J. Cosmet. Sci., **61,** 133–145 (March/April 2010)

Comparison of hydration, tyrosinase resistance, and antioxidant activation in three kinds of pearl powders

DONG-ZI SHAO, CHENG-KUN WANG, HANN-JANG HWANG, CHING-HSIA HUNG, and YU-WEN CHEN, *Department of Cosmetics Application and Management, Chung Hwa University of Medical Technology, 89 Wenhwa 1st St., Tainan, Taiwan 717 (D.-Z.S., H.-J.H.), E-Chyun Dermatology Clinic, No.70, Sec. 3, Jhonghua E. Rd., East District, Tainan City, Taiwan 701 (C.-K.W.), Department of Physical Therapy, National Cheng Kung University, Tainan, Taiwan (C.-H.H.), and Department of Physical Therapy, China Medical University, Taichung, Taiwan (Y.-W.C.).*

Accepted for publication August 24, 2009.

Synopsis

In recent years, people have bred freshwater pearls as a substitute for natural pearls that occur in seawater, and they have also developed water-soluble pearl powder (P-w) and ultra-micro (P-μ) and ultra-nano pearl powder (P-n) products. However, neither the scientific value of pearl powder, nor the differences in efficiencies of different pearl powder products is still unknown. In this study, the effectiveness of three kinds of pearl powder products in various applications was compared. Tests for transepidermal water loss (TEWL) and evaluations of the skin surface hydration of test subjects showed that pearl powder has a satisfactory moisturizing effect on skin and that P-μ has a distinctly stronger moisturizing effect than P-w. The three pearl powder products can also significantly reduce the activation of tyrosinase and free radicals. In tests for reducing power and 1,1-diphenyl-2-picrylhydrazyl (DPPH) for scavenging free radicals, P-n and P-μ showed better performance than P-w. These results provide a reliable scientific basis for the use of pearl powder in beauty treatment, resistance to aging, and clinical medical treatment.

INTRODUCTION

In accordance with volume 46 of the *Compendium of Materia Medica* by Li Shizhen of the Ming Dynasty (1578 AD), "applying the pearl to the face can make the skin moisturized and glossy…comfort the mind and eliminate the poxes and the toxicity." In the *Encyclopedic Dictionary of Chinese Medicine* Xie Guan *et al.* wrote in 1921: "The pearl can pacify people and brighten the eyes; if applied to the face, it can make the skin moisturized and glossy." For more than 2000 years, pearls have been used in China, and their value in beauty treatment and medicine has been often recognized. Since ancient times, the pearl

Address all correspondence to Dong-Zi Shao.

has been the favorite choice of the "imperial family" for beauty treatment and health care, and still, people consider the pearl as a symbol of beauty, youth, and eternity, and have great esteem for it.

Pearl powders ground from natural pearls are considered pure products and are used for beauty treatment and health care (internal or external). They are rich in nutrients and mainly contain the following ingredients: calcium carbonate ($CaCO_3$), proteins stimulating cell regeneration, nearly 20 kinds of amino acids, some trace elements (1,2), and conchiolin. Conchiolin is the general designation for organic substances contained in shells and pearls, and they endow pearls with their beauty and health-care efficacy (3).

In 1905, Mikimoto Kokichi, from Japan, called the "father of pearl breeding," was the first to successfully breed a pearl in seawater. In 1940, when "outer membrane nucleus insertion technology" was developed (4), freshwater pearl-breeding industries emerged at a tremendous pace. In 1968, nucleus insertion technology was promoted and improved, providing favorable conditions for the development of freshwater pearl-breeding industries.

Before its use, water-soluble pearl powder (P-w) receives biotechnical treatment. In general, acid (most frequently lactic acid) or enzyme is used to turn the $CaCO_3$, which is insoluble, into soluble calcium lactate in pearl powders. Insoluble components are discarded, and the water-soluble products are obtained through recrystallization. As these products are soluble, they are conveniently absorbed both internally and externally by the human body.

Compared with traditional pearl powders, nano pearl powder is ground to microscopic dimensions. These molecules are small, which enables high-efficiency absorption.

Evaporation of moisture from human skin mainly proceeds through noninductive evaporating excretion, sweating, and external factors. Noninductive evaporating excretion is mainly through epidermal water loss (i.e., transepidermal water loss [TEWL]). TEWL measures skin barrier function; high loss of water from the skin implies lower stratum corneum water-holding capacity. Using moisturizing cosmetics, the water-holding capacity of the stratum corneum can be improved, and loss of water from the skin itself can be reduced. The hydration state of the skin is used to evaluate the water content of the skin surface and the water-holding capacity of the skin.

In the epidermis, pigmentation is due to an excess of melanin. Tyrosinase is a rate-limiting enzyme used for the ultimate formation of melanin. Therefore, it is possible for tyrosinase depressors to reverse abnormal pigments and to be used as a skin whitener (5).

Reactive oxygen species (ROS), such as superoxide anion, hydroxyl radicals, and hydrogen peroxide, are chemically reactive molecules derived from oxygen. They are by-products produced in living organisms through many metabolic pathways. Formation of excessive free radicals in the human body can damage proteins, lipids, cell membranes, nucleic acids, and cells. Damage produced by free radicals can accumulate in the human body, which may cause aging, cancers, cardiovascular disease, Alzheimer's disease, and Parkinson's disease. Natural antioxidants in the daily diet can bind to unstable free radicals in the human body and protect the body from the damage caused by active oxygen as well as by oxidation due to other free radicals (6,7). Consequently, antioxidants that can neutralize direct ROS attacks and terminate free radical-mediated oxidative reactions would have benefits in protecting the human body from such diseases (8).

In recent years, people have bred freshwater pearls as a substitute for natural pearls that occur in seawater. They have also developed water-soluble pearl (P-w) as well as ultra-micro (P-μ) and ultra-nano pearl powder (P-n) products, which have appeared in the cosmetics, health care, and medicine markets. However, there have been no scientific data to verify the efficacy of these products until now. In this study, the P-w, P-μ, and P-n products were compared using moisturizing efficiency analysis, a tyrosinase blocking test, and multiple tests for oxidation resistance.

MATERIALS AND METHODS

CHEMICALS

Ascorbic acid (L-Vit. C), butylated hydroxyanisole (BHA), 1,1-diphenyl-2-picrylhydrazyl (DPPH), ethylenediaminetetraacetic acid (EDTA), ferrous ammonium sulfate, ferrozine, tyrosine, and tyrosinase were purchased from Sigma Chemical Co. (St. Louis, MO). Ferric chloride, iron chloride, potassium ferricyanide, potassium hydroxide, sodium carbonate, sodium phosphate, sodium sulfate, and trichloroacetic acid were purchased from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). Dimethylsulfoxid (DMSO) was obtained from Germany. One percent sodium hyaluronate (Hya) was obtained from Union Chemical Works (Tainan, Taiwan). Arbutin and seaweeds were obtained from SETALG® (France), whereas *Spiraea formosana* hayata powder, ginkgo powder, taraxacum powder, and *Angelica dahurica* powder were purchased from an herbal market in Taiwan.

MATERIALS

(a) The P-w and the P-μ (sieve size = 8000) were purchased from an important freshwater pearl-breeding center in Zhejiang, China.

(b) The P-n was purchased from Qing Dynasty Medicine King Tongrentang of Taiwan. It was examined by Elements Bio-tech Co., Ltd. with an atomic force microscope, and the results showed that the particle diameter was 117.1 ± 19.5 nm on average.

(c) *Spiraea formosana* hayata (Spi) powders, produced by grinding, were diluted to 10 mg/ml. In traditional Chinese medicine, *Spiraea* species have been used as detoxifying, analgesic, and anti-inflammatory agents (9). These were used as a negative comparison for humidification in this assay.

(d) Hya, a high-molecular-weight glycosaminoglycan of the extracellular matrix of skin, also contributes to the hydration of skin and is a natural moisturizing factor (10).

(e) Ginkgo powders were produced by grinding ginkgo. The superoxide-scavenging effect and the antioxidant activity of the ginkgolides were adopted as a control in the antioxidant activity assays (11).

(f) Taraxacum (Tara), ground into powder, has been widely used as a folkloric medicine to treat diverse diseases and is also a naturally occurring antioxidant (12). It is used in the antioxidant activity assays as a control.

(g) Arbutin, named p-hydroxyphenyl-β-D-glucopyranoside, can be extracted from plants. It is a well-known tyrosinase inhibitor and has been widely used for the whitening of skin (13).

(h) *Angelica dahurica* (Ange-da) powders were produced by grinding *Angelica dahurica.* It has been reported that the ethyl acetate extract of *Angelica dahurica* has potent inhibitory activity against mushroom tyrosinase. The chemical structure of the compound was identified as 9-hydroxy-4-methoxypsoralen (14).

(i) Seaweeds: There are a number of antioxidant and antiproliferative activities of extracts found in a variety of edible seaweeds (15).

ANALYSIS OF THE EFFICACY OF MOISTURIZING

A total of 16 healthy female students (20.0 ± 0.8 yr) were selected. Measurements of the hydration state of the skin were performed at 23° ± 2°C room temperature and at a relative humidity of 45 ± 5%. The TEWL was measured using an evaporimeter (Tewameter MPA5, Courage & Khazaka, Germany), whereas the hydration state of the skin was obtained using the CORNEOMETER® CM825 (Courage & Khazaka). Both TEWL and the hydration state were measured in a marked area on the front of the left hand. Ultra-micro (sieve size = 8000) pearl powders and P-w were diluted with distilled (DI) water. The arm was cleansed one hour before the test, and seven areas with a diameter of 1.5 ± 0.2 cm were defined on the surface of the lower arm. One test was carried out five minutes before applying the sample, and then a test was carried out every five minutes for 30 minutes.

TYROSINASE INHIBITION ASSAY

Tyrosinase inhibitory activity test was performed according to the method proposed by Rout and Banerjee (16). with minor modifications. Briefly, the reaction mixture contained 1 ml of 0.03% L-tyrosine, 0.9 ml of 25 mM phosphate buffer (pH = 6.8), and 1 ml of different concentrations of samples. After ten minutes of incubation, 0.1 ml of 350 U/ml mushroom tyrosinase was added. The optical density was taken at 475 nm after 30 minutes of incubation. The percentage of inhibition of tyrosinase activity was calculated as:

$$\% \text{ inhibition} = \frac{(A-B)-(C-D)}{A-B} \times 100$$

where A is the absorbance of a blank solution after incubation, B is the absorbance of the blank solution before incubation, C is the absorbance of the sample solution after incubation, and D is the absorbance of the sample solution before incubation.

ANTIOXIDANT ACTIVITY ASSAYS

Reducing power. Reducing power was determined according to the method of Oyaizu (17). Three kinds of pearl powders were mixed with 10 ml of DMSO to prepare samples with weight-to-volume ratios of 1, 5, 10, and 20 mg/ml. Two millilitiers of the above samples were then mixed with 2.5 ml of phosphate buffer (0.2 M, pH = 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated in a 50°C water bath for 20 minutes, then rapidly cooled, mixed with 2.5 ml of 10% trichloroacetic acid for five minutes, and centrifuged at 3000 rpm for ten minutes. After centrifugation, 5 ml of the supernatant was mixed with 5 ml of DI water and 1 ml of 0.1% ferric chloride, and this was left to stand for ten minutes. Absorbance at 700 nm was used as the indicator of

reducing power. A higher absorbance indicates a higher reducing power. Ginkgo and dandelion were used as positive controls and diluted to 1, 5, and 10 mg/ml for further use. (A concentration of P-w, P-n, ginkgo, and dandelion exceeding 20 mg/ml would lead to sedimentation.)

Free-radical scavenging activity of DPPH. A method described by Shimada *et al.* (1992) was used to detect the DPPH radical scavenging activity (18). To start, 2 ml of each test solution, including three kinds of pearl powders, ascorbic acid, and ginkgo solutions, was mixed with 2 ml of 0.2 mM freshly prepared DPPH methanolic solution. The mixture was shaken vigorously and left to stand for 30 minutes in the dark. Absorbance was then measured at 517 nm against a blank solution. Ascorbic acid and ginkgo solutions were used as positive controls for comparison to P-μ and P-n diluted to 1, 5, and 10 mg/ml, as well as for comparison to P-w diluted to 1, 5, 10, 20, and 50 mg/ml. The percentage of DPPH scavenging activity is expressed as [1 − (test sample absorbance/blank sample absorbance)] × 100 (%).

Ferrous ion chelating ability. Chelating ability was determined according to the method proposed by Dinis *et al.* (19). Each sample dilution (1, 5, 10, and 20 mg/ml in water or DMSO) was mixed with 0.1 ml of 0.1 mM $FeCl_2$ and 0.2 ml of 5 mM ferrozine solutions. After ten minutes at room temperature, the absorbance of the solution was determined at 562 nm and the values were measured and recorded. The percentage of ferrous ion chelating ability is expressed as [1 − (test sample absorbance/blank sample absorbance)] × 100 (%). EDTA and BHA were used as positive and negative controls.

SAMPLING AND STATISTICAL ANALYSIS

The results were obtained from three or more individual examinations. Values were expressed as means ± SEM. Group means were compared by one-way ANOVA followed by Scheffe's test. Probability values less than or equal to 0.05 or 0.01 were considered significant or very significant, respectively.

RESULTS

THE EFFICACY OF PEARL POWDER MOISTURIZING

The time course of TEWL is shown in Figure 1A. For both TEWL and the hydration moisturizing test, 1% Hya diluted with 30%, 50%, 80%, and 30% of glycerite was used. The results showed that 50% Hya had a better moisturizing effect than that of 30% or 80% Hya, which was similar to that of 30% glycerite. Fifty percent Hya was considered as the positive comparison for the experimental group, and 10 mg/ml Spi was considered as the negative comparison. Pearl powders were diluted with DI water as the blank. The P-μ (sieve size = 8000) and the P-w were used at 1 mg/ml and 10 mg/ml for the test, and the results showed that from five to 30 minutes after application of the samples, the P-μ and the P-w had almost the same moisturizing performance as that of soluble Hya. The three groups showed significant differences ($p < 0.01$ and 0.001) compared with the blank and the Spi.

The effect of hydration on skin is shown in Figure 1B. There were no distinct differences between the test substances in the first 15 minutes. From 15 to 30 minutes, there were distinct differences among the 1 mg/ml or 10 mg/ml P-μ and the 50% Hya, the blank, and

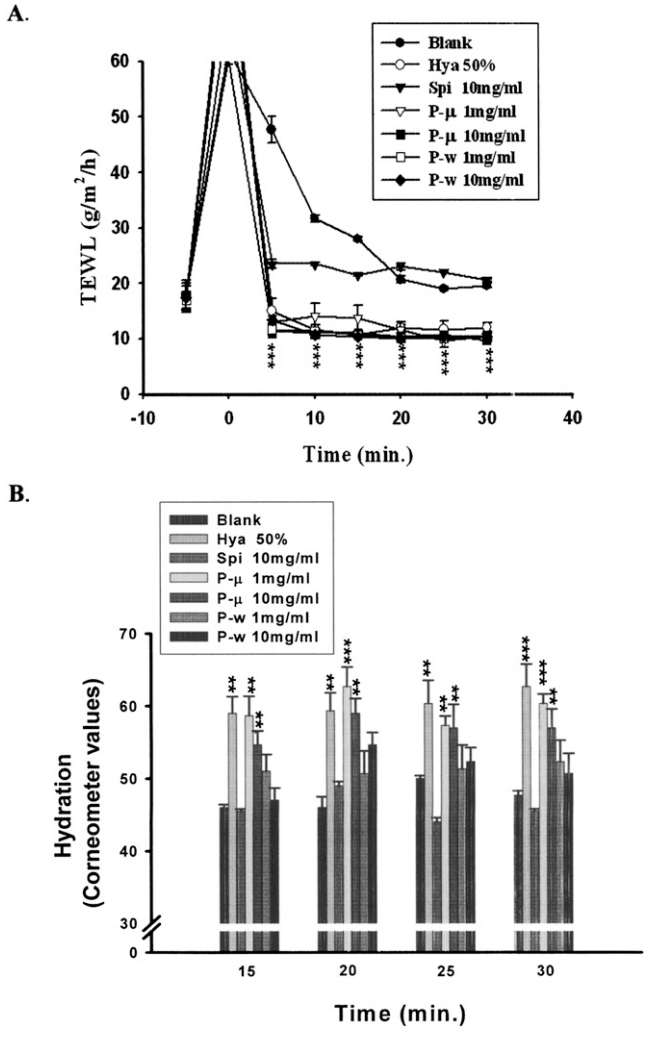

Figure 1. (A,B) Transepidermal water loss (TEWL) and effect of hydration on skin over time. Values represent the means ± SEM for three separate experiments. ***Significantly different from blank and *Spiraea formosana*, $p < 0.001$. **$p < 0.01$ (one-way ANOVA followed by Scheffe's test).

the Spi ($p < 0.01$ and 0.001). However, there were no differences among the P-w, the blank, and the Spi.

INHIBITORY EFFECTS OF TYROSINASE ACTIVITY

Ange-da and arbutin were originally adopted as positive comparisons in the test. However, it was found that the seaweed had a low ability to resist activation of tyrosinase. The three kinds of pearl powders were diluted to 5, 10, and 20 mg/ml, as shown in Figure 2. It can be observed that the three pearl powders at 5 mg/ml have a higher capacity for tyrosinase resistance than Ange-da. At the concentration of 20 mg/ml, the tyrosinase resistance of these three kinds of pearl powders was the same as that of Ange-da and

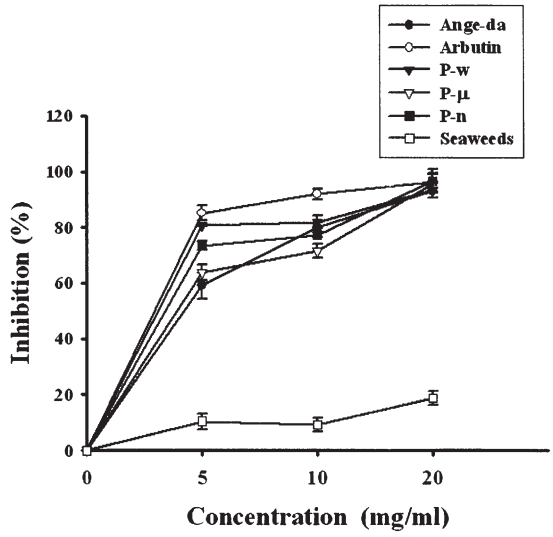

Figure 2. Inhibitory effects of water-soluble pearl powder, ultra-micro pearl powder, and ultra-nano pearl powder on tyrosinase activity with L-tyrosine as a substrate.

arbutin, even reaching a tyrosinase resistance of 90-100%. In comparison, seaweed had a tyrosinase resistance of only 18.7 ± 2.3%.

ANTIOXIDANT ACTIVITY

Reducing power. The reducing powers of the three kinds of pearl powders compared with ginkgo and Tara are shown in Figure 3A. Gingko and Tara were adopted as positive comparisons. The three kinds of pearl powders (P-w, P-μ, P-n) have a stronger reducing power with increasing concentration. From the bar diagram, Figure 3B, it can be observed that the Tara at 10 mg/ml has a stronger reducing power than ginkgo but that P-μ and P-n have a stronger reducing power nearly equal to that of ginkgo. Meanwhile, at concentrations of 5 mg/ml and 10 mg/ml, P-μ, P-n, ginkgo, and Tara all have a significantly ($p < 0.01$) stronger reducing power than P-w.

Determination of free-radical scavenging activity by DPPH. Ascorbic acid and ginkgo were adopted as positive comparisons. As shown in Figure 4A, the ability of P-μ and P-n to scavenge the DPPH free radical is observed, whereas P-μ does not increase with increasing concentration. Though rather weak, the P-w free-radical scavenging capacity also rises with increasing concentration; at 50 mg/ml, its DPPH free-radical scavenging capacity can reach 58.2 ± 0.9%. From the bar diagram in Figure 4B, it can be seen that at 1 mg/ml, the DPPH free-radical scavenging capacities of P-μ and P-n are 33.8 ± 4.6%, and 34.4 ± 6.1%, respectively, which are even stronger than the 24.4 ± 0.2% of ginkgo (positive comparison). At the three different concentrations (1, 5, and 10 mg/ml), except for P-μ at 10 mg/ml, P-μ, P-n, and ginkgo have a distinctly stronger DPPH free-radical scavenging capacity than P-w ($p < 0.001$).

Ferrous ion chelating ability. The three kinds of pearl powders, as well as EDTA for positive comparison and BHA for negative comparison, were diluted to various concentrations according to the above-mentioned method. As shown in Figure 5, the chelating ability of

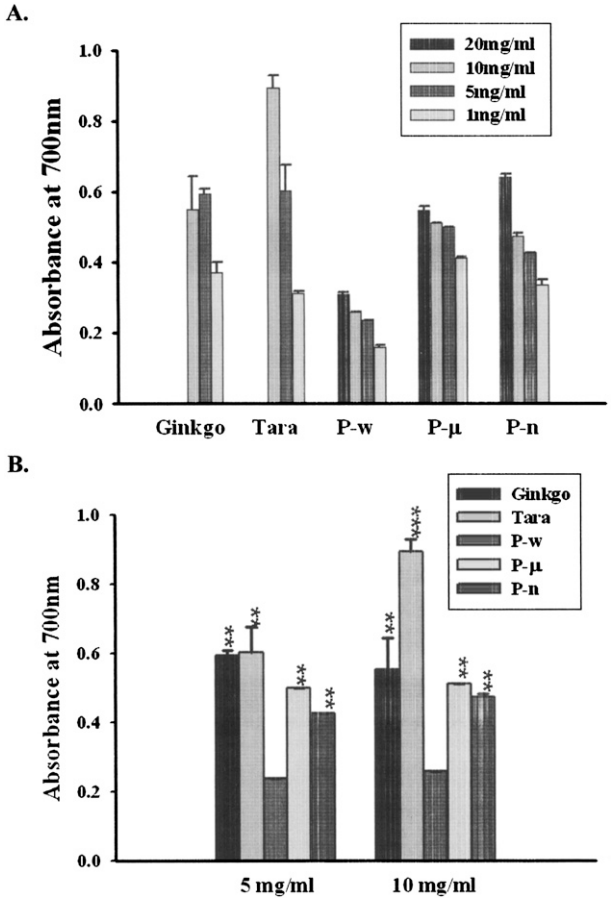

Figure 3. (A,B) Reducing power of the P-w, P-μ, and P-n compared with ginkgo and Tara. All samples were diluted five times with DMSO and distilled (DI) water before absorbance measurement. Values represent the means ± SEM for three separate experiments. ***Significantly different from P-w, $p < 0.001$. **$p < 0.01$ (one-way ANOVA followed by Scheffe's test).

1 mg/ml EDTA is 68.8 ± 3.3%, lower than that of any of the three pearl powders (at 78.3 ± 1.7–79.5 ± 1.4%). However, the three pearl powders at other concentrations (5, 10, and 20 mg/ml) have almost the same chelating ability as EDTA, which is between 75% and 80%. When compared with the solutions of 1–20 mg/ml of BHA in 96% ethanol, their chelating abilities are only 0.2% and 2.0%.

DISCUSSION

In general, analysis of skin quality should include at least two parameters: the lipid content and the moisturizing capacity of the skin. The lipid content of normal skin can not only make the skin glossy and beautiful, but can also help in retaining the water content of the skin (20,21). It is very important to maintain a certain amount of water in the corneum, as it provides the skin with healthy softness, smoothness, plasticity, and fine barrier functions. The water-holding capacity of the skin significantly declines as the skin ages (22,23).

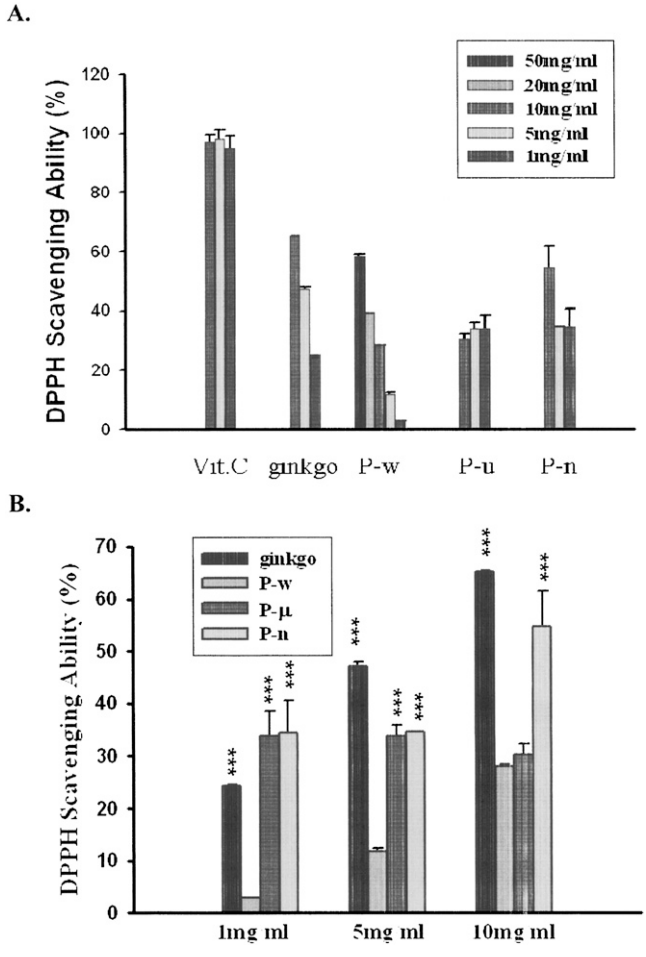

Figure 4. (A,B) Scavenging ability of three kinds of pearl powders on 1,1-dipheny1-2-picrylhydrazyl. Each value is expressed as mean ± SEM (n = 3). ***Significantly different from P-w, $p < 0.001$ (one-way ANOVA followed by Scheffe's test).

Evaluation of the moisturizing capacity of the skin, in general, encompasses (a) the capacity of water to pass through the skin, (b) the water content on the surface of the skin, and (c) the water-holding capacity of the skin. TEWL can be used to determine the ability of water to pass through the skin, whereas the water content is determined by the hydration state of the skin. The results of these two tests can be used to evaluate the water-holding capacity of the skin. There is no change in lipid content due to the pearl powder (data not shown). In evaluating the moisturizing efficiency of the pearl powders, the results of TEWL showed that P-μ and P-w, similarly to Hya (Figure 1), show fine moisturizing performance and stratum corneum barrier function. P-μ has a distinctly stronger moisturizing performance than P-w. This has provided scientific proof for the high efficiencies of pearl powder in moisturizing, enhancing the water-holding capacity of the skin, and supporting skin care and resistance to aging.

Tyrosinase (EC 1.14.18.1) catalyzes conversion of tyrosine to 3,4-dihydroxyphenylalanine (L-DOPA) and further oxidizes this to dopaquinone, which is ultimately used for the formation

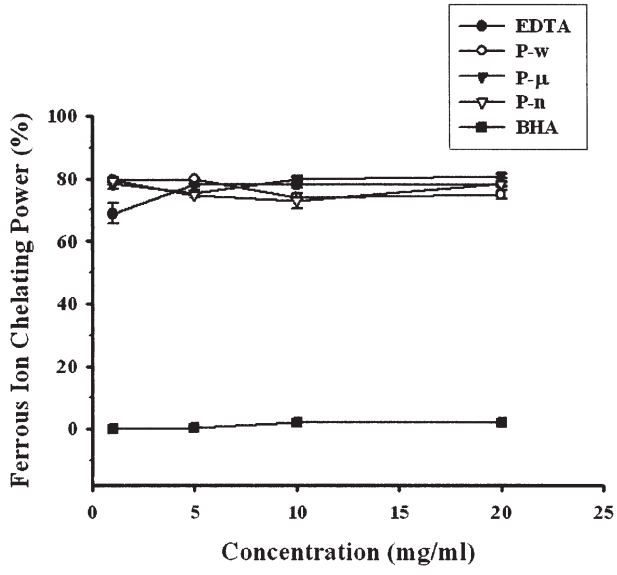

Figure 5. Chelating ability of three kinds of pearl powders on ferrous ion. Each value is expressed as mean ± SEM (n = 3).

of melanin (14,24). Melanin formation is the most important determinant of mammalian skin color intensity. Consequently, inhibiting the formation of melanin may result in a reduction in skin darkness. Therefore, tyrosinase inhibitors have a proven potential for treating abnormal pigmentation disorders and as skin-whitening agents in the cosmetics industry. Several tyrosinase inhibitors, including arbutin and Ange-da, have been widely used for the purpose of whitening the skin, and some plant extracts, such as glycyrrhizae radix and morus radix, have also been used for the same purpose (24). There has been a concerted effort to search for naturally occurring tyrosinase inhibitors from plants. Plants represent a rich source of bioactive chemicals, many of which are largely free from harmful adverse effects (25,26), but their individual activity is not sufficiently potent to be of practical use. However, pearl powder showed a strong inhibitory effect on the activity of mushroom tyrosinase, and any one of P-w, P-μ, or P-n can produce a tyrosinase resistance of 90% to 100%.

Recently, safe and effective tyrosinase inhibitors have become important for their potential applications in improving the quality of food, preventing pigmentation disorders, and preventing other melanin-related health problems in human beings (27,28). Furthermore, tyrosinase inhibitors are also important in cosmetic applications for the whitening of skin because many men and women prefer a lighter skin color. Natural pearl powder may be able to effectively meet these demands.

ROS are continuously produced during normal physiological events, and they can easily initiate the peroxidation of membrane lipids, leading to accumulation of lipid peroxides. However, they are removed by antioxidant defense mechanisms. Under pathological conditions, ROS are overproduced and result in oxidative stress. ROS are formed when endogenous antioxidant defense is inadequate. The imbalance between ROS and antioxidant defense mechanisms leads to oxidative modification in cellular membranes or intracellular molecules (29). There are many antioxidants that

can be introduced to minimize the effects of ROS, and natural antioxidants are of high interest.

Reducing power can be attributed mainly to the bioactive compounds associated with antioxidant activity. These bioactive compounds include the vitamin E derivative Trolox, flavonoids from the bark of Pinus Pinaster Pycnogenol, the leaves of *Ginkgo biloba* (EGb 761) (30), and pyridine derivatives that were originally isolated from the fresh roots of Taraxacum (31). This study show that P-w, P-μ, P-n, ginkgo, and Tara have been shown to be good electron donors and could terminate radical chain reactions by converting free radicals to more stable products.

DPPH has been widely used to evaluate the free-radical scavenging effects of various antioxidant substances. Unsaturated lipids in cell membranes are susceptible to peroxidation. This chain reaction is initiated by hydroxyl radicals attacking lipids, and it is extended by the generated lipid hydroperoxide free radicals. It has been reported that extract of ginseng exhibits scavenging activity against DPPH radicals *in vitro*, and that this extract shows scavenging activity for hydroxyl radicals to prevent lipid peroxidation (32). In this study, the three kinds of pearl powders and ginkgo showed similar scavenging activity against DPPH radicals, and the free-radical scavenging effects of P-μ and P-n were greater than those of P-w.

Iron is known as the most important lipid oxidation pro-oxidant due to its high reactivity. The ferrous state of iron accelerates lipid oxidation by breaking down hydrogen and lipid peroxides into reactive free radicals. In the presence of chelating agents, the complex formation is disrupted, resulting in a decrease in oxidation reactions. It is reported that the methanolic extracts (MEs) of various processed tomatoes at a concentration of 2 mg/ml could reach more than 65% ferrous ion chelating ability (FICA). The use of soybean sprout extract at a concentration of 3 mg/ml was required to obtain the same level of FICA. MEs from medicinal mushroom of Chang-chih could reach a FICA of 64.4–74.5% at 5 mg/ml (33). In comparison, three kinds of pearl powders at a concentration of 1 mg/ml reach a FICA of 80%.

CONCLUSIONS

From the above, it can be understood that the pearl powder has fine moisturizing efficiency for human skin. It can improve not only the barrier action for the moisturization of the skin but also the hydration of the skin. P-μ, in particular, has better hydration than P-w. The three kinds of pearl powders can also significantly inhibit the activation of tyrosinase. Moreover, the three kinds of pearl powders have strong capacities for restraining and removing free radicals. More remarkably, in tests of reducing power and DPPH for scavenging free radicals, P-μ and P-n showed better performance than P-w, whereas P-n showed better performance than P-μ. These results provide sufficient scientific proof for the efficacy of pearl powders for beauty treatments, resistance to aging, and clinical treatments, among other applications, and help us to use natural pearl powders to benefit the people.

REFERENCES

(1) D. Tsukamoto, I. Sarashina, and K. Endo, Structure and expression of an unusually acidic matrix protein of pearl oyster shells, *Biochem. Biophys. Res. Commun.,* **320,** 1175–1180 (2004).

(2) C. Zhang, L. Xie, J. Huang, X. Liu, and R. Zhang, A novel matrix protein family participating in the prismatic layer framework formation of pearl oyster, Pinctada fucata, *Biochem. Biophys. Res. Commun.,* **344,** 735–740 (2006).
(3) L. H. Zhang, J. L. Wang, H. P. Mei, and B. Luo, Beauty effect and health care efficacy of pearl, *Deterg. Cosmet.,* **25,** 177–180 (2002).
(4) C. P. Suja and S. Dharmaraj, *In vitro* culture of mantle tissue of the abalone Haliotis varia Linnaeus, *Tissue Cell.,* **37,** 1–10 (2005).
(5) Y. Tokiwa, M. Kitagawa, and T. Raku, Enzymatic synthesis of arbutin undecylenic acid ester and its inhibitory effect on mushroom tyrosinase, *Biotechnol. Lett.,* **29,** 481–486 (2007).
(6) J. Busciglio and B. A. Yankner, Apoptosis and increased generation of reactive oxygen species in Down's syndrome neurons in vitro, *Nature,* **378,** 776–779 (1995).
(7) G. R. Zhao, Z. J. Xiang, T. X. Ye, Y. J. Yuan, and Z. X. Guo, Antioxidant activities of Salviamiltiorrhiza and Panax notoginseng, *Food Chem.,* **99,** 767–774 (2006).
(8 B. H. Havsteen, The biochemistry and medical significance of the flavonoids, *Pharmacol. Ther.,* **96,** 67–202 (2002).
(9) T. S. Wu, C. C. Hwang, P. C. Kuo, T. H. Kuo, A. G. Damu, and C. R. Su, New neolignans from Spiraea formosana, *Chem. Pharm. Bull.,* **52,** 1227–1230 (2004).
(10) L. J. Meyer and R. Stern, Age-dependent changes of hyaluronan in human skin, *J. Invest. Dermatol.,* **102,** 385–389 (1994).
(11) H. Scholtyssek, W. Damerau, R. Wessel, and I. Schimkek, Antioxidative activity of ginkgolides against superoxide in an aprotic environment, *Chem. Biol. Interact.,* **106,** 183–190 (1997).
(12) H. J. Jeon, H. J. Kang, H. J. Jung, Y. S. Kang, C. J. Lim, Y. M. Kim, and E. H. Park, Anti-inflammatory activity of Taraxacum officinale, *J. Ethnopharmacol.,* **115,** 82–88 (2008).
(13) K. K. Song, L. Qiu, H. Huang, and Q. X. Chen, The inhibitory effect of tyrosinase by arbutin as cosmetic additive, *J. Xiamen. Univ.*, **42,** 791–794 (2003).
(14) X. L. Piao, S. H. Baek, M. K. Park, and J. H. Park, Tyrosinase-inhibitory furanocoumarin from Angelica dahurica, *Biol. Pharm. Bull.,* **27,** 1144–1146 (2004).
(15) Y. V. Yuan and N. A. Walsh, Antioxidant and antiproliferative activities of extracts from a variety of edible seaweeds, *Food Chem. Toxicol.,* **44,** 1144–1150 (2006).
(16) S. Rout and R. Banerjee, Free radical scavenging, anti-glycation and tyrosinase inhibition properties of a polysaccharide fraction isolated from the rind from Punica granatum, *Bioresour. Technol.,* **98,** 3159–3163 (2007).
(17) Y. L. Lee, M. T. Yen, and J. L. Mau, Antioxidant properties of various extracts from Hypsizigus marmoreus, *Food Chem.,* **104,** 1–9 (2007).
(18) K. Shimada, K. Fujikawa, K. Yahara, and T. Nakamura, Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion, *J. Agric. Food Chem.,* **40,** 945–948 (1992).
(19) T. C. Dinis, V. M. Maderia, and L. M. Almeida, Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers, *Arch. Biochem. Biophys.,* **315,** 161–169 (1994).
(20) P. G. Sator, J. B. Schmidt, and H. Honigsmann, Comparison of epidermal hydration and skin surface lipids in healthy individuals and in patients with atopic dermatitis, *J. Am. Acad. Dermatol.,* **48,** 352–358 (2003).
(21) S. A. Wissing and R. H. Muller, The influence of solid lipid nanoparticles on skin hydration and viscoelasticity—*in vivo* study, *Eur. J. Pharm. Biopharm.,* **56,** 67–72 (2003).
(22) Y. Sunwoo, C. Chou, J. Takeshita, M. Murakami, and Y. Tochihara, Physiological and subjective responses to low relative humidity, *J. Physiol. Anthropol.,* **25,** 7–14 (2006).
(23) H. Tagami, K. Yoshikuni, K. Inoue, M. Yamada, and Y. Iwase, Functional analysis of factors influencing the water content of the stratum corneum in vivo, *Nippon. Hifuka. Gakkai. Zasshi.,* **92,** 1363–1367 (1982).
(24) A. Sanchez-Ferrer, J. N. Rodriguez-Lopez, J. N. Garcia-Canovas, and F. Garcia-Carmona, Tyrosinase: A comprehensive review of its mechanism, *Biochim. Biophys. Acta,* **1247,** 1–11 (1995).
(25) I. Kubo, Y. Yokokawa, and I. Kinst-Hori, Tyrosinase inhibitors from Bolivian medicinal plants, *J. Nat. Prod.,* **58,** 739–743 (1995).
(26) Y. Shi, Q. X. Chen, Q. Wang, K. K. Song, and L. Qiu, Inhibitory effects of cinnamic acid and its derivatives on the diphenolase activity of mushroom (Agaricus bisporus) tyrosinase, *Food Chem.,* **92,** 707–712 (2005).
(27) K. Maeda and M. Fukuda, *In vitro* effectiveness of several whitening cosmetic components in human melanocytes, *J. Soc. Cosmet. Chem.,* **42,** 361–368 (1991).

(28) S. Y. Seo, V. K. Sharma, and N. Sharma, Mushroom tyrosinase: Recent prospects, *J. Agric. Food Chem.,* **51,** 2837–2853 (2003).
(29) I. Gulcin, Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid), *Toxicology,* **217,** 213–220 (2006).
(30) P. Voss, L. Horakova, M. Jakstadt, M. Kiekebusch, and T. Grune, Ferritin oxidation and proteasomal degradation: Protection by antioxidants, *Free Radic. Res.,* **40,** 673–683 (2006).
(31) Y. L. Leu, Y. L. Wang, S. C. Huang, and L. S. Shi, Chemical constituents from roots of Taraxacum formosanum, *Chem. Pharm. Bull.,* **53,** 853–855 (2005).
(32) C. H. Jung, H. M. Seog, I. W. Choi, H. D. Choi, and H. Y. Cho, Effects of wild ginseng (Panax ginseng C. A. Meyer) leaves on lipid peroxidation levels and antioxidant enzyme activities in streptozotocin diabetic rats, *J. Ethnopharmacol.,* **98,** 245–250 (2005).
(33) C. H. Chang, H. Y. Lin, C. Y. Chang, and Y. C. Liu, Comparisons on the antioxidant properties of fresh, freeze-dried and hot-air-dried tomatoes, *J. Food Eng.,* **77,** 478–485 (2006).