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Determination of Total Polyphenolic Compounds in Organically and Conventionally Produced Fruits of *Cucurbitaceae* Family

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Abstract: *Cucurbitaceae* family, which contain a wide range of vegetables, have been commonly used as a food and it is a part of our Mediterranean cuisine, however, in some other regions its products including seeds and oils have been consumed as a dietary supplement for medicinal purposes. Also, it is considered rich in total phenolic compounds that benefit human health. Organic farming has been known to increase the accumulation of total phenolic compounds in the food. An experiment was conducted during 2017/2018 seasons on *Cucurbitaceae* family plants; seedlings were transplanted in raised beds in an open field as organic and conventional systems; fruit samples at two stages of growth (mature and immature), were taken to determine the total phenolic compounds. Results obtained showed that the levels of total polyphenols in immature are higher than that in mature vegetable fruits in both systems, however, the differences between the immature fruits in the systems investigated were insignificant. On the other hand, the effect of the interaction between the planting system and the harvesting stage on the levels of the entire polyphenols in the harvested vegetables were investigated; immature organic fruits contain higher content of total phenolic compounds compared to immature conventional produced fruits.

Key words: Bottle gourd · Colocynth · Cucumber · Pumpkin · Squash

INTRODUCTION

Polyphenols are secondary plant intermediates that play a vital role in plant colours. They include phenolic acids, flavonoids and anthocyanins. Research studies have shown that polyphenols are linked to many beneficial health effects including antifungal, antimicrobial and antioxidant effects [1], in addition to their anticancer property [2, 3]. However, the anti-inflammatory properties are poorly investigated. Polyphenols can be found in different plant-based food sources, such as vegetables of the *Cucurbitaceae* family, which include a varied range of vegetables like watermelon, cantaloupe, squash, cucumber, pumpkin seeds and pumpkins [4].

Pumpkins have been typically used as a food and it is a part of our Mediterranean meals, however, in some other counties other pumpkin products, such as oils and seeds have been used as dietary supplements for therapeutic purposes including the treatment of bladder and prostate problems [5, 6]. Investigations on pumpkin extracts and oil, indeed, have revealed beneficial health effects. It has been shown that the water extract of pumpkin seeds has positive effects against colorectal, prostate cancer and breast cancer cells [7].

There are numerous environmental factors that can alter the polyphenols content in the plant, such as climatic conditions, growing locations, harvest seasons and cultivars [8-10]. These factors are drawing the world's attention. Many research studies are carried out in order to develop and improve practical procedures that have a reduced influence on the environment and human health [11-13]. Conventional farming procedures require chemical fertilizers and pesticides [8, 14, 15], whereas organic farming essentially excludes the use of many pesticides and fertilizers associated with modern farming and is becoming more and more common worldwide [16].

Research studies have found that total phenolic levels were significantly higher in crops where organic farming was used compared with those which were grown in conventional farming [8]. This finding along with the known health beneficial effects of phenolic compounds in preventing chronic diseases, such as cancer and

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cardiovascular diseases in human [17] support the assumption that organically grown crops serves the human health better than the corresponding conventionally grown crops [18].

It has been shown that the maximum levels of total soluble phenols can be reached during the first stage of fruit development, then the levels decrease during the ripening stage [19, 20]. In organic farming in which insecticides, pesticides and herbicides are not used, in addition of applying nutrients without the use of inorganic fertilizers, the synthesis of phenolic compounds might be enhanced [21].

There are different approaches by which polyphenols can be separated and quantified, such as HPLC method [22], or Folin-Ciocalteu assay [15]. HPLC specifically measures simple phenols, while, Folin-Ciocalteu assay is a general method that measures simple polyphenols, phenols, tannins, flavonoids and some easily oxidized substances like ascorbic acid [23].

However, to the best of our knowledge, there are no study that have investigated and compare the levels of total phenolic compounds in organically and conventionally grown *Cucurbitaceae* Family, during mature and immature harvesting stage of the fruits. Therefore, the current study investigated the total levels of polyphenols in the extracts of various organically and conventionally produced vegetables of *Cucurbitaceae* family, such as Pumpkin (*Cucurbita maxima*), Cucumber (*Cucumis sativus*), Squash (*Cucurbita pepo*), Bottle Gourd (*Lagenaria vulgaris*) and Colocynth (*Citrullus colocynthis*) and in both harvesting stage of the fruits of the vegetables.

MATERIALS AND METHODS

The study experiments was performed during 2017/2018 seasons at Al-Balqa Applied University, As-Salt, Jordan; on *Cucurbitaceae* Family includes: Bottle Gourd (*Lagenaria vulgaris*), Colocynth (*Citrullus colocynthis*), Squash (*Cucurbita pepo*), Cucumber (*Cucumis sativus*) and Pumpkin (*Cucurbita maxima*), seedlings were transplanted in raised beds in an open field as organic farming (in which all chemical compounds were excluded) and as conventional systems (in which chemical compounds were included).

At the two stages of growth (mature and immature), two samples of the fruits were taken during the performance of the experiment in order to determine the total phenolic compounds, which was according to the method published by Asami *et al*. [15]. Fresh fruits of *Cucurbitaceae* family were grinded using pestle and mortar and homogenized. Three grams of the powder was then extracted with 40 ml of a solvent consisting of acetic acid, water and acetone (0.5:29.5:70) and vortexed with Vortex Mixer (Biosan, V1 Plus-Spain). The samples were allowed to stand for 1 h at room temperature for complete solvent extraction. Extracts were centrifuged for 15 minutes at 4000 round/minute using the centrifuge (Hettich EBA-20, Tuttlingen-Germany). Using a rotary evaporator (Heidolph, Laborota 4001, Schwabach-Germany), the samples were filtrated and concentrated under partial vacuum at 40°C and brought up to a total volume of 25 ml. Total phenolic concentrations were determined by Folin- Ciocalteu assay (Sigma, Steinheim-Germany), where 0.5 ml of sample was mixed with 0.5 ml of Folin-Ciocalteu reagent and allowed to stand for 5-8 min. A 10 ml of a 7 % sodium carbonate solution was then added, followed by distilled water to reach a final volume of 25 ml. Solutions were mixed and allowed to stand for 2 h. Total phenolic concentration was determined by measuring absorbance at 750nm using a spectrophotometer (Biotech Engineering Management CO. LTD., UV-9200, Nicosia- Cyprus). The results were expressed as milligrams Gallic acid equivalent (GAE) per 100 g fresh weight [15, 24, 25].

Statistical Analysis: A Completely Randomized Design (CRD) were used with five treatments (five types of plants); Bottle Gourd, Colocynth, Squash, Cucumber and Pumpkin. The treatments were performed in triplicates. Analysis of variance (ANOVA) was used to analyze the results according to Steel and Torrie [26]. Mean separation was performed by the Least Significant Difference (LSD) using SAS program. Data were considered significant when the differences with probability value were equals to and higher than 0.05.

RESULTS AND DISCUSSION

Data obtained; showed that generally in vegetables grown organically, the levels of the total phenolic compounds was higher than those grown conventionally, except with bottle gourd fruits; that showed an opposite trend (Table 1).

These results are in agreement with the previous findings that obtained by Oliveira [27]; in which organically produced fruits contain more phenolic compounds in compare to conventionally produced fruits.

Table 1: The effect of the planting system on the levels of total phenolic compounds (mg GAE/100g fresh weight) of the fruits of the *Cucurbitaceae* family plants

Planting system	Squash	Pumpkin	Cucumber	Colocynth	Bottle Gourd
Organic *	459.47[a]	81.667[a]	29.960[a]	165.577[a]	194.98[b]
Conventional *	420.23[a]	62.370[b]	24.673[a]	146.008[a]	294.11[a**]
LSD 0.05	77	10.98	6.63	22.4	40.94

* Values representing the means of triplicates

** Means in each column with different letters are significantly different according to LSD at 5 % level

Table 2: The effect of harvesting stage on the levels of total phenolic compounds (mg GAE/100g fresh weight) of the fruits of the *Cucurbitaceae* family plants

Harvesting stage	Squash	Pumpkin	Cucumber	Colocynth	Bottle Gourd
Immature *	493.18[a**]	80.983[a]	28.913[a]	160.500[a]	291.41[a]
Mature *	386.52[b]	63.053[b]	25.720[a]	151.085[a]	197.68[b]
LSD 0.05	77	11	6.2	22.4	40.94

* Values representing the means of triplicates

** Means in each column with different letters are significantly different according to LSD at 5 % level

Table 3: The effect of the interaction of planting system and harvesting stage on the levels of total phenolic compounds (mg GAE/100g fresh weight) of the fruits of the *Cucurbitaceae* family plants*

Treatments interaction	Squash	Pumpkin	Cucumber	Colocynth	Bottle Gourd
Organic immature *	530.57[a]	92.0833[a]	32.3133[a]	153.39[a]	295.02[a]
Conventional immature *	455.78[a]	69.8833[b]	25.5133[a]	167.61[a]	287.80[a]
Organic mature *	388.36[a]	71.2500[ab]	27.6067[a]	138.62[a]	94.9367[b]
Conventional mature *	384.67[a]	54.8567[b]	23.8333[a]	163.55[a]	300.42[a]

* Values representing the means of triplicates

** Means in each column with different letters are significantly different according to LSD at 5 % level

In this study, the detected levels of total phenolic compounds of pumpkin were very close to the levels reported in pumpkin grown in the household stated in the study of Zdunić *et al.* [28]. Nawirska-Olsza *et al*. [29], however, detected lower amounts (24 mg/100g FW) [29]. Interestingly, similar amounts of total phenolic compounds reported in this study was detected in another species of pumpkin (*Cucurbita moschata*) [30]. There were no previous studies on the levels of total phenolic compounds in bottle gourd, therefore, the results of bottle gourd in this study represent the first report of the levels of total phenolics.

The results obtained showed that squash has the highest levels of total phenolics compared to the other vegetables, while pumpkin and cucumber have the lowest amount. The levels of total phenolic compounds were affected by the harvesting stage, this effect was obvious in squash, pumpkin and bottle gourd; they all contain significant higher amounts of total phenolics in immature harvested vegetable fruits compared to their mature counterpart vegetables (Table 2).

Both cucumber and colocynth only have statistically similar amounts of total phenolics. According to previous studies; the total content of phenolic compounds in vegetables is affected by the harvesting stage. Generally, it has been found that immature vegetable fruits have higher content of total phenolic compounds compared to mature harvested vegetable fruits [31, 32]. The higher accumulation of the phenolic compounds in immature fruit and vegetables have an important role in the plant resistance against pathogenic attack [31].

The results observed in Table 2 showed that immature squash has the highest levels of the total phenolic compounds, followed by bottle gourd. While Colocynth, pumpkin and cucumber were all have similar amounts.

In the current study, the effect of the interaction between the planting system and the harvesting stage on the levels of total phenolic compounds in the vegetables were investigated (Table 3).

Immature organic pumpkin contains significantly higher content of total phenolic compounds compared to immature conventional pumpkin and mature conventional pumpkin, indicating the remarkable effect of the organic farming on increasing the levels of the total phenolic compounds in pumpkin. Whereas in bottle gourd, the total content of the total phenolic compounds was significantly higher in mature bottle gourd grown in conventional way compared to mature organic counterpart, which is opposite to that observed in pumpkin. The content of the mature conventional bottle gourd was statistically similar to that of the immature conventional counterpart, indicating the importance of the planting system on the levels of total phenolics in this vegetable. However, in the organically farmed vegetable, the levels of the total phenolic compounds were higher in immature vegetable compared to that in the mature vegetable, nevertheless, the differences did not reach significant levels. In bottle gourd the levels of the total phenolic compounds seem to increase as the vegetable get matured. In cucumber, squash and colocynth, both planting system and harvesting stage did not induce a significant influence on the levels of the total phenolic compounds in these vegetables.

Comparing the interaction of both planting system and harvesting stage between all of the harvested vegetable fruits; immature organically produced vegetable fruits have the highest levels of total phenolic compounds, such as squash, followed by bottle gourd, colocynth, pumpkin and cucumber.

While in mature organically grown vegetables, the highest amount was in squash, colocynth, bottle gourd, pumpkin and cucumber. In immature vegetables grown in conventional farming system, the highest amount was in squash, bottle gourd, colocynth, pumpkin and cucumber, which is similar to the arrangement observed for the organic immature vegetables. Whereas, the highest content in mature vegetables grown conventionally was squash and bottle gourd, followed by colocynth, pumpkin and cucumber.

It is important to note that other parameters, such as color, physical and chemical characteristics, could not be measured due to no funding resources and the limited time for conducting this study.

This study represented for the first time, the levels of total phenolic compounds in terms of milligrams/100 GAE of fresh weight in immature and mature vegetable fruits belonging to *Cucurbitaceae* family produced under organic and conventional systems and also reported the effect of the interaction of both planting system and harvesting stage on the levels of the total phenolics for all investigated vegetable fruits. Therefore, further studies are needed to confirm the results of this study and investigate further the individual phenolic compounds that characterise this specific plant vegetable of the *Cucurbitaceae* family.

CONCLUSION

The results of the present study showed that in organic and conventional systems, immature vegetable fruits contain more total polyphenols compared to the mature vegetable fruits. The total polyphenols increased in immature fruits grown organically compared to those grown conventionally.

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