Antioxidant Assessment of Schizochytrium Meal Protein Enzymatic Hydrolysate and Its Potential Application

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\textbf{ABSTRACT}

\textit{Schizochytrium} meal protein (SMP) extracted from \textit{Schizochytrium} meal was hydrolyzed by flavourzyme. Response surface methodology (RSM) was used to optimize the extraction conditions for the protein extraction yield from \textit{Schizochytrium} meal. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (DRSA) was used to evaluate the antioxidant activity of hydrolysates. The orthogonal test was used to investigate the effects of three independent variables, namely protease dosage, hydrolysis time, and pH. The optimum conditions obtained were as follows: protease dosage of 7\%, hydrolysis time of 1.5 h, pH of 6, under which, DRSA at the concentration of 2 mg/mL was 89.38\%. Aspartic and glutamic acid constituted approximately 26.32\% of the total amino acids, and glutamic acid was the most abundant amino acid of \textit{Schizochytrium} meal protein hydrolysate (SMPH) by amino acid composition analysis, which may have contributed to the scavenging activity of SMPH. Moreover, SMPH was made into chewable tablets with suitable formula and high humidity stability. These findings indicate that \textit{Schizochytrium} meal can be reused as a raw material for preparation of antioxidant peptides.

\textbf{KEYWORDS}

\textit{Schizochytrium} meal; antioxidant; hydrolysis; DPPH radical scavenging activity; chewable tablets

\textbf{Introduction}

Oxidative metabolism exists in all aerobic organisms. It provides oxygen (O\textsubscript{2}) for energy production and simultaneously generates some free radicals with unpaired electrons (Baratzadeh et al., 2013; Pihlanto, 2006). These free radicals are unstable and highly reactive in the human body and may damage biological macromolecules, including DNA, membrane lipids, and proteins. The human body has various antioxidant mechanisms that can scavenge part of free radicals. However, the balance of the generation and elimination of free radicals may be damaged during illness (Martim et al., 2003), giving rise to a number of conditions including cancer, cardiovascular disease, and diabetes (Kumar and Nazeer, 2012). This brings about the need for synthetic and natural antioxidants that can relieve oxidative stress (Dong et al., 2010). Because of the risk existing in the application of synthetic antioxidants, investigators are interested in natural antioxidants from food resources.

It has been reported that peptides and protein hydrolysates of plant and animal origins, including the hydrolysates of peanut meal, whey protein, and goose egg white proteins, possess outstanding antioxidant activity (Beatriz et al., 2014; Baratzadeh et al., 2013; Cheison et al., 2007; Lin et al., 2012; Silvestre et al., 2012). Depending on the sequence of amino acids, these bioactive peptides show diverse activities, including antihypertensive, antioxidant, chelating, and immunomodulatory activity.
Furthermore, protein hydrolysates display better solubility and heat stability than intact protein (Xiao et al., 2014). *Schizochytrium* is a type of spherical marine fungi popularly used for the extraction of unsaturated fatty acid. Since the 1990s, people gradually focused on *Schizochytrium*, and its oil accounts for over 70% of the dry weight (Dang et al., 2013; Morita et al., 2006; Ren et al., 2014a; Wu et al., 2005; Xia et al., 2012). Because of its simple fatty composition, *Schizochytrium* has become the main raw material in the industrial production of docosahexaenoic acid (DHA) (Lian et al., 2010; Lippmeier et al., 2009; Qu et al., 2010; Ren et al., 2013; Yan et al., 2013; Yokochi et al., 1998). However, after the manufacture of DHA, *Schizochytrium* meal has become a burden in the production process. So far, there has been little research on the antioxidant activity of protein hydrolysate from *Schizochytrium* meal. Hence, hydrolysate preparation with antioxidant properties from *Schizochytrium* meal could effectively increase its value in the marketplace.

Response surface methodology (RSM), an effective technique for investigation of a multivariable system, has been successfully employed to obtain optimum conditions of biochemical and biotechnological processes (Puri et al., 2002). RSM has also been used to evaluate the interaction of various parameters, and the mathematical model generated can accurately describe the overall process (Yu et al., 2008).

*Schizochytrium* meal, like other microalgae, is rich in protein. The purpose of this study was to prepare antioxidant peptides from extraction of *Schizochytrium* meal by proteolysis. RSM was used to optimize the SMP extraction conditions, and orthogonal experiment was employed for optimizing the hydrolysis conditions. The SMPH obtained from enzymatic hydrolysis was evaluated for antioxidant properties using different *in vitro* assay systems and produced into chewable tablets.

### Material and methods

#### Materials

*Schizochytrium* meal was obtained from Fujian Fisheries Research Institute (Xiamen, China). Flavourzyme was purchased from Guangzhou Hakey Biological Co., Ltd (Guangzhou, China). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sigma-Aldrich Co., Ltd (Pasadena, TX, USA). All other chemicals and reagents were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd (Fuzhou, China).

#### Extraction of schizochytrium meal protein

*Schizochytrium* meal was ground into flour and passed through a 60-mesh sieve. The protein extraction conditions were subsequently optimized: the flour was suspended in sodium hydroxide solution and incubated at designated time and temperature in a shaking water bath. The protein extraction conditions, including liquid/solid, extraction time, and sodium hydroxide concentration, were optimized by experimental design. The extraction was centrifuged at 10,000 rpm for 30 min at 4°C using a XZ-21K refrigerated centrifuge (Xiangzhi Centrifuge Instrument Co., Ltd, Changsha, China) after incubation. Then, the supernatant was collected. The optimal sedimentation pH is presented in Figure S2. The sedimentation rate increased with the decrease of pH at pH 3.5–5.0, while the sedimentation rate remained at 35% at pH below 3.5. Therefore, the pH of the supernatant was adjusted to 3.0 with 6 M HCl. The pH was maintained at 4°C for 30 min to allow protein aggregation, and then the suspension was centrifuged at 10,000 rpm (15 min, 4°C). After separation, the protein precipitate was collected, lyophilized (FD-1C-50, Boyikan Experimental Instrument Co., Ltd, Beijing, China), and stored at −20°C until used (Zhao et al., 2014).
Preparation of SMPH

As shown in Figure S1, the hydrolysate enzyme produced with flavourzyme obtained the highest antioxidant activity and degree of hydrolysis (DH). Therefore, flavourzyme was selected as the enzyme in this study. The SMPH was obtained by hydrolysis using flavourzyme, and the protease dosage, hydrolysis time, substrate concentration, and pH were determined by orthogonal experimental design. The SMP was mixed with distilled water and homogenized before hydrolysis. It was then heated in a water bath to the appropriate temperature before the addition of flavourzyme to achieve the same enzyme activity. Each mixture was incubated with stirring (SKY-110WX, Sukun Industry & Commerce Co., Ltd, Shanghai, China) and then heated in a boiling water bath for 10 min to terminate the hydrolysis reaction. It was cooled down to room temperature and centrifuged at 10,000 rpm for 30 min (4°C). The supernatant was stored at −20°C until use.

Determination of protein

The protein was quantitatively analyzed using the Lowry method.

Degree of hydrolysis

Degree of hydrolysis was defined as the percentage of free amino groups cleaved from proteins and calculated from the ratio of α-amino nitrogen (AN) to total nitrogen (TPN). The degree of hydrolysis of hydrolyzed protein was measured by formaldehyde titration method according to Morais et al. (2013).

Antioxidant activity in vitro

The DPPH radical scavenging activity (DRSA) was measured using the method described by Shimada et al. (1992). The hydroxyl radical scavenging activity (HRSA) was measured using the method described by Yen and Chen (1995). The ABTS radical cation (ABTS+) decolorization assay was determined as previously described (Wang et al., 2012). The reducing power decolorization assay was performed according to Gülcin et al. (2005) with slight modifications. Briefly, 0.2 mL of different concentrations of sample was mixed with 0.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 0.5 mL of 1% (w/v) potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Then, 0.5 mL of 10% trichloroacetic acid was added into the mixture. After centrifugation at 4,000 r/min for 10 min, 0.5 mL of the supernatant were collected and mixed with 0.5 mL of distilled water and 0.1 mL of 0.1% (w/v) ferric chloride. After keeping the mixture at room temperature for 10 min, the absorbance was measure at 700 nm. One milliliter of distilled water was used as blank group.

Experimental design and optimization of protein extraction conditions

On the basis of single factor experiment, response surface methodology was applied to identify the optimum levels of three variables, namely liquid/solid (v/w) (X₁), extraction time (X₂), and sodium hydroxide concentration (X₃). A Box-Behnken design with three factors and three levels, including five replicates at the center point, was used for fitting a second-order response surface. As shown in Table 1, the designated parameters were selected using software Design Expert 8.0 (Stat-Ease Inc., Minneapolis, MN, USA). A total of 17 experimental designs (i.e., 12 factorial points and 5 central points) were carried out (Table 1). In addition, protein extraction yield (Y) was taken as the response.

The second order polynomial regression equation was given, including the effects of the linear, the quadratic, and the interaction of the three variables (X₁, X₂, and X₃) on the response value (Y). The final desirability function equation is shown below:
where $Y$ is the response variable, $\beta_0$ is the model constant, and $\beta_i$, $\beta_{ii}$, and $\beta_{ij}$ are the model coefficients, respectively. $X_i$ and $X_j$ are levels of the independent variables, representing the linear, quadratic and interaction effects of the variables, respectively. The optimum levels for each variable were obtained from solution of the regression equation and analysis of response surface contour plots.

**Experimental design and optimization of enzymatic hydrolysis conditions**

The effect of the hydrolysis parameters (i.e., protease dosage, hydrolysis time, substrate concentration, and pH) were studied under different combinations of hydrolysis conditions as follows.

Effect of protease dosage: The hydrolysis of SMP was carried out at varying protease dosages (1%, 3%, 5%, 7%, 9%) (w/w), while the hydrolysis time, pH, and substrate concentration were fixed at 4 h, 7.0, and 2% (g/100 mL), respectively.

Effect of hydrolysis time: The hydrolysis of SMP was carried out at varying times of hydrolysis (30, 60, 120, 180, 240, 300, 360 min), while the extraction substrate concentration, pH, and protease dosage were fixed at 2%, 7.0, and 7% (w/w), respectively.

Effect of substrate concentration: The hydrolysis of SMP was carried out at varying substrate concentrations (1%, 2%, 3%, 4%, 5%, 6%, 7%), while the hydrolysis time, pH, and protease dosage were fixed at 1 h, 7.0, and 7%, respectively.

Effect of pH: The hydrolysis of SMP was carried out at varying pH (4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0), while the hydrolysis time, substrate concentration, and protease dosage were fixed at 1 h, 2%, and 7%, respectively.

On the basis of single factor experiment, the orthogonal test was applied to identify the optimum conditions of enzymatic hydrolysis. The designated parameters are shown in Table 2, and the antioxidant activity of the hydrolysate, determined by DRSA, was taken as the response.

**Amino acid composition analysis and determination of molecular mass distribution of SMPH**

Samples were submitted to the Fujian Academy of Agricultural Sciences (Fuzhou, China) for amino acid composition analysis by acid hydrolysis. The hydrolysis used 6 M HCl for 20 or 24 h at 110°C in vacuo (Fountoulakis and Lahn, 1998). The tryptophan of SMPH was not detected. The molecular mass distribution of SMPH was determined by high performance liquid chromatography (HPLC)
with a Waters TM 650E Advanced Protein Purification System (Waters Corporation, Milford, MA, USA) assembly running 10 mL sample at 0.5 mL/min. The molecular weight distribution was calculated by comparing the elution curve of SMPH with the standard (Wu, 2008).

**Preparation of chewable tablets**

The proportion of other flavor materials was determined according to the raw material properties and taste. The SMPH and various materials were weighed and mixed uniformly. The materials included apple powder, pineapple powder, soluble starch, β-cyclodextrin, and magnesium stearate. Next, 75% of alcohol was added into the mixture as wetting agent for granulation. The mixture was passed through a 16-mesh sieve and dried at 65°C. Then, the mixture powder was poured into the tablet press and compressed into tablets.

**Statistical analyses**

All of the DH, DRSA, HRSA, ARSA, and RP assays in this study were conducted with three replicates, and the data analysis was performed using SPSS 19.0 (SPSS, Chicago, IL, USA). The significance in differences was determined by Duncan’s multiple range test (p < 0.05). Analysis of variance (ANOVA) was conducted for the analysis of the response values obtained by the RSM model.

**Results and discussion**

**Optimization of protein extraction conditions**

Results of different runs of protein extraction experiments are shown in Table 1. The protein extraction yields were found to be between 48.47 and 73.85% under different tested conditions. In addition, multiple linear regression analysis of the experimental data generated a second order polynomial model for predicting protein extraction yield. ANOVA was conducted to determine significant effects of process variables on response (Table 3). The probability value (p-value) of the regression model was less than 0.0001, suggesting that the model was significant and could be used to monitor the optimization. Among the three independent variables, the largest effect on the protein extraction yield (Y) was linear term of sodium hydroxide concentration (X_3), followed by quadratic term of sodium hydroxide concentration (X_3^2), linear term of extraction time (X_2), and linear term of liquid/solid (X_1), respectively. On the other hand, the interaction terms of X_1X_2 and X_1X_3, as well as the quadratic terms of liquid/solid (X_1^2), were not significant (p > 0.05). Furthermore, effects that were not significant (p > 0.05) were removed from the model without damaging the model hierarchy. The regression equation coefficients of the proposed models and the revised p-value are shown in Table 3.

**Table 2.** Experimental design and response variables for orthogonal experimental design.

| No. | K1j  | K2j  | K3j  | R    |
|-----|------|------|------|------|
| 1   | 220.18 | 221.10 | 223.30 | 220.83 |
| 2   | 226.88 | 222.75 | 226.51 | 221.10 |
| 3   | 223.30 | 226.51 | 258.90 | 223.21 |
| 4   | 220.83 | 226.33 | 223.21 | 220.83 |
| 5   | 221.10 | 222.75 | 258.90 | 221.10 |
| 6   | 226.88 | 222.75 | 268.62 | 226.33 |
| 7   | 223.30 | 226.51 | 258.90 | 223.21 |
| 8   | 220.18 | 221.10 | 223.30 | 220.83 |
| 9   | 226.88 | 222.75 | 268.62 | 226.33 |
| K1j | 220.18 | 221.10 | 142.84 | 220.83 |
| K2j | 226.88 | 222.75 | 268.62 | 226.33 |
| K3j | 223.30 | 226.51 | 258.90 | 223.21 |
| R   | 6.70  | 5.41  | 125.78 | 5.50  |
The lack of fit was used to determine the failure of the model to represent the data in the experimental domain that were not included in the regression. After revising, the insignificant p-value of 0.0854 ($p > 0.05$) is shown in Table 3, indicating that lack of fit was not significant relative to the pure error. $R^2$ (0.9882) was close to 1, and Adj-$R^2$ was 0.9730, which indicates that the model can explain 97.30% of the variation in the data, and the experimental error was very small (Table 3). Adeq. precision measured the signal to noise ratio, and a ratio greater than 4 was desirable (Erbay et al., 2015). This model had a high ratio of 28.818, indicating an adequate signal. Therefore, this model can be used to navigate the design space.

To visualize the interaction effects of two factors on any response, the response surface and contour plots were drawn for the fitted model (Figure 1). To obtain high protein extraction field values, higher sodium hydroxide concentrations should be used for short extraction time and moderate sodium hydroxide concentrations should be chosen for long extraction time. In other words, the sodium hydroxide concentration affected protein extraction field values more than extraction time, and low sodium hydroxide concentrations should be avoided.

### Optimization of enzymatic hydrolysis conditions—effect of protease dosage

The hydrolysates of flavourzyme have the highest antioxidant activity and DH compared to other enzymes (Figure S1). The protease dosage had significant impact on the DRSA and DH, as demonstrated in Figure 2. The DH increased gradually with the increase of protease dosage. In addition, DRSA was also significantly ($p < 0.05$) increased with the increase of protease dosage up to 7%. However, the DRSA was reduced slightly, and the changes were not significant ($p < 0.05$) at 9%. This could be due to a large number of enzymes hydrolyzing the original antioxidant peptides into amino acids or smaller peptides with no or less antioxidant activity. Hence, to obtain peptides with high antioxidant activity, the protease dosage of 7% was selected.

### Optimization of enzymatic hydrolysis conditions—effect of hydrolysis time

The DH was increased with the increase of hydrolysis time (Figure 3), while the DRSA was significantly ($p < 0.05$) increased in the first 60 min. Additional hydrolysis to 300 min did not result in higher values in DRSA. This suggested that a longer hydrolysis time did not improve the DRSA efficiency. Similarly,
Ren et al. (2008) found that hydrolysis released antioxidant peptides, resulting in an increase in DRSA in first 5.0 h, while further treatment (from 6.0 to 12.0 h) caused a decrease of the DRSA. The present results corroborated well with their results. The reason may be because there were no extra hydrophobic residues exposed in the hydrolysates after 2.0 h. In this study, DRSA was used as the main index. Hence, to improve efficiency and save resources, hydrolysis time of 60 min was selected.

Optimization of enzymatic hydrolysis conditions—effect of substrate concentration

Influence of the substrate concentration is illustrated in Figure 4. With the increase of substrate concentration, the total DRSA of hydrolysate was proportionally enhanced. This might be caused by the increasing yield of active peptide. Simultaneously, the DH values of SMP were slowly increased. This could be explained by the increased substrate concentration leading to more substrate used in
hydrolysis. The DH value per unit of SMP increased while the total hydrolysate increased. There was no difference in DRSA in 6–7% of substrate concentration. An amount of 6% of substrate concentration was used in the optimization study.

**Optimization of enzymatic hydrolysis conditions—effect of pH**

The pH had significant impact on the DRSA and DH, as demonstrated in Figure 5. The DRSA significantly ($p < 0.05$) increased with the increase of pH up to 6. However, the DRSA fell sharply, and the changes were also significant ($p < 0.05$) from 6 to 10. This consequence could be related to the activity of the enzymes being sensitive to the pH of the environment. Namely, an overly basic or acidic environment had a negative influence on the hydrolysis, which led to a decrease in the
generation of the antioxidant peptides. Second, the change in DH was similar to the DRSA, and the value of DH reached the maximum at pH 6. Hence, pH 6 was selected.

**Optimization of enzymatic hydrolysis conditions—orthogonal test**

Results of optimization of enzymatic hydrolysis conditions are shown in Table 2. The DRSA were found to be between 44.86% and 89.82% under different tested conditions. In addition, the order and contribution rate of every experiment factor on DRSA was determined by range analysis. As shown in Table 2, the order of influence degree on the antioxidant activity was C > A > B; the influence of pH on antioxidant activity was largest, followed by the protease dosage, and the influence of hydrolysis time was minimum. Therefore, the optimal combination was A2B3C2: protease dosage 7%, hydrolysis time 1.5 h, pH 6.

**DPPH radical-scavenging activity**

DPPH is a relatively stable free radical at room temperature, and the maximum absorbance value of DPPH in ethanol is 517 nm. SMPH showed a dose-dependent effect on DPPH scavenging activity (Figure 6a). SMPH had scavenging ability at a concentration of 1.0 mg/mL of approximately 86.84%. The 50% inhibition concentration (IC50) value of SMPH was 420 μg/mL, based on nonlinear regression. Pan et al. (2011) reported that the IC50 of hydrolysate of rapeseed protein was 710 μg/mL, which was much higher than the value of SMP. However, the IC50 value of SPH hydrolyzed by alcalase and flavourzyme from Schizochytrium sp. was about 0.5 mg/mL (Cai et al., 2017). This result was similar to the IC50 of SMPH.

**Hydroxyl radical-scavenging activity**

Hydroxyl is one of the main free radicals in the human body and reacts easily with other substances. Moreover, hydroxyl is the most destructive of the free radicals that can cause aging and pathological changes in the body. Therefore, hydroxyl radical-scavenging activity is used frequently as the main determinant of antioxidant capacity. As shown in Figure 6b, SMPH had certain hydroxyl radical-scavenging activity, and its clearance rate increased with the increase of concentration. The 50% inhibition concentration (IC50) value of SMPH was calculated based on nonlinear regression to be 3.0 mg/mL. It has
been reported that the free radicals scavenging activity of fraction 1 of chickpea protein hydrolysate at 1.5 mg/mL was 64.74%, which was much lower than the value of SMPH (Li et al., 2008).

**ABTS radical-scavenging activity**

ABTS generates a relatively stable blue-green aqueous solution after oxidation. When some free radical scavengers act on ABTS radical, the color of the solution faded and the stronger the antioxidant, the more obvious the decoloring degree. SMPH has strong ABTS radical-scavenging activity (Figure 6c). When the SMPH concentration increased, ABTS radical clearance gradually increased. The 50% inhibition concentration (IC$_{50}$) value of SMPH was calculated based on non-linear regression to be 35 µg/mL. Noh and Suh (2015) reported that the IC$_{50}$ of hydrolysate of egg white liquid hydrolysate protein was 0.21 mg/mL on ABTS radical, which was much higher than the value of SMPH. This indicated that SMPH had stronger antioxidant activity.

**Reducing power**

There is a direct relationship between the antioxidant activities and reducing power of SMPH. The higher the absorbance at 700 nm, the greater the reducing power. The absorbance value increased
with an increase in the SMPH concentration in a range of 0–4 mg/mL. Reducing power values of SMPH determined at 700 nm were dose-dependent (Figure 6d). When reducing power absorption value reached 0.5, the concentration of SMPH was 3.20 mg/mL. It has been reported that the reducing power absorption value of fraction 1 of chickpea protein hydrolysate at 2.5 mg/mL was 0.175, which was much lower than the value of SMPH (Li et al., 2008). Cai et al. (2017) reported that when the concentration was 1 mg/mL, the absorbance at 700 nm of SPH, SPH-I, and SPH-II was 0.43, 0.54, and 0.33, respectively. This result indicates that the three fractions had stronger ability to inhibit the transformation of Fe$^{3+}$ to Fe$^{2+}$.

**Chemical composition**

The chemical composition of SMPH is shown in Table S1. The protein content, moisture content, and ash content were 57.77%, 3.66%, and 10.66%, respectively. There was no significant difference between the ash, moisture, and lipid content of SMP and the contents of SMPH (data not shown).

**Molecular mass distribution and amino acid composition analysis**

The molecular mass distribution of SMPH was determined by HPLC and was distributed from 71 to 21814 Da (Figure 7a). The molecular mass distribution in <5,000 Da reached 57.2%, which suggests that SMPH mainly consisted of peptides with a molecular weight lower than 5,000 Da.

The amino acid compositions of SMPH are presented in Table 4. The results show that acidic amino acids such as aspartic and glutamic acid constituted approximately 26.32% of the total amino acid, and glutamic acid was the most abundant amino acid in SMPH. It was reported that sulfur-containing acidic and hydrophobic amino acids had strong positive effects on scavenging of DPPH, using chemometric analysis (Udenigwe and Aluko, 2011). Therefore, a large number of acidic amino acids in SMPH may have a greater contribution to the scavenging activity on free radical of SMPH. In addition, leucine was revealed as the third abundant constituent amino acid among the 18 amino acids. As reported by many studies, peptides containing basic amino acids, aromatic ring, or hydrophobic amino acids, such as histidine, lysine, tyrosine, tryptophan, phenylalanine, and leucine, may have good antioxidant activity (Chenison et al., 2007). Hence, leucine may be the major factor to cause high antioxidant activity of SMPH. It was found that the hydrophobic amino acid content reached 45.83%.

**Production process of chewable tablets**

In the process of establishing the recipe for SMPH chewable tablets, the granulating of molding conditions were the primary index. Soluble starch with β-cyclodextrin was chosen as filling agent. Soluble starch is
often used as a filling agent because of its stable nature and affordability. However, the forming results are not desirable. Therefore, soluble starch was combined with β-cyclodextrin so that the structure of the tablets was not too loose. Apple powder and pineapple powder were chosen as the main excipients to retain the unique flavor of SMPH. Finally, magnesium stearate was selected as lubricant.

The chewable tablet formulation was as follows: SMPH 4%, apple powder 10%, pineapple powder 5%, soluble starch 15%, β-cyclodextrin 10%, and magnesium stearate 1%. The chewable tablets are shown in Figure 8a. The appearance of the chewable tablet met the demands of tablets because the organization was not loose and the shape was intact.

### High humidity stability of chewable tablets

In the preparation, transportation, and storage processes, stability is very important. The high humidity stability was tested, and the moisture absorption curve of the chewable tablets in 90% environmental humidity is shown in Figure 8b. At time of 1–6 day, the moisture absorption of the chewable tablets was strong, and hygroscopicity grew fast. At time of 6–10 day, there was a constant balance in the chewable tablets and the environment so that the hygroscopicity was in a fluctuating state. The average of hygroscopicity was about 28%. Hence, the chewable tablets should not have contact with moisture to prevent spoilage.

### Conclusion

The hydrolysate with antioxidant activity from proteins in various types of food and its by-products has been investigated by many researchers. The optimum hydrolysis condition of *Schizochytrium*

![Figure 8. Product of chewable tablets and moisture absorption curve.](image-url)
meal protein hydrolysates with excellent DRSA was studied using the orthogonal test. The hydrolysate of SMP is a rich and safe source that could be used in chewable tablets that possess antioxidant activity. This study provides a new prospect for the use of *Schizochytrium* meal.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

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**References**

Baratzadeh, M. J., Asoodeh, A., and Chamani, J. 2013. Antioxidant peptides obtained from goose egg white proteins by enzymatic hydrolysis. Int. J. Food Sci. Tech. 48: 1603–1609. doi:10.1111/jifs.12130

Beatriz, G. M., Maria, E. J. F., Luis, C. G., and David, B. A. 2014. Comparison of physicochemical properties, antioxidant and metal-chelating activities of protein hydrolysates from *Phaseolus lunatus* and hard-to-cook Phaseolus vulgaris. Int. J. Food Sci. Tech. 49: 1859–1868. doi:10.1111/jifs.12495

Cai, X. X., Yan, A. N., Fu, N. Y., and Wang, S. Y. 2017. In vitro antioxidant activities of enzymatic hydrolysate from *Schizochytrium* sp. and its hepatoprotective effects on acute alcohol-induced liver injury in vivo. Mar. Drugs. 15(4): 115–128. doi:10.3390/md15040115

Cheison, S. C., Wang, Z., and Xu, S. Y. 2007. Use of macroporous adsorption resin for simultaneous desalting and debittering of whey protein hydrolysates. Int. J. Food Sci. Tech. 42: 1228–1239. doi:10.1111/j.1365-2621.2006.01461.x

Chenison, S. C., Wang, Z., and Xu, S. Y. 2007. Preparation of whey protein hydrolysates using a single- and two-stage enzymatic membrane reactor and their immunological and antioxidant properties: characterization by multivariate data analysis. J. Arg. Food Chem. 55: 3896–3904. doi:10.1021/jf062936i

Dang, H., Dinh, T., and Le, T. 2013. Biodiesel production from vietnam heterotrophic marine microalga *Schizochytrium mangrovei* PQ6. J. Biosci. Bioeng. 116: 180–185. doi:10.1016/j.jbiosc.2013.02.002

Dong, X. P., Zhu, B. W., Zhao, H. X., et al. 2010. Preparation and in vitro antioxidant activity of enzymatic hydrolysates from oyster (*Crassostres talienwhannensis*) meat. Int. J. Food Sci. Tech. 45: 978–984. doi:10.1111/j.1365-2621.2010.02223.x

Erbay, Z., Koca, N., Kaymak-Ertekin, F., and Ucuncu, M. 2015. Optimization of spray drying process in cheese powder production. Food Bioprod. Process. 93: 156–165. doi:10.1016/j.fbp.2013.12.008

Fountoulakis, M., and Lahm, H. W. 1998. Hydrolysis and amino acid composition analysis of proteins. J. Chromatogr. A: 826: 109–134.

Ghassem, M., Arihara, K., and Babji, A. S. 2012. Isolation, purification and characterization of angiotensin I-converting enzyme-inhibitory peptides from catfish (*Clarias batrachus*) muscle protein thermolysin hydrolysates. Int. J. Food Sci. Tech. 47: 2444–2451. doi:10.1111/j.1365-2621.2012.03122.x

Gülcin, I., Alici, H. A., and Cesur, M. 2005. Determination of in vitro antioxidant and radical scavenging activities of propofol. Chem. Pharm. Bull. 53: 281–285.

Kumar, N. S. S., and Nazeer, R. A. 2012. In vivo antioxidant activity of peptide purified from viscera protein hydrolysate of horse mackerel (*Magalaspis cordyla*). Int. J. Food Sci. Tech. 47: 1558–1562. doi:10.1111/j.1365-2621.2012.03002.x

Li, Y., Jiang, B., Zhang, T., Mu, W., and Liu, J. 2008. Antioxidant and free radical-scavenging activities of chickpea protein hydrolysate (CPH). Food Chem. 106: 444–450. doi:10.1016/j.foodchem.2007.04.067

Lian, M., Huang, H., and Ren, L. J. 2010. Increase of docosahexaenoic acid production by *Schizochytrium* sp. through mutagenesis and enzyme assay. Appl. Microbiol. Biot. 162: 935–941.

Lin, S. J., Tian, W. N., Li, H., Cao, J. K., and Jiang, W. B. 2012. Improving antioxidant activities of whey protein hydrolysates obtained by thermal preheat treatment of pepsin, trypsin, alcalase and flavourzyme. Int. J. Food Sci. Tech. 47: 2045–2051. doi:10.1111/j.1365-2621.2012.03068.x

Lippmeier, J., Crawford, K., and Owen, C. 2009. Characterization of both polyunsaturated fatty acid biosynthetic pathways in *Schizochytrium* sp. Lipids. 44: 621–630. doi:10.1007/s11745-009-3311-9

Martim, A. C., Sanders, R. A., and Watkins, J. B. 2003. Diabetes, oxidative stress, and antioxidants: A review. J. Biochem. Mol. Toxicicol. 17: 24–38. doi:10.1002/jbt.10058

Morais, H. A., Silvestre, M. P. C., and Silveria, J. N. 2013. Action of a pancreatin and an *Aspergillus oryzae* protease on whey proteins: correlation among the method of analysis of enzymatic hydrolysates. Braz. Arch. Biol. Techn. 56: 985–995. doi:10.1590/S1516-89132013000600014
Morita, E., Kumon, Y., Nakahara, T., Kagiwada, S., and Noguchi, T. T. 2006. Docosahexaenoic acid production and lipid-body formation in *Schizochytrium limacinum* SR21. Mar. Biotechnol. 8: 319–327. doi:10.1007/s10126-005-5060-y

Noh, D. O., and Suh, H. J. 2015. Preparation of egg white liquid hydrolysate (ELH) and its radical-scavenging activity. Prev. Nutr. Food Sci. 20: 183–189. doi:10.3746/pnf.2015.20.3.183

Pan, M., Jiang, T. S., and Pan, J. L. 2011. Antioxidant activities of rapeseed protein hydrolysates. Food Bioproc. Tech. 4: 1144–1152. doi:10.1007/s11947-009-0206-y

Pihlanto, A. 2006. Antioxidative peptides derived from milk proteins. Int. Dairy J. 16: 1306–1314. doi:10.1016/j.idairy.2006.06.005

Puri, S., Beg, Q. K., and Gupta, R. 2002. Optimization of alkaline protease production from *Bacillus* sp. by response surface methodology. Curr. Microbiol. 44: 286–290.

Qu, L., Ji, J., and Ren, L. J. 2010. Enhancement of docosahexaenoic acid production by *Schizochytrium* sp using a two-stage oxygen supply control strategy based on oxygen transfer coefficient. Lett. Appl. Microbiol. 52: 22–27.

Ren, L. J., Zhao, M., Shi, J., et al. 2008. Optimization of antioxidant peptide production from grass carp sarcoplasmic protein using response surface methodology. LWT-Food Sci. Technol. 41: 1624–1632. doi:10.1016/j.lwt.2007.11.005

Ren, L. J., Li, J., and Hu, Y. W. 2013. Utilization of cane molasses for docosahexaenoic acid production by *Schizochytrium* sp. CCTCC M209059. Korean J. Chem. Eng. 30: 787–789. doi:10.1007/s11814-013-0020-0

Ren, L. J., Sun, L. N., and Zhuang, X. Y. 2004. Regulation of docosahexaenoic acid production by *Schizochytrium* sp.: effect of nitrogen addition. Bioproc. Biosyst. Eng. 37: 865–872. doi:10.1007/s00449-013-1057-5

Ren, X. F., Pan, D. D., Wu, Z., et al. 2014b. Limited hydrolysis of β-casein by cell wall protease and its effect on hydrolysates’s conformational and structural properties. Int. J. Food Sci. Tech.. 50: 1–6.

Shimada, K., Fujikawa, K., and Yahara, K. 1992. Antioxidative properties of xanthan on the antioxidantant of soybean oil in cyclodextrin emulsion. J. Agr. Food Chem. 40: 945–948. doi:10.1021/jf00018a005

Silvestre, M. P. C., Motaís, H. A., Silva, M. R., Souza, M. W. S., and Silva, V. D. M. 2012. Preparation and analysis of hydrolysates from whey protein concentrate using the proteases from *Bacillus licheniformis* and *Aspergillus oryzae*. Int. J. Food Sci. Tech. 47: 1532–1539. doi:10.1111/j.1365-2612.2012.03003.x

Udenigwe, C. C., and Aluko, R. E. 2011. Chemometric analysis of the amino acid requirements of antioxidant food protein hydrolysates. Int. J. Mol. Sci. 12: 3148–3161. doi:10.3390/ijms12053148

Wang, B., Li, Z. R., and Chi, C. F. 2005. Study on the preparation of sericin peptides, their biological activities and structure. Ph.D. Thesis, Jiangnan University.

Wu, J. H. 2008. Effect of culture conditionson docosahexaenoic acid production by *Schizochytrium* sp. S31. Process Biochem. 40: 3103–3108. doi:10.1016/j.procbio.2005.03.007

Xia, X. L., Yang, H. L., and Zhang, L. 2012. Effect of C/N ratio on DHA production by *Schizochytrium* sp. JN-3 and its pilot-scale fermentation. Agric. Biotechnol. 1: 51–53.

Xiao, C. Q., Zheng, L., Su, G. W., and Zhao, M. M. 2014. Effect of solution pH and activated carbon dosage on the decolourization ability, nitrogen components and antioxidant activity of peanut meal hydrolysate. Int. J. Food Sci. Tech. 49: 2571–2577. doi:10.1111/ijfs.12587

Yan, J. F., Cheng, R. B., and Lin, X. Z. 2013. Overexpression of acetyl-CoA synthetase increased the biomass and fatty acid proportion in microalgae *Schizochytrium*. Appl. Microbiol. Biot. 97: 1933–1939. doi:10.1007/s00253-012-4481-6

Yen, G. C., and Chen, H. Y. 1995. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J. Agric. Food Chem. 43: 27–32. doi:10.1021/jf00049a007

Yokochi, T., Honda, D., and Nakahara, T. 1998. Optimization of docosahexaenoic acid production by *Schizochytrium limacinum* SR21. Appl. Microbiol. Biot. 49: 72–76. doi:10.1007/s002530051539

Yu, B., Lu, Z. X., Bie, X. M., Lu, F. X., and Huang, X. Q. 2008. Optimisation of the medium composition of protease and soybean peptides by *Bacillus subtilis* SHZ using response surface methodology. Int. J. Food Sci. Tech. 43: 1143–1151. doi:10.1111/j.1365-2621.2007.01580.x

Zhao, L. N., Huang, S. L., Cai, X. X., and Hong, J. 2014. A specific peptide with calcium chelating capacity isolated from whey protein hydrolysates. J. Funct. Foods. 10: 46–53. doi:10.1016/j.jff.2014.05.013