Potravinarstvo, vol. 10, 2016, no. 1, p. 444-451
doi:10.5219/658
Received: 7 September 2016. Accepted: 10 October 2016.
Available online: 16 October 2016 at www.potravinarstvo.com

ISSN 1337-0960 (online)

CONTENT OF TOTAL POLYPHENOLS AND ANTIOXIDANT ACTIVITY IN VARIETIES OF ONION AND GARLIC

Ismael Sulaiman Dalaram

ABSTRACT

Garlic as onion family and the most important Allium species consumed all over the world. Garlic possesses potential health promoting effects due to its high phenolic content and a good source of vitamins, minerals and major component like polyphenols, flavonoids, thiosulfinates and other sulfur compounds. Red onion and garlic are among the important parts of diet in many world populations, and there is also a long-held belief in their health enhancing properties. In this work evaluated content of total polyphenols and antioxidant activity in onion and garlic. Samples of plant material (onion, garlic) were collected at the stage of full maturity in the village (Harmota) in Koysinjac town in Erbil city. The content of the total polyphenols was determined by using the Folin-Ciocalteu reagent (FCR). Antioxidant activity was measured by using a compound DPPH (2.2-diphenyl-1-picrylhydrazyl). Phenolic content and antioxidant activities of one variety of garlic and four varieties of onions have been studied. In the present experiment it was detected, that total polyphenols content in samples onion ranges from 626.61 – 322.83 mg.kg^{-1} (onion) and 506.7 mg.kg^{-1} for (garlic). Statistically significant highest value of total polyphenols was recorded in red onion the lowest content of total polyphenols was recorded in white onion 322.83.mg.kg^{-1}, and statistically significant highest value of antioxidant activity was recorded in red onion (33.42%) the lowest value was in white onion (22.68%). The value of antioxidant activity of garlic was (28.47%). Data analysis showed that the red variety onion presents highest value of total polyphenolic compounds. Consequently, antioxidant capacity was highest for red variety compared to other variety of onion and garlic. The order value of TPC was follow: Red Onion (626.61) >Garlic (506.70) >yellow onion (423.94) >pink onion (345.36) >white onion (322.83). Based on the measured values of AOA in onion and garlic samples can be classified as follows: Red Onion (33.42) >Garlic (28.47 >yellow onion (25.95) >pink onion (23.74) >white onion (22.68).

Keywords: onion; garlic; total polyphenols; antioxidant activity; ethanolic extracts

INTRODUCTION

Plants and plant-derived produce are among the prime utilities of mankind for food, shelter and cure since the dawn of civilization and it wouldn't be inappropriate to state that the use of medicinal plants predates written human history **(Harrison et al., 2015)**. Among these medicinal plants, garlic (*Allium sativum* L.) has secured its repute of a therapeutic panacea. Onion and garlic are the most important Alliums species consumed all over the world. Onion (*Allium cepa* L.) is one of the most consumed vegetable planted widely across the world. Huge quantities of onions are consumed all over the world, as it is very popular flavoring agent. However, available information on their free radical scavenging activities is scanty. In Europe Onion is one of the main vegetables consumed either raw or processed in different ways **Benitez et al. (2011a)**. Medicinal plants are important in pharmacological research and drug de- elopement **(Li and Vederas, 2009)** Polyphenols are natural substances in plants that are antioxidants with the potential to protect the body from some diseases. Interest in natural antioxidants and particularly in dietary antioxidants which, are present in vegetables and contribute to protection against oxidative stress in humans, is increasing. Antioxidant is defined as any substance that when present at low concentration compared to those of an oxidisable substrate significantly delays or prevents oxidation of that substrate **Li et al. (2007)**. The essential oil reveals interesting properties, such as antimicrobial agent and moderate reducing power feasible to implement in food **Ye et al. (2013)**. Also, phenolic extracts obtained from wastes of onion were used to evaluate the capacity inhibiting of processes inflammatory and oxidation of low-density lipoprotein (LDL) **Albishi et al. (2013)**. Onions (*Allium cepa* var. cepa) are an important source of bioactive compounds including phenolic compounds, flavonoids, fructooligosaccharides (FOS), thiosulfinates and other sulfur compounds, and many of these compounds have potential beneficial properties for human health **Soinen et al. (2012)**. Onion is one of the highly rich sources of main flavonols, quercetin in human diet **(Sellappan, Akoh, 2002)**. Onion is a source of minerals such as iron, selenium, iodine, potassium, calcium, sulfur, and many others. Onion is characterized by not only rich in vitamins and minerals, but is characterized by a strong content of biologically active substances, especially polyphenolic compounds. Many groups of polyphenolic compounds contained in onion e.g. phenolcarboxylic acids, such as protocatechuic, caffeic, ferulic, p-coumaric, p-hydroxybenzoic and vanillic acid. Many reports have indicated that onions have a wide range of beneficial properties for human health, such as anti-mutagenic **Singh et al. (2009)**, and antioxidant capacity **(Lu et al., 2011; Pérez-Gregorio et al., 2011)**. Epidemiological studies suggest that long term consumption of diets rich in plant polyphenols offer protection against development of cancers, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases **(Pandey and Rizvi, 2009)**.

Garlic (*Allium sativum* L.) is one of the most commonly produced vegetables worldwide. Fruits and vegetables are the major functional foods because they are the main sources of nutraceuticals such as vitamins, minerals and phenolic compounds **(Tomás-Barberán and Espin, 2001; Rupasinghe and Clegg, 2007)**. Garlic possesses potential health-promoting effects due to its high phenolic phytochemical content and is a source of natural antioxidants **Nuutila et al. (2003)**. The health properties of garlic depend on its bioactive compounds and especially on phenolic compounds **(Lanzotti, 2006; Corzo, et al., 2007)**, which have interesting pharmacological properties, are present in relatively high amounts **Beato, et al. (2011)**. Although researchers have paid considerable attention to isolate and identify bioactive compounds of garlic which account for its marvelous therapeutic repute, less reports are available regarding the diversity for abundance of the active allelochemicals within different garlic ecotypes and very few literature documents the considerable differences accounted for total phenolic compounds **Chen et al. (2013)**. The wealth of scientific literature supports the proposal that garlic consumption have significant effects on lowering blood pressure, prevention of atherosclerosis, reduction of serum cholesterol and triglyceride, inhibition of platelet aggregation, and increasing fibrinolytic activity **(Chan et al., 2013)**. Antioxidants are absorbed and metabolized in the body in a variety of ways and some antioxidants are more bioavailability than others **(Kalt, 2005)**.The natural antioxidants in foods, fruits, vegetables, beverages, spices and supplements have received much attention for their nutritive value in recent years and various synthetic antioxidants have also been in commercial use. There is an increasing demand for natural antioxidants to replace synthetic additives in the food industry. Natural antioxidant substances are presumed to be safe since they occur in plant foods. Antioxidants can scavenge radicals by three major mechanisms: hydrogen atom transfer, electron transfer, and combination of both these transfers **Prior et al. (2005)**. Antioxidants are vital substances, which possess the ability to protect the body from damages caused by free radical induced oxidative stress. A variety of free radical scavenging antioxidants is found in a number of dietary sources **Qusti et al. (2010)**. The main characteristic of antioxidant is its ability to trap free radicals. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidants scavenge free radicals that reduce risk of cancer and cardiovascular diseases. According to **Benkeblia (2005)**, Allium species are revered to possess anti-bacterial and anti-fungal activities, and they contain the powerful antioxidants, sulphur and other numerous phenolic compounds which have aroused great interests for food industries. A mong the species of onion, the red onion is abundant in polyphenols, flavonol and tannin **(Gorinstein et al., 2010)**. The essential oil from Allium cepa may be anew agents applied in food system **(Ye et al., 2013)**. The aim of this study was to determine levels of total polyphenolic and antioxidant activities among local commonly consumed vegetables (Onion and garlic) in Kurdistan region Iraq.

MATERIAL AND METHODOLOGY

Chemicals and reagents

Folin–Ciocalteu reagent, 2, 2- diphenyl-1- picrylhydrazyl (DPPH), Sodium carbonate (Na_2CO_3), Methanol (CH_3OH).

Sample preparation

Four onion varieties, namely red onion, white onion, pink onion, yellow onion and garlic. Samples of plant material for this experiment were harvested at full maturity stage from area in the village (Harmota) in Koysinjac town in Erbil city in Iraq Republic.

Extraction of polyphenols

Samples of fresh onion and garlic were homogenized and were prepared an extract: 25 g cut onion, garlic extracted by 50 mL 80% ethanol according 24 hours. These extracts were use for analyze. Extractions were performed in light exposure was avoided during the extraction process.

Determination of TPC

Total phenolic content of each extract was determined by the Folin-Ciocalteu procedures according compounds in to the method of **Lachmann et al. (2003)** and expressed as mg of gallic acid equivalent per kg fresh mater(GAE) which corresponds to the mean response of all the major phenolic fruits and vegetables **(Georgé et al., 2005)**. And Gallic acid is usually used as a standard unit for phenolics content determination because a wide spectrum of phenolic compounds. The total polyphenol content was estimated using Folin-Ciocalteau assay. The Folin-Ciocalteau phenol reagent was added to a volumetric flask containing 100 μL of extract. The content was mixed and 5 mL of a sodium carbonate solution (20%) was added after 3 min. The volume was adjusted to 50 mL by adding of distilled water. After 2 hours, the samples were centrifuged for 10 min. and the absorbance was measured at 765 nm of wavelength against blank (Shimadzu UV/VIS-1240, Japan). The concentration of polyphenols was calculated from a standard curve plotted with known concentration of garlic acid (Sigma Aldrich). The average content of polyphenol compounds in the samples was obtained from six replicates.

Determination of antioxidant activity (TAC)

The antioxidant capacity (TAC) of the onion and garlic extracts was measured using a DPPH method described by **Brand and Williams et al. (1995)** method-using a compound $DPPH^{\cdot}$ (2.2-diphenyl-1-pikrylhydrazyl)).

2.2-diphenyl-1-pikrylhydrazyl (DPPH) was pipetted to cuvette (3.9 cm^3) then the value of absorbance, which corresponded to the initial concentration of $DPPH^{\cdot}$

solution in time Ao, was written. Then 0.1 cm³ of the followed solution was added and then the dependence A = f (t) was immediately started to measure. The absorbance of 0 and 10 minutes at 515.6 nm in the Spectropho- tometer Shimadzu UV/VIS-1240 was mixed and measured. The percentage of inhibition reflects how antioxidant compound are able to remove DPPH· radical at the given time.
Inhibition (%) = (A0 - At / A0) x 100

Statistical analysis

Results were statistically evaluated by the Analysis of Variance. All the assays were carried out in triplicates and results are expressed as mean ±SD. The data were subjected to the F-test in the one-way analysis of variance (ANOVA) If the *p*-value of the F-test is less than 0.05, there is a statistically significant difference between the means at the 95% confidence level; the Multiple Range Tests will tell which means are significantly different from which others. The method currently being used to discriminate among the means of Fisher's least significant difference (LSD) procedure. Analysis was conducted using SAS software 9.4.

RESULTS AND DISCUSSION

On the base of reached results there were estimated changes in the total polyphenols content and also changes in total antioxidant capacity values in dependence on garlic and onion.

Evaluation of total polyphenol content and values of antioxidant capacity in onion and garlic

The distribution of polyphenolic content and antioxidant properties among different varieties onion and one variety garlic. The frequency distributions of TPC and TAC are shown in Figure 1, 2 and Table 1, 2. In the present experiment it was detected, that total polyphenols content in samples ranged from 322.83 mg.kg^{-1} GAE (in white variety of onion) to 626.61mg.kg^{-1} GAE (in red variety of onion) (Table. 1). According to the obtained results, the polyphenols content (TPC) in the tested was significantly different and was influenced by variety and color. Statistically the highest value of total polyphenols (626.61 mg.kg^{-1} GAE) was recorded in variety Red onion. The lowest content of total polyphenols was recorded in white variety (322.83 mg.kg^{-1} GAE). According to the average contents of total polyphenols in fresh matter of onion and garlic there is the following line in present work: red onion (626.61 mg.kg^{-1} GAE) >garlic (506.70 mg.kg^{-1} GAE) >yellow onion (423.94 mg.kg^{-1} GAE) >pink onion (345.36 mg.kg^{-1} GAE) >white onion (322.83 mg.kg^{-1} GAE). **Andrejiová et al. (2011)** found that the content of total polyphenols in onion was in the interval from 105 to 134 mg.kg^{-1}. **Amin et al. (2013)** reffered that the content of polyphenols was 132.2 mg.kg^{-1}. **Brat et al. (2006)** published that the content of total polyphenols in onion was761 mg.kg^{-1}. comparision to our determined values of polyphenols in onion with their results our results are higher than the values of **(Andrejiová et al., 2011: Amin et al., 2013)** but lower than 761 mg.kg^{-1} for **Brat et al. (2006)**. **Bystrická et al. (2014)** found the values of total polyphenols in onion were in the range from 389.64 ±20.31 mg.kg^{-1} to 429.58 ±17.44 mg.kg^{-1}. **Karadeniz et al. (2005)** reported that the polyphenols in onion was in the amount 536 mg.kg^{-1}. **Benkeblia et al. (2005)** reported that the highest content of polyphenols was in red onion (473 mg.kg^{-1} GAE), followed by yellow variety (347 mg.kg^{-1} GAE). In comparision to our determined values of red and yellow onion, our result was higher content polyphenols in red onion (626.61 mg.kg^{-1} GAE) followed by yellow variety (423.94 mg.kg^{-1} GAE). **Kavalcová et al. (2015)** published that the content of polyphenols in the case of yellow varieties of onion Sherpa (455.22 mg.kg^{-1} GAE), Bingo (451.71 mg.kg^{-1} GAE) and Boston (441.32 mg.kg^{-1} GAE). In comparision to our determined values of polyphenols in yellow onion, their results were in similar interval. **Armand et al. (2012)** reported that the content of polyphenols in onion was 620 mg.kg^{-1}. This report is in agreement with the values of polyphenols content in red variety of our result. **Kavalcová et al. (2015)** published that the content of polyphenols in the case of white dry variety of onion (142.01 mg.kg^{-1} GAE). In comparison to our determined values (322.83 mg.kg^{-1} GAE) of polyphenols content in white variety, their results were lower. Our results correspond to the results of **Armand et al. (2012)**, which reported the highest values of total polyphenols in variety of red onion followed by yellow variety and white variety. **Andrejiová et al. (2011)** also reported the content of total polyphenols in red onion 1088.51 mg.kg^{-1}, followed by yellow onion 652.15 mg.kg^{-1} and in white onion 105.19 mg.kg^{-1}.

In the present work antioxidant activity in samples ranges from 22.68% to 33.42%. The DPPH method is frequently used to determine the antioxidant activity. DPPH assay is a primary antioxidant activity test that determines the free radical scavenging activity of the respective samples. When comparing onions and garlic from the antioxidant capacity (TAC%) point of view (Figure 1 and 3; Table1, 2). *p*-value <0.05 there is a statistically significant difference between the means of the 4 varieties onion and one variety garlic at the 95.0% confidence level. The highest value was reached in case of red onion variety with the value 33.42%, the lowest value was found in case of white variety with the value 22.68%, in the case of garlic(28.47%) was recorded. Based on the measured values of AOA in garlic and onion varieties samples can be classified as follows: red onion (33.4242%) garlic (28.47%) >yellow onion (25.95%) >pink onion (23.74%) >white onion (22.68%). **Prakash et al. (2007)** published that the value of antioxidant activity in red onion was 50.6% and in white onion 13.6%. In comparison to our measured values of antioxidant activity in red onion was higher but in white onion was lower than our result. **Kavalcová et al. (2014)** published that the value of antioxidant activity in yellow onion was (25.7%). In comparison to our measured values (25.95) of antioxidant activity in yellow onion their results were in similar interval. **Škerget et al. (2009)** published that the value of antioxidant activity in yellow onion was (35%) there result were higher than our value (25.95) measured in yellow variety of onion. **Cheng et al. (2013)** determined that red onion extracts showed good antioxidant activity varying from 53.36% to 85.53% and better than in the yellow

Table 1. Total phenolic content and antioxidant capacity (mean and standard deviation values). In chosen variety Onion and Garlic (mg.kg^{-1}).

Variety	TAC%	TPC
Garlic	28.47 ±1.59 b	506.70 ±17.67 b
Pink onion	23.74 ±0.86 d	345.36 ±38.79 d
Red onion	33.42 ±0.87 a	626.61 ±11.22 a
White onion	22.68 ±1.14 d	322.83 ±11.37d
Yelow onion	25.95 ±0.51 c	423.94 ±9.21c

Note: Data expressed as means of six replications ± standard deviation. Values in the same column with the different letters present significant differences *p* <0.05 using F-test for independent samples.

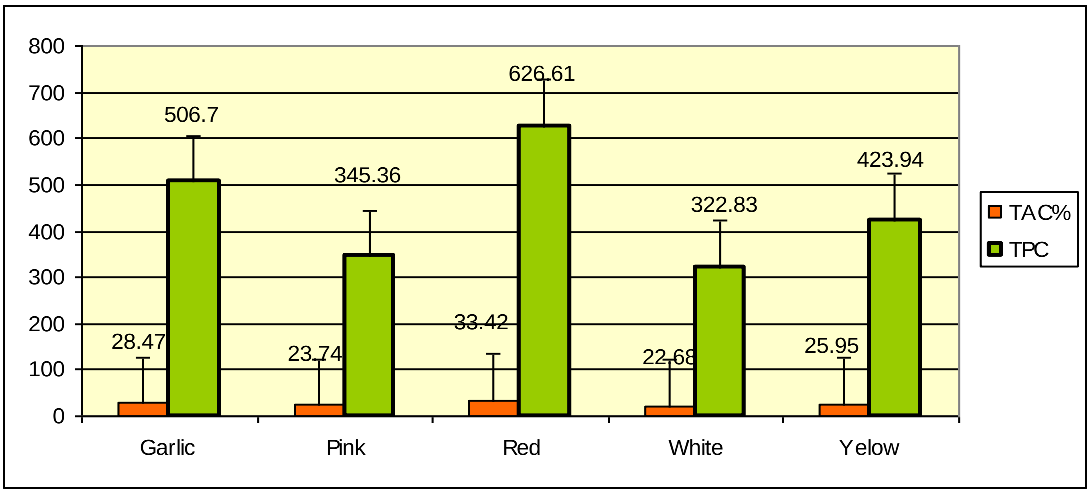

Figure 1 Average content of total polyphenols TPC (mg.kg^{-1}) and Average content of total antioxidant capacity TAC (%) in chosen varieties of onion and garlic.

Table 2 Total phenolic content and antioxidant capacity (mean and standard deviation values with the group). In chosen variety Onion and Garlic (mg.kg^{-1}).

	TAC (%)			TPC		
Variety	Mean	Std Dev	Group	Mean	Std Dev	Group
Garlic	28.47	1.59	b	506.70	17.67	b
Pink onion	23.74	0.86	d	345.36	38.79	d
Red onion	33.42	0.87	a	626.61	11.22	a
White onion	22.68	1.14	d	322.83	11.37	d
Yellow onion	25.95	0.51	c	423.94	9.21	c

Note: All the assays were carried out in triplicates and results are expressed as mean ±SD. The data were subjected to one-way analysis of variance (ANOVA) and the differences between various concentrations were determined Fisher LSD test using SAS software.

variety ranging from 52.32% to 72.25%. In comparison to our measured values their results were higher.

In case of garlic, **Priecina et al. (2013)** reported that the polyphenols in garlic was in the amounts from (272.28 to 1818.81 mg.kg^{-1}). In comparison to our determined values (506.70 mg.kg^{-1}), their results were higher. **Bystrická et al. (2014)** published that the content of polyphenols in the case of garlic (260.62 to 279.74 mg.kg^{-1}). In comparison to our determined values, their results were lower. **Manach et al. (2004)** noted that environmental and genetic factors have a major effect on polyphenols content. The content of polyphenolic compounds in onion also can be affected by the type of variety and color of bulb of onion. Several factors may affect the polyphenol content of plants, such as ripeness at the time of harvest, genotype, environmental factors, processing and storage **Picchi et al. (2012)**. Various factors, such as genotype of cultivar, growing season, post harvest treatment, and cultivation place are responsible for the variation in contents of such photochemical in garlic **Beato et al. (2011)**.

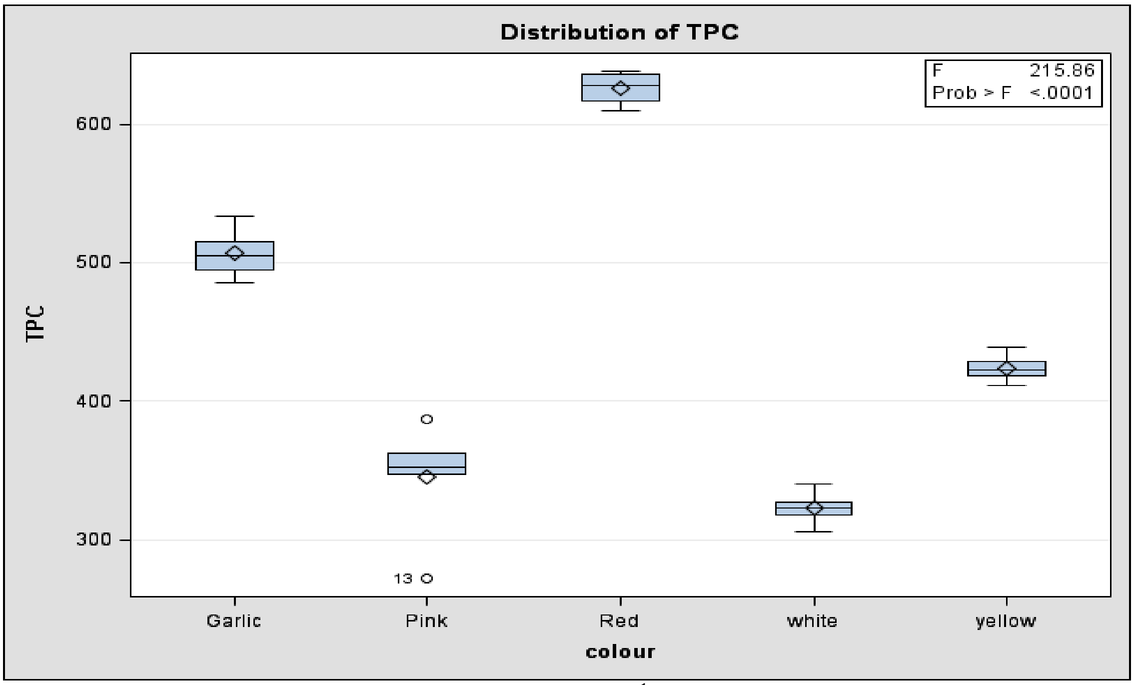

Figure 2 Box-plot of the content of total polyphenols TPC (mg.kg^{-1}) in chosen varieties of onion and garlic.

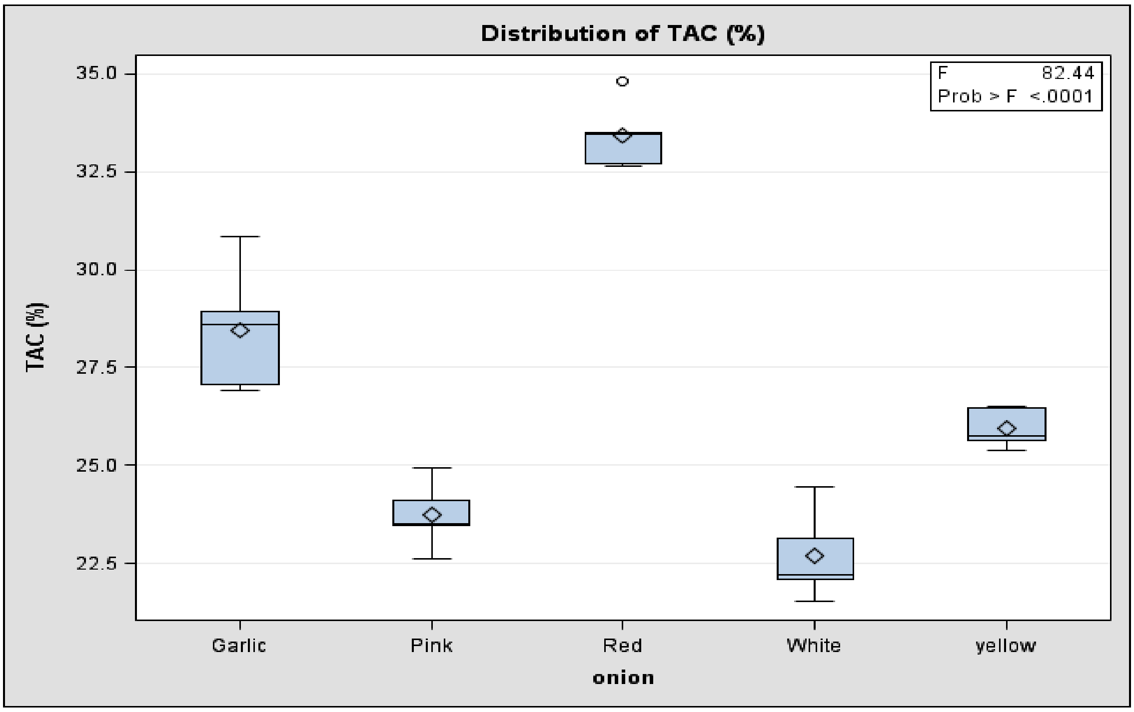

Figure 3 Box-plot of the content of total antioxidant capacity TAC (%) in chosen varieties of onion and garlic.

Statistical evaluation of total polyphenol (TPC) content differences significance within of chosen varieties

When comparing average content of total polyphenols TPC (mg.kg^{-1}) in four onion varieties and garlic in Table 2 and Figure 2 there were significant differences according to used statistical methods on the all types. Four types onion (red, yellow, pink, white) and garlic, observed confidence levels between almost the all observed varieties. Effect of varieties analysis (Table 2, Figure 2) for the phenolic compounds contents (TPC) of varieties onion and garlic of tested showed the presence of significant variety differences (p <0.05).

As shown in Figure 2. Garlic has higher content of total polyphenols TPC than yellow, pink and white but lower content of TPC than red variety onion.

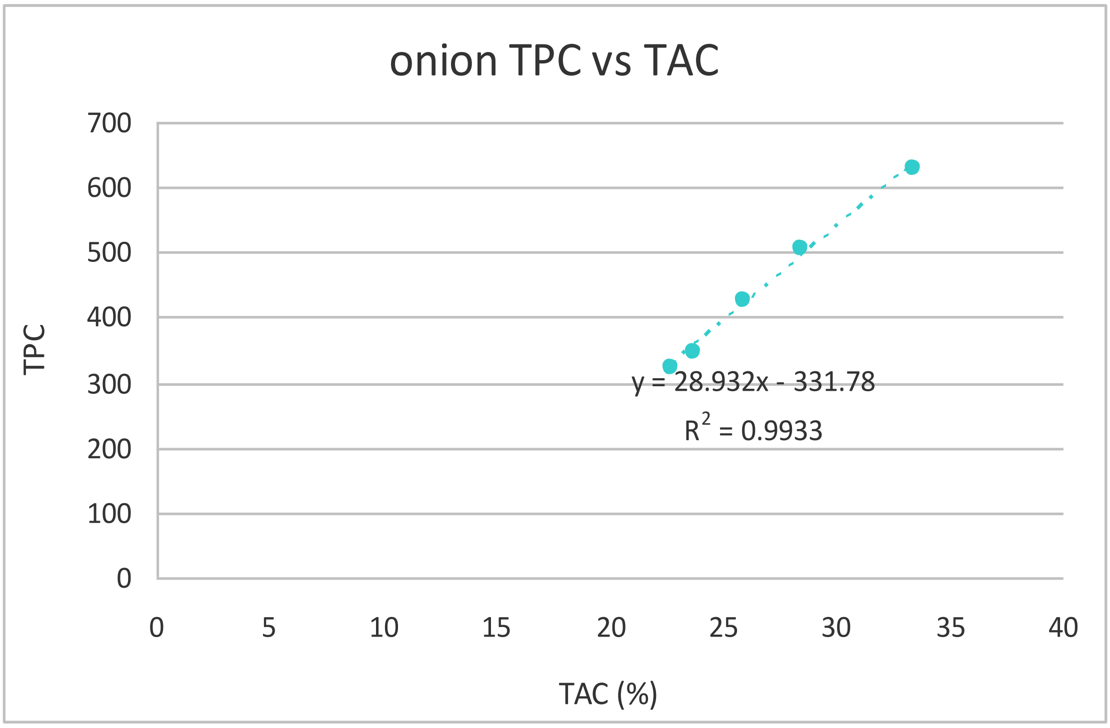

Figure 4 Correlation between TP and TAC of varieties of onion and garlic.

In the present work it was detected, that antioxidant activity in samples ranges from (22.68% to 33.42%). Statistically significant highest value of antioxidant activity was recorded in red onion the lowest value in white onion. **Kavalcova et al. (2014)**, published the interval of statistically significant highest value of antioxidant activity was recorded in onion from 20.22 ±0.53% to 25.76 ±0.53% and statistically significant the lowest value of antioxidant activity was recorded in garlic from 4.05 ±0.20% to 5.07 ±0.47%, our measured values of AOA in onion and garlic samples can be classified as follows: Red Onion (33.42%) >Garlic (28.47% >yellow onion (25.95%) >pink onion (23.74%) >white onion (22.68%). In comparison to our measured values incase of garlic and Onion our value of AOA was higher.

Correlation between the total antioxidant activity values and total phenolics contents

ANOVA linear correlation coefficients were used to assess the relationships between TPC and TAC, Correlation: Our result confirmed a strong statically correlations between total polyphenol content and total antioxidant capacity values. A statistically strongly significant correlation (R = 0.9966; p <0.05) was found (Figure 5). **Amarowicz et al. (2005)** analyzed the extracts of fababean, broad bean, adzuki bean, red bean, pea, red lentil and green lentil seeds using 80% (v/v) acetone and confirmed a statistically significant correlation between the total antioxidant activity values and total phenolics (p = 0.01). A strong correlation between total polyphenol content and antioxidant activity (R = 0.86; p <0.05) was observed also by **Akond et al. (2011)** in common bean and a statistically strongly significant correlation (p-value 2.391E-06; R = 0.802) was found between total polyphenol content and total antioxidant capacity values by **Dalaram et al. (2013)** in lentil. According these authors this finding suggests that total polyphenol content is a good predictor of in vitro antioxidant activity.

CONCLUSION

The phenolic content, antioxidant activity, and the correlation between phenolic content and antioxidant capacity were studied in varieties onion and garlic, Cultivar significantly affected phenolic accumulation and antioxidant capacity. Current results of total phenolic content (TPC) assay indicate that TPC is higher in red onion, also (TAC); the positive interrelationship between these two parameters demonstrates that the antioxidant activity depends mainly on polyphenols contents. Onions were rich sources of polyphenols and flavonoids, and showed the promising antioxidant and free radical scavenging activities. The results suggest that statistically the highest value of total polyphenols and antioxidant activity was in red onion. In the case of white varieties of onion, determined significantly the lower content of total polyphenols and antioxidant activity was recorded. The value of total polyphenols content and antioxidant activity of garlic were lower than red variety onion but higher value of total polyphenols and antioxidant activity than other varieties onion(yellow, pink, white).The content of total polyphenols and antioxidant activity may be affected by many factors for example postharvest (storage) and climatic conditions (altitude, rainfall, mean annual temperature) and the agrochemical composition of the soil (humus of content, nutrients) and type varieties.

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Contact address:

Ismael Sulaiman Dalaram, Department of Food Technology, College of Agriculture, Salahaddin University in Erbil, Iraq Republic, Tel. +42144989270, +9647504360364, E-mail: dlaram.dizayee@gmail.com.