不同水生生物來源所得之膠原蛋白物理與生物化學相關特性
Biochemical and physical properties of collagen extracted from aquatic animals

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摘要

膠原蛋白大量存在脊椎動物中，動物性蛋白質約 30%是由膠原蛋白構成，含量會隨著年紀及季節的不同而改變。膠原蛋白是一種纖維蛋白可分為 28 型：第 I 型至第 XXVIII 型，主要分佈在細胞外之外基質，其穩定器官與結締組織以維持它們的結構完整性。膠原蛋白提取製品已廣泛應用於醫藥、保健食品加工與化妝保養品等眾多領域，但由於動物疫情持續爆發，使其源自於陸生動物的膠原蛋白之安全性受到質疑，因此以水生動物如淡水、海水魚與軟體動物類之膠原蛋白作為替代來源受到高度重視，而不同生物來源與不同組織所得膠原蛋白之生物化學與物理相關特性，如熱穩定性會有所差異。另外，近十年來膠原蛋白與明膠酵素水解物更受到重視，其具有不同特性與功能性可符合不同顧客之需求並已開發多種商品，因此本篇將探討不同水生動物來源所

得膠原蛋白之特性與其水解物機能性之應用。

ABSTRACT

Collagen is the most abundant protein in vertebrates and constitutes about 30% of total proteins. It provides the major structural and mechanical support to tissues. There are at 28 different types of collagen, named from type I to type XXVIII, which varies considerably in their complexity and diversity of their structure. Collagen has been widely used in fields including foods, medicines, cosmetics and tissue engineering due to its excellent biocompatibility or biodegradability. Generally, the main sources of type I collagen are limited to those of bovine or porcine dermis. However, bovine spongiform encephalopathy (BSE), transmissible spongiform encephalopathy (TSE) and foot and mouth disease (FMD) are threat to collagen products based on these materials. As a consequence, the alternative sources of collagen, especially from aquatic animals including freshwater and marine fish and mollusks have received increasing attention. Biochemical properties of collagen, such as thermal stability, might be different between the different sources. On the other hand, enzyme-hydrolyzed collagen plays an increasingly important role in various products and applications. Its different properties and functionalities benefit the end consumer now in ways which were not present ten years ago. Over the past decade, a large

number of studies have investigated enzymatic hydrolysis of collagen or gelatin for the production of bioactive peptides. The aim of this review was to characterize collagen from aquatic animals and to investigate the functionalities of the enzyme-hydrolyzed collagen.

一、前言

膠原蛋白主要構成動物支持組織的結構蛋白，其提取製品經由適當的處理後，可依應用的需要而製備成不同型態，已廣泛應用於醫藥、生物科學研究、攝影工業、保健食品加工與化妝保養品等眾多領域，其總產量自 1979 年(130 tons)至 2005 (305 tons)間增加 2.35 倍[(1)],膠原蛋白的需求量及原料價格日益高漲,雖至 1950 年起已開始進行膠原蛋白與明膠相關研究，但多以陸生哺乳動物為主，近年由於動物疫情持續爆發，使其源自於陸生動物的膠原蛋白與明膠之安全性受到質疑，因此陸續研究方向以從水產品加工廢棄物中去找尋替代方案。水生動物來源可由魚皮、魚骨、鰭、鱗、鰾及頭足類外皮等萃取出[(2)(3)]，膠原蛋白大量存在脊椎動物中，動物性蛋白質約有 30%是由膠原蛋白構成，含量會隨著年紀及季節的不同而改變，存在腱、皮膚、骨、血管、結締組織等[(4)]。膠原蛋白(collagen)分子是由三條多胜肽鏈(polypeptide chain)相互纏繞而成的三股螺旋結構(triple-helical structure)，平均分子量為 285 kDa，長度為 289 nm，直徑為 1.5 nm。而每一條多胜肽鏈(α chain)的組成相似，但不一定完全相同，有兩條相同而另一條不同者如 Type Ⅰ，有由三條完全相同之 α chain 所組成如 Type Ⅱ，也有三條完全不同如 Type Ⅵ,因此所組成的膠原蛋白特性也不相同且在不同部位組織各為不同含量及型態的膠原蛋白，目前已知膠原蛋白種類有 28 種[(5)]。其在電子顯微鏡下呈橫紋構造，一條 α 鏈

大約含一千個胺基酸，膠原蛋白含有豐富的甘胺酸、丙胺酸、脯胺酸和羥脯胺酸，特別是甘胺酸占全部胺基酸 30%，每三個胺基酸殘基就有一個甘胺酸，是膠原蛋白中豐富的胺基酸，此外甘胺酸為較小的胺基酸，側鏈只有一個氫原子，使三條 α 鏈互相纏繞成右螺旋狀的三股螺旋結構。而膠原蛋白中半胱胺酸、甲硫胺酸、酪胺酸與組胺酸的含量則非常低[(6)]。吡咯環的脯胺酸和羥脯胺酸會促使加強三股螺旋結構，羥脯胺酸的含量取決於魚生長的溫度且會影響膠原蛋白熱變性溫度之穩定性[(7)]。魚種生長在較冷環境比生長在較溫暖的環境，其羥脯胺酸低，熱變性溫度也較低；另外高亞胺酸(Pro+Hyp)含量，特別是羥脯胺酸含量，其會影響膠原蛋白轉變為明膠的功能特性[(8)]。

明膠(gelatin)為膠原蛋白熱變性後的產物，膠原蛋白是由三條胜肽鏈以分子內氫鍵及其他共價鍵形成三股螺旋且不溶於水之結構，因此要使膠原蛋白轉為明膠，就須切斷膠原蛋白分子間及分子內鍵結，且不使胜肽鏈水解，當以 40℃以上之熱水萃取時即可破壞膠原蛋白三股螺旋結構，並轉化為水溶性之明膠，而胺基酸組成方面類似膠原蛋白，仍以甘胺酸、脯胺酸和羥脯胺酸為主。按萃取方法尚可分為以酸(Type A gelatin, pI at pH 6-9)或鹼(Type B gelatin, pI at pH 5)萃取，可得不同性質之明膠以配合不同用途所需[(5)]。而魚皮明膠多先以弱酸處理來分解酸不穩定型的交聯分子，因為膠原蛋白中存在著對熱與酸安定的交聯分子，則會降低明膠之萃取率，之後經約 40℃溫熱水萃取得可溶性明膠[(9)]。

二、水生動物膠原蛋白產率與相關物理生化特性

(一) 膠原蛋白產率

水生動物膠原蛋白來源為魚皮、魚骨、鰭、鱗、鰾及頭足類外皮等。膠原蛋白可直接利用有機酸(醋酸、氯乙酸、檸檬酸、乳酸)或無機酸(鹽酸)從組織分離萃取出，而萃取膠原蛋白的效果取決於選取的動物種類、年齡和特徵。已知利用0.5 M醋酸溶液萃取不同水產動物膠原蛋白，會有2%-90%不等的溶解度[10][11][12]，將原料以0.5 M醋酸溶液持續攪拌萃取後，以離心方式去除不可溶之殘渣，上清液進行鹽析後所得之沈澱物收集後，以0.5 M醋酸溶解，注入透析膜中，以0.1 M醋酸溶液進行透析，最後將膜內溶液凍結乾燥即得酸可溶性膠原蛋白(acid-soluble collagen, ASC)，而上述以0.5 M醋酸浸泡萃取後，以離心方式所去除之不可溶之殘渣，將殘渣收集再以0.5 M的醋酸含胃蛋白酶之溶液持續攪拌重複上述ACS萃取步驟，即可萃取出胃蛋白酶可溶性膠原蛋白成份(pepsin-soluble collagen, PSC)如表1與2所示，已知以乾基(dry basis)計算之魚皮膠原蛋白萃取產率有鬚石首魚(black drum) (ASC: 2.3%, PSC: 15.8%)、鰩魚(skate) (ASC: 35.6%)、紅鰭多紀魨(ocellate puffer fish) (ASC: 10.7%, PSC: 44.7%)、大鰭鱯(largefin longbarbel catfish) (ASC: 16.8%, PSC: 28.0%)、鯰魚(channel catfish) (ASC: 25.8%, PSC: 38.4%)、花腹鯖(chub mackerel) (ASC: 49.8%)、斑紋異齒鮫(bullhead shark) (ASC: 50.1%)、日本真鱸(Japanese sea bass) (ASC: 51.4%)和黑鯖河魨(brown backed toadfish) (ASC: 54.3%) 等

[3][13][14][15][16][17][18]。而少數文獻以濕基(wet basis)表達如：縱帶笛鯛(brownstripe red snapper) (ASC: 9%, PSC: 4.7%)、鰻鯰(striped catfish) (ASC: 5.1%, PSC: 7.7%)、skate (ASC: 8.9%)和點紋斑竹鯊(brownbanded bamboo shark) (ASC: 9.4%, PSC: 8.86%)等之膠原蛋白產率[13][19][20][21][22]。由以上可知鬚石首魚、紅鰭多紀魨、大鰭鱯和鯰魚魚皮所得之PSC產率大於ASC，Zhang等[3]指出由於魚皮膠原蛋白間的共價鍵與在末端胜肽(telopeptide)區之醛基團促使分子交聯作用程度較高，使得膠原蛋白無法完全溶解於醋酸中，需經過pepsin作用打斷末端胜肽區之交聯分子但並不破壞膠原蛋白三股螺旋結構，進而提高膠原蛋白的溶解度並降低其免疫活性。主要的pepsin來源多為豬肚，近年來學者由幾種魚類，如：大眼鯛(bigeye snapper)、長鰭鮪魚(albacore tuna)、小黃鰭鮪(tongol tuna)及正鰹(skipjack tuna)等胃部所得到的pepsin被應用在膠原蛋白的萃取[9][23][24][25]。

(二) 膠原蛋白之熱變性溫度與胺基酸組成

膠原蛋白之熱穩定性與亞胺酸(imino acid) (hydroxyproline 及 proline)之含量成正相關，通常陸上膠原蛋白亞胺酸含量高於水產膠原蛋白，而水產膠原蛋白的亞胺酸含量會受物種生長的溫度影響。亞胺酸總含量會影響三股螺旋穩定性，因為每個 Pro 及 Hyp 的吡咯環可行成三個氫鍵以穩定螺旋結構，故亞胺酸含量高者熱穩定性越高[26]。目前最常使用的測定膠原蛋白熱變性溫度方法有下列兩種：(1) 以熱示差掃瞄卡量計 (differential scanning calorimeter, DSC)來測量膠原蛋白的熱變性溫度，其原理為升溫過程中，當加熱至某一溫度時蛋白質結構因熱而被破壞後(蛋白質結構的穩定鍵結被打斷)會產生吸熱反應(熱流變化)，所以可由電腦監測圖譜之熱流變化情形來找出蛋白質的熱變性溫度(maximum temperatures, *Tmax*)；(2)以黏度計測量膠原蛋白在加熱過程中膠原蛋白溶液的黏度變化，熱變性溫度即為膠原蛋白溶液黏度變化達二分之一改變量時所測得的溫度(denaturation temperature, *Td*)[27]。

以 DSC 測量其 *Tmax*，可知哺乳類動物的變性溫度一般都較水產動物來的高，豬皮膠原蛋白與牛皮膠原蛋白變性溫度經 DSC 測定皆 37.0℃[15][17]，一般由冷水域所萃出的水生動物膠原蛋白僅有少數，如：海鱒(ASC: 10.0℃)[10]、波羅的海鱒(ASC: 15.0℃)[12]與鯽魚(ASC: 15.7℃)[25]，大多的魚種都屬溫帶與熱帶水域，如: 點紋斑竹鯊(ASC, PSC: 34.5℃)[20]、黑邊鰭白眼鮫(ASC: 36.3℃; PSC: 34.6℃)[21]、金線鰱 (ASC: 33.5℃)[25]與鰻鯰 (ASC: 35.3℃; PSC: 35.3℃)[22] (表 3)。以黏度計測量其 *Td*，冷水域魚種之 *Td* 較熱帶與亞熱水域魚種低，如：大馬哈鮭(skin, muscles) (ASC: 16.4℃, 20.6℃)[7]、尖吻平鮋(skin, bone, scale) (ASC: 16.1℃, 17.5℃, 17.7℃)[28]與波羅的海鱒(ASC: 15.0℃)[29]，一般溫水與熱水魚種，如：尼羅河尖吻鱸(young fish, adult fish) (ASC: 36.0℃, 36.5℃)[4]、小鬚鯨(ASC: 31.5℃)[30]、銀鯉(ASC: 29.0℃)[29]、六斑刺河魨 (ASC: 29.0℃; PSC: 30.0℃)[31] (表 4)，顯示由不同物種所得之膠原蛋白，其變性溫度會受其所棲息環境溫度之影響。

ASC 與 PSC 具有相似的胺基酸組成，兩者膠原蛋白中最主要的胺基酸為 Gly 佔總胺基酸中的 1/3，此外，Pro 約佔 12%、Ala 約 11%與 Hyp 約佔 10%，海洋生物來源之 ASC 與 PSC 之亞胺酸(Pro+Hyp)含量與熱穩定性有正相關，已知點紋斑竹鯊(ASC: 20.3 %; PSC: 20.4 %)、黑邊鰭白眼鮫(ASC: 19.6%; PSC: 19.7%)[20]、尼羅口孵魚(21.0%)[3]、長尾大眼鯛(19.3 %)[32][33]、縱帶笛鯛(21.2 %)[19]、與老鼠斑(PSC: 18.4%)[34]、金線鰱(ASC: 18.8 %)[25]、草魚(PSC: 18.6%) [3][33]、鯉魚(ASC: 19.0%)[29]、舵魚(ASC: 18.3%)[2]、狹鱈(ASC: 18.4%)[35]、擬目烏賊(ASC: 18.0%)[36]、比目魚(ASC: 18.0%)[8]，Gómez-Guillén 等[37][38]表示一般冷水魚種之變性溫度約 4-12℃ 或小於 17℃ 以下，如海鱈與波羅的海鱈魚皮(ASC: 15.0℃)[29]與阿拉斯加鱈魚(ASC: 16.8℃)[78]，比溫水水域魚種(~18-19℃和~24-29℃)來的低[37]，而鱈魚之亞胺酸含量為 15-18% 小於溫水域魚種，亦小於牛皮 ASC 亞胺酸含量 21.5%[2] (表 5)，顯示不同的動物來源所含 imino acid 含量有所不同，而亞胺酸含量與膠原蛋白之熱穩定性有正相關。

(三) 膠原蛋白之物理特性

於全波長掃描下，顯示魚皮 ASC 和 PSC 的最高吸收波約為 210-240 nm，如六斑刺河魨魚皮約 210-240 nm[31]與尼羅口孵魚：220 nm[39]、大鰭鱴：233 nm[39]、阿拉斯加鱈魚：220 nm[35]、河運鯰魚：232 nm[14]、鯊魚皮：230 nm 與牛蛙(bullfrog)膠原蛋白所測得波長 236 nm[40]相似，主要因為膠原蛋白分子完全不含 Trp，可

由 280 nm 下無明顯吸收波鋒，表示所萃取之此蛋白質為膠原蛋白。膠原蛋白僅含有微量的 Tyr、His 與 Met[33][29]，而一般 Tyr 與 Phe 的吸光值為 283 和 251 nm[41]，因此於 250-280 nm 間僅觀察到有微小之吸收波鋒。

鯉魚[29]、比目魚[34]、點紋斑竹鯊[20,21]、星蟲[42]、尼羅口孵魚[39]、小鬚鯨[30]、尼羅河尖吻鱸[4]、紅鰭多紀魨[15]等，皆屬於典型第一型膠原蛋白。SDS-PAGE 圖譜顯示 type I collagen 都具有 β 及 α1、α2-chain，但部分魚種之魚皮如長尾大眼鯛[32]、點紋斑竹鯊[20]、尼羅河尖吻鱸[4]、紅鰭多紀魨[15]、鬚石首魚與羊頭鯛[17]、黑鯖河魨[18]、鰻鯰[22]、狭鱈[35]和大鰭鱯[3]尚具有 γ-chain，γ-chain 為 α2-chain 之三聚體，而 β-chain 的組成由兩個 α-chain 組成。而 ASC 之 β-chain 顏色較 PSC 深，則因胃蛋白酶的添加可分解末端胜肽區之交聯分子，使 β-chain 變成兩個 α-chain，故 PSC 之 β-chain 強度小於 ASC[43]。Mizuta 等[44]將烏賊套膜經 pepsin 水解後萃取 type I collagen 和 type V collagen，並經由 SDS-PAGE 圖譜顯示 α1-chain 的強度會因 pepsin 水解減弱，而伴隨 α2-chain 的強度增加，表示 α1-chain 比 α2-chain 易受到 pepsin 分解所影響。另外，Hwang 等[13]並指出軟骨魚中的 red stingray 和 yantai stingray 的 α2-chain 與 β-chain 分子量比硬骨魚小。

已知當pH值高於或低於pI時，蛋白質分別會帶正電或負電，則改變蛋白質分子與分子間的排斥力增加，當於pI相同時疏水性與疏水性作用力增加，導致蛋白質凝聚沉澱，不同魚皮膠原蛋白

中所含胺基酸組成不同，因而造成溶解度之差異。不同pH下鰻鯰[22]、六斑刺河魨[31]、剝皮魚[45]、比目魚[34]、尼羅口孵魚[39]、虎河魨、燕魟、刺魟、褐籃子魚、光土魟[13]與長尾大眼鯛[32]在pH 2-4具有最佳溶解度，最低溶解度範圍則在pH 7-10。Kittiphattanabawon等[32]表示不溶解的蛋白質會沉澱其黏度會相對增加，而溶解度最大與最小兩者pH值之差異，主要是因膠原蛋白不同的分子特性和構造所影響。

將魚皮膠原蛋白溶於 0.5 M 醋酸中，調整 NaCl 的濃度測定其溶解度變化，鰻鯰(striped catfish)[22]、六斑刺河魨[31]、剝皮魚[45]、比目魚[34]、尼羅口孵魚[39]、虎河魨、燕魟、赤土魟、褐籃子魚、光土魟[2]、大眼鯛魚[32, 19]、縱帶笛鯛[19]、鱈魚[46]與鱒魚魚皮之膠原蛋白[47]結果顯示，ASC 及 PSC 會隨著 NaCl 的濃度上升，溶解度因而下降，其中以 NaCl 濃度大於 3%，溶解度下降速率最為明顯。魚皮膠原蛋白溶於醋酸溶液中，會隨著 NaCl 的濃度上升，造成溶解度下降，主要因鹽析現象的發生，離子強度增加會導致蛋白質溶解度下降，因蛋白質-蛋白質間作用力較大，而蛋白質-水分子間之作用力較小，使蛋白質呈現不溶之狀態，造成蛋白質沉澱。而六斑刺河魨[31]、鰻鯰[22]、比目魚[34]和縱帶笛鯛[19]之 ASC 與 PSC 溶於 NaCl 濃度 1-3%時具有較高的溶解度，但大於 3%時 PSC 比 ASC 有較佳的溶解度，可能因 pepsin 水解部份高交聯分子，導致 PSC 具有較佳的溶解度[31]。

保水力是蛋白質吸收水分並保留在蛋白質組織中的能力，由

於膠原蛋白之三股螺旋胜肽鏈富含保水性，已被廣泛應用於化妝品與醫療產品[48]。Nam 等[49]測定魷魚外皮之膠原蛋白其保水力為 98.5 H_2O µL/mg of collagen，內皮為 99.7 H_2O µL/ mg of collagen，而牛腱之膠原蛋白保水力僅 51.0 H_2O µL/mg，其指出魷魚皮膠原蛋白除應用於化妝品與醫療產品外，亦可以應用於食品系統中作為體積增加劑與安定劑。保水力之差異是受到胺基酸中所含親水性胺基酸的多寡影響[50]，而膠原蛋白的三股螺旋多胜肽鍵間的水合網，是藉由多胜肽鍵分子與分子間之羧基(COOH)與氨基(NH_2-)所形成之氫鍵來調控水分子。而 Balti 等[50]指出烏賊皮明膠與牛皮明膠相比較下，其具有較高油脂吸附能力與較低保水力，油脂吸附能力與疏水性基暴露的多寡有關，如酪胺酸、白胺酸與異白胺酸等。

三、 水產品膠原蛋白與明膠水解物之機能性與應用

蛋白質經酸或蛋白質水解酵素在適當的作用條件下具有許多機能性的物質，如由魷魚皮所得之膠原蛋白以3.5％之鹼性蛋白酶(Alcalase 2.4 L)於pH 7.0、60℃下水解85分鐘可得最大水解度，其膠原蛋白水解物(collagen hydrolysates, CH)經由Sephacry S-100管柱純化目標片段具有抗氧化、酪胺酸酶抑制力與抗彈性蛋白酶活性[49]。由鯰魚、鱈魚和大鰭鱯所得之膠原蛋白之紫外光吸收測定得知ASC和PSC的最高吸收波為210-240 nm[3][14][35]與牛蛙膠原蛋白測得波長236 nm[40]相似。已知紫外線照射皮膚會使得相關的活

性氧物質(reactive oxygen species, ROS)大量產生，Zhang等[3]至水母萃取所得之膠原蛋白經鹼性蛋白酶後續經胰蛋白酶水解後，每天以口餵食小鼠體重之50、200 mg/kg之劑量，研究顯示CH隨著口服劑量增加，可更有效減緩小鼠裸露皮膚組織中因UV照射而造成超氧化物歧化酶(SOD)、穀胱甘肽過氧化物酶(GSH-Px)、觸酶(CAT)等抗氧化酵素下降之現象，顯示CH可避免光照射引起之傷害，推論為水解胜肽中所含之甘胺酸、脯胺酸與殊水性胺基酸提供抗氧化之效力[51]。而未給予水解物小鼠經UV照射後皮膚組織中之穀胱甘肽(GSH)、Hyp含量與給予水解物之小鼠相較之下呈明顯減少而丙二醛(MDA)含量明顯增加，顯示餵食水解物可以抑制小鼠受光照後ROS之產生並抑制油脂過氧化反應因而減少MDA量，亦可抑制因光照使得纖維母細胞之膜內脂質產生過氧化使真皮層纖維母細胞衰亡而使膠原蛋白含量減少之情形。

Li等[51]指出犛牛骨經酵素水解所得之膠原蛋白胜肽(11.7-25.3 kDa)可應用於食品與化妝品，因其與豬皮膠原蛋白多胜肽相較下更具有良好之水吸收力、保水力、油吸收力與乳化力($P<0.05$)，推論為其具有較高比例之親水性基團。Nam等[49]指出魷魚皮膠原蛋白經Alcalase水解後具抗氧化力與酪胺酸抑制力，並已有相關文獻指出其他海洋來源所得之活性胜肽具有腫瘤抑制、高血壓與降膽固醇外[52]，Zhu等[53]以海洋物種為原料製備所得之CH胜肽(130-3000 Da)給予患有糖尿病合併高血壓之患者服用後，已知游離脂肪酸、cytochrome P450和高感度C-反應性蛋白(high-sensitivity

CRP, hs-CRP)指標與糖尿病有正相關性，由各生化指標顯示服用膠原蛋白活性胜肽可一提高血管舒緩激酶(bradykinin)與降低前列環素(prostacyclin)，並顯著減少游離脂肪酸、cytochrome P450和hs-CRP含量，因而可利用活性胜肽來預防與輔助治療高血壓與第2型糖尿病患者。取白鮭魚皮膠原蛋白胜肽餵食20月齡之老年雌性小鼠，可減少大腦氧化傷害與增加BDNF (Brain-derived neurotrophic factor) 與PSD95 (postsynaptic density protein 95)等神經滋養因子之表現，有助於調節神經介質傳導、參與神經元生長、分化及重塑，且由被動逃避測試(passive-avoidance task)與莫氏水迷宮(Morris water maze)來作為動物「學習與記憶」之行為測試結果可知，餵食0.44％與1.32％ (wt/wt)白鮭魚皮膠原蛋白胜肽與幼齡小鼠控制組相比較下，各指標無差異顯示CH可開發為預防抑鬱、失憶與相關老年神經退化性疾病[54]。由於構成骨骼中骨質的主要蛋白質成分，在缺乏膠原蛋白的情況下，不易固定鈣質，使得骨骼中的鈣質逐漸流失，減低骨質密度，終至產生骨質疏鬆症。Han等[55]指出以鯊魚明膠餵食去卵巢鼠，顯示可預防因雌激素缺乏所造成之骨鬆，而Cuneo等[56]為要瞭解膳食中補充膠原蛋白胜肽對人體而言是否有益於骨質健康，挑選停經婦女每日服用10 g CH持續24週，由造骨與破骨指標顯示與控制組無差異，由於受試婦女多有鈣攝取不足與過重之情形，因而仍須進一步闡明不同型態膠原蛋白胜肽、不同營養吸收受試者與長期攝取條件下，膠原蛋白胜肽是否有益於骨質健康。

經膠體層析、離子交換層析及逆相層析法所純化之阿拉斯加鱈魚魚皮明膠胜肽(G-P-H-G-P-H-G-P-H-G-P-H-G)具有抑制亞麻油酸過氧化反應[57]。將皮氏叫姑魚(*Johnius belangerii*)魚皮明膠以胰蛋白酶(Trypsin)進行水解，得到的797 Da之胜肽(H-G-P-L-G-P-L)具有抑制linoleic acid氧化的能力且高於tocopherol[58]。海鱺魚皮明膠酵素水解物(<3 kDa)與經121℃下萃取30分鐘可得分子量小於700 Da之明膠胜肽皆具有亞麻油酸自氧化抑制之能力與捕捉DPPH自由基之能力[59]。但吳郭魚皮熱水解產物雖亦具抗氧化性，但分子量小於690 Da之水解物並非呈現最高之抗氧化力，Yang等[59]指出除分子量分佈會影響抗氧化力外，萃取條件中以磷酸萃取比例與熱萃取時間亦會影響所得之胜肽種類而影響抗氧化力。吳郭魚鱗明膠酵素水解物所得之EPALATZPEPMPF (1382.57Da)胜肽無細胞毒性，對清除氫氧、DPPH與過氧化自由基之IC50分別為7.56、8.82、17.83 μM。Gimenez等[60]亦指出烏賊明膠經Alcalase水解所得之胜肽具有起泡性與乳化力，且經由FRAP、ABTS與金屬螯合力測試，顯示其具有抗氧化力雖比BHT小，但其為天然安全添加物建議可應用於食品系統。已有相關文獻指出不同海洋物種所得之明膠經不同酵素水解後，經膠體層析、離子交換層析及逆相層析法進一步分離與純化亦可得抑制Angiotensin-I-converting enzyme (ACE)之胜肽且經餵食高血壓大鼠與控制組相較下可有效降低血壓，由海參所得MW 840 Da之胜肽，其胺基酸組成主要為Glu、Asp、Pro、Gly和Ala，其IC_{50}(抑制酵素活性50％所需之濃度)

為0.0142 mg/ml[61]。鱈魚皮明膠水解物之G-P-L及G-P-M的ACE抑制肽，IC_{50}分別為2.6與17.13 μM[62]，而yellowtail fish魚骨膠原蛋白水解後的ACE抑制肽IC_{50}為0.16 mg/ml[63]。

經攝食膠原蛋白水解物後，人體血液中可測得Pro-Hyp、Pro-Hyp-Gly、Ala-Hyp、Ala-Hyp-Gly、Ser-Hyp、Ser-Hyp-Gly、Leu-Hyp、Ile-Hyp和Phe-Hyp之胜肽片段，其中以Pro-Hyp為主要胜肽，其已被證實可刺激小鼠纖維組織母細胞增生[64]與玻尿酸之合成[65]，建議可長時間每天攝取5克之膠原蛋白水解物可改善皮膚狀況[66]，Nakatani等[67]指出Pro-Hyp可抑制因攝取高磷飲食所誘發之骨關節炎。Shigemura等[68]藉由有別於傳統之分離方法鑑定人體攝食膠原蛋白水解物後血液中胜肽含量，證實除存在著Pro-Hyp外，亦測得Hyp-Gly，且Hyp-Gly較Pro-Hyp不易受到血清中胜肽酶之影響且更有助於纖維組織母細胞增生。

四、結語

膠原蛋白具有保護膠體的特性，並有分散作用、乳化效果及膠質化功能，已廣泛應用於保健食品、醫藥與化妝保養品等眾多領域，能改善產品的物性、化性、外觀、風味，也是一種天然的抗氧化劑，目前已有訴求防止肌膚老化、關節退化及抑制骨質疏鬆效果之膠原蛋白產品上市[69]。近年陸生動物來源的膠原蛋白之安全性受到質疑，因此以水生動物類之膠原蛋白作為替代來源受到歡迎，充分利用水生動物加工後所棄之的魚皮、魚骨、鰭、鱗、

鰾及頭足類外皮等廢棄物來萃取膠原蛋白，可減少環境污染與提高附加價值，而水產物膠原蛋白與明膠酵素水解物具有不同特性與功能性，可符合不同消費者之需求，未來其應用於保健食品、醫藥與化妝品之商品開發將更為多元豐富。

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表 1、不同水產品中酸可溶膠原蛋白 (acid-soluble collagen, ASC) 的產率
Table 1. The yields of ASC from different aquaculture products

Source	The yield of ASC (%)	Wet/Dry	Reference
Skin			
小鬚鯨 Minke whale	0.9%	wet	30
擬目烏賊 Cttlefish	2.0%	dry	15
鬚石首魚 Black drum	2.3%	dry	17
羊頭鯛 Sheephead seabream	2.6%	dry	17
舵魚 Sea chub	3.4%	dry	2
褐籃子魚 Dusky spinefoot	3.9%	dry	2
六斑刺河魨 Balloon fish	4.0%	dry	31
剝皮魚 Unicorn leatherjacket	4.2%	wet	45
鰻鯰 Striped catfish	5.1%	wet	22
船蛸 Paper nautilus (outer skin)	5.2%	dry	11
燕魟 Eagle ray	5.3%	dry	2
光土魟 Yantai stingray	5.5%	dry	2
赤土魟 Red stingray	5.7%	dry	2
縱帶笛鯛 Brownstripe red snapper	9.0%	wet	19
點紋斑竹鯊 Brownbanded bamboo shark	9.4%	wet	20
黑邊鰭白眼鮫 Blacktip shark	9.5%	dry	21
紅鰭多紀魨 Ocellate puffer fish	10.7%	dry	15
長尾大眼鯛 Bigeye snapper	11.0%	wet	32
大鰭鱯 Largfin longbarbel catfish	16.8%	dry	3
尼羅河尖吻鱸 Nile perch	25.0%	-	4
鯰魚 Channel catfish	25.8%	dry	14
花鯽魚 Arabesque greenling	30.3%	dry	25
鰩魚 Skate	35.6%/8.9%	dry/ wet	13
尼羅口孵魚 Nile tilapia	39.4%	dry	39
鯉魚 Carp	41.3%	dry	29

波羅的海鱈 Cod	42.5%	-	29
尖吻平鮋 Deep-sea redfish	47.5%	dry	28
花腹鯖 Chub mackerel	49.8%	dry	70
斑紋異齒鮫 Bullhead shark	50.1%	dry	70
日本真鱸 Japanese sea-bass	51.4%	dry	70
黑鯖河魨 Brown backed toadfish	54.3%	dry	18
比目魚 Flatfish	57.3%	-	34
尼羅河尖吻鱸 Nile perch (adult fish)	58.7%	dry	4
尼羅河尖吻鱸 Nile perch (young fish)	63.1%	dry	4
Bone			
鯉魚 Carp	1.1%	dry	29
長尾大眼鯛 Bigeye snapper	1.6%	wet	32
尖吻平鮋 Deep-sea redfish	10.3%	dry	28
黃錫鯛 Yellow sea bream	40.1%	dry	70
日本真鱸 Japanese sea bass	40.7%	dry	70
正鰹 Skipjack tune	42.3%	dry	70
竹筴魚 Horse mackerel	43.5%	dry	70
香魚 Ayu	53.6%	dry	70
Fin			
七星鱸 Japanese sea-bass	5.2%	dry	70
Scale			
鯉魚 Carp	1.4%	dry	29
尖吻平鮋 Deep-sea redfish	6.8%	dry	28
Other			
章魚 Octopu (arm)	10.4%	-	71
大赤魷 Jumbo suid (mantle)	15.0%	-	72
星蟲 Sipunculida (coelomic well)	45.6%	dry	42
麗蛸 Callistoctopus arakawai (arm)	62.9%	dry	71

表 2、不同水產品中胃蛋白酶可溶膠原蛋白 (pepsin-soluble collagen, PSC) 的產率

Table 2. The yields of PSC from different aquaculture products

Source	The yield of PSC (%)	Wet/Dry	Reference
Skin			
點紋斑竹鯊 Brownbanded bamboo shark	1.04%	dry	20
縱帶笛鯛 Brownstripe red snapper	4.7%	wet	19
鰻鯰 Striped catfish	7.7%	wet	22
點紋斑竹鯊 Brownbanded bamboo shark	8.9%	wet	20
黑邊鰭白眼鮫 Blacktip shark	10.3%	dry	21
牛蛙 Bullfrog	12.6%	dry	40
花鯽魚 Arabesque greenling	14.0%	dry	25
鬚石首魚 Black drum	15.8%	dry	17
六斑刺河魨 Balloon fish	19.5%	dry	31
羊頭鯛 Sheephead seabream	29.3%	dry	17
大鰭鱯 Largfin longbarbel catfish	28.0%	dry	3
小鬚鯨 Minke whale	28.4%	wet	30
鯰魚 Channel catfish	34.8%	dry	14
紅鰭多紀魨 Ocellate puffer fish	44.7%	dry	15
草魚 Grass carp	46.6%	dry	33
花腹鯖 Chub mackerel	49.8%	dry	70
船蛸 Paper nautilus	50.0%	dry	11
斑紋異齒鮫 Bullhead shark	50.1%	dry	70
比目魚 Flatfish	85.5%	-	34
Bone			
紫海膽 Purple sea urchin test	35.0%	dry	71
竹筴魚 Horse mackerel	43.5%	dry	73
Mesogloea			
傘水母 Jellyfish exumbrella	46.4%	dry	74

Reizostomous jellyfish	35.2%	dry	27
Other			
章魚 Octopu (arm)	62.9%	-	71
烏賊 Cuttlefish (outer)	35.0%	dry	75

表 3、不同來源的膠原蛋白之變性溫度 (*Tmax*)

Table 3. Denaturation temperature (*Tmax*) of different collagen source

Source	ASC/PSC	T*max* (℃)	Reference
海鱈 Hake	ASC	10.0	10
波羅的海鱈 Baltic cod	ASC	15.0	12
花鯽魚 Arabesque greenling	ASC	15.7	25
尖吻平鮋 Deep-sea redfish	ASC	16.1	28
尖吻平鮋 Deep-sea redfish (bone)	ASC	17.5	28
大西洋白鱈 Pacific whiting	ASC	21.7	76
草魚 Grass carp	PSC	24.6	33
Rainbow trout (muscle)	ASC	24.9	13
剝皮魚 (單角革單棘魨) Unicorn leatherjacket	ASC	27.2	45
虎河魨 Tiger puffer	ASC	28.4	2
褐籃子魚 Dusky spinfoot	ASC	28.7	2
長尾大眼鯛 Bigeye snapper	ASC	28.7	32
鰩魚 Skate	ASC	28.8	13
舵魚 Sea chub	ASC	29.2	2
六斑刺河魨 Balloon fish	ASC	29.6	31
六斑刺河魨 Balloon fish	PSC	30.3	31
縱帶笛鯛 Brownstripe red snapper	ASC/PSC	30.5	19
長尾大眼鯛 Bigeye snapper (bone)	ASC	30.8	32
大鰭鱯 Largfin longbarbel catfish	PSC	31.6	3
尼羅口孵魚 Nile tilapia	ASC	32.0	39
大鰭鱯 Largfin longbarbel catfish	ASC	32.1	3
光魟 Yantai stingray	ASC	32.2	2
長尾大眼鯛 Bigeye snapper	ASC	32.5	23
赤土魟 Red stingray	ASC	33.2	2
金線鰱 Ornate threadfin bream	ASC	33.5	25
燕魟 Eagle ray	ASC	34.1	2
黑鼓魚 Black drum	ASC	34.2	17
點紋斑竹鯊	ASC	34.5	20

Brownbanded bamboo shark			
點紋斑竹鯊 Brownbanded bamboo shark	PSC	34.5	20
黑邊鰭白眼鮫 Blacktip shark	PSC	34.6	21
鰻鯰 Striped catfish	ASC	35.3	22
鰻鯰 Striped catfish	PSC	35.3	22
點紋斑竹鯊 Brownbanded bamboo shark	PSC	36.0	20
黑邊鰭白眼鮫 Blacktip shark	ASC	36.3	21
點紋斑竹鯊 Brownbanded bamboo shark	ASC	36.7	20
牛皮 Calf	ASC	37.0	17
豬皮 Porcine	ASC	37.0	39
老鼠 Rat	ASC	40.1	13
雞胸軟骨 Chich sternal cartilage	ASC	43.8	77
星蟲 Sipunculida	ASC	54.5	42

表 4、不同來源的膠原蛋白之變性溫度 (Td)

Table 4. Denaturation temperature (Td) of different collagen source

Source	ASC/PSC	*Td* (℃)	Reference
波羅的海鱈 Cod (skin)	ASC	15.0	29
尖吻平鮋 Deep-sea redfish (skin)	ASC	16.1	28
阿拉斯加鱈魚 Alaka Pollack (skin)	ASC	16.8	78
尖吻平鮋 Deep-sea redfish (bone)	ASC	17.5	28
尖吻平鮋 Deep-sea redfish (scale)	ASC	17.7	28
阿拉斯加鱈魚 Alaka Pollack (swim bladder)	ASC	18.4	78
大馬哈鮭 Chum salmon (skin)	ASC	19.4	7
大馬哈鮭 Chum salmon (muscles)	ASC	20.6	7
海星 Starfish (body wall)	ASC	23.0	79
秋刀魚 Saury (skin)	ASC	23.0	7
秋刀魚 Saury (muscles)	ASC	24.0	7
狭鱈 Walleye Pollock (skin)	ASC	24.6	35
斑紋異齒鮫 Bullhead shark (skin)	ASC	25.0	70
花腹鯖 Chub mackerel (skin)	ASC	25.0	27
花腹鯖 Chub mackerel (skin)	ASC	25.6	70
傘水母 Jellyfish exumbrella (body wall)	ASC	26.0	74
鯖魚 Mackerel (skin)	ASC	26.1	7
日本鱸魚 Japanese sea bass (skin)	ASC	26.5	70
比目魚 Flatfish (skin)	ASC	26.6	34
比目魚 Flatfish (skin)	PSC	26.7	34
鯖魚 Mackerel (muscles)	ASC	26.9	7
擬目烏賊 Cuttlefish (outer skin)	ASC	27.0	75
虎皮蛙 *Rana tigerina* (skin)	ASC	27.0	80
Callistoctopus arakawai arm (bone)	ASC	28.0	75
紫海膽 Purple sea urchin test (bone)	ASC	28.0	71
紅鰭多紀魨 Ocellate puffer fish (skin)	PSC	28.0	15
黑鯖河魨 Brown backed toadfish (skin)	ASC	28.0	18
嘉臘魚 Red sea bream (skin)	ASC	28.0	81
鯉魚 Carp (scale)	ASC	28.0	29

草魚 Grass carp (skin)	PSC	28.4	33
沙丁魚 Sardine (scales)	ASC	28.5	81
Rhizostomous jellyfish mesogloea (mesogloea)	PSC	28.8	27
銀鯉 Silver carp (skin)	ASC	29.0	29
六斑刺河魨 Balloon fish (skin)	ASC	29.0	31
日本鱸魚 Japanese sea bass (fin)	ASC	29.1	70
鰻魚 Eel (skin)	ASC	29.3	7
黃錫鯛 Yellow sea bream (bone)	ASC	29.5	70
竹筴魚 Horse mackerel (bone)	ASC	29.5	70
正鰹 Skipjack tuna (bone)	ASC	29.7	70
香魚 Ayu (bone)	ASC	29.7	70
日本鱸魚 Japanese sea bass (bone)	ASC	29.7	70
六斑刺河魨 Balloon fish (skin)	ASC	30.0	31
鰻魚 Eel (muscles)	ASC	30.2	7
牛蛙 Bullfrog (skin)	PSC	30.3	40
剝皮魚 Unicorn leatherjacket (skin)	ASC	31.2	45
小鬚鯨 Minke whale (skin)	PSC	31.5	30
鯉魚 Carp (skin)	ASC	31.7	7
鯉魚 Carp (muscles)	ASC	32.5	7
鯰魚 Channel catfish (skin)	ASC	32.5	14
尼羅河尖吻鱸 Nile perch (young fish)	ASC	36.0	4
尼羅河尖吻鱸 Nile perch (adult fish)	ASC	36.5	4
豬皮 Porcine (skin)	ASC	37.0	15
牛皮 Calf (skin)	ASC	40.8	26

表5、膠原蛋白中亞胺基酸(Proline + Hydroxyproline)含量

Table 5. Imino acid (Proline + Hydroxyproline) content of collage

Source	Imino acid (Pro+Hyp)	Reference
ASC		
褐籃子魚 Dusky spinefoot	13.5%	2
鰩魚 Skate	15.3%	13
鰩魚 Skate (muscle)	15.3%	44
波羅的海鱈 Cod	15.4%	8; 29
尖吻平鮋 Deep-sea redfish (scale)	16.0%	28
赤魷 Squid (inner skin)	16.1%	49
尖吻平鮋 Deep-sea redfish (bone)	16.3%	28
尖吻平鮋 Deep-sea redfish	16.5%	28
烏賊 Squid (outer skin)	16.9%	49
羊舌 Megrim	17.0%	8
綠鱈 Hake	17.0%	8
美洲大赤魷 Squid	17.0%	8
鯰魚 Channel catfish	17.1%	14
六斑刺河魨 Balloon fish	17.9%	31
比目魚 Japanese flounder	18.0%	8
擬目烏賊 Cuttlefish	18.0%	36
舵魚 Sea chub	18.4%	2
狹鱈 Walleye pollock	18.4%	35
金線鰱 Ornate threadfin bream	18.8%	25
鯉魚 Carp	19.0%	29
比目魚 Flatfish	19.0%	34
鯉魚 Carp	19.0%	29
鯉魚 Siver Carp	19.2%	29
長尾大眼鯛 Bigeye snapper	19.3%	32
尼羅河尖吻鱸 Nile perch (young fish)	19.3%	4
燕魟 Eagle ray	19.3%	2
黑邊鰭白眼鮫 Blacktip shark	19.6%	21

光魟 Yantai stingray	19.7%	2
尼羅河尖吻鱸 Nile perch (young fish)	20.1%	4
點紋斑竹鯊 Brownbanded bamboo shark	20.3%	20
鰻鯰 Striped catfish	20.6%	22
尼羅口孵魚 Nile tilapia	21.0%	39
星蟲 Sipunculida	21.0%	42
虎皮蛙 *Rana tigerina*	21.1%	80
縱帶笛鯛 Brownstripe red snapper	21.2%	19
大鰭鱯 Largefin longbarbel catfish	21.3%	3
牛皮 Calf skin	21.5%	3
赤土魟 Red stingray	21.6%	2
豬皮 Pig skin	22.0%	82
PSC		
傘水母 Edible jellyfish	12.2%	74
牛蛙 Bullfrog	16.7%	40
紅鰭多紀魨 Ocellate puffer fish	17.0%	15
六斑刺河魨 Balloon fish	17.5%	31
鯰魚 Channel catfish	17.7%	14
比目魚 Flatfish	18.4%	34
草魚 Grass carp	18.6%	33
黑鼓魚 Black drum (bone)	19.4%	17
羊頭鯛 Sheepshead seabream (bone)	19.5%	17
黑邊鰭白眼鮫 Blacktip shark	19.7%	21
黑鼓魚 Black drum (scale)	19.9%	17
小鬚鯨 Minke whale	19.9%	30
羊頭鯛 Sheepshead seabream (scale)	19.9%	17
點紋斑竹鯊 Brownbanded bamboo shark	20.4%	21
點紋斑竹鯊 Brownbanded bamboo shark	20.7%	20
鰻鯰 Striped catfish	21.1%	22

虎皮蛙 *Rana tigerina*	21.3%	80
小牛皮 Calf	21.5%	83
縱帶笛鯛 Brownstripe red snapper	22.1%	19