



Title	Inhibitory effect of grape skin extracts on DNA damage caused by UV irradiation and development of cosmetics using the waste skin [an abstract of dissertation and a summary of dissertation review]
Author(s)	宋, 雨桐
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# 学位論文内容の要旨

博士 (環境科学)

氏名 宋雨桐

## 学位論文題名

Inhibitory effect of grape skin extracts on DNA damage caused by UV irradiation and development of cosmetics using the waste skin

(紫外線照射によるDNA損傷に対するブドウの皮の抽出物の抑制効果および  
廃棄果皮を用いた化粧品の開発)

Continuously increased environment pollution caused by human activities leads to human are more likely to suffer from ultraviolet (UV) radiation due to the impacts on appearance of hole in the ozone layer. UV irradiation is the main cause of skin damage, and this damage eventually contributes to some health diseases, including rosacea, urticaria, and cancer. In early studies, antioxidant or anti-inflammatory agents were effective on suppress the damage in skin caused by overexpose to UV radiation. Since it have been extensively reported, phenolic compounds which rich contain in fruits, plants, and herbs has aroused quite a lot of concern. Because there is increasing evidence that phenolic compounds has excellent properties in inflammation remission via impact biochemical pathways, DNA mutagenesis decrease, scavenge activity of free radical affiliated with disease prevention. Unfortunately, it is un-hardly to concern the uncertainty risk about long-term use of synthetic phenolic compounds led to plant extracts such as strawberry, grape, bean received more expectation due to a mounts of phenolic compounds are also contained. Grape is one of the most widely cultivated plants and popular for richest polyphenols which mainly use for wine-making. During the industrial manufacture of wine, large amount of grape skin and seed are remained, and then become industrial wastes. Grape skin extract (GSE) come from these grape residues can be expected act as a natural source with potential antioxidant effect in protect skin avoid from damage of UV light. Therefore, the aim of this study is evaluation the inhibition effect of GSE in damage of skin caused by UV overexposure and development GSE participate in cosmetic formulation.

Normal human epidermal keratinocytes (NHEK) cells added with extraction of Zweigelt (a species of red grape) and Niagara (a piece of white grape) skins expose to UV irradiation at 10 mJ / cm<sup>2</sup> (250 nm) and 15 mJ / cm<sup>2</sup> (290 nm) were examined. The data showed decreased cell viability due to UV irradiation was improved by adding Niagara or Zweigelt skin extract. Generation of cyclobutane pyrimidine dimer (CPD) is attributing to triggered DNA mutagenesis by absorbed UV radiation. It makes CPD is regard as an indicator of

DNA damages induced by UV irradiation. The ELISA result of Chapter 2 means the CPD formation was increased by UV irradiation, and the increased DNA damage was improved significantly by addition of Niagara or Zweigelt extract. After then, in Chapter 3, to deeply confirm the effects of GESs in diminishing UV-induced cytotoxicity in NHEK cells. Four different kinds of UV doses: 10 mJ/cm<sup>2</sup> at 250 nm, 10 mJ/cm<sup>2</sup> at 270 nm, 15 mJ/cm<sup>2</sup> at 290 nm and 500 mJ/cm<sup>2</sup> at 310 nm were employed. As results, it was confirmed again that GSE protects against DNA damage-induced cell death. Optimal extract condition of GSE by compare the level of CPD formation was determined as 80% ethanol for 24 h at 60 °C using Zweigelt grape skin. Zweigelt (red grape) skin improved significantly DNA damage compared as Niagara (white grape) skin, and in preventing (before irradiation) better than repairing (after irradiation). UV-induced upregulations of Bax/Bcl-2 ratio and cytochrome c in cytosol were evaluated by western blotting, and reduced by GSE treatment. It means that mitochondria pathway in the cells relates to GSE protect mechanism.

Base on the effect of Zweigelt grape skin extract in suppressing the DNA damage caused by UV irradiation, applying it to cosmetics and pharmaceuticals was considered. To accomplish this aim, in Chapter 4 grape seed extract (GEE) was studied for comparison the suppression ability of GSE in remission DNA damages caused by UV irradiation. For further practical industrial application, GSE and GEE were collected in different days of producing wine process: days 0, 5, 8, and 10. It was shown that GSE (Day 0) was effective for the expression level of longevity gene and suppressing CPD generation, but not as much as GEE (Day 5). Using the GSE, a prototype cream with UV protection effect was made. Its protection effect of UV against was comparing with two commercially available products which have reported, then the prototype showed a sufficient protecting effect.

In conclusion, the extract from grape skin, which was a waste product of the wine manufacturing process, has the effect of suppressing DNA damage by UV irradiation. The cream martial with GSE could be an efficiently and securely application in protect skin avoid from skin issues caused by emitted UV from the sunlight.