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Research Article

Comparison of the Antioxidant Activity of Garlic Cloves with Garlic Husk and Stem: Determination of Utilization Potential of Garlic Agricultural Wastes

Deren TAHMAS KAHYAOĞLU

Kastamonu University, Faculty of Engineering and Architecture, Department of Food Engineering, Kastamonu
Corresponding Author: dtkahyaoglu@kastamonu.edu.tr

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Abstract

During the harvest period, garlic heads are collected and processed, while stems and leaves are discarded. Processing of these sections, forming 10% (w/w) of the total weight of garlic, has great importance in terms of both gains for the country's economy and to reduce the amount of solid waste. In our study, some physicochemical analyses were performed, along with total phenolic compound, total flavonoid matter, total antioxidant activity, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azinobis-3-ethylbenzthiazoline-6-sulfonate (ABTS), ferric reducing/antioxidant power (FRAP) tests to determine antioxidant capasity in garlic cloves, and the husk and stem of the dried form of this garlic obtained from 25 different fields in Taşköprü county, Kastamonu province in Turkey. When mean values are noted, the highest total phenolic compounds, total flavonoid matter, total antioxidant activity, DPPH, ABTS and FRAP values were found for garlic cloves; this was followed by garlic husk and garlic stem. The total bioactive compounds and antioxidant activity determined in garlic husks and garlic stems are promising.

Key words: *Allium sativum* L., total bioactive compounds, DPPH, ABTS, FRAP

Sarımsak Dişinin Antioksidan Aktivitesinin Sarımsak Kabuğu ve Sapı ile Karşılaştırılması: Sarımsak Tarımsal Atıklarının Kullanım Potansiyelinin Belirlenmesi

Öz

Hasat döneminde sarımsak başları toplanıp işlenirken, sapları ve yaprakları atılır. Toplam sarımsak ağırlığının %10'unu (m/m) oluşturan bu kısımların işlenmesi, hem ülke ekonomisi açısından kazanımlar sağlaması hem de katı atık miktarının azaltılması açısından büyük önem taşımaktadır. Çalışmamızda Türkiye'nin Kastamonu İlinin Taşköprü İlçesinde bulunan 25 farklı tarladan temin edilen sarımsakların dişi ve bu sarımsakların kurumuş formdaki kabuk ve saplarında bazı fizikokimyasal analizler yapılmış ve antioksidan kapasitenin belirlenmesi için toplam fenolik bileşik, toplam flavonoid madde, toplam antioksidan aktivite, 2,2-difenil-1-pikrilhidrazil (DPPH), 2,2′-azinobis-3-etilbenztiazolin-6 sülfonat (ABTS) ve demir indirgeyici/antioksidan güç (FRAP) testleri yapılmıştır. Ortalama değerler gözönüne alındığında, en yüksek toplam fenolik bileşik, toplam flavonoid madde, toplam antioksidan aktivite, DPPH, ABTS ve FRAP değerleri sarımsak dişlerinde tespit edilmiştir; bunu sarımsak kabuğu ve sarımsak sapı takip etmiştir. Sarımsak kabuğu ve sarımsak saplarında belirlenen toplam biyoaktif bileşikler ve antioksidan aktivite umut vericidir.

Anahtar kelimeler: *Allium sativum* L., toplam biyoaktif bileşikler, DPPH, ABTS, FRAP

Introduction

A balanced diet should contain enough fruits and vegetables to keep the human organism functioning properly and reduce the risk of degenerative disease (Piechowiak et al., 2019). Garlic contains many nutrient components such as carbohydrates, organosulfur compounds, protein, free amino acids, vitamins and trace elements (Zhang et al., 2018). It is well known that garlic have antioxidant (Nencini et al., 2011; Amagase, 2006), anticancer (Agarwal, 1996), cholesterol-lowering (Yeh and Liu, 2001), and immune-enhancing (Amagase, 2006; Amagase et al., 2001) properties from human and animal studies. Reactive oxygen species are a by-product in the aerobic metabolism process. In vivo, if reactive oxygen species continues at a high level, the organism is exposed to oxidative stress, resulting in protein and nucleic acid damage, lipid peroxidation, and even necrocytosis (Liu et al., 2015). Up to a certain level, oxidant molecule increases can be counteracted by natural antioxidants always found at certain levels in the body (Mates et al., 1999). In some situations, the antioxidant defense mechanisms which organisms use to protect themselves may be insufficient and exogenous antioxidants are required (Kasapçopur-Özel and Birdane, 2014). The garlic stem is considered waste, but it should be considered a useful biological resource. It is necessary to use this product efficiently to reduce environmental pollution (Han et al., 2017). Garlic husk is also an important biological waste. In a study was reported that the extract obtained from garlic husk contains phenolic compounds with antioxidant and antibacterial activities (Kallel et al., 2014). Garlic is produced around the world in two different ways, fresh and dry. Fresh garlic, also referred to as 'green' garlic, is garlic with green leaves, harvested when the body and head surface are fresh and the cloves are not fully mature. Dried garlic is a product in which leaves, stems and cloves are completely dried by natural curing after harvest. Dry garlic has a wider usage area (food, medicine, cosmetics, feed, etc.) and processing procedure due to the longer storage time, the higher the nutrient and aromatic compound content. Therefore, dried garlic is preferred by consumers and the industry, and this is reflected in its economic value (Akan and Ünüvar, 2020). In garlic processing factories in Kastamonu province, in Turkey, the stem and husk of garlic are removed and garlic is packaged for using canning technology, some meat product, some food ingredient and the market. The stems and husks are not used but are left in the environment as agricultural waste. This study aimed to reveal the potential to use the agricultural waste of the dried forms of husks and stems of garlic in industry.

Materials and Methods

For this study, naturally dried garlic samples (husk, stem, clove of garlic parts) obtained from 25 different fields in Taşköprü-Kastamonu. Chemical materials used for analyses were obtained from Sigma Chemical Company.

Dry matter, pH and titratable acidity of the garlic samples were determined according to the methods used by Cemeroğlu (1992), while ash was determined according to the methods used by Ağbaş et al. (2013). Ash content in dry matter was calculated from the formulae.

After disintegrating 20 g sample in a blender it was mixed with 50 mL ethanol and placed in an ultrasonic bath (Elma, Germany) for 30 minutes. Mixtures were centrifuged (Hermle Z 326 K, Germany) at 10000×g (approximately 9970 rpm) at 4 ºC for 20 minutes and the supernatant above the precipitate was obtained and centrifuged again at the same conditions for 10 minutes. The extract obtained was used to determine total phenolic compounds, total flavonoid matter, total antioxidant activity and DPPH inhibition activity, ABTS radical scavenging capacity and FRAP value.

Total phenolic content, total flavonoid content and total antioxidant activity were determined according to the methods used by Slinkard and Singleton (1977), by Jia et al. (1999) and by Prieto et al. (1999), respectively. DPPH, ABTS and FRAP values were determined according to the methods used by Wang et al. (2015).

The trial pattern in the research was 25 (sample from different fields) × 3 (sample type; garlic cloves, dry garlic husk, dry garlic stem) completed according to the full chance-dependent factorial trial pattern. Data obtained as a result of laboratory analyses completed in parallel for a total of 75 samples are given as tables. Statistical analysis was performed using SPSS 17.0 package program.

Results and Discussion

Identification of water amounts in a food is necessary in terms of commerce, but is very important in terms of evaluating the storage stability of a food (Cemeroğlu, 2013). The dry matter values minimum and maximum results of garlic cloves, husk and stem samples are given in Table 1. According to Duncan's Multiple Range test results, the dry matter values in garlic cloves, garlic husk and garlic stem samples are given in Table 2. When mean values are considered, the highest dry matter was in garlic stems. This was followed by garlic husk and garlic cloves. The mean dry matter amounts in samples were significant statistical difference from each other ($P<0.05$) (Table 2). In our study, the lowest and highest dry matter values determined for garlic cloves showed similarity the standardized values of the dry matter values stated in the Taşköprü Garlic geographical indications certificate (Anonymous, 2009). Our mean values for garlic cloves were found to be higher than values stated in a research result (Khalid et al., 2014) lower than Kallel et al. (2014) and similar with values stated in other (Zor, 2006).

Table 1. Dry matter, ash content, ash content in dry matter, pH and titratable acidity minimum and maximum results of garlic cloves, husk and stem samples.

Samples	Results	Dry matter (%)	Ash content (%)	Ash content in dry matter (%)	pH	Titratable acidity (%)
	Min.	35.48±0.02	1.14±0.00	2.76±0.01	5.93±0.02	0.483±0.00
A	Max	42.76±0.04	1.54±0.01	4.13±0.01	6.21±0.03	0.500±0.00
	Min.	84.40±0.06	5.74±0.00	6.52±0.00	5.49±0.00	0.478±0.00
B	Max	89.95±0.00	9.96±0.00	11.49±0.00	6.18±0.02	0.591±0.00
	Min.	90.13±0.02	6.08±0.00	6.63±0.00	5.31±0.01	0.479±0.00
C	Max	91.89±0.01	9.76±0.00	10.65±0.00	6.35±0.01	0.595±0.00

A: clove of garlic B: garlic husk C: garlic stem

Table 2. Duncan's Multiple Range test results of dry matter, ash content, ash content in dry matter, pH and titratable acidity in garlic cloves, garlic husk and garlic stem samples*.

Samples	Dry matter	Ash content	Ash content in dry matter	pH	Titratable acidity
Clove	$38.80±2.05^a$	$1.32±0.10^a$	$3.42±0.37^a$	$6.04±0.06^c$	$0.493±0.00^a$
Husk	$87.12±1.34^b$	$7.84±1.10^b$	$9.00±1.27^c$	$5.83±0.17^b$	$0.503±0.01^b$
Stem	$91.08±0.47^c$	$7.89±0.98^c$	$8.65±1.07^b$	$5.75±0.27^a$	$0.516±0.02^c$

(*) Differences between the averages with the same letters are statistically indistinguishable from each other (P<0.05)

Just as with any organic material, an inorganic remnant called ash remains when food is combusted. The ash amount remaining after combustion of foods and composition of this ash provide information about food (Cemeroğlu, 2013). The ash values minimum and maximum results of garlic cloves, husk and stem samples are given in Table 1. According to Duncan's Multiple Range test results, ash values in garlic cloves, garlic husk and garlic stem samples are given in Table 2. When the mean values are considered, the highest ash amount was in garlic stems, followed by garlic husk and garlic cloves. The mean ash amounts in samples were significant statistical difference from each other (P<0.05) (Table 2). In our study, the lowest and highest ash amounts determined for garlic cloves showed similarity the standardized values of the ash values stated in the Taşköprü Garlic geographical indications certificate (Anonymous, 2009). Our values for garlic cloves were similar to values found in some studies (Ağbaş et al., 2013; Khalid et al., 2014).

However, in our study for a comparison of garlic cloves with the agricultural waste of the dried form of garlic husks and garlic stems, it will be more accurate to examine the ash content in dry matter. The ash content in dry matter values minimum and maximum results of garlic cloves, husk and stem samples are given in Table 1. According to Duncan's Multiple Range test results, the ash contents in dry matter in garlic cloves, garlic husk and garlic stem samples are given in Table 2. In our study, the highest ash content in dry matter was found in garlic husk. This was followed by garlic stem and garlic cloves. The mean ash content in dry matter for samples were significant statistical difference from each other (P<0.05) (Table 2). A research result is higher than the value found in our study (Kallel et al., 2014).

Acidity is one of the most commonly measured and most important characteristics of foods. Many processes are regulated according to the acidity of food (Cemeroğlu, 2013). Like the pH value directly reflecting the acidity and alkalinity of more than one product, physicochemical conditions have important effects on the final quality and chemical composition of the harvested product, and as a result biologic potential (Martins et al., 2016). The pH values minimum and maximum results of garlic cloves, husk and stem samples are given in Table 1. According to Duncan's Multiple Range test results pH values in garlic cloves, garlic husk and garlic stem samples are given in Table 2. In the results of our study, while the garlic cloves had the highest pH, this was followed by garlic husk and garlic stem. The mean pH values of samples were significant statistical difference from each other (P<0.05) (Table 2). In our study, the lowest and highest pH values determined for garlic cloves were lower than the pH values stated in the Taşköprü Garlic geographical indications certificate (Anonymous, 2009). The mean value found for garlic cloves in our study were lower than (Zor, 2006) and higher than the value stated in other (Khalid et al., 2014).

Titratable acidity is the total acid concentration in a food (Cemeroğlu, 2013). The dominant acid in garlic is citric acid (Cemeroğlu, 1992), and according to analyses, titratable acidity is given as anhydrous citric acid. The titratable acidity values minimum and maximum results of garlic cloves, husk and stem samples are given in Table 1. According to Duncan's Multiple Range test results titratable acidity values in garlic cloves, garlic husk and garlic stem samples are given in Table 2. When the mean values are noted, the highest titratable acidity was found for garlic stem, this was followed by garlic husk and garlic cloves. The mean titratable acidity values of samples were significant statistical difference from each other (P<0.05) (Table 2). In our study, the lowest and highest titratable acidity determined for garlic cloves showed similarity the standardized values of titratable acidity stated in the Taşköprü Garlic geographical indications certificate (Anonymous, 2009). Our mean values found for garlic cloves were higher than values determined in some study (Khalid et al., 2014) and lower than other (Zor, 2006).

Garlic has high antioxidant activity due to phenolic compounds in its structure (Pascual-Teresa et al., 2010). Garlic is a rich source of phytochemicals encouraging health including antioxidants like phenolics, flavonoids and allicin (Lanzotti, 2006). Table 3 gives minimum and maximum results of total phenolic compounds detected in garlic cloves, garlic husk and garlic stems. According to Duncan's Multiple Range test results, the total phenolic compounds values in garlic cloves, garlic husk and garlic stem samples are given in Table 4. The highest phenolic content was found in garlic cloves. This was followed by garlic husk and garlic stem. The total phenolic compound amount in garlic husks is more than half of the amount found in garlic cloves. Garlic stems have nearly half the amount found in garlic cloves. All samples (garlic cloves, garlic husk and garlic stem) contain phenolic in their composition and total phenolic have a significant effect on content in the samples (P<0.05) (Table 4). The reason for more phenolic compounds being found in the husk of garlic compared to the stem may be that garlic husk is in contact with garlic cloves. While the results obtained in a research carried out by Nuutila et al. (2003) was higher than the value found in our study, some research results (Kallel et al., 2014; Bozin et al., 2008; Cai et al., 2004; Naheed et al., 2017) were lower. Some research results (Selvan et al., 2018; Chhouk et al., 2017) were similar with the result of our study. Evaluation of this agricultural-industrial byproduct with phenolic compound extraction may be considered a very interesting alternative.

Table 3. Total phenolic, total flavonoid and total antioxidant activity minimum and maximum results of garlic cloves, husk and stem samples.

Samples	Results	Total phenolic (mg GAE g^{-1})	Total flavonoid (mg QUE g^{-1})	Total antioxidant activity (mg AE g^{-1})
A	Min.	20.48±0.90	0.81±0.07	122.82±0.31
	Max	25.40±0.38	0.91±0.11	146.52±1.81
B	Min.	12.88±0.19	0.44±0.07	74.25±0.82
	Max	16.74±0.61	0.51±0.04	94.85±0.24
C	Min.	6.36±0.36	0.22±0.04	35.63±0.45
	Max	12.40±0.21	0.32±0.04	70.71±0.34

A: clove of garlic B: garlic husk C: garlic stem

Table 4. Duncan's Multiple Range test results of the total phenolic content, total flavonoid content and total antioxidant activity amounts in garlic cloves, garlic husk and garlic stem samples*.

Samples	Total phenolic	Total flavonoid	Total antioxidant activity
Clove	23.32±1.26^{c}	8.63±0.75^{c}	134.74±6.51^{c}
Husk	14.01±0.97^{b}	4.76±0.44^{b}	80.70±4.94^{b}
Stem	10.34±1.74^{a}	2.89±0.33^{a}	59.88±10.09^{a}

(*) Differences between the averages with the same letters are statistically indistinguishable from each other (P<0.05)

Flavonoids may prevent injury due to free radicals in a variety of ways and one of these is by direct scavenging of free radicals. Flavonoids enter reactions with the reaction compound of the radical stabilizing reactive oxygen species. Due to the high reactivity of the hydroxyl group on flavonoids, radicals are inactivated (Panche et al., 2016). Table 3 gives minimum and maximum results of total flavonoid values detected in garlic cloves, garlic husk and garlic stems. According to Duncan's Multiple Range test results total flavonoid values in garlic cloves, garlic husk and garlic stem samples are given in Table 4. Our results showed that the highest flavonoid content was in garlic cloves, followed by garlic husk and garlic stems. The total flavonoid amount in husks is more than half of the amount in garlic cloves. Garlic stems contain nearly 1/3 of the amount in garlic cloves. All samples (garlic cloves, garlic husk and garlic stem) contain flavonoid in their composition and total flavonoid have a significant effect on content in the samples ($P<0.05$) (Table 4). Some research results (Kallel et al., 2014; Selvan et al., 2018) were similar with the value found in our study and a research result is lower (Bozin et al., 2008).

The antioxidant potential of different plant extracts and pure compounds can be measured using countless in vitro analyses (Bozin et al., 2008). Table 3 gives minimum and maximum results of total antioxidant activity values detected in garlic cloves, garlic husk and garlic stems. Total antioxidant activity is examined; the husks contain more than half of the total flavonoid amount in garlic cloves. Garlic stems contain nearly half of the amount in garlic cloves. According to Duncan's Multiple Range test results the total antioxidant activity values in garlic cloves, garlic husk and garlic stem samples are given in Table 4. In this study determined that clove of garlic exhibits the highest total antioxidant activity, followed by garlic husks and garlic stems. All samples (garlic cloves, garlic husk and garlic stem parts) showed antioxidant activities and there was significant statistical difference ($P<0.05$) in total antioxidant activities (Table 4).

Organisms have several different antioxidant systems that play a role in various interactions; therefore, the methods used to analyze antioxidant activity should accurately reflect all antioxidants (Chen et al., 2013). For this reason, DPPH, ABTS and FRAP analyzes were done in the study. Minimum and maximum results of antioxidant capacities determined in garlic cloves, garlic husk and garlic stem samples are given in Table 5. According to Duncan's Multiple Range test results DPPH, ABTS, FRAP values in garlic cloves, garlic husk and garlic stem samples are given in Table 6. All samples (garlic cloves, garlic husk and garlic stem) had DPPH inhibition capacity and there was significant statistical difference ($p<0.05$) in DPPH inhibition. ABTS inhibition was observed in garlic cloves and garlic husk, but ABTS inhibition was not observed in most of the garlic stem. ABTS inhibition showed a significant statistical difference ($p<0.05$) in all parts of garlic. All samples (garlic cloves, garlic husk and garlic stem) showed FRAP activity and there was significant statistical difference ($p<0.05$) in FRAP activities. When the average values are taken into consideration, the highest DPPH, ABTS and FRAP values found garlic cloves. This was followed by garlic husk and garlic stem (Table 6). DPPH, ABTS, FRAP activities of garlic husk were higher than garlic stem. This situation might be arised from that the garlic husk is in contact with the garlic cloves. In another study about Kastamonu garlic, DPPH % inhibition value was found as 64.67 (Zor, 2006). DPPH scavenging activity was reported between 3.60-45.63% and FRAP value ranged between 0.27-0.51% in 43 varieties of garlic obtained from different countries (Chen et al., 2013). Some research results (Petropoulos et al., 2018; Kallel et al., 2014) were similar to our findings. On the other hand, our results were higher than the some studies (Queiroz et al., 2009; Locatelli et al., 2017) and lower than others (Bhatt and Patel, 2013; Nuutila et al., 2003; Bozin et al., 2008).

Table 5. DPPH, ABTS, FRAP values minimum and maximum results of garlic cloves, husk and stem samples.

Samples	Results	DPPH (%)	ABTS (%)	FRAP (mM ml^{-1} extract)
	Min.	41.13±0.68	18.09±0.37	0.36±0.00
A	Max	59.54±0.42	52.52±0.25	0.55±0.00
	Min.	31.17±0.65	1.97±0.16	0.20±0.00
B	Max	44.77±1.44	6.44±0.33	0.37±0.01
	Min.	14.22±0.85	ND	0.10±0.01
C	Max	29.45±0.57	0.68±0.05	0.21±0.00

A: clove of garlic **B:** garlic husk **C:** garlic stem, ND: not defined

Table 6. Duncan's Multiple Range test results of DPPH, ABTS, FRAP values in garlic cloves, garlic husk and garlic stem samples*.

Samples	DPPH	ABTS	FRAP
Clove	$47.87±4.40^{c}$	$36.30±7.76^{c}$	$0.45±0.06^{c}$
Husk	$34.69±3.11^{b}$	$3.35±1.06^{b}$	$0.26±0.05^{b}$
Stem	$24.57±4.32^{a}$	$0.12±0.18^{a}$	$0.13±0.02^{a}$

(*) Differences between the averages with the same letters are statistically indistinguishable from each other ($P<0.05$)

Conclusions

The total bioactive compounds and antioxidant activity determined in garlic husks and garlic stems are promising. Considering these results, the agricultural waste of garlic husks and garlic stems appears to have the potential to be used in industry. The processing of these parts, which make up 10% of the total weight of garlic, is of great importance both in terms of bringing it to the national economy and also in terms of reducing the amount of solid waste. Considering all these beneficial effects, the determination of antioxidant activity in clove of garlic, husk, and stem will help to increase competitiveness and thus contribute to the national economy.

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