Journal of Functional Foods 44 (2018) 112–117

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

Effect of oral administration of collagen hydrolysates from Nile tilapia on the chronologically aged skin

Lin Wang[a], Yu Jiang[a], Xiaoxiao Wang[a], Jing Zhou[b], Haiying Cui[a], Weidong Xu[a], Yuanqing He[a], Haile Ma[a], Ruichang Gao[a,*]

[a] School of Food and Biological Engineering, Jiangsu University, Zhenjiang 212013, PR China
[b] Product Quality Supervision and Inspection Center in Lianyungang, Lianyungang 222006, PR China

ARTICLE INFO

Keywords:
Collagen hydrolysates (CHs)
Skin properties
Chronological aging
Oxidative stress
Nile tilapia (*Oreochromis niloticus*)

ABSTRACT

The oral ingestion of collagen hydrolysates (CHs) seems popular nowadays, but there has been little report on the chronological aging of skin so far. A CHs (average molecular weight = 624.72 Da) was prepared from the skin of Nile tilapia by simulated gastrointestinal digestion in this study. It exhibited high yield (97.04%), digestibility (98.12%) and antioxidant activity towards 1,1-diphenyl-2-picrylhydrazyl radical (half maximal inhibitory concentration, IC_{50} = 2.57 mg/mL) and linoleic acid peroxidation (IC_{50} = 0.36 mg/mL). The safety (4.07 g/kg bw·d) was also confirmed through the feeding of 9-month-old mice for 180 days. More importantly, the visual appearance, histological structure and matrix homeostasis of mice skin were remarkably improved. Furthermore, oxidative stress was alleviated by enhancing the activities of antioxidant enzymes, which was an important reason for the improvement of skin properties. These suggested its potential to be exploited into new functional foods with the effect against chronological aging of skin.

1. Introduction

The skin is the largest organ of the human body and protects the organism against various external damages as a barrier. It is also an organ of sense and endocrine, a regulator of temperature and fluid-electrolyte balance, a biofactory for synthesis, processing and metabolism (Zouboulis & Makrantonaki, 2011). During aging, the skin suffers from progressive morphologic, structural and functional deterioration, and becomes more susceptible to damages and diseases, which include pruritus, ulcer, lichen, herpes, dermatitis, vitiligo, psoriasis, lupus, tumor (eg, basal cell carcinoma, squamous cell carcinoma, malignant melanoma), as well as common lesions (eg, dryness, telangiectasia, purpura, freckling, lentigines, pseudoscars, milia).

Nowadays, the incidence of age-associated skin diseases is rapidly rising due to the aging of the world's population. As far as non-melanoma skin cancers (NMSC) is concerned, more than one million new cases were identified in United States, which is roughly estimated to account for 50% of all cancers (Owczarek, Majewski, & Schwartz, 2011). The incidence was more than five times that of all other cancers combined in Australia (Staples et al., 2006). The incidence rate was 98.86/100000 person-years in England (Lomas, Leonardi-Bee, & Bath-Hextall, 2012). Besides, 78.8% of inhabitants aged 60 years and older in the mainland of China were suffering from skin diseases (Gu, Xiong, & Zhu, 1999). Consequently, the age-associated skin diseases have become a serious financial burden for many countries, and will be an important issue in the following years.

It is a practical and effective approach to fight against the age-associated skin diseases through retarding the aging process, which can be achieved by topical care, caloric restriction, hormone therapy, nutritional supplementation, etc. Nutritional supplementation, such as protein, vitamin, trace mineral and fatty acid, has been demonstrated to have beneficial effects on aging skin (Zague, 2008). Among the nutrients, collagen and its hydrolysates have been attracting particular attention for years. The food enriched in collagen is usually favored by Asian with the expectancy of anti-skin-aging. Nowadays, CHs seems more and more popular, but their anti-skin-aging effect is still not clearly understood.

Skin aging can be attributed to extrinsic (UV-induced) and intrinsic (chronological) factors. The effect of CHs against photoaging has been widely reported in recent years. It could reduce wrinkle, hyperplasia and erythema, increase hydration, elasticity and immunity, inhibit matrix metalloproteinases (MMPs) activities, repair collagen and elastic fibers in photoaging skin (Fan, Zhuang, & Li, 2013; Hou et al., 2012; Pyun et al., 2012; Song, Meng, Cheng, Li, & Wang, 2017; Sun, Zhang, & Zhuang, 2013; Tanaka, Koyama, & Nomura, 2009). It was also reporeted that the CHs could stimulate the proliferation, migration and

* Corresponding author.
E-mail address: ruichanggao@126.com (R. Gao).

https://doi.org/10.1016/j.jff.2018.03.005
Received 21 October 2017; Received in revised form 15 December 2017; Accepted 2 March 2018

hyaluronic acid synthesis of dermal fibroblasts *in vitro* (Haratake et al., 2015; Ohara et al., 2010; Shigemura et al., 2009, 2011). However, there has been little report about their effect on the chronological aging of skin so far, which is a separate process from photoaging and results markedly differences in terms of morphology, structure and function.

Tilapia is the second most important group of farmed fish after carps (Zeng et al., 2009). It is usually processed to fillets accompanied by large amount of by-products, such as skin, scale, bone, etc. In this study, we prepared the CHs from the skin of Nile tilapia (*Oreochromis niloticus*) by simulated gastrointestinal digestion and evaluated the protective effect on the chronologically aged skin of mice. The obtained results suggested that the CHs had significant protective effect on the chronologically aged skin, and could be developed into new nutraceuticals or functional foods.

2. Materials and methods

2.1. Materials

Nile tilapia skin was provided by Haideyi Food, Lianyungang, China. Test kits were bought from Jiancheng Bioengineering Institute, Nanjing, China. AIN-93M purified diet powder was obtained from Trophic Animal Feed High-tech, Nantong, China. Healthy female ICR mice (38 ± 4 g, 9-month-old) were provided by the Laboratory Animal Research Center of Jiangsu University, Zhenjiang, China. Pepsin and pancreatin were purchased from Sigma-Aldrich, St Louis, MO, USA. Whey protein was obtained from Tongji Biologics, Shanghai, China. All the other chemicals were from Sinopharm Chemical Reagent, Shanghai, China.

2.2. Preparation of collagen and CHs

The collagen and CHs were prepared according to our previous reports (Liang et al., 2014; Wang, An, Xin, Zhao, & Hu, 2007). Briefly, the skin was pretreated by 20% NaCl, then the collagen was extracted with 0.5 mol/L acetic acid containing 0.1% (w/v) pepsin at the ratio of 1:100 (w/v) for 48 h under 4 °C. After centrifuging, the collagen was collected by salting-out with NaCl, dialyzed with dialysis tubing (diameter = 28 mm, molecular weight cut off = 8–14 kDa) against 0.1 mol/L acetic acid for 72 h, and then freeze-dried.

The collagen was hydrolyzed at 1:20 (w/v) for 4 h under 37 °C in a shaking incubator of 150 rpm with simulated gastric fluid, which was confected by dissolving 3.2g of pepsin in 7.0 mL of HCl and sufficient water to make 1000 mL (pH = 1.2). Then, pancreatin was added at 1:100 (w/v) after adjusting the pH to 6.8 with NaOH, and the mixture was further incubated for 6 h. After inactivating enzymes and centrifuging, CHs was obtained by spray-drying of supernatant. The CHs was mixed with normal AIN-93M purified diet powder, granulated, and stored at −20 °C until used. The controls were conducted in the same manner, with distilled water and whey protein used instead of collagen.

2.3. Characterization of CHs

The hydroxyproline (Hyp) was determined using the colorimetric method recommended by ISO 3496. The yields of collagen and CHs were estimated by Hyp recovery (Wang et al., 2014). The degree of hydrolysis was determined using the *o*-phthaldialdehyde (OPA) method (Marambe, Shand, & Wanasundara, 2008). The average chain length (ACL) and average molecular weight (AMW) of CHs were calculated by the equation (Mišún, Čurda, & Jelen, 2008): ACL = 100/DH, AMW = ACL × 90. The activities of scavenging 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and inhibiting linoleic acid peroxidation were conducted according to Liang's reports (2014). The amino acid profile was analyzed with an amino acid analyzer (Sykam S-433D, Berchtesgaden, Germany), after hydrolyzing under vacuum with 6 mol/L HCl at 110 °C for 24 h in the presence of 1% phenol (v/v).

2.4. Determination of digestibility of CHs

The mice were randomly divided into two groups of 25 each: control group (normal diet) and treated group (10% CHs in normal diet). They were housed in metabolic cages of 5 each and allowed access to water and food *ad libitum*. After acclimatizing for 2 weeks, the faeces were collected every day for five consecutive days to determine the Hyp content. The digestibility (D) was calculated as follows: $D = (Hyp_i - Hyp_{e(f)})/Hyp_i \times 100$. Where Hyp_i is Hyp intake in CHs on the test diet. $Hyp_{e(f)}$ is the difference between the Hyp excreted in faeces whilst on the test diet and the Hyp excreted in faeces not from ingested CHs.

2.5. Treatment of mice

The mice were randomly divided into 5 groups of 20 each: blank control group (BC, normal diet), whey protein control group (WC, 10% whey protein hydrolysates), low- (LD, 2.5% CHs), medium- (MD, 5% CHs) and high-dose group (HD, 10% CHs). Additionally, 20 newly weaned mice were used as young control group (YC, normal diet). They were allowed access to water and food *ad libitum*. Body weight and food intake were recorded monthly. After 6 months, the mice were sacrificed by cervical dislocation, and viscera and dorsal skin were collected. The viscera index was calculated by the equation: index (g/kg) = visceral weight/body weight. All procedures and ethics were reviewed in advance by the Laboratory Animal Management Committee of Jiangsu University and also met the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.6. Analysis of composition and elasticity of skin

The ash, nitrogen, moisture and fat were determined by ISO 936, 937, 1442 and 1443, respectively. The protein content was calculated from the nitrogen using a conversion factor of 5.95 (Wang et al., 2007). The collagen content was obtained from Hyp with a conversion factor of 7.46 (Kong et al., 2015). The elasticity was measured using a skin elasticity meter (Cutometer SEM 575, Courage and Khazaka, Cologne, Germany) as described by Edwards et al. (2001).

2.7. Assay of oxidative stress of skin

The skin was homogenized with normal saline (1:9, w/v) in ice bath using a homogenizer (Werke IKA T10, Staufen, Germany), and then centrifuged at 2000*g* for 10 min at 4 °C. The supernatant was collected for the assays of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) activities and malondialdehyde (MDA) content with test kits according to instructions.

2.8. Histochemical staining of skin

The skin was fixed in 10% neutral-buffered formalin, embedded in paraffin and sectioned at 5 μm with a slicer (Leica RM2245, Wetzlar, Germany). The slices were subjected to hematoxylin-eosin (H-E), Masson's trichrome and Sirius red staining, respectively. The samples were observed using an ordinary optical or polarized light microscopy (Nikon EPIPHOT 300, Tokyo, Japan). The dermis thickness, density of collagen fiber and ratio of type III to type I collagen were evaluated by the imaging processing software HALCON 12.0 (MVTec, Munich, Germany).

2.9. Statistical analysis

The data were presented as mean value ± standard deviation. Statistical analyses were performed using one-way analysis of variance. Multiple comparisons of means were done by the Duncan's multiple-range test. A P value of < 0.05 was considered significant. All