

Research on Detection Methods and Applications of Natural Whitening Components in Cosmetics

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Abstract. With the improvement of people's living standards, cosmetics have been widely used. The addition of whitening ingredients in cosmetics is necessary. Natural whitening ingredients have the advantages of small side effects, strong efficacy and high safety, but excessive addition is harmful to the human body. Therefore, the detection of natural whitening ingredients in cosmetics is very important. This paper summarizes the research on natural whitening ingredients detection technology, and compares the advantages and disadvantages of high-performance liquid chromatography, pre-column/ post-column derivatization high performance liquid chromatography, thin layer chromatography and thin layer scanning method. We extract polyphenols from natural plants and thymine from natural animals as ingredients of cosmetics.

1. Research background

1.1 Introduction of natural whitening ingredients

Cosmetics are chemical industrial products or fine chemical products that are spread to any part of the human body surface, such as skin, hair, lips and teeth, by smearing, spraying or other similar methods, for the purpose of cleaning, maintaining, cosmetic, modification and change of appearance, or to correct human odor and maintain good condition.

Natural cosmetics have a long history and are well documented in Li Shizhen's *Materia Medica* and some ancient texts. It is reported that more than 500 kinds of Chinese herbal medicines are used in cosmetics developed in China alone, and cosmetics advertisements are also branded as "from natural essence." Natural components refer to the components obtained from plants, animals, microorganisms and minerals, which are processed by crushing, drying, distillation and fermentation without changing the chemical structure of the material. The whitening mechanism is mainly divided into three categories: inhibition of melanin production, blocking melanin transport, and accelerating epidermal cell metabolism. At present, most of the whitening ingredients used in cosmetics are mainly tyrosinase inhibitors. The inhibition mechanism is mainly non-destructive inhibition of tyrosinase, that is, by inhibiting the synthesis of tyrosinase or replacing the substrate of tyrosinase, the purpose of inhibiting melanin formation is achieved. Such as commonly used domestic arbutin, vitamin C and its derivatives, plant flavonoids

and other chemical syntheses of whitening agents have high safety, stable properties, not easily damaged by acid and alkali advantages [1]. Nicotinamide can not only accelerate metabolism, promote the shedding of melanocytes, but also activate an enzyme in the cell, and the product of this enzyme stimulation can reduce the production of melanin [2]. It is a natural antioxidant, and its special physical and chemical properties and specific distribution make its stability significantly better than common antioxidants. This kind of chemical whitening agent has good whitening effect and is relatively safe for the skin.

1.2 The necessity of natural whitening ingredients detection

Beauty is the unremitting pursuit of all women, is the so-called "a white cover all ugly". The history of Chinese women 's pursuit of white skin can be traced back at least a thousand years. Whitening cosmetics were born. These whitening products are directly used for human eyes, oral mucosa and skin, which is related to human health and hygiene. Excessive addition will also endanger human health. Therefore, the quality inspection of cosmetics before they are put on the market is particularly important. The Ministry of Health has set up a special cosmetics safety assessment agency, which has issued detailed national standards for the health and safety of cosmetics. The quality inspection of cosmetics is an important indicator for assessing health and safety. Heavy metal is one of the important safety indexes of cosmetics. The " Regulations on the Supervision and Management of Cosmetics " issued by China in 2020

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divides cosmetics into special cosmetics and ordinary cosmetics. Special cosmetics include cosmetics for hair coloring, perming, freckle whitening, sunscreen, hair loss prevention, and cosmetics that claim new effects. Speckle whitening cosmetics into special use cosmetics implementation management reflects the importance that the regulatory authorities attach to their safety [3].

Commonly used detection methods for natural whitening ingredients include high-performance liquid chromatography, high-performance liquid chromatography tandem mass spectrometry, thin layer chromatography and thin layer scanning. Among them, liquid chromatography is the most common method for the detection of whitening ingredients reported in the literature. Since the determination time of high-performance liquid chromatography is relatively long, and all components may not be completely separated, the advantage of liquid chromatography mass spectrometry that does not require complete separation of

chromatographic peaks is reflected. The pretreatment of liquid chromatography mass spectrometry is basically consistent with that of liquid chromatography. Except for a few functional components such as arbutin, most of the whitening ingredients are difficult to gasify, so there are few reports on the use of gas chromatography or gas chromatography-mass spectrometry [3].

2. Detection methods

We introduce the analytical methods according to the characteristics of different types of substances, such as small molecular natural compounds and large molecular biological substances, etc. Natural whitening ingredients commonly used in detection methods are high-performance liquid chromatography, liquid chromatography, thin layer chromatography, thin layer scanning method. The details are shown in Table 1.

Table 1 Common detection methods of natural whitening ingredients

Method	Components	Mobile phase condition	Results	Literature
High performance liquid chromatography	Nicotinamide	Methanol : water = 1 : 9	Nicotinamide absorbs at both 264 and 214 nm.	[4]
Pre-column / post-column derivatization high performance liquid chromatography	Ergothioneine	Methanol : water = 1 : 9	The optimum reaction temperature after column was 25oC, and the mass concentration of 2-Py-S-S-2-Py solution was 5015 mL-1. The optimum pH of derivatization reaction was 1.	[5]
Thin layer chromatography	Astaxanthin	n-Hexane : acetone = 7 : 2	The recovery rate of astaxanthin was 98.53 %.	[6]
Thin layer scanning method	Arbutin	Methanol : water = 1 : 9	The color was developed by baking with 10% sulfuric acid ethanol solution, and the arbutin showed brown spots.	[7]

2.1 High-Performance Liquid Chromatography

Lin et al. developed a method for the determination of arbutin in whitening cosmetics by online microdialysis sampling combined with high-performance liquid chromatography. The optimal analysis conditions for microdialysis sampling were probe length of 10 mm and dialysis flow rate of 5ul min⁻¹. The accuracy of intra-day (n=6) and inter-day (n= 30, 5 consecutive days) analysis ranged from-8.9 % to 11.5 %, and the relative standard deviation (RSD) of precision was less than 7.64 %. The linear range was 0.1-20 mM (R2 = 0.9989). The detection limit is 15pM. By comparing the content of arbutin in whitening cosmetics determined by this method with the results of no net flux method, it is concluded that the online microdialysis-high performance liquid chromatography system proposed in

this paper has good accuracy. The robustness of the morning best conditions was evaluated by Plackett-Burman design. Except for the effect of low perfusion flow rate (increased by 12.52 + 2.31 %), no significant changes were observed in the analysis after changing the level of any other parameters. Because the online method is simple and reliable, without tedious sample pretreatment process and reducing the use of organic solvents, it is considered to be suitable for the daily analysis of commercial cosmetics. [8]

Sun Weihong et al. established a high-performance liquid chromatography method for accurate determination of astaxanthin content in Antarctic krill and its products. Firstly, the sample was removed by anhydrous MgSO₄, and acetone was used as the extraction solvent. The extract of astaxanthin compounds was purified by dispersive solid phase extraction with N-propyl ethylenediamine filler, saponified by

NaOH-methanol solution, YMC-Carotenoid C3 was used as the chromatographic column, methanol, tert-butyl methyl ether and 1% phosphoric acid aqueous solution were used for purification. The mobile phase was subjected to gradient elution and determined by UV detector [9].

2.2 Thin layer chromatography

Victor et al. reported a method for the simultaneous determination of eight water-soluble B-vitamins in cosmetics. The method is based on liquid chromatography with ultraviolet detection (LC-UV analysis with simple water leaching of the analytes from the cosmetic matrix. No organic solvent is required except ethanol used in the chromatographic mobile phase. The results showed that the method was well linear with detection limits in the low $\mu\text{g mL}^{-1}$ range. (0.14~0.43 $\mu\text{g mL}^{-1}$) with good reproducibility (relative standard deviation less than 11%). The accuracy of the method has been confirmed by the analysis of laboratory samples (i.e. creams and gels) with known analyte concentrations, providing a low relative error (less than 12%). Finally, the method was successfully applied to four different formulations of cosmetic samples without significant matrix effects. The results indicate that the method is environmentally friendly and can be used for pre- and post-market quality control of final cosmetic products [10].

Huang et al. used high-performance thin layer chromatography to determine the content of astaxanthin in Antarctic krill oil. In this study, n-hexane: acetone (7:2) as mobile phase, high-performance silica gel as stationary phase, the silica gel can be completed within 10 min separation, combined with the scanner for optical density analysis, astaxanthin recovery was 98.53 % [11].

2.3 Thin-layer scanning method

Tang Xinwen et al. used thin-layer scanning to determine the content of arbutin in Mao Dading grass. Silica gel G thin-layer plate was spot-spotted, and the spotting volume was 2 μL ; the developing solvent was ethyl acetate butanone-formic acid-water (10:1:1:1), and the spread was 8.0 cm; sprayed with 10% ethanol sulfate solution and baked at 105°C for 3 min to locate the brownish spots. The sample solution was prepared and chromatographed by reflection method with double wavelength sawtooth scanning, the size of the sawtooth was 8mm*0.05mm, the slit was 0.4mm*0.4mm; $\lambda_s=420\text{nm}$, $\lambda_R=700\text{nm}$, and the sample solution was prepared and chromatographed by taking 2.00g of crude powder of Mao Da Ding Cao in a triangular flask with stopper, adding 40ml of methanol, and after ultrasonic treatment for 30 min, the solution was weighed at room temperature, and the lost mass was supplemented with methanol, and the subsequent filtrate was collected. Precisely measure 10 ml of the subsequent filtrate, evaporate to dryness in a water bath, transfer the residue with methanol, and dilute to a 5 mL volumetric flask as

the test solution. Under the proposed TLC analysis conditions, the thin-layer chromatographic separation of Mao Da Ding Cao was carried out, and then sprayed with 10% sulfuric acid ethanol solution for baking and positioning. The R_f value of arbutin was 0.33 and scanned at wavelengths of $\lambda_s=420\text{nm}$ and $\lambda_R=700\text{nm}$. A variety of developing systems were selected in the experiment. A small amount of acid in the developing agent has a certain influence on the separation of arbutin on the silica gel G plate. and the spots trailed in the unfolding system without acid, and the spots were concentrated and did not trail after adding 1% formic acid, and the separation was good. Since arbutin is a phenolic glucoside, color developers such as 3% ferric chloride reagent and 5% phosphomolybdic acid reagent were selected, but some spots faded and were unstable after color development, and some backgrounds were too dark and not visible. Conducive to thin layer scanning, these two color developers are not suitable. Finally, the color was developed by baking with 10% sulfuric acid ethanol solution, and the arbutin was brown spots. In the test, the baking temperature and time must be strictly controlled to ensure uniform baking, so as to obtain a better positioning effect [7].

2.4 Pre-column/post-column derivatization high performance liquid chromatography

In recent years, pre-column/post-column derivatization high-performance liquid chromatography has been increasingly used in the determination of ergothioneine. The main applications of derivatization techniques in the determination of ergothioneine are pre-column derivatization and post-column derivatization. The pre-column derivatization method has the advantages of a wide range of derivatization reagents, short derivatization time, simple operation and high sensitivity, but the derivatization by-products can interfere with the chromatographic separation. The post-column derivatization method effectively avoids the interference of other substances and is suitable for the analysis and determination of ergothioneine in complex samples, with the advantages of good repeatability and stable derivative products, but the detection sensitivity is low. Nguyen et al. used 2-Py-S-S-2-Py as a derivatization reagent to establish a post-column derivatization high-performance liquid chromatography method for the quantitative determination of ergothioneine in the fruitbodies of Songkou, Agaricus bisporus, Auricularia auricular, Grifola frondosa, Tabby mushroom, Lentinula edodes, Pholiota nameko and Pleurotus eryngii. The mobile phase was 10 % methanol, the flow rate was 0.15 ml min⁻¹, and the optimum reaction temperature after the column was 25 ° C. The optimum pH for the derivatization reaction of 2-Py-S-S-2-Py solution at a mass concentration of 50 $\mu\text{g mL}^{-1}$ is 1 [5].

3. Application of natural whitening ingredients in cosmetics

3.1 Application of natural plant ingredients in cosmetics

Polyphenol is a plant component which can effectively inhibit tyrosinase activity and has a phenolic hydroxyl structure. Most plant extracts or monomeric components are studied to inhibit mTYR activity, rather than hTYR activity. Since the homology and structural differences between mTYR and hTYR are significant, and the inhibition activities of most commonly used tyrosinase inhibitors differ greatly between the two, many plant extracts or monomer components are used as whitening ingredients in cosmetics. Further experiments are needed to determine the whitening effect more accurately. In addition, due to the components of plant extracts are complex, and most of them have relatively poor inhibition of tyrosinase activity or cannot inhibit its activity; even though a variety of components in plant extracts have a synergistic effect on the inhibition of tyrosinase activity, due to their relatively low content, the inhibition of tyrosinase activity by plant extracts is usually less than that of monomeric components. [12].

3.2 Application of natural animal components in cosmetics

Thymidine can be extracted from calf thymus or porcine thymus, which is clinically used to help treat primary and secondary immunodeficiency diseases and diseases caused by immune dysfunction, and has better anti-aging [13], anti-stress and anti-viral effects [14]. With social progress, technological development and improvement of living standards, people are more in pursuit of natural and green health, and cosmetics have become an indispensable part of people's daily life. The efficacy of functional cosmetics is developed with the targeted skin problems of the audience groups, mainly targeting the sub-health problems of the skin. Therefore, animal thymus extracts, which are widely used clinically to increase immunity, were explored in a refined manner. It was demonstrated that animal thymus extracts are non-cytotoxic: they have some reactive oxygen species inhibition ability and gradually increase antioxidant capacity as the content increases: they have some whitening effect at lower levels and have good ability to increase body immunity, so they can be used as a penetration aid to accelerate the absorption rate of whitening ingredients and are a cosmetic ingredient with multifunctional properties [15].

In summary, natural whitening ingredients are popular and widely used because of their high safety. In order to avoid the harm to human beings caused by excessive addition of ingredients, the detection methods of natural whitening ingredients should be continuously improved. The efficacy and safety of natural cosmetic applications should be improved.

4. Conclusion

With the development of modern society and the economy, people pay attention to whitening cosmetics not only in their efficacy, but also in safety. Cosmetics containing natural whitening ingredients have the advantages of anti-aging, whitening and safety, and thus are popular among the public. This paper reviews the detection methods and necessity of natural whitening ingredients, as well as the application of natural whitening ingredients in cosmetics in plants and animals that have far-reaching implications for future development. Therefore, it is very important to further explore the application of natural whitening ingredients as well as to improve their detection methods, which are important directions and topics for the development of natural whitening ingredients in cosmetics.

References

1. Liu Wei. 2016. Research on the application of natural whitening ingredients in whitening cosmetics. 13(47): 47-53.
2. Hou Cong, Ji Xuejiao. 2020. Uncovering the Mystery of "Niacinamide". Meikai. 115-118.
3. Gu Yuxiang, Zheng Yi, Yang Yimei. 2022. Detection methods and usage of commonly used whitening ingredients in cosmetics. *Perfume, Flavor and Cosmetics*. 1: 81-85.
4. Cheng Peng, Chen Meilan, Zhu Yan. 2010. Determination of α -B-arbutin and nicotinamide in cosmetics by high performance liquid chromatography. *Chromatography*. 1: 89-92.
5. Feng Lulu, E Hengchao, Zhang Yanmei, Li Xiaobei, Zhou Changyan, Zhao Xiaoyan, Ren Jiali. 2021. Research progress on extraction, separation and detection methods of ergothioneine in edible fungi. *Journal of Edible Fungi*. 28(1): 115-123.
6. Gao Yan, Xing Lihong, Sun Weihong, Wu Xuqian, Zu Lu. 2020. Extraction, purification and quantitative detection of astaxanthin from different sources. *Journal of Food Safety and Quality Inspection*. 11(51): 1414-1423.
7. Tang Xinwen, Liu Tingjiang. 2009. Determination of arbutin in *Gerbera gerbera* by thin-layer scanning method. *Drug Identification*. 18(2): 29.
8. Cheng-Hui Lin, Hsin-Lung Wu, Yeou-Lih Huang. 2005. Microdialysis sampling coupled to on-line high performance liquid chromatography for determination of arbutin in whitening cosmetics. *Journal of Chromatography B*. 829 (1 -2): 149-152.
9. Sun Weihong, Xing Lihong, Leng Kailiang, etc. 2017. Determination of Astaxanthin in Krill and its Products by High Performance Liquid Chromatography. *Journal of Food Safety and Quality Inspection*. 8(4): 1248-1253.
10. Victor Vallez- Gomis, Guillem Peris- Pastor, Juan L. Bened e, Alberto Chisvert, Amparo

- Savador. 2021. Green determination of eight water-soluble B vitamins in cosmetic products by liquid chromatography with ultraviolet detection. *Journal of Pharmaceutical and Biomedical Analysis*. 205:114308.
11. Huang M, Liu D. 2013. High-performance thin layer chromatography determination of astaxanthin in *Euphausia superba* oil. *Chem Nat Compd*. 49(1): 145-147.
 12. [12] Ren Qianqian, Wu Hua, Jin Jianming. 2021. Cosmetic plant raw materials - research and development of plant whitening raw materials that inhibit tyrosinase activity. *Daily Chemical Industry*. 51(3): 178-185.
 13. [13] Wang Huiyan, Dao Jian, Geng Xiaofeng. 1999. The effect of thymosin on the reduction of immune function in aged rats caused by psychological stress. *Acta Psychologica Sinica*. 03: 330-336.
 14. [14] Lu Hongzhi. 2008. Research on the extraction process of thymus makeup and the stability of its preparation. *Journal of Yueyang Vocational and Technical College*. 05: 90-94.
 15. [15] Zhang Yao, Wu Zonghan, Li Gaocong, Qiu Yan, Zhang Yuchen. 2022. Study on the cytotoxicity, antioxidant capacity and whitening effect of (animal) thymus extract. *Guangdong Chemical Industry*. 7(49): 77-80.