

海參體壁中膠原蛋白的萃取與特性分析

鍾朝淳、柯文慶

E-mail: 364878@mail.dyu.edu.tw

摘要

本研究以黑赤星海參體壁為原料萃取胃蛋白? ‘ i溶性膠原蛋白 (pepsin – solubilized collagen, PSC) , 並與吳郭魚魚皮和豬皮所萃取之膠原蛋白進行特性分析及比較。一般成分分析中，黑赤星海參之水分含量最高，達 85.84 %，而粗脂肪與粗蛋白質含量分別為 0.30 % 與 10.29 %，可以看出海參屬於一種高蛋白、低脂肪的無脊椎動物。以粗膠原蛋白產率來看，豬皮最高，達 26.12 %，其次為魚皮，海參最低。市販海參大多為乾品復水，本研究發現乾品復水後的海參無法萃出膠原蛋白，推測可能乾燥過程或復水過程受熱，造成蛋白質變性所致。以 SDS – PAGE 分析三物種萃取之膠原蛋白皆屬於第 I 型，就分子量言，海參膠原蛋白最小 (80~90 kD)；紫外光 – 可見光光譜分析顯示三物種皆在 230 nm 出現最大吸收波峰，此乃蛋白質含有 C=O, COOH 和 CONH₂ 之官能基產生的；以傅立葉轉換紅外線光譜顯示三物種萃取之膠原蛋白皆有 amide A、I、II、III，此為蛋白質主要官能基產生的吸收波峰；胺基酸組成分析顯示三物種萃取之膠原蛋白，均以甘胺酸 (31 %)、脯胺酸 (9~12 %) 以及丙胺酸 (10~12 %) 之含量最高；示差掃描熱分析顯示三物種中以黑赤星海參膠原蛋白熱穩定性最低，此應與其生長溫度有關；另三物種膠原蛋白之保濕性及吸水性均較對照組 (甘油) 為佳，表示萃取之膠原蛋白其親水性基團含量較多。綜合以上結果顯示，海參膠原蛋白之萃取雖然成本高、產率低，但就其基本特性言，海參膠原蛋白優於其他兩物種。唯需進一步研究，使於加工或其他應用上均能保持所萃膠原蛋白的特性，以最佳利用。

關鍵詞：海參、膠原蛋白、萃取、特性分析

目錄

封面內頁 簽名頁 中文摘要iii 英文摘要v 誌謝vii 目錄ix 圖目錄xii 表目錄xiv 1. 前言 1.2. 文獻回顧 3.2.1 膠原蛋白之簡介 3.2.2 膠原蛋白之萃取 4.2.3 膠原蛋白之基本結構 4.2.4 膠原蛋白之應用 8 4.2.4.1 食品領域 8 4.2.4.2 化妝品領域 8 4.2.4.3 生物醫學領域 9 2.5 海參的介紹 11 2.5.1 海參分類與介紹 11 2.5.2 海參採集與乾燥品製造 12 2.5.3 近年海參的研究 15 2.5.4 黑赤星海參 17 3. 材料與方法 18 3.1.1 黑赤星海參 18 3.1.2 吳郭魚 18 3.1.3 豬皮 18 3.2 試藥 20 3.3 儀器 21 3.4 實驗方法 23 3.4.1 海參膠原蛋白萃取 23 3.4.2 吳郭魚魚皮膠原蛋白萃取 23 3.4.3 豬皮膠原蛋白萃取 24 3.4.4 基本成分分析 28 3.4.4.1 粗蛋白質 28 3.4.4.2 粗脂肪 28 3.4.4.3 水分 29 3.4.4.4 灰分 29 3.4.5 產率 30 3.4.6 SDS-polyacrylamide gel electrophoresis 30 3.4.6.1 試劑配製 30 3.4.6.2 蛋白質變性 32 3.4.6.3 鑄膠 32 3.4.6.4 電泳 32 3.4.6.5 膠體染色及脫色 33 3.4.7 胺基酸分析 (Amino acid composition) 35 3.4.8 傅立葉轉換紅外線光譜儀分析 (FTIR) 35 3.4.9 紫外光 - 可見光光譜 35 3.4.10 示差掃描量熱分析法 (DSC) 36 3.4.11 膠原蛋白之溶解度測定 36 3.4.11.1 pH 值對膠原蛋白溶解性之影響 36 3.4.11.2 NaCl 對膠原蛋白溶解度之影響 36 3.4.12 保濕性及吸水性測定 37 4. 結果與討論 38 4.1 成分分析 38 4.2 膠原蛋白之產率 (%) 40 4.3 膠原蛋白分子量測定 (SDS-PAGE) 42 4.4 紫外光 – 可見光光譜 44 4.5 胺基酸組成分析 48 4.6 傅立葉轉換紅外線光譜分析 50 4.7 膠原蛋白之溶解度測定 55 4.8 示差掃描量熱儀分析 (DSC) 58 4.9 吸水性及保濕性測定 62 5. 結論 67 參考文獻 68 圖目錄 圖1. 膠原蛋白分子之三股螺旋結構 7 圖2. 海參乾品製作流程 14 圖3. 黑赤星海參之圖鑑 19 圖4. 黑赤星海參、魚皮以及豬皮膠原蛋白分析之架構圖 22 圖5. 海參體壁之膠原蛋白萃取流程 25 圖6. 吳郭魚魚皮膠原蛋白萃取之流程圖 26 圖7. 豬皮膠原蛋白萃取之流程圖 27 圖8. 黑赤星海參體壁、吳郭魚魚皮以及豬皮膠原蛋白之SDS-PAGE 43 圖9. 黑赤星海參膠原蛋白之紫外光 – 可見光光譜 45 圖10. 魚皮膠原蛋白之紫外光 – 可見光光譜 46 圖11. 豬皮膠原蛋白之紫外光 – 可見光光譜 47 圖12. 牛腱之第 I 型膠原蛋白之傅立葉轉換紅外線光譜圖譜 51 圖13. 黑赤星海參膠原蛋白之傅立葉轉換紅外線光譜 52 圖14. 吳郭魚魚皮膠原蛋白之傅立葉轉換紅外線光譜 53 圖15. 豬皮膠原蛋白之傅立葉轉換紅外線光譜 54 圖16. 黑赤星海參、魚皮及豬皮膠原蛋白於不同 pH 值下之溶解度測定 56 圖17. 黑赤星海參、魚皮及豬皮膠原蛋白於不同鹽濃度下之溶解度測定 57 圖18. 黑赤星海參膠原蛋白之示差掃描量熱儀分析 59 圖19. 吳郭魚魚皮膠原蛋白之示差掃描量熱儀分析 60 圖20. 豬皮膠原蛋白之示差掃描量熱儀分析 61 圖21. 海參體壁、魚皮及豬皮膠原蛋白之吸水性 (RH 43 %) 63 圖22. 海參體壁、魚皮及豬皮膠原蛋白之吸水性 (RH 81 %) 64 圖23. 海參體壁、魚皮及豬皮膠原蛋白之保濕性 (RH 43 %) 65 圖24. 海參體壁、魚皮及豬皮膠原蛋白之保濕性 (RH 81 %) 66 表目錄 表1. 膠原蛋白之分類及分佈 6 表2. SDS-PAGE 分析所用試劑之比例 34 表3. 黑赤星海參體壁之一般成分分析 39 表4. 黑赤星海參體壁、吳郭魚魚皮以及豬皮膠原蛋白之產率 41 表5. 黑赤星海參體壁、吳郭魚魚皮以及豬皮膠原蛋白之胺基酸 49

參考文獻

1. 王文政。1982。海參之乾製。豐年雜誌 32 (5), 44-45。2. 王靖鶴。2008。海參皂素溶血活性分析及 PCR-RFLP 技術在其鑑種上之應用

。國立台灣海洋大學食品科學系碩士論文。基隆。台灣。3.李小勇，李洪軍，杜虹霞，穆莎茉莉。2006。膠原蛋白的最新研究及應用進展。西南大學食品科學學院。重慶。4.李玉蓮。2011。膠原蛋白的發展與展望。國立虎尾科技大學生物科技系碩士論文。雲林。台灣。5.肖楓。2005。海棒槌膠原蛋白的物理特性及其酶解的生物活性研究。中國海洋大學食品科學系。碩士論文 6.喬聚海。1988。刺參池塘養殖研究。海洋科學4 , 1-5。7.趙世民。1998。台灣礁岩海岸的海參。臺北市:國立自然科學博物館。8.劉禧賢。2003。海參體蛋白多糖之分離及其抗氧化、抗致突變性與降血脂之研究。國立中興大學食品科學系博士論文。台中。台灣。9.鄧達祺，陳章波。1990。海參養殖與繁殖技術。中國水產月刊 565 , 3-28。10.鴻巢章二，橋本周久編，郭曉風，鄒勝祥譯。1994。水產用化學。北京。中國農業出版社:268-279。11.Aberoumand, A. 2011. Isolation of collagen from some fishes skins in Iran. Journal of Agricultureal Technology 7, 783-788.12.Agafonova, I. G., Aminin, D. L., Avilov, S. A. and Stonik, V. A. 2003. Influence of Cucumariosides upon intracellular [Ca²⁺] and lysosomal activity of macrophages. Journal of Agricultural and Food Chemistry 51: 6982-6986. 13.Anonymoys, M. 1979. Beche-demer of the tropical pacific: a handbook for fishermen. South Pacific Commision Noumea, New Caledonia. 14.AOAC. 1984. Official Methods of Analysis. 14th ed. Association of Official Analytical Chemists, Washington, D.C.USA 15.AOAC. 1995. Official Methods of Analysis. 16th ed. Association of Official Analytical Chemists, Washington, D.C.USA 16.AOAC. 2000. Official Methods of Analysis 17th ed Association of Official Analytical Chemists, Washington, D.C.USA 17.Auger, F. A., Rouabchia, M., Goulet, F., Berthod, F., Moulin, V., Germain, L., 1998. Tissue-engineered human skin substitutes developed from collagen populated hydrated gels: clinical and fundamental applications. Medical & Biological Engineering & Computing 36, 801-812. 18.Bae, I., Osatomi, K., Yoshida, A., Osako, K., Yamaguchi, A., & Hara, K. 2008. Biochemical properties of acid-solubilized collagens extracted from the skins of 19.Barbani, N., Giusti, P., Lazzeri, L., Polacco, G., Pizzirani, G., 1995. Bioartificial materials based on collagen: 1. Collagen cross-linking with gaseous glutaraldehyde. Journal of Biomaterials Science Polymer Edition 7, 461-469. 20.Barriga M. I., Piette J. P. G. 1996. Reduction of adhesion of a Lactobacillus sp. to collagen sausage casing by proteins. Food Research International 29, 249-257. 21.Baumann, E., Stoya, G., V?zker, A., Richter, W., Lemke, C. and Linss, W. 2000. Hemolysis of human erythrocytes with saponin affects the membrane structure. Acta Histochemica 102: 21-35. 22.Belitz, H., W. Grosch and P. Schieberle. 2004. Food Chemistry. 3rd ed., Springer-Verlag Berlin Heideberg. Germany. 1070p. 23.Bradley, W. G., Wilkes, G. L., 1977. Some mechanical property considerations of reconstituted collagen for drug release supports. Biomaterials, Medical Devices, and Artificial Organs 5, 159-175. 24.Brandl, U., Meiser, B., Thiery, J., Seidel, D., Reichart, B. 1998. Heparin-induzierte extrakorporale LDL-Pr?ipitation nach Herztransplantation. Zeitschrift f Herz-, Thorax-Ge?r?anstrich 12, 160-166.. 25.Brouns F., Vermeer C. 2000. Functional food ingredients for reducing the risks of osteoporosis. Trends in Food Science and Technology 11 : 22-33. 26.Browder, I. W., Litwin, M. S. 1986. Use of absorbable collagen for hemostasis in general surgical patients. Am. Surg. 52, 492-494. 27.Cameron, W. J., 1978. A new topical hemostatic agent in gynecological surgery. Obstetrics and Gynecology 51, 118-122. 28.Cheeke, P. R. 2000. Actual and potential applications of Yucca schidigera and Quillaja saponaria saponins in human and animal nutrition. Proceed American Society of Animal Science 77: 1-10 29.Chen, L., Du, Y., and Zeng, X. 2003. Relationships between the molecular structure and moisture-absorption and moisture-retention abilities of carboxymethyl chitosan II. Effect of degree of deacetylation and carboxymethylation. Carbohydrate Research 338(4), 333-340. 30.Chithra, P., Sajithlal, G. B., Chandrasekaran, G. 1985. Influence of Aloe vera on the glycosaminoglycans in the matrix of healing dermal wounds in rats. J. Ethnopharmacol 59, 179-186. 31.Chvapil, M., Speer, D. P., Holubec, H., Chvapil, T. A., King, D. H. 1993. Collagen fibers as a temporary scaffold for replacement of ACL in goats. Journal of Biomedical Material Research 27 (3), 313-325. 32.Cui, F., Xue, C., Li, Z., Zhang, Y., Dong, P., Fu, X., Gao X. 2007. Characterization and subunit composition of collagen from the body wall of sea cucumber *Stichopus japonicus*. Food Chemistry 100(3), 1120-1125. 33.Doillon, C. J., Silver, F. H., 1986. Collagen – based wound dressing effects of hyaluronic acid and fibronectin on wound healing. Biomaterials 7, 3-8. 34.Edward, B.; Krzysztof, S.; Lech, R.; Chyczewski, L.; Jaworski, S. 1996. Collagen and glycosaminoglycans of Wharton's and their alterations in EPH-gestosis. European Journal Obstetrics and Gynecology 66, 109-117. 35.Elsner, P., Berardesca, E., & Maibach, H. I. 1994. Bioengineering of the skin: Water and the stratum corneum. Boca Raton: CRC Press LLC. 36.Engelmann, S. Ebeling, O. Schwartz-Albiez, R. 1995. Modulated glycosylation of proteoglycans during differentiation of human B lymphocytes. Biochimica et Biophysica Acta .1267, 6-14. 37.Engelmann, S., Schwartz-Albiez, R. 1997. Differential release of proteoglycans during human B lymphocyte maturation. Carbohydrate Research 302,85-95.. 38.Fonseca, M. J., Alsina, M. A., Reig, F. 1996. Coating liposomes with collagen (Mr 50000) increases uptake into liver. Biochimica et Biophysica Acta 1279 (2): 259-265. 39.Friess, W., 1998. Collagen-biomaterial for drug delivery. European Journal of Pharmaceutics and Biopharmaceutics 45, 113-136. 40.Gee, J. M. and John, I. T. 1988. Interactions between hemolytic saponins, bile salts and small intestinal mucosa in the rat. Journal of Nutrition 118:1391-1387. 41.Gelse, K., E. P?ochl, T. Aigner. 2003. Collagens—structure, function, and biosynthesis. Advanced Drug Delivery Reviews 55(12): 1531-1546. 42.Hamamoto, T., Kisiel, W. 1998. The effect of cell glycosaminoglycans (GAGs) on the inactivation of factor VIIa- tissue factor activity by antithrombin II. International Journal of Hematology 68, 67-78. 43.Harkness, R. D., 1961. Biological functions of collagen. Biological Review 36: 399-463. 44.Haug, Y., Kjuul, A. K., Styrvold, O. B. and Sandsdalen, E. 2002. Antibacterial activity in *Strongylocentrotus droebachiensis* (Echinoidea), *Cucumaria frondosa* (Holothuridea), and *Asterias rubens* (Asteroidea). Journal of Invertebrate Pathology 81: 94-102. 45.Hirooshi, K., Guang, W. S., Yu, T., Takshi, K., Takshi, T. 1999. Serum hyaluronic acid levels during pregnancy and labor. Obstetrics and Gynecology 93, 480-484. 46.Huang, Y. R., Shiao, C. Y., Chen, H. H., Huang, B. C., 2011. Isolation and characterization of acid and pepsin-solubilized collagens from the skin of balloon fish (*Diodon holocanthus*). Food Hydrocolloids, V.25, P.1507-1513. 47.Huynh, T., Abraham, G., Murray, J., Brockbank, K., Hagen, P.-O., Sullivan, S. 1999. Remodeling of an acellular collagen graft into a physiologically responsive neovessel. Nature Biotechnology 17, 1083-1086. 48.Ikoma, T., H. Kobayashi, J. Tanaka, D. Walsh, and S. Mann. 2003. Physical properties of type I collagen extracted from fish scales of *Pagrus major* and

rechromis niloticas. International Journal of Biological Macromolecules 32(3 – 5): 199-204. 49.Jacobi, C. A., Wildbrett, P. T., Volk, J. M. M. 1999. Influence of different gases and intraperitoneal instillation of antiadherent or cytotoxic agents on peritoneal tumor cell growth and implantation with laparoscopic surgery in a rat model. International Journal of Angiology 13, 1021-1025. 50.Jakobsen, R. J., L. L. Brown, T. B. Hutson, D. J. Fink, and A. Veis. 1983. Intermolecular interactions in collagen self-assembly as revealed by fourier transform infrared spectroscopy. Science: 220:1288-1290. 51.Jongjareonrak, A., Benjakul, S., Visessanguan, W., & Tanaka, M. 2005. Isolation and characterization of collagen from bigeye snapper (*Priacanthus macracanthus*) skin. Journal of the Science of Food and Agriculture 85, 1203 – 1210. 52.Jongjareonrak. A., 2006. Characterization and functional properties of collagen and gelatin from bigeye snapper (*Priacanthus macracanthus*) and brownstrip red snapper (*Lutjanus vitta*) skins. Ph. D. Thesis. Prince of Songkla University, pp: 155. 53.Jurgen E. and H. P. Bachinger. 2005. Structure, Stability and Folding of the Collagen Triple Helix. Topics in Current Chemistry 247: 7-33. 54.Kaufman, H. E., Steinemann, T. L., Lehman, E., Thompson, H. W., Varnell, E. D., Jacob-La Barre, J. T., Gerhardt, B. M., 1994. Collagen based drug delivery and artificial tears. Journal of Ocular Pharmacology 10 (1), 17-27. 55.Kazuhiko, M., Shigeki H., Akio A., Kazuko Y., Youji N., Naoyuki T., Chifumi K., Takaaki K., Yoshiyuki, K. 1998. Heparin-binding epidermal growth factor-like growth factor stimulates mitogenic signaling and is highly expressed in human malignant gliomas. Acta Neuropathologica 96, 322-328. 56.Kemp, P. D., 2000. Tissue engineering and cell populated collagen matrices. In: Streuli, C., Grant, M. (Eds.), Methods in Molecular Biology vol. 139, pp. 287-293. 57.Khantaphant, S. and Benjakul, S. 2010. Purification and characterization of trypsin from the pyloric caeca of brownstripe red snapper (*Lutjanus vitta*). Food Chemistry 120, p 658-664. 58.Kimura, S. and Ohno, Y. 1987. Fish type I collagen: Tissue specific existence of two molecular forms, (- 1)2 2 and 1 2 3 in Alaska pollack. Comparative Biochemistry and Physiology 88 B(2), 409-413. 59.Kittiphattanabawon, P., Benjakul, S., Visessanguan, W., Nagai, T., & Tanaka, M., 2005. Characterisation of acid-soluble collagen from skin and bone of bigeye snapper (*Priacanthus tayenus*). Food Chemistry 89 (3), 363-372. 60.Lefebvre, F., Gorecki, S., Barelli, R., Amedee, J., Bordenave, L., Rabaud, M. 1992. New artificial connective matrix-like structure made of elastin solubilized peptides and collagens: elaboration, biochemical and structural properties. Biomaterials 13, 28-33. 61.Lefebvre, F., Pilet, P., Bonzon, N., Daculsi, G., Rabaud, M. 1996. New preparation and microstructure of the Endo-Patch elastin-collagen containing glycosaminoglycans. Biomaterials 17, 1813-1818. 62.Li, S. T. 1993. Collagen biotechnology and its medical applications. Biomedical Engineering Applications Basis Communications 5: 646-657. 63.Lima A. M., Cerqueira M. A., Souza B. W. S., Santos E.C.M., Teixeira J. A., Moreira R. A., Vicente A. A. 2010. New edible coating composed of galactomannans and collagen blends to improve the postharvest quality of fruits-Influence on fruits gas transfer rate. Journal of Food Engineering 97: 101-109. 64.Love, R. M., K. Yamaguchi, Y. Cr?縱c'h, J. Lav?膺y. 1976. The connective tissues and collagens of cod during starvation. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry 55(4): 487-492. 65.Lucas, P. A., syftestad, G T., Goldberg, V. M., Caplan, A. I. 1989. Ectopic induction of cartilage and bone by water soluble proteins from bovine bone using a collagenous delivery vehicle. Journal of Biomedical Materials Research 23, 23-39. 66.Maeda, M., Tani, S., Sano, A., Fujioka, K. 1999. Microstructure and release characteristics of the minipellet, a collagen based drug delivery system for controlled release of protein drugs. Journal of Controlled Release 62, 313-324. 67.McPherson, J. M., Sawamura, S., Armstrong, R. 1986. An examination of the biologic response to injectable, glutaraldehyde cross-linked collagen implants. Journal of Biomedical Materials Research 20, 93-107. 68.Miller, J. M., Zoll, D. R., Brown, E. O. 1964. Clinical observation on use of an extrude collagen suture. Archives of Surgery 88, 167-174. 69.Muyonga, J. H., C. G. B., Cole, Duodu, K. G. 2004. Characterisation of acid soluble collagen from skins of young and adult Nile perch (*Lates niloticus*). Food Chemistry 85(1): 81-89. 70.Nagai, T. and N. Suzuki. 2000. Isolation of collagen from fish waste material-skin, bone and fins. Food Chemistry 68: 277-281. 71.Nagai, T. and Suzuki, N. 2000. Isolation of collagen from fish waste material-skin, bone and fins. Food Chemistry 68, pp. 277-281. 72.Nagai, T., and Suzuki, N. 2002. Preparation and partial characterization of collagen from paper nautilus (*Argonauta argo*, Linnaeus) outer skin. Food Chemistry 76 : 149 -153. 73.Nalinanon S., S. Benjakul, W. Visessanguan, H. Kishimura. Use of pepsin for collagen extraction from the skin of bigeye snapper (*Priacanthus tayenus*). Food Chemistry 104, 593-601. 74.Nicholas, F. L., Gagnieu, C. H. 1997. Denatured thiolated collagen. II. Crosslinking by oxidation. Biomaterials 18, 815-821. 75.Nimni, M.E., Harkness, R. D. 1988. Molecular structures and functions of collagen. In: Nimni, M. E. (Ed.), Collagen-Bio-chemistry, vol. I. CRC Press, Boca Raton, FL, pp. 1-79. 76.Noitup, P., W. Garnjanagoonchorn and M. T. Morrissey. 2005. Fish skin type I Collagen characteristic comparison of albacore tuna (*Thunnus alalunga*) and silver-line grunt (*Pomadasys kaakan*). Journal Aquatic Food Product Technology 14 (1): 17-27. 77.Normura Y., Oohashi K., Watanabe M., Kasugai S. 2005. Increase in bone mineral density through oral administration of shark gelation to ovariectomized rats. Nutrition 21: 1120-1126. 78.Nussinovitch A. 2009. Biopolymer Films and Composite Coatings. Modern Biopolymer Science, pp. 295-326. 79.O ' Leary, M. J., Quinton, N., Ferguson, C. N., Preedy, V. R., Ross, R. J. M., Hinds, C. J. 2000. In rats with sepsis, the acute fall in IGF-I is associated with an increase in circulating growth hormone- binding protein levels. Intensive Care Medicine 26, 1547-1552. 80.Ogawa, M., M. W. Moody, R. J. Portier, J. Bell, M. A. Schexnayder and J. N. Losso, 2003. Biochemical properties of black drum and sheepshead seabream skin collagen. Journal of Agricultural and Food Chemistry 51: 8088-8092. 81.P. Kittiphattanabawon, S. Benjakul, W. Visessanguan, H. Kishimura, F. Shahidi. 2010 Isolation and Characterisation of collagen from the skin of brownbanded bamboo shark (*Chiloscyllium punctatum*). Food Chemistry 119, 1519-1526. 82.Petite, H., Rault, I., Huc, A., Menasche, P. H., Herbage, D. 1990. Use of the acyle azide method for crosslinking collagen rich tissues such as pericardium. Journal of Biomedical Materials Research 24, 179-187. 83.Rao, K. P. 1995. Recent Developments of Collagen-based materials for medical applications and drug delivery systems. Journal Biomaterials Science 7 (7), 623-645. 84.Reuter, G., Gabius, H. J. 1999. Eukaryotic glycosylation: whim of nature or multipurpose tool? Cellular and Molecular Life Sciences 55, 368-422. 85.Rodziewicz-Motowidlo, A., A. ?ladewska, E. Mulkiewicz, A. Ko?odziejczyk, A. Aleksandrowicz, J. 2008. Isolation and

characterization of a thermally stable collagen preparation from the outer skin of the silver carp Hypophthalmichthys molotrix. Aquaculture 285, 130-134. 86.Rossler, B., Kreuter, J., Scherer, D. 1995. Collagen microparticles: preparation and properties. Journal of Microencapsulation 12, 49-57. 87.Rousseau, C. F. and Gagnieu C. H. 2002. In vitro cytocompatibility of porcine type I atelocollagen crosslinked by oxidized glycogen. Biomaterials 23, 1503 – 1510. 88.Ruderman, R. J., Wade, C. W. R., Shepard, W. D., Leonard, F. 1973. Prolonged resorption of collagen sponges: vaporphase treatment with formaldehyde. Journal of Biomedical Materials Research 7, 263-265. 89.Sadowska M., I. Kolodziejska and C. Niecikowska. 2003. Isolation of collagen from the skins of Baltic cod (*Gadus morhua*). Food Chemistry 81, 257-262. 90.Saito, M., Kunisaki, N., Urano, N. and Kimura, S. 2002. Collagen as the major edible component of sea cucumber (*Stichopus japonicus*). Journal of Food Science, 67(4), 1319-1322. 91.Shen, X. R., Kurihara, H., & Takahashi, K. 2007. Characterisation of molecular species of collagen in scallop mantle. Food Chemistry 102, 1187-1191. 92.Sidhu, G. S. and Oakenfull, D. G. 1986. A mechanism for the hypocholesterolaemic activity of saponins. British Journal of Nutrition 55: 643-649. 93.Stonik, V. A., Kalinin, V. I. and Avilov, S. A. 1999. Toxin from sea cucumbers (Holothuroids): chemical structures, properties, taxonomic distribution, biosynthesis and evolution. Journal of Natural Toxins 8: 235-248. 94.Surewicz, W. k. and H. H. Mantsch. 1988. New insight into protein secondary strcuture from resolution enhanced infrared spectra. Biochimica et Biophysica Acta 952:115-130. 95.Suzuki, S., Kawai, K., Ashoori, F., Morimoto, N., Nishimura, Y., Ikada, Y. 2000. Long-term follow-up study of artificial dermis composed of outer silicone layer and inner collagen sponge. British Journal of Plastic Surgery 53 (8), 659-666. 96.Tian, F., Zhang, X., Tong, Y., Yi, Y., Zhang, S., Li, L., Sun, P., Lin, L. and Ding, J. 2005. PE, a new sulfated saponin from sea cucumber, exhibits anti-angiogenic and anti-tumor activities in vitro and in vivo. Cancer Biology and Therapy 4: 847-882. 97.Tu, R., Lu, C. L., Thyagarajan, K., Wang, E., Nguyenm, H., Shen, S., Hata, C. Quijano, R. C. 1993. Kinetic study of collagen fixation with polyepoxy fixatives. Journal of Biomedical Materials Research 27, 3-9. 98.Vojdani, F. 1996. Solubility In G. M. Hall (Ed.), Methods of testing protein functionality : pp. 11 – 60. St. Edmundsbury Press. Great Britain. 99.Ward, A. G. and A. Courts. 1977. The Science and Technology of Gelatin. Academic Press, London, pp:564. 100.Wong, D. W. S. 1989. Mechanism and theory in food chemistry. New York: Van Nostrand Reinhold. 101.Zhang, M., W. Liu and G. Li. 2009. Isolation and characterisation of collagens from the skin of largefin longbarbel catfish (*Mystus macropterus*). Food Chemistry 115: 826-831. 102.Zhang, Z. K., Li, G. Y., Shi, B. 2006. Physicochemical properties of collagen, gelatin nad collagen hydrolysate derived from bovine limed split wastes. Journal of the Society of Leather Technologists and Chemists 90, pp. 23-28. 103.Zhu, B. W., Dong, X. P., Zhou, D. Y., Gao Y., Yang, J. F., Li, D. M., Zhao, X. K., Ren, T. T., Ye, W. X., Tan, H., Wu, H. T., and Yu, C. 2012. Physicochemical properties and radical scavenging capacities of pepsin-solubilized collagen from sea cucumber *Stichopus japonicus*. Food Hydrocolloids 28, 182-188.