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硕 士 学 位 论 文

鱼类胶原蛋白肽的制备及其功能的研究

Studies on the Preparation of Collagen Peptides from Fish
and Their Functions

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中文摘要

本论文主要利用酶工程技术，以渔业加工的下脚料鲨鱼皮和真鲷鱼鳞为原料，通过选择合适的蛋白酶和控制酶解过程，研究开发鱼胶原蛋白肽制品。

首先，分别以鲨鱼皮和真鲷鱼鳞为原料，以对血管紧张素转化酶（ACE）的抑制率为指标，利用单因素实验优化菠萝蛋白酶、胰蛋白酶和中性蛋白酶酶法制备胶原蛋白肽的最佳工艺条件，表明：在各自最适温度和最适 pH 条件下，三种酶酶解鲨鱼皮的最适酶量（酶与鲨鱼皮重量之比）都为 0.2%，最适时间都为 5 h。三种酶酶解真鲷鱼鳞的最适酶量（酶与真鲷鱼鳞重量之比）除胰蛋白酶为 0.1%，其他酶都为 0.2%，最适时间都为 5 h。然后，制得在各种最佳条件下的胶原蛋白肽，把菠萝蛋白酶酶解鲨鱼皮所得肽标记为 S1，胰蛋白酶酶解鲨鱼皮所得肽标记为 S2，中性蛋白酶酶解鲨鱼皮所得肽标记为 S3，菠萝蛋白酶酶解真鲷鱼鳞所得肽标记为 Z1，胰蛋白酶酶解真鲷鱼鳞所得肽标记为 Z2，中性蛋白酶酶解真鲷鱼鳞所得肽标记为 Z3。测定它们对 ACE 的半抑制浓度（ IC_{50} ），S1，S2，S3，Z1，Z2，Z3 对 ACE 的 IC_{50} 分别为 4.8，8.4，8.2，6.5，12.0，7.2 mg/ml。再把它们分别经 Sephadex G-25 凝胶过滤柱层析进一步分离纯化，测定各个峰值部分的肽液对 ACE 的抑制效果，只有 S1 过 G25 的第一个峰值部分和 Z1 过 G25 的第二个峰值部分的肽液对 ACE 的抑制率超过 50%。将这两个组分的肽液冷冻干燥，得到干品，分别标记为样品 A 和样品 B，样品 A 和样品 B 对 ACE 的半抑制浓度分别为 4.0 mg/ml 和 2.2 mg/ml。对 ACE 的抑制效果比过柱前分别提高了 1.2 和 3 倍。虽然样品 B 对 ACE 具有最好的抑制效果，但是它得率很低。如果要生产胶原蛋白肽保健品，S1 是最合适的。

得到 6 种胶原蛋白肽干品之后，选取鲨鱼皮胶原蛋白肽中对 ACE 具有最佳抑制效果的肽 S1 及真鲷鱼鳞胶原蛋白肽中对 ACE 具有最佳抑制效果的肽 Z1，对它们进行了基本成分的测定。结果显示，胶原蛋白肽中粗蛋白含量丰富，分别达到 87.5%和 86.3%，粗脂肪含量较少，分别为 0.15%和 0.12%，水分含量分别为 11%和 5.5%，灰分含量分别为 1.0%和 0.4%；胶原蛋白肽中对人体有益的元素含量丰富，S1 中含量从高到低依次为 K、Na、Ca、Fe、Cu、Zn，Z1 中从高到低依次为 Na、K、Ca、Fe、Cu、Zn，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；氨基酸种类丰富，人体必需氨基酸分别为 21.41% 和 13.36%，是质量较好的蛋白源；用此方法得到的胶原蛋白肽的分子量范围为 2-3kDa 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。鲨鱼皮胶原蛋白肽吸湿率和保湿率分别为 26.9%和 123%，真鲷鱼鳞胶原蛋白肽吸湿率和保湿率分别为 29.5%和 117%，具有良好的吸湿和保湿性能，胶原蛋白肽对皮肤和头发有较大的亲和力，使其在化妆品开发方面有独特的应用价值。

关键词：胶原蛋白肽；ACE抑制肽；成分分析

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Abstract

The whole research study was aimed at investigating the preparation and bioactivities of collagen peptides from the castoff of marine processing, such as shark skin and red porgy scales. The appropriate protease and condition of hydrolysis was selected to produce collagen peptides.

First, taking the inhibition rate of ACE as the criterion, using the shark skin and red porgy scales as the raw materials, the technical parameters of preparation of collagen peptides with pineapple proteinase, trypsin and litmusless proteinase were optimized by the single factor experiments, which were as follows: Under the condition of the optimum temperature and pH of every protease, the best amount of three proteinase used in the enzymolysis of shark skins were all 0.2% and the optimum times on the hydrolysis of shark skins were all 5 h; the best amount of pineapple proteinase, trypsin and litmusless proteinase used in the enzymolysis of red porgy scales were 0.2%, 0.1%, 0.2%, respectively; the optimum times on the hydrolysis of red porgy scales were all 5 h.

Then, we prepare the collagen peptides under the best conditions of every protease. The peptide made from shark skin with pineapple proteinase was marked as S1; peptide made from shark skin with trypsin was marked as S2; peptide made from shark skin with litmusless proteinase was marked as S3; peptide made from red porgy scales with pineapple proteinase was marked as Z1; peptide made from red porgy scales with trypsin was marked as Z2; peptide made from red porgy scales with litmusless proteinase was marked as Z3.

Their IC_{50} were measured, the IC_{50} on ACE of S1, S2, S3, Z1, Z2, Z3 were 4.8 mg/ml, 8.4 mg/ml, 8.2 mg/ml, 6.5 mg/ml, 12.0 mg/ml, 7.2 mg/ml, respectively. They were purified by using column chromatography on Sephadex G-25. The effect on ACE of every peak was studied. The first peak of S1 and the second peak of Z1 had good inhibiting activities on ACE, and their inhibition rates are bigger than 50%.

We prepare the peptides of the two peaks by freeze drying. They were marked as sample A and sample B and their IC_{50} were 4.0 mg/ml and 2.2 mg/ml, respectively.

Their inhibiting effects on ACE were increased 0.2 and 2 times after they were purified by using column chromatography on Sephadex G-25. Although sample B has the best inhibiting activity on ACE, it is low-yield. So if we want to yield collagen peptide health care product, S1 is the most appropriate.

We analyze the basic components of S1 and Z1, which has the best inhibiting activity on ACE among the shark skin collagen peptides and porgy scale collagen peptides, respectively. The result shows that: protein is rich in collagen peptide and the amount is 87.5% in S1 and 86.3% in Z1, respectively; fat is poor and the amount is 0.15% and 0.12%, respectively; the amount of moisture is 11% and 5.5% and the amount of ash is 1% and 0.4%, respectively. Beneficial metal element is very rich that K、Na、Ca、Fe、Cu、Zn are in sequence less in S1 and Na、K、Ca、Fe、Cu、Zn are in sequence less in Z1. Heavy metals are very poor and much lower than the safe concentrations in national food test criteria. The categories of amino acids are abundant, in which the amount of human indispensable amino acids is 21.41% and 13.36%, respectively. The collagen peptide is one of the source of protein. The molecular weights of collagen peptide were between 2 kD and 3 kD. So the collagen peptide is easy for human body to absorb and it also proves the very good performance of proteinase. The moisture absorption and maintenance ratio of S1 is 26.9% and 123%, respectively. The moisture absorption and maintenance ratio of Z1 is 29.5% and 117%, respectively. It has good performance on moisture absorption and maintenance. Collagen peptides are compatible with skin and hair, so it has particular application value on the development of cosmetic.

Key words: collagen peptide; ACE inhibitory peptide; ingredient analysis

第 1 章 前言

1.1 水产品市场概况

在近 20 年间，全世界的水产总产量一直保持低速持续增长，由上个世纪 80 年代初的 9000 万吨左右增长到 2005 年的 1.3 亿吨；同时，中国的水产品产量一直保持着高速增长势头，以 2001 年为例，中国水产品产量在 2005 年已经达到约 4900 万吨，占世界水产品产量的 35%，位居世界第一位，尤其是养殖产量已占到世界养殖总产量的 70% 以上，是世界上唯一一个养殖产量超过捕捞产量的国家；2005 年，全世界人均水产品占有量约 20 kg；而中国 2005 年的人均水产品占有量已达 33.8 kg，超过了世界人均占有量，但人均食用量仍很低，不到 13 kg。目前，中国亿元以上 72 个水产批发市场，全年成交金额为 447 亿元，比 2004 同期增长 11.93%。是肉、禽、蛋成交额 119 亿元的 4.1 倍。水产品是继蔬菜之后，中国农产品批发市场中，成交量最大的商品。

1.1.1 水产品综合开发利用方面存在的不足之处

多年来，我国水产品加工业发展迅速。我国已能生产各种水产加工品数百种，紫菜、鱼干、鱼油和保健品等几十种水产加工品的质量，已达到或接近世界先进水平。然而，我国水产品加工和综合利用方面存在的问题仍然很多，与世界水平相比差距还十分明显。在水产品的加工过程中往往会产生许多废弃物，例如鱼类产品加工的时候会有内脏，鱼鳞，鱼皮和鱼骨等废弃物，蟹、虾类加工往往会有大量的虾头和蟹、虾壳产生。对这些废弃物的利用，目前我国主要用来生产饲料鱼粉，对其中很有价值的成分尚未充分利用。

1.1.2 水产品综合开发利用概况

对于水产品加工废弃物的综合利用研究始于二十世纪四十至五十年代，最初的研究主要集中在鱼粉、鱼油等方面。从二十世纪七、八十年代开始，此领域的研究主要集中在水解蛋白、胶原及明胶、皮革、软骨素及生物活性肽等方面。近

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