Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2014, 6(8):97-101

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Preparation of anti-aging collagen face mask

Zibin Shu, Siyao Zou and Hua Yang*

College of Chemistry and Materials Science, Sichuan Normal University, 5 Jing An Road, Jin Jiang District, Chengdu, Sichuan, China

---

ABSTRACT

Collagen was extracted from pig skin, and mixed with antioxidants, a moisturizing agent, and a crosslinking agent to prepare anti-aging face mask. The antioxidant activity of olive oil and tea polyphenols (TP) was studied using DPPH• assay. The results show that both olive oil and TP present a clearance effect towards DPPH• radicals. The best clearance effect was obtained when the ratio of olive oil to TP is 1g: 0.6mg. Single factor experiment shows that when the amount of added polyvinyl pyrrolidone was 0.03 g, the antioxidants can be uniformly dispersed in the film. The optimal formulation and processing parameters were obtained for the fabrication of anti-aging face mask from collagen, olive oil, tea polyphenols, lactic acid and glutaraldehyde. The resulting collagen anti-aging face mask present outstanding moisturizing, repairing, whitening, anti-oxidation, anti skin aging effects.

Keywords: collagen, olive oil, tea polyphenols, face mask, antioxidant activity

---

INTRODUCTION

As China gradually entering the era of aging population, research on anti-aging products, and especially cosmetic products has attracted much attention. Among the various cosmetic products, face mask presents many intensive care properties such as whitening, moisturizing, and removing freckles. With the increasing environmental pollution, the face mask with intensive care properties is becoming more and more popular, and its use is fast growing [1]. The skin is located at the surface of the body, and is thus one of the most significant tissues during the body aging process. With people ageing, degradation and crosslinking of collagen fibers and elastic fibers within the dermis will occur, leading to loss of skin moisturizing ability, skin wrinkling and elasticity decrease. Among the mechanisms of ageing, free radical theory and crosslinking theory are currently the most widely adopted one. Radicals can cause lipid peroxidation in the body, oxidation or crosslinking of DNA and RNA, oxidation of polysaccharides, thus leading to tissue damage. Antioxidants exhibit good anti-aging effects since they are able to eliminate these radicals and slow down oxidation.

Hydrolyzed collagen presents similar chemical structure as skin collagen, and good affinity to many compounds. It has been used as additive in many high end cosmetics. Collagen molecules contain a large number of hydrophilic groups such as hydroxyl and amino groups, and thus present excellent moisturizing effect. Hydrolyzed collagen can promote fibroblast proliferation and reduce wrinkles, thus slowing down skin aging. It also presents a whitening effect by reducing UV-induced skin color staining [2].

Olive oil is composed of more than 99% saponified compounds (triglycerides), and some efficient antioxidants. Extra virgin olive oil has been shown to effectively remove superoxide anions from different tissues and reduce their fat peroxides by 70%. It can form an artificial protective layer of sebum which can be easily absorbed by the body, thus effectively maintaining skin elasticity and moist. Olive oil is rich in monounsaturated fatty acids and vitamin E, K, A, D, squalene and phenolic antioxidants, which can eliminate facial wrinkles and prevent skin aging [3]. On the other hand, as one of the most active component of tea polyphenols, catechin presents outstanding antioxidant, anti-aging and anti-radiation activities. Its antioxidant capacity is 4 to 6 times that of synthetic antioxidants such as

butylated hydroxy toluene (BHT), butylated hydroxyanisole (BHA), or tertiary butyl hydroquinone (TBHQ), 6 to 7 times that of vitamin E, and 5 to 10 times that of vitamin C. Moreover, tea polyphenols present no side effects and no smell, in contrast to artificial antioxidants [4].

In this work, we developed a novel anti-aging collagen face mask by addition of tea polyphenols and olive oil in collagen. This face mask presents no side effects to human skin. The anti-oxidants contained in the face mask can be efficiently absorbed due to the good affinity between collagen and skin, thus allowing to remove free radicals and slowing down skin aging.

EXPERIMENTAL SECTION

2.1 Materials

Collagen (laboratory prepared); glutaraldehyde (AR); sodium carboxymethyl cellulose (CP); soluble starch (AR); lactic acid (AR); absolute ethanol (AR); acetic acid (AR); Na_2CO_3(AR); Na_2HPO_4 (AR); NaH_2PO_4 (AR); olive oil (Olivoila extra virgin olive oil)；tea polyphenols (Hangzhou hetian biological technology Co., LTD)；DPPH• (Sigma D9132).

2.2 Methods

2.2.1 Extraction of collagen

Residual fat was first removed from fresh pig skin which was then chopped into small pieces. Degreasing of pig skin was realized in a 10% Na_2CO_3 solution, followed by hydrolysis in a pH 8.0 enzyme solution at 40℃ for 2h. The resulting solution was filtered, and the filtrate was centrifuged.

The collagen solution was finally lyophilized to yield a powder [5][6].

2.2.2 DPPH• assay

1,1-Diphenyl-2-picrylhydrazyl radical (DPPH•) is a nitrogen-centered radical which is stable in organic solvents. DPPH• assay is a simple and rapid method for the analysis of free radicals. In fact, DPPH• solution in ethanol appears dark purple with a strong absorption at a wavelength of 517 nm. The color gradually disappears when radicals in clearance agent make pair with DPPH• in solution. Therefore, the absorption will disappear if other substances provide a radical to make a pair with that of DPPH•. The decrease of absorption is proportional to the quantity of paired radicals [7]. Thus the change in absorbance can be used to assess the ability of antioxidants to eliminate free radicals. DPPH• assay is a rapid, sensitive and simple method to evaluate the antioxidant activity of natural antioxidants, and can be achieved using spectrophotometer.

(1) Preparation of reagents

a) Preparation of tea polyphenols solution

0.3 g tea polyphenols was dissolved in 50 mL of anhydrous ethanol, yielding a TP solution of 6 mg/mL.

b) Preparation of DPPH• solution

0.028 g DPPH• was dissolved in ethanol in a small beaker. The solution is introduced into a 1000 mL volumetric flask, and diluted to a total volume of 1000 mL by addition of ethanol. A 7.1×10^{-5} M DPPH• solution was thus obtained, and conserved in a refrigerator before use.

c) Preparation of olive oil-TP solution

3 g olive oil was separately introduced in 9 small beakers. A predetermined amount (0, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35, 0.4, 0.45 mL) of TP solution previously prepared was then added and homogenized. 0.1 mL of each solution was taken and introduced in a 50 mL conical flask. 5 mL ethanol was added to yield an olive oil-TP solution.

(2) Determination of the clearance rate

2 mL of the various olive oil-TP solutions was separately introduced in a test tube. 2 mL DPPH• solution was then added and homogenized for 30 min. The absorbance of the solutions was measured at 517 nm to give A_1. Similarly, the absorbance of 2 mL olive oil-TP solution mixed with 2 mL ethanol was measured to give A_2, and the absorbance of 2 mL DPPH• mixed with 2 mL ethanol was measured to give A_0. The clearance rate was determined using the following equation:

$$\text{Clearance rate (\%)} = [1-(A_1-A_2)/A_0]\times 100\%$$

Where: A_0 = absorbance of 2 mL DPPH• solution + 2 mL ethanol solution,
A_1 = absorbance of 2 mL DPPH• solution + 2 mL olive oil-TP solution,
A_2 = absorbance of 2 mL olive oil-TP solution + 2 mL of ethanol.

The absorbance was measured using a UV-2802 UV-visible spectrophotometer (Shanghai UNICO Instrument Co., Ltd.).

2.2.3 Preparation of collagen anti-aging face mask

1.0 g sodium carboxymethyl cellulose solution was dissolved in 18 mL water to yield an aqueous solution. 0.1 g collagen was introduced in a beaker, followed by addition of 0.1 g starch, 2 mL lactic acid, 0 ~ 0.04 g PVP, and 3.0 mL glutaraldehyde. The mixture was stirred at room temperature for homogenization. 9.0 g of sodium carboxymethyl cellulose solution was then added in the mixture. The resulting solution was stirred in a 40 ℃ water bath to yield a transparent gel. 0.2 ~ 0.6 mL olive oil-TP solution was added to the gel and homogenized. The viscous mixture was spread on a template, followed by drying at 60℃ in an oven. Finally the film was detached from the template.

2.2.4 Measurements

Gel permeation chromatography (GPC) was realized using 1100/1200 HPLC (Agilent Technologies Inc. USA) equipped with 18 angle laser light scattering (Wyatt Technology Company USA) and Optilab T-rEX differential refractive index detectors, 100 000 aqueous phase column (Japan Shodex OHpak SB-803 HQ), and a guard column (Japan Shodex OHpak SB-G). A pH = 6.0 phosphate buffer of 25 mM was used as the eluent. Measurements were realized at a flow rate of 0.5 mL/min at 35°C.

RESULTS AND DISCUSSION

3.1 Characterization of collagen

The molecular weights of collagen were determined by GPC. The weight-average molecular weight (Mw) and number-average molecular weight (Mn) were 7930 and 7400, respectively. And the polydispersity index (Ip = M_w/M_n) is 1.07.

3.2 Determination of the reaction time between antioxidants and DPPH•

The rate and efficiency of antioxidants to eliminate DPPH• radicals depend on their intrinsic properties. The clearance effect of antioxidants towards DPPH• can be dynamically monitored by measuring the change in absorbance at 517 nm. Olive oil-TP solutions were added in DPPH• ethanol solution, and the absorbance of the mixed solutions was measured until stabilization of the absorbance in order to determine the clearance rate and the reaction time of antioxidants solutions towards DPPH•.

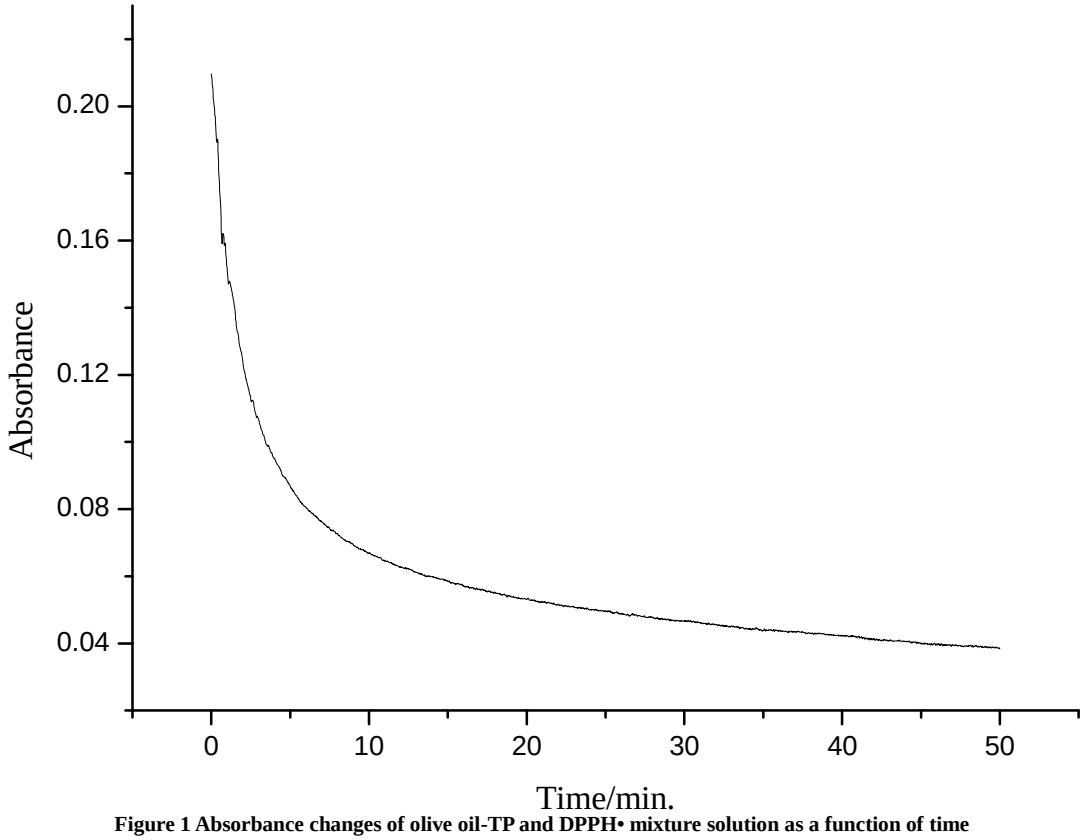

Figure 1 Absorbance changes of olive oil-TP and DPPH• mixture solution as a function of time

Figure 1 shows the absorbance changes of olive oil-TP and DPPH• mixture solution as a function of time. It appears that after addition of antioxidants, the absorbance decreases very rapidly in the first 5 minutes. Later on, the absorbance decrease gradually slows down. In the following experiments, a reaction time of 30 min was selected for the measurements of absorbance.

3.3 Determination of the optimal amount of tea polyphenols

A series of olive oil-TP solutions were prepared, and then mixed with a DPPH• solution. The clearance rate of olive oil-TP mixture towards DPPH• radicals was measured after 30 min.

Table 1 Relationship between the clearance rate and the amount of added TP

No	1	2	3	4	5	6	7	8	9
olive oil/g: TP/mg	1:0	1:0.2	1:0.3	1:0.4	1:0.5	1:0.6	1:0.7	1:0.8	1:0.9
Clearance rate/%	70.6	76.9	80	82.8	85.3	88.2	88.1	87.8	87.3

Table 1 summarizes the clearance rate data. It appears that both olive oil and TP present clearance effect towards DPPH•. In fact, olive oil contains a small amount of vitamin E, polyphenols and squalene which are efficient antioxidants. And catechin as a component of tea polyphenols is a highly active antioxidant. Moreover, olive oil-TP mixture presents better clearance effect to DPPH• than olive oil itself. The clearance rate increases with the increase in the ratio of olive oil to TP, reaching 88% when the ratio of olive oil to TP is 1: 0.6. Beyond, the clearance rate levels off. Therefore, it is assumed that tea polyphenols present a synergic antioxidant effect with olive oil.

3.4 Effect of antioxidants and PVP on the preparation of face mask

During the preparation of the face mask, it was observed that antioxidants leak out to the surface of the film, especially when a large amount of antioxidants was used. Moreover, uneven distribution of antioxidants in the film was detected. That is why polyvinyl pyrrolidone (PVP) was used as a dispersant so that antioxidants can be uniformly dispersed in the film. The optimal amounts of antioxidants and PVP were determined using single factor experiment.

Table 2 Influence of antioxidants and PVP amounts on the preparation of face mask

No	1	2	3	4	5	6	7	8	9
Antioxidants/mL	0.6	0.5	0.4	0.3	0.2	0.3	0.3	0.3	0.3
PVP/g	0	0	0	0	0	0.01	0.02	0.03	0.04
Leakage at surface*	+ + + +	+ + +	+ + +	+ +	+ +	+ +	+ +	+	+
Distribution	uneven	uneven	uneven	uneven	uneven	uneven	even	even	even

** Leakage at surface is noted using "+": more "+" means more leakage.*

As shown in Table 2, the obtained face mask appeared non-greasy with uniform distribution when the amount of added anti-oxidant was 0.2 ~ 0.3mL, and that of added PVP was 0.03 ~ 0.04g.

3.5 Role of other ingredients in the face mask

The other components also play important roles in the preparation of the collagen face mask. Sodium carboxymethyl cellulose is the main component of the film. Starch contributes to enhance the strength and toughness of the material. Lactic acid is added to greatly improve the moisturizing properties of the face mask, and glutaraldehyde plays the role of crosslinker to improve the film strength.

CONCLUSION

The present work examined the antioxidant activity of olive oil-TP using DPPH• assay. It was observed that both olive oil and tea polyphenols present clearance effect towards DPPH• radicals. The highest clearance rate and antioxidant effect were obtained when the ratio of olive oil to TP is 1g: 0.6mg.

In this experiment, PVP was used as a solid dispersant to improve the distribution of antioxidants. Single factor experiments show that when the amount of added PVP was 0.03 g, the antioxidants can be uniformly dispersed in the film.

This work studied the preparation process of anti-aging collagen face mask by mixing collagen with olive oil, TP, lactic acid and PVP. The optimal formulation and preparation conditions were obtained as follows: 0.1 g collagen, 0.1 g starch, 2 mL lactic acid, 0.03 g PVP, and 3.0 mL glutaraldehyde are mixed by stirring at room temperature; 9.0 g sodium carboxymethyl cellulose solution is then added to the mixture. A transparent gel is obtained by stirring at 40℃. 0.3 mL antioxidants (olive oil: TP =1g: 0.6mg) is added to the gel. The mixture is homogenized and spread on

the template, followed by drying in an oven at 60 ℃ to yield an anti-aging collagen mask.

The novel anti-aging collagen face mask presents outstanding moisturizing, repairing, whitening, anti-oxidation, anti skin aging effects, and is a promising anti-aging and repairing face mask.

Acknowledgments
This work was supported by the Scientific Research Fund of the Education Department of Sichuan Province (Grant No. 11ZA095).

REFERENCES

[1] Y Yang; ZY Li; XY Li, *Detergent and Cosmetics*, **2013**, 36(6), 3-5.
[2] ZF Zhao; AQ Liu, *China Bio-Beauty*, **2010**, (1), 58-62.
[3] CY Zhong, *Journal of Chemical Industry of Forest Products*, **2005**, 39(6), 34-38.
[4] CL Wang; JD Li, *Chemical Engineer*, **2012**, (9), 57-59.
[5] H Yang; XX Chen; ZB Shu; XF Guo; RH Gao; SY Zhou; YC Yan, *J. Chem. Pharm. Res*., **2013**, 5(11), 655-658.
[6] H Yang; Y Jiang; SL Ding; H Wang; J Liu; LM Bi; ZB Shu, *J. Chem. Pharm. Res.*, **2014**, 6(5), 1035-1039.
[7] J Wei; JJ Lu; HT Mo; LP Guo, Guang Xi *Journal of Light Industry*, **2006**, 22(1), 11-14.