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Total Polypheonls,Flavonoid Content, Kaempferol Concentration And Antioxidant Activity Of Two Onion Syrian (Spring And White)

Issa, M.*, karabet, F., and ALJOUBBEH, M

Department of Chemistry - Faculty of Science - University of Damascus - Syria .

***Corres.author: mayadaissa99@hotmail.com**

Abstract: The content of polyphenols and antioxidant activity for spring and white of Syrian's Onion have been studied. The concentration of polyphenols in spring onion is higher than white onions, with values ranged (1665.7 ± 13.6 to 2288.9 ± 12.5) mg gallic acid equvalents / 100g dry weight) and (297.9 ± 2.6 to 538.5 ± 3.2) mg GAE / 100 g DW respectively. The total flavonoid content was measured and the values was (380.3 ± 3.4to 569.1 ± 2.6) mg Qu eg/100g DW for the spring onion and from (80.2 ± 1.5 to 180.0 ± 2.4) mg Qu eg/100g DW for the white onion.

The extracts of spring onion show higher antioxidant activity (68.8 ± 0.9 to 83.1 ± 2.3 %DPPH) than white onion (60.4 ± 0.26 to 75.3 ± 0.14 %DPPH),so the higher polyphenols content was associated with higher antioxidant activity.

Concentrations of keampferol was determined using HPLC analysis in spring onion ranged from (49.2 ± 0.7 to 188.4 ± 2.3) µg/100g DW. And in the white onion was from (16.0 ± 0.4 to 66.5 ± 0.8) µg/100g DW.

Key Words: White onion, Spring onion, polyphenols content, flavonoid antioxidant activity, Keampferol.

1. Introduction

The synthesized flavonols in onion plants (Allium cepa) are a class of flavonoid with high ant oxidative effect to protect cells against damaging effects of Ultra violet radiation and hydrogen peroxide[1].

Onions are one of the most widely consumed vegetables. Fresh and dehydrated are widely used in the human diet, not only as a spicy garnish but as a source of nutriets and non-nutritive health- promotion compounds. Worldwide production of onions is estimated at ~64 million tons. The largest producers (in millions of tons) are in china (19.8) India (9.5), the united states (3.4) Turkey (2.2), South Korea (1.8) and Japan (1.6). Components of onions (in percent) include water (89.1), carbohydrates (9.3), Protein (1.1), Fat (0.11), Vitamins, minerals, and Sulfur compounds which are responsible for Pungency, Saponins and flavonoids[2].

Approximately ≈ 15.5 kg of onions are consumed worldly wide annually per person. And there are relatively large amounts of quercetin and keampferol in onions[3].

One group of total polyphenolic compound are secondary metabolites widely distributed across the Syrian plant with a great variety of structures they from the significant constituents of human diet.

Plants of Allium family are an important source of dietary flavonols[4] quercetin and kaempferol further more kaempferol is a natural flavonoid. Previous studies have reported that kaempferol has anti-proliferation activities and induces apoptosis in many cancer cell lines Kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one) is a flavonoid found in many edible plants (e.g. tea, broccoli, cabbage, kale, beans,

endive, leek, tomato, strawberries and grapes)., onions is important and commonly consumed uncooked, which minimizes loss of flavonoids[5]. The aim of this research is to quantify the total total polyphenol content,total flavonoids and antioxidant activity of two typical Syrian onion varieties (white and spring onion) of the most widely consumed in Syrian, and determine the content of keampferol by HPLC.

2- Material and Methods

2.1. Chemicals

Methanol for HPLC, Gallic acid, Folin-Ciocaltey reagent, Sodium Carbonate, Sodium Nitrite, Aluminum Chloride, Sodium Hydroxide, Hydrochloric acid, quercetin, kaempferol, 2,2-diphenyl- 1-picryl hydrazyl (DPPH) and ascorbic acid analytical grade were purchased from fluka Chem. Co.

2.2. Preparation of extracts

Plant material was collected in March(2011) from region of Syria white onion (1:Alppo 2:Hama 3:ALrastan 4:ALgaab) and spring onion

(5:Parzph 6:Gota 7:Daria 8:Kafarsosa). The collected plant material were stored in darkness at room temperature +20C°.Dried plant parts were cut up and stored in dark containers until needed. Aground dried sample of 5 gr, weighted and phenolic and flavonoid compounds were extracted with 50 ml 80% aqueous methanol (80%) using ultrasonic bath for 20 min at room temperature. An a liquid of the extracts was ultra-centrifuged for 5 min at 14000 rpm[6].

2.3. Total polyphenols contents (TPC)in the onion extracts

The total polyphenols contents (TPC) of the onion was determined with Folin-Cico calteau assay. An aliquot (1ml) of extracts or a standard solution of gallic acid ranging from (0 to 60 mg/L Final concentration and r^2=0.999) was added to 25ml volumetric Flask, containing 9 ml of distilled deminirised water (dd H_2O). Areagent blank using (dd H_2O) was also prepared. One milliliter of folin-Ciocalteu's phenol reagent was added to mixture and shaken. After 5 min, 10 ml of 7% Na_2CO_3 solution was added to the mixture. Reagent blank using (dd H_2O).The solution was added to 25 ml with dd H_2O and mixed. After in cubation for 90 min at room temperature, the absorbance against the prepared reagent blank was determined at 765 nm with an (UV-VIS Spectrophotometer Jasco model 7500 Japan). The data for the total polyphonls contents of white birch leaves were expressed as milligrams of gallic acid equivalents (GAE) per 100 grammas dry mass (mg GAE/100g DW)[6]. All samples were analyzed in triplicates.

2.4. Total flavonoid contents (TFC)

The total flavonoid content (TFC) was determined according to (Atanassova, et al., 2011)[6], using a method based on formation of a complex flavonoid - aluminum chloride. An aliquot (1 ml) of extracts or a standard solution of quercetin ranging from (10 to 100 mg/L) final concentration and r^2=0.998) was added to a 10 ml volumetric flask, containing 4 ml of distilled deionized water (dd H_2O). To the flask was added 0.3 ml 5% $NaNO_2$. After 5 min, 0.3 ml 10% $AlCl_3$ was added. After six minutes 2 ml 1 M NaOH was added and the total volume was made up to 10 ml with dd H_2O. The solution was mixed well and the absorbance was measured against a prepared reagent blank at 433 nm with an UV-VIS spectrophotometer. The data of total flavonoid contents of the onion were expressed as milligrams of quercetin equivalent (QE) per 100 grammas dry mass (mg QE/100g DW). All samples were analyzed in triplicates.

2.5. DPPH Free radical scavenging activity

The free radical scavenging activity of extracts was evaluated with DPPH (1.1-diphenyl-2 picrylhydrazyl) using mouthed[7]. One ml of sample was added to (4ml) of methanol solution of DPPH(0.4 m Mol^{-1}) and the mixture was kept at room temperature. After (30 min) the absorbance was measured at 515 nm with spectra photometer(UV-VIS-Jasco model 7500 Japan). The antiradical activity(%) was calculated using the ratio

$I\% = [(A_{control} - A_{sample}) / A_{control}] \times 100$

Where:$A_{control}$: is the absorbance of $DPPH^{\cdot}$Solution.

A_{sample}: is the absorbance of $DPPH^{\cdot}$Solution after the addition of the sample[6].

The IC_{50} value of DPPH scavenging activity was Calculated from the graph of antioxidant activity percentage against extract concentrations.

2.6. Kaempferol content

samples Preparation for HPLC analysis 1g of dried and powdered plant material was extracted with(90%)methanol water (1×10 ml) for 2h at room temperature. The plant extract was hydrolyzed with 1.2 N HCl by refluxing on a water bath at 85 C° for one hour in (The hydrolysis was processed and subjected to qualitative and quantitative analysis by using HPLC system).The extract was cooled, filtered using a Büchner filter made to 5 ml with methanol and filtered again with 0.45 µm Whitman membrane filter before injection into the HPLC. The extracts were kept in air light amberbettles and stored in the freezer until analyzed[8].

HPLC condition column, Nova pak C_{18} (150 × 3.9 mm, 4µm) mobile phase: mixture of methanol , water and 0.025M phosphate buffer (45/55, V/V) (PH=2.4).

Results (µg/100g DW) were obtained by comparison of peak areas (λmax 370 nm) of samples with those of standards[9].

3- Results and discussion

3.1. total polyphenol content (TPC)

The total polyphenol content (TPC) of the onion extracts determined by folin-Ciocaltey method was in the range (297.9±2.6 to 583.5 ± 3.2) mg GAE/100g DW for the white onions and the range (1665.7 ± 13.6 to 2288.9 ± 12.5) mg GAE/100g DW for the spring onions from the green part. While the values ranging from (390.9 ± 11.3to 479.4 ± 1.5) mg GAE eg/100g DW for the white parts of the spring onion. There for, the TPC of the spring onions was significantly higher than that of the white onions (table 1). Results obtained for these varieties are consistent with those reported for other onion varieties from other countries. The TPC of white onions was similar to the range reportedly[10] for unhdrolysed extracts. Several onion varieties contained (TPC) in a similar rang to that of the Spanish onions[11].

Table (1) total polyphenol content in dry extract.

White onion	total polyphenol content (mg GAE/100gDW)	Spring onion		Total polyphenol content (mg GEA/100g DW)
1	583.5 ± 3.2	5	white part	391.0 ± 11.3
			Green part	1665.7 ± 13.6
2	350.7 ± 4.1	6	white part	408.2± 4.3
			Green part	1907.0 ± 10.5
3	456.8± 3.7	7	white part	415.7 ± 3.2
			Green part	1873.8 ± 4.6
4	297.9 ± 2.6	8	white part	479.4 ± 1.5
			Green part	2288.9± 12.5

* Data are mean ± SD(n=3)

3.2. Total flavonoids content (TFC)

The flavonoid content of the white onion extracts was also determined, to compare with (TFC) in the spring onion. The results confirmed to a literature report[12,13] that flavonoids represent the main group of total polyphenol compounds in onion this can be seen in (table 2) with values ranging from (80.2 ± 1.5 to 180.0 ± 2.4) mg Qu eg/100g DW the white onion. While the values ranging from (380.3 ± 3.4to 569.1 ± 2.6) mg Qu eg/100g DW for the spring onion but the white parts from the spring onion the values ranging from (100 ± 2.6to 126.1 ± 3.1) mg Qu eg/100 g DW.

Table (2) total flavonoids content in dry extract

White onion	Flavonoid contents mg Qu/100 g DW	Spring onion		mg Qu/100 g DW
1	180.0 ± 2.4	5	white part	100.0 ± 2.6
			Green part	380.3 ± 3.4
2	80.2 ± 1.5	6	white part	103.8 ± 1.5
			Green part	456.2 ± 3.7
3	97.0 ± 0.9	7	white part	103.8 ± 2.2
			Green part	446.1 ± 1.9
4	110.0 ± 1.1	8	white part	126.1 ± 3.1
			Green part	569.1 ± 2.6

* Data are mean ± SD(n=3)

3.3. Antioxidant activity

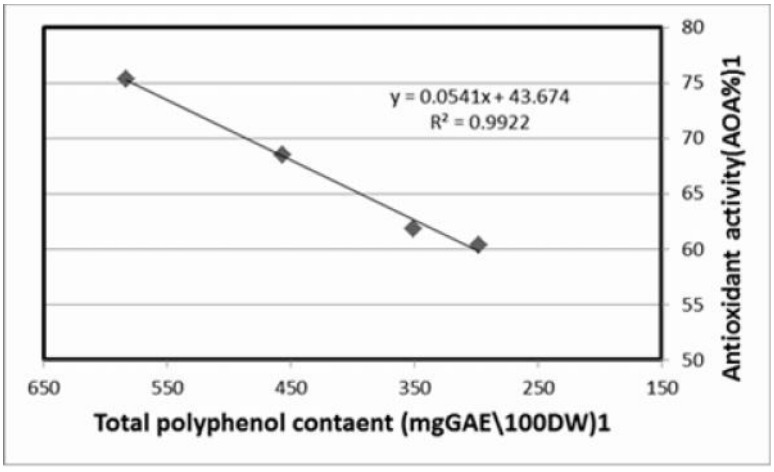
The variation of the TPC of onion samples are reflected in the antioxidant activity of extracts as reported previously[11]. The antioxidant activity of onion extracts determined by this method is in good agreements with recent reports for onions from other countries[7,14]. Table (3) the values of spring onion extracts are slightly higher than values reported in the range of (68.8 ± 0.9 to 83.1 ± 2.3) %in the green part while in the white onion give us in the range (60.4 ± 1.5 to 75.3 ± 0.1) %.

Table (3) antioxidant activity (AOA)%

White onion	AOA%	Spring onion		AOA%
1	75.3 ± 0.1	5	white part	64.3± 1.2
			Green part	68.8 ± 0.9
2	61.8 ± 0.2	6	white part	65.2 ± 0.3
			Green part	80.3 ± 1.6
3	68.5 ± 2.5	7	white part	68.5 ± 0.5
			Green part	75.5 ± 0.3
4	60.4 ± 1.5	8	white part	70.3 ± 1.3
			Green part	83.1 ± 2.3

* Data are mean ± SD(n=3)

Fig(1)linear concentrationsbetween the total polyphenol content and antioxidant activity (AOA)%in white onion



Linear concentrations between the amount of total polyphenol and antioxidant activity in white onion correlation coefficient Y=0.0541X+43.674, coefficient of delamination R2 =0.9921.

4.3 Kaempferol concentration

Spring onion contained quite high levels of flavonoids in their green part, with the high a mounts of kaempferol in the range (49.2 ± 0.7 to 188.4± 2.3) µg / 100g DW, and in the white part the range is(2.7 ± 0.3 to18.6 ± 0.5) µg / 100g DW ,while the white onion the range (16.0 ± 0.4 to 66.5 ± 0.8) µg / 100g DW.

Several onion varieties contained keampferol in a similar rang (Hui, K and Mohamed, 2001) so we can see the results in table (4) and Figure (1).

Table (4) concentration of Kaempferol

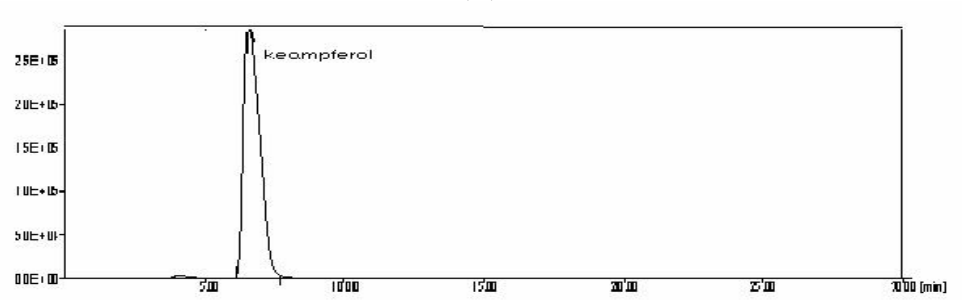
White onion	Kaempferol («g/100g)	Spring onion		Kaempferl («g/100g)
1	66.6 ± 0.8	5	white part	18.6 ± 0.5
			Green part	102.1± 1.4
2	16.1 ± 0.4	6	white part	2.7 ± 0.3
			Green part	90.7 ± 1.1
3	18.5 ± 0.6	7	white part	16.7 ± 0.6
			Green part	188.4 ± 2.3
4	54.0 ± 0.7	8	white part	10.0 ± 0.3
			Green part	49.2 ± 0.7

* Data are mean ± SD(n=3)

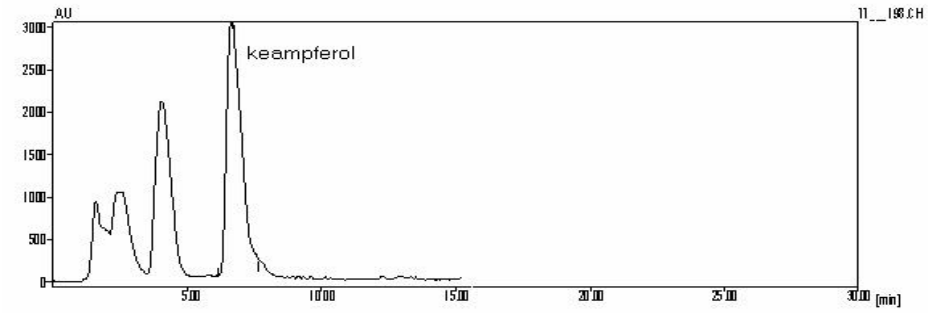
That the spring onion has content the higher test of values table (4)

Fig.2. Typical Chromatograms of(A) standard solution of keampferol at 160 («g/ml)and(B)Spring onion green part extract(C)white onion extract

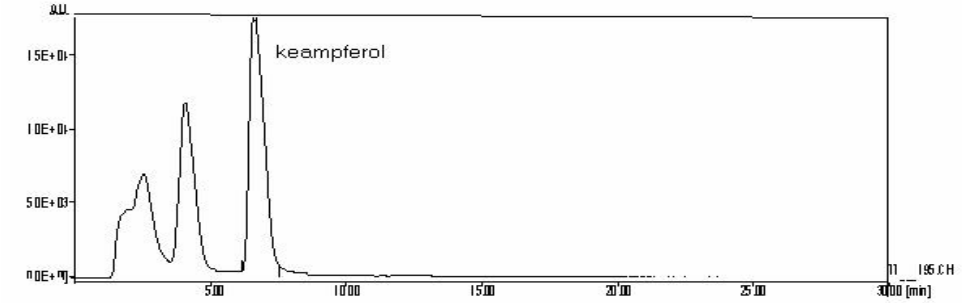
(A)



(B)



(C)



Conclusions

Our study has shown that the spring onion variety has higher concentrations of TPC, TFC and keampferol than the white onion variety. There was a good correlation between TPC content and antioxidant activity. So give us the high amounts of kaempferol was found in spring onion .We recommend including spring onion(white part and green part)in human diet because their high amounts of antioxidants.

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