

Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology

Effects of Organic Fertilizer and Drying Methods on Total Phenolic Content and Antioxidant Capacity of Organic White Tea (*Camellia sinensis* (L.) Kuntze)

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ARTICLEINFO	ABSTRACT
Research Article	The aim of this study was to determine the effect of different fertilizer doses and drying methods on total phenolic contents and antioxidant activity of organic white tea (<i>Camellia</i> sinensis (L.) Kuntze) obtained from Hemşin Valley, region of Rize/Turkey. The determination of phenolic constituents
Received : 29/05/2019 Accepted : 04/09/2019	was done by the Folin-Ciocalteau method and the determination of antioxidant activity by the FRAP method. The results showed that the phenolic content of white tea was as follows: 443,7- 506,2 µg GAE/g DW in the drying oven, 421,4- 473,6 µg GAE/g DW in shadow and 434,4- 485,2 µg GAE/g DW in the liofilizator. According to the FRAP method the antioxidant activity was as follows: 1130,1-1176,9 mg FeSO4/gr DW in drying oven, 1141,2-1157,1 mg FeSO4/gr DW in shadow and
<i>Keywords:</i> Camellia White tea Polyphenol Antioxidant Phenolic constituents	1005,3-1183,2 mg FeSO4/gr DW in lyophylizator. The results revealed that different fertilizer doses and drying methods had important effects on the total phenolic content and antioxidant activity of white tea.
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Introduction

Compared with the World the tea plant has a shorter adaptation, acceptation and cultivation history in Turkey. However, the tea plant (*C. sinensis*) has been accepted by the local farmers at the Black Sea Region rapidly beginning from the 1930s. Tea is probably the most widely consumed beverage in the world after water (Muktar and Ahmad, 2000). On the World tropical and subtropical areas with adequate rainfalls, good drainage, and a slightly acidic soil are the suitable conditions where this plant can be grow best (Graham, 1999).

The common tea plant is the evergreen shrub, there are several varieties of this species: one being the Indian Assam tea (*C. sinensis var. assamica (J.W.Mast.) Kitam.*) and the the China-type (*Camellia sinensis* L. Kuntze). Traditionally, tea is prepared from its dried young leaves and leaf buds, made into a beverage by steeping the leaves in boiling water. China is credited with introducing tea to the world, though the evergreen tea plant is in fact native to Southern China, North India, Myanmar and Cambodia (Hicks, 2001).

Tea contains a wide range of phenolic compounds. Tea's polyphenols include flavanols, flavandiols, flavonoids, and phenolic acids; these compounds may account for up to 30% of the dry weight of the tea leaves according to the literature (Hilal and Engelhardt, 2007). Further, tea is one of the richest sources of antioxidants and the three major forms of antioxidant tea are green tea, oolong tea, and black tea. These teas are differing in their production methods and chemical composition (Balentine et al., 1997; Lambert and Elias, 2010).

Tea production are practised in the Eastern Black Sea Region, in a zone beginning from the Georgian border up to the Fatsa district in Ordu. In this region tea production is mainly located in Rize, Ordu, Giresun, Trabzon and Artvin (Anonymous, 2016). If we are considering the tea production areas in the World these regions are located in the top zone. In Asian countries like China, India and Sri Lanka temperature does not fall up to minus degree in tea production areas and tea production is covering the whole year. But in our country where we can feel four climates tea plantations are in fallow four six months. The fact that snow falls on Turkish tea plantations bring them an extra important characteristic. Because of this character pesticides are not applicated in our tea production areas. This gives Turkish tea compared with teas in the World "the most natural tea" characteristic. Parallel to the developments regarding organic tea cultivation in the world ÇAYKUR initiated in 2003 studies to increase organic tea farming in our country. Within the context of organic tea farming Borçka/Artvin and Çamlıhemşin and Hemşin/Rize was chosen as organic tea production areas.

Organic Fertilizers in Tea Production

The objective of organic tea cultivation is to have an eco-friendly plantation; although aiming the conservation of ecology and natural habitat without polluting soil, air and water and yet maintaining sustainable tea production. In organic tea cultivation tea is produced in the absence of synthesized chemicals like pesticides, fungicides, herbicides, growth regulators and concentrated fertilizers (Shamsul et al., 2017).

Ren et al. (1999) and Sun et al. (2003) reported that application of organic fertilizers is one of important practical measures to improve soil fertility. In addition to providing necessary nutrients for crops and improving soil physicochemical properties, organic fertilizer is able to enhance soil microbial activity of soil, such as improving activity of soil enzymes and increasing soil microbial biomass. Organic fertilizers have traditionally been used in agricultural areas, especially in view of their benefits for the soil biological and chemical properties (Queiroz et al., 2004). It is worth remembering that the addition of organic residues is fundamental for carbon (C) recycling in the soil and can improve its physical quality Brancalião and Moraes (2008). Organic manures can be used as an alternative for the inorganic fertilizers. They release nutrients rather slowly and steadily over a longer period and also improve the soil fertility status by activating the soil microbial biomass (Ayuso et al., 1999). For decades researchers have noted the benefits of manure additions to soil, from renovating eroded sites to improving soil physical properties and fertility following centuries of manuring (Latham, 1940).

White Tea

White tea is made mainly from newly grown buds and young leaves with tiny, silvery hairs not exposed to sunlight to prevent chlorophyll production. Buds and leaves for white tea production are harvested only once a year in the early spring. Buds are plucked before they are open, then withered and air dried in the shade, under sunshine, or in a temperature-controlled room. White tea is the least processed type of tea. It is considered as a nonfermented type, however, a slight fermentation occurs since the processing lacks the step of enzyme deactivation (Kosinska and Andlauer, 2014). The tea takes its name from the silver fuzz that covers the buds which turn white after drying. Oxidation is completely avoided by ensuring there is no rolling and bruising (Owuor and Kwach, 2012).

Phenolic Content in White Tea

White tea, the least processed tea, is one of the less studied and is ascribed to have the highest content of phenolic compounds (Dias et al., 2003). White tea is composed of polyphenols, alkaloids, amino acids, carbohydrates, proteins, chlorophyll, volatile organic compounds, fluoride, aluminum, minerals and trace elements (Cabrera et al., 2003). White tea's polyphenols include flavanols, flavandiols, flavonoids, and phenolic acids, these compounds may account for up to 30% of the dry weight of the tea leaves.

White tea, obtained from the same *C. sinensis* plant as black and green varieties, may have a lower TPC due to the fact that oxidation by polyphenol oxidase is prevented in white tea processing (Astorino et al., 2012). The total phenolic content of white tea was determined by Shannon et al. (2017) as 190.24 ± 7.73 . Further the extraction efficiency of different concentrations of aqueous ethanol, temperature and time for the extraction of total polyphenols from white tea leaves was investigated using a central composite design. The total phenolic content in the white tea extracts ranged from 20.93 to 178.70 mg as GAE/g DW (Peiró et al., 2014).

Antioxidant Activity in White Tea

Tea polyphenols have great medicinal and health benefits and they are potent source of antioxidants (Sharangi, 2009). In spite of numerous data about the phenolic constituents. antioxidant activity and ameliorating effects of green and black tea on human health, little is known in this sense about white tea, which is the rarest and the least processed tea (Rusak et al., 2008). White tea is just made by drying only without any other normal fermentation process which is adopted for other tea varieties. The biochemical components like flavonoids, total polyphenols, tannins and catechins are prominent in white tea. Catechin content is higher than tannin content. All those active components are higher in methanolic extracts than their corresponding aqueous extracts. The higher antioxidant activities are manifestations of all those active components (Saha et al., 2017).

Effect of different factors on tea quality have been conducted like the analysis of chemical constituents analysis of white tea of different qualities and different storage times (Ning et al., 2016), but studies on the effect of organic fertilizer and drying method together on phenolic content and antioxidant activity on white tea samples have not been conducted.

A tea plantation in the Apso settlement, located in the Hemşin valley, Rize was chosen as study area. Organic fertilizer was applied as 1000 kg/ha, 1500 kg/ha and 2000 kg/ha as sole doses (March) compared with control (no organic fertilizer) application. The aim of this study was to determine the effect of organic fertilizer and different drying methods on yield, total phenolic content and antioxidant capacity in white tea. As far we know it's the first study in Turkey investigating the effect of different drying methods and organic fertilizer applications on phenolic content and antioxidant activity of white tea.

Material and Methods

Material

An organic fertilizer with pH= 8.2 and containing N-P-K at ratios of 7-2-6% respectively was used as sole doses (0 kg/ha, 5000 kg/ha, 1000 kg/ha, 1500 kg/ha and 2000 kg/ha) in the present study. A tea plantation in the organic tea plantation area in Hemşin, Rize/Turkey was used as research area.

Methods

A field trial according to randomized block design with three replications was conducted. Organic fertilizer doses were applied in March 2017. First shoot harvest was performed in May 2017, which were used for phenolic content and antioxidant activity analysis.

Drying Methods

- Drying oven: Fresh white tea samples were dried in a drying oven trademark Binder at 35°C for up to balanced dry weight.
- Shadow: Fresh white tea samples were dried in shadow for up to balanced dry weight.
- Lyophilizator: Lyophilizators are used to remove water from fast putrescent products to extend their validity life and to simplify their transportation. Lyophilization process is known as - freeze-drying is based on sublimation. The sample in chamber is freezed and the pressure reduced, water from the sample is removed without disrupting the structure of the material. Fresh white tea samples were dried for 3-4 hours at -80°C de in a lyophilizator trademark Labomar up to balanced dry weight.

Statistical Analysis

Analysis of variance (ANOVA) was used to assess the influence of different drying methods and organic fertlizer doses on the phenolic content and antioxidant activity of tea samples obtained from the present research conducted in the Apso settlement, Pazar/Rize, Turkey. All analyses were conducted with SPSS 20.0 and the F ratio was considered significant at P=0.05 and high significant at P=0.01. Graphics were created using Excel 2016.

Determination of Total Phenolic content

The total phenolic content of collected samples were determined using UV-Vis spectrophotometer as mg GAE/gr DW. The pre-treatment of samples was the same as described in the FRAP method. Gallic acid was used as standard, according to the method described by the International Organization for Standardization (ISO) 14502-1. Sample extract as 1/10 of the total volume and 300 μ L Na₂CO₃ was added to tubes containing water involving Folin reagent and all tubes were keep waiting in a ultrasonic shaker (50°C) for 15 min. The measurement were done using a UV spectrophotometer device at a wave length of 765 nm to obtain the absorbance values.

Determination of Antioxidant Activity

A modified version of the FRAP assay described by Izzreen and Fadezelly (2013) was used to determine the antioxidant activity of collected samples as mg FeSO4/gr DW.

For the determination of antioxidant content of the samples as pre-treatment, 0.1 g of each dried sample was completed with methanol (80%) to reach 10 ml volume. Samples were mixed first in the water bath (50° C) for a duration of 20 minutes and the samples were keep waiting after this procedure for 1 h in the dark. The mixture was centrifuged after that for a 20 min, 4000 cycle/min process for obtaining the extracts, which are used for the determination of phenolic content and antioxidant activity of the investigated samples.

Collected samples were analysed regarding their antioxidant activity values. White tea leaves were dried in the drying oven at 40°C and its antioxidant activity was determined using the UV-spectrophotometer by the FRAP method. The determination of antioxidant capacity of investigated samples (pre-treatments completed) was done using the FRAP method. The FRAP method bases on the colourization after the degradation of the Fe+3 ion, bounded to TPTZ in an acid environment, to Fe+2. 300 mM acetate buffer (pH 3,6), 10 mM 2,4,6-tripyridyl-striazine (TPTZ) and 20 mM FeCl3.6H2O solutions were mixed at a proportion of 10:1:1 as FRAP (ferric reducing / antioxidant power) reactive to obtain a buffer solution. A FeSO4.H2O solution was used to prepare different standard probes to obtain a calibration curve. The final samples were obtained with a mix of 1980 µL FRAP dispersive + 20 µL sample and keep waiting after that for 3 min n a ultrasonic shaker (50°C). The measurements were done using a UV Spectrophotometer device at a wavelength of 595 nm to obtain the final absorbance values.

Results and Discussion

As far we know there no research about effect of organic fertilizer and drying methods on the phenolic content and antioxidant activity of white tea. If we talk about the chemical composition of tea we can say that it is complex and includes polyphenols, alkaloids (caffeine, theophylline and theobromine), amino acids. carbohydrates, proteins, chlorophyll, volatile compounds, minerals, trace elements and other unidentified compounds. Among these substances, mentioned polyphenols constitutes the most interesting group and are the main bioactive molecules in tea (Cabrera et al., 2003). The major polyphenolic compounds in tea are the flavan3ols called catechins which include: (-)-epicatechin (EC), (-)-Epigallocatechin (EGC), (-)-epicatechin gallate (ECG), (-)-epigallocatechingallate (EGCG), (-)-Gallocatechins (GC) and (-)-gallocatechin gallate (GCG). Catechins are present in large amounts in green tea (Peterson et al., 2005). Based on their chemical structure, catechins that contain three hydroxyl groups in the B ring (positions 3', 4' and 5') are called gallocatechins while gallic acid substitution in position 3 of the ring is characteristic of catechin gallate (Pellilo et al., 2008). Catechins account for 6 - 16% of the dry green tea leaves with EGCG constituting 10 - 50% of catechins and being the most potent due to its degree of gallation and hydroxylation (Stewart et al., 2009). TFs and TRs are another group of polyphenolic compounds found in both black and oolong teas (Oband et al., 2001). The tea beverage has continued to be considered a medicine since the ancient times because of its polyphenols. Research on the effects of tea on human health has been fuelled by the growing need to provide naturally healthy diets that include plant-derived polyphenols. In line with this, there is need to elucidate how known functional components in foods could expand the role of diet in disease prevention and treatment (Mandel et al., 2006). There is already growing evidence that tea polyphenols reduce the risk of heart diseases and cancer in humans (Vanessa and Williamson, 2004). In some studies, tea has been associated with antiallergic action (Jamamoto 1643

et al., 2004) and antimicrobial properties (Paola et al., 2005). Further studies have demonstrated that the coadministration of drugs with catechins (EC and EGCG) inhibits glucuronidation and sulfonation of orally administered drugs thereby increasing the bioavailability of such drugs (Hang et al., 2003). Moreover some epidemiological studies have associated consumption of tea with a lower risk of several types of cancer including those of the stomach, oral cavity, oesophagus and lungs (Cabrera et al., 2003; Hakim and Chow, 2004). Therefore, tea appears to be an effective chemo preventive agent for toxic chemicals and carcinogens.

Statistical ANOVA analysis of total phenol content (mg GAE/g DW) of tea samples dried with different drying methods and obtained from organic fertilizer applications can be seen in Table 1, and corresponding total phenol content values can be seen in Graphic 1, in Table 2. The ANOVA analysis of phenolic content values of tea samples revealed high significant differences between drying methods, applied organic fertilizer doses and drying method x drying method interaction (Table 1).

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Table LANUVA OT	phenolic content v	vnite tea samples	using values	obtained from d	rving methods a	nd organic tertilize	tr doses
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Source	Degree of Freedom	Sum of Squares	Mean of Square	F
Total	44	27178.87		
Replication	2	66.323	33.16	0.89
Combination	14	26075.76	1862.55	50.30**
Drying method (DM)	2	9630.09	4815.04	130.04**
Organic fertilizer Dose(F)	4	15406.86	3851.71	104.02**
$\mathbf{D}\mathbf{M} \times \mathbf{F}$	8	1038.81	129.85	3.506*
Error	28	1036.77	37.027	

CV=1.32%

Table 2 Phenolic content values (mg GAE/g DW) of white tea samples obtained from different drying methods and organic fertilizer doses

Durvin a Mathad	Fertilizer Doses					
Drying Method	Control	500 kg/ha	1000 kg/ha	1500 kg/ha	2000 kg/ha	Mean
Drying oven	443.74 ^{fg}	462.93 ^{de}	479.66 ^{bc}	478.42 ^{bc}	506.29 ^a	474.21 ^{a**}
Shadow	421.43 ⁱ	424.54 ^{hi}	435.68 ^{gh}	442.50 ^g	473.46 ^{cd}	439.52 ^{b**}
Lyophilizator	434.45 ^{gh}	454.88 ^{ef}	475.32 ^{bc}	473.46 ^{cd}	485.23 ^b	$464.67^{a^{**}}$
Mean	433.20 ^{d**}	447.45 ^{c**}	463.55 ^{b**}	464.79 ^{b**}	488.32 ^{a**}	

*Means with the same letter are not significantly different from each other (P>0.05), **Means with the same letter are not significantly different from each other (P>0.01)



Graphic 1 Phenolic content of white tea samples obtained from different drying methods and organic fertilizer doses

The calculated coefficient of variation (1.32%) is relatively low, because the allowed top level in field trials is 20%.

The results showed that the phenolic content of white tea samples was as follows: 443.74-508.15 mg GAE/g DW in the drying oven, 419.0-479.0 mg GAE/g DW in shadow and 431.35-487.71 mg GAE/g DW in the lyophilizator (Table 2).

As can be seen in Table 2 and Graphic 1 total phenol content of white tea samples increased in every used drying method beginning from control up to every organic fertilizer dose of 500 kg/ha, 1000 kg/ha, 1500 kg/ha and 2000 kg/ha. Drying oven and lyophilizator methods were located in the same group, differing from shadow drying. Regarding fertilizer doses the dose 2000 kg/ha was very different from the other fertilizer doses, 1000 kg/ha and

1500 kg/ha were in the same group. The dose 500 kg/ha and control were grouped separately in the groups c and d respectively.

Regarding drying method \times organic fertilizer interaction the highest phenolic content value was obtained in samples applied with 2000 kg/ha organic fertilizer and dried in the lyphilizator (506.29 mg GAE/g DW).

Shannon et al. (2017) compared the total phenolic content (TPC), total flavonoid content (TFC), ferric reducing antioxidant power (FRAP), DPPH radical scavenging capacity, and caffeine content of teas (black, green, white, chamomile, and mixed berry/hibiscus) over a range of infusion times (0.5–10 mins) at 90°C. In this study green tea had the highest total phenolic content of 557,58±74,98; followed by black 499.19±46.56; white 190.24±7.73; berry 98.86±14.72; and chamomile 75.31±3.65 µg GAE/g tea. Unachukwu et al. (2010) quantified a TPC of 1.17mg GAE/g in green tea; and 0.96 mg GAE/g in white tea. In this study green tea was found to have the highest level of total phenolics (Komes et al., 2010; Anissi et al., 2012), followed by black tea; while the chamomile, berry, and white teas were significantly lower

(P≤0.05). White tea, although from the same *C. sinensis* plant as black and green varieties, may have a lower TPC due to the fact that oxidation by polyphenol oxidase is prevented in white tea processing (Astorino et al., 2014). Therefore, the phenolic monomers in the fresh leaves are not complexed into polyphenols such as catechins in green tea, or thearubigin in black tea. (Rusak et al., 2008) reported that the extraction of total phenolics from white tea leaves occurred at a significantly slower rate than that of other teas. The range of determined phenolic content was between 421.44 and 506.29 µg GAE/g DW for all applications and these values are higher as in above given values.

Antioxidant Activity

Statistical ANOVA analysis of antioxidant activity values (mg FeSO4/g DW) of tea samples dried with different drying methods and obtained from organic fertilizer applications can be seen in Table 3, and corresponding antioxidant values can be seen in Graphic 2, in Table 4.

Table 3 ANOVA of white tea samples using values obtained from drying methods and organic fertilizer doses

			2	
Source	Degree of Freedom	Sum of Squares	Mean Square	F
Total	44	74311.61		
Replication	2	17.80	8.90	0.218
Drying Method (DM)	2	3330.06	1665.03	40.667**
Organic Fertilizer Dose (F)	4	36481.60	9120.40	222.77**
DM x F	8	33335.79	4166.97	101.78**
Error	28	1146.35	40.94	

CV=0.558%

Table 4 Antioxidant activity values of white tea samples obtained from different drying methods and organic fertilizer doses

DM	Fertilizer Doses						
DIVI	Control	500 kg/ha	1000 kg/ha	1500 kg/ha	2000 kg/ha	Mean	
DO	1130.13 ^{i**}	1145.85 ^{gh**}	1161.96 ^{c-f**}	1168.96 ^{bc**}	1176.89 ^{ab**}	1156.76 ^{a**}	
S	1141.23 ^{hi**}	1142.42 ^{hi**}	1145.59 ^{gh**}	1156.02 ^{e-g**}	1157.08 ^{d-g**}	1148.51 ^{b**}	
L	1005.31 ^{j**}	1159.19 ^{c-f**}	1163.02 ^{c-e**}	1168.30 ^{b-d**}	1183.36 ^{a**}	1135.84 ^{c**}	
Mean	1092.22 ^{d**}	1149.15 ^{c**}	1156.86 ^{bc**}	1164.43 ^{b**}	1242.44 ^{a**}		

DM: Drying Method, DO: Drying oven, S: Shadow, L: Lyophilizator, **Means with the same letter are not significantly different from each other (P>0.01)



Graphic 2 Antioxidant capacity of white tea samples obtained from different drying methods and organic fertilizer doses

The ANOVA analysis of antioxidant activity values of tea samples revealed very significant differences between drying methods, applied organic fertilizer doses and drying method x drying method interaction (Table 3).

According to the FRAP method the antioxidant activity was as follows: 1124.585-1181.25 mg FSO4/gr DW in drying oven, 1132.907-1160.249 mg FSO4/gr DW in shadow and 997.3859-1185.609 mg FeSO4/gr DW in liyofilizator.

As can be seen in Table 4 and Graphic 2 antioxidant activity of white tea samples increased in every used drying method beginning from control up to every organic fertilizer dose of 500 kg/ha, 1000 kg/ha, 1500 kg/ha and 2000 kg/ha.

All drying methods; drying oven, shadow and lyophilizator were grouped in the groups a, b and c respectively. Regarding statistical analysis results the fertilizer dose 2000 kg/ha was determined as the most effectice dose in group a.

Natural antioxidants are increasingly appreciated by consumers due to both their inherent positive effects and to the possibility of using them as a source of natural additives to replace synthetic ones (Perumalla and Hettiarachchy, 2011; Gülçin, 2012; Oh et al., 2013; Silva-Weiss et al., 2013; Sevindik et al., 2017; Sevindik et al., 2018; Sevindik, 2019). Tea is a natural plant that is a rich source of natural antioxidants and provides a high free radical scavenger activity (Gramza and Korczak, 2005; Almajano et al., 2008). In our study antioxidant activity of white tea samples obtained from different drying methods and organic fertilizer doses showed high amounts of antioxidant activity.

Conclusion

Organic tea production will be a weak issue in Turkey in the future decades. Governmental institutions like ÇAYKUR and the private sector are searching for suitable and high yield performing organic fertilizers in Rize, Turkey. The primary focus is the income, which of course means total fresh leaf yield, of local farmers, because tea is the biggest income in this region.

But yield only is not sufficient for the production of different tea types, also their phenolic contents and antioxidant activity values are very important. In the present study the effect of different drying methods and organic fertilizers on the phenolic content and antioxidant activity of white tea were investigated for the first time in Rize, Turkey. Regarding both investigated quality characters 2000 kg/ha was the best organic fertilizer dose, but the highest values regarding drying methods were obtained from the drying oven in all fertilizer doses considering phenolic content. If we look to obtained antioxidant activity values, it can be stated that all investigated samples displayed high amounts of these investigated quality character.

Based on obtained first year results it can be stated that high potential exists in organic tea production regarding the increase of phenolic content and antioxidant activity in tea. To develop arguments for the whole tea plantation area in Turkey, samples from different locations in these regions should be investigated.

Acknowledgement

The present study was conducted on the field with private means. For the quality analysis the laboratory facilities of the Faculty of Agriculture and Natural Sciences in Pazar, Rize/Turkey were used.

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