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Improvement of skin condition on skin moisture and anti-melanogenesis by collagen peptides from milkfish (*Chanos chanos*) scales

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Abstract. Milkfish Chanos chanos is considered as the fourth largest aquaculture product. Collagen peptides from milkfish scales (MSCP) were used to evaluate the characteristics including moisturizing, transepidermal water loss rate (TEWL), anti-skin aging estimation by the matrix metalloproteinase (MMP) activity, anti-melanogenesis, and sun protection factor (SPF). MSCP revealed the excellent capacity of moisture absorption and was increased to 20% moisture absorption rate after 4 h at 44% and 65% relative humidities. This result also corresponds to the result of TEWL. On gelatin zymography assay, the inhibition of MMPs treated with MSCP at 1 mg/mL was observed, preventing the gelatin from degradation. Moreover, the effectiveness results of MSCP in inhibiting tyrosinase activity and melanin production were 752.4 and 887.1 μg/mL, respectively, at the half maximal inhibitory concentration (IC_{50}). However, the SPF of MSCP spread at a dose of 20 mg/cm^2 was only 1.09, thereby indicating that MSCP was not suitable as a sunscreen alone. MSCP was recognized as safe according to the cell cycle distribution analysis of flow cytometry. Consequently, MSCP could be modulated as moisturizers, anti-skin aging and skin-whitening agents for applications of cosmeceuticals. Thus, the milkfish scales can be converted into the useful by-products and decrease the environmental pollution.

1. Introduction

Marine fish-derived peptides and proteins have applied into various cosmeceuticals due to their antioxidant ability, metalloproteinase inhibitory activity, as well as anti-photoaging activity [1]. Collagen is a major marine fish protein and has been extensively utilized in cosmeceuticals, tissue

engineering, and wound healing owing to its low antigenicity, good biocompatibility, biodegradability, antioxidant and moisturizing properties [2]. Therefore, a volume of research has been published regarding the collagen from by-products of fish processing such as fish bones, skins and scales [1-3].

The control of skin aging, i.e., typically wrinkles and sagging of the face and hyperpigmentation, is a challenge in cosmetic industry. The depletion of hyaluronic acid (HA), which can be restored by replenishing HA in skin, mainly resulted in skin laxity [4]. Thus, in addition to HA, several materials, such as collagens, ceramides, chitosan derivatives, and algal extracts, used in hydrolifting for a dermal hydration have been the focus of many researches [1, 5, 6]. Collagen hydrolysate exhibits excellent water-holding capacity, moisture absorption, and retention [7, 8]. This result is consistent with the effects of collagen hydrolysate from jellyfish on UV-induced skin damage of mice, increasing the moisture retention ability [9]. Thus, collagen hydrolysate can efficiently reduce the hydration loss of the skin to tighten skin wrinkles.

Skin whitening agents that contribute to depigmentation are gradually increasing in the cosmetic industry. The accumulation of melanin produced by melanocytes mainly results in skin pigmentation and is conducted by regulating tyrosinase, responsible for the melanin synthesis [10]. Several depigmenting agents, such as arbutin, corticosteroids, hydroquinone, and kojic acid, are used as the tyrosinase inhibitors [11]. However, the effects of pigmented contact dermatitis and skin irritation have been raised for safety concerns [12].

The milkfish *Chanos chanos* is considered as a high-value marine food and the fourth largest aquaculture product [13]. Consequently, much waste that contains fish skin, bones, and scales aggravates the environmental pollution. Useful proteins via fish processing waste have been widely utilized for various applications. Therefore, pepsin-soluble collagen method was performed in this research to extract the collagen peptide from milkfish scales (MSCP). The moisture absorption of MSCP was estimated and observed. Moreover, MSCP-suppressed metalloproteinase and tyrosinase activities were further analyzed. This research will present evidence to show the potential of MSCP as a cosmeceutical application.

2. Moisture absorption of MSCP

2.1 Assessment of moisture absorption and moisturizing rate of MSCP

The MSCPs from milkfish scales were used to estimate the ability of moisture absorption under 44%, 65%, and 80% relative humidities during 48 h. At both 44% and 65% relative humidities, the moisture absorption of MSCP was increased to 20% by degrees and exhibited stationarity after 4 h analysis (Figure 1). Furthermore, the MSCP gradually revealed the excellent capacity of moisture absorption over time at 80% relative humidity. However, the moisture absorption of aqua has completely disappeared at 8 h between 44% to 80% relative humidities. Overall, the MSCP displayed higher moisture absorption than HA for 10%.

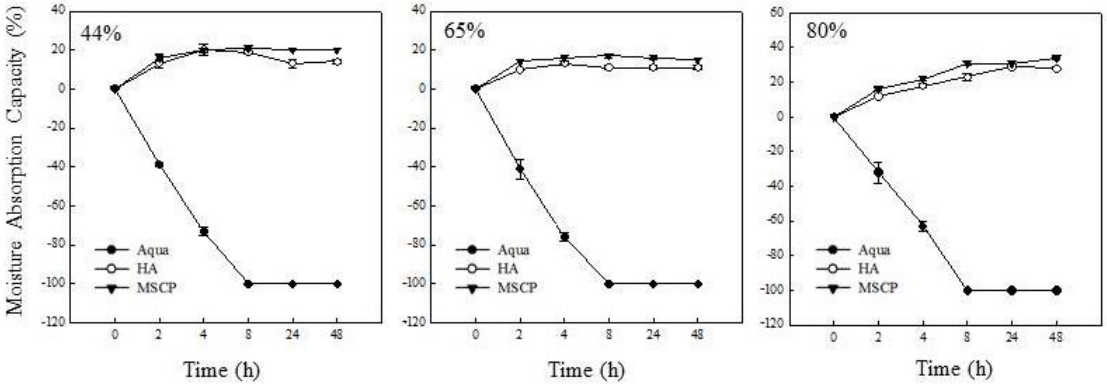

Figure 1 The moisture absorption of MSCP. Moisture absorption rates of MSCP, HA and aqua were measured by the percentage of dry weight increase in a constant temperature of desiccator with relative humidity values of 44%, 65% and 80% for 48 h at 37°C, respectively. MSCP, collagen peptide from milkfish; HA, hyaluronic acid.

2.2 Evaluation of TEWL rate by MSCP

TEWL has been used to survey the efficiency of the skin barrier function and considered as the most important physiological property [14]. The participants (n = 6) with an average age of 25 years were divided into three groups treated with MSCP, HA, and aqua. TEWL was measured by the time course after the samples were laid on the arm with four spots. TEWL complied with the order, HA < MSCP < aqua (Table 1). The mean values of TEWL in HA, MSCP, and aqua were 8.7, 12.1, and 14 g/h/m^2, respectively, after 60 min of treatment.

Table 1 TEWL was estimated by a Tewameter® TM 300 with six participants for 60 min.

	TEWL (g/h/m^2)					
Sample	5 min	10 min	20 min	30 min	45 min	60 min
MSCP	12.5	12.9	14.1	12.3	12.7	12.1
HA	9.8	9.5	9.3	9.1	9.0	8.7
Aqua	14.8	15.2	13.7	15.1	14.9	14.0

Each value is presented as the mean from independent experiments of six participants. MSCP, collagen peptide from milkfish; HA, hyaluronic acid.

The lack of moisture on epidermis results in dry skin, characterized by fine lines, itching, and scaling [6]. The potential causes of dry skin are as follows: weather, reducing humidity, abrasive friction of skin, frequent bathing, detergent utilization, sun exposure and aging [6]. Thus, several methods, such as cleansing, skin care products, emollients, and moisturizers, are proposed for the prevention of dry skin. Much moisturizers, such as HA, lactic acid, glycolic acid, urea, glycerine, propylene glycol and ceramides, have been utilized. MSCP also can be presented as another moisturizer with high moisture absorption. Furthermore, the moisture absorption capability of MSCP was in agreement with the collagen peptides grafted *N*-succinyl chitosan and hydroxypropyl chitosan [7, 8].

3. Effect of MSCP on MMPs, tyrosinase activity and melanin content

3.1 Effect of MSCP on MMPs activity

The MMPs are involved in extracellular matrix degradation in the epidermal and dermal layers during skin aging progression. Collagen breakdown is regulated by MMPs including collagenases (MMP-1, -8, -13, -18), gelatinases (MMP-2, -9), stromelysins (MMP-3, -10), matrilysins (MMP-7, MMP-26), membrane-type MMPs (MMP-14, -15, -16, -17, -24, -25), and others (MMP-12, -19, -20, -21, -23, -27, -28)[15]. The effect of MSCP on MMP activity was detected using gelatin zymography. On gelatin

zymography assay, the gelatin was significantly degraded, presenting in the control with the clear bands (Figure 2). However, a dose-dependent manner of MSCP prevented the gelatin from degradation by the MMPs including the MMP-2 and MMP-9. The inhibition rate of MMPs treated with MSCP was represented as the band intensity by the Image J software. The MMP-2 and MMP-9 bands had 2.2-fold and 1.7-fold greater intensities in the 1 mg/mL MSCP treatment than those in the 0.5 mg/mL MSCP treatment, respectively. This demonstrated that MSCP can prevent gelatin from degradation by MMP-2 and MMP-9. In addition, MMP-2 and MMP-9 had a potential as a stimulator on tumor growth, while MMP-9 contributed to progression of breast cancer [16]. The relationship between MCSP and MMPs must be further investigated for medical development.

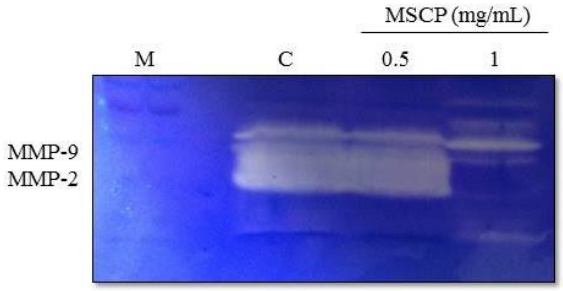

Figure 2 Ability of MSCP to inhibit MMP-2 and MMP-9 activities. According to the gelatin zymography assay, the proteins of fibroblast cells treated with MSCP were subjected to 10% non-denaturing Tris-Glycine gel containing 0.1% w/v gelatin. The gel was stained with Coomassie blue. M, marker; C, control; MSCP, collagen peptide from milkfish.

3.2 Effect of MSCP on tyrosinase activity and melanin content

Tyrosinase is involved in the melanogenesis process driven by the hydroxylation of monophenol to *o*-diphenol and the subsequent oxidation of an *o*-diphenol to *o*-quinone [10]. These tyrosinase inhibitors usually have the ability as antioxidants to bind the copper of the enzymatic activity site and arrest the oxidative pathway [11]. Therefore, the inhibition of tyrosinase activity by MSCP was observed, and ascorbic acid was used as the positive control. The result revealed that 500 and 1000 μg/mL MSCP can repress the tyrosinase activity by 44.8% and 55.1%, respectively (Figure 3). A dose-dependent trend of MSCP on the tyrosinase inhibition was obviously found. To calculate the half maximal inhibitory concentration (IC_{50}), the effectiveness of the MSCP in inhibiting tyrosinase activity was 752.4 μg/mL. MSCP may be able to chelate the copper of tyrosinase. Our result indicated that MSCP exhibited higher tyrosinase inhibition than the collagen peptides in ranges of 3-10 and 1-3 kDa from squid skin (*Todarodes pacificus*) and jellyfish (*Rhopilema esculentum*), which showed 39.65% and 53.9% inhibition at 1 and 5 mg/mL, respectively [17].

To determine the effect of MSCP on the melanogenesis, B16 cells were incubated with 500 and 1000 μg/mL MSCP for 72 h. The result revealed that 20% inhibition of extracellular melanin content was found as 500 μg/mL MSCP addition (Figure 3). When MSCP was increased to 1000 μg/mL, 51% inhibition rate of melanin content was observed in a dose-dependent manner. To estimate the IC_{50}, the effectiveness of the MSCP in prohibiting melanin was 887.1 μg/mL. Our findings were consistent with epidermal growth factor as rh-oligo-peptide-1, which is widely used in cosmeceuticals as a moisturizer and anti-melanogenic agent [18].

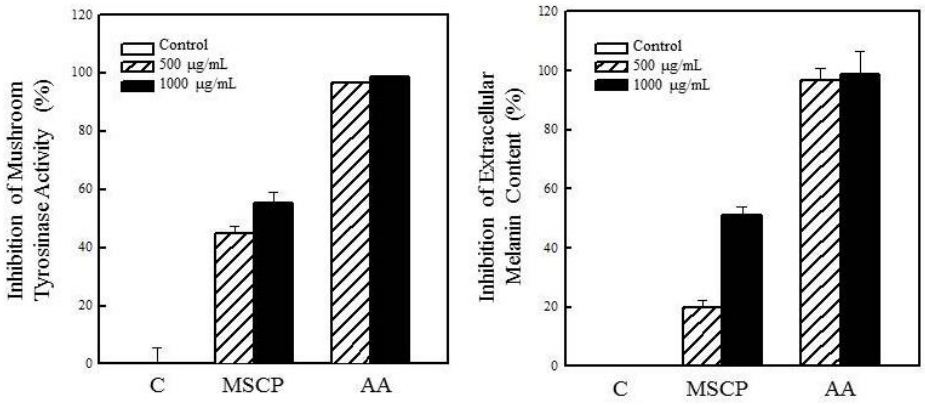

Figure 3 Ability of MSCP to inhibit tyrosinase activity and extracellular melanin level. The inhibition of mushroom tyrosinase with addition of MSCP was determined using a spectrophotometer at OD490. Changes in the extracellular melanin level indicated by α-MSH in phenol red free medium with B16 cells treated with MSCP. The extracellular melanin generation level of B16 cells was measured using a spectrophotometer at OD405. Ascorbic acid (AA) was used as the positive control. C, control; MSCP, collagen peptide from milkfish. Results are the means ± S.D. (n = 3).

4. UV absorption assessment of MSCP

Sun protection factor (SPF) is usually used to label the sunscreen products according to their protective benefits from UV damage. The Labsphere UV-1000S Ultraviolet Transmittance Analyzer was utilized to detect the SPF of MSCP *in vitro*. The result revealed that the SPF of MSCP spread at a dose of 20 mg/cm^2 was only 1.09 (Table 2). The transmittance values of UVA and UVB for MSCP were 95.94% and 89.7%, respectively. Boots Star Rating system represents the protection against UVA ray [19]. In this study, MSCP achieved a rating of 2 stars. An SPF of sunscreens above 10 evidently achieved UVB protection. However, no outstanding SPF was observed by the MSCP treatment. This finding was in agreement with the result of Peres et al. [20]. These natural compounds were triggered by UV photodegradation, resulting in low sun protection and SPF value detection. Considering the utilization of MSCP-based skin care products, high SPF compounds were necessary to append into the formulation.

Table 2. SPF of MSCP was predicted by a LabsphereTM UV-1000S UV Transmission Analyzer *in vitro*

Units	SPF[a]	T[UVA][b]	T[UVB]
Mean	1.09 ± 0.01	95.94 ± 0.29%	89.7 ± 0.59%
COV[c]	0.60%	0.30%	0.66%
UVA/UVB Ratio[d]		0.45	
Boots Star Rating[e]		**	

[a]SPF is calculated as follows: $SPF = \int_{280\,nm}^{400\,nm} E\lambda \cdot S\lambda \cdot d\,\lambda \Big/ \int_{280\,nm}^{400\,nm} E\lambda \cdot S\lambda \cdot T\lambda \cdot d\,\lambda$, where $E\lambda$ = CIE erythemal spectral effectiveness, $S\lambda$ = solar spectral irradiance, and $T\lambda$ = spectral transmittance of the sample determined by a LabsphereTM UV-1000S UV Transmission Analyzer.

[b]T[UVA] and T[UVB] are the average transmittances for UVA and UVB.

[c]COV is an average variation from the mean of SPF values.

[d]UVA/UVB Ratio is calculated as follows: $aUVA/aUVB = \int_{320\,nm}^{400\,nm} A\lambda \cdot d\,\lambda \Big/ \int_{290\,nm}^{320\,nm} A\lambda \cdot d\,\lambda$ according to the reference of LabsphereTM UV-1000S UV Transmission Analyzer.

[e]The star rating associated claim for UVA protection is detected by UVA/UVB ratio.

5. Effect of MSCP on cell-cycle distribution

To estimate the effect of MSCP on the cell cycle progression of HaCaT cells, the results of apoptosis rate were calculated using propidium iodide staining and flow cytometry (Figure 4). After treatment of HaCaT cells with MSCP for 72 h, the MSCP did not influence the percentage of apoptotic cells in M1 phase with 3.8% at 1 mg/mL compared with the control (3.1%). MSCP had no significant influence on cell cycle distribution.

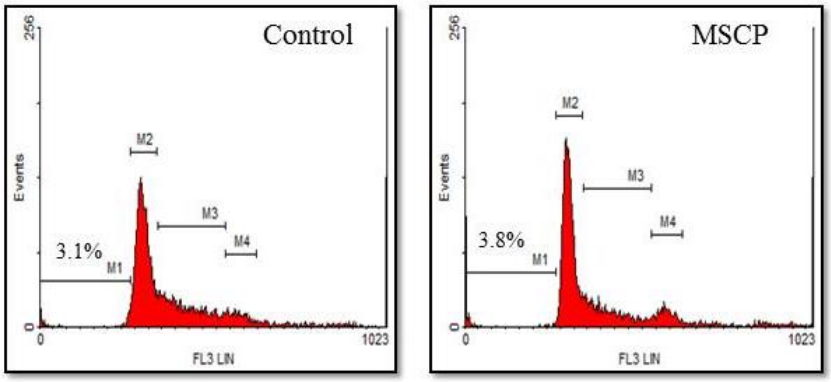

Figure 4 Effect of MSCP on cell-cycle distribution of HaCaT cells. HaCaT cell was treated with 1 mg/mL MSCP for 72 h and stained with propidium iodide for flow cytometry assay. Ascorbic acid (AA) was used as a positive control. C, control; MSCP, collagen peptide from milkfish.

6. Conclusion

Milkfish *Chanos chanos* is the fourth largest aquaculture commodity. Much fish scale waste from milkfish results in environmental pollution problem. Therefore, the high value of collagen peptides generated from marine fish has attracted much attention and utilized in skin-targeting cosmeceuticals. Our results verified that milkfish scales can be converted into the useful and functional collagen peptides. It not only decreases the environmental pollution but also increases the values of by-products. MSCP showed significant moisture absorption, anti-skin aging, and anti-melanogenic capacities. It had potential as a major active ingredient of skin care products.

Acknowledgments

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