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Antioxidant peptides from corn gluten meal: Orthogonal design evaluation

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ABSTRACT

Protamex catalyzed corn gluten meal (CGM) hydrolysis peptides (CHP) were prepared. Orthogonal design L_{16} (4^5) was used to optimize processing variables of CGM concentration, CGM heat pretreatment (121 °C) time, and enzymolysis pH, temperature, and time. Degree of hydrolysis (DH), undigested residue ratio, molecular weight (MW) distribution and DPPH radical inhibition were selected as analysis indicators. Optimum variables were CGM concentration of 18%, heat pretreatment time of 40 min, and enzymolysis pH, temperature and time of 7.5, 55 °C and 24 h, respectively. Verification test showed that CHP IC_{50} for scavenging hydroxyl radical was the best and then followed by reducing power. Oligopeptides improved after hydrolysis at the expense of di- and tripeptides, suggesting formation of soluble aggregates from low MW peptides. The increase in the DH, oligopeptides, Alanyl-Tyrosine, and antioxidant free amino acids coincided with the improvement in the antioxidant activity of CHP.

1. Introduction

In recent years, natural antioxidant peptides have drawn the attention of researchers due to their strong activity and their low public health concerns compared to synthetic antioxidants (Pan, Jiang, & Pan, 2011; Suh, Whang, Kim, Bae, & Noh, 2003). Natural antioxidant peptides are characterized by relatively low molecular weights and they are easily digested and absorbed by the body (Saiga, Tanabe, & Nishimura, 2003). They also have anti-aging effect by inhibiting the hydroxyl radical, and superoxide anion radical activities, inhibiting lipid peroxidation and ability to chelate metal ions (He et al., 2012). Reactive oxygen species, including superoxide anion radicals, hydroxyl radicals and so on, can lead to a variety of biochemical and physiological lesions, and often result in metabolic impairment and cell death (He, Girgih, Malomo, Ju, & Aluko, 2013). Hence, to provide protection against

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various diseases, inhibiting the formation of free radicals occurring in the living body is very important. In addition, free radical-induced lipid oxidation is a major concern in the food industry, because the oxidation of fats and oils during processing and storage of food products has a negative effect on the quality and nutritive value of lipids (Rajapakse, Mendis, Jung, Je, & Kim, 2005). Corn gluten meal (CGM), a by-product of corn wet milling, contains about 60% (w/w) protein. However, its low water solubility and severely imbalanced amino acid composition makes it difficult to be used as a food additive (Zhuang, Tang, & Yuan, 2013). The amino acids profile of the CGM protein contains considerable amounts of hydrophobic amino acids such as leucine, alanine and phenylalanine. Therefore it is thought to be a good source of antioxidant peptides. Corn gluten meal peptides (CHP), is a food derived bioactive peptide obtained by enzymolysis technology (Suh et al., 2003; Zheng et al., 2015). The food derived bioactive peptides, in general, are typically small in size, highly active, safe for consumption and easily absorbable (Escudero, Aristoy, Nishimura, Arihara, & Toldrá, 2012). Compared with the CGM, not only the CHP is taken up easily by the human body where it performs special physiological functions, but also has high water-solubility and high utilization (Zhou, Sun, & Canning, 2012). Several studies have reported that the antioxidative activity of protein hydrolysates and isolated peptides prepared from natural sources, in some cases, is similar or higher than that of commonly used synthetic antioxidants, such as butylated hydroxytoluene (BHA), butylated hydroxylanisole (BHT) and propylgallate (Zhou, Yu, Zhang, He, & Ma, 2012; Zhou et al., 2013). The optimal value of different factors can be easily determined by the single factor experiment, but this method cannot distinguish whether the difference between the data fluctuation of each factor level is caused by experimental conditions or by experimental errors (Meng, Zhang, Zhao, Qiu, & Yang, 2013). Comprehensive balance method is used to assess the impact of each factor separately for each indicator. Then the method is comprehensively analyzed and compares the data based on the influential degree of the indicators on the factors (Wang, Chen, Wang, & Xing, 2014). According to the above method, the experiment can determine the best level, from where the optimum test program can be obtained. In this paper, the effects of five processing parameters (CGM concentration, CGM heat pretreatment time, enzymolysis pH, enzymolysis temperature, enzymolysis time) on the antioxidant activity as well as other characteristics of peptides from CGM were investigated based on L_{16} (4^5) orthogonal design evaluation. The orthogonal design had been applied successfully in optimization processes in order to extract bioactive components from various materials, such as phenolics (Wu et al., 2012) and protein hydrolysate peptides (Wang, Wang, Dang, Zheng, & Zhang, 2013). Due to the random nature of such experimental process, it is very essential to optimize the process parameters in determining the antioxidant activity of CHP. The purpose of optimizing this process was to gain more target product. Conventional method deals with single response optimization only and it may give different set of optimal combinations. In this approach, the multiple craft can be converted into single craft. Then it is easy to obtain the optimal set of process parameters (Muthuramalingam & Mohan, 2014). And based on the optimum test program, some characteristic indictors were determined, which were degree of hydrolysis (DH), content of Ala-Tyr, molecular weight (MW) distribution, content of CHP, and crude yield of CHP. This paper, however, can provide a theoretical and experimental basis for the development of highly efficient CGM bioactive peptides, regarding their commercial yield as well as their potential applications. Therefore, the primary objective of this study was to evaluate the antioxidant activities of CHP using different *in vitro* methods. The secondary objective was to evaluate other characteristics of CHP.

2. Materials and methods

2.1. Materials and chemicals

CGM, with 58.6–60% protein, was purchased from Yishui Earth Corn Development Co., Ltd. (Shandong, China). Protamex protease was purchased from Nanning Doing Higher Bio-tech Co., Ltd. China and had an activity of 3.0×10^5 U/g. L-Alanayl-L-Tyrosine (Ala-Tyr, 99%, 252.27 Da) was purchased from Aladdin Industrial Corporation (Shanghai, China). Cytochrome C (99%, 12,500 Da), aprotinin (99%, 6500 Da) and L-tryptophan (99%, 204 Da) all were purchased from Sigma Chemical Co., St. Louis, MO, USA. Amino acid standard solution was purchased from SYKAM Corporation (Germany). All other chemicals used were of analytical grade.

2.2. Preparation of CGM hydrolysis peptides

Dispersions of CGM in deionized water at different concentrations (6–24%, w/v) were prepared. The dispersions were pre-heated (121 °C) for different times between 20 to 50 min. After pre-heating, the mixtures were cooled to 55 °C, and then adjusted the pH 7.0–8.5. One percent (w/w) protamex was added to the mixture and hydrolysis was done, aided by constant agitation, at different temperatures ranged between 45 and 60 °C. The pH of the mixture was maintained constant during the hydrolysis by frequent addition of 1.0 mol/L NaOH. At the end of the reaction, the enzyme was deactivated by heating the mixture for 10 min at 85 °C. The mixture was then centrifuged at $5000 \times g$ for 15 min and the supernatant was lyophilized (CHP) and kept for subsequent analysis.

2.3. Orthogonal design

In this study, the experiments were based on an orthogonal design L_{16} (4^5) where the following five factors were analyzed: CGM concentration (*A*), heat pretreatment time (*B*), enzymolysis pH (*C*), enzymolysis temperature (*D*) and enzymolysis time (*E*). Four levels of each factor were chosen for the experiment (Table 1).

2.4. Characterization of the hydrolysate peptides

2.4.1. Determination of degree of hydrolysis

The degree of hydrolysis (DH) was calculated as a proportion (%) of free amino groups ($-NH_2$) released after hydrolysis with respect to total amino groups in each sample. The DH was measured by reaction of free amino groups with 2,4,6-trinitrobenzenesulfonic acid (Orsini Delgado, Tironi, & Añón, 2011; Zhou et al., 2013). The DH was calculated as follows:

$$\mathrm{DH}\ (\%) = (L_t - L_0)/(L_{max} - L_0) \times 100$$

where L_t is the amount of α-NH_2 released at time t, L_0 is the amount of α-NH_2 in original homogenate and L_{max} is the total α-NH_2 in original homogenate obtained after acid hydrolysis (6 N HCl at 110 °C for 24 h).

2.4.2. Determination of content of L-Alanyl-L-Tyrosine

The content of Ala-Tyr of CHP was determined by high performance liquid chromatography; using C_{18} (carbamate column 4.6×250 mm) reversed phase packing as the stationary phase. Samples were separated in accordance with the difference in sizes of the sub-molecular polarity. Analysis was performed by a gradient elution method [Mobile phase A: water:trifluoroacetic acid, 100:0.1 (volume ratio) and mobile phase B: acetonitrile:water:trifluoroacetic acid, 80:20:0.1 (volume ratio)]. The gradient elution conditions were 0–10 min, 0–5% B, 10–25 min, 5–9% B; 25–