Journal of Industrial and Engineering Chemistry 25 (2015) 216–221

Contents lists available at ScienceDirect

Journal of Industrial and Engineering Chemistry

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

Antioxidative activity and antiaging effect of carrot glycoprotein

Mi-Jin Lee [a,*], Noh-Hee Jeong [b], Boo-Sik Jang [a]

[a] CNABiotech., Co., Ltd., Cheongwon-gun, South Korea
[b] Department of Engineering Chemistry, Chungbuk University, Cheongju 361-763, South Korea

ARTICLE INFO

Article history:
Received 30 July 2014
Received in revised form 20 October 2014
Accepted 25 October 2014
Available online 3 November 2014

Keywords:
Glycoprotein
Collagen peptide
Antioxidant
Anti-aging
Functional raw material

ABSTRACT

The objectives of this study were to investigate the antioxidant activity and antiaging effect of carrot glycoprotein (hereinafter called as CG), it is compared with the fish scale collagen peptide (hereinafter called as SCP). As a result of analysis of the content of the total flavonoid which is one of ingredients of anti-oxidative action, CG and SCP was 1.35 mg/g and 1.46 mg/g, respectively. In the experiment of oft the radical elimination ability by means of 1,1-diphenyl-2-picryhydrazyl (hereinafter called as DPPH), CG expressed anti-oxidative effect depending on the concentration considering SCP. The Lipid per-oxidation of CG and SCP expressed changes of 9.37%, 36.72%, 51.64% and 0%, 28.93%, 63.84%, respectively, according to the passage of time of 1 day, 3 days and 5 days, so CG and SCP expressed a higher lipid per-oxidation than that of comparison group butylated hydroxyl anisole (hereinafter called as BHA) and vitamin E. In vitro the cytotoxic experiment using the fibroblasts of the dermis of human body, the toxicity didn't appear in the concentration less than CG 1.0 mg/ml, SCP 0.75 mg/ml. In the efficacy experiment to promote the generation of collagen type-1, it appeared that CG promoted the generation of collagen type-1 in more than 0.5 mg/ml concentration and SCP did so in more than 0.3 mg/ml concentration. In the inhibition experiment of matrix metalloproteinase-1 (hereinafter called as MMP-1) expression, CG was reduced meaningfully and statistically all in the concentration of 0.25 mg/ml ($p < 0.05$), 0.5 mg/ml ($p < 0.01$), 1 mg/ml ($p < 0.01$), and SCP showed the inhibition of MMP-1 expression in 0.3 mg/ml ($p < 0.01$). These results express that CG eliminates reactive oxygen species (hereinafter called as ROS), protect cell membrane, and can act as antioxidants and antiaging article in the skin exposed to solar ultraviolet light. Therefore, it is thought that CG can be applied to new material related to antioxidants and antiaging.

Introduction

The skin impaired by UV reduces the quality of collagen is reported, because the manifestation of matrix metalloproteinase (hereinafter called as MMPs) increases in the skin by means of UV, and MMPs plays an important role in photoaging [1]. As aging proceeds in the dermis of the skin, the manifestation of MMPs increases, the breakdown of collagen type-1 is promoted out of extracellula matrix proteins (hereinafter called as ECMPs) composing the dermis of the skin by increased MMP-1 and the quantity of collagen decreases in the skin and fine wrinkle, thick wrinkles, and the decrease of skin elasticity etc. appear. Especially the skin is exposed to solar UV and incurs photooxidative damage continuously induced by ROS of a great reaction. ROS contains $^1O_2$ (single oxygen) •OH (hydroxy radical), O_2^- (superoxide anion radical), H_2O_2 (hydrogen peroxide), and ROO•, RO•, ROOH and HOCl etc. [2,3]. These can be generated in the cells and textures through a diverse process containing high energy radiant ray, photosensitized reactions and some enzymatic reactions [4]. Out of these ROS, $^1O_2$ •OH is known to play an important role in the skin Enzymatic reactions. These speed up skin aging by participating in the destruction of skin oxidants, commencement of lipid peroxidation reaction, oxidation of protein and DNA, side chain cleavage of connective tissue ingredients and the process of wrinkle generation by normal intersect and melanin generation process etc. [5].

Carrot is used for valuable material for plant breeding or the study of cell physiology, and recently the study of physiological activity β-carotene has proceeds continuously, attracting attention. Vitamin A promotes the growth of animals, has relation with the stabilization of skin and mucous membrane, maintains good

* Corresponding author at: R&D Center, 5 Hojuk-Ri, Ocksan-Myun 363-912, Cheongwon-Gun Chungcheong, South Korea. Tel.: +82 0 0432128522; fax: +82 0 0432128524.
E-mail addresses: shuduc@hanmail.net, shuduc@naver.com (M.-J. Lee).

http://dx.doi.org/10.1016/j.jiec.2014.10.037
1226-086X/

health, increases the resistance against germ, becomes the material of the creation of visual purple in rod cell of retina and engages in the visual function to adjust the brightness to be seen in the darkness [6]. In deficiency of vitamin A, night blindness, conjunctival xerosis, ulcer, loss of appetite, keratosis etc. appear according to the lack, It becomes sensitive to infection or shows the keratinization of ependymocyte of respiratory organ or other organs, brings the suspension of growth of bones and changes of shapes, joint pain, chasm of teeth, cavities, the decline of the function of cells forming the dentin of teeth, diarrhea owing to the change of the surface inside a digestive organ, abnormality of bladder and the infection of kidney and bladder [7,8]. And according to the research of Han and others [9], The layer of ethyl acetate fraction out of 5 kinds of fractions of carrot was excellent in the control effect of the proliferation of cancer cells and cancer preventive effect, so it was confirmed that physiologically active substances exist in this fraction layer, and it was reported through various studies that β-carotene had the correlation with the decrease of the risk of chronic diseases like cancer [10–16]. Therefore, in this research we progressed the study on the activation of antioxidative activity such as total flavonoid content, radical scavenging by 1,1-diphenyl-2-picryhydrazyl (hereinafter called as DPPH), lipid peroxidation control effect etc. to enhance utilization value of carrot glycoprotein extracted from carrot. And we attempted to measure antiaging effect with the efficacy experiments to promote the creation of collagen type-1 and MMP-1 manifestation control experiments with the reference of the measurement of cell toxicity using dermal fibroblasts of human body and its results and check the utility as an important material in the functional material industry.

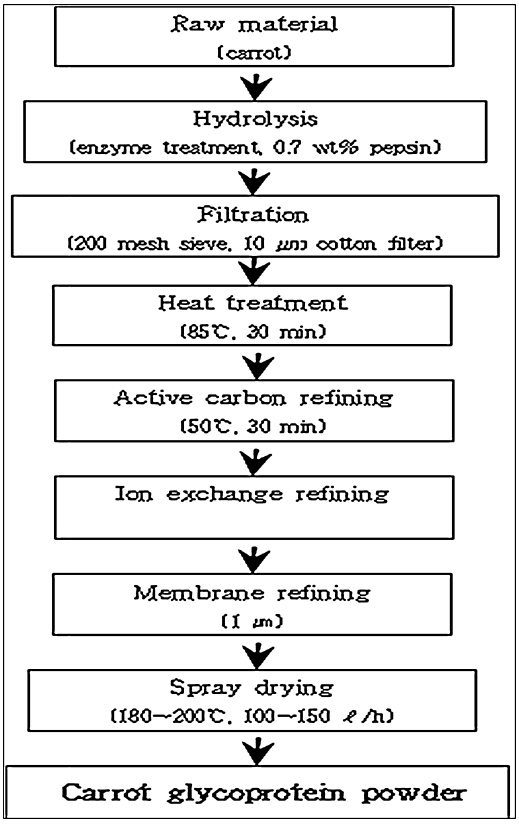

Fig. 1. Preparation process of glycoprotein.

Experimental

Manufacture of carrot glycoprotein

The manufacture of carrot glycoprotein was done as follows; considered Lee's method [17,18], smashed carrot of raw materials, used 10 wt% HCl (pH 2.0) aqueous solution, soaked it at the rate of 1:3, added pepsin of 0.5 wt% versus raw material carrot, stirred it in vibration thermostat on the condition of 50 °C, 200 rpm for 3 h into hydrolysis. With the samples after finishing enzyme hydrolysis we progressed 1st filtration to separate them into extract and residue using 200 mesh sieve. Next we did 2nd decompression filtration of 1st filtration solution by using a filter of the pore size 10 μm, removed impurities, heated the filtrate at 85 °C for 30 min, inactivated remaining enzyme and manufactured carrot glycoprotein. We added activated carbon of 0.3 wt% versus the weight of liquid measure to the extract of manufactured carrot glycoprotein, stirred it in shaking thermostat on the conditions of 50 °C, 200 rpm for 30 min and decolored and deodorized it. We dealt with the deionization of the extract of carrot glycoprotein refined first with the activated carbon treatment using ion-exchange resin with 2nd refinement process to remove the base such as free amino acid and Na^+, H^+, Cl^-, OH^- etc. contained in extract. We decompressed and filtered the extract of carrot glycoprotein of deionized treatment using the membrane filter of pore 1 μm, removed poorly soluble impurities, refined pure carrot glycoprotein, melted maltodextrin in refined solution, dried the water of 100–150 l/h using a spray drier in the condition of the temperature of 180–200 °C, used the carrot glycoprotein powder gained from powdering as the material of this experiment, and the manufacture process was expressed in Fig. 1.

The collagen peptide used as control group was provided by C&A Biotech Co., Ltd. with the collagen peptide extracted from the scale of the tilapia, Vietnamese farmed fish.

Measurement of antioxidative activity of carrot glycoprotein

Measurement of total flavonoid content

The measurement of total flavonoid content is to transform Teresa et al.'s method, add distilled water 1 ml and 5 wt% $NaNO_2$ 75 μl to extract 250 μl, and add 10 wt% $AlCl_3$ $6H_2O$ 150 μl after 5 min, leave it for 6 min and added 1 N NaOH 500 μl. After 11 min, we measured absorbance value of reaction solution at 510 nm [19]. Total flavonoid quantity was to use (+)-catechin hydrate (Sigma Chemical Co. USA) with standard substance like Fig. 2, calculate it from measured calibration curve with the same method as above and get the flavonoid content of carrot glycoprotein and collagen peptide.

Measurement of DPPH radical scavenging activity

DPPH (1,1-diphenyl-2-picrylhydrazyl) is a kind of dyes tinged with violet, and is the method used often in measuring

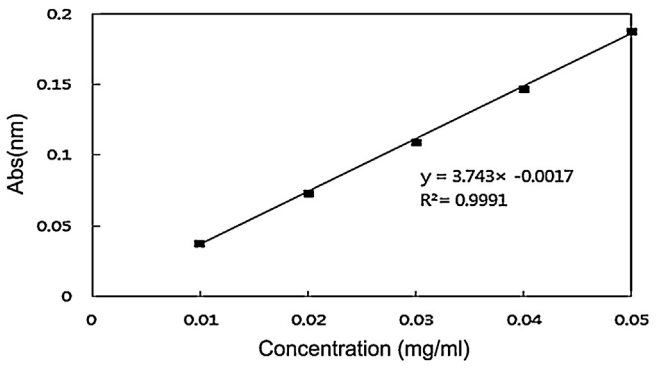

Fig. 2. Calibration curve for (+)-catechin hydrate.