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硕 士 学 位 论 文

文蛤多肽保健品的研究与开发

Study and exploitation of *Meretrix meretrix Linnaeus* polypeptide health product

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摘　　要

文蛤(*Meretrix meretrix Linnaeus*) 具有很高的营养价值和药用价值，其食用的营养价值之高和用途之广居各种贝类之首被誉为”天下第一鲜”。本文通过六种蛋白酶酶解文蛤蛋白的产物比较、成分分析和生理功能研究，以期获得具有质量稳定、颜色风味俱佳、多肽分子量小含量高、生理功能丰富的文蛤多肽保健品。

本文以酶解后产物中的游离氨基酸含量的多寡即降解度为标准，研究了菠萝蛋白酶、木瓜蛋白酶、复合胰蛋白酶、胰蛋白酶、中性蛋白酶和碱性蛋白酶六种蛋白酶各自酶解文蛤的 pH、温度、酶量和时间等条件，发现它们酶解文蛤的最适 pH 依次为 6.5，7.0，8.0，8.0，7.0，8.0；最适酶解温度除了菠萝蛋白酶和胰蛋白酶为 45 ℃外均为 40 ℃；最适酶用量（酶重比上文蛤肉重）依次为 0.7 %，0.7 %，0.5 %，0.3 %，1.1 %，0.5 %；最适酶解时间除了胰蛋白酶和中性蛋白酶为 6 h 外均为 5 h。比较了在各自最佳条件下，这六种蛋白酶酶解文蛤的效果，发现复合胰蛋白酶在 pH 8.0，40 ℃，酶量 0.5 %，酶解 5 h 后，其酶解作用的效果在六种蛋白酶中最好，其酶解产物中游离氨基酸的浓度达到 59.71 μmol/mL，因此在后面的实验中本文选用复合胰蛋白酶来酶解文蛤。复合胰蛋白酶的酶解产物通过喷雾干燥工艺，获得白色、干燥粉末状产物即文蛤多肽保健品。

分析该保健品的主要成分、金属元素、氨基酸组成和多肽分子量的范围，结果显示该保健品中蛋白质和氨基酸的含量丰富分别达到 30.3 %和 9.6 %，总糖含量也很高为 8.3 %；文蛤酶解产物中对人体有益的金属元素含量丰富，含量从高到低依次为 Na、K、Ca、Mg、Fe、Cu，对人体有害的重金属含量都大大低于国家食品检验标准的安全剂量；氨基酸种类齐全，其中酸性氨基酸的含量最高可达 10.37 %，人体必需氨基酸（含胱氨酸、酪氨酸）丰富高达 14.09 %，占氨基酸总量的 36.64 %，是质量较好的蛋白源；多肽分子量均小于 5200 Da，分子量越小多肽的含量越高，分子量小于 1000 Da 的多肽占总多肽含量的 69.03 %。，更容易为人体所吸收，同时也进一步验证了极佳的蛋白酶酶解效果。

研究本品对血管紧张素转化酶的抑制作用、抗氧化作用、对肝癌细胞的抑制作用、对昆明种小鼠的急性毒理作用和免疫调节作用，结果表明该保健品对

血管紧张素转化酶的抑制作用呈明显的量效关系其 IC_{50} 为 9.0 mg/mL，具有显著的降血压作用；对 DPPH 自由基和羟自由基都具有很强的清除作用，在较低的质量浓度下即有较高的清除效果，且随其质量浓度的增加而不断增强，其 IC_{50} 分别为 1.4 mg/mL 和 1.1 mg/mL 抗氧化作用作用明显；对体外培养的肝癌细胞 SMMC-7721 具有明显的杀伤作用，使细胞变形以至破裂从而抑制癌细胞的生长，抗肿瘤作用明显；根据检验标准国标 GB1519.3-2003 食品急性毒性试验中最大耐受剂量法，对清洁级 KM 小鼠无毒害作用，作用后小鼠均健康无异常反应，解剖无明显病变，其 LD_{50}>15g/kg 体重；文蛤多肽保健品给药组的胸腺指数和脾脏指数较空白对照组明显升高，提示文蛤多肽保健品能促进小鼠胸腺和脾脏的生长发育,增强免疫力。

关键词：文蛤；多肽；保健品；蛋白酶；成分；生物活性

Abstract

Meretrix meretrix Linnaeus has high nutrition value and medicine value, its edible nutrition value and usage is the best in all kinds of seashells and is honored as "the most delicious food in the world". By comparing the product of 6 proteinases hydrolyzing and studying the product biology functions, in this paper we try to get *Meretrix meretrix Linnaeus* health care product with stable quality, good color, good flavor, high polypeptide content and rich biological activities.

On the criterion of dissociating amino acid in enzyme hydrolyzing product, we respectively study the conditions including pH, temperature, enzyme quantity and hydrolyzing time of bromelain, papain, composite trypsin, trypsin, neutral proteinase and alkaline proteinase. The results show that their best pH in sequence are 6.5，7.0，8.0，8.0，7.0，8.0, that their best temperatures are all 40 ℃ except that those of papain and trypsin are both 45 ℃, that their best enzyme quantities in sequence are 0.7 %，0.7 %，0.5 %，0.3 %，1.1 %，0.5 % and that their best hydrolyzing time are all 5 h except that those of neutral proteinase and trypsin are both 6 h. On their their own best hydrolyzing conditions, we compared their hydrolyzing performance and find that the performance of composite trypsin on the pH 8.0，40 ℃，enzyme quantity 0.5 %，hydrolyzing time 5 h is the best, and the amino concentration of composite trypsin hydrolysate is 59.71 μmol/mL. So we chose composite trypsin for hydrolyzing in latter experiments. Throught spray dryness, we can obtain white, dry powder that is *Meretrix meretrix Linnaeus* polypeptide health product.

By analyzing the main component, metal content, amino acid composition and the range of polypeptide molecular weight, we found that in the health product protein, amino acid and saccharoid are all very rich that protein is 30.3 %, dissociated amino acid is 9.6 % and total saccharoid is 8.3 %. Metal element is very rich that Na、K、Ca、Mg、Fe、Cu are in sequence less. Heavy metals are very poor and much lower than the safe concentrations in national food test criterial. The categories of amino acids are complete, in which acidic amino acids are the richest and are 10.37 %, human indispensable amino acids are very rich and are 14.09 %. The polypeptide molecular weights are less than 5200 Da, polypeptide less in

molecular weight is richer and polypeptide which molecular weight is less than 1000 Da is 69.03 % of total polypeptide. So the health product is easy for human body to absorb and it also proves the very good performance of proteinase.

After studying the inhibition on ACE, the antioxidation effect, the inhibition on liver cancer cell, the effect on Kunmin mice tumour of the powder product and Kunmin mice immune regulation, the results show that the inhibition of health product on ACE is concentration dependent, its IC_{50} is 9.0 mg/mL, so it has very high biological activities of decreasing blood pressure. Even in low concentration the health product can strongly clean DPPH- and OH-. The cleanup is concentration dependent, and their IC_{50} are 1.4 mg/mL and 1.1 mg/mL respectively. So it has very high biological activities of antioxidation. In vitro the health product can obviously kill cancer cell SMMC-7721, makes cell distortion and breaking. So it has very high biological activities of tumor inhibition. On the national test criterion of GB1519.3-2003 for the most enduring dosage in food urgent toxicity experiment, health product is not harmful to clear Kunming mice. After treated by the product, mice are normal in health and there is no illness in mice, the LD_{50} is higher than 15 g/kg weight. After treated by the product, the thymus index and splenic index obviously increase, which indicates that *Meretrix meretrix Linnaeus* polypeptide health product can promote the growth of mice thymus and spleen, thus improves mice immunity.

Key words: *Meretrix meretrix Linnaeus*; polypeptide; health care product；proteinase; component; biology activity