Amino Acid Composition and Functional Properties of Different Molecular Weight Segments of Macadamia Peptides

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Abstract. In this study, macadamia peptides were produced by ultrasonic-assisted alkaline protease, and five components were separated by ultrafiltration centrifuge with different molecular weight (1kDa, 3kDa, 10kDa, 30kDa). The amino acid composition, solubility, water absorption capacity and emulsifying properties of five components were compared. Results showed that all macadamia peptide components were rich in amino acid species, the contents of essential and hydrophobic amino acids ranged from 189.59 to 281.10mg/g and 204.26 to 258.78mg/g, respectively. Meanwhile, the small molecular weight components (MP-1) showed the best solubility and water absorption capacity, large molecular weight components (MP-5) had the best emulsifying properties. This result indicated that the distribution of molecular weight had significant effects on the amino acid composition and functional properties of macadamia peptide components. This study provides a theoretical basis for the further development of macadamia peptide products.

Keywords: Macadamia peptides, Ultrafiltration, Amino acid composition, Functional properties.

1. Introduction

The macadamia (Macadamia integrifolia) is an evergreen native tree indigenous to the coastal rainforests of Australia [1]. Macadamia processing by-product is rich in protein. It is generally discarded or used as animal feed or fertilizer, which pollutes the environment and increases costs. It is well known that proteins are poorly absorbed in the human body due to their high molecular weight. After degradation into peptides with small molecular weight, the absorption rate and some new functions will be improved. It has been reported that the physicochemical and functional properties of peptide components are related to their molecular weight. Deng et al. (2006) studied the functional properties of four molecular weight segments of soybean peptides obtained by ultrafiltration. The results showed that the functional properties of soybean peptides with different molecular weight segments were different in solubility, foaming and emulsification [2]. In recent years, there have been many reports on the functional properties of different molecular weight components of common peptides [3-4]. However, there are few reports on the functional properties of different molecular weight components of macadamia peptides.
In this study, five components of macadamia peptides obtained by ultrafiltration were used as raw materials to evaluate their amino acid composition and functional properties, including solubility, water absorption capacity and emulsifying properties, in order to provide guidance for the development and application of macadamia peptide products.

2. Experimental

2.1. Materials, reagents and instruments
Macadamia protein is provided by the National Engineering Research and Development Center for Important Tropical Crops. Alkaline protease (Activity≥4000U/mg) was purchased from Aladdin (Shanghai) Chemical Reagent Co., Ltd. All other reagents were of analytical reagent grade. The instrument includes high speed refrigerated centrifuge (ST40, Thermo Fisher Technology Co., Ltd), high-speed dispersion machine (T25, Shanghai jiangyi Instrument Co., Ltd), pH-meter (S210 Seven Compact, Mettler-Toledo), et al.

2.2. Methods

2.2.1. Pretreatment of macadamia peptides. The macadamia peptides was based on previously reported methods [5]. Five kinds of macadamia peptide components (MP-1, MP-2, MP-3, MP-4 and MP-5) with different molecular weights were obtained by ultrafiltration of the enzymatic hydrolysate with the centrifuge tubes with the intercepted molecular weights of 1kDa, 3kDa, 10kDa and 30kDa, respectively.

2.2.2. Analysis of functional properties. Functional properties, including solubility, water absorption capacity, and emulsifying properties, were determined according to the method reported by Zhu et al (2014) [3].

2.2.3. Amino acid composition analysis. The sample was hydrolyzed with 6.0mol/L HCl at 110°C for 24h, and then determined by automatic amino acid analyzer.

2.3. Statistical analysis
All the experiments were repeated in triplicate, and the date were expressed as means ± standard deviations. Statistical analysis was performed on Origin 2020 and SPSS 25.0.

3. Results and discussions

3.1. Amino acid composition
The amino acid composition of different macadamia peptide components were shown in Table 1. All the samples analyzed contained seven essential amino acids (except for tryptophan) ranging from 189.59 to 281.10mg/g. MP-3 had the highest value, and the content of essential amino acids increased first and then decreased with the decrease of molecular weight. The contents of total and hydrophobic amino acids were also showed the same trend. It is generally believed that hydrophobic amino acids were related to the antioxidant activity of peptides. Therefore, macadamia peptide components had high nutritional value and can be used as a potential resource for antioxidant development.
Table 1. Amino acid composition of different macadamia peptide components.

| Species         | MP-1     | MP-2     | MP-3     | MP-4     | MP-5     |
|-----------------|----------|----------|----------|----------|----------|
| Aspartic acid (Asp) | 72.37±0.11 | 77.63±0.00 | 83.68±0.05 | 84.19±0.11 | 86.78±0.05 |
| Threonine (Thr) | 21.01±0.02 | 24.99±0.05 | 27.20±0.03 | 24.53±0.01 | 24.02±0.03 |
| Serine (Ser)    | 36.19±0.10 | 43.40±0.06 | 46.02±0.16 | 44.44±0.20 | 40.06±0.11 |
| Glutamic acid (Glu) | 150.58±0.18 | 157.87±0.24 | 167.42±0.13 | 155.04±0.16 | 152.27±0.09 |
| Glycine (Gly)   | 35.60±0.10 | 43.40±0.06 | 46.02±0.16 | 44.44±0.20 | 40.06±0.11 |
| Alanine (Ala)   | 26.85±0.00 | 16.38±0.01 | 26.51±0.06 | 17.93±0.01 | 23.34±0.01 |
| Cystine (Cys)   | 5.25±0.07  | 35.53±0.03 | 38.37±0.01 | 2.62±0.00  | 1.37±0.02  |
| Valine (Val)    | 33.27±0.06 | 10.53±0.11 | 13.94±0.02 | 35.73±0.05 | 32.66±0.07 |
| Methionine(Met) | 9.34±0.01  | 28.94±0.04 | 31.38±0.02 | 10.62±0.01 | 5.38±0.01  |
| Isoleucine (Ile) | 25.10±0.00 | 53.26±0.03 | 55.79±0.11 | 28.52±0.01 | 25.40±0.06 |
| Leucine (Leu)   | 46.69±0.13 | 42.75±0.00 | 47.44±0.16 | 47.09±0.15 | 50.07±0.10 |
| Tyrosine (Tyr)  | 40.86±0.10 | 24.35±0.08 | 32.79±0.11 | 44.41±0.18 | 44.07±0.09 |
| Phenylalanine (Phe) | 37.35±0.08 | 38.15±0.02 | 42.56±0.03 | 27.19±0.02 | 24.71±0.05 |
| Histidine (His) | 62.45±0.01 | 33.55±0.03 | 31.39±0.02 | 38.45±0.03 | 38.73±0.01 |
| Lysine (Lys)    | 30.35±0.08 | 103.93±0.11 | 94.17±0.26 | 29.88±0.01 | 32.73±0.05 |
| Arginine (Arg)  | 92.22±0.03 | 77.63±0.09 | 83.68±0.33 | 112.76±0.19 | 106.86±0.08 |
| Proline (Pro)   | 32.10±0.05 | 28.94±0.04 | 41.16±0.11 | 43.79±0.06 | 42.70±0.03 |
| Total           | 757.58±0.69 | 835.31±0.12 | 900.46±0.88 | 781.01±1.07 | 765.87±0.87 |
| EAA*            | 193.77±0.33 | 273.61±0.26 | 281.10±0.22 | 192.94±0.19 | 189.59±0.22 |
| HAA*            | 210.70±0.19 | 218.95±0.20 | 258.78±0.31 | 210.87±0.33 | 204.26±0.22 |

*Essential amino acid (EAA): Lys, Phe, Met, Thr, Ile, Leu, Val. Hydrophobic amino acid (HAA): Phe, Val, Leu, Ile, Ala, Pro, Met.

3.2. Solubility
The solubility of different macadamia peptide components were shown in Figure 1. The results showed that MP-1, MP-2 and MP-3 had higher solubility (>90%) at different pH values. With the decrease of molecular weight, the solubility of macadamia peptide gradually increased, and MP-1 had the highest value. These results were similar to those reported by Zhu et al. (2014) for the solubility of Sea Bass Collagen peptides with different molecular weights [3]. This may be due to the increase of polar residues with increasing degree of hydrolysis, and these residues form hydrogen bonds with water, thus increasing their solubility [6]. And it is widely known that proteins and their hydrolysates generally exhibit the lowest solubility at their isoelectric points. Thus, it can be seen from Figure 1 that the isoelectric point of MP-4 and MP-5 were around pH=6.
3.3. Water absorption capacity
The water absorption capacity of different macadamia peptide components were shown in Figure 2. It can be seen that the water absorption capacity of macadamia peptide gradually increased with the decrease of molecular weight. At 0~2h, the water absorption capacity of all samples were the most significant, and almost saturated after 6h. Compared with the other components, MP-1 had the highest value (127.13%), but was weaker than glycerol. This may be due to the exposure of more hydrophilic groups by enzymatic hydrolysis. The tendency was consistent with the results reported by Zhu et al. (2014) [3].

![Figure 2. Water absorption capacity of different macadamia peptide components.](image)

3.4. Emulsifying properties
Emulsification activity index (EAI) is a measure of the ability of proteins to contribute to emulsion formation and stabilization. As shown in Figure 3, the EAI of macadamia peptide components decreased with the decrease of molecular weight. Compared with the other components, MP-5 had the highest value (14.88cm²/mg). This may be due to the overexposure of hydrophobic groups in the macadamia peptide of small molecular weight, which broke the hydrophilic-hydrophobic balance and could not reduce the interfacial tension, thus reducing the emulsification.

![Figure 3. Emulsifying properties of different macadamia peptide components.](image)

4. Conclusion
In this study, the amino acid composition and functional properties of five macadamia peptide components were studied. The result showed that all macadamia peptide components were rich in amino acid species. And the small molecular weight components (MP-1) showed the best solubility and water absorption capacity, large molecular weight components (MP-5) had the best emulsifying properties. This result indicated that the distribution of molecular weight had significant effects on the
amino acid composition and functional properties of macadamia peptide components. This study provides a theoretical basis for the further development of macadamia peptide products.

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