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ISSN 6299 -0975

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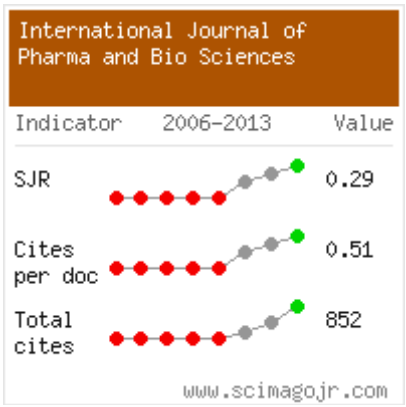

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Int J Pharm Bio Sci 2015 July; 6(3): (P) 133 - 140

Research Article **Natural Chemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

PHENOLIC PROFILE, ANTIOXIDANT CAPACITY AND SULFORAPHANE CONTENT DURING THE STORAGE OF BROCCOLI SPROUTS

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ABSTRACT

The aim of this investigation was to determine the phenolic profile, antioxidant capacity and sulforaphane content in 3-day-old sprouts during storage at 5°C for 14 days. The content of total polyphenols and antioxidant capacity were determined by spectrophotometric methods; while that phenolic compounds and sulforaphane were quantified by HPLC-DAD. The polyphenol content were present in a range of 6.48 to 13.07 mg GAE/g fresh weight. The DPPH radical scavenging capacity occurred in a range of 21.78 to 29.10 Mmol TE/g fresh weight. Chlorogenic and caffeic acid were found in larger concentration. Sulforaphane content in the sprouts stored at 5ºC showed a reduction of 47% at day 14. The bioactive compounds content in broccoli sprouts may vary in accordance with the time and storage conditions. Broccoli sprouts are an important source of phenolic compounds and sulforaphane.

KEYWORDS: **DPPH, edible sprouts, Functional foods, Polyphenols, Storage stability**

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INTRODUCTION

Recently, consumers have increased the interest to include in their diet, certain foods or physiologically active food components that improve the health. The potential health benefits attributed to the consumption of certain vegetables, are due to the presence of bioactive compounds known as phytochemicals in these foods. Into which can be mentioned the carotenoids, phenolic acids, flavonoids, and glucosinolates. These are not essential to the nutrition, but in sufficient quantities play an important role to promote the consumer health [1]. Which are known as functional foods and these have inherent health benefits and reduce the risk of specific diseases, such as anticancerous effect, antioxidant activity, anti-inflammatory, hypocholesterolemic and hypoglycemic properties [2]. In recent years, broccoli sprouts have been widely consumed in different parts of the United States and Europe as complements in salads, sandwiches and soups. In Latin America, the benefits of broccoli sprouts consumption are unknown, and the advantages about the practice of seed germination, both in nutrition and disease prevention [3]. According to Sangronis and Machado[4], sprouts provide multiple nutritional and therapeutical benefits for those who consume them, such as vitamins, minerals, proteins, carbohydrates, fatty acids and enzymes, these compounds are also available in the body. Broccoli sprouts are considered as a functional food [5]; since in addition to essential nutrients provide diverse secondary metabolites or phytochemicals. The phenolics compounds, especially flavonoids and anthocyanins, show a great ability to capture free radicals that leading to oxidative stress, to these compounds are attributed a beneficial effect in the prevention of cardiovascular diseases, circulatory problems, neurological disorders and cancer [6]. Isothiocyanate sulforaphane (1-isothiocyanate-4-(methylsulfinyl)-butane) is a phytochemical of great interest, since numerous studies have been attributed chemopreventive antimicrobial, and anticarcinogenic properties; its precursor glucoraphanin constitutes over 80% of total glucosinolates present in broccoli [7]. The glucoraphanin is converted to sulforaphane by an enzyme released from the consumption of broccoli called myrosinase. It has been shown that the broccoli sprouts have 20 to 30 fold higher concentration of glucoraphanin than mature plant [8]. Indicating that 1 oz. of broccoli sprouts may have the amount of antioxidant present in 20 oz. of mature broccoli. Furthermore, broccoli sprouts are sold in sealed plastic containers and are destined to be stored in domestic refrigerators for consumption. Although the concentration of bioactive compounds may be high at the time of harvest, there is not accurate data that document the stability of these phytochemicals during the storage [9]. Several researches have shown that the temperature and storage time are the main factors responsible of the concentration changes of diverse compounds, such as glucosinolates [10]. Therefore, it is necessary to know the stability of sulforaphane in the food matrix under refrigeration storage. The present study focuses to report the phenolic compounds, antioxidant capacity and sulforaphane stability of 3-day-old sprouts during storage at 5°C. This, this shows its potentiality as a source of bioactive compounds and nutrients that contribute to its functional properties, to improve consumer health.

MATERIALS AND METHODS

HPLC-grade methanol, acetonitrile, ethyl acetate and dichloromethane were obtained from EMD Chemicals (Darmstadt, Hessen, Germany). DL-sulforaphane standard, quercetin standard, rutin standard, chlorogenic acid, caffeic acid, ferulic acid, 4, 5-trihydroxybenzoic, 2,2-diphenyl-1-picrylhydrazyl radical, Folin-Ciocalteu reagent 2N, formic acid, potassium carbonate were obtained from Sigma (St. Louis, Missouri, USA). Bakerbond SPE silica gel (SiOH) 3 ml disposable columns was obtained from J.T. Baker (J.T. Baker, Xalostoc, Mexico).

(i) Plant material and germination condition

Broccoli seeds (*Brassica oleracea L. var. Italica cv. Waltham 29*) of supplier Edena Seeds (California, USA) were used. Germination of broccoli seeds was carried out

according to Pérez-Balibrea et al[11]. The seeds were soaked in a sodium hypochlorite solution at 5 g/l for 15 min. Then were drained and rinsed with distilled water and were placed over inert substrate in a germination tray. The tray was placed in a seed germinator model S-6920 (Seedburo Equipment Company, Chicago, IL, USA). Germination conditions were controlled at 22±2°C with a cycle of 16 h light and 8 h darkness, and a relative humidity (RH) between 80-90%.

(ii) Preparation of methanolic extracts

Extraction was performed under dark conditions using as solvent methanol/water at a ratio 80:20; specifically weighed 300 mg of broccoli sprouts were added 5 ml of methanol 80%, was homogenized for 30 sec vortexed and sonicated for 5 min. The mixture was filtered using Whatman no. 41 paper.

(iii) Determination of total polyphenols

The total phenolic content was determined by the spectrophotometric method using the Folin-Ciocalteu reagent [12]. The absorbance was measured at 760 nm. Results were expressed as mg of gallic acid equivalents (GAE) per 100 gram of fresh weight (FW).

(iv) Measurement of DPPH radical scavenging capacity

2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity was determined by Brand-Williams et al[13]. A working solution of 0.1 mM DPPH radical was prepared in methanol 80%, which shows an absorbance of 1.237 at 515 nm. The DPPH radical scavenging capacity of the sample was expressed as mM Trolox equivalent (TE) per 100 gram FW.

(v) Determination free phenolic acids and flavonoids determined by HPLC-DAD

Phenolic compounds were determined using a modified version of Ćetković et al[14]. Each methanolic extract was vortexed for 30 sec and filtered with a membrane of 0.45 µm. A 20 µl sample of this solution was injected onto the column (analytical Zorbax SB-C18 column 25 x 0.46 cm i.d., particle size of 5µm) of the HPLC-DAD (Waters, Milford, MA, USA). The analysis were carried out at a flow of 1 ml/min with a mobile phase mixture of 1% formic acid in water v/v (A) and 100% methanol (B). The method consisted of a step gradient starting at 0-40 min of 20% (B) and 80% (A) to 65% (B) and 35% (A); 92% (B) and 8% (A) 45 min and 100% (B) 0% (A) at 50 min. The absorbance was recorded at 254 and 324 nm. The compounds were identified through comparison of their UV spectrum data with an established database of reference standards. The phenolic content was calculated using standard curves and was expressed as mg per gram of dry weight (DW).

(vi) Quantification of sulforaphane

Sulforaphane was determined by Campas-Baypoli et al[15]. This method include the conversion of glucoraphanin to sulforaphane with acidic water (pH 6) at 45±2°C for 2.5 h, the extraction with dichloromethane and finally the purification using Bakerbond SPE silica gel (SiOH) columns [16]. The purified extract was evaporated to dryness and redissolved in acetonitrile, and filtered with a membrane of 0.45 µm. A 20 µl sample of this solution was injected onto the column (analytical SS Exil ODS C18 column, 25 x 0.46 cm i.d., particle size of 5µm) of the HPLC-DAD (GBC, Dandenong, Australia). The analysis were carried out isocratically at a flow rate of 0.6 ml/min, employing a mixture of acetonitrile:ultrapure water (30:70, v/v) as the mobile phase. The column was thermostated at 36°C. The sulforaphane was detected at 202 nm. For quantification, peak areas were correlated with the concentrations according to the calibration curve. All samples were analyzed in duplicate. The sulforaphane content was expressed as µg per gram FW.

(vii) Changes during the storage of 3-day-old sprouts

Changes during storage were evaluated using fresh sprouts harvested at three days, which were placed in plastic packaging securely closed to prevent moisture loss. They were then placed in cold storage for a period of 14 days at 5°C. Sampling was conducted in consecutive periods of two days for the quantification of sulforaphane, total polyphenols and DPPH radical scavenging capacity.

(viii) Statistical analysis

A completely randomised design was used to analyze changes during the germination

process and the broccoli sprouts storage. Data was analyzed using ANOVA procedures. Means were compared using Tukey test at a level of significance of 0.05. Statistical analyses were performed using the Statgraphics Plus software, version 4.0 (STSC Inc., Rockville, MD, USA). All data was expressed as average ± SD (standard deviation).

RESULTS AND DISCUSSION

1. Total polyphenols content and DPPH radical scavenging capacity

The results of the total polyphenols content in broccoli sprouts during storage at 5°C for 14 days are presented in Table 1. It can be seen a gradual increase in the total polyphenol content, reaching a maximum value at day 4 (13 mg GAE/g FW) and subsequently this concentration decreased to 8.91 mg GAE/g FW at day 8, and remained constant until day 14. According to our knowledge not reports were found of the total polyphenol content during storage of broccoli sprouts. However, some authors confirm that low storage temperature causes an accumulation of total polyphenols [17]. Additionally the DPPH radical scavenging capacity was determined during the storage period (Table 1). The results of antioxidant capacity increased significantly until day 10, then remained constant until day 12 and finally decrease 17% at day 14. These results differ from those reported by Nath et al.[18] who observed a steady decrease in the antioxidant capacity for 144 hours of storage of broccoli inflorescences. The above behavior may be due to the constant changes in plant metabolism during storage as a result of oxidative stress, which may include structural changes in synthesis or antioxidant compounds [19].

2. Free phenolic acids and flavonoids determined by HPLC-DAD

The content of gallic acid, chlorogenic acid, caffeic acid, ferulic acid, rutin and quercetin in seeds and 3-day-old sprouts are presented in Figure 1. Chlorogenic acid and caffeic acid were found in higher concentrations, ranging from 0.552 to 1.12 and from 0.305 to 0.588 mg/g (DW from 0.305 to 0.588 mg/g), respectively. Ferulic acid was found in very low concentrations in all samples. While those flavonoids as rutin and quercetin were found in higher concentrations in 3-days-old sprouts, with values of 0.116 and 0.039 mg/g DW, respectively. It should be mentioned that each phenolic component may contribute differently in the antioxidant capacity, according to their concentration in the food and the nature of its chemical structure [20]. Okawa et al.[21] reported that when the capacity to scavenge free radicals is evaluated is not always important the polyphenol content, but rather by position of the hydroxyl group. The results obtained in this study are in agreement with the phenolic profile reported by Pająk et al.[22] for broccoli seeds and sprouts, who found higher amounts of chlorogenic acid and caffeic acid (12.02 and 3.25 mg/100 g DW, respectively) compared with the amount reported for gallic acid (1.57 mg/100 g DW), ferulic acid (1.17 mg/100 g DW) and quercetin (0.38 mg/100 g DW). The results of this study with reference to the composition of free polyphenols show that fresh broccoli sprouts are a viable option for their consumption as source of antioxidants.

3. Sulforaphane content

Figure 2 shows the variation in the sulforaphane concentration in 3-days-old sprouts during refrigerated storage. The results of the samples analyzed showed significant differences with respect to storage time ($p \leq 0.05$). The sulforaphane content during the storage at 5 °C presented values in the range from 195 to 372 µg/g of FW. A gradual decrease is observed up to a concentration of 195 µg/g on day 14, decreasing its level by 47%. This behavior is consistent with experiments conducted by Howard et al.[23], who reported a decrease of 50% on day 14 of storage at 4°C in broccoli sprouts. Force et al.[24] also identified significant changes in the content of sulforaphane in broccoli samples under refrigerated storage for 3 weeks at 4°C, however, sulforaphane content was constant after the sixth day. Evidently, sulforaphane is very unstable and tends to degrade rapidly in the food matrix. Cultural practices, handling and storage conditions, as well as the vegetable preparation, have a potential impact on the glucosinolates content, causing a change in the rate of formation of sulforaphane [25]. However, it is suggested that

low temperatures below 4°C and high relative humidity of 98-100% are the best preservation processes to maintain a high content of glucosinolates in *Brassica* crops and their nutritional quality [26].

Table 1
Total polyphenols and DPPH radical scavenging capacity of 3-day-old sprouts storage at 5°C by 14 days.

Storage time (Days)	Total Polyphenols (mg GAE/g FW)	DPPH (Mmol TE/g FW)
0	6.48 ± 0.34^{d}	21.78 ± 0.82^{b}
2	6.65 ± 0.27^{d}	21.87 ± 0.44^{b}
4	13.07 ± 0.65^{a}	21.68 ± 0.91^{b}
6	9.50 ± 0.28^{b}	28.77 ± 0.69^{a}
8	8.91 ± 0.28^{c}	29.10 ± 0.17^{a}
10	8.40 ± 0.34^{c}	28.40 ± 0.53^{a}
12	8.20 ± 0.31^{c}	28.20 ± 1.25^{a}
14	8.14 ± 0.40^{c}	24.21 ± 1.09^{b}

Data expressed as the mean ± SD of three assays (in triplicate).

Different superscripts in the same column denote significant differences.

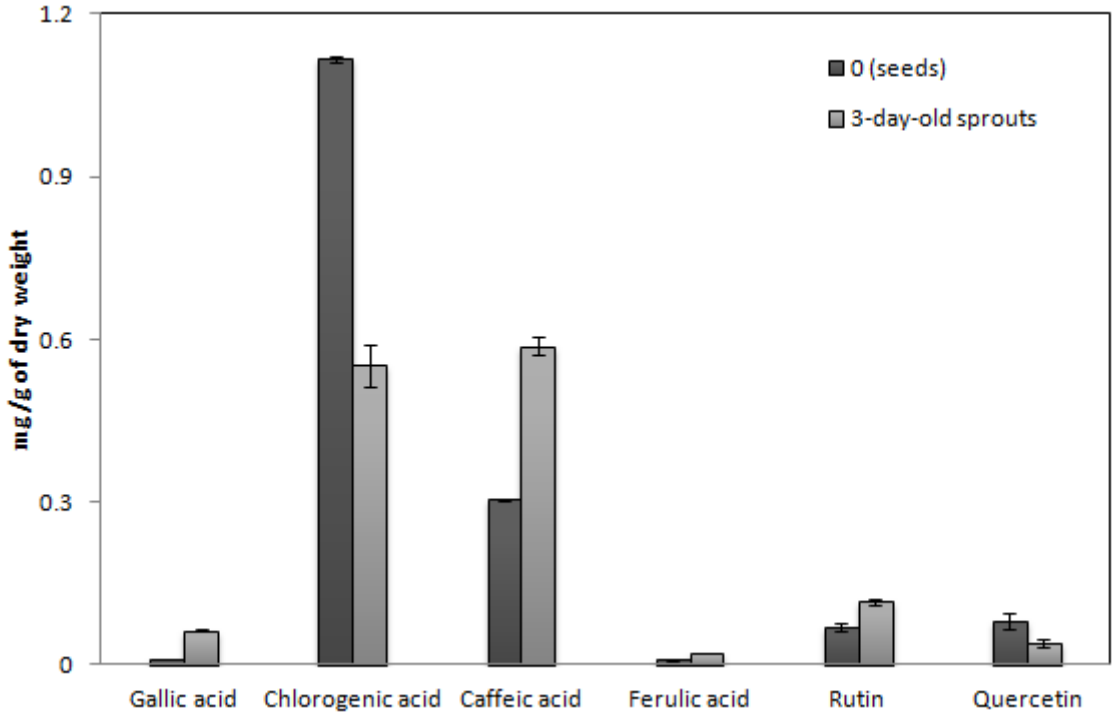

Figure 1
Polyphenols profile of broccoli seeds and 3-day-old sprouts (HPLC-DAD).

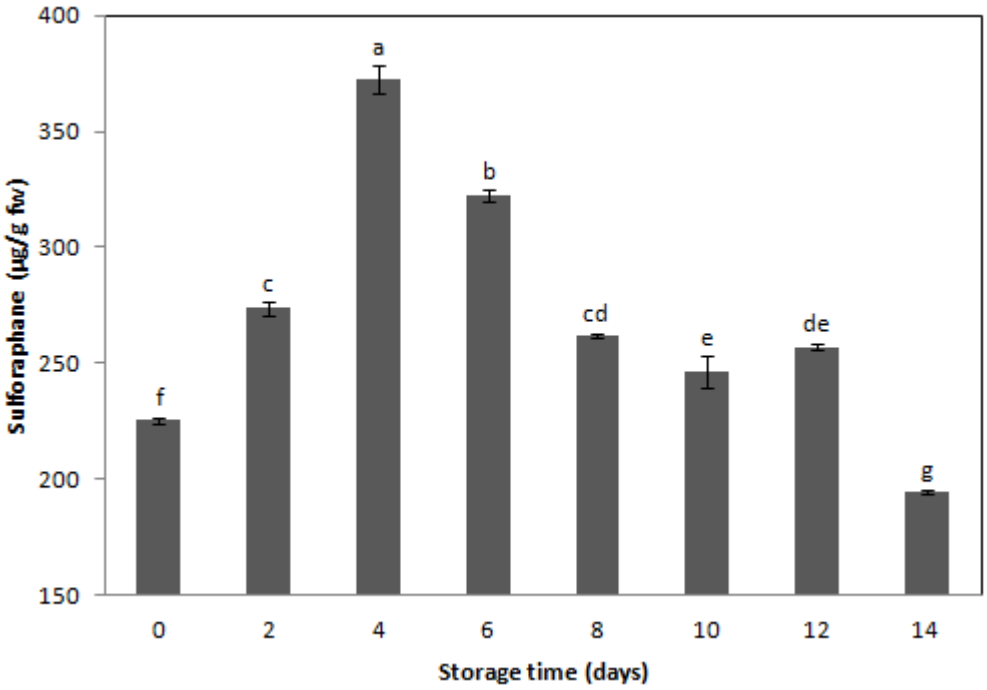

Figure 2
Sulforaphane content of 3-day-old sprouts versus storage time at 5°C.

CONCLUSION

The results of this study show that the content of phytochemicals such as sulforaphane, and polyphenols in broccoli sprouts, is variable in relation to the maturity and storage conditions. However, it can be said that broccoli sprouts studied here are an economical source of antioxidants. Furthermore, it was found that refrigerated storage (5°C) is a suitable method to preserve at least 50% of sulforaphane in broccoli sprouts up to two weeks. Therefore, the consumption of this food can play an important role in the prevention of related diseases with free radical generation, considering to broccoli sprouts as a functional food.

ACKNOWLEDGMENT

This research was financed under Project PROFAPI_00362 from Technological Institute of Sonora.

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