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Biologically Important Thiols in Various Vegetables and Fruits

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Biological thiols are important antioxidants, and recent studies showed that their contents vary depending on the groups of foodstuffs. Therefore, we investigated the levels of some biological thiols in various vegetables and fruits by using a sensitive high-performance liquid chromatography (HPLC) technique. Biological thiols measured in some vegetables and fruits include glutathione (L-glutamyl-L-cysteinly glycine, GSH), N-acetylcysteine (NAC), captopril [CAP ($C_9H_{15}NO_3S$)], homocysteine (HCYS), cysteine (CYS), and γ-glutamyl cysteine (GGC). Our results show that biological thiol contents are between 3−349 nM/g wet weight in vegetables and 4−136 nM/g wet weight in fruits. CAP is only found in asparagus (28 nM/g wet weight). Furthermore, none of the biological thiols analyzed were found in cabbages, red grapes, blackberries, apples, and peaches. Therefore, various vegetables and fruits differ significantly in their thiol contents. Oxidation of these important thiols may occur and result in the production of toxic byproducts, if they are exposed to radiation and ozone treatment for sterilization purposes. Further studies should be performed to monitor the levels of these biological thiols.

KEYWORDS: Thiol; free radicals; vegetables; fruits

INTRODUCTION

There have been several studies indicating that there is a strong correlation between the development of various chronic disorders (e.g., cancer, diabetes, and Alzheimer's) and nutritional habits (*1*). Fruits and vegetables are essential food groups for a healthy life, and routine consumption has shown that they decrease the risk of the occurrence of cancer and cardiovascular diseases (*2*).

The phytochemicals found in fruits and vegetables (along with minerals and vitamins) seem to enhance the nutritional value of these foods. The preventive function of fruits and vegetables against cancer and other chronic disorders might be due to their phytochemical content rather than their vitamin content (vitamin A, vitamin C, and β-carotene) (*3*, *4*). In addition, phytochemicals are assumed to provide an even greater antioxidant action than these vitamins do in the same foods.

Thiols are a type of mercaptan characterized by having a sulfhydryl functional group. Biothiols (or biologically derived thiols) are the most important antioxidants that protect cells from any kind of oxidative damage (*5*, *6*).

One of the widely studied biothiols, glutathione (L-glutamyl-L-cysteinyl-glycine, GSH), is an antioxidant that protects cells against oxidative stress (*7*). It is synthesized from γ-glutamyl cysteine (GGC) by glutathione synthetase. GSH also plays a role in the reductive processes that are essential for the synthesis of proteins and DNA. Its other physiological roles include storage and transport of cysteine (CYS), plus a coenzymatic role in several reactions with foreign compounds (*7*, *8*). CYS and GSH delivery compounds have been used to protect normal cells from antitumor agents and radiation (*9*). Homocysteine (HCYS), a thiol amino acid, is generated from the metabolism of the essential amino acid, methionine. It has recently been suggested that HCYS exhibits its role in the pathogenesis of atherosclerosis through mechanisms involving oxidative stress-induced damage (*10*). Another thiol compound, N-acetylcysteine (NAC), is a precursor of GSH and is an antioxidant as well. NAC has long been used as a mucolytic agent for treating chronic bronchitis as well as being an effective antidote for acetaminophen poisoning and the drug of choice for paracetamol intoxication (*9*, *11*, *12*). Captopril [CAP ($C_9H_{15}NO_3S$)], a synthetic thiol and inhibitor of angiotensin converting enzyme (ACE), has also been postulated as a free radical scavenger because of its terminal sulfhydryl group (*13*).

Various analytical studies of these biological thiols have been reported as well as investigations of their efficacy in preventing and treating numerous disorders (*11*, *12*). These studies have also provided some medical applications of biological thiols. However, because there is insufficient data on the thiol content of fruits and vegetables, this study proposes to determine the thiol concentrations in fruits and vegetables consumed daily.

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MATERIALS AND METHODS

Reagents and Chemicals. Acetonitrile, acetic acid, water, and phosphoric acid [all high-performance liquid chromatography (HPLC) grade] were purchased from Fisher (St. Louis, MO). NAC, CAP, GSH, GGC, CYS, and HCYS were purchased from Sigma (St. Louis, MO). N-(1-Pyrenyl)maleimide (NPM) was purchased from Aldrich (Milwaukee, WI).

Samples. Fruits and vegetables were purchased from major chain supermarkets in Rolla, MO, from December 2003 to March 2004.

Determination of NAC, CAP, GSH, CYS, HCYS, and GGC by the HPLC Method. A new method for determining GSH was developed in this laboratory to analyze γ-glutamyl cycle intermediates (*14*, *15*). Vegetables and fruits (0.5 g/mL) were placed in a serine-borate buffer (100 mM Tris-HCl, 10 mM borate, 5 mM serine, and 1 mM diethylenetriaminepentacetic acid, pH 7.0) to prevent artifactual oxidation. The samples were homogenized with tissue tearor (model 985-370, type 2, Biospec Products Inc.) on ice for 2 min, with 5 s intervals of homogenization and then centrifuged at 2000 $\times$ *g* for 15 min. The supernatant (20 μL) was derivatized with NPM. This compound reacts with free sulfhydryl groups to form fluorescent derivatives. Each sample was first diluted with distilled water to make the volume up to 250 μL, and then 750 μL of NPM (1 mM in acetonitrile) was added. The resulting solution was mixed and incubated at room temperature for 5 min. Ten microliters of 2 N HCl was added to stop the reaction. After filtration through a 0.2 μm acrodisc, the derivatized samples were injected onto a 5 μm C_{18} column in a reverse phase HPLC system. NAC, CAP, GGC, CYS, and HCYS were determined concurrently with GSH since they also form fluorescent derivatives with NPM.

HPLC System. The HPLC system (Shimadzu) was comprised of a model LC-10A pump, a Rheodyne injection valve with a 20 μL injection filling loop, and a model RF535 fluorescence spectrophotometer operating at an excitation wavelength of 330 nm and an emission wavelength of 375 nm. The HPLC column was 100 mm $\times$ 4.6 mm and packed with 5 μm particles of C_{18} packing material. The mobile phase was 30% water and 70% acetonitrile, containing 1 mL/L acetic acid and 1 mL/L *o*-phosphoric acid. The NPM derivatives were eluted from the column isocratically at a flow rate of 1 mL/min. Quantitation of the peaks from the HPLC system was performed with a Chromatopac, model C-R601 (Shimadzu).

Calibration Curves. Calibration curves were plotted by using concentration as the *x*-axis vs peak areas as the *y*-axis. Linearity was obtained for NAC, CAP, GSH, GGC, CYS, and HCYS concentrations ranging from 0 to 2500 nM with a regression constant of $r^2 = 0.999$.

RESULTS

In situ derivatization of the vegetables and fruits with NPM produced NPM−NAC, NPM−CAP, NPM−GSH, NPM−GGC, NPM−CYS, and NPM−HCYS adducts, which were rapidly separated by the HPLC system employed for this study. **Figure 1** shows a chromatogram of the mix standards using the NPM method. Chromatograms prepared from asparagus (**Figure 2**) and strawberry (**Figure 3**) samples further illustrate the well-defined separation of the NPM−NAC, NPM−CAP, NPM−GSH, NPM−GGC, NPM−CYS, and NPM−HCYS adduct peaks achieved by this method. Retention times are found to be 3.36, 4.09, 9.17, 10.67, 12.54, and 15.12 for NAC, CAP, GSH, GGC, CYS, and HCYS, respectively.

Results of Vegetable Samples. The NAC, CAP, GSH, GGC, CYS, and HCYS concentrations in various vegetables were reported in **Table 1**. Our results show that asparagus has the highest levels of GSH and NAC, 349 and 46 nM/g wet weight, respectively, among biological thiols. Red pepper has the highest level of CYS, 349 nM/wet weight. HCYS is found only in asparagus and red pepper; GGC is found only in spinaches and green beans. The level of HCYS was determined to be 11 nM/g wet weight, which is significantly higher than that of red pepper. The level of GGC was determined to be 23 nM/g wet weight,

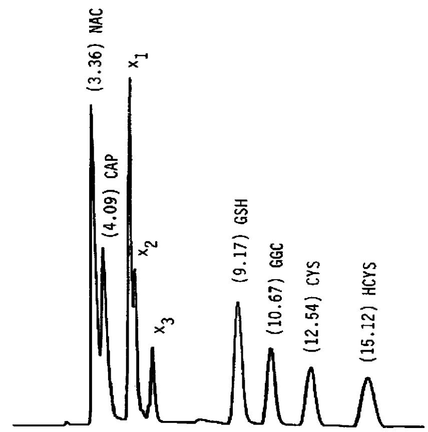

Figure 1. Chromatogram of a mixture of NAC, CAP, GSH, GGC, CYS, and HCYS. X_1, X_2, and X_3 represent NPM hydrolysis peaks. Retention times are given in minutes in parentheses next to the corresponding peaks.

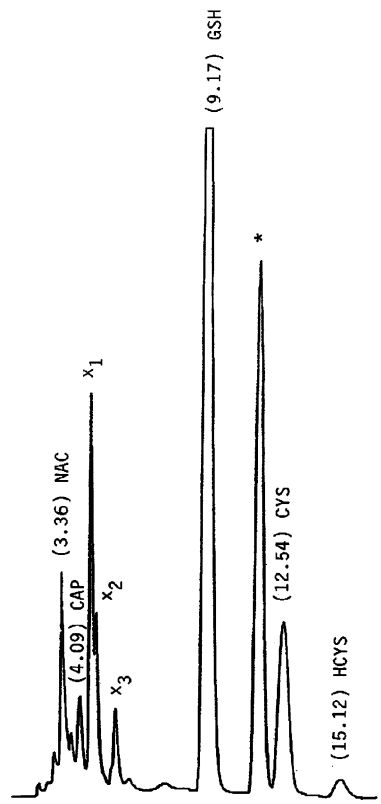

Figure 2. Chromatogram of NPM-derivatized asparagus sample showing NAC, CAP, GSH, CYS, and HCYS. X_1, X_2, and X_3 represent NPM hydrolysis peaks. Retention times are given in minutes in parentheses next to the corresponding peaks. An unidentified peak is denoted as "*".

which is also higher than that of green beans. The lowest levels of thiols among vegetables observed are as follows: The NAC level was the lowest in tomatoes (3 nM/g wet weight), the GSH level was the lowest in carrots and broccoli (4 nM/g wet weight), the CYS level was the lowest in avocados (4 nM/g wet weight), the HCYS level was the lowest in red peppers (5 nM/g wet weight), and the GGC level was the lowest in spinaches (4 nM/g wet weight). Furthermore, none of the biological thiols analyzed was found in cabbages, and CAP was only found in asparagus (28 nM/g wet weight).

Results of Fruit Samples. The NAC, GSH, GGC, and CYS concentrations in various fruits were reported in **Table 2**. Our results showed that strawberries have the highest levels of NAC and CYS, 5 and 59 nM/g wet weight, respectively, among

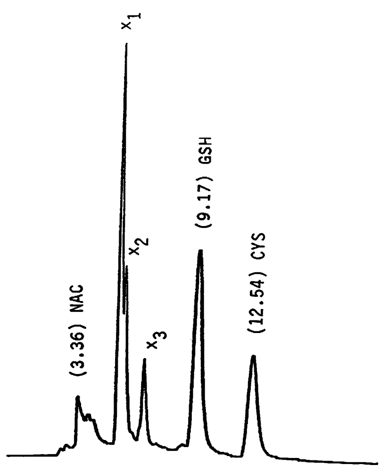

Figure 3. Chromatogram of NPM-derivatized strawberry sample showing NAC, GSH, and CYS. X_1, X_2, and X_3 represent NPM hydrolysis peaks. Retention times are given in minutes in parentheses next to the corresponding peaks.

Table 1. Thiols Concentrations in Vegetables (nM/g Wet Weight)[a]

	mean ± SD ($n = 3$)				
vegetables	NAC	GSH	CYS	HCYS	GGC
asparagus	46 ± 1	349 ± 26	122 ± 1	11 ± 1	ND
spinach	ND	313 ± 33	84 ± 2	ND	4 ± 0
broccoli	ND	4 ± 1	ND	ND	ND
cauliflower	ND	6 ± 1	7 ± 1	ND	ND
green squash	ND	47 ± 11	6 ± 1	ND	ND
yellow squash	ND	39 ± 8	27 ± 6	ND	ND
cucumber	6 ± 1	123 ± 38	11 ± 3	ND	ND
carrot	ND	4 ± 0	ND	ND	ND
parsley	9 ± 1	17 ± 9	8 ± 1	ND	ND
tomato	3 ± 1	64 ± 10	55 ± 3	ND	ND
red pepper	25 ± 4	42 ± 2	349 ± 18	5 ± 1	ND
green pepper	12 ± 2	8 ± 1	9 ± 1	ND	ND
potato	ND	5 ± 0	ND	ND	ND
avocado	ND	339 ± 10	4 ± 1	ND	ND
green beans	ND	230 ± 2	67 ± 11	ND	23 ± 1

[a] ND, not detectable. CAP was only detected in asparagus (28 ± 6 nM/g wet weight). The results are given as means ± standard deviations of three samples of each vegetable.

Table 2. Thiols Concentrations in Fruits (nM/g Wet Weight)[a]

	mean ± SD ($n = 3$)		
fruits	NAC	GSH	CYS
orange	ND	5 ± 11	41 ± 2
lemon	4 ± 0	5 ± 0	6 ± 0
grapefruit	4 ± 0	13 ± 3	15 ± 2
mango	ND	59 ± 6	10 ± 0
papaya	ND	136 ± 12	58 ± 5
banana	ND	ND	7 ± 0
strawberry	5 ± 1	39 ± 8	59 ± 5

[a] ND, not detectable. GGC was only detected in papaya (42 ± 3 nM/g wet weight). The results are given as means ± standard deviations of three samples of each fruit.

biological thiols. The highest level of GSH was 136 nM/g wet weight in the papaya. GGC was only found in the papaya (42 nM/g wet weight). CYS was the only thiol found in banana (7 nM/g wet weight). Among biological thiols, the lemon has the lowest levels of NAC, GSH, and CYS (4, 5, 6 nM/g wet weight, respectively). Furthermore, none of the biological thiols analyzed were found in red grapes, blackberries, apples, or peaches.

DISCUSSION

It has been proposed that reactive oxygen species might be involved in the development of cancer, stroke, Alzheimer's disease, cardiovascular diseases, and aging, as well as in iron overload. According to recent studies, the lack of an antioxidant defense in cells may be a risk factor for carcinogenesis (*16*). Free radical scavengers can be found in fruits and vegetables, along with phytochemicals and antioxidant vitamins. It has been suggested that consumption of fruits and vegetables should be increased because they have substantial amounts of antioxidants and phytochemicals (*3*). Moreover, the biological thiols found in plants can also function as an antioxidant, an antimutagen, and an anticarcinogen (*16*). Biological thiols, such as GSH, NAC, CYS, HCYS, CAP, and GGC, have strong antioxidant properties that allow them to protect cells from oxidative stress. Besides being potent antioxidants, these thiols act as therapeutic agents and indicators of diseases (*17*).

To determine the amounts of thiols in various fruits and vegetables, we used a sensitive HPLC technique involving an initial derivatization with NPM to react with the free sulfhydryl groups and form fluorescent derivatives in the foods (*14*, *15*). This NPM method was developed in our lab and has been used for several years to conduct analyses of cells and biological tissue. For the first time, however, it was used in this study to determine the levels of various important thiols in food.

Our study showed that the content of various thiols in a variety fruits and vegetables differed significantly. For example, asparagus had the highest levels of GSH and NAC (**Table 1**). Recent studies have reported that the GSH contained in various foods may increase the elimination of oxidized lipids by either enhancing their excretion or decreasing their absorption. Moreover, consumption of foods with a high GSH content has been shown to decrease the risk of having pharyngeal and oral cancers by 50% (*18*).

The levels of thiols depend on various conditions such as climate, region, storage method, duration of storage, and cooking style. One study showed that the mean GSH concentration decreases significantly (87% decrease) when various foods (grapefruit, tomatoes, spinach, carrots, apples, peaches, and sweet potatoes) are processed (*19*). However, in the same study, frozen strawberries and reconstituted frozen oranges have retained high levels of GSH (*19*). This study indicated that GSH levels should be carefully studied, particularly in processed foods.

The biological importance of the differences found in the content of each thiol in each vegetable and fruit has yet to be investigated since previous studies (*8*, *11*−*13*) only show the effects of GSH, NAC, and CAP on various disorders. These thiols were given as pure chemicals in these studies to determine their biological effects. Our future studies will include feeding animals with some of the vegetables that (for example) have the highest GSH and monitoring the outcome to determine the effects.

In summary, this study determined the various levels of thiols in certain fruits and vegetables. The high levels of thiols identified reinforced the concept that these foods are very healthy and are valuable because of their protective and therapeutic contributions.

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