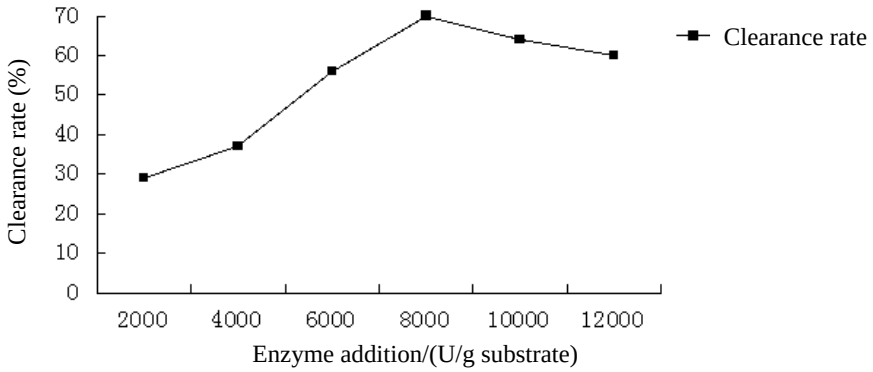


**Figure 2.** Effect of protease addition on clearance rate

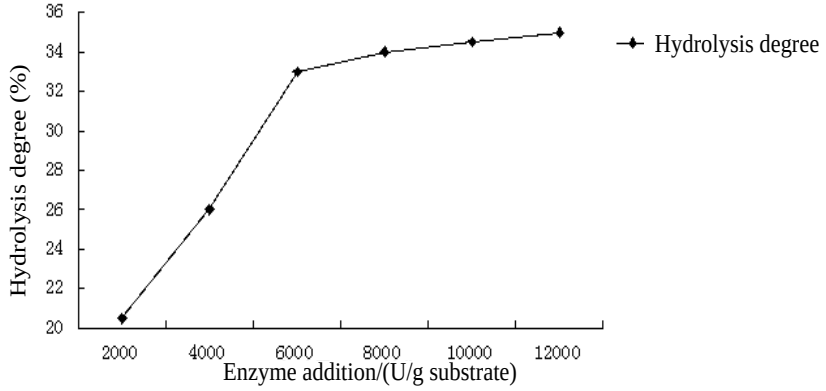


**Figure 3.** Effect of protease concentration on hydrolysis degree

Hydrolysis degree of soybean peptide increased with enzyme addition, thus distribution range of hydrolysate molecular weight decreased. Since hydrolysate only showed high antioxidant activity within certain molecular weight range, antioxidant activity of hydrolysate was relatively lower with insufficient or excessive hydrolysis (James, et al., 2009).

### 3.4. Effect of different substrate concentrations on hydrolysis degree and antioxidant activity

On condition that enzyme amount was 8000 U/g substrate, reaction time was 3 hours, reaction temperature was 55 °C, and the pH value was 11, defatted soybean pulp solutions of different concentrations (2%, 2.5%, 5%, 7.5%, 10%, 12.5%) were prepared for enzymolysis. The results are shown in Figures 4 and 5. According to Figure 4 and Figure 5, it can be concluded that when substrate concentration was low, substrates were not enough to combine with all the enzymes, in which case some dissociative enzymes did not play catalytic roles. Therefore, production speed increased with the concentration of substrates. Namely, under the condition of first-order reaction, more soybean peptides could be generated, thus the clearance rate of removing hydroxyl free radical was higher. However, when all the enzymes combined with substrates, reaction rate reached saturation condition (zero order reaction state) regardless of the increase in substrate concentration.

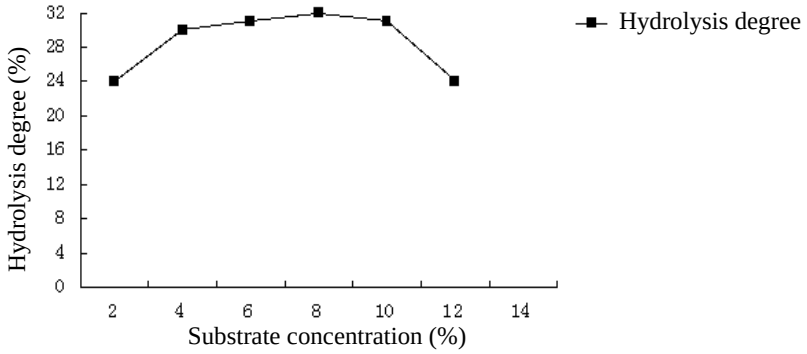


**Figure 4.** Effect of different substrate concentrations on hydrolysis degree

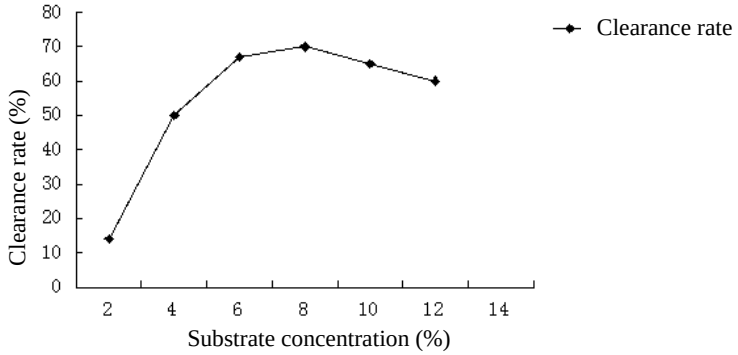


**Figure 5.** Effect of different substrate concentrations on clearance rate

### 3.5. Effect of different pH on hydrolysis degree and antioxidant activity

When the substrate concentration was 7.5%, enzyme amount was 8000 U/g substrate, the reaction temperature was 55 °C, and the reaction time was 3 hours, different pH values were selected for hydrolysis. The results are shown in Figures 6 and 7. It can be seen that when pH value was 11, the obtained soybean peptide had the highest hydrolysis degree; and hydroxyl radical clearance rate of the hydrolysate was the highest (Fréchette, et al., 2016). Therefore, the most suitable pH value was 11 for proteases to take effect.

### 3.6. Optimal process parameters of enzymatic hydrolysis

Response surface analysis method was applied. On the basis of single factor experiment, taking substrate concentration, enzyme addition and pH value as the factors, soybean meal hydrolysate of hydroxyl radical clearance rate as the response values, we designed a three-factor and three-level quadratic regression equation for fitting of the functional relationship between factors and indexes. Experimental factors and levels are shown in Table 3 while experimental results are shown in Table 4.

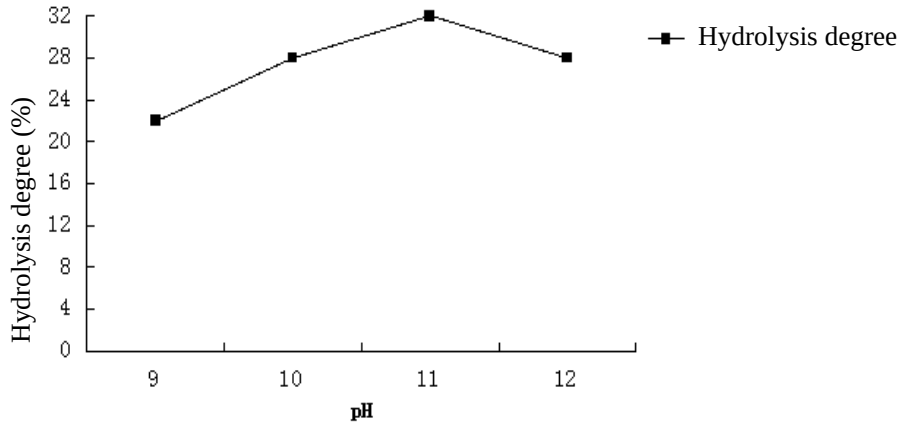


**Figure 6.** Effect of different pH values on hydrolysis degree

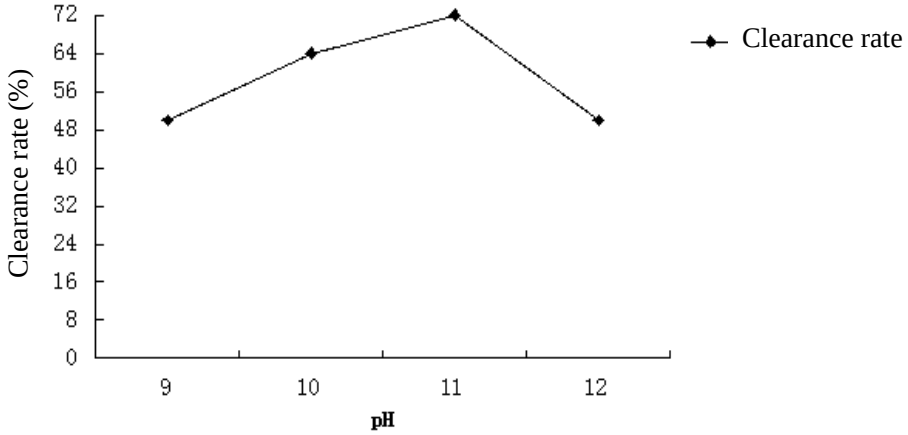


**Figure 7.** Effect of different pH values on clearance rate

Each experiment was carried out for three times; the mean value of three experiment results was selected as the corresponding response value. The experimental data were given quadratic regression analysis with Design-Expert software based on the equation:

$$Y_{DH} = +63.3 + 7.2 * X1 + 8.6 * X2 + 1.3 * X3 + 0.9 * X1 * X2 - 5.2 * X1 * X3 - 4.9 * X2 * X3 - 10.3 * X1^2 - 9.3 * X2^2 - 11.0 * X3^2 \quad (2)$$

**Table 3.** Experimental factors and levels

| **Factors** | **Levels** | | |
|---|---|---|---|
| | **-1** | **0** | **+1** |
| Enzyme addition/(U/g substrate) | 6000 | 8000 | 10000 |
| Substrate concentration (%) | 5 | 7.5 | 10 |
| pH | 10 | 11 | 12 |

**Table 4.** Experimental design and results

| Number | Enzyme addition | Substrate concentration (%) | pH | Clearance rate (%) |
|---|---|---|---|---|
| 1 | -1 | -1 | 0 | 29.6 |
| 2 | 1 | -1 | 0 | 40.6 |
| 3 | -1 | 1 | 0 | 45.2 |
| 4 | 1 | 1 | 0 | 59.6 |
| 5 | -1 | 0 | -1 | 28.1 |
| 6 | 1 | 0 | -1 | 54.5 |
| 7 | -1 | 0 | 1 | 39.9 |
| 8 | 1 | 0 | 1 | 45.6 |
| 9 | 0 | -1 | -1 | 27.8 |
| 10 | 0 | 1 | -1 | 54.6 |
| 11 | 0 | -1 | 1 | 41.2 |
| 12 | 0 | 1 | 1 | 48.5 |
| 13 | 0 | 0 | 0 | 63.2 |
| 14 | 0 | 0 | 0 | 62.9 |
| 15 | 0 | 0 | 0 | 63.8 |

where: X1, X2 and X3 respectively represent enzyme addition, substrate concentration and pH value.

Variance analysis was adopted to verify the significance of models and parameters, as shown in Table 5.

**Table 5.** Variance analysis of response surface

| Source of variation | Quadratic sum | Degree of freedom | Mean square | F value | Prob>F |
|---|---|---|---|---|---|
| Models | 2221 | 9 | 247 | 146 | <0.0001 |
| X1 | 414 | 1 | 414 | 245 | <0.0001 |
| X2 | 589 | 1 | 589 | 349 | <0.0001 |
| X3 | 13 | 1 | 13 | 8 | 0.0386 |
| X1X2 | 3 | 1 | 3 | 2 | 0.2361 |
| X1X3 | 106 | 1 | 106 | 63 | 0.0005 |
| X2X3 | 95 | 1 | 95 | 56 | 0.0007 |
| $X1^2$ | 391 | 1 | 391 | 231 | <0.0001 |
| $X2^2$ | 317 | 1 | 317 | 187 | <0.0001 |
| $X3^2$ | 445 | 1 | 445 | 263 | <0.0001 |
| Lack of fit | 8 | 3 | 3 | 12 | 0.0781 |

As can be seen from Table 5, if Prob > F value of model was less than 0.0001, it means the model was extremely significant. Lack of fit represented the probability of model's predictive value not fitting with the actual value (Gragg and Yang, 2016). Coefficient of variation (CV) reflected the confidence coefficient of models: the lower the CV value, the higher the model confidence coefficient. In the experiment, CV value was 2.77%, indicating that the model equation could reflect the actual experimental value efficiently (Wan-Kai, et al., 2005; Bucher, et al., 2016). Therefore, the model was used to analyze changes of response values.

## 4. Conclusions

Through single factor experiment combined with response surface analysis (Qingxiang, et al.,

2013), this study carried out an optimization research on enzymolysis technology of antioxidative soybean peptides. Soybean pulp was used as substrate, and different proteases were selected for hydrolysis (Virunanon, et al., 2013). We determined the optimal enzymolysis conditions for preparing soybean peptide of high antioxidant activity with alkaline protease: substrate concentration was 8.50%; enzyme addition was 8750 U/g substrate; pH was 11; temperature was 55 °C; reaction time was 3 hours. Under such conditions, we obtained the soybean peptide with a hydroxyl radical clearance rate of 65.37%.

In summary, as a kind of bioactive peptide, soybean peptide plays a role in regulating physiological function of organisms, of which protein is not capable. Therefore, it is of great value to develop soybean peptide into food and health products which can benefit the recovery of basketball players' physical fitness.

## 5. References


Alaskar A.A., Rashad Y.M., Hafez E.E. et al. (2015). Characterization of alkaline protease produced by Streptomyces griseorubens E44G and its possibility for controlling Rhizoctonia root rot disease of corn. *Biotechnology & Biotechnological Equipment*, 29(3), 457-462.

Altendorfer I., König S., Braukmann A. et al. (2015). Quantification of αS1-casein in breast milk using a targeted mass spectrometry-based approach. *Journal of Pharmaceutical & Biomedical Analysis*, 103, 52-58.

Bamba A., Gaillot D.P., Tanghe E. et al. (2015). Assessing Whole-Body Absorption Cross Section For Diffuse Exposure From Reverberation Chamber Measurements. *IEEE Transactions on Electromagnetic Compatibility*, 57(1), 27-34.

Black D. M., Delmas P. D., Richard E. et al. (2007). Once-yearly zoledronic acid for treatment of postmenopausal osteoporosis. *New England Journal of Medicine*, 356(8), 1809-22.

Bucher R., Jeffrey H., Bryden I. G. et al. (2016). Creation of investor confidence: The top-level drivers for reaching maturity in marine energy. *Renewable Energy*, 88, 120-129.

Cwalina C.D., Wagner N.J. (2016). Rheology of non-Brownian particles suspended in concentrated colloidal dispersions at low particle Reynolds number. *Journal of Rheology*, 60(1):47-59.

Cherif M., Said M., Chaatani S., et al, (2012). The Effect of a Combined High-Intensity Plyometric and Speed Training Program on the Running and Jumping Ability of Male Handball Players. *Asian Journal of Sports Medicine*, 3(1), 21-28.

Corral S., Leitner E., Siegmund B. et al. (2016). Determination of sulfur and nitrogen compounds during the processing of dry fermented sausages and their relation to amino acid generation. *Food Chemistry*, 190, 657-664.

Fréchette M., Urquiza J.M., Daigle G. et al. (2016). Clearance rate regulation in mussels: Adding the effect of seston level to a model of internal state-based regulation. *Journal of Experimental Marine Biology & Ecology*, 475,1-10.

Gragg J., Yang J. (2016). Predicting the probability of slip in gait: methodology and distribution study. *Computer Methods in Biomechanics & Biomedical Engineering*, 19(1), 1-8.

Hoffman S.J., John-Arne R. (2012). Assessing implementation mechanisms for an international agreement on research and development for health products. *Bulletin of the World Health Organization*, 90(11), 854-863.

James P., Anne-Marie B., Elisabeth G. et al. (2009). Phosphate (Pi) Starvation Effect on the Cytosolic Pi Concentration and Pi Exchanges across the Tonoplast in Plant Cells: An in Vivo P-31-Nuclear Magnetic Resonance Study Using Methylphosphonate as a Pi Analog. *Plant Physiology*, 151(3), 1646-1657.

Koji N., Fumihiro A., Toshio F. et al. (2011). Highly clear and transparent nanoemulsion

preparation under surfactant-free conditions using tandem acoustic emulsification. *Chemical Communications*, 47(20), 5765-5767.

Kohsokabe, T., Kaneko, K. (2016). Evolution-development congruence in pattern formation dynamics: Bifurcations in gene expression and regulation of networks structures. *Journal of Experimental Zoology Part B Molecular & Developmental Evolution*, 326(1), 61-84.

Liping, S., Yumin, D., Xiaowen, S. et al. (2006). A new approach to chemically modified carboxymethyl chitosan and study of its moisture-absorption and moisture-retention abilities. *Journal of Applied Polymer Science*, 102(2), 1303-1309.

Monnier S., Zakhireh J., Esserman L.J. (2012). The Current Role of Endocrine Therapy in Locally Advanced Breast Cancer to Improve Breast Conservation Rates. *Current Breast Cancer Reports,* 4(2), 139-147.

Nikolaidis P., Calleja-González J., Padulo J. (2014). The effect of age on positional differences in anthropometry, body composition, physique and anaerobic power of elite basketball players. *Sport Sciences for Health*, 10(3), 225-233.

Palani K., Harbaum-Piayda B., Meske D. et al. (2016). Influence of fermentation on glucosinolates and glucobrassicin degradation products in sauerkraut. *Food Chemistry*, 190, 755-762.

Qiang, G., Wujun, X., Yao, X. et al. (2008). Amino acid adsorption on mesoporous materials: influence of types of amino acids, modification of mesoporous materials, and solution conditions. *Journal of Physical Chemistry B*, 112(7), 2261-2267.

Qingxiang, L., Yang, Z., Xin, Z. et al. (2013). Optimization on extraction technology for Eucommia ulmoides by single-factor experiment combined with response surface methodology. *Chinese Journal of Pharmaceutical Analysis, volume* 33, 859-865(7).

Quezada M.Y., Moreno J., Vázquez M.E. et al. (2006). Hermetic storage system preventing the proliferation of Prostephanus truncatus Horn and storage fungi in maize with different moisture contents. *Postharvest Biology & Technology*, 39(3), 321-326.

Rafiee A., Riazi-Rad F., Darabi H. et al. (2014). Ferroportin-encapsulated nanoparticles reduce infection and improve immunity in mice infected with Leishmania major. *International Journal of Pharmaceutics*, 466(1-2), 375-381.

Rhodes, D.C.J. (2006). Importance of carbohydrate in the interaction of Tamm-Horsfall protein with complement 1q and inhibition of classical complement activation. *Immunology & Cell Biology*, 84(84), 357-65.

Ueki N., Wan J., Watabe S. (2016). Deterioration of white croaker (Pennahia argentata) meat thermally-induced gel products caused by proteolytic enzymes in the contaminated intestine and kidney. *Food Chemistry*, 199, 416-422.

Virunanon C., Ouephanit C., Burapatana .V, et al, (2013). Cassava pulp enzymatic hydrolysis process as a preliminary step in bio-alcohols production from waste starchy resources. *Journal of Cleaner Production*, 39(1):273-279.

Wan-Kai, P., Ping-Kei, L., Wei-Kwang, H. et al. (2005). On interval estimation of the coefficient of variation for the three-parameter Weibull, lognormal and gamma distribution: A simulation-based approach. *European Journal of Operational Research*, 164(2), 367-377.

Zhou, A.J., Liu, H.L., Du, Z.Q. (2015). Secondary structure estimation and properties analysis of stretched Asian and Caucasian hair. *Skin Research & Technology*, 21(1), 119-128.