ISSN:1306-3111
e-Journal of New World Sciences Academy
2012, Volume: 7, Number: 3, Article Number: 3A0055

NWSA-PHYSICAL SCIENCES
Received: January 2012
Accepted: July 2012
Series : 3A
ISSN : 1308-7304

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INVESTIGATION OF WATER SOLUBLE COMPOUNDS IN STRAWBERRY *(FRAGARIA VESCA)*

ABSTRACT

Amount of reduced glutathione (GSH), oxidized glutathione (GSSG), ascorbic acid (Vitamin C), thiamine hydrochloride (Vitamin B1), riboflavin (Vitamin B2), nicotinic acid (Vitamin B3), pyridoxine hydrochloride (Vitamin B6) and folic acid (Vitamin B9) were investigated in strawberry (*Fragaria vesca)* fruits, grown in Elazig city region by using high performance liquid chromatography. The amounts of GSH, GSSG, vitamin C, vitamin B1, vitamin B2, vitamin B3, vitamin B6 and vitamin B9 were obtained to be 722.25 ± 23.19; 17.13 ± 2.59; 386.55 ± 15.98; 1.05 ± 0.24; 1.40 ± 0.30; 26.95 ± 3.46; 14.66 ± 1.87 and 7.21 ± 0.51 μg/g, respectively. From these results, it may be concluded that, strawberry is rich in GSH, vitamin C, vitamin B3, and vitamin B6 and also it contains considerable amount of vitamin B9.

Keywords: Strawberry, Glutathione, Vitamins B, Vitamin C, HPLC

ÇİLEKDE (*FRAGARIA VESCA*) SUDA ÇÖZÜNEN BİLEŞİKLERİN İNCELENMESİ

ÖZET

İndirgenmiş glutatyon (GSH), yükseltgenmiş glutatyon (GSSG), askorbik asit (C vitamini), Tiyamin hidroklorür (B1 vitamini), riboflavin (B2 vitamini), nikotinik asit (B3 vitamini), piridoksin hidroklorür (B6 vitamini) ve folik asit (B9 vitamini) miktarları yüksek performanslı sıvı kromatografisi (HPLC) ile belirlendi. GSH, GSSG, C, B1, B2, B3, B6 ve B9 vitaminlerinin miktarları sırası ile 722.25 ± 23.19; 17.13 ± 2.59; 386.55 ± 15.98; 1.05 ± 0.24; 1.40 ± 0.30; 26.95 ± 3.46; 14.66 ± 1.87 ve 7.21 ± 0.51 μg/g olduğu gözlendi. Elde edilen bu verilerden, çilekte GSH, C, B3 ve B6 vitaminleri açısından çok iyi bir kaynak olduğu, ayrıca önemli miktarda da B9 vitamini ihtiva ettiği söylenebilir.

Anahtar Kelimeler: Çilek, Glutatyon, B Vitaminleri, C Vitamini, HPLC

1. INTRODUCTION (GİRİŞ)

Strawberries are popular fruit with rich nutrients, high visible appeal and desirable flavor but are highly perishable and have relatively high physiological postharvest activities [1]. Thus strawberries are consumed mainly as fresh fruit, and many other strawberry products such as juice, nectar, puree, and juice concentrate as well as jam are commercially available [2].

Strawberries are a good source of ascorbic acid (AA) [3] Ascorbic acid is the water-soluble vitamin. The major part of the water-soluble vitamins comprises the B-complex. In fact, the term B-complex refers to all known essential water-soluble vitamins except for vitamin C. Each member of the B-complex has a unique structure and performs unique functions in the human body [4].

Strawberries contain high levels of antioxidant compounds such as anthocyanins and flavonoids, and phenolic acids, and glutathione [3 and 5]. Glutathione (GSH) is a cysteine-containing tripeptide that plays a wide variety of physiological roles, including regulation of signal transduction [6], intracellular defense against oxidative stress [7 and 8].

Strawberries are one of the rich sources of natural antioxidants among fruits [9]. Previous research showed that strawberry extracts exhibited a high level of antioxidant capacity against free radical species [5 and 10]. Antioxidants can scavenge free radical and nonradical reactive oxygen species, which have been associated with several cellular toxic processes including oxidative damage to proteins and DNA, membrane lipid oxidation, enzyme inactivation and gene mutation, which may lead to carcinogenesis [3, 9, 11 and 13].

2. RESEARCH SIGNIFICANCE (ÇALIŞMANIN ÖNEMİ)

The aim of this study is to determine the amount of GSH, GSSG, vitamin C, vitamin B1, vitamin B2, vitamin B3, vitamin B6 and vitamin B9 in fresh strawberry (*Fragaria vesca)* samples.

3. EXPERIMENTAL METHOD (DENEYSEL METOD)

The GSH, GSSG, vitamin C, vitamin B1, vitamin B2, vitamin B3, vitamin B6 and B9 levels in strawberry fruits were determined. Fresh strawberry fruits samples were mashed in a homogenizer and 1.5 g of homogenate paste per sample was taken for extraction of vitamin C, reduced and oxidized glutathione. 1.0 mL Aliquot of 1.0 mol/L $HClO_4$ was added into the homogenate, thus precipitating the proteins. Total volumes were made up to 4.0 mL with adding distilled water. The mixture was centrifuged at 4000 rpm for 5 min at 4 °C. The supernatant was filtered by Whatman No. 1 paper (Whatman Limited, UK) and vitamin C levels were determined with the method proposed by Tavazzi *et al*.[14] in HPLC on a Tecopak C18 reversed-phase column (Mundells Industrial Centre; 250 mm, 3.9 mm ID, 10 µm particle size). For vitamin C analysis, mobile phase of 3.7 mM phosphate buffer with pH = 4.0 at a flow rate of 1 mL/min was used, with detection at 245 nm. GSH and GSSG levels were determined using the method proposed by Dawes and Dawes [15]. In order to determine the GSH and GSSG, a SGE Walkosil II 5C18 RS column (150 mm × 4.6 mm ID, 5 µm particle and 120 Å pore size) packed having 50 mM $NaClO_4$ in 0.1% H_3PO_4 as a mobile phase with pH = 4.0 at a flow rate of 1.0 mL/min was used, with detection at 215 nm.

Vitamin B1, vitamin B2, vitamin B3, vitamin B6 and B9 levels were determined using the method proposed by Amidzic *et al*. [16] Markopoulou *et al*. [17] and Ivanovic *et al*. [18].

Supelcosil LC-18-DB (15 cm - 4.6 mm ID; particle size 5 µm) was used for the determination of vitamin B1, vitamin B2, vitamin B3, vitamin B6 and vitamin B9. The experiments were conducted at 25 °C

for the determination of all vitamins. In this process, methanol - 5mM heptanesulphonic acid sodium salt / 0.1% triethylamine (TEA) (25:75, v:v) mixture was used as mobile phase. The pH of mobile phase was adjusted with orthophosphoric acid as 2.8. The flow rate was 0.70 mL / min and the injected volume was 20 µL. Vitamins B1, B2 and B3 were detected at 260 nm while vitamins B6 and B9 were detected at 290 nm. All the prepared mobile phases were degassed with an ultrasonic bath. Each run was repeated three times to check repeatability. Results are mean values of ± SD of three replicated analyses.

In high-performance liquid chromatography separations were accomplished at room temperature with a Cecil liquid chromatography system (Series 1100) consisting of a sample injection valve (Cotati 7125 CA, USA) with a 20 µL sample loop, an ultraviolet (UV) spectrophotometric detector (Cecil 68174, UK) and an integrator (HP 3395A, China).

The chemicals and reagents used in this work were of analytical grade and purchased from Sigma Chemical Co. (Darmstadt, Germany). All glassware was acid washed and rinsed with doubly distilled deionized water.

4. FINDINGS AND DISCUSSIONS (BULGULAR VE TARTIŞMA)

The amounts of GSH, GSSG, vitamin C, vitamin B1, vitamin B2, vitamin B3, vitamin B6 and B9 in Strawberry fruits cultivated in Elazig city at Turkey were investigated (Table 1).

Table 1. The amounts of GSH, GSSG, vitamin C, B1, B2, B3, B6 and B9 in Strawberry
(Tablo 1. Çilekteki, GSH, GSSG, C, B1, B2, B3, B6 ve B9 vitamin miktarları

Water Soluble Compounds	Amount (µg/g + SD)
Reduced glutathione (GSH)	722.25 ± 23.19
Oxized glutathione (GSSG)	17.13 ± 2.59
Ascorbic acid (Vitamin C)	386.55 ± 15.98
Thiamine hydrochloride (Vitamin B1)	1.05 ± 0.24
Riboflavin (Vitamin B2)	1.40 ± 0.30
Nicotinic acid (Vitamin B3)	26.95 ± 3.46
Pyridoxine hydrochloride (Vitamin B6)	14.66 ± 1.87
Folic acid (Vitamin B9)	7.21 ± 0.51

It is well known that, ascorbic acid (vitamin C) and reduced glutathione (GSH) are the non enzymatic antioxidant defense system in plants [19]. Glutathione (GSH) is the most abundant non enzymatic antioxidant in the cells, where it plays a significant role as a redox regulator and an important role against oxidative stress- induced cell injury [20].

Vitamin C is an important quality component of many fruits, particularly in some berry fruits. Strawberry fruits are considered one of the richest sources of vitamin C. In the present study, it was found that the amounts of vitamin C in strawberry is 386.55 ± 15.98 µg/g (Table 1). Vitamin C in strawberries were previously as 345-600 µg/g determined [21 and 24].

Vitamins are essential for the normal growth, self-maintenance and functioning of human and animal bodies. They play different specific and vital functions in metabolism, and their deficiency or redundancy produces specific diseases [25].

Previous studies have shown that strawberries have vitamin B1, vitamin B2, vitamin B3, vitamin B6 and vitamin B9; 0.24; 0.22; 3.86; 0.47 and 0.24 µg/g, respectively [24].

The difference of the worked water soluble compounds (GSH, GSSG, vitamin C, vitamin B1, vitamin B2, vitamin B3, vitamin B6 and vitamin B9) might be explanation by a number of factors for example; cultivation, variety, fertilization, altitude, maturity level, soil, region and weather conditions as well as sampling time and degree of ripeness considerably affect the nutritive value of strawberries [26 and 28].

5. CONCLISION (SONUÇLAR)

As a result, it can be said that, strawberry is a healthy fruit that rich in GSH, vitamin C, vitamin B3 and vitamin B6. These properties of strawberry are an important factor for consumer knowing that strawberry is rich in vitamins and antioxidant.

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