GLUTATHIONE LEVELS IN THE FIVE HEALTHY FRUITS FOR SKIN CARE

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ABSTRACT

Glutathione, a tripeptide composed of glycine, cysteine, and glutamic acid, is one of the most abundant low molecular weight cellular thiols in mammalian tissues. It is an important endogenous antioxidant in many organisms including human. In addition to its many biological functions including maintenance of intracellular redox activities, xenobiotic metabolism, signal transduction, and gene regulation, glutathione has been associated with skin whitening ability. The role of glutathione as a skin whitener was discovered as a side effect of large doses of glutathione. So, the objective of this work was to study the glutathione level and compare between the five fruits known as healthy fruits for skin care by using Ellman's reagent, a chemical used to quantify the concentration of thiol groups in a sample. The results showed that the glutathione levels (mg/ 100g fresh mass) were 11.58 ± 1.32, 10.5 ± 3.28, 5.5 ± 1.06, 2.46 ± 0.13 and 1.46 ± 0.09 for orange, strawberry, lemon, avocado, and tomato respectively.

Keywords—Ellman's reagent, Glutathione, Healthy fruits

INTRODUCTION

Skin is the largest organ of the human body, and its functions include protection, sensory detection, and thermal regulation. People suffer from many skin problems, such as mottled pigmentation, wrinkles, photoaging, acne, and skin cancer. Skin diseases are usually caused by overexposure to ultraviolet radiation. However, one of the most dermatologic concerns is skin aging that involves degradation of extracellular matrix in both the epidermal and dermal layers that leave visible signs on the surface of the skin. The detailed mechanisms involved in the age-dependent decline of tissue function to produce harmful effects during proteolytic degradation of fiber network [1]. So, in to slow down it, people, especially women, have always taken good care of it. Skin-care is the group of practices that support skin integrity including nutrition, avoidance of excessive sun exposure, and appropriate use of emollients that enhance appearances such as the use of cosmetics, exfoliation, and fillers. Although there are several synthetic skin-care products containing ingredients active against skin aging, they can produce adverse reactions such as allergic contact dermatitis, phototoxic and photoallergic reactions. Therefore, it is necessary to seek out new safe and effective skin-care cosmetic compositions from natural resources. The properties of natural products as good resources for developing skin-care cosmetic agents included their non-toxic and environmentally friendly properties compared to artificial synthetic chemicals. Many herbs, vegetables, fruits and whole grains are currently in demand worldwide due to their impact on skin aging. Notably, fruits and vegetables constitute an important source in the search for metabolites active against skin aging due to their antioxidant capacity and their richness in phenolic compounds, carotenoids, and ascorbic acid. Plants and their secondary metabolites have been widely used in the cosmetic industry as antioxidants, sunscreen agents, and skin whitening agents [2-3]. Glutathione, a tripeptide composed of glycine, cysteine, and glutamic acid, is one of the most abundant low molecular weight cellular thiols in mammalian tissues. It is an important endogenous antioxidant in many organisms including human. In addition to its many biological functions including maintenance of intracellular redox activities, xenobiotic metabolism, signal transduction, and gene regulation [4], glutathione has been associated with skin whitening ability [5]. The role of glutathione as a skin whitener was discovered as a side effect of large doses of glutathione [6]. In plants, glutathione is crucial for biotic and abiotic stress management. It is a crucial component of the glutathione-ascorbate cycle. To monitor the glutathione level, several analytical methods have been developed for many years [7]. The current methods for analyzing GSH in biological samples included high performance liquid chromatography (HPLC), enzymatic method [8] and colorimetric method in which a derivatization step was required to introduce on glutathione either a chromophore moiety (Elman's reagent) [9-10], or a fluorescent one (monobromobimane) permitting its detection by a diode array or fluorescence detector or by combination with electroanalytical method [11]. In this work, to understand more about the relation of glutathione level between the five fruits (Orange, strawberry, lemon, avocado, and tomato) that have been reported as healthy fruits for skin-care [3, 12-14], The colorimetric method by using Elman' reagent was used for GSH level determination.

METHODS

Chemicals and Reagents

5,5'-dithiobis-2-nitrobenzoic acid (DTNB), sulphosalicylic acid (SSA) and reduced glutathione (GSH) were purchased from Sigma-Aldrich Co., Ltd. All other solvents and chemicals were of analytical grade.

Sample preparations

The glutathione content in the following fruits was measured, orange (*Citrus sinensis*), strawberry (*Fragaria × ananassa*), lemon (*citrus × limon*), avocado (*Persea americana*), and tomato (*Solanum lycopersicum*). Sample preparation included the preparation of juice. Fruit juice was obtained by squeezing (orange and lemon) or mixing (strawberry, avocado, and tomato) the fruit. After centrifuging the supernatant was guided through a filter (0.45µm) and stored immediately in the freezer.

Glutathione (GSH) assay

A modified M.W. Korir [10] method was used to assay glutathione (GSH) levels in the fruits. Fruit juice was mixed with a solution containing sulphosalicylic acid (5.0% w/v) and 0.25 mM EDTA and the mixture centrifuged at 8000g for 10 min. 200 µM glutathione (GSH) standard solution was prepared in 0.5% sulphosalicylic acid (SSA) and serial dilutions prepared using the same solution (0.5% SSA) to reach final concentrations of 200, 100, 50, 25, 12.5 and 6.25 µM. Ellman's reagent (5,5'-dithiobis-2-nitrobenzoic acid) was prepared by dissolving in 0.1 M potassium phosphate buffer and 5 mM EDTA disodium salt, pH 7.5, to a final concentration of 0.6 mg/ml. 0.5 ml of standard solution or sample were mixed with freshly prepared Ellman's reagent after that the absorbance was measured at 412 nm. A calibration GSH standard curve was prepared which was used to extrapolate the GSH levels from the biological samples.

RESULTS AND DISCUSSION

The existence of GSH caused a yellow color development by the reaction between thiol and DTNB, so the GSH can be detected via measuring the amount of yellow color product (TNB^{2-}). Figure 1 showed the UV-Vis spectra of Ellman 's reagent solutions with the concentrations of 0.0, 6.25, 12.5, 25.0, 50.0, 100.0, and 200.0 µM GSH. It is obvious that the absorbance value at 412 nm (λ_{max}) gradually increase along with GSH concentration.

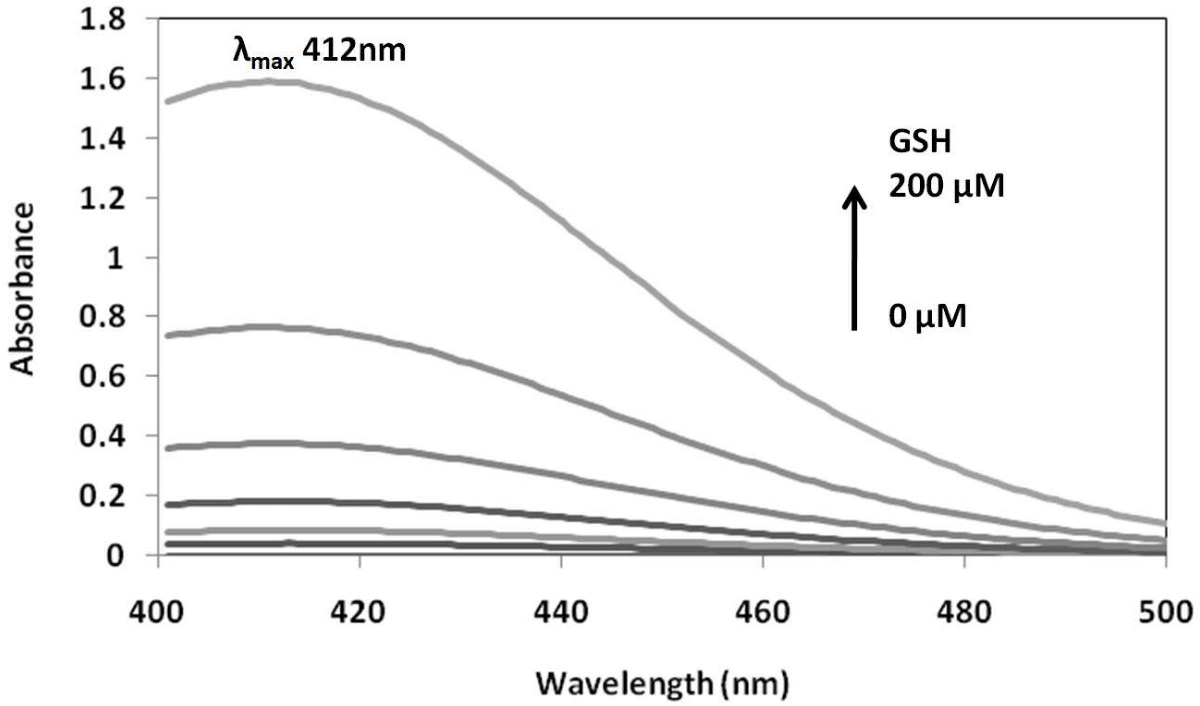

Figure 1 UV-Vis spectra of Ellman 's reagent solutions with the concentrations of 0.0, 6.25, 12.5, 25.0, 50.0, 100.0, and 200.0 μM GSH.

The calibration curve for GSH determination was shown in figure 2. The result indicated that the concentration of detectable GSH could be 6.25 μM with a linear range from 6.25 to 200.0 μM and correlation coefficient (R^2) 0.999.

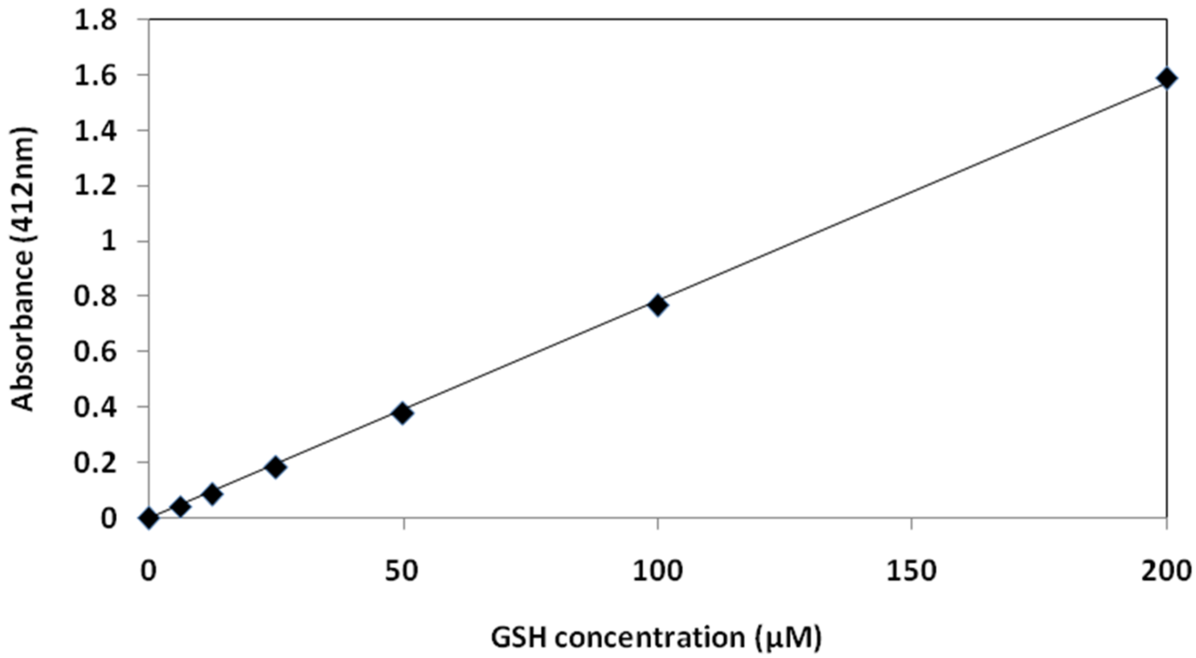

Figure 2 The linear calibration plot for GSH detection.

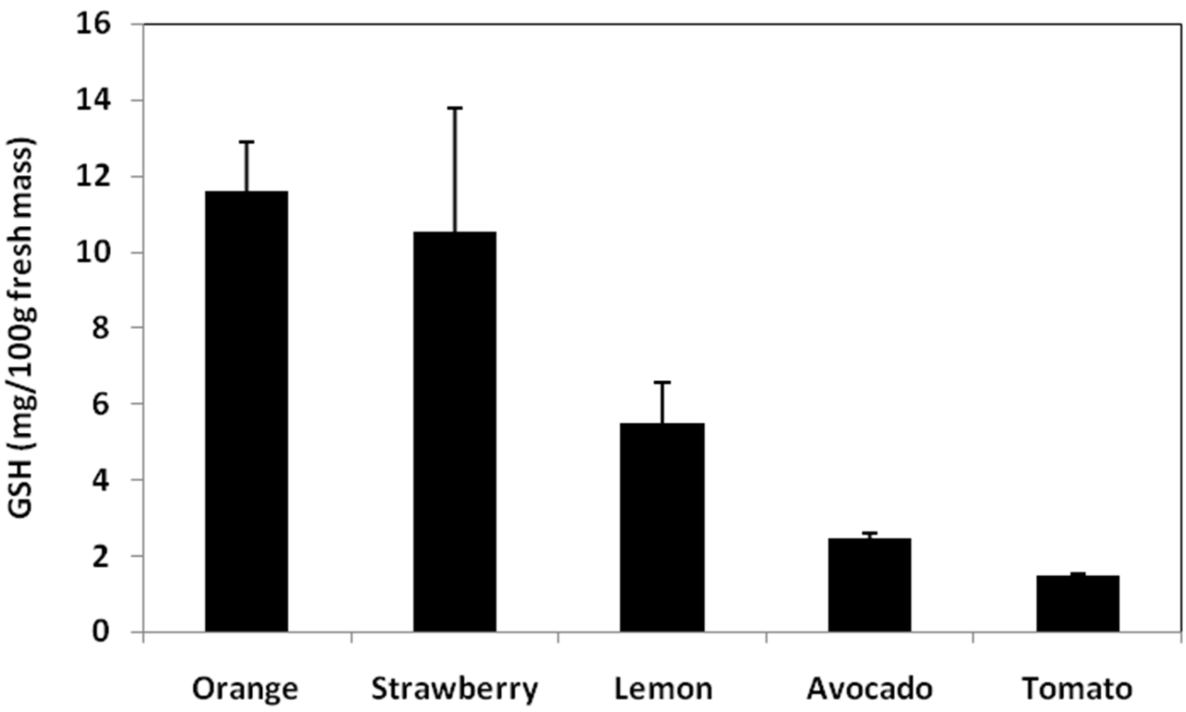

Figure 3 Glutathione levels in the five healthy fruits.

The results from figure 3 showed that the glutathione levels (mg/ 100g fresh mass) were 11.58 ± 1.32, 10.5 ± 3.28, 5.5 ± 1.06, 2.46 ± 0.13 and 1.46 ± 0.09 for orange, strawberry, lemon, avocado, and tomato respectively. These results are in agreement with the report from D.P. Jone [15] except the glutathione levels in avocado and tomato that was too low in this work.

CONCLUSION

To determine the glutathione levels in the five healthy fruits for skin-care by using Ellman 's reagent. The results showed that the glutathione levels were too low when compared to the amount that can be effectively used as the whitening agent.

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