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Bioactive compounds of strawberry tree (*Arbutus unedo* L.) genotypes grown in the East Black Sea and Marmara regions

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ABSTRACT

The research was carried out to determine the total phenolics, total flavonoids and antioxidant activity (according to DPPH and FRAP assays) of 21 strawberry tree genotypes grown naturally in Piraziz (Giresun, East Black Sea) and Gebze (Kocaeli, Marmara regions) districts. A wide variation was determined between strawberry tree genotypes in terms of the properties investigated. In the strawberry tree genotypes examined, total phenolics was determined from 528 to 985 mg GAE 100 g^{-1}, while total flavonoids was detected from 21 to 134 mg QE 100 g^{-1}. According to DPPH and FRAP tests antioxidant activity was determined from 2.1 to 15.5 mmol TE 100 g^{-1} and 20.5 to 50.9 mmol TE 100 g^{-1}, respectively. According to the principal component analysis result, the first two components explained 88.1% of the total variation. PC1 was related to total phenolics and antioxidant activity (both DPPH and FRAP), while PC2 was associated with total flavonoids. As a result, the G-14 genotype had remarkable results in terms of the properties investigated. This genotype, which stands out in terms of beneficial substances on human health, is thought can be used as genetic material in future breeding programs.

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1. Introduction

Strawberry tree (*Arbutus unedo* L.), which belongs to the Ericaceae family, is a fruit species with a wide distribution area in the world. It has found the opportunity to grow in the temperate climatic conditions of Asia, Europe and Africa in the world (Karadeniz et al., 2003). Apart from that, it grows quite extensively in the Mediterranean basin, which covers a large area (Miguel et al., 2014). In Türkiye, it has the opportunity to grow naturally in the Black Sea, Mediterranean, Marmara and Aegean regions (Yaltırık and Erdinç, 2002). In this regard, Türkiye, which has suitable climatic characteristic for the cultivation of strawberry tree, contributes significantly to genotype richness (Anşin and Özkan, 1993).

The strawberry tree is a species with an evergreen plant habitus that can be between 1.5 and 9.0 m tall (Yarılgaç and Pekdemir, 2019) and round or nearly round orange-red fruits (Özcan and Hacıseferoğulları, 2007). Because of the fruit resembles a strawberry, it's also known as the strawberry tree (Anşin and Özkan, 1993). In Türkiye, according to the regions, different names have been given such as "dağ çileği, dağ yemişi, ayı yemişi, kocakarı yemişi, kara yaprak, andrana, andıra, davulga, davulgı üzümü, piridim, yağma, endirek and zefre yemişi" (Şeker, 2004; Erboğa, 2016; Koyu et al., 2019). The strawberry tree plant has many uses, both with its visual and nutritive properties. The evergreen plant forms showy bell-shaped flowers that can be seen on the plant for a long time (Soufleros et al., 2005), with colorful fruits ranging from yellow to red in the period from fruit set to fruit ripening (Gilman and Watson, 1993). It is considered an ornamental plant (Maleš et al., 2006; Celikel et al., 2008; Yıldız, 2014). In addition, shrub-like strawberry tree plants are used as hedge plants (Yarılgaç and Pekdemir, 2019). Fruits are used primarily for fresh consumption, jelly, marmalade, jam, ice cream, pastry, alcoholic beverage production and medical fields (Ayaz et al., 2000; Şeker 2004; Soufleros et al., 2005; Ganhão et al., 2010; Oliveira et al., 2011; Aloğlu et al., 2018).

The fruiting period of the strawberry tree is much longer compared to many other species (Anşin and Özkan, 1993; Şeker, 2004). It provides an alternative food source for people, especially since its ripe fruits are harvested in the winter or close to winter (Soufleros et al., 2005; Yıldız,

2014). In addition, it has been reported that it provides a protective effect against many diseases thanks to its high biochemical content. These have been stated disease such as neurological disorders, cardiovascular diseases, preventing the formation of cancer cells, atherosclerosis, reducing fever, kidney disorders, rheumatism, hypertension, relieving constipation, diuretic and anti-infection (Ziyyat et al., 1997; Jouad et al., 2001; Tuzlacı and Aymaz, 2001; Bnouham et al., 2007; Pallauf et al., 2008; Oliveira et al., 2011; Molina et al., 2011; Erboğa, 2016).

The strawberry tree has been used for centuries both as a food ingredient and in folk medicine. Today, it still has an important position with its similar uses. So much so that with the increase in the world population, the importance of foods with high nutritional value has increased even more. In addition, people's perspectives on healthy life have changed due to epidemics that have been common in recent years. In this case, people have directed the consumption of fruits and vegetables that increase their quality of life, are more economical, more accessible and have high bioactive content. The strawberry tree is one of these fruit species with its rich nutritional content.

This study aimed to determine the bioactive components that support human health in strawberry tree genotypes grown naturally in two different regions.

2. Materials and methods

2.1. Plant materials

The research was carried out in Piraziz district (Giresun, Türkiye), located in the Eastern Black Sea region and Gebze district (Kocaeli, Türkiye), located in the Marmara region. The research material consisted of 4 strawberry tree genotypes grown in Piraziz district and 17 strawberry tree genotypes grown in Gebze district, a total of 21 genotypes. Each plant selected in the study was accepted as a genotype.

2.2. Methods

In the selection of the strawberry tree genotypes, fruit size was taken into account. Approximately 250 g of fruit samples were collected from the genotypes at harvest time. The fruits were stored at -20°C until biochemical analysis. Total phenolics, total flavonoids and antioxidant activity (according to DPPH and FRAP assays) were determined as biochemical properties.

2.2.1. Bioactive compounds

Spectrophotometric measurements for biochemical properties were performed in a UV-Vis spectrophotometer (UVmini-1240, Shimadzu, Japan). Total phenolics were measured according to the method described by Aglar et al. (2019). Total flavonoids were determined according to defined Chang et al. (2002). Total phenolics and flavonoids were expressed as mg GAE (gallic acid equivalent) 100 g^{-1} fw and mg QE (quercetin equivalent) 100 g^{-1} fw, respectively. The antioxidant activity of strawberry tree genotypes was detected according to two different procedures of DPPH (Blois, 1958) and FRAP (Benzie and Strain, 1996) assays. According to both DPPH and FRAP, the antioxidant activity was expressed as mmol trolox equivalent (TE) 100 g^{-1} fw.

2.2.2. Statistical analysis

To evaluate the data were used Minitab 17 and JMP 14 (trial) statistical package programs. The difference between the genotypes in terms of the properties investigated was determined according to the Tukey multiple comparison method at the 5% significance level. Principal component analyzes and hierarchical clustering analysis were performed using biochemical properties.

3. Results and discussion

3.1. Total phenolics

It has been reported that many diseases such as cancer and cardiovascular diseases, especially chronic diseases that significant impact on human health, can be prevented thanks to the biochemicals found in fruits and vegetables (Doré, 2005). In this sense, antioxidants have been stated to contribute significantly to the body's defense system by preventing the harmful effects of irregular reactive oxygen-nitrogen species and free radicals known to cause diseases (Ratnam et al., 2006). However, it has been stated that the antioxidant produced in the human body has a limited protective effect, and the antioxidants taken from food have a higher protective capacity (Thomas et al., 2010). This situation increases the importance of consuming edible wild fruit species with high biochemical content. In this sense, the strawberry tree has an important place with its rich nutritional content.

Significant differences were determined between the strawberry tree genotypes in terms of total phenolics ($p<0.05$). While the highest total phenolics was detected in the G-14 genotype with 985 mg 100 g^{-1}, the lowest was determined in the G-6 genotype with 528 mg 100 g^{-1}. The genotypes G-14, G-8, and G-15 with the highest total phenolic content were statistically grouped (Table 1). Total phenolics was reported as 1428 mg 100 g^{-1} in *Arbutus unedo* specie grown in Lapseki (Çanakkale) region (Isbilir et al., 2012), from 483 to 627 mg 100 g^{-1} in strawberry tree genotypes grown in the Muğla province (Colak, 2019), from 567 to 818 mg 100 g^{-1} in strawberry tree genotypes grown in Akçabat (Trabzon) region (Sagbas et al., 2020), from 479.62 to 850.02 mg 100 g^{-1} in strawberry tree grown in Croatia (Sic Zlabur et al., 2020). In terms of total phenolics, the findings obtained are compatible with the results of many researchers, while Isbilir et al. (2012) were lower than the findings. Differences in total phenolics are thought to be due to genotype, ecological conditions, and fruit ripeness.

3.2. Total flavonoids

The difference between the total flavonoids of the strawberry tree genotypes investigated was significant ($p<0.05$). Total flavonoids varied between 21 (G-10) and 134 (P-4) mg 100 g^{-1}. The P-4 genotype with the highest total flavonoids was followed by P-1 (129 mg 100 g^{-1}), G-3 (120 mg 100 g^{-1}) and G-8 (114 mg 100 g^{-1}) genotypes, respectively (Table 1). Sic Zlabur et al. (2020) determined the total flavonoids between 235.39 and 466.88 mg 100 g^{-1} in strawberry tree genotypes grown in Croatia. The findings obtained in terms of total flavonoids were found to be lower than the results of Sic Zlabur et al. (2020). It can be stated that the observed differences may be due to the genetic structure and ecological conditions, as well as the sunshine period and overnight temperature during the maturing period.

Table 1. Total phenolics, total flavonoids and antioxidant activity (according to DPPH and FRAP assays) of strawberry tree genotypes investigated

Genotypes	Total phenolics (mg GAE 100 g^{-1})	Total flavonoids (mg QE 100 g^{-1})	Antioxidant activity (mmol TE 100 g^{-1})	
			DPPH	FRAP
G-1	685±30.8 ^{cde*}	88±4.0 ^{ef}	9.2±0.5 ^{ef}	34.8±1.7 ^{de}
G-2	555±24.9 ^{f}	60±2.7 ^{jk}	2.1±0.1 ^{ı}	22.0±1.1 ^{hı}
G-3	670±30.1 ^{cde}	120±5.4 ^{bc}	8.5±0.4 ^{ef}	42.0±2.1 ^{bc}
G-4	602±27.1 ^{def}	72±3.2 ^{ghı}	3.5±0.2 ^{hı}	25.7±1.3 ^{ghı}
G-5	680±30.6 ^{cde}	66±3.0 ^{hıj}	10.7±0.5 ^{cde}	37.6±1.9 ^{cde}
G-6	528±23.7 ^{f}	46±2.1 ^{lm}	2.7±0.1 ^{ı}	20.5±1.0 ^{ı}
G-7	701±31.5 ^{cd}	63±2.8 ^{ijk}	9.5±0.5 ^{ef}	32.9±1.6 ^{ef}
G-8	968±43.5 ^{a}	114±5.1 ^{cd}	12.1±0.6 ^{bc}	50.9±2.5 ^{a}
G-9	667±30.0 ^{cde}	78±3.5 ^{fg}	9.2±0.5 ^{ef}	34.8±1.7 ^{de}
G-10	697±31.3 ^{cd}	21±1.0 ^{n}	13.1±0.7 ^{b}	38.9±1.9 ^{cd}
G-11	628±28.2 ^{def}	53±2.4 ^{kl}	11.8±0.6 ^{bc}	38.1±1.9 ^{cde}
G-12	589±26.5 ^{ef}	69±3.1 $^{g-j}$	4.3±0.2 ^{h}	27.5±1.4 ^{fgh}
G-13	835±37.5 ^{b}	72±3.2 ^{ghı}	9.6±0.5 ^{ef}	35.2±1.8 ^{de}
G-14	985±44.3 ^{a}	84±3.8 ^{ef}	15.5±0.8 ^{a}	40.0±2.0 ^{bcd}
G-15	963±43.3 ^{a}	35±1.6 ^{m}	10.1±0.5 ^{de}	41.0±2.1 ^{bc}
G-16	760±34.2 ^{bc}	77±3.5 ^{fgh}	9.5±0.5 ^{ef}	45.1±2.3 ^{b}
G-17	611±27.4 ^{def}	41±1.8 ^{m}	6.9±0.3 ^{g}	28.4±1.4 ^{fg}
P-1	700±31.5 ^{cd}	129±5.8 ^{ab}	11.5±0.6 ^{cd}	38.0±1.9 ^{cde}
P-2	693±31.1 ^{cd}	90±4.0 ^{e}	8.2±0.4 ^{fg}	26.4±1.3 ^{gh}
P-3	834±37.5 ^{b}	103±4.6 ^{d}	11.6±0.6 ^{bc}	42.7±2.1 ^{bc}
P-4	762±34.2 ^{bc}	134±6.0 ^{a}	11.9±0.6 ^{bc}	37.6±1.9 ^{cde}

*The differences among mean values shown on the same line with the same letter is not significant ($p<0.05$).

3.3 Antioxidant activity (DPPH and FRAP)

Significant differences were determined in terms of antioxidant activity among strawberry tree genotypes investigated ($p<0.05$). According to the DPPH test, the highest antioxidant activity was determined as 15.5 mmol TE 100 g^{-1} (G-14), while the lowest was determined as 2.1 mmol TE 100 g^{-1} (G-2). The G-14 genotype with the highest antioxidant activity was followed by the G-10 (13.1 mmol TE 100 g^{-1}), G-8 (12.1 mmol TE 100 g^{-1}) and P-4 (11.9 mmol TE 100 g^{-1}) genotypes, respectively. According to the FRAP test, the antioxidant activity was determined between 20.5 (G-6) and 50.9 mmol TE 100 g^{-1} (G-8). The G-8 genotype, in which the highest antioxidant activity was determined, was followed by the G-16 (45.1 mmol TE 100 g^{-1}), P-3 (42.7 mmol TE 100 g^{-1}) and G-3 (42.0 mmol TE 100 g^{-1}) genotypes, respectively (Table 1).

Serce et al. (2010) reported as 2.7 mmol TE 100 g^{-1} the antioxidant activity in the *Arbutus andrachne* specie according to the FRAP assay, while Isbilir et al. (2012) found 4.4 mmol TE 100 g-*1* in the *Arbutus unedo* specie according to the DPPH assay.

In addition, according to the TEAC assay, antioxidant activity was determined from 1.6 to 2.9 mmol TE 100 g^{-1} in strawberry tree genotypes grown in Akçabat region (Sagbas et al., 2020) and 1.8 to 3.3 mmol TE 100 g^{-1} in strawberry tree genotypes grown in the Muğla province (Colak, 2019). When the antioxidant activity results determined according to the DPPH and FRAP assays by different researchers were examined, the findings obtained from the FRAP assay were high; in contrast, the results obtained from the DPPH assay were low. Some differences observed are thought to be due to genetic structure, ecological conditions, and fruit ripeness.

According to principal component analysis results, the first two components (PC1 and PC2) explained 88.1% of the total variation. PC1 was related to total phenolic and antioxidant activity (both DPPH and FRAP), explaining 66.9% of the total variation. PC2 was associated with total flavonoids and accounted for 21.2% of the total variation (Figure 1).

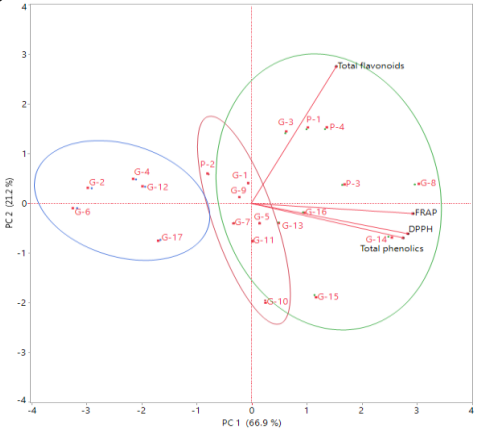

Figure 1. Biplot of the first two principal components (PC1 and PC2) in the strawberry tree genotypes based on bioactive compounds

Two main groups (A and B) were formed in the dendrogram, which was created using the biochemical properties of the strawberry tree genotypes investigated. The first main group (A) was divided into 2 subgroups (A-1 and A-2) and consisted of 16 genotypes. The first subgroup (A-1) included 7 genotypes (G-1, G-9, G-7, P-2, G-5, G-11 and G-10), while the second subgroup (A-2) consisted of 9 genotypes (G-3, P-1, P-4, G-8, G-14, G-13, G-16, P-3 and G-15) were formed. The second main group (B) included 5 genotypes (G-2, G-6, G-4, G-12 and G-17). When evaluated generally, the genotypes in the first main group had higher values than the genotypes in the second main group in terms of biochemical properties investigated. The G-14 in the first main group gave remarkable results in terms of total phenolics and antioxidant activity (according to DPPH assay). Also, the P-4 stood out with regards to the total flavonoid, while G-8 was remarkable in terms of antioxidant activity (according to FRAP assay) (Figure 2).

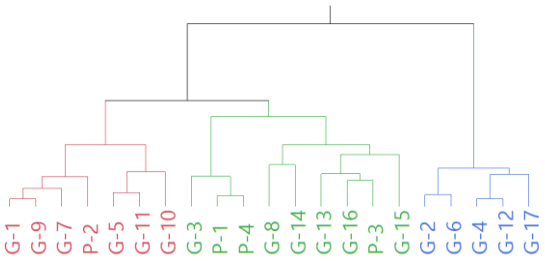

Figure 2. Dendrogram grouping of strawberry tree genotypes based on bioactive compounds

4. Conclusion

In the research examining the total phenolics, total flavonoids and antioxidant activity (DPPH and FRAP) properties of strawberry tree genotypes grown in two different regions (Piraziz and Gebze), a wide variation was determined among genotypes in terms of these properties. Among the genotypes investigated, G-14 gave remarkable results with regard to total phenolics and antioxidant activity (according to DPPH assay), while the P-4 stood out in terms of total flavonoids. As a result, G-14, which stands out in terms of beneficial substances on human health, can be used as genetic material in breeding programs. In addition, more detailed research is recommended carried out on the examined genotypes in the future.

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Umut Ates: Investigation, Conceptualization, Validation, Writing - original draft, Visualization, Review and editing. **Orhan Karakaya:** Investigation, Conceptualization, Writing - original draft, Visualization, Validation, Review and editing. **Süleyman Muhammed Çelik:** Methodology, Formal analysis, Data curation. **Ahmet Haseb Faizy:** Formal analysis, Data curation

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Data availability

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Changes in microbial quality of fruit juices, syrups, and ready-to-serve carbonated drinks produced with different processing parameters and stored in different conditions within six months

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ABSTRACT

The research was carried out during 2015-2017, aimed to consider the microbial quality of juice, syrup, and ready-to-serve (RTS) carbonated beverage prepared from mandarin fruit (Nagpur cultivar). Fully ripened, mature, fresh fruits were washed and peeled, then the juice was extracted using a screw-type pulper. The syrup and ready-to-serve carbonated beverage prepared from the extracted juice. Microbial analysis of juice was carried out by using Potato Dextrose Agar (PDA) culture medium. The results revealed no microbial growth in the ready-to-serve carbonated beverage up to 60 d of storage, after that from 90 to 180 d of storage was negligible. In the syrup, up to 90 d was no detection of microbes; after that, up to 180 d of storage was negligible. In the juice samples under cold storage (S2), all the treatments were within acceptable levels for 180 d. But under room temperature (S1), eight treatments (T1, T2, T3, T4, T5, T6, T8, T9) showed microbial colonies more than acceptable level, and only one treatment (T7-S1P3B1= juice sample in the room storage (SI) which added 350 ppm sodium benzoate as chemical preservative (P3) and packed in the glass bottle (B1) was remained safe for consumption during 180 d of storage. The microbial quality, viz. yeast and mold count were increased during 180 d of storage in the ready-to-serve carbonated beverage, syrup and juice. The microbial growth was observed within the acceptable level in all treatment combinations of ready-to-serve carbonated beverage, syrup; and juice in cold storage and T7 of juice under ambient conditions.

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1. Introduction

The mandarin fruits are juicy, and the fresh mandarin juice has refreshing flavor characteristics, pleasing aroma, and thirst-quenching properties. Besides that, it is the main source of important phytochemical nutrients, antioxidants, vitamins, minerals, soluble and insoluble dietary fibers that help reduce the risk for cancers as well as many chronic diseases, for example arthritis, obesity, and coronary heart diseases (Kamaljeet, 2002). A single mandarin is said to have about 170 phytonutrients and over 60 flavonoids with anti-tumor, anti-inflammatory, and blood clot inhibiting properties (Aslin, 2014; Etebu et al., 2014; Faizi and Mahen, 2020). Kumar (2009) revealed that for juice processing, it is essential to use cultivars with high juice content, good Brix-acidity balance, and having juice with attractive color. Vadakkan et al. (2010) mentioned that fruit juice has a low shelf life and needs preservation via various methods. Carbonation is the best one with few changes in quality parameters. Adding sufficient CO_2 into beverages was reported to enhance the appearance, flavor, taste and overall acceptability due to increased acidity, taste, sparkle and unique taste of CO_2 gas. Sandhan (2003) observed an increasing trend in the microbial count during the storage of carbonated beverages. Ahire (2007) reported that juice stored in cold storage (5.0 °C) condition in glass bottles could be stored satisfactorily for up to 3 months compared to ambient storage. Glevitzky et al. (2009) noticed that the enzymatic and microbiological reactions take place faster, leading to faster degradation and increased microbial potential in the beverages at room temperature storage. Beuchat (1982) studied the thermal inactivation of five yeasts (*Candida krusei, Hansenula anomala, Saccharomyces bailii, S. cerevisiae* and *Torulopsis magnolia*) suspended in five fruit juices (apple, apricot, grape, orange, and pineapple) with potassium sorbate, sodium benzoate, and sucrose. Yeasts were most sensitive to heat when suspended in orange juice. Both preservatives, at a concentration as low as 100 ppm, enhanced the inactivation rate in juices containing no added sucrose. Grandall et al. (1982) noted that preventing microbial spoilage is also an important element of storage stability. Covadonga et al. (2002) revealed that economic losses due to juice spoilage are minimized by good sanitation procedures before and

during citrus processing. Pasteurization, concentration, or low-temperature storage protocols help reduce the number of micro-organisms in the final product. Citrus juices are acidic beverages with high sugar content. Under these conditions, acidolactic bacteria, molds, and yeasts comprise the typical microbiota present in citrus juices. Lactic acid bacteria are the primary spoilage bacteria in fruit drinks; however, their numbers are greatly reduced after pasteurization, concentration, and refrigeration. Molds and yeasts tolerate high-osmotic and low-pH conditions and grow at refrigeration temperatures and can therefore cause spoilage in the processed product. Polydera et al. (2003) determined that yeasts, mold, and lactic acid bacteria are the micro-organisms responsible for the spoilage of orange juice during storage. Himani (2003) studied microbiological evaluation of kinnow squash, RTS, and concentrates and found that yeast and mold counts were found to be much below the permissible limits and were detected higher than permissible limits only in the long storage period. Ashurst (2010) mentioned that all beverages are at risk of suffering deterioration due to microbial action. Almost all fruit drinks are acidic in nature; the principle risk is microbial spoilage rather than contamination by a pathogenic organism. The main effects of microbial contamination are likely to be the development of off-flavors and changes in physical appearance. Bhardwaj (2011) studied microbiological analysis of stored juice samples of Kinnow mandarin for six months and observed that all the samples were contaminated with a large variety of bacterial, fungal, and mold species but within the acceptable limit. Kumar et al. (2011) noted that there was no microbial growth in the RTS beverages prepared with sweet lime juice; the increase in microbial load after 45 d of storage was negligible and safe for consumption. Chukwumalume (2012) carried out studies on microbiological assessment of preservative methods for African star apple juice. The juice sample was pasteurized and preserved with sodium benzoate 0.1% at ambient and refrigeration temperature for six weeks. The results showed that the combination of pasteurization, use of sodium benzoate, and storage at refrigeration temperature gave the best storage stability with a minimum microbial load. Oranusi et al. (2012) mentioned that most fruit juices are acidic enough and have sufficient sugar to favor yeast growth. Molds are generally considered the least important group of micro-organisms causing spoilage in fruit juice because of their limitation and inability to grow in the absence of air (anaerobic conditions) except for a few molds such as *Penicillium* and spore-forming *Aspergillus*. The presence of microbial contaminants in all the products could reflect the quality of the raw materials, processing equipments, environment, packaging materials and the personnel in the production process. Bhardwaj (2013) found that the untreated fruit juices and pulp were highly contaminated with bacteria, yeast, and mold. The minimum increase in bacteria, yeast, and mold population was observed when juice was processed at 85°C temperature for 15 min with the addition of preservative chemicals. Verma et al. (2014) conducted an experiment on the utilization of aonla (Indian gooseberry) and lime for the development of fruit-based carbonated soft drinks, studied the microbial evaluation of the carbonated fruit drinks, and found a non-significant presence of microbes in the blends. The yeasts-mold count ranged from 0.30×101 to 0.35×101 cfu mL^{-1}. Carbonation removes air, creating anaerobic conditions, and hence controls mold and yeast. Ogodo et al. (2016) observed *Aspergillus* species, *Rhizopus* species and *Penicillium* species in commercially packed fruit juices. *Penicillium* sp. and *Aspergillus* sp. could produce mycotoxins, which could lead to health hazards for the consumers. The research was done to consider the microbial quality (yeast and mold) of juice, syrup, and ready-to-serve (RTS) carbonated beverages of Nagpur mandarin fruit at different processing parameters which stored in the two storage conditions within 180 days of storage in 2015-2017.

2. Materials and methods

2.1. Materials

The research was conducted at the Postharvest Technology Center of Horticultural Crops and Microbial Quality Analysis in the Laboratory of the Mycology Department of Mahatma Phule Krishi Vidyapeeth (MPKV) Agricultural University during 2015-17. Fully ripened (horticultural maturity = ready to eat), mature (physiological maturity = ready to harvest), fresh, and good mandarin fruits of Nagpur mandarin cultivar from an orchard located in Ahmednagar district were provided. The juice was extracted, the syrup prepared (Faizi et al., 2020) from extracted juice, and ready-to-serve (RTS) carbonated beverage was made from previously prepared syrup by dilution (Faizi, 2022). The packing materials such as pet bottles, glass bottles and standy pouches were obtained from Postharvest Technology Centre, MPKV, Rahuri for packing the juice, syrup, and carbonated RTS drinks. Food grade and analytical grade chemicals obtained from manufacturers M/s. Thermo Fisher Scientific India Pvt. Ltd., Mumbai; M/s. Qualigens Fine Chemicals, Mumbai; M/s. E. Merck (India) Ltd., Mumbai and M/s. S. D. Fine-Chem Ltd., Mumbai, was used. All the glass wares used were obtained from manufacture M/s. Borosil Glass Works Ltd. (BGWL), Ahmedabad, Gujarat. Carbonation cum crown corking machine (Make: M. G. Industries, Coimbatore) was used for carbonation and crown corking of the ready-to-serve beverage. The screw-type pulper machine (Make: M.G. Industries, Coimbatore) was used to extract juice from fruits.

2.2. Methods

Mandarins were washed with cold tap water and peeled (Figure 1A). Then the juice was extracted using a screw-type pulper. The juice and the pomace were collected separately in two outlets. The juice was filtered through a clean muslin cloth. The extracted juice was pasteurized at 65°C for 15 min by adding sodium benzoate as the preservative. Then, at that temperature juice was filled in the pre-sterilized 200 mL glass bottles, 200 mL pet bottles, and 200 mL stand pouches (Figure 1B) and sealed with a crown cork and pouch sealer. All packed juice samples were sterilized (were kept in the boiling water for 10-20 min) as well after closing. Sugar syrup was prepared by adding water to sugar to boiling at a temperature of 90°C. Then the sugar syrup temperature decreased to 60°C, and the juice was mixed well. The syrup was bottled in the presterilized 200 mL transparent glass bottles and pet bottles (Figure 1C) and then sealed. After bottling, all syrup samples were sterilized.

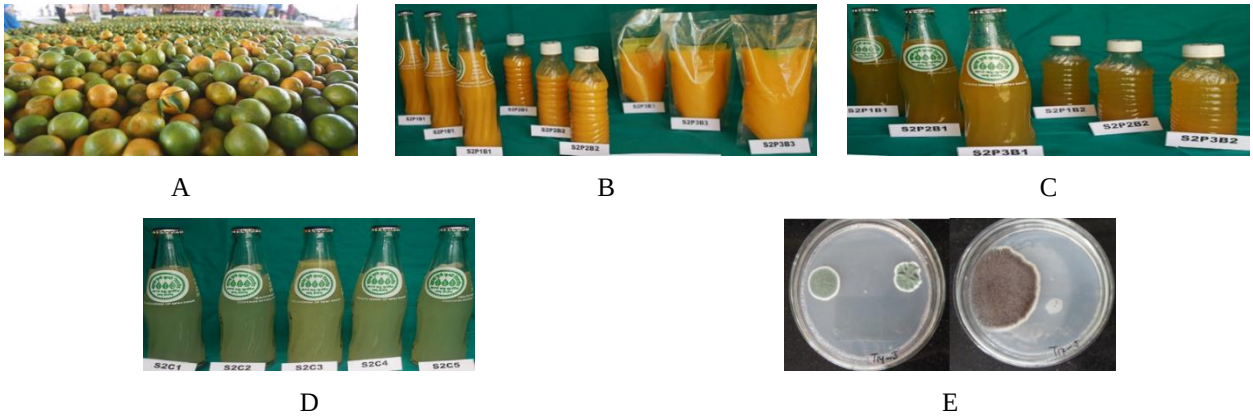

Figure 1. Fresh mandarin cultivar Nagpur which used for juice extraction (A). Prepared juice for microbial analysis in glass bottles, pet bottles, and standy pouch (B). Prepared syrup for microbial analysis in glass bottles and pet bottles (C). Prepared ready-to-serve (RTS) carbonated beverage for microbial analysis in glass and pet bottles (D). Mold colony forming of the spoiled juice samples in the PDA medium (E).

Table 1. Treatment details of juice, syrup and ready to serve (RTS) carbonated beverages

Tre. No.		Treatment Combinations
		Juice
TI	S1P1B1	Room Storage (19.80 - 27.60°C and 43.00 - 70.60% RH) + 150 ppm Sodium Benzoate + Glass Bottle.
T2	S1P1B2	Room Storage + 150 ppm Sodium Benzoate + Pet Bottle.
T3	S1P1B3	Room Storage + 150 ppm Sodium Benzoate + Stand Pouch.
T4	S1P2B1	Room Storage + 250 ppm Sodium Benzoate + Glass Bottle
T5	S1P2B2	Room Storage + 250 ppm Sodium Benzoate + Pet Bottle
T6	S1P2B3	Room Storage + 250 ppm Sodium Benzoate + Stand Pouch.
T7	S1P3B1	Room Storage + 350 ppm Sodium Benzoate + Glass Bottle.
T8	S1P3B2	Room Storage + 350 ppm Sodium Benzoate + Pet Bottle.
T9	S1P3B3	Room Storage + 350 ppm Sodium Benzoate + Stand Pouch.
T10	S2P1B1	Cold Storage (5.0±2.0°C and 92-95% RH) + 150 ppm Sodium Benzoate + Glass Bottle.
T11	S2P1B2	Cold Storage + 150 ppm Sodium Benzoate + Pet Bottle.
T12	S2P1B3	Cold Storage + 150 ppm Sodium Benzoate + Stand Pouch.
T13	S2P2B1	Cold Storage + 250 ppm Sodium Benzoate + Glass Bottle
T14	S2P2B2	Cold Storage + 250 ppm Sodium Benzoate + Pet Bottle
T15	S2P2B3	Cold Storage + 250 ppm Sodium Benzoate + Stand Pouch.
T16	S2P3B1	Cold Storage + 350 ppm Sodium Benzoate + Glass Bottle.
T17	S2P3B2	Cold Storage + 350 ppm Sodium Benzoate + Pet Bottle.
T18	S2P3B3	Cold Storage + 350 ppm Sodium Benzoate + Stand Pouch.
		Syrup
TI	S1P1B1	Room Storage (19.80 - 27.60°C and 43.00 - 70.60% RH) + 150ppm Sodium Benzoate + Glass Bottle.
T2	S1P1B2	Room Storage + 150 ppm Sodium Benzoate + Pet Bottle.
T3	S1P2B1	Room Storage + 250 ppm Sodium Benzoate + Glass Bottle
T4	S1P2B2	Room Storage + 250 ppm Sodium Benzoate + Pet Bottle
T5	S1P3B1	Room Storage + 350 ppm Sodium Benzoate + Glass Bottle.
T6	S1P3B2	Room Storage + 350 ppm Sodium Benzoate + Pet Bottle.
T7	S2P1B1	Cold Storage (5.0±2.0°C and 92-95% RH) + 150 ppm Sodium Benzoate + Glass Bottle.
T8	S2P1B2	Cold Storage + 150 ppm Sodium Benzoate + Pet Bottle.
T9	S2P2B1	Cold Storage + 250 ppm Sodium Benzoate + Glass Bottle
T10	S2P2B2	Cold Storage + 250 ppm Sodium Benzoate + Pet Bottle
T11	S2P3B1	Cold Storage + 350 ppm Sodium Benzoate + Glass Bottle.
T12	S2P3B2	Cold Storage + 350 ppm Sodium Benzoate + Pet Bottle.
		Ready to Serve (RTS) Carbonated Beverage
TI	S1C1	Room Storage (19.80 - 27.60°C and 43.00 - 70.60% RH) + Carbonation Pressure (C)= 70 psi (Pound Per Square Inch).
T2	S1C2	Room Storage + Carbonation Pressure (C)= 80 psi (Pound Per Square Inch).
T3	S1C3	Room Storage + Carbonation Pressure (C)= 90 psi (Pound Per Square Inch).
T4	S1C4	Room Storage + Carbonation Pressure (C)= 100 psi (Pound Per Square Inch).
T5	S1C5	Room Storage + Carbonation Pressure (C)= 110 psi (Pound Per Square Inch).
T6	S2C1	Cold Storage (5.0±2.0°C and 92-95% RH) + Carbonation Pressure (C)= 70 psi (Pound Per Square Inch).
T7	S2C2	Cold Storage + Carbonation Pressure (C)= 80 psi (Pound Per Square Inch).
T8	S2C3	Cold Storage + Carbonation Pressure (C)= 90 psi (Pound Per Square Inch).
T9	S2C4	Cold Storage + Carbonation Pressure (C)= 100 psi (Pound Per Square Inch).
T10	S2C5	Cold Storage + Carbonation Pressure (C)= 110 psi (Pound Per Square Inch).

Carbonated RTS beverage was prepared from the syrup. The 40 mL of syrup were added to 200 mL transparent glass bottles (Figure 1D), and bottles were filled with chilled water and different carbon dioxide levels (carbonation). The bottles were sealed simultaneously by a crown corking machine and sterilized. All the juice, syrup, and carbonated RTS beverage were stored at ambient and cold conditions and evaluated at an interval of 30 d for 180 d for microbial quality. Treatment details of juice, syrup and ready to serve (RTS) carbonated beverages is given in the Table 1.

2.3. Microbial analysis

The microbial analysis (yeast and mold count) of juice was carried out accordingly (Adedeji and Oluwalana, 2013; Michael, 2021). Potato Dextrose Agar (PDA) culture medium was used. Ingredients of the PDA culture medium used for analysis were included Potato infusion from peeled potato 200 g, Dextrose 20 g, agar 20 g, water 1 L, and antibiotic (chlortetracycline or streptomycin or chloramphenicol) 25-40 mg. For preparation of the medium, potatoes peeled, then put in 1000 mL distilled water added on it then boiled for 10-20 min. After that sieving by gauze and refill distilled water to 1000 ml. Then added in dextrose and agar and melted down. After that, the medium sterilized at 121°C for 20 min. In the following, a small amount of ethanol was used to dissolve Chloromycetin and put into the culture medium before pouring it into the flat plates. It is mentionable that antibiotics were used to prevent bacteria growth and allowing easy growth of yeast and molds in medium during incubation. An aseptic micropipette used to pipet 1 mL of sample into a test tube containing 9 mL of sterile distilled water. Then, another 1 mL aseptic pipette was used to repeat dilution for 6 times. One mL of each from appropriate dilution was plated (pour plating) in the required medium (PDA), then incubated at 28±1°C for 5-7 d. Then observed and took records. The yeasts and molds colonies expressed as cfu (colony forming units) per mL. Counted the yeasts and molds respectively according to their appearance in the medium when the molds covered the whole plate the average of 2 plates counted for colony count. Calculated the average value based on two plate counts. If the colony counts from all plates were two or more than two cfu/mL, the sample reported as a spoiled sample; if less than two, it was reported acceptable and saved for consumption.

3. Results

3.1. Microbial quality (yeast and mold) of ready-to-serve (RTS) carbonated beverage

The data presented in Table 2 showed that there was no microbial (yeast and mold) growth in the carbonated RTS beverage of Nagpur mandarin up to 60 d of storage in ambient and cold storage situations prepared under relatively hygienic conditions condition. Increase in microbial load after 60 d from 90 to 180 d of storage was negligible and within the acceptable level (less than 2.00 colony forming units per mL) and safe for consumption.

3.2. Microbial quality (yeast and mold) of syrup

The data presented in Table 3 indicated no detection (ND) of microbes (yeast and mold) up to 90 d of storage. There was no microbial detection in treatments T10, T11, and T12 under cold storage conditions during 120 and 150 d compared to other treatments. From 120 to 180 d of storage, there was microbial detection in all the treatments, both in ambient condition/room temperature (RT) and cold storage (CS). Still, it was negligible, within an acceptable level (less than 2.00 colony forming units per mL), and safe for consumption.

3.3. Microbial quality (yeast and mold) of juice

The data presented in Table 4 indicated that there was no detection (ND) of microbes (yeast and mold) in cold storage and treatment T7 under ambient conditions. There was microbial detection in treatments T1, T2, T3, T4, T5, T6, T8, and T9 at ambient conditions up to 90 d of storage. Still, it was negligible, within the acceptable level (less than 2.00 colony forming units per mL), and safe for consumption. From 90 to 180 d of storage, there was microbial detection in ambient conditions (S1) and cold storage (S2). Under CS (S2), all the treatments were within acceptable level during 180 days of storage, but under RT (S1), eight treatments (T1, T2, T3, T4, T5, T6, T8, T9) showed microbial colony (Figure 1E) more than acceptable level (2 or more than two colony forming unit per mL), and only one treatment (T7-S1P3B1) was remained safe for consumption during 180 d of storage.

Table 2. Effect of storage conditions and carbonation levels on microbial (yeast and mold) quality of carbonated RTS Beverage and their treatment combinations. count (cfu/mL)

Treatment Combinations		Storage Period/ Count Period						
		0 days	30 days	60 days	90 days	120 days	150 days	180 days
TI	S1C1	ND	ND	ND	1.34	1.42	1.48	1.52
T2	S1C2	ND	ND	ND	1.3	1.38	1.44	1.48
T3	S1C3	ND	ND	ND	ND	ND	1.42	1.46
T4	S1C4	ND	ND	ND	ND	1.31	1.37	1.41
T5	S1C5	ND	ND	ND	1.23	1.27	1.33	1.37
T6	S2C1	ND	ND	ND	1.19	1.25	1.31	1.35
T7	S2C2	ND	ND	ND	1.17	1.21	1.27	1.31
T8	S2C3	ND	ND	ND	ND	ND	1.23	1.27
T9	S2C4	ND	ND	ND	1.04	1.12	1.18	1.22
T10	S2C5	ND	ND	ND	1.03	1.11	1.17	1.21

S1= ambient storage/room storage. S2= cold storage. C1= 70 psi. C2=80 psi. C3= 90 psi. C4= 100 psi. C5= 110 psi. ND= not detected. cfu= colony forming unit. C= Carbonation Pressure. Psi= Pound Per Square Inch.

Table 3. Effect of storage conditions, preservative levels, and packing materials on syrup's microbial (yeast and mold) quality and their treatment combinations. count (cfu/mL)

Treatment Combinations		Storage Period/ Count Period						
		0 days	30 days	60 days	90 days	120 days	150 days	180 days
TI	S1P1B1	ND	ND	ND	ND	1.39	1.42	1.46
T2	S1P1B2	ND	ND	ND	ND	1.42	1.45	1.49
T3	S1P2B1	ND	ND	ND	ND	1.28	1.31	1.35
T4	S1P2B2	ND	ND	ND	ND	1.32	1.35	1.39
T5	S1P3B1	ND	ND	ND	ND	1.21	1.24	1.28
T6	S1P3B2	ND	ND	ND	ND	1.26	1.29	1.33
T7	S2P1B1	ND	ND	ND	ND	1.15	1.18	1.22
T8	S2P1B2	ND	ND	ND	ND	1.17	1.2	1.24
T9	S2P2B1	ND	ND	ND	ND	1.07	1.1	1.14
T10	S2P2B2	ND	ND	ND	ND	ND	ND	1.11
T11	S2P3B1	ND	ND	ND	ND	ND	ND	1
T12	S2P3B2	ND	ND	ND	ND	ND	1.02	1.05

S1= ambient storage/room storage. S2= cold storage. P1= 150 ppm sodium benzoate. P2= 250 ppm sodium benzoate. P3= 350 ppm sodium benzoate. B1= glass bottle. B2= pet bottle. ND= not detected. cfu= colony forming unit.

Table 4. Effect of storage conditions, preservative levels, and packing materials on microbial (yeast and mold) quality of juice and their treatment combinations. count (cfu/mL)

Treatment Combinations		storage period/ count period						
		0 days	30 days	60 days	90days	120 days	150 days	180 days
TI	S1P1B1	ND	1.78	1.81	1.84	1.87	3.5*	
T2	S1P1B2	ND	1.82	1.85	1.89	2.6*		
T3	S1P1B3	ND	1.89	1.92	1.98	14*		
T4	S1P2B1	ND	ND	1.67	1.7	1.76	1.77	3*
T5	S1P2B2	ND	1.67	1.7	1.73	1.76	3*	
T6	S1P2B3	ND	1.74	1.77	1.8	1.8	2*	
T7	S1P3B1	ND	ND	ND	ND	1.54	1.6	1.63
T8	S1P3B2	ND	ND	ND	1.67	1.73	1.78	4*
T9	S1P3B3	ND	ND	1.74	1.77	1.78	2.3*	
TI0	S2P1B1	ND	ND	ND	ND	1.41	1.47	1.5
T11	S2P1B2	ND	ND	ND	ND	1.46	1.52	1.55
T12	S2P1B3	ND	ND	ND	ND	1.5	1.56	1.59
T13	S2P2B1	ND	ND	ND	ND	1.27	1.33	1.36
T14	S2P2B2	ND	ND	ND	ND	1.31	1.37	1.4
T15	S2P2B3	ND	ND	ND	ND	1.37	1.43	1.46
T16	S2P3B1	ND	ND	ND	ND	1.2	1.26	1.29
T17	S2P3B2	ND	ND	ND	ND	1.24	1.3	1.33
T18	S2P3B3	ND	ND	ND	ND	1.35	1.41	1.44

S1= ambient storage/room storage. S2= cold storage. P1= 150 ppm sodium benzoate. P2= 250 ppm sodium benzoate. P3= 350 ppm sodium benzoate. B1= glass bottle. B2= pet bottle. B3= standy pouch. ND= not detected. *= discarded samples /terminated shelf life. Cfu= colony forming unit.

4. Discussions

The data revealed that the microbial detection was negligible and within the acceptable level (less than 2.00 colony forming units per mL) in all treatment combinations at ambient storage up to 90 d in juice, up to 180 d in syrup, and carbonated RTS. Fruit beverages that are sold commercially are consumed by individuals of various ages all around world as they are nutritious, also the flavonoids of fruit beverages inhibit cancer cell development (Pinto et al., 2022; Faizi, 2022), but if improperly prepared, that could be harmful to people's health due to microbial growth in it, so to guarantee the protection of the public's health, manufacturing procedures need to be substantially stricter (Ahmed et al., 2018). The microbial detection was negligible and within an acceptable level in all treatment combinations at cold storage up to 180 d in juice, syrup, and carbonated RTS. The microbial growth was found to be within an acceptable level in the juice, syrup and carbonated RTS beverage, which might be due to the acid environment, high sugar level, chemical preservative, packaging materials, and CO_2 gas maintaining the beverage at a safe level and has prevented microbial growth. Similar results were reported by Lotha et al. (1994) on Kinnow mandarin juice; Covadonga et al. (2002) on orange juice; Polydera et al. (2003) on orange juice; Himani (2003) on Kinnow mandarin juice; Kumar et al. (2011) on sweet lime juice; Chukwumalume (2012) on African star apple juice; Oranusi et al. (2012) and Ogodo et al. (2016) on different fruit juices grown in Nigeria. In our research, the findings were acceptable that rely upon strictly applied health standards and regulations during processing, the same idea mentioned by Kumar (2009) and Strano et al.

(2022); otherwise, the product will be exposed to contamination by bacteria, mold and yeasts.

5. Conclusion

Microbial quality (yeast and mold) count was increased during 180 d of storage in the ready-to-serve carbonated beverage, syrup, and juice samples. The microbial growth was recorded within the acceptable level and saved for consumption in all treatment combinations of ready-to-serve carbonated beverage, syrup and juice in cold and room storage. But in the room storage, only T7 of juice remained within the acceptable level, and remained all discarded at different periods. Not only storage conditions but also packaging materials and preservative quantities had shown an important stability of the samples as the juice sample in the cold storage (SI) which added highest (350 ppm) level of sodium benzoate as chemical preservative (P3), and packed in the glass bottle (B1) remained save for consumption. So, for production fruit beverages free of mold and yeast specially in case of Nagpur cultivar of mandarin beverages, always glass bottle + cold storage + using ideal level of preservative is a need and should take it serious at industrial production level.

Compliance with Ethical Standards

Conflict of Interest

As the author of article declare that there are no conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' Contributions

All the procedures including research conception and design, draft manuscript controlling and corrections, data collection and analysis of the manuscript as well as finalization of the manuscript was done by **Zaki Ahmad FAIZI**.

Ethical approval

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Metabolomic exploration of CTC tea manufacturing waste validates its potentiality as organic fertilizer

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ABSTRACT

Valorization of agro-industrial waste resources is today's main focus for agribiotechnologists. This research work was designed to valorise tea industrial waste, i.e., manufactured by-products from crush-tear-curl (CTC) tea factory. Physicochemical analysis has been carried out to characterize tea waste treated soil. Pot experiment with cowpea [*Vigna unguiculata* (L.) Walp.] was considered to study the impact of tea waste on plant growth. Morphological parameters such as length of plants and pods, and girth diameter were considered for growth study. Effect of tea factory waste on soil nutrition was found remarkable with increased organic carbon, organic matter, nitrogen, phosphorus, potassium and sulphur content. Pot culture revealed impact of tea waste composted soil on boosted plant growth. GC-MS based metabolite profiling revealed xanthosine and caffeine as major compounds in tea waste extract. A possible pathway has been proposed to explain the role of xanthosine and caffeine breakdown in fertilization of soil and plant growth. Disposal of tea wastes produced during tea manufacturing can be managed in a sustainable manner if this research is implemented industrially. This research portrays a notable nutrient richness in tea waste treated soil. Detection of purine metabolites revealed remarkable fertilizing and plant growth promoting properties of CTC tea waste.

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1. Introduction

Fertilization of soil is an essential factor that provides nutrients for boosting crop productivity and quality. Substantial employment of chemical fertilizer has caused depletion in soil health by affecting the function of soil microorganisms. Excessive use of such chemicals has given rise to serious problems on soil fertility, crop production, environment and human health which have stressed scientists, biotechnologists, activists, and policy makers. So, presently, organic manure and bio fertilizers have emerged as alternative and effective options for fertilizing soils without damaging the environment (Mekki et al., 2017; Ngan and Riddech, 2021). Green waste management and organic farming are two sides of the same coin because organic or biodegradable waste exhibits its necessity as far as organic agriculture is concerned. Compost manuring is an interesting agronomic practice as well as an attractive waste management strategy. Crop residues, manures and compost from organic wastes not only fertilize the soil but also improve its physical properties that have been affected due to overuse of inorganic substances since ages (Annabi et al., 2017). Proper utilization would have rather portrayed waste as a solution, not a problem. Recycling and effective valorization of different agro-industrial waste resources are reported by researchers around the world. Recovery of value-added compounds and conversion of wastes into various processed materials are such economic and environmental approaches, which can reduce the problems regarding conventional disposal of waste.

From producers to consumers, plantation workers to tea stalls, the whole tea industry connecting chain produces vast number of bio-wastes, i.e., pruning litters from gardens, post-processing fiber rich waste tea from factories and spent or leftover tea or remainders from tea stall and households. Tea factory waste is a resource of stalks, hard leaves, stems, and other fiber rich parts of the tea plant (Majumder et al., 2022). Extraction of caffeine from industrial tea waste is referred to as the most popular and effective method so far which is used by multiple tea factories to make profit (Shalmashi et al., 2010; Khan et al., 2018). Moreover, it was reported that tea factory waste could be utilized as a source of renewable energy to produce biochar and bio methane (Majumder et al., 2022). Being a novel adsorbent of toxic pollutants, industrial tea wastes are often used to remove toxic substances like heavy metal ions, dyes, phenols, antibiotics, benzene etc. from wastewater (Hussain et., 2018; Kabir et al., 2021; Majumder et al., 2022). Feeding livestock i.e., pigs, poultry and ruminants (Jayasuriya et al., 1978; Angga et al., 2018) recovery of tannin and antioxidant polyphenolic compounds (Abdeltaif et al., 2018; Rajapaksha et al., 2020) and production of bioactive probiotic beverage kombucha (Majumder et al., 2022) are various other ways to recycle the

fiber rich tea factory waste as demonstrated by agribiotechnologists and food scientists. As organic fertilizer and priming agent, scientists (Gurav and Sinalkar, 2013; Gammoudi et al., 2021) proved functionality of leftover tea (spent tea waste). People around the world who have been using this kitchen waste as compost for houseplants and gardens since ages. According to Tea Board of India, "tea waste" means tea sweepings, tea fluff, tea fiber or stalks or any article purporting to be tea which does not conform to the specification for tea laid down under Prevention of Food Adulteration Act. 1954 (37 of 1954) but does not include green tea or green tea stalks and minimum volume of tea waste and made tea should be at the ratio of 2:100 kilograms (Anonymous, 2022a).

In this context, the aim of this research was to study the effect of tea factory waste on enhancement of soil nutrition and its impact on plant growth. To achieve these goals, physicochemical analysis on tea waste treated soil were conducted along with a simple pot assay. GC-MS analysis of CTC (crush-tear-curl, a type of processed black tea) waste extract has also been carried to detect the metabolites responsible for soil fertilizing and promoting plant growth. Furthermore, results of soil analysis and pot assay have also been elucidated with a metabolomic discussion.

2. Materials and methods

2.1. Collection of tea waste, soil preparation and composting of tea waste for pot experiment

Post-manufacturing tea waste was collected from CTC tea factory of Jayantika Tea Estate, Darjeeling (26°32'06.0"N, 88°16'18.0"E). For pot experiment, garden soil was taken from a barren land and sun-dried for three days. The soil was ground, sieved through 2 mm filter and homogenously mixed (Ngan and Riddech, 2021). Cylindrical plastic pots having 15 cm depth and 5 cm diameter were taken. One and half kilogram of dried soil was placed in each pot.

A total of twenty pots were prepared with dried soil and out of which ten were treated with 5% (w/w) tea waste, i.e., seventy-five grams of dried tea waste in each pot, for composting tea waste to prepare tea waste treated soil (TWS). Other ten replicas were considered as control soil or CS. All the CS and TWS pots were irrigated on a regular basis with equal amount of tap water and tilled or mixed daily up to three months using a tilling fork to avoid any weed growth. Just before sowing seeds, soil samples were collected from all the pots to examine nutritional values as described below.

Table 1. Physicochemical characters of control soil (CS) and tea waste treated soil (TWS)

Physicochemical parameters	Sample	Results
pH	CS	6.14 ± 1.02^{b}
	TWS	4.5 ± 0.87^{a}
EC (mS/m)	CS	78 ± 14^{a}
	TWS	196 ± 6^{b}
Moisture content (%)	CS	18.66 ± 3.91^{a}
	TWS	31.23 ± 2.17^{b}
Organic carbon (%)	CS	0.98 ± 0.26^{a}
	TWS	5.06 ± 0.6^{b}
Organic matter (%)	CS	1.69 ± 0.31^{a}
	TWS	8.71 ± 0.87^{b}
Total nitrogen (%)	CS	0.13 ± 0.02^{a}
	TWS	0.52 ± 0.1^{b}
Available phosphorus (ppm)	CS	51 ± 4^{a}
	TWS	72 ± 2^{b}
Available potassium (ppm)	CS	25 ± 6^{a}
	TWS	201 ± 24^{b}
Available sulphur (ppm)	CS	10 ± 2^{a}
	TWS	29 ± 1^{b}

The superscript letters in the same column represent statistically ($p\leq0.05$) different groups for each parameter.

2.2. Analysis of soil nutrients with selected physicochemical parameters

CS and TWS samples from all twenty pots (ten replicates for each sample) were air dried and sieved through 2 mm filter. For organic carbon determination, the samples were further grind and sieved through a 0.5 mm filter. pH, electrical conductivity (EC), moisture content, organic carbon (OC), total nitrogen (N), available phosphorus (P), available potassium (K) and available sulphur (S) were assessed on both samples. The pH and EC were measured in water extracts (20 g soil in 100 mL double distilled water) using a pre-calibrated pH meter and EC meter respectively (Ghosh et al., 2022). Moisture content percentage of soil samples were determined following protocol of Ghosh et al. (2022). OM (%) and OC (%) were assessed following the titration method developed by Walkley and Black (Walkley and Black, 1934). Total N (%) was measured by Kjeldahl method (Kirk, 1950). Available P was extracted and measured using the colorimetric method with molybdenum (Bray and Kurtz, 1945). Estimation of available K was done by using a flame photometer (Jackson and Smith, 1956). Available S was figured out following the protocol of Williams and Steinbergs (1962). Values of available P, K and S were

calculated based on respective standard curves and results have been expressed as parts per million or ppm.

2.3. Pot experiment

Cowpea [*Vigna unguiculata* (L.) Walp.] seeds were bought from a local market of Siliguri (Darjeeling district of West Bengal, India). Seeds were dipped in warm tap water (60 °C) for 1 h (sinker-floater test) to determine healthy seeds. Twenty of those healthy seeds were taken up and sown in CS and TWS pots which were being prepared for three months as described earlier. The incubation was conducted for seventy days and during that period, every pot was irrigated with equal amount of tap water. From vegetative growth to reproductive development- a detailed plant growth study was done through morphological characterization. Germination period, foliation and flowering stages, leaf count, plant height, girth diameter, fruit setting period and fruit length were recorded regularly on every fifth alternate day. Single or only one fruit bearing plants were considered standard for this comparative study. So, data of three of such pots (for each CS and TWS) were taken from the records for statistical analysis. After one month of pot culture, plants were harvested and post-harvest morphological characters like weight (both fresh and dry) and length of a whole plant and its parts (stem, root, and the fruit); number of seeds inside the fruit; and number and weight of nodules present in root were recorded.

2.4. GC-MS analysis on tea waste extract

Tea waste (1% w/v) was soaked in freshly boiled hot water for fifteen minutes. After this hot-water extraction, 1 mL of the extract was dried and dissolved in 1 mL of methanol to prepare the sample for GC-MS analysis. GC-MS analysis was done following the protocol of Majumder et al. (2021). One microliter of sample (in 20:1 split ratio) was injected in GCMS-QP2010 Plus (Shimadzu Co., Japan). DB-5 fused-silica capillary column (0.25 µm x 0.25 mm x 30 m) was used. Interface and source temperature was set to 270°C and 230°C, respectively. Helium was used as carrier gas. Total flow rate was 16.3 mL/min and column flow rate stood at 1.21 mL/min. Mass spectra were recorded at 5 scan/sec with a scanning rate of 40-650 m/z. Compounds were identified after comparing the spectral configurations obtained with that of available mass spectral databases (NIST08s.LIB and WILEY8.LIB). The chromatogram (TIC or Total Ion Chromatogram) is based on the intensity of fragments produced by the ionization. Quantification of the amount (area %) of each compound was done based on peak areas (Majumder et al., 2020; Acharyya et al., 2021). The data obtained from GCMS analysis were further studied from available reported scientific literature to find out responsible growth promoting components or priming agents present in tea waste.

2.5. Statistical analysis

Data obtained from the various experiments during this research were analyzed statistically using Microsoft Excel. Results have been expressed as mean ± SD (n = 3). The test for statistical difference was performed using the Student's t-test to compare the means between two treatments (CS and TWS). Differences were considered significant at $P < 0.05$.

3. Results and discussion

3.1. Effect of tea waste on soil nutrients

Results of soil nutrient analysis represented in Table 1, exhibits a noticeable variation of physicochemical parameters between CS and TWS. Soil was turned into more acidic due to tea waste treatment as the pH value of TWS was found 4.5±0.87 while for CS it was recorded 6.14±1.02. Moisture content in TWS was as high as 31.23±1.17% and for CS it was only 18.66±0.91%, despite the same amount of irrigation. Composting of CTC tea waste in soil has enriched other nutrient values too. Amounts of N, P, K and S for CS were 0.13±0.02%, 51±4 ppm, 25±6 ppm and 10±2 ppm respectively while after composting, the same soil showed very much high values i.e., 0.52±0.1%, 72±2 ppm, 201±24 ppm and 29±1 ppm for N, P, K and S respectively.

3.2. Effect of tea waste on growth and development of cowpea plant

Experimental data collected during pot-culture and postharvest periods have been studied to evaluate the effect of tea waste on plant growth and development which have been discussed below.

Various stages such as seed germination period, sprouting, flowering, fruit-setting, and fruit maturation phases were noted since sowing of seeds to determine time taken by each plant to accomplish each event. Table 2 has been provided to express the results that shows, from germination to fruit maturation, plants grown on tea waste compost (TWS) took lesser time to succeed every growth level compared to the plants grown on control soil (CS). On TWS, germination was happened after 7.57±0.76 d while for CS, it took 9.5±0.36 d. Similarly, sprouting of leaf on CS plants was observed on 12±0.4 d where TWS plants took only 10±0.9 d. Moreover, in CS plants, no flowering was there till thirty days of pot experiment while on TWS, the first flower was already bloomed on 28.2 d. Both fruit-setting and fruit maturation stages were faster in TWS plants with 33.87±0.21 d and 49.60±1.31 d accordingly compared to CS plants where maturation process was detected even after sixty days of pot culture (Table 2, Figure 1). Maturation or ripening was determined by the change in pod's color (turning into whitish yellow from green) and raise in pod's length which was immobilized after a certain period (Figure 1).

Being deciduous, leaves of bean plants were seen to be shed during growth resulting an irregular foliage which was contradictory in this relative study. However, leaves produced by each plant during the whole pot culture period were counted. TWSP recorded high with a total of 47.17±2.33 leaves production in each plant while each CSP produced only 29.29±1.07 leaves. After seventy days of pot culture, plant height development was found higher in plants grown in TWS (from 1.8±0.36 cm to 23.13±3.53 cm) compared to the CS plants (from 1±0.12 cm to 16.33±0.25 cm). Results of girth diameter did not differ significantly CS and TWS plants, but the graphical representation (Figure 1) never failed to keep TWS ahead of CS. In case of TWS fruits, the rate of pod enlargement was lofty upto fifteen days from fruit set before attaining maturity. Meanwhile, CS fruits took about twenty-five days to achieve the same and the growth was also nowhere near to TWS as shown in the graph (Figure 1). Fresh and dry weight, moisture content and length of different parts i.e., stem, root and fruit from harvested plants

were recorded and the results have been given in Table 3. A lower content of moisture signifies a higher mass or dry matter content, which was seen in TWS plants. Moreover, not only growth and development but also production was influenced by tea waste. In TWS plants, 16-18 seeds or beans were found in each pod while the numbers were lower in CS plants with 8-10 beans only.

Table 2. Effect of tea waste treated soil on germination period, sprouting, flowering, fruit-setting, and fruit maturation phases compared to control soil.

	Germination		Sprouting		Flowering		Fruit-Setting		Fruit Maturation	
	CS	TWS	CS	TWS	CS	TWS	CS	TWS	CS	TWS
Mean	9.5±0.4^{b}	7.6±0.7^{a}	12±0.4^{d}	10±0.9^{c}	31.2±1.6^{e}	29.5±1.5^{e}	35.8±1.6^{g}	33.9±0.2^{f}	58.2±2.7^{i}	49.6±1.3^{h}
Min.	9.1	6.7	11.6	9.1	30.3	28.2	34.9	33.7	55.2	48.2
Max.	9.8	8.1	12.4	10.9	33.1	31.1	37.7	34.1	60.3	50.8

The test for statistical difference was performed using ANOVA (analysis of variance). Values are means ± standard deviation, means with different superscripts in are significantly different (P < 0.05).

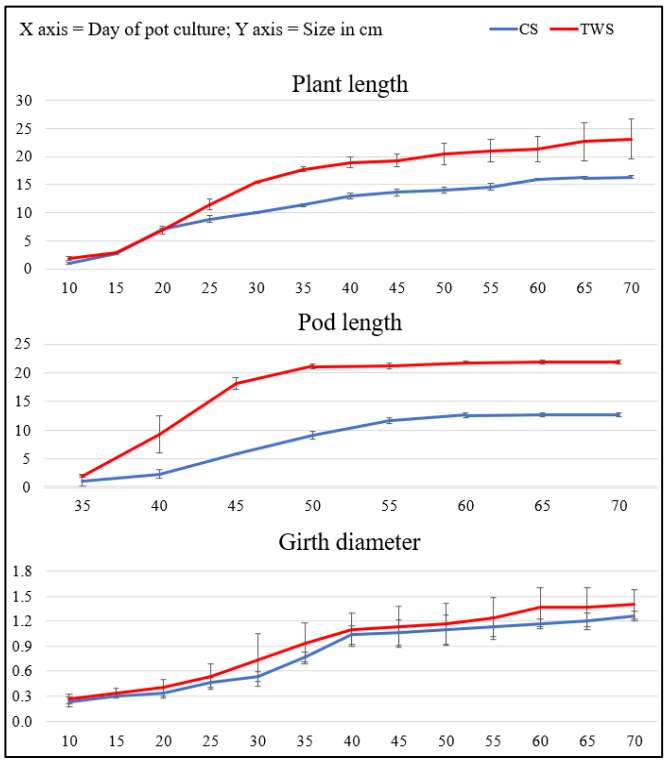

Figure 1. Impact of tea waste compost (TWS) on plant growth i.e., plant length, pod length and girth diameter. Values are means ± SD (n = 3).

13-18 nodules (158.33±49.74 mg of total weight) were obtained from each CS plant while in TWS, the count was ranged between 8 to 11 (73.33±11.72 mg of total weight) for each plant. Most certainly, tea waste composting left an effect on soil nitrogen status as described earlier. Moreover, not only soil nitrogen availability but also accumulation of that nitrogen was high in TWS plants which was clearly reflected by other results of this pot experiment. Additionally, GC-MS based metabolomics was considered to evaluate the results mentioned above.

3.3. Responsible plant growth promoting compounds in tea waste

Metabolite profiling of the waste extract revealed twelve different components (Table 4) dominated by xanthosine with a share of 68.89% peak area and caffeine (5.19%) (Figure 2). Detection and isolation of caffeine from tea waste were already recounted in various research papers (Shalmashi et al., 2010; Khan et al., 2018; Majumder et al., 2022). Xanthosine is just another metabolite of the same pathway by which caffeine is biosynthesized, i.e., tea's purine metabolic pathway. Xanthosine is the precursor of caffeine as well (Majumder et al., 2022). Presence of these two substantial nitrogenous components in tea waste extracts surely gives an account of TWS's fertilizing properties. Moreover, by freeing ribose (five-carbon sugar), xanthosine certainly degrades into xanthine which is reported as a plant growth promoting agent. Perhaps soil microbes helped this purine degradation process during composting of tea waste.

Table 3. Effect of tea waste treated soil (TWS) on selected parameters considered to evaluate harvested plants in comparison with control soil (CS).

	Stem		Root		Fruit	
	CS	TWS	CS	TWS	CS	TWS
Fresh weight (g)	6.87±0.23^{a}	9.76±1.34^{b}	3.20±0.65^{c}	4.61±1.32^{d}	5.9±0.36^{e}	9.86±0.47^{f}
Dry weight (g)	1.98±0.02^{e}	3.01±0.8^{f}	0.79±0.02^{a}	1.38±0.52^{c}	0.91±0.02^{b}	1.83±0.05^{d}
Moisture content (%)	71.15±1.2^{a}	69.41±2.44^{a}	75.17±0.77^{c}	70.02±2.21^{b}	84.37±0.8^{e}	81.54±0.66^{d}
Length (cm)	16.33±0.25^{b}	23.13±3.52^{e}	16.01±0.39^{b}	20.1±2.36^{c}	12.47±0.38^{a}	21.6±0.36^{d}

Values are means ± standard deviation, means with different superscripts in each row are significantly different (P < 0.05).

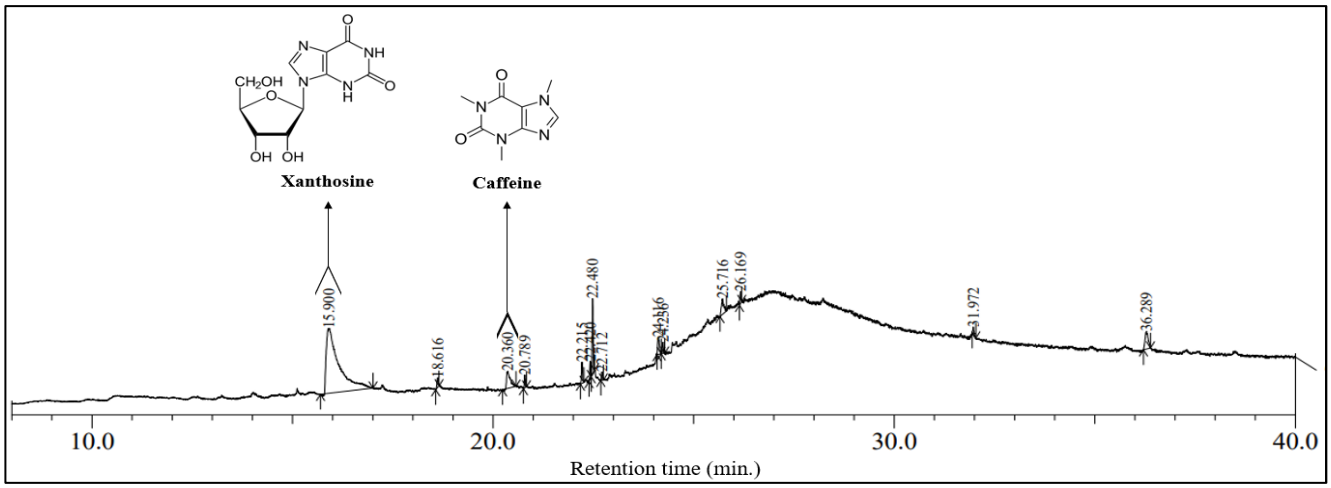

Figure 2. GC-MS total ion chromatogram of tea waste extract indicating peaks of different compounds listed in Table 4. Peaks representing major compounds xanthosine and caffeine are labelled.

Table 4. Components of tea waste extract revealed through GC-MS analysis.

Peak index	Retention time	Area%	Name of compound
1	15.9	68.89	Xanthosine
2	18.616	1.04	Octadecanal
3	20.36	5.19	Caffeine
4	20.789	1.09	Stearic acid methyl ester
5	22.215	2	13-Hexyloxacyclotridec-10-en-2-one
6	22.42	1.28	Linolelaidic acid, methyl ester
7	22.48	6.41	Petroselinic acid methyl ester
8	22.712	0.73	Stearic acid methyl ester
9	24.116	2.41	(Z,Z)-3,9-cis-6,7-epoxy-nonadecadiene
10	24.236	1.19	Lauric acid, chloride
11	25.716	3.35	1-oleoylglycerol
12	26.169	1.1	Dinonyl Phthalate
13	31.972	0.81	beta-Sitosterol
14	36.289	4.53	beta-Sitosterol

3.4. Possible metabolic breakdown of xanthosine and caffeine in TWS

A set of probable pathways directing breakdown of tea waste metabolites have been proposed through a pictorial demonstration in Figure 3. Metabolic breakdowns of major tea waste compounds xanthosine and caffeine are proportional to increased nitrogen availability in soil and nitrogen accumulation by TWS plants as reflected through soil fertility status and faster growth of plants on TWS. During composting of tea waste, xanthosine and caffeine have produced xanthine which further bio-transformed into the final product of purine catabolism i.e., urea. During this conversion, compounds such as uric acid, hydroxyisourate, OHCU (2-oxo-4-hydroxy-4-carboxy-5-ureidoimidazoline), allantoin, allantoic acid etc. are also produced as intermediates (see Figure 3). Urea then enters nitrogen metabolism pathway by producing ammonia using the enzyme urease (EC 3.5.1.5). Moreover, ammonia is transformed to absorbable forms (NH_4^+ and NO_3^- ions) that can be uptaken by plants to accomplish their nitrogen needs. From production of urea to nitrification of ammonia, a series of enzymatic reactions are involved where different soil microbes take their parts.

Certainly, results of this research have shown effect tea waste on soil nutrient profile. Naturally, tea is found to be acidic (Das et al., 2020) due to presence of various organic acids and phenolics, which can be a reason behind the low pH of TWS. Moreover composting, especially food waste composting obviously lowers the pH of soil, which indicates an increased soil fertility (Sundberg et al., 2013). According to Ritchie and Dolling (Ritchie and Dolling, 1985) soil acidification is a sign of degradation of organic matter. Previously, Porter (1980) described how added organic matter lowers soil pH. According to the study, organic matter releases hydrogen ions that were associated with organic anions and helps to improve the process of nitrification (Porter, 1980). So, results of OC and OM were quite satisfying as those were observed boosted (Table 1) after composting of tea waste in soil and the negative relation with the trend of pH was also there. Soil EC, much like pH, is also a good overall indicator of soil fertility which does not directly affect plant growth but indicates the amount of nutrients available for plant uptake (Anonymous, 2022b).

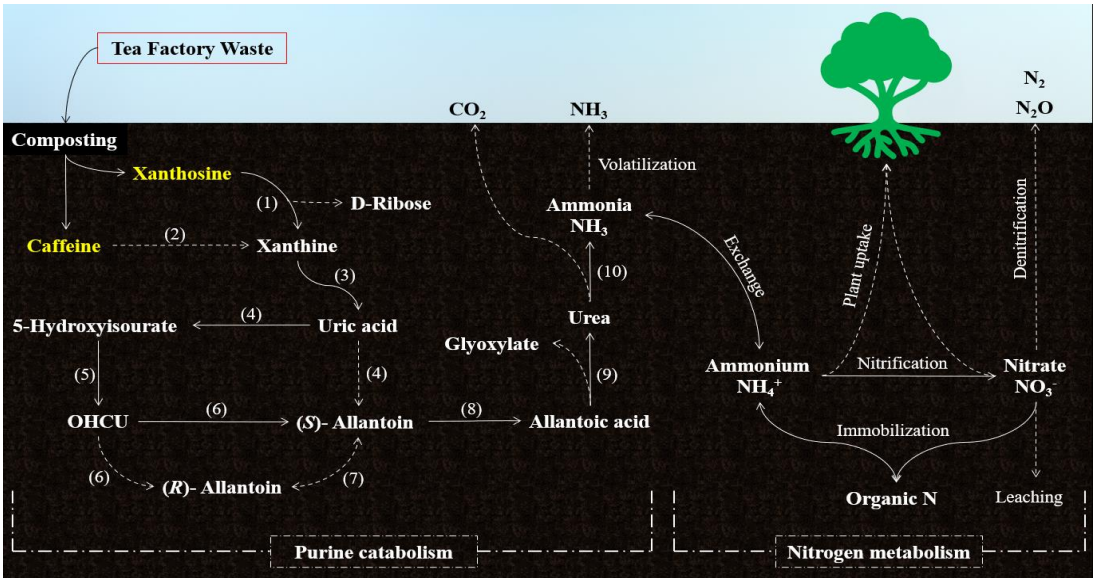

Figure 3. Possible metabolic pathways of xanthosine and caffeine catabolism and nitrogen metabolism in tea waste treated soil (TWS). Numbers indicate the reaction steps catalysed by the enzymes mentioned below:

(1) Purine (xanthosine) nucleosidase (EC 3.2.2.1); (2) xanthine oxidase; (3) xanthine dehydrogenase (EC 1.1.1.204); (4) uricase or urate oxidase (EC 1.7.3.3); (5) HIU (5-hydroxyisourate) hydrolase (EC 3.5.2.17); (6) OHCU (2-oxo-4-hydroxy-4-carboxy-5-ureidoimidazoline) decarboxylase (EC 4.1.1.97); (7) allantoin racemase (EC 5.1.99.3); (8) allantoinase (EC 3.5.2.5); (9) allantoicase (EC 3.5.3.4); (10) urease (EC 3.5.1.5).

It is reported that non-saline soils having a higher EC value have more available nutrients than those that have a lower EC value (Anonymous, 2022c) and here, EC of TWS (196±6) was detected to be higher than the EC of CS (78±14). Beside acidification, organic matter can improve the water-holding capacity of soil (Hudson, 1994) that was also clearly reflected in the results. Interestingly, promising amount of OC, N, P and K have been reported in left over green tea waste (Iqbal et al., 2007) and left-over black tea composted soil (Gurav and Sinalkar, 2013). In this research, tea factory waste composted soil also exhibited remarkable results.

Multiple growth parameters and plant development attributes were considered in this research to evaluate tea waste's effect on plant growth and development. Post-harvest characterization also helped to evaluate the effect of tea waste on plant's growth and development. On the other hand, parameters linked to nodule characterization indicated the soil fertility status indirectly by conveying the nitrogen demand and availability (Xia et al., 2017). Certainly, results were higher for TWS plants compared to CS plants, with only moisture percentage as exception.

Root nodules are found primarily in legumes because of symbiosis with nitrogen-fixing bacteria. Under nitrogen-limiting conditions, plants create a symbiotic relationship with a nitrogen fixing bacteria called rhizobia through nodulation (Bordeleau and Prévost, 1994; Xia et al., 2017). Interestingly, the nodulation process in a plant is controlled by nitrogen availability in soil and nitrogen demand of that plant. A limiting nitrogen status in soil increases the rate of nodulation (Xia et al., 2017). Low pH soil also inhibits nodulation process (Ferguson et al., 2013) and it has been reflected in results of research as plants grown in lower pH of TWS had less amount and weight of nodules compared to plants of higher pH- i.e., CS plants. Likewise, in this research, available nitrogen in CS samples was lower than TWS (Table 1) where significant nodulations have occurred. The objective of GC-MS analysis was to find out any nitrogen rich fertilizing agent or priming agent or any other growth-promoting factor among the tea waste metabolome, which may be responsible for TWS's positive outcome. Result of this analysis have demonstrated presence of nitrogen rich xanthosine and caffeine, which were introduced as abundant components of tea waste (Table 4). Previously, Brychkova et al. (2008) applied xanthine and some other purines exogenously as a nitrogen source and reported an improved rate of seed germination and plant growth while, here, the tea waste extract itself is rich in such components. Yi et al. (2021) reported the effect of xanthine on both seedling growth and early senescence of cotyledons and that too under a nitrogen deficient condition. According to their study, seedlings treated with xanthine as the only nitrogen source grew faster and more cotyledon chlorophyll was broken down, compared to seedlings without xanthine. Moreover, xanthine oxidase catalyzes the oxidation of xanthine to produce ureides, urea and other metabolically active intermediates which are effective soil fertilizing components (Brychkova et al., 2008; Kostić et al., 2015). Therefore, exposures of materials in soil that source xanthine may help plants to grow healthy (Nakagawa et al., 2007). Caffeine, the other key component of the tea waste extract, can also degrade into xanthine and further urea (Mazzafera, 2002) just like xanthosine. Caffeine has already been recognized as a potential priming agent for seed germination (Ransom, 1912) and plant growth promoter as well, but there are mixed opinions (Montes, 2014).

Breakdown of tea waste metabolites as shown in Figure 3 was based on two reference pathways i.e., purine catabolism pathway (Anonymous 2022d) and nitrogen metabolism pathway (Powlson, 1993; Ashihara, 2012). There are some pioneer works which established microbial

metabolic pathways involved in purine catabolism. Triplett et al. (1980) reported synthesis of allantoic acid in root nodule cytosol of legume plants via enzyme xanthine dehydrogenase. Activity of uricase in root nodules of cowpea (plant used in this pot experiment) was demonstrated by cytochemical methods, which confirmed the ureide biogenesis in cowpea (Webb and Newcomb, 1987). High allantoinase activity was also reported inside the nodules of this plant (Webb and Newcomb, 1987). Sun et al. (2021) have stated that exogenous application of xanthine promotes not only the plant growth but also the root elongation which has been observed in this research as well. Sun et al. (2021) and Cunliffe et al. (2016) have reported microbial purine catabolism pathway in the environment where production of urea and ammonia by utilizing xanthine has clearly been mentioned.

5. Conclusion

Disposal of huge quantity of solid tea wastes produced during tea manufacturing is considered as a problem without looking into its potential application in enhancing soil fertility. This research portrays a notable nutrient richness in tea waste treated compost (TWS) over control soil (CS). Organic carbon, nitrogen and other nutrient content were remarkably highly in TWS which was also reflected in the results of pot assessment. Detection of major peaks of purine metabolites like xanthosine and caffeine in tea waste extract has validated results of soil analysis and pot experiment where TWS revealed remarkable fertilizing properties, seed germination property, plant growth and beans production. These two metabolites were reported as plant growth promoting factors whose possible breakdown pathways have been graphically explained in this research. Conclusively, soil physicochemical analysis, pot experiments and GC-MS based metabolomics together established the fertilizer potentiality of CTC tea waste. An integrated approach to process design is therefore recommended for the utilization of CTC tea factory waste, which will help tea industry to shift towards a carbon neutral, sustainable and zero waste industry.

Compliance with Ethical Standards

Conflict of Interest

As the author of article declare that there are no conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' Contributions

Sahadeb Sarkar: Conceptualization, Participated in material collection, soil analysis and pot experiment, Review and editing. **Soumya Majumder:** Methodology, Conceptualization, Formal analysis, Metabolomics, Writing - original draft, **Arindam Ghosh:** Formal analysis, Review and editing. **Sumedha Saha:** Participated in material collection, Formal analysis **Sukanya Acharyya:** Review and editing. **Sourav Chakraborty:** Data curation and statistical analysis. **Malay Bhattacharya:** Participated in supervision of the work starting from the proposal up to final draft, Conceptualization, Validation, Review and editing.

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Data availability

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Consent for publication

Not applicable.

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The short term effect of tillage on soil physicochemical properties in Bayelsa State, Nigeria

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ABSTRACT

The objective of the research was to determine the effect of different tillage methods on some soil physicochemical properties in Niger Delta University Teaching and Research Farms. The land was divided into plots measuring 5 x 5 m with three replicates per treatment. Five tillage treatments considered were: No Till, hoeing, digging, hoeing + digging once, and hoeing + digging twice. Two samples were collected per plot at 0-15 cm and 15-30 cm depth. A total number of thirty (30) samples were collected from the field. The research was laid out using the Randomized Complete Block Design. Results showed that the tillage methods had no significant effect on the soil's chemical properties but influenced the physical. Mean pH across all plots ranged from 4.42 – 4.49 indicating strongly acidic state, electrical conductivity ranged 65.67-82 dS m^{-1} indicating no salinity stress, detrimental to crops; organic carbon and organic matter were moderate with a range of 18.52-21.55 g kg^{-1} and 37.03-43.10 g kg^{-1} respectively. Total nitrogen was at its moderate range of 7.88-12.17 g kg^{-1}. Exchangeable acidity was low with a range of 1.52-1.71 cmol kg^{-1}. The tillage methods influenced soil bulk density and porosity; the highest bulk density was recorded in the NT zone (1.18 g cm^{-3}) which decreased steadily with an increase in tillage intensity. With the hoe+digging (twice) recording the lowest value of 0.94 g cm^{-3}. Similarly, the highest porosity value was found in the tillage method with the lowest bulk density value (0.94 g cm^{-3}: 64.7%), while the lowest was observed in the tillage method with the highest bulk density value (1.18 g cm^{-3}: 55.7%). pH had positive correlation ($P<0.05$) with electrical conductivity (r=0.62), organic carbon (r=0.94), organic matter (r=0.90), and negative correlation with exchangeable acidity (r=-0.52). Organic carbon had strong positive correlation with organic matter (r=0.99), effective cation exchange capacity (r=0.69), clay (r=0.50), and total nitrogen (r=0.61). Silt (r=0.74) and clay (r=0.70) showed positive relationship with bulk density, while bulk density (r= -0.99) showed strong negative correlation with porosity. It is therefore recommended that crude tillage methods be used for sustainable and conservative agriculture.

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1. Introduction

There are several definitions of tillage. According to Lal et al. (2007), it is defined as physical, chemical or biological soil manipulation to optimize conditions for germination, seedling establishment and crop growth. Tillage is the mechanical manipulations of soil to make it favorable for plant growth eliminating weeds during the growth of the plant (Sahay, 2008). Tillage affects the physical, chemical, and biological properties of soils as observed from research results on soils in several parts of Africa where it was seen to affect soil aggregate, temperature, water infiltration, and retention (Ofori, 2009).

Tillage systems are often divided into two types: conservation tillage and conventional tillage (Mohammed and Umogbai, 2014). Reduced soil compaction, economically viable crop rotations, and the creation of surface residue cover are three key components of conservation agriculture (Kienzler et al., 2012), which ensure long-term crop productivity while reducing environmental concerns (Valbuena et al., 2012). Conservation tillage is now used on an estimated 125 million hectares, or about 9% of the world's arable land (Kassam et al., 2012). Over several decades, the conservation agricultural system was reviewed and applied in several

climatic regions around the world, including the tropical, subtropical, and temperate zones. Conservation tillage leaves at least 30% of crop residue on the soil surface, which equates to at least 1.100 kg ha^{-1} of small grain residue (Mohammed and Umogbai, 2014).

Soil tillage is among the important factors affecting soil's physical, chemical properties and crop yield. Among the crop production factors, tillage contributes up to 20% (Khurshid et al., 2006). Tillage method affects the sustainable use of soil resources through its influence on soil properties (Alvarez and Steinbach, 2009). Jabro et al. (2016) reported that tillage depth and intensity alter the soil's physical and chemical properties that affect plant growth and crop yields.

The proper use of tillage can improve soil-related constraints, while improper tillage may cause a range of undesirable processes, e.g. destruction of soil structure, accelerated erosion, depletion of organic matter and fertility, and disruption in cycles of water, organic carbon, and plant nutrient. The use of excessive and unnecessary tillage operations is often harmful to the soil. It has been observed that more tillage is being done than necessary leading to soil loss by wind and water erosion. In some instances, too many passes of tractor machine aggregate lead to the formation of soil pan thereby reducing air and water infiltration and circulation. Therefore, currently, there is a significant interest and emphasis on the shift to conservation and NT age methods to control the erosion process (Costa et al., 2015). This study aims to assess the impact of Tillage on Soil Physico-Chemical Properties in Niger Delta University Teaching and Research Farm, Bayelsa State, Nigeria, and to determine the correlation coefficient between some physical and chemical properties of the soil under the different tillage methods. The tillage implements and methods were considered because they have been used by subsistent farmers in Bayelsa State for decades without scientifically researching their impact to soils.

2. Materials and methods

2.1. Study area

The study was carried out in the Niger Delta University Teaching and Research Farm, located in Amassoma Community, Southern Ijaw Local Government Area in Bayelsa State. It lies within 4058'47.85" N and 606'19.51" and is situated in the southern part of the Niger Delta of Nigeria. In Amassoma, the wet season is warm and overcast, the dry season is hot and mostly cloudy, and it is year-round. For the year, the temperature typically varies from 21°C to 30°C and is rarely below 17°C or above 32°C. Average annual rainfall of the study region is 2,250 mm. The topography doesn't change. Shrubs found in the area include elephant grass (*Pennisetum purpureum* L.), *Jatropha tanjorensis* Ellis &Saroja, Costus afer Ker Gawl, Goat weed (*Ageratum conyzoides* L.). Other trees found in the area include plantain (*Musa paradisiaca* L.), oil palm (*Elaeis guineensis* Jacq.) etc. Sandy loam and sandy clay loam were the predominant surface and subsurface textures in the NT zone. In the hoed area, the digging zone, and the H+D (1) zone, it ranged from loamy sand to sandy loam, and in the H+D (2) zone, it ranged from sandy loam to loamy sand.

2.2. Field layout

The research field was carried out was divided into five treatment plots of 5 x 5 m and three replicates with a walkway of 1m. The five conservational tillage methods (No till (NT), hoeing, digging, hoeing + digging once (H+D 1) and hoeing + digging twice –H+D 2) were carried out in each plot with the aid of a hoe (20 x 20 cm blade) and digger (30 cm length), to the depths of 0-15 cm and 15-30 cm. The area for the experiment was a land kept aside for student research and had not been tilled before hand. The tillage practices were completed within an interval of one week. The soil texture of the area is mainly coarse textured, inherently from the parent material found in the region.

Plate 1. Tillage implements utilized for the research

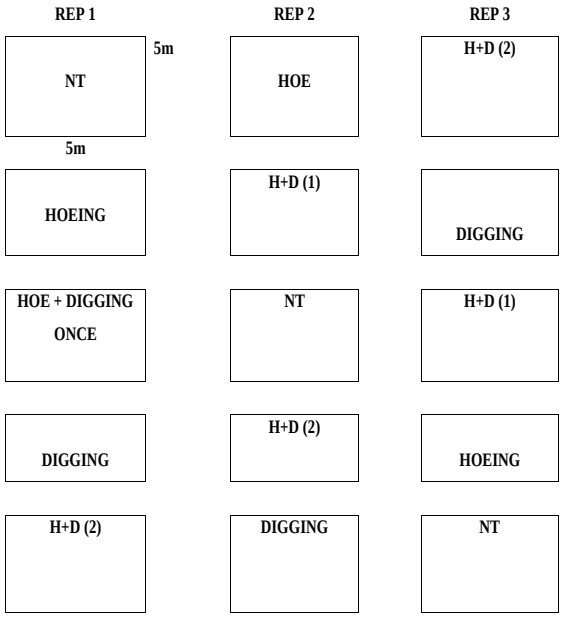


Figure 1. Experimental layout of the study site

2.3. Sample collection

Soil samples were taken from the 0-15cm and 15-30cm depth with the use of a soil auger. Two samples were collected per plot with a total of thirty (30) samples from the field. Each sample was put into a clean polythene bag and then properly labeled with an indelible marker. The samples were transferred to the Soil Science Laboratory where they were air dried, crushed, and passed through a 2mm sieve. The air-dried samples were analyzed to determine pH, electrical conductivity, organic carbon, organic matter, total nitrogen, exchangeable acidity, Na, K, Ca, Mg, available P, cation

exchange capacity, effective cation exchange capacity, and base saturation.

Soil cores were used to take samples for bulk density and porosity. The core was carefully hammered into different depths using a block of hardwood, after the different tillage exercises. Excess soil was then cut off using a knife to create equilibrium between the soil column and the core.

2.4. Laboratory analysis

Soil pH and electrical conductivity were determined in a soil-water medium at a ratio of 1:1 using Coleman's pH and EC meter. Particle size analysis was carried out using the hydrometer method according to the method of Bouyoucous (1962). Soil organic carbon (SOC) was determined by the Walkley and Black (1934) procedure according to Nelson and Sommers (1996); Soil organic matter was estimated as organic carbon multiplied by 1.724. Exchangeable cations, Ca, Mg, K and Na were extracted with 1N ammonium acetate solution (1N NH4OAc) buffered at pH 7.0 the Ca and Mg were determined from the extract using 0.01m EDTA (ethylenediaminetra-acetic acid) titration method as described by Black (1965), while K and Na were determined using flame photometer (Jackson, 1962). Total nitrogen was determined using the regular micro Kjeldahl method as reported by Bremmer and Mulvaney (1982). Available P was determined by Bray's P1 method (Bray and Kurtz, 1945) and read on the atomic absorption spectrophotometer, while exchangeable cations (K, Ca, Na and Mg) were first extracted using the method of Jackson (1962), thereafter, K, Na and Ca were determined by the flame photometer while Mg was read from the Atomic Absorption Spectrophotometer (AAS). The titration method was used to determine exchangeable acidity as described by McLean (1982). Soil bulk density in two layers was determined using the core method. The soil samples were randomly taken per plot using a stainless teel core sampler. The collected soil cores were trimmed to the exact volume of the cylinder and oven dried at 105 °C for 24 h. Precautions were taken to avoid compaction inside the core sampler. The bulk density was determined from the ratio of the mass of dry soil per unit volume of soil cores (Aikins and Afuakwa, 2012).

$$\text{Bulk density} = \frac{mass\ of\ oven\ dried\ soil\ (g)}{total\ volume\ of\ soil\ (cm^3)}$$

The total porosity was calculated from the values of the bulk density and an assumed particle density of 2.65 g cm^{-3} using the following Equation (Aikins and Afuakwa, 2012).

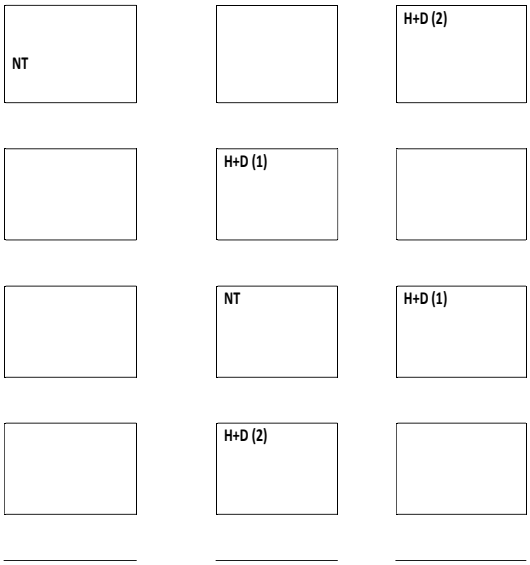
$$TP = 1 - \left(\frac{Bulk\ density}{particle\ density}\right) \times 100$$

2.5. Experimental design and statistical analysis

Randomized Complete Block Design (RCBD) was used to arrange the experiment. Analysis of variance (ANOVA) was conducted on the collected data to check the differences between the treatments. Duncan Multiple Range Test on a grand mean data at the 5% level of probability ($p<0.05$) based on the F-test of the analysis of variance was used to determine the difference in the means, while correlation analysis was carried out to check the relationship between some physical and chemical properties.

3. Results and discussion

Table 1 contains the values of some physical and chemical properties of the soils and their effects under the considered crude tillage practices.



3.1. pH

Both surface and subsurface soils under the no till (NT) zone had very acidic pH values (4.33). The surface and underlying soils remained very acidic after hoeing (4.60 and 4.37). Digging (D) had no discernible impact ($P<0.05$) on soils with pH values of 4.30 and 4.40, either on the surface or below. The pH remained significantly acidic (4.37 and 4.47) at both levels despite [H+D (1)] having no significant impact ($P<0.05$). Additionally, (H+D (2) had no discernible impact on the soils' pH status in the surface and subsurface zones, which were 4.20 and 4.63 respectively.

According to Table 1, there was no discernible difference in the pH of the soil when the amount of tillage was increased (NT, hoe, digging, H+D (1), and H+D (2). The University's Teaching and Research Farm's soils are regularly cultivated by students for academic, practical, and research purposes, which "may" be the cause of the low pH. The heavily acidic condition of the soils can be linked to high cropping intensity, which caused the crops to absorb most of the basic cations, and excessive rainfall, which causes the basic cations in the soil to leach (Nta et al., 2017).

3.2. Electrical conductivity (EC)

The EC was 0.094 dS m^{-1} at the surface soil and 0.062 dS m^{-1} at the subsurface soil following hoe use. After digging, the EC at the surface and subsurface soils was 0.065 dS m^{-1}. The surface soil at the H + D (1) practice had an EC of 0.083 dS/m, while the subsurface soil had an EC of 0.081 dS m^{-1}. After twice hoeing and digging, the soil's subsurface EC was 0.078 dS m^{-1} and the surface EC was 0.070 dS m^{-1}. The average values for the five tillage methods revealed that the H + D (1) site had the greatest EC value of 0.082 dS m^{-1} and the H + D (2) zone had the lowest (0.074 dS m^{-1}). All five tillage methods had electrical conductivities below 4, which indicated that neither soil structure aggregation nor saline

restriction to root and seed sprouting existed (Ganjegunte et al., 2018).

3.3. Organic carbon and organic matter

Organic carbon content of the surface and subsurface soils in the NT zone ranged from 16.43 to 21.53 g kg^{-1}, with a mean of 18.98 g kg^{-1}. The organic matter concentration in the surface and subsurface soils of the NT Zone ranged from 32.87 to 43.07 g kg^{-1}, with a mean of 37.97 g kg^{-1}. Surface and subsurface values during hoeing were 49.73 and 36.47 g kg^{-1} respectively, with a mean of 43.10 g kg^{-1}. H+D (2). The outcome demonstrates that none of the tillage methods had a significant impact ($P<0.05$) on organic carbon and organic matter (Table 1). Under the tillage methods, the organic matter was of an amount. Despite frequent use, the moderate level of organic carbon and organic matter could be attributable to students' gradual application of organic manure for crop production. Heavy tillage machinery can excessively widen soil pores (Szostek et al., 2022), facilitating the illuviation of organic materials and clay to the subsoils under heavy rains. Therefore, the light mechanical structure of the rudimentary implement as compared to heavy-duty machinery was the cause of the steady, immutable nature of the organic carbon and matter (Nta et al., 2017).

3.4. Total nitrogen

The average total nitrogen for NT, digging, hoeing, H+D (1) and H+D (2) was 8.87, 12.17, 11.63, 7.88, and 10.87 g kg^{-1} respectively (Table 1). The average mean separation demonstrates that the tillage methods had no appreciable impact on total nitrogen. Since the total nitrogen and organic carbon are correlated, the moderate state of the total nitrogen under the various tillage methods results from this (Brady and Weil, 2005).

3.5. Exchangeable acidity

With 1.65 and 1.76 cmol kg^{-1} at the surface and subsoil of the NT, exchangeable acidity was low. When hoeing, the values were 1.50 (surface) and 1.60 (subsurface), 1.50 and 1.70 (after digging), 1.59 and 1.63 (hoeing and digging), and 1.41 and 1.63 (hoeing and digging twice). There was no significant difference between the two depths for any of the five tillage methods at $P<0.05$.

In the cases of NT, Hoeing, Digging, H+D (1), and H+D (2), the mean exchangeable acidity was 1.71, 1.55, 1.60, 1.61, and 1.52 cmol kg^{-1}, respectively. The results showed that there were no significant differences between results, indicating that the use of crude tillage implements at higher intensities had no impact on the soil's exchangeable acidity (Table 1). The higher organic matter preceded the low exchangeable acidity.

There were no appreciable differences in Na levels across the five-tillage method. The methods supported it further by obtaining results of 0.21 cmol kg^{-1} in the NT area, 0.19 cmol kg^{-1} while hoeing, 0.13 cmol kg^{-1} when digging, 0.28 cmol kg^{-1} when H+D (1), and 0.20 cmol kg^{-1} when H+D(2). With averages of 0.51, 0.33, 0.24, 0.62, and 0.54 cmol kg^{-1} under NT, hoeing, digging, H+D (1), and H+D (2), respectively, a similar trend was seen in the K values.

However, the results demonstrate that the mean calcium (Ca) levels at the NT, H+D (1), and H+D (2), as well as hoe (0.80 cmol kg^{-1}) and digging (0.55 cmol kg^{-1}), were identical.

The decline in Ca upon tillage could be attributed to disintegration of soil particles and leaching of bases by rainfall. This would inevitably cause a reduction in Ca availability. Also, Mg indicated significant difference ($P<0.05$) with H+D (1) - 1.51 cmol kg^{-1} and H+D (2) - 1.21 cmol kg^{-1} at the NT, Hoeing (0.50 cmol kg^{-1}), and digging locations. This finding suggests that increasing tillage techniques may decrease the availability of magnesium (Agbede and Ojeniyi, 2009).

3.6. Bulk density

The average bulk density was 1.17 g cm^{-3}, 1.07 g cm^{-3} in the hoeing zone, 1.09-1.12 g cm^{-3} in the digging zone, 0.92-1.0 g cm^{-3} in the H+D (1) zone and 0.90 – 0.98 g cm^{-3} in the H+D (2) Zone.

The mean values showed that the crude tillage methods had a significant effect on the bulk density. There was a steady decline in bulk density values with increasing intensity of the tillage practices. NT registered the highest mean bulk density of 1.18 g cm^{-3}, hoe (1.07 g cm^{-3}), digging (1.11 g cm^{-3}), H+D (1) (0.96 g cm^{-3}), while the lowest was found in the H+D (2) zone (0.94 g cm^{-3}). There was no significant difference between the bulk density of hoeing and digging, and between H+D (1) and H+D (2). Additionally, the results showed that hoeing and digging had no impact ($P<0.05$) on bulk density, as was also observed between H+D (1) and H+D (2). The bulk densities of the "methods" of all the different tillage techniques fell below the critical criterion of 1.80 g cm^{-3} (Weil et al., 2016). The result in Table 1 shows that increased tillage methods can affect soil bulk density, i.e., that bulk density decreases as tillage methods are increased (Agbai and Kosuowei, 2022).

3.7. Soil porosity

Porosity was inversely related to the measured bulk densities. The following porosity values were detected as a result of decreasing bulk density values: NT (55.7%), hoe (59%) and digging (58.4%), H+D (1) (63.9), and H+D (2) (64.7). The increased tillage methods damaged the soil's structure, increasing the amount of pore space. The maximum porosity values were recorded by H+D (1) and H+D (2), while the lowest values were found in the NT zone. Elder and Lal (2008) found that tilled plots had higher overall porosity than no-tillage plots for organic soils.

3.8. Correlation matrix

Table 2 shows the correlation between some physical and chemical properties. Soil pH correlated positively with EC, OC, OM and negatively with EA (r=0.62), organic carbon (r=904), organic matter (r=0.905), and negative correlation with exchangeable acidity at r = -0.522. An increase in organic matter and carbon caused an increase in pH and electrical conductivity of the soils while an increase or decrease in pH caused a decline or increment in effective cation exchange capacity.

Organic carbon showed strong positive correlation with organic matter at r=0.99, Effective Cation Exchange Capacity (r = 0.697), clay (r = 0.502), total nitrogen at r = 0.607, and negative relationship with exchangeable acidity at r=-0.819. Organic matter showed a similar positive correlation with organic matter, effective cation exchange capacity, clay, and total nitrogen; with exchangeable acidity exhibiting the negative correlation. This result indicates that

as organic matter increases so will total nitrogen (Brady and Weil, 2005). Total nitrogen was negatively correlated with exchangeable acidity at r = -0.601. Effective cation exchange capacity showed negative correlation with sand and silt at r = -0.678 and -0.509 and positive correlation with clay, bulk density and porosity (r = 0.888, 0.508 & 0.698). A decrease in effective cation exchange will cause a decrease in the availability of total nitrogen in the soil. As clay increases, there is an inverse movement in the relative proportion and sand; which further significantly increases effective cation exchange capacity, bulk density, and porosity.

Silt and clay showed positive correlation with clay and bulk density with a correlation index at r= 0.737, 0.705, while bulk density negatively correlated with porosity with r = -0.995. The result showed that bulk density and porosity are inversely proportional to one another; therefore, an increase in bulk density will amount to a decrease in porosity.

4. Conclusion

The research showed that the tillage methods had no significant effect on soil chemical properties such as pH, electrical conductivity, organic carbon, organic matter, total nitrogen, and exchangeable acidity. It further showed that intrinsic soil properties like texture cannot be easily changed by mechanical implements over a short time. However, physical parameters such as bulk density and porosity were significantly impacted by the intensification of the tillage methods. It was therefore observed that tillage implements can significantly impact soil physical characteristics but have no significant effect on their chemical characteristics.

From the results discussed, it is therefore recommended that crude tillage implements such as hoe and diggers can be used in the practice of conservative and sustainable agriculture in Niger Delta University Teaching and Research Farm.

Compliance with Ethical Standards

The authors declare that there is no conflict of interest.

Authors' Contributions

Agbai Williams Perekekeme: Validation, Investigation, Writing - original draft, Methodology, Conceptualization. **Joseph Oyinbrakemi Tate**: Review and editing. Validation, Formal analysis, Data curation

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

We humbly give consent for this article to be published

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Table 1. Physical and chemical properties of the soils under different crude tillage measures

TRTS	depth	pH	EC	Org.C	Org.M	TN	EA	Na	K	Ca	Mg	Av.P	ECEC	Sand	Silt	Clay	Texture	BD	POR
			dS m^{-1}		g kg^{-1}					cmol kg^{-1}					g kg^{-1}			g cm^{-3}	%
NT	0-15cm	4.33a	75.67a	16.43a	32.87a	6.30a	1.65a	0.19a	0.48a	1.20a	0.93a	1.97b	4.47a	615	144.4	240.6	Sandy loam	1.17a	56.0a
	15-30cm																Sandy Clay		
		4.43a	77.00b	21.53b	43.07b	11.43b	1.76a	0.23a	0.54a	1.25a	0.96a	1.24a	4.73a	655	124.4	220.6	loam	1.18a	55.3a
Mean		**4.38A**	**76.34C**	**18.98A**	**37.97A**	**8.87B**	**1.71A**	**0.21A**	**0.51A**	**1.23B**	**0.95A**	**1.61B**	**4.60C**	**635**	**134.4**	**230.6**	**Sandy loam**	**1.18C**	**55.7A**
Hoe	0-15cm	4.60a	93.67b	24.87b	49.73b	14.63b	1.50a	0.25b	0.42a	1.03b	0.66a	0.39a	3.87b	855	64.4	80.6	Loamy sand	1.06a	59.9a
	15-30cm	4.37a	61.67a	18.23a	36.47a	9.70a	1.60a	0.12a	0.24b	0.56a	0.34a	0.23a	2.87a	695	114.4	190.6	Sandy loam	1.07a	59.5a
Mean		**4.49A**	**77.67C**	**21.55B**	**43.10B**	**12.17E**	**1.55A**	**0.19A**	**0.33A**	**0.80A**	**0.50A**	**0.31A**	**3.37B**	**775**	**89.4**	**135.6**	**Loamy sand**	**1.07B**	**59.0B**
Digging	0-15cm	4.30a	65.67a	17.57a	35.13a	10.93a	1.50a	0.12a	0.24a	0.56a	0.45a	0.28a	2.87a	735	114.4	120.6	Loamy sand	1.09a	59.0a
	15-30cm	4.40a	65.67a	19.47b	38.93b	12.33b	1.70a	0.13a	0.24a	0.53a	0.46a	0.32a	3.07b	745	84.4	170.6	Loamy sand	1.12a	57.7a
Mean		**4.35A**	**65.67A**	**18.52A**	**37.03A**	**11.63A**	**1.60A**	**0.13A**	**0.24A**	**0.55A**	**0.46A**	**0.30A**	**2.97A**	**740**	**99.4**	**145.6**	**Loamy sand**	**1.11B**	**58.4B**
H+D (1)	0-15cm																		
		4.37a	82.67b	18.03a	36.07a	6.13a	1.59a	0.29a	0.63a	1.52a	1.14a	2.52a	5.20a	855	64.4	80.6	Loamy sand	0.92a	65.4a
	15-30cm	4.47a	81.33a	23.40b	46.80b	9.63b	1.63a	0.27a	0.61a	1.50a	1.18a	2.32a	5.20a	735	94.4	170.6	Loamy sand	1.00a	62.4a
Mean		**4.42A**	**82D**	**20.72B**	**41.44B**	**7.88A**	**1.61A**	**0.28A**	**0.62A**	**1.51B**	**1.16B**	**2.42C**	**5.20D**	**795**	**79.4**	**125.6**	**Loamy sand**	**0.96A**	**63.9C**
H+D (2)	0-15cm																		
		4.20a	70.00a	20.53b	41.07b	11.73b	1.41a	0.21a	0.47a	1.19a	1.14a	1.10a	4.40a	695	124.4	180.6	Sandy loam	0.90a	66.2a
	15-30cm	4.63a	78.00b	17.83a	35.67a	10.00a	1.63a	0.19a	0.60b	1.37a	1.28a	1.46b	5.13b	735	144.4.	120.6	Loamy sand	0.98a	63.1a
Mean		**4.42A**	**74B**	**19.18A**	**38.37A**	**10.87C**	**1.52A**	**0.2A**	**0.54A**	**1.28B**	**1.21B**	**1.28B**	**4.77C**	**715**	**134.4**	**150.6**	**Loamy sand**	**0.94A**	**64.7C**

Letters a, b and c depict similarities or differences at the different depths while A, B, and C represent similarities or differences of the depth means. Value(s) with the same letters(s) in the column are not significantly different from one another at a 5% level of probability in each tillage methods using Duncan Multiple Range Test. EC – Electrical Conductivity, Org. C – Organic carbon, Org.M – Organic Matter, TN – Total Nitrogen, EA – Exchangeable acidity, Na – Sodium, Ca – Calcium, Mg – Magnessium, Av. P – Available Phosphorus, ECEC – Effective Cation Exchange Capacity, BD – Bulk Density, POR - Porosity

Table 2. Correlation Matrix of selected physical and chemical properties

	pH	EC	Org.C	Org.M	TN	EA	ECEC	Sand	Silt	Clay	BD	POR
pH	1											
EC	0.623*	1										
Org. C	0.904	0.728	1									
Org.M	0.905	0.729	0.999**	1								
TN	0.244	-0.204	0.617	0.616	1							
EA	-0.522	0.094	-0.819	-0.885	-0.601	1						
ECEC	0.268	0.478	0.697	0.648	0.397	0.255	1					
Sand	0.482	0.2442	0.364	0.362	0.147	-0.598	-0.678	1				
Silt	-0.329	-0.259	0.252	-0.349	0.001	0.192	-0.509	-0.887	1			
Clay	-0.410	-0.065	0.502	-0.699	-0.286	0.769	0.888**	-0.957	0.737	1		
BD	-0.371	-0.349	-0.313	-0.393	0.116	0.721	0.508	-0.596	0.214	0.705	1	
POR	0.282	0.3117	0.229	0.289	-0.163	-0.679	0.698	0.551	-0.171	0.669	-0.995	1

EC – electrical conductivity, Org.C – organic carbon, Org.M – organic matter, TN – total nitrogen, EA –exchangeable acidity, ECEC – effective cation exchange capacity, BD – bulk density, POR - porosity

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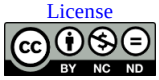

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Damage ratio of the Asian chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu, 1951 (Hemiptera: Cynipidae) in Samsun Province of Türkiye: First Report

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ABSTRACT

The Asian chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu (*Hymenoptera, Cynipidae*) is a critical pest that threatens chestnut cultivation. The chestnut gall wasp causes yield losses by deforming the buds of the infected trees. This pest, which was first detected in Italy in 2002 in Europe, was seen in Gacık village of Yalova province in 2014 in Türkiye. In the following years, it spread to Bursa, Istanbul, Sakarya, Kocaeli, Balıkesir, Bilecik, Düzce, Giresun, Bartın, Zonguldak, Sinop and İzmir provinces. In this study, the orchard of chestnut gene resources belonging to the Ali Nihat Gökyiğit research station in Samsun province was examined for the presence of the Asian chestnut gall wasp. As a result of the study, the presence of the Asian chestnut gall wasp was determined in the cultivars Marigoule' and 'Macit 55'. As a result of the counts made in 50 cm parts of the branches, it was determined that the presence of chestnut gall wasp is in a higher population in the 'Marigoule' cultivar than in the other cultivars. However, the invasion in Samsun is at the early stage.

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1. Introduction

Chestnut (*Castanea sativa* Mill.) is an important nut that grows worldwide (Soylu, 2004). It affects human life in the past as it is today (Conedera et al., 2004). In historical periods, chestnut culture in various parts of Europe has been so indispensable for the survival of mountain communities that these cultures have been described as 'chestnut civilizations' (Gabrielli, 1994). People can benefit from its nuts, timber, and honey (Soylu, 2004). However, there are some pests and diseases that threaten chestnut cultivation. One of the significant and most popular pests is *Dryocosmus kuriphilus Yasumatsu* (Hymenoptera: Cynipidae). It is commonly known as the Asian chestnut gall wasp (ACGW).

The first damage of the ACGW was recorded in China in 1941 (Zhang et al., 2009). It was first noted in Europe in Italy in 2002 (Brussino et al., 2002). After 12 years, in 2014 the ACGW damage was observed in Yalova province of Türkiye for the first time (Çetin et al., 2014). As of 2022, gall wasp has been noted in the provinces of Yalova, Bursa, İstanbul, Sakarya, Kocaeli, Balıkesir, Bilecik, Düzce, Giresun, Bartın, Zonguldak, Sinop and İzmir in Türkiye (Çetin et al., 2014; Mıcık and İpekdal, 2021; Mıcık et al., 2021; Yıldız et al., 2020). Also, other province are in the risk to face with this pest.

In this study, chestnut genetic research orchard of the Ali Nihat Gökyiğit research station in Samsun was observed for the ACGW.

2. Materials and methods

The field study was conducted in 2022 in the Samsun province of Türkiye. The chestnut orchard is in the Ali Nihat Gökyiğit research station (coordinates: 41° 23' 53'' N; 36° 03' 34'' E). In the orchard, 'Akyüz', 'Ali Nihat', 'Bouche de Betizac', 'Macit 55', 'Marigoule' cultivars and BL genotype were examined for the ACGW. For each cultivar and genotype, 15 plants were observed. The galls on ten branches, 50 cm were randomly counted in the trees where the gall wasp was detected.

The study was designed with three replications and each replication had five plants. Percent values were subjected to AsinH transformation. Data were analyzed statistically in RStudio (2021.09.0+351 Release for macOS) package program, and Duncan Multiple Range Test determined the significance level of the differences between the means.

3. Results and discussion

The collected galls were examined and *D. kuriphilus* was identified. The ACGW was only observed in the cultivars 'Macit 55' and 'Marigoule' cultivars (Figure 1). No galls were observed on other cultivars or genotypes (Table 1). However, Çil (2018) tested these cultivars and genotypes in the Yalova province of Türkiye and found that only the 'Akyüz' cultivar was resistant to the ACGW (Çil, 2018). Furthermore, the 'Marigoule' cultivar was the most sensitive cultivar among the tested ones, as Sartor et al. (2015) and Çil (2018) stated. Unlike, Çil (2018), no galls were observed on the 'Ali Nihat' cultivar and BL genotype (Table 1). However, in the study of Çil (2018), the actual damage was observed in the second year.

Table 1. Infected plant ratio according to the cultivars and genotype

Cultivar/Genotype	Infected plant ratio (%)
Akyüz	0.00 b*
Ali Nihat	0.00 b
BL	0.00 b
Bouche de Betizac	0.00 b
Macit 55	25.00 a
Marigoule	60.00 a
P	***

*Different letters within the same column are statistically significant (Duncan test, P<0.01).

In addition, the orchard in the the Ali Nihat Gökyiğit research station was designed mainly for adaptation studies. In the orchard, there are other cultivars and genotypes. As the invasion is in the beginning stage, the ACGW population is low. Therefore, they could be attracted to more sensitive cultivars like 'Marigoule'.

Figure 1. The appearance of the galls in the 'Marigoule' cultivar.

The number of galls was only counted in damage plants. The 'Marigoule' cultivar had the highest number of galls, 10.82. It was followed by 'Macit 55' cultivar with 1.04. In the 'Marigoule' cultivar, the number of galls is higher than in other cultivar (6 galls/twig) (Battisti et al., 2013) (Table 2). 'Macit 55' cultivars number of galls in 50 cm branch is low due to the ACGW damage is in the beginning stage. In Sinop city, a neighbor of Samsun, 17 galls per 50 cm chestnut branch was counted (Mıcık and İpekdal, 2021). Also, this data shows that the ACGW damage in Samsun is in the beginning stage.

Table 2. Number of average galls on a 50cm branch according to the cultivars and genotype

Cultivar/Genotype	number of average galls on a 50 cm branch (pieces)
Akyüz	0.00 c*
Ali Nihat	0.00 c
BL	0.00 c
Bouche de Betizac	0.00 c
Macit 55	1.04 b
Marigoule	10.82 a
P	***

*Different letters within the same column are statistically significant (Duncan test, P<0.01).

4. Conclusion

The ACGW is a harmful invasive pest, and it spreads quickly. As it is the first report in Samsun, the invasion is in the beginning stage. Even no galls were observed in sensitive cultivars or genotypes. Therefore, the development in the coming years should be followed closely and biological control should be started. Biological control with *Torymus sinensis* Kamijo (Hymenoptera Torymidae) is promising however this parasitoid infects the ACGW eggs. So it should be released after the invasion of the ACGW. Also, in early stage of the invasion, green pruning can be applied to reduce damage ratio.

Compliance with Ethical Standards

Conflict of Interest

As the author of article declare that there are no conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' Contributions

Burak Akyüz: Methodology, Investigation, Conceptualization, Writing - original draft. **İslam Saruhan:** Methodology, Review and editing. **Ümit Serdar:** Methodology, Review and editing.

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Data availability

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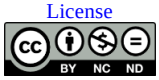

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Comparison of hardaliye produced by different starters: Back-slopping and kombucha

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ABSTRACT

During traditional hardaliye production by fermentation from grape juice, mustard seeds are insufficient to inhibit yeast activities and alcohol formation. Chemical preservatives are used for the production of hardaliye of standard quality in traditional and industrial production. Today, consumers prefer natural products that do not contain chemical preservatives and additives. For this reason, in this study, considering that different production techniques should be tried in order to prevent alcohol formation, hardaliye production was carried out with two different methods as back-slopping (BH) and addition of kombucha mushrooms (KH). These methods were tried for the first time on hardaliye. Fermentation continued for 7 days (d) and storage for 14 d. During fermentation, pH and reducing sugar, L* and a* values of samples decreased, while phenolic compounds' concentration, viscosity, and b* values increased. The pH continued to drop during storage. No significant changes were observed in reducing sugar contents. During storage, phenolic content of KH sample decreased and viscosity and L* values increased. The titratable acidity increase was greater in BH sample compared to the KH sample. While 5.5% alcohol formation was observed in the BH sample on the 7th day of fermentation, no alcohol formation was detected in the KH sample At the end of fermentation and storage, Total mesophilic aerobic bacteria (TMAB), yeast, *Lactobacillus* spp. and lactic streptococci numbers were found to be higher in BH sample than in KH sample. According to the results of the research, thanks to the metabolic activities of the kombucha mushroom microorganisms and their symbiotic association, natural fermentation takes place without any preservative chemicals in KH and more durable hardaliye production is provided compared to BH.

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1. Introduction

There are two main fermentation methods in the fermentation of foods. The first is the spontaneous fermentation that occurs spontaneously without external intervention with the microorganisms present in the natural structure of the raw material or in the processed environment, as was the case in the period when fermentation was discovered. Another fermentation method is culture dependent fermentation and can be performed by adding a known starter culture (i) to the raw material. Fermented products such as kefir, kombucha and natto can be given as an example to this method (Rezac et al., 2018; Dimidi et al., 2019). Another way of culture-dependent fermentation is the back-slopping method (ii). Fermentation is initiated by taking a small amount of pre-fermented food and adding it to the raw material. For example, fermented products such as sourdough bread, beer, various cheeses, natto and tempeh can be produced by back-slopping method (Marco et al., 2017; Dimidi et al., 2019). This method is preferred over spontaneous fermentation to reduce fermentation time and minimize the risk of unsuccessful fermentation (Harris, 1998; Leroy and De Vuyst, 2004).

Hardaliye is a traditional beverage of the Thrace Region of Türkiye, produced from grape juice. It is a non-alcoholic, acrid, characteristic drink produced as a result of lactic acid fermentation with the addition of mustard seeds and cherry leaves to dark-colored and fragrant grapes (Faikoğlu, 2012). Mustard seeds are preferred to prevent yeast activities and alcohol fermentation during fermentation, and for the same purpose 0.1% benzoic acid is used. It has also been observed that 0.1% benzoic acid and sorbic acid mixtures are used in traditional productions. In addition to giving the local drink its name, mustard seeds also contribute to the unique taste and smell of hardaliye. The leaves of the cherry trees grown in the same region are used to give aroma to hardaliye (Aşkın and Atik, 2016; Aydogdu et al., 2016; Arici et al., 2017). In a study conducted by Arici et al. (2017) to determine lactic acid bacteria in hardaliye samples, it was determined that 23 isolates (46%) were *Lactobacillus plantarum*, 20 isolates (40%) were *Lactobacillus pentosus*, 4 isolates (8%) were *Lactobacillus brevis* and the remaining 3 isolates (6%) were *Lactobacillus collinoides*.

Kombucha; It is an acidic and comforting beverage that is formed by fermenting sweetened tea with the addition of a cellulosic film layer that is formed as a result of the symbiotic association of yeast and bacteria. The cellulosic layer formed by yeast and bacteria is called SCOBY (Symbiotic Culture of Bacteria and Yeast) (Sreeramulu et al., 2000). The botanical nomenclature of kombucha was made by Lindau in 1965 as *Medusomyces gisevii* (Hesseltine, 1965). In the cellulosic film layer formed by the symbiotic

association of bacteria and yeasts of Kombucha mushroom, Gram-negative aerobic bacilli belonging to the *Acetobacteraceae* family (*Acetobacter xylinum, A. xylinoides, A. aceti, A. pasteurianus, Bacterium gluconicum* and *Gluconobacter oxydans*), yeasts (*Saccharomyces cerevisiae, S. ludwigii, Zygosaccharomyces bailii, Z. rouxii, Z. kombuchaensis* sp.*nov., Schizosaccharomyces pombe, Torulaspora delbrueckii, Brettanomyces bruxellensis, B. lambicus, B. custerii, Candida krusei, C. albicans, Kluyveromyces africanus, Pichia membranaefaciens, Kloeckera apiculata, Torulopsis* sp., *Dekkera* sp.) and lactic acid bacteria (*Lactobacillus* sp., *Lactococcus* sp., *Leuconostoc* sp., *Bifidobacterium* sp.) were detected (Jarrell et al., 2000; Kurtzman et al., 2001; Goh et al., 2012; Velicanski et al., 2014). Bacteria in kombucha fermentation mainly produce acetic acid, gluconic acid and cellulose (Greenwalt et al., 2000). It has been reported that ethanol and acetic acid, which are produced as a result of the symbiotic association of yeasts and bacteria during fermentation, have antimicrobial activity against pathogenic bacteria (Liu et al., 1996). Today, kombucha has been tested with new substrates and some of the new substrates have been reported to stimulate fermentation better and complete fermentation in a shorter time compared to the original kombucha tea (Vitas et al., 2013).

In a study, it was determined that the number of lactic acid bacteria (LAB) and species diversity in the product produced by the back-slopping method was higher than in self-fermentation (Wirawati et al., 2019). The back-slopping method has positive effects on flavor and textural stability (Kim et al., 2018).

In the production of hardaliye, alcohol formation can be observed in the later stages of storage. In order to prevent this, hardaliye productions with different starters have been tried in recent years. In this study, physical, chemical and microbiological properties of hardaliye produced with different starters were investigated during production and storage. As a starter culture, kombucha mushrooms and back-slopping were used for the first time in the production of hardaliye.

2. Materials and methods

2.1. Materials

The research materials consist of hardaliye samples produced in the laboratory, using traditionally obtained hardaliye (back-slopping) and kombucha mushrooms as starters. The hardaliye obtained by the back-slopping method was named "BH", and the hardaliye produced by adding kombucha mushrooms was named "KH". Grape juice produced from Öküzgözü grapes used in production from Şarköy Mursallı Agricultural Development Cooperative, kombucha mushroom from Shaman's Secret A.Ş., mustard seeds and cherry leaves to be used in aromatization of hardaliye from Arpaş Arifoğlu Pazarlama Dağıtım ve Ticaret A.Ş., hardaliye which has completed the fermentation required in the production of traditional hardaliye by the back-slopping method was obtained from Karlıbağ Hardaliye A.Ş.

2.2. Methods

2.2.1. The production of hardaliye

Two different methods were used in the production of hardaliye. In two different methods, fermentation was continued until the pH of the hardaliye samples decreased to 3.10-3.25. Some physical, chemical and microbiological analyzes were applied to the produced hardaliye samples on the 0^{th} day when fermentation started, on the 7^{th} day when fermentation was completed, and on the 14^{th} day of storage at +4 ° C. It was added ground mustard seeds (1% of total weight of grape juice+hardaliye whose fermentation was completed or SCOBY) and cherry leaf (1% of total weight of grape juice+hardaliye whose fermentation was completed or SCOBY) to grape juice (90%). Then pasteurization was applied (72 °C, 20 min). After the cooling process (22-25 °C), hardaliye (10%), whose fermentation was completed, was added to the first sample where the first method will be applied. SCOBY (10%) was added to the sample where the second method will be applied. Fermentation of both samples took 7 d at 23 °C. After the samples were filtered using a 1 mm diameter mesh strainer, and then bottled in glass bottles. They were stored at 4°C for 14 d (Roussin, 1996).

2.2.2. Physical and chemical analyses

HANNA pH211 model pH meter was used to measure the pH of the samples (Wirawati et al., 2019). In the acidity analysis, 10 mL of the sample was taken, and 10 mL of distilled water was added. After the mixture became homogeneous, 0.5 mL of phenolphthalein indicator was added and titrated with 0.1 N NaOH until the pH reached 8.1 (Mbaeyi-Nwaoha and Ajumobi, 2015). Luff-Schoorl method was used to determine the reducing sugar content of hardaliye samples (Cemeroğlu, 2004). In alcohol analysis, 2.9 mL of Glycine buffer solution was poured into the tubes containing NAD, 0.1 mL of distilled water was placed on it and after 0.1 mL of sample was added, reading was taken at 340 nm in the spectrophotometer (Shimadzu Corporation UV- 1208, Japan) (Boehringer-Mannheim, 1989). The amount of ethanol (mg/dL) was calculated from the absorbance value read according to the formula below.

ABV- Control ABV= ΔA340
ΔA340 x 223=…..mg dL^{-1} ethanol
ABV= Absorbance Value

The color of the grape juice and hardaliye samples used in the production of hardaliye was determined using the Konica Minolta Chroma Meter CR-5 color measuring device. Hardaliye samples were placed in the glass container of the device and their readings were performed. The results were given as L*, a* and b* values (Utoiu et al., 2018). The total amount of phenolic substances was determined according to the method reported by Cemeroğlu (2007). The commonly used Folin-Ciocalteu reagent was used to calculate the total phenolic content. Total phenolic content was calculated as gallic acid equivalent (GAE) using the gallic acid calibration curve (Cemeroğlu, 2007). Viscosity analysis of grape juice and hardaliye samples was carried out using TA Discovery HR-20 rheometer device. Viscosity values were expressed as Pa.s.

2.2.3. Microbiological analyses

The dilutions were prepared into tubes containing 9 mL of 0.85% sterile physiological water and 0.1 mL of the dilutions

were dispersed on the surface of agar plate using the spread-plate method. For total aerobic mesophilic bacteria count, PCA Agar medium was used and the colonies (cfu mL^{-1}) formed after 48 h of incubation at 30 °C were counted (Temiz, 2002). PDA Agar medium was used for total yeast and mold count, incubation lasted 5 d at 25 °C (Bergmann et al., 2010; Maturin and James 2001; Tournas et al., 2001). Mold growth was observed morphologically. For *Lactobacillus* spp. enumeration, MRS Agar medium was used and incubation was continued at 37 ° C under anaerobic conditions for 72 h (De Man et al., 1960; Bergmann et al., 2010) M17 Agar medium was used for lactic streptococci counting, incubation continued for 72 h in aerobic environment at 37 °C (Bergmann et al., 2010).

2.2.4. Statistical analysis

Hardaliye samples were analyzed in triplicate. The results obtained were evaluated by using the JMP 5.0.1 (SAS Institute) program and applying binary ANOVA analysis. Significant differences between results were determined by Tukey's multiple comparison test, with a grade of $P<0.05$.

3. Results and discussions

3.1. Physicochemical properties

During the fermentation, the pH decrease was 0.27 unit in hardaliye produced by back slopping method and 0.21 unit in hardaliye produced with kombucha mushrooms ($P<0.05$). The decrease during storage was less than during fermentation ($P<0.05$). Ayed et al., (2017) stated in their research that the pH values decreased during the fermentation of grape juice with kombucha mushrooms. While the pH value was 3.95 on the first day of that study, it was measured as 3.18 on the 6th day. It is seen that the values obtained in that study are similar to the values of the KH samples in this study. In the study of Coşkun et al. (2018), the pH values of hardaliye produced from different grape varieties varied between 3.33 and 3.73 at the end of fermentation. In this study, the pH decreases were higher than in the study of Coşkun et al. (2018) because benzoic acid or sorbic acid was not used as a preservative in hardaliye produced by the back slopping method. Mustard seeds do not show sufficient effect in preventing alcohol formation. In order to prevent the formation of alcohol, preservatives must be used together with mustard seeds. When the results of the study of blueberry tea carried out using sucrose, glucose, fructose carbon sources are examined; pH values were measured as 3.30, 3.27 and 3.09, respectively on the 0th day of fermentation, and as 3.01, 2.94 and 2.91 on the 8th day, respectively (Tarhan, 2017). Based on these studies (Arici and Coskun, 2001; Tarhan, 2017), it is thought that the acidity development ability of kombucha culture is similar to hardaliye.

Although there is a partial relationship between pH and total acidity, the degree of this relationship varies according to the type of acid formed (Amerine et al., 1965). While pH is important for assessing the ability of a microorganism to grow in a particular food, titratable acidity is a better indicator than pH of how organic acids in the food affect flavor (Tyl and Sadler, 2017). The total acidity of the BH sample increased during production and storage. In the KH sample, there was no increase in the total acidity value during fermentation, but an increase was observed during storage (Figure 1). In a study conducted without using preservatives; in the sample using 1% mustard seeds and starter culture *(L. plantarum*), the total acidity (as tartaric acid) value on the 7th day of fermentation was measured as 6.10 g L^{-1} (Gürbüz, 2018). It is seen that the values are close to the total acidity (as tartaric acid) value on the 7th day of the BH sample in this study.

The amount of reducing sugar in hardaliye samples decreased with fermentation and increased slightly during storage (Table 1). Arici and Coskun (2001) reported that these reductions in reducing sugar during fermentation are due to the microorganisms in the environment using reducing sugar as a substrate and breaking it down into lactic acid, ethyl alcohol, CO_2 and some other organic acids. The increase in the amount of reducing sugar in Hardaliye samples on the 14th day of storage was associated with the release of sugar bound to anthocyanidins as a result of the degradation of anthocyanins, as stated by Coskun et al. (2012). It was determined that the amount of reducing sugar in the KH sample at the end of the fermentation (7th day) as in the hardaliyes (17.5-16.88%) produced by Coskun et al. (2012). The amount of reducing sugar on the 7th day of the BH sample in this study seems to be close to the values of hardaliye (8.81 g L^{-1}-10.91 g L^{-1}) produced by Faikoğlu (2012) with different grape varieties.

Alcohol was not detected at the beginning of Hardaliye production (Table 1). While 5.5% alcohol formation was observed at the end of fermentation (7th day) in the BH sample, no alcohol formation was observed in the KH sample during production and storage. Alcohol could not be detected in hardaliye samples produced in laboratory by Coşkun et al. (2018). Researchers detected alcohol (maximum 6%) in hardaliye samples collected from the people of Kırklareli, as they may be in the advanced stages of storage. Sometimes producers prefer producing hardaliye containing some alcohol and do not use chemical preservatives other than mustard during the first few days of fermentation. It is seen that the alcohol content of those samples is close to the amount of alcohol on the 7th day of the BH sample in this study. In the study of Ayed et al. (2017), changes in ethanol were observed during kombucha fermentation in grape juice. In that study, the amount of ethanol was measured as 0.52 g 100 mL^{-1} on the 6th day, and 0.29 g 100 mL^{-1} on the 12th day. In the study of Tarhan (2017), as a result of kombucha fermentation carried out by using different carbon sources in coffee and various herbal and fruit teas, alcohol was not detected as in the KH sample in our study. This has been associated with the conversion of ethanol produced by yeasts to acetic acid, where the main thing in kombucha fermentation is acetic acid fermentation.

In the BH sample, while a rapid decrease is observed in the amount of reducing sugar during fermentation, there is a simultaneous increase in the amount of alcohol. This situation can be explained as the microorganisms in the environment using reducing sugar as a substrate and breaking it down into lactic acid, ethyl alcohol, CO_2 and some other organic acids (Arici and Coşkun, 2001). Alcohol could not be detected in the KH sample during production and storage.

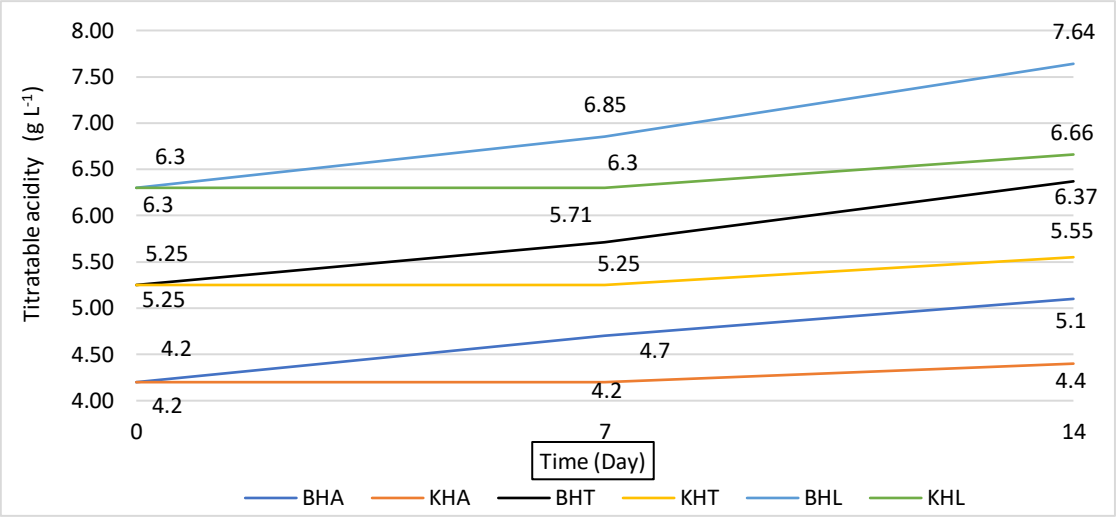

Figure 1. Titratable acidity (g L^{-1}) values of BH and KH samples. *BHA:BH acetic acid, KHA: KH acetic acid, BHT:BH tartaric acid, KHT: KH tartaric acid, BHL: BH lactic acid, KHL: KHL lactic acid*

This can be explained as a symbiotic association of yeasts and acetic acid bacteria found in kombucha mushrooms. While glucose in grape juice is converted to gluconic acid by acetic acid bacteria in the environment, fructose is converted to ethanol by yeasts. Ethanol produced by yeast is converted to acetic acid by acetic acid bacteria (Arıkan, 2018). Acetic acid bacteria (AAB) are well-known microorganisms found in fruits such as grapes (Valera et al., 2011). Since the alcohol formed in hardaliye is broken down by acetic acid bacteria and the conversion of hardaliye into vinegar is in the later stages of storage, it was thought that the number of AABs transferred from grapes to mustard is less than that of kombucha. According to the Turkish Food Codex Communiqué on Non-Alcoholic Beverages (Communiqué No: 2007/26), it is stated that the amount of ethyl alcohol that may arise from the nature of production in the beverages covered by the Communiqué should be at most 3.0 g L^{-1} (approximately 0.4% v/v) (TGK 2007). In this study, the alcohol content of the BH sample is well above the specified limit. In this respect, it is very important to prevent the formation of alcohol during the fermentation of hardaliye.

The highest amounts of phenolic substances were determined as 792.33 mg GAE L^{-1} in BH sample and 830.19 mg GAE L^{-1} in KH sample on the 7th day of fermentation ($P<0.05$). The phenolic content of hardaliye samples stored at +4 ° C for 14 days decreased by 7.6% in BH samples and 6.6% in KH samples ($P<0.05$) (Table 1). Total phenolic content was determined in the range of 368-2727 mg L^{-1} in twenty-three hardaliye samples collected from local people by Coşkun et al. (2018).

In the study of Gündüz et al. (2019), the total phenolic content of grape juice was determined as 1515.27 mg L^{-1}. In that study, the total phenolic content of hardaliye produced by fermenting grape juice was determined as 2029.20 mg GAE L^{-1} in homemade hardaliye and 2193.08 mg GAE L^{-1} in commercial hardaliye. As a result of the processing of grape juice into hardaliye, the total amount of phenolic compounds increased significantly. In this study, an increase of 6.7% in the BH sample and 11.7% in the KH sample was observed in the total amount of phenolic substances with fermentation (Table 1). In a study examining the changing parameters of grape juice during fermentation with kombucha, it was observed that 6 d after the start of fermentation, the total phenolic content of grape juice increased by 40% with fermentation (Ayed et al., 2017). *Gluconobacter* has been identified as a key bacterial species that increases the bioavailability of polyphenols and the antioxidant activity of beverages (Dufresne and Farnworth 2000). However, in Ayed et al. (2017), a slight decrease in phenolic content was observed from the 10th d. This phenomenon can be explained by the polymerization of some phenolic compounds into higher molecular weight molecules, which leads to the detection of lower polyphenol content. A similar situation was observed in this study as well. Phenolic substances can be hydrolyzed by the microflora (Ozcan et al., 2021). The difference in the amount of phenolic substances in KH and BH samples during fermentation and storage can be explained by the research they have done by Çam and Yıldırım (2018). In that research; they suggested that the selection of appropriate cultures for fermentation may affect the phenolic profile and phenolic substance content of the product formed at the end of fermentation, that mixed cultures should be used to obtain a good phenolic profile, and that the phenolic profile of the product can be controlled by using different cultures.

The desired red color in grape juices is due to the presence of anthocyanins. Anthocyanin content of grapes varies depending on many factors such as grape variety, maturity, harvest year, environmental conditions, etc. (Mazza and Francis, 1995). The color values of the hardaliye samples in this study are given in Table 1. It was determined that there were statistically significant losses in the brightness level of both hardaliye samples during the fermentation ($P<0.05$). While no significant change was observed in the BH sample during storage ($P>0.05$), the change in the KH sample was found to be statistically significant ($P<0.05$). It was thought that the increase in the microorganism counts during fermentation caused a decrease in the L* value by increasing the turbidity in the grape juice. A similar result was obtained in the study of Bayram et al. (2015). Watawana et al. (2016) suggested in their study with kombucha that the decrease in L* value was

due to the degradation of color pigments and polyphenolic components as a result of the decrease in pH due to fermentation and microorganism growth.

During the fermentation, a significant decrease of 3.67 units in the a* value of the BH sample and 9.63 units in the a* value of the KH sample was detected (P<0.05). Although there was an increase in the a* value of the KH sample and a decrease in the a* value of the BH sample during storage, these changes were not found to be statistically significant (P>0.05) (Table 1). When KH and BH samples were compared, the difference in a* values determined on the 7th day of production and 14th day of storage was found to be statistically significant (P<0.05). The color tone of the hardaliyes produced using red grape juice is red at the beginning and it is desired to have less yellow tone. On day 0, b* values were measured as 33.69. The differences between b* values of hardaliye samples during production and storage were significant (P<0.05). No significant change was observed in the b* value of the KH sample during production and storage (P>0.05). The highest b* value in the BH sample was measured as 36.57 on the 7th day, and then a slight decrease was observed during storage and was measured as 36.05 on the 14th day of storage. The changes in the b* value of the BH sample during production and storage were significant (P<0.05).

Table 1. Physicochemical properties of hardaliye samples during fermentation and storage.

Properties	Samples	Fermentation time (day)		Storage time(14th day)
		0	7	14
pH	BH	3.41 ± 0^{Aa}	3.14 ± 0.01^{Bb}	2.97 ± 0.16^{Ca}
	KH	3.41 ± 0^{Aa}	3.2 ± 0.01^{Ba}	3.00 ± 0.06^{Ca}
Reducing sugar (%)	BH	24.96 ± 1.59^{Aa}	7.93 ± 0.32^{Bb}	8.16 ± 0.45^{Bb}
	KH	24.96 ± 1.59^{Aa}	$16.45\pm1,32^{Ba}$	16.8 ± 1.42^{Ba}
Ethyl alcohol (%)	BH	0 ± 0^{B1}	$5.5\pm0,3^{A}$	5.45 ± 0.24^{A}
	KH	0±0	0±0	0±0
Phenolic compound (mg GAE L^{-1})	BH	743.52 ± 1.79^{Ba}	792.33 ± 6.84^{Ab}	732.52 ± 6.28^{Cb}
	KH	743.52 ± 1.79^{Ca}	830.19 ± 7.36^{Aa}	775.15 ± 7.56^{Ba}
Viscosity (Pa.s)	BH	2.05 ± 0.02^{Ba}	3.82 ± 1.46^{Aa}	3.79 ± 1.28^{Ab}
	KH	2.05 ± 0.02^{Ca}	2.77 ± 0.055^{Ba}	5.62 ± 0.43^{Aa}
Color				
L*	BH	32.81 ± 0.61^{Aa}	26.04 ± 2.7^{Bb}	26.65 ± 2.32^{Bb}
	KH	32.81 ± 0.61^{Aa}	30.12 ± 2.44^{Ba}	33.76 ± 1.65^{Aa}
a*	BH	52.73 ± 0.10^{Aa}	49.06 ± 0.94^{Ba}	48.93 ± 0.93^{Ba}
	KH	52.73 ± 0.10^{Aa}	43.1 ± 0.29^{Bb}	45.76 ± 2.81^{Bb}
b*	BH	33.69 ± 0^{Ca}	36.57 ± 0.51^{Aa}	36.05 ± 0.01^{Ba}
	KH	33.69 ± 0^{Aa}	33.00 ± 1.50^{Ab}	32.21 ± 1.49^{Ab}

There is no statistically significant difference between the values shown with the same lowercase letters in each column (P>0.05). There is no statistically significant difference between the values shown with the same capital letters in each row (P>0.05).

Tarhan (2017) investigated the differences in the growth of kombucha mushroom with the use of various sugar sources in different plants-fruits and coffee. In the study, it was determined that the b* value in all carbon sources of pomegranate tea was not affected by fermentation. In the same study, it was determined that there was a decrease in the L* and b* values of only xylose sugar samples due to fermentation in blueberry and rosehip teas. While no significant difference was observed in the b* value during fermentation and storage in the KH sample in this study, a difference was detected in the BH sample. Watawana et al. (2016), in their study with kombucha, thought that the reason for the changes in a* value and the decrease in L*, b* value was the decrease in pH due to fermentation and the degradation of color pigments and polyphenolic components by microorganisms growing in the environment. Color stability is highly dependent on pH and anthocyanin structure (Torskangerpoll and Andersen, 2005).

Viscosity values of BH and KH samples at the beginning of fermentation were measured as 2.048 Pa.s. The increase in viscosity of the BH sample during fermentation was greater than that of the KH sample. During storage, a statistically insignificant (P>0.05) decrease was observed in the BH sample. In the KH sample, however, the increase continued (P<0.05) (Table 1). In study conducted by Watawana et al. (2016), coconut water with kombucha mushrooms was fermented for 7 days. As in this study, they reported that the viscosity increased due to fermentation. This may be due to the secretion of exopolysaccharides formed during fermentation (Zhao et al., 2015; Vivek et al. 2019). Exopolysaccharides (EPS) from LAB have been reported to be used as stabilizing, viscosity modifying and gelling agents in foods (Ahmed et al., 2013; Altay et al. 2013). Exopolysaccharides can be produced by many microorganisms other than LAB (Ergene and Avcı, 2016).

3.2. Microbiological properties

Grape juice, mustard seed and cherry leaf mixture used as substrate in this study was pasteurized to prevent microbial contamination from raw materials. TMAB was not found at the beginning of fermentation after heat treatment (Table 2). In the BH sample, the TMAB count was 5.58 log cfu mL^{-1} at day 7. It was determined as 4.49 log cfu mL^{-1} after 14 d of storage at +4 °C and a significant decrease was observed during storage ($P<0.05$). In the KH sample, it was determined as 4.55 log cfu mL^{-1} on the 7th day, and 3.1 log cfu mL^{-1} with a significant decrease on the 14th day of storage ($P<0.05$). In the study of Arici and Coskun (2001) the total bacterial count of hardaliye samples produced by the traditional method was at least (pH 3.21) 2.04 log cfu mL^{-1}, at most (pH 4.12) 5.9 log cfu mL^{-1} average (pH 3.58) was determined as 4.9 log cfu mL^{-1}. The results of that study appear to be similar to those of this study. The decrease in TMAB numbers of KH and BH samples during storage may be due to the increase in acidity in the products. As a matter of fact, Arici and Coskun (2001) suggested in his study that the low pH of hardaliye may cause a lethal and/or inhibitory effect against many microorganisms.

Yeast was not detected in the product consisting of pasteurized grape juice, mustard seed and cherry leaf mixture at the beginning of fermentation (Table 2). Mold growth was not observed in Hardaliye samples in analyzes made during production and storage. While yeast growth was observed during fermentation, a decrease was observed in the number of yeast during storage (Table 2). Gürbüz (2018), in his study on hardaliye, stated that the optimum growth temperature of yeasts is between 20-30 ºC, and suggested that a decrease in the number of yeasts can be observed depending on the temperature when the hardaliye samples are stored at +4 ºC. In this study, we can associate the decrease in yeast numbers of KH and BH samples with temperature during storage at +4 ºC (Table 2). In the BH sample, an increase in the amount of ethyl alcohol and a decrease in the amount of invert sugar were observed with the growth of yeast during fermentation. Yeast growth was lower in the KH sample than in the BH sample. While an increase was observed in the number of yeast during fermentation in the KH sample, the amount of reducing sugar decreased less than in the BH sample. Alcohol formation was not observed. This is thought to be due to the symbiotic association of yeasts and acetic acid bacteria during KH fermentation. Glucose in grape juice is converted to gluconic acid by acetic acid bacteria in the environment, while fructose is converted to ethanol by yeasts. Ethanol produced by yeast is converted to acetic acid by acetic acid bacteria (Arıkan, 2018). The fact that the number of acetic acid bacteria transferred from grapes to mustard may be less than that of kombucha, suggested that the ethyl alcohol content of the BH sample may have been higher than that of KH.

Table 2. Microbiological properties of hardaliye samples during fermentation and storage.

Properties	Samples	Fermentation time (day)		Storage time (14th day)
		0	7	14
TMAB (log cfu mL^{-1})	BH	0±0^{C}	5.58±0.06^{Ab}	4.49±0.49^{Ba}
	KH	0±0^{C}	4.55±0.02^{Ab}	3.1 ±0.14^{Bb}
Total yeast (log cfu mL^{-1})	BH	0±0^{C}	5.00±0.07^{Aa}	4.65±0.19^{Ba}
	KH	0±0^{C}	3.7±0.11^{Ab}	2.69±0.15^{Bb}
Total mold (log cfu mL^{-1})	BH	<DL	<DL	<DL
	KH	<DL	<DL	<DL
Lactobacillus spp. (log cfu mL^{-1})	BH	0±0^{C}	5.44±0.05^{Aa}	4.32±0.19^{Ba}
	KH	0±0^{C}	4.15 ±0.13^{Ab}	3.86 ±0.49^{Aa}
Lactic streptococci (log cfu mL^{-1})	BH	0±0^{C}	4.75±0.06^{Aa}	3.91±0.05^{Ba}
	KH	0±0^{C}	3.9±0.09^{Ab}	2.97±0.09^{Bb}

There is no statistically significant difference between the values shown with the same lowercase letters in each column ($P>0.05$). There is no statistically significant difference between the values shown with the same capital letters in each row ($P>0.05$). DL: Less than detection limit (10 cfu g^{-1})

In the BH and KH samples, on the 7th day of the fermantation, *Lactobacillus* spp. development was observed and the numbers were determined as 5.44 and 4.15 log cfu mL^{-1}, respectively. *Lactobacillus* spp. numbers decreased at the end of storage. In the study of Arici and Coskun (2001), the change in the number of lactic acid bacteria during the fermentation period (7 d) of hardaliye produced by inoculating different mustard seeds with different *Lactobacillus* ssp. was investigated. The number of lactic acid bacteria on the 7th day of fermentation in hardaliye with the addition of 1% black mustard seeds and *Lb. sanfansisco* was found to be 5.46 log cfu mL^{-1}. The number of *Lactobacillus* determined is similar to the results of the BH sample in this study. It is seen that the LAB number of the KH sample is low compared to that study. This may be because the number of lactic acid bacteria in SCOBY added to grape juice for the KH sample was lower than that of hardaliye added to the grape juice for the BH sample. Also, there may be a difference in the fermentation metabolism in the KH sample to which SCOBY was added. In a study investigating the metabolic activity of kombucha in milk and its ability to be a functional beverage; milk products fermented with kombucha were stored at +4 °C for 30 d at the end of fermentation and their biochemical and microbiological changes in this process were investigated. It has been reported that the number of *Lactobacillus* spp. decreased in the first 10 d of storage (Şarkaya, 2019). It was

observed that the *Lactobacillus* spp. numbers of the KH sample in this study also decreased during storage.

On the 7th day of fermentation, the streptococcal numbers of Hardaliye samples were determined as 4.75 log cfu mL^{-1} in BH sample and 3.91 log cfu mL^{-1} in KH sample. A statistically significant decrease occurred in both samples at the end of 14 d of storage ($P<0.05$) as in the study of Şarkaya (2019). In their study, Akarca and Tomar (2020) performed kombucha fermentation with red and purple vegetables for 21 days. On the 7th day of fermentation, the *Streptococcus* spp. numbers were determined as 3.75 log cfu mL^{-1} in the kombucha sample produced with red carrots, and 3.63 log cfu mL^{-1} in the kombucha sample prepared with red beet. The number of *Streptococcus* spp. on the 7th day of fermentation in the KH sample in this study is similar to the study.

4. Conclusion

When the results obtained were evaluated, it was determined that hardaliye, a fermented product with high nutritional value, can be produced with kombucha mushrooms without using preservatives. It has been observed that yeast growth cannot be prevented only with mustard seeds during BH production and alcohol will form in the product. Back-slopping production method should not be preferred in hardaliye production without using preservatives. It is thought that the preference of vigorous fermentation in the production of hardaliye with kombucha mushrooms will positively affect the total phenolic content and color values of the product. There are few studies on the production of hardaliye without preservatives. There is no legal standard for this traditionally produced product. In this context, studies on hardaliye are of great importance for a legal regulation to be made about hardaliye in the future. Physical, chemical and microbiological similarity of hardaliye produced using kombucha mushroom to hardaliye produced using preservative should be investigated, studies should be carried out on the identification of the microflora of hardaliye produced with kombucha mushroom.

Compliance with Ethical Standards

Conflict of Interest

As the author of article declare that there are no conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' Contributions

Ayşenur Pekcan: Validation, Writing - original draft, Methodology, Investigation, Formal analysis, Data curation. **Fatma Coşkun**: Writing - original draft, Methodology, Investigation, Conceptualization, Validation, Review and editing. **Ömer Öksüz**: Methodology, Investigation, Conceptualization, Validation, Writing - original draft, Review and editing, Visualization, Data

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Effect of drying methods on the sensory attributes of hazelnut cultivars in different sizes throughout the storage

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ABSTRACT

The main aim of this study was to evaluate the effect of nut sizes and drying methods on the sensory attributes of hazelnut cultivars (*Corylus avellana* L. cvs. Çakıldak, Palaz and Tombul) during the storage. Shelled hazelnut were dried under sun, shadow at room conditions and cold dried at 2 °C and 7 °C and stored at 20±5 °C and 80±5 % relative humidity. Evaluation of sensory attributes was carried out quarterly (harvest, 3, 6, and 9 months). In the Çakıldak cultivar (in 16 mm size), the odor of cold-dried hazelnuts at 2°C was higher than other drying methods in the last two measurements. It was observed that shadow-dried hazelnuts and at 7°C cold, dried hazelnuts had lower rancidity. No significant effect of drying methods was observed on the cultivars' flavor, firmness and color. As a result, it is revealed that drying methods have an effect on the rancidity in hazelnut kernels during storage.

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1. Introduction

In addition to being consumed as dried nut, hazelnut is used as a raw material in the production of many foods such as chocolate, dessert, cake and cream. Therefore, it can be stored for 1-2 years in ordinary warehouses as raw materials. Nutriment quality is lost during storage. It is even stated that there are losses in sensory properties such as taste, flavor and aroma (Turan, 2019; Chen et al., 2021). These losses can be caused by early or late harvest, maturity stage, transportation and storage conditions, and diseases and pests (Borompichaichartkul et al., 2009; Qu et al., 2016; Turan and İslam, 2019). As in all agricultural products, some applications are made with hazelnut to prevent losses and maintain quality in the postharvest period (Güler et al., 2017). At the beginning of these is the reduction of the moisture content, which is necessary for maintaining the quality loss for a long time. Failing to dry hazelnuts to international standards causes product and market loss (Aktaş et al., 2004; Turan and İslam, 2016). For this reason, many drying methods (Bostan, 2000) and technologies (Ceylan and Aktaş, 2008) are used for drying hazelnuts. Researchers have reported that drying methods affect hazelnut bioactive content and sensory properties (Fennema, 1985; Akçin and Bostan, 2019; Wang et al., 2020; Tu et al., 2021).

While hazelnuts are dried by machine in countries such as the USA, Spain and Italy, they are widely dried in the sun in Türkiye. Sun-dried nuts are laid on concrete, wood and grass and dried to the desired humidity level (Bostan, 2000; Turan and İslam, 2019). Drying in the sun is preferred because of its low energy cost. However, due to the rainy season in the hazelnut growing regions, the drying process is very laborious for the producers and increases the drying costs. Machine drying is not common in Türkiye. Especially when machine drying costs are added to sustainable costs, profitability in production decreases. In this context, alternative drying methods should be investigated. Cold drying can be one of these methods. As a result of high drying temperature, loss of color, taste, flavor, vitamin content and structural deterioration are more. However, the losses in products dried at low temperatures may be lower (Hürdoğan et al., 2013). This study aimed to determine the effects of sun, shadow and low-temperature drying methods on the sensory properties of Tombul, Palaz and Çakıldak hazelnut cultivars of different sizes stored under ordinary conditions.

2. Materials and methods

2.1. Plant materials

The plant material of this study was obtained from Tombul, Palaz and Çakıldak (Balık et al., 2016) hazelnut cultivars grown in commercial orchard in the Altinordu, Ordu. After the nuts were separated from the husk manually, each hazelnut variety was first passed through 2 different sorting sieves of 16 mm and 18 mm.

Sun drying was carried out under sun under the weather conditions of the harvest season. Drying in shadow conditions was carried out in an environment with air movement, where the nut were not exposed to sun. Drying at 2 and 7 °C was carried out in a cooler (Arçelik, Türkiye) at

70±5% RH, where temperature control could be achieved at 0-10 °C. Nuts were placed in grids with 10 cm intervals in order not to prevent the air movement created in the cooler. The distance between the grids was 25 cm. A fan assembly was installed to provide cold air movement at a speed of 0.5 m s^{-1} in the environment and the air mobility was continuous. Drying was terminated when the kernel moisture content fell to 6% at all. Afterward, all nuts were immediately transferred to ordinary conditions (to the merchant warehouse). Nuts were stored during the 270 days (9 months) and sensory analyses were performed at 90-day intervals.

2.2. Methods

Approximately 100 g of shelled nuts was taken as a coincidence for each application in each measurement period. The experiment was designed with three replications. Details of the applications are given in Table 1.

2.2.1. Sensory evaluation

The analyses were carried out by 6 panelists aged between 30-45, who had been cultivating hazelnuts for many years, could distinguish the sensory properties of hazelnuts, and had high school and undergraduate education levels, and did not smoke were preferred. In the study conducted on Çakıldak, Palaz and Tombul hazelnut cultivars, each sample was given a code number, and the panelists evaluated the hazelnuts they ate by filling out the form given to them. In the study, sensory characteristics (flavor, rancidity, odor, firmness and color) evaluated according to the hedonic scale. Flavor, odor, firmness and color characteristic in the scale were scored from 0 to 5 (0: Unacceptable, 1: Very Bad, 2: Bad, 3: Acceptable, 4: Good, 5: Very Good) and averaged. The rancidity was described as 0: Excellent 1: Very good, 2: Good, 3: Acceptable, 4: Bad, 5: Very bad.

Table 1. Details of treatments in the study

Çakıldak/Palaz/Tombul							
Sun		Shadow		2 °C		7 °C	
16 mm	18 mm	16 mm	18 mm	16 mm	18 mm	16 mm	18 mm

2.3. Statistical analysis

The normal distribution of data (Kolmogorov-Smirnov) and homogeneity of variance (Levene) tests were checked. ANOVA was performed on the data that met the conditions. Tukey's multiple comparison test was used to determine whether there was a difference between the treatments ($p \leq 0.05$). Statistical analyzes were performed in Minitab® 17 software (Minitab Inc., State College, PA, USA).

Table 2. Effect of drying methods on the flavor of hazelnut cultivars in different sizes throughout the storage

Cultivars	Sizes	Drying methods	Flavor			
			Harvest	90 d	180 d	270 d
Çakıldak	16 mm	Shadow	4.00 ab	3.83 a	4.66 a	4.00 a
		Sun	3.66 b	4.16 a	4.33 a	4.16 a
		2 °C	4.66 a	4.16 a	4.33 a	4.50 a
		7 °C	4.00 ab	3.83 a	4.00 a	4.00 a
	18 mm	Shadow	4.00 ab	3.83 ab	4.00 a	3.83 b
		Sun	3.66 b	3.83 ab	4.33 a	3.83 b
		2 °C	4.33 a	3.67 b	4.33 a	3.83 b
		7 °C	4.16 ab	4.33 a	4.33 a	4.16 a
Palaz	16 mm	Shadow	4.00 a	4.00 a	4.16 a	3.83 a
		Sun	4.16 a	4.00 a	4.00 a	4.00 a
		2 °C	4.33 a	3.83 a	4.16 a	4.16 a
		7 °C	4.66 a	3.66 a	4.00 a	4.16 a
	18 mm	Shadow	4.16 b	3.50 a	3.83 a	3.83 ab
		Sun	4.16 b	4.00 a	3.88 a	3.66 b
		2 °C	4.83 a	4.00 a	4.00 a	4.11 ab
		7 °C	4.66 ab	4.83 a	4.16 a	4.50 a
Tombul	16 mm	Shadow	3.83 ab	4.00 b	4.33 a	4.16 a
		Sun	3.66 b	3.50 c	4.33 a	4.00 a
		2 °C	4.33 a	3.66 c	4.33 a	4.16 a
		7 °C	3.66 b	4.33 a	4.55 a	4.00 a
	18 mm	Shadow	3.33 b	3.83 a	4.16 a	3.66 a
		Sun	4.00 ab	4.00 a	4.33 a	4.00 a
		2 °C	4.16 a	4.50 a	4.00 a	4.33 a
		7 °C	4.66 a	4.00 a	4.16 a	4.16 a

The differences among mean values shown on the same column with the same letter is not significant ($p<0.05$).

3. Results

3.1. Flavor

When Çakıldak nut of different sizes was evaluated, the flavor of the nut (a size16 mm) dried at 2 °C was found to be higher compared to dried only in the sun at harvest. In the evaluation made in the third month, the flavor of the nut (a size 18 mm) dried at 7 °C was higher than those dried only at 2 °C. In addition, in the examinations made in the ninth month, it was determined that the flavor of the nuts dried at 7 °C was higher than the other drying methods.

Table 3. Effect of drying methods on the rancidity of hazelnut cultivars in different sizes throughout the storage

Cultivars	Sizes	Drying methods	Rancidity			
			Harvest	90 d	180 d	270 d
Çakıldak	16 mm	Shadow	0.00 b	0.50 a	0.50 a	0.30 b
		Sun	0.00 b	0.00 b	0.00 b	0.41 b
		2 °C	0.00 b	0.00 b	0.00 b	0.22 b
		7 °C	0.50 a	0.33 a	0.00 b	1.00 a
	18 mm	Shadow	0.00 b	0.00 b	0.00 a	0.50 ab
		Sun	0.00 b	0.00 b	0.00 a	0.83 a
		2 °C	0.00 b	0.50 a	0.00 a	0.50 ab
		7 °C	0.33 a	0.17 ab	0.00 a	0.39 b
Palaz	16 mm	Shadow	0.28 a	0.00 a	0.00 b	0.16 b
		Sun	0.00 a	0.00 a	0.16 ab	0.16 b
		2 °C	0.33 a	0.00 a	0.33 a	0.66 a
		7 °C	0.00 a	0.16 a	0.00 b	0.16 b
	18 mm	Shadow	0.00 b	0.00 b	0.50 a	0.50 bc
		Sun	0.00 b	0.33 a	0.66 a	1.00 ab
		2 °C	0.50 a	0.50 a	0.66 a	1.66 a
		7 °C	0.00 b	0.00 b	0.00 b	0.00 c
Tombul	16 mm	Shadow	0.00 a	0.00 a	0.00 b	0.00 b
		Sun	0.00 a	0.00 a	0.66 a	0.66 a
		2 °C	0.00 a	0.00 a	0.00 b	0.00 b
		7 °C	0.00 a	0.00 a	0.00 b	0.00 b
	18 mm	Shadow	0.50 a	0.00 a	0.00 b	0.50 bc
		Sun	0.00 b	0.00 a	0.00 b	0.00 c
		2 °C	0.00 b	0.00 a	0.16 b	1.16 a
		7 °C	0.16 b	0.00 a	0.50 a	0.66 ab

The differences among mean values shown on the same column with the same letter is not significant ($p<0.05$).

Table 4. Effect of drying methods on the odor of hazelnut cultivars in different sizes throughout the storage

Cultivars	Sizes	Drying methods	Odor			
			Harvest	90 d	180 d	270 d
Çakıldak	16 mm	Shadow	3.83 b	4.00 a	4.00 ab	3.83 b
		Sun	4.00 ab	4.16 a	3.83 b	3.66 b
		2 °C	4.50 a	4.00 a	4.66 a	4.50 a
		7 °C	4.00 ab	4.00 a	3.83 b	3.66 b
	18 mm	Shadow	3.83 a	3.83 a	3.83 a	3.66 b
		Sun	4.00 a	4.33 a	4.00 a	4.00 a
		2 °C	4.33 a	3.66 a	4.00 a	4.00 a
		7 °C	4.16 a	4.16 a	4.16 a	4.16 a
Palaz	16 mm	Shadow	4.33 a	4.16 ab	4.33 a	4.16 a
		Sun	3.66 b	4.50 a	4.16 a	4.16 a
		2 °C	4.33 a	3.83 ab	4.16 a	4.00 a
		7 °C	4.66 a	3.50 b	4.00 a	4.16 a
	18 mm	Shadow	4.16 a	3.50 b	4.00 ab	3.50 b
		Sun	4.16 a	3.50 b	3.50 b	3.66 ab
		2 °C	4.33 a	3.83 ab	4.00 ab	3.66 ab
		7 °C	4.33 a	4.16 a	4.50 a	4.16 a
Tombul	16 mm	Shadow	4.00 a	4.33 a	4.33 a	4.00 a
		Sun	4.00 a	4.50 a	4.00 a	4.16 a
		2 °C	4.50 a	4.50 a	3.83 a	4.00 a
		7 °C	4.00 a	4.00 a	4.33 a	4.00 a
	18 mm	Shadow	3.83 a	4.50 a	4.66 a	4.16 a
		Sun	4.16 a	4.66 a	4.16 ab	4.16 a
		2 °C	4.00 a	4.16 a	3.83 b	3.83 a
		7 °C	4.66 a	4.50 a	4.16 ab	4.16 a

The differences among mean values shown on the same column with the same letter is not significant ($p<0.05$).

When Palaz nuts with a size of 18 mm were examined, it was determined that the flavor of the nuts dried at 2 °C was higher than those dried in the shadow and the sun. Considering the values obtained in the 9th month, it was determined that the flavor of the nuts dried at 7 °C was higher than those dried only in the sun. In the measurements made during the harvest period of 16 mm sized Tombul nuts, the flavor of the nuts dried at 2 °C was higher than those dried at 7 °C and the sun. In the evaluations made in the 3rd month, the flavor of the nuts dried at 7 °C was higher than the other drying methods. When Tombul nuts with a size of 18 mm were evaluated, it was determined that the flavor of the nuts dried at 2 and 7 °C at harvest was higher than those dried in the shadow (Table 2).

3.2. Rancidity

In the examinations of Çakıldak nuts with a size of 16 mm in the harvest period and the 9th month, the rancidity rate of nuts dried in shadow, sun and at 2°C was found to be lower compared to the nuts dried at 7°C. Considering the values obtained in the 3rd month, the rancidity rate of nuts dried in the sun and at 2°C was lower than those dried at 7°C and in the shadow. In the evaluations made in the 6th month, the rancidity rate of the nuts dried in the sun at 2 and 7°C was lower than those dried in the shadow. When Çakıldak nuts with a size of 18 mm were evaluated, the rancidity rate of the nuts dried at 2°C was found to be lower than the dried nuts at 7°C at harvest. Considering the data obtained in the 3rd month, the rancidity rate of the nuts dried in the shadow and in the sun was lower than those dried at 2°C. The evaluations made in the 9th month determined that the rancidity rate of nuts dried at 7°C was lower than those dried only in the sun. In the 6th month examination of 16 mm long Palaz nuts, the rancidity rate of nuts dried at 7°C and shadow was lower than those dried at 2°C. In the evaluations made in the 9th month, it was determined that the rancidity rate of the nuts dried at 7°C and shadow sun was lower than those dried at 2°C. During the harvest period, it was determined that the rancidity ratio of the 18 mm-sized Palaz nuts in shadow, sun and dried at 7°C was lower than those dried at 2°C. In the 3rd month of storage, the rancidity rate of nuts dried in shadow and at 7°C was lower than those dried at 2°C and in the sun. It was determined that the rancidity rate of the nuts dried at 7°C in the 6th month of storage was lower compared to the other drying methods, and in the 9th month, the rancidity rate of the nuts dried at 7°C was lower than those dried in the sun and at 2°C. Tombul nutswith a size of 16 mm had a higher rancidity rate than the others in only sun drying (6th and 9th months). The rancidity rate of 18 mm sized Tombul nuts, dried in the shadow at harvest, dried at 7°C in the 6th month and 2°C in the 9th month, was significantly higher than the others (Table 3).

3.3. Odor

When Çakıldak nuts with a size of 16 mm were evaluated, the odor rate of the nuts dried at 2°C during the harvest period was higher than those dried only in the shadow. Considering the odor changes during storage, nuts dried at 2°C; It was found that the rate of the odor was higher in the 9th month compared to other methods, while it was higher than those dried in the sun and at 7°C in the 6th month. Çakıldak nuts (in the 18 mm sized) odor rate was found the significantly lower in shadow drying only at 9th months. According to the measurements made at the harvest of Palaz nuts with a size of 16 mm, the odor rate of the nuts dried in the sun was lower than the others. However, the odor rate of the nuts dried at 7°C in the 3rd month was measured lower. Considering the odor rate of the Palaz nuts (in the 18 mm sized), the nuts dried in the sun in the 6th month and in the shadow in the 9th month of storage were found to be lower than those dried by the other methods. Whereas, in the 3rd month of storage, sun and shadow drying were measured lower. There was no odor change in the 16 mm sized Tombul nuts during storage. However, in 18 mm sized Tombul nuts, it was determined that different odors in the 9th month of storage (the lowest value was at 2°C drying) (Table 4).

3.4. Firmness

When Çakıldak nuts with a size of 16 mm were evaluated, the firmness rate of the nuts dried at 2 and 7°C during the harvest period was higher than those dried in the shadow and sun. In the examinations made in the 9th month of Çakıldak nuts with a size of 18 mm, the firmness rate of the nuts dried at 7°C was found to be higher than those dried only in the shadow. In addition, drying methods did not have any effect on firmness in Çakıldak hazelnut storage. The measurements made during the harvest period of Palaz nuts with a size of 16 mm determined higher than the firmness ratio of the nuts dried at 7°C compared dried in the shadow and in the sun. In the 6th month of storage, nuts dried at 7°C had higher firmness than nuts dried only at 2°C. The measurements made in the 3rd month of Palaz nuts with a size of 18 mm determined that the firmness ratio of the nuts dried at 2 and 7°C was higher than those dried in the shadow and in the sun. When we look at Tombul nuts with a size of 16 mm, the firmness of the nuts dried at 2°C at harvest and in the 3rd month of storage was determined at the highest level. However, the firmness of 18 mm at Tombul nuts was found to be at a similar level. (Table 5).

3.5. Color

The drying methods did not affect Çakıldak nuts with a size of 16 mm (harvest and storage). However, in Çakıldak nuts with a size of 18 mm, the effect of drying methods was determined only in the 9th month (nuts dried at 7°C had a higher color value). The drying effect was not determined in both Palaz nuts sizes during harvest and storage. The highest color value in 16 mm sized Tombul nuts was determined from the nuts dried at 2°C during the harvest period. However, drying methods had no effect during storage. Similarly, the drying effect was not detected in 18 mm sized Tombul nuts during the harvest period. However, the color change of nuts dried at 2°C in the 6th month of storage (18 mm sized) was significantly lower than those dried in shadow and sun. (Table 6).

4. Discussion

Sensory evaluation is a discipline that measures, analyzes and explains the effects of various properties of foods on the senses of sight, smell, taste, touch and/or hearing (Dermirci Ercoşkun, 2009).

Table 5. Effect of drying methods on the firmness of hazelnut cultivars in different sizes throughout the storage

Cultivars	Sizes	Drying methods	Firmness			
			Harvest	90 d	180 d	270 d
Çakıldak	16 mm	Shadow	3.66 b	3.83 a	4.16 a	3.66 a
		Sun	4.00 b	4.00 a	4.66 a	3.83 a
		2 °C	4.66 a	3.83 a	4.61 a	4.00 a
		7 °C	4.50 a	4.00 a	4.00 a	4.16 a
	18 mm	Shadow	3.83 a	4.16 a	4.33 a	3.66 b
		Sun	4.33 a	4.16 a	4.33 a	3.83 ab
		2 °C	4.16 a	3.83 a	4.16 a	3.83 ab
		7 °C	4.00 a	4.16 a	4.66 a	4.00 a
Palaz	16 mm	Shadow	3.66 c	3.66 a	4.00 ab	3.66 a
		Sun	4.00 bc	4.16 a	4.00 ab	3.83 a
		2 °C	4.33 ab	4.16 a	3.50 b	3.83 a
		7 °C	4.66 a	3.83 a	4.50 a	3.83 a
	18 mm	Shadow	3.94 a	3.50 b	4.00 a	3.50 a
		Sun	4.16 a	3.66 b	4.00 a	4.00 a
		2 °C	4.50 a	4.33 a	3.83 a	4.00 a
		7 °C	4.66 a	4.16 a	3.83 a	4.00 a
Tombul	16 mm	Shadow	3.50 b	3.66 b	3.50 a	3.66 a
		Sun	3.50 b	4.16 ab	3.50 a	3.50 a
		2 °C	4.33 a	4.50 a	3.83 a	4.16 a
		7 °C	3.83 ab	4.33 ab	4.16 a	3.83 a
	18 mm	Shadow	3.33 a	4.00 a	4.00 a	3.66 a
		Sun	4.00 a	4.33 a	4.16 a	4.16 a
		2 °C	4.33 a	4.16 a	3.83 a	4.00 a
		7 °C	4.16 a	4.33 a	4.00 a	4.16 a

The differences among mean values shown on the same column with the same letter is not significant ($p<0.05$).

Table 6. Effect of drying methods on the color of hazelnut cultivars in different sizes throughout the storage

Cultivars	Sizes	Drying methods	Color			
			Harvest	90 d	180 d	270 d
Çakıldak	16 mm	Shadow	4.16 a	4.16 a	4.50 a	4.33 a
		Sun	4.33 a	4.16 a	3.83 a	4.00 a
		2 °C	4.66 a	4.33 a	4.50 a	4.50 a
		7 °C	4.16 a	4.16 a	4.16 a	4.33 a
	18 mm	Shadow	4.00 a	4.16 a	4.33 a	4.16 b
		Sun	4.00 a	4.50 a	4.00 a	4.00 b
		2 °C	4.33 a	4.77 a	4.16 a	4.16 b
		7 °C	4.50 a	4.50 a	4.66 a	4.66 a
Palaz	16 mm	Shadow	4.50 a	4.00 a	4.33 a	4.16 a
		Sun	4.16 a	4.33 a	4.50 a	4.16 a
		2 °C	4.33 a	4.00 a	4.50 a	4.50 a
		7 °C	4.66 a	4.16 a	4.50 a	4.50 a
	18 mm	Shadow	4.00 a	4.16 a	4.33 a	4.00 a
		Sun	4.33 a	3.66 a	4.16 a	4.00 a
		2 °C	4.66 a	4.16 a	4.33 a	4.50 a
		7 °C	4.33 a	4.16 a	4.66 a	4.16 a
Tombul	16 mm	Shadow	4.00 b	4.16 a	4.16 a	4.16 a
		Sun	3.66 b	4.16 a	4.33 a	4.00 a
		2 °C	4.66 a	4.16 a	4.16 a	4.33 a
		7 °C	3.83 b	4.16 a	4.50 a	4.16 a
	18 mm	Shadow	4.00 a	3.83 a	4.50 a	4.00 a
		Sun	4.16 a	4.50 a	4.50 a	4.33 a
		2 °C	4.00 a	4.66 a	3.50 b	4.00 a
		7 °C	4.50 a	4.00 a	4.00 ab	4.16 a

The differences among mean values shown on the same column with the same letter is not significant ($p<0.05$).

Quality in nuts is a feature sought by both producers and consumers. The first thing that comes to mind when talking about quality is the appearance of the nuts. In addition, essential quality parameters consist of sensory characteristics. So much so that consumers demand high taste, aroma and nutritional content in marketable nuts. Each type of nuts has a different sensory quality (Noguera-Artiaga et al., 2019). However, postharvest drying processes (Mokhtarian et al., 2017) or storage time (Güler et al., 2017) can affect sensory properties. In fact, hard-shelled nuts are preserved by drying after being harvested. Some sensory properties may also change depending on this drying process and method. Changes in some sensory properties were detected in hazelnuts that we preserved by applying different drying methods. Like our research findings, Bostan (2000) reported that the shell and inner color of hazelnuts dried on concrete, grass, wood and plastic materials varies depending on the methods. İn this research also stated that the best drying method is drying on concrete, that the hazelnuts dry faster in this process, and this situation significantly affects the sensory properties. Güler et al. (2017) reported that the sensory properties (taste, smell, rancidity, color and firmness) of hazelnuts stored for 18 months by applying different doses of radiation (0.5, 1.0 and 1.5) could vary depending on the storage period. Researchers especially stated that the rancidity is at its highest in the 12th month of storage. Bostan and Güler (2016) determined that the initial brightness of hazelnut kernel decreased during the more extended storage period. Also, emphasizing that this situation differs between cultivars, Palaz and Çakıldak cultivars were determined to darken more than Tombul and Kalınkara cultivars. Considering the drying studies on other nut species, Kashani Nejad et al. (2003) and Ghazanfari et al. (2003) reported significant differences in flavor and firmness of pistachio nuts dried by different methods. They found that especially sun-dried nuts were more delicious than other drying methods. In addition, it was stated that the firmnessdrying method's flavor and firmness decreased with the hot air flow, which created a higher moisture content. Similarly, Mokhtarian et al. (2017) reported differences between the sensory properties of pistachio nuts dried in different methods, especially nuts traditionally dried directly in the sun with stronger flavor and aromatic odor. On the contrary, research findings (Kader et al., 1982) state that the drying method does not affect the sensory quality. According to Kader et al. (1982) stated that depending on the increase in drying temperature, rancidity decreased, and nut firmness and flavor increased. Whereas, Kashani Nejad et al. (2003) stated that the drying method did not have a significant effect on rancidity in pistachio. However, our study found lower rancidity in nuts dried in shadow and at 7 °C. While no significant effect of drying methods on the color was observed in our study, Chen et al., (2020) reported that there is darkening due to rapid moisture loss in nuts that survived at high temperatures in their study on walnuts. Wu et al. (2014) stated that darkening causing color change might be related to moisture content, while Xiao et al. (2017) stated that this may be due to enzymatic/non-enzymatic reactions.

5. Conclusion

It was observed that shadow-dried hazelnuts and at 7 °C cold, dried hazelnuts had lower rancidity. As a result, it is revealed that drying methods affect the rancidity in hazelnut kernels during storage. No significant effect of drying methods was observed in the cultivars' flavor, firmness and color. But, nuts dried in different sizes (16-18 mm) was effective on the sensory properties. More detailed studies are needed to increase the widespread impact of the results and ensure consistency among the findings.

Compliance with Ethical Standards

Conflict of Interest

As the author of article declare that there are no conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' Contributions

Kader Sali: Formal analysis, Data curation **Burhan Ozturk**: Investigation, Conceptualization, Validation, Writing - original draft, Visualization, Review and editing. **Mithat Akgün**: Methodology, Formal analysis, Data curation. **Umut Ates**: Investigation, Conceptualization, Writing - original draft.

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Consent for publication

We humbly give consent for this article to be published

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