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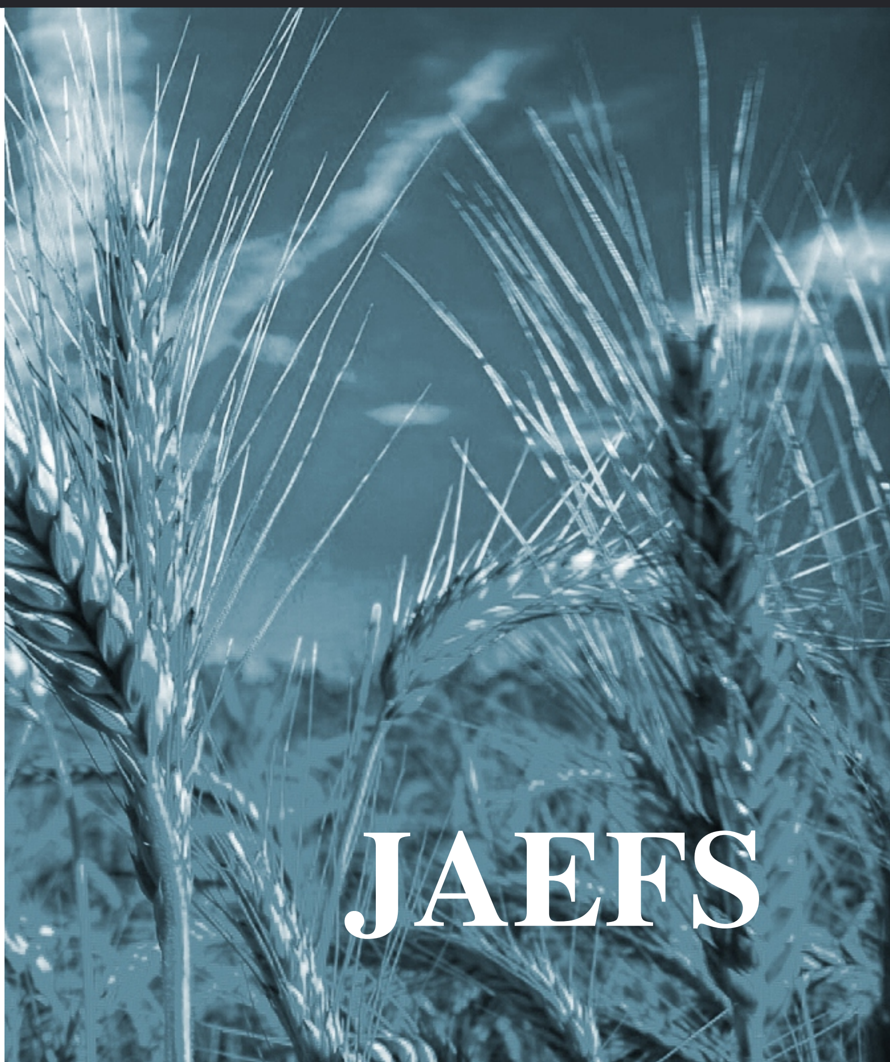

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Research Article Int J Agric Environ Food Sci 4 (1):1-6 (2020)

Possible effects of priming on germination performance of white clover (*Trifolium repens* L.) seeds in hypothermia condition

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Abstract

The aim of the study is investigate the effects of hidropriming on germination performance of white clover (*Trifolium repens*) seedsunder cold stress, optimum temperature and dark condition. The seeds were germinated applying hidropriming with dH_2O in different periods (2, 4, 6, 8, 16, 18, 20, 22, 24 and 48 hours) in dark at 4 ^{o}C. The study was applied in according to randomized controlled trials as 4 replications to measure radicular lengths, germination rate and homogeneity parameter in the seeds. The results of the study was indicated that radicle length, germination rate, rate and the homogeneity parameter were positive and statistically significant ($P<0.001$) comparing with control group in short period (2 and 4 hours). On the other hand radicle length, germination rate, the homogeneity parameter were found negative and statistically significant when compare with control group in the other periods ($P<0.001$). In addition, this study was discussed possible physiological effects to the priming process implemented with dH_2O in hypothermia condition at white clover seeds.

Keywords: Cold stress, Germination, Hidropriming, White clover

Introduction

White clover (*Trifolium repens*) which widely cultivated in the Marmara, Black Sea and transition zones in Turkey is quite valuable for animals. This plant which is short, abundant leaf, thin handle, soil surface covering, and high quality production is a high quality meadow pasture and forage crop (Manga et al. 1995). Germination of plants varies between varieties but it is highly influenced by environmental factors such as light, water, temperature, oxygen etc. Water and temperature factors are the most commonly used environmental factors investigated to start germination of the seeds. Theoretically, it is possible to stimulate the germination of the seeds with these applications to get fast and high germinate percentage (Karakurt et al. 2010). For this purpose, humidification / hydropriming applications on plants have demonstrated beneficial effects on the rate of germination such as Basu and Pal (1980) in rice seeds, Rao et al. (1987) in lettuce seeds, Sivritepe and Dourado (1995) in pea seeds, Sivritepe and Demirkaya (2002) in onion seeds, Demirkaya (2006) in Çetinel-150 pepper seeds (Karakurt et al. 2010). Özdemir (2006) observed a positive effect on the germination rate of the kiwi (Hayward varieties) seed with low germination rate by applying hdropriming with pure water at different temperatures and different durations.

The damage of plants due to low temperature has been seen varies between species and variety. According to Oquist (1983), low temperature is a relative expression. Plants adapted

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to cold climates may photosynthesize at temperatures below 0 °C and may develop normally, while the photosynthesis mechanism may undergo irreversible damage at temperatures below 10 °C in tropical and semi-tropical climatic plants. According to Raison and Lyons (1986), the term cold damage defines the physiological damage that occurs when most tropical and semi-tropical plants are exposed to a low but not freezing temperature (usually up to 15 - 0 °C). Inhibition of photosynthesis is a precursor reaction to low temperature. The effect of cold damage photosynthesis is increases with medium or high light intensity. The damage to the cold is partly increased by free oxygen radicals that cause secondary damage to membranes and photosystems (McKersie and Leshem 1994). The increase in electrolyte output with membrane damage in tissues exposed to low temperature is the most commonly known effect of low temperature (Belous and Bondarenko 1982; Zauralov and Lukatkin 1985). Rylski (1973) in his study on pepper has been reported the flowering time and growing and development time of vegetative organs in plants increased with the decrease in air and soil temperature. Polowick and Sawhney (1985) conducted a study on Vinedale type. According to results, the low temperature (18 °C day / 15 °C at night) cause functional male infertility by abnormal such as petal leaf, male organ and female organ formation, male organ deterioration, in some cases partially carpel-like male organs have the ability to abnormal flower powder production. Researchers who determine that the ovary is larger than normal and the style extension is prevented were reported plants that grow at low temperatures produce small fruits, which are seedless, but are pollinated with flower dust from plants growing at normal and high temperatures. Aloni et al. (2001) reported that low temperature affected the carbohydrate mechanism negatively. It negatively affects the characteristics of the pollen and the germination rate and also accordingly fruit shape, shape and development are affected (Shaked et al. 2004). Farrell et al. (2006) reported that low temperature application on rice decrease the amount of flower powder and starch it accumulates. In the study on chickpea of Nayyar et al. (2005), low temperatures on the reproductive organ causes abortion and flowering and fruit attitude were negatively affected. During cold stress, due to disruption of the membrane function (cytoplasmic, mitonodrial and chloroplast membrane), photosynthesis is reduced, carbohydrate transport slows, respiration slows, protein synthesis is inhibited, the rate of disintegration of existing proteins increases, the dissolved substance exudes from the cell. Low temperature affects the activity of integral membrane proteins that regulate the transport of H+-ATPaz' carriers, enzymes, ions and other dissolved contents of required for metabolism (Taiz and Zieger 2008). In cold sensitive plants, the double layer oils have a high proportion of saturated fatty acid chains. This type of membrane reaches the semi-crystalline phase at temperatures above 0 °C. As the membrane loses its fluid, the protein components are no longer working. The membrane lipids of cold-resistant plants have generally higher unsaturated fatty acids than cold-sensitive plants (Kaçar et al. 2006). A gene was transferred from Escherichia coli to Arabidopsis in order to increase the high-melting (saturated) membrane lipids. This gene has greatly increased the resistance of gene-modified plants to low temperature (Taiz and Zieger 2008).Moran et al. (1989) have studied that effect of the study of growth and root growth of lentil (*Lens culinaris Medik*. Cv. Castellana) plant under water and alternative temperature (4-20 °C, 16/8 hours). According to this study, they determined that the osmotic potential was initially high but it was lower controls than in the alternative temperature regimen. The cellulose content of the stem cell wall was similar in all applications on the 3rd day of development, but lower than the controls. In all cases during the study period, natural non-cellulosic sugars in the cell wall decreased but in stressful roots were higher than controls. While uronic acids and protein content were higher in roots at control groups, the amount of uronic acids increased and differences of protein content was not found significant.

In this study, the application of hydropriming and the times of this application were investigated of the effects of rate and speed of the seed germination besides the development of the plant under the cold stress.

Materials and Methods

In the study, white clover (*Trifolium repens*) seedswere used as trial material. The study was carried out in the Department of Field Crops, Faculty of Agriculture, Kahramanmaras Sutcu Imam University.

Hydro-Priming Application

White clover seeds used as test material were soaked with dH2O at different times (0, 2, 4, 6, 8, 16, 18, 20, 22, 24 and 48 hours) and 4 ± 1 ºC in dark condition. Then, all seeds taken to the drying paper in the room conditions. The main aim in there is to remove the water from the seed surface. The dried seeds were placed in glass petri dishes (5x5.5 cm) in which two layers of drying paper.

Petri dishes with seeds was added 3 ml of distilled water and then these were germinated in a climatic cabinet at 15 ± 1 ºC temperature and in the dark condition. Also, untreated seeds were used as control seeds. The study was carried out with 4 replications according to the experimental design of randomized plots.

In this study, germinated seeds were counted daily and removed from petri dishes and then their radicle length was measured. This process continued until the number of seeds germinated in all petri dish was zero for 3 consecutive days (9 days). In the experiment, 1-2 mm root outlet was accepted as germinated seed.

This study was examined traits such as percentage of germination, angular transformation of germination percentage, time to germinate 50% of seeds (day), time to germinate 90% of seeds (day), time for germination of 10% of seeds (day), span value (the number of days required for seeds to reach 90% germination by 10%), total radicle length, average radicular length.

Statistical Analysis

The last germination percentage (GerY) and its angular transformation (arcsine√GerY) and also the root length of germinated seeds were determined in germinated seeds. Then, the data of obtained were analysed by SAS (SAS 1997) statistical package program. The differences between the means were tested by Fisher's smallest significant difference (LSD) test at grade of $p < 0.05$.

Results and Discussion

The results of the research have shown Table 1 and Figure 1-2 belong to white clover seeds which are germinated after hydropriming application at different times under cold stress. When the results were examined, it was observed statistically significant differences between the seeds applied of hydropriming at different times under cold stress. Accordingly, it has been observed a small but continuous decrease due to increased hydropriming time at the germination rates. It was determined that the highest germination rate with 95% was obtained from the seeds which were applied hydropriming for 0 and 2 hours while the lowest germination rate was obtained from the seeds applied hydropriming for 48 hours with 37%. The germination rates of the seeds which were applied hydropriming for other periods (4, 6, 8, 16, 18, 20, 22 and 24 hours) were 82%, 78.5%, 68.5%, 68%, 56%, 54.5%, 49.5% and 57.5%, respectively (Fig. I).

The highest root length value was obtained from seeds which were applied of hydropriming for 2 hours with 176 mm. Total root length of the seeds of control (0 hour) was obtained 149.5 mm. In the other periods (4, 6, 8, 16, 18, 20, 22, 24 and 48 hours), the total root length values from seeds which were applied of hydropriming were determined as70.75, 48.50, 44.50, 40.75, 33.50, 30.50, 32.25, 31.75 and 20.00mm, respectively (Fig. 1). At the application of hydropriming for 4 hours and more time, there have been significant decreases in the proportions total root lengths. According to obtained data, the ratio of the total root length to the total number of germinated seeds and the average root length per seed germinated (ARL) were calculated. When the ARL data were compared between the applications, the maximum root length was determined as 1.85 mm and 1.73 mm values from seeds which were applied of hydropriming for 2 hours and 4 hours , respectively, while this value was determined as 1.57 mm in the control seeds. The ARL data for other applications (6, 8, 16, 18, 20, 22, 24 and 48 hours) were determined as 1.24, 1.30, 120, 1.20, 1.12, 1.30, 1.10 and 1.08 mm, respectively (Figure 2).

At the seeds which were hydropriming applicated at different times under cold stress, germination parameters such as speed of germination, TRL, ARL and germination rate of seeds were found different ($p<0.01$). The most rapid germination (G50 = 1.55 days) in seeds germinated depending on the duration of the application was obtained from the seeds the applied of hydropriming for 2 hours. In addition, this value was determined as 1.67 days in any untreated control seeds. In the seeds germinated depending on the application time, the slowest germination was determined as 2.52 (for 22 hours), 2.50 (for 18 hours) and 2.47 days (for 20 hours), respectively. In other applications, the speed of germination which 4, 48, 16, 8, 6 and 24-hours applications was determined to 1.74, 1.85, 2.09, 2.13, 2.14 and 2.20 days, respectively. According to these results, the other times except for 24-hour application showed slower germination than control and the 2-hour application, it was determined that the germination rate was slowed continuously with little difference in increasing time. But the 24-hour application showed faster germination than 18, 20 and 22 hours application.

In the light of this data, the highest values of all germination parameters were obtained from the seeds which were subjected to hydropriming for 2 hours, and then were obtained from the seeds which were subjected to hydropriming for 4 hours. When compared with the control group, it was determined that these applications were higher than the control group. It was determined that the other applications had less but gradually decreased values in the control group.

The purpose of the application of hydropriming was to provide water transition to inside of the seed to start germination. In this study, we used hidropriming of applications under cold stress (4 ºC) and applicated different time periods in order to determine how it promote germination. According to these results, the 2-hour hydropriming application caused an increase in speed of germination, germination rate, TRL and ARL values compared to control. However, we found that the other applications decreased continuously. The germination and development in seeds is slowing due to O_2 deficiency in excess water stress. However, it changed death in plants according to the duration of exposure to excessive water stress. On the other hand, seed and plants under cold stress are getting slowing water intake and damage due to O_2 shortage usually occurs in plants after 24 hours depending on the variety and species (Taiz and Zieger 2008). This situation made us think that this hydropriming application under cold stress did not cause any damage due to O2 scarcity in seeds. The white clover seeds which the most suitable germination temperature of 20-25 ºC were determined that didn't adversely affect the seeds up to 4 hours in temperature of 4 ºC.

Even, it has been observed that hydropriming positively promotes germination but the negative effects of the 4 ºC temperature on the germination. And root growth in the white clover seeds were found to be lower gradually after 4 hours. In the light of previous studies by many researchers, this situation has been disclosed as increase in electrolyte output by membrane destruction in tissues exposed to low temperatures (Belous and Bondarenko 1982; Zauralov and Lukatkin 1985), negative effects on carbohydrate metabolism (Aloni et al. 2001), decreased photosynthesis, slow down transport of carbohydrates, slowing of respiration, inhibition of protein synthesis, increasing the rate of fragmentation of existing proteins, leakage of dissolved substances from cells, the physical properties of the lipids involved in the membrane structure affect the activity of H+-ATPaz' carriers, integral membrane proteins that regulate the transport of enzymes, ions and other dissolved substances necessary for metabolism (Taiz and Zieger 2008), osmotic potential during root growth and reduction of cellulose content of root cell wall (Moran et al. 1989).

Table 1. Data on germination performance of white clover seeds of cold stress pre-treatment at different times

Application	Germination Rate (GerY)							
	%	[GerY]	G_{50}	G_{90}	G_{10}	Span	TRL	ARL
0 hour	95.00	61.22^{A}	1.67^{CD}	2.94^{DE}	1.10^{AB}	1.84^{E}	74.75^{B}	1.57^{B}
2 hours	95.00	62.97^{A}	1.55^{D}	2.62^{E}	1.00^{B}	1.62^{E}	88.00^{A}	1.85^{A}
4 hours	82.00	53.43^{B}	1.74^{BCD}	3.60^{CD}	1.09^{AB}	2.51^{DE}	70.75^{B}	1.73^{AB}
6 hours	78.50	51.80^{B}	2.14^{ABC}	4.11^{BC}	1.14^{AB}	2.97^{CD}	48.50^{C}	1.24^{CDE}
8 hours	68.50	48.17^{BC}	2.13^{ABC}	4.48^{ABC}	1.03^{AB}	3.44^{BCD}	44.50^{C}	1.30^{C}
16 hours	68.00	48.03^{BC}	2.09^{ABC}	4.12^{BC}	0.91^{B}	3.22^{BCD}	40.75^{CD}	1.20^{CDE}
18 hours	56.00	43.85^{CD}	2.50^{A}	4.99^{AB}	1.19^{AB}	3.80^{ABC}	33.50^{D}	1.19^{CDE}
20 hours	54.50	43.40^{CD}	2.47^{A}	4.78^{AB}	1.31^{A}	3.48^{BCD}	30.50^{D}	1.12^{CDE}
22 hours	49.50	41.83^{DE}	2.52^{A}	5.13^{AB}	1.09^{AB}	4.03^{AB}	32.25^{D}	1.28^{CD}
24 hours	57.50	44.28^{CD}	2.20^{AB}	4.85^{AB}	1.06^{AB}	3.79^{ABC}	31.75^{D}	1.11^{DE}
48 hours	37.00	37.44^{E}	1.85^{BCD}	5.19^{A}	0.46^{C}	4.73^{A}	20.00^{E}	1.09^{E}
p>0.001		**	**	**	**	**	**	**

**: significant at p <0.01; §, GerY: Percentage of germination; [GerY]: Angular transformation of germination percentage; G50: Time to germinate 50% of germinated seeds (day); G90: Time to germinate 90% of germinated seeds (day); G10: Time for germination of 10% of germinated seeds (day); Span: The number of days required for germinating seeds to reach 90% germination by 10%; TRL: Total radicle length of germinated seeds; ARL: Average radicular length of germinated seeds

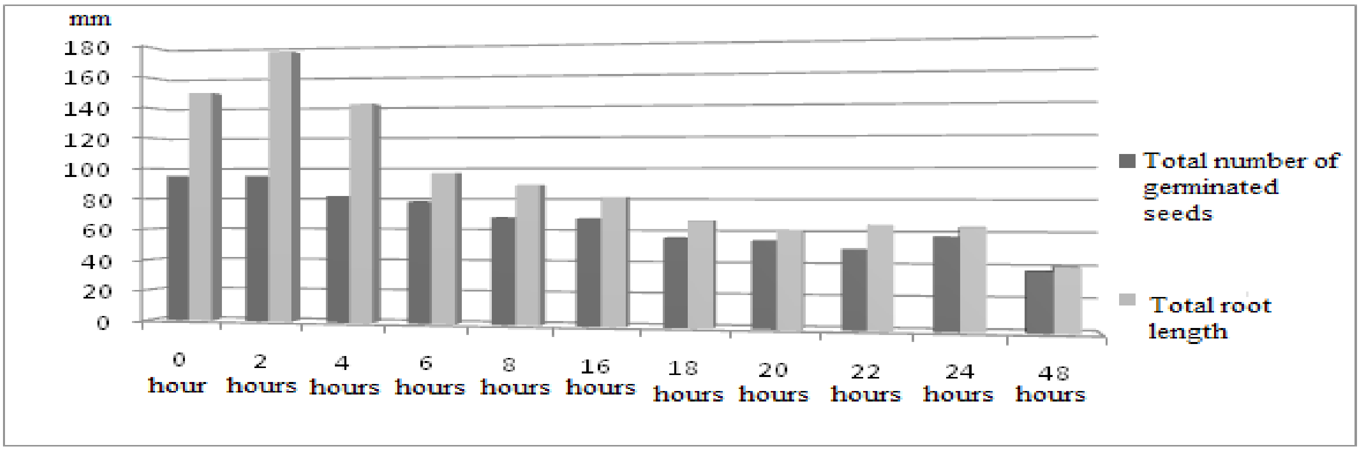

Figure 1. The means values of total germinated seeds and total root length data of germinated seeds

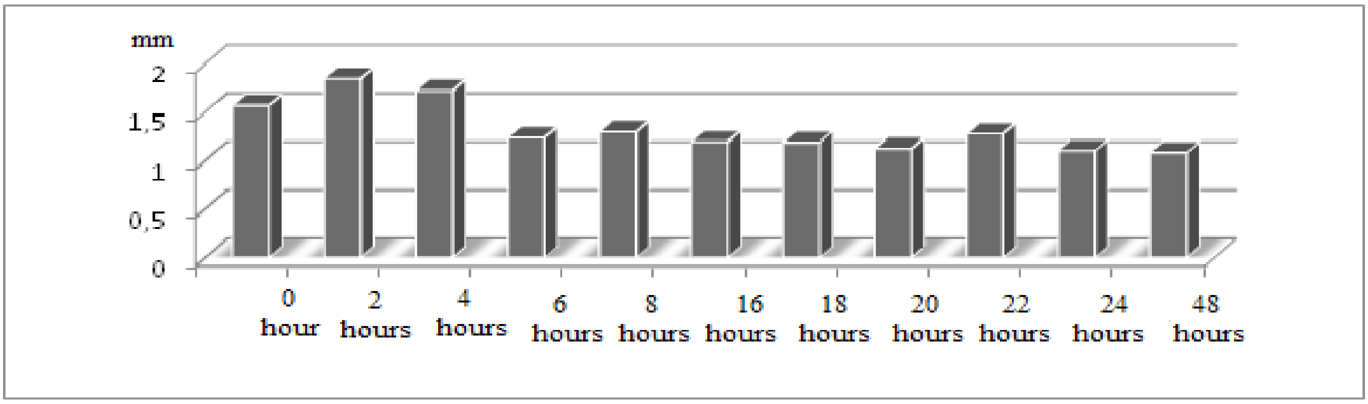

Figure 2. The means of average root length data per seed germination

Compliance with Ethical Standards

Conflict of interest
The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution
The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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Research Article

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Determination of fatty acids profiles and volatile compounds of cows' and goats' butters

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Abstract

In this study, the fatty acid composition and volatile components of cows' and goats' yayik butters were analyzed. For this purpose, analyzes in cows' and goats' yayik butter obtained from local markets in Adıyaman province were performed. Saturated fatty acids (SFAs) were determined as dominant fatty acids in the butter samples. While the highest SFA content was detected in goats' butters, the highest unsaturated fatty acid (UNSFA) and monounsaturated fatty acid (MUFA) contents were in cow samples. Furthermore, some individual fatty acid concentrations (tridecanoic, behenic and α-linolenic) of cows' and goats' butters were significantly different. Additionally, the total 27 volatile compounds including alcohols, aldehydes, acids, esters, hydrocarbons, ketones and terpenes were quantified in samples. The most important volatile compounds in samples were oxylene, mxylene, butyric, caprylic and valeric acids.

Keywords: Yayik butter, Fatty acids, Volatile components, Cows' milk, Goats' milk

Introduction

Cream and yogurt are used as raw material in butter production and the butter made from yoghurt is called "yayik butter". Yayik butter is one of the popular dairy products in Turkey due to its unique flavor and aroma and it is traditionally produced by farmers in Anatolia for many centuries. "Yayik" is the name given to the churning of the yogurt during the production. Yogurt, which is raw material in the production of yayik butter, is obtained from different animal milk such as goats' and cows' milk (Sağdiç et al., 2002; Sağdıç et al., 2004). Goat breeding is still being maintened in southeastern region of Anatolia and goat's milk butter is produced in this region. It is well known that the butters produced from goats' milk have some characteristic features. Although, goat's butter is more preferred by some consumers than butter of other mammal milks (Hayaloglu and Karagul-Yuceer, 2011), it is not commercially produced in significant amount (Haenlein, 2004).

Milk fat contains more than 400 different fatty acids including saturated fatty acids, monounsaturated fatty acids and polyunsaturated fatty acids (Méndez-Cid et al., 2017). The certain fatty acids have benefical or potentially harmfull effects on human health (Pegolo et al., 2016). In the evaluation of the quality properties of butter, it is critical to define the content of fatty acids. Furthermore, milk fat is important in the formation of aroma components in butter because of the distinct flavor and low aroma thresholds of fat-derived compounds (Vagenas and Ioannis, 2012).

There has been some studies about butter and yayik butter produced from milk of different species of mammals (Sağdıç et al., 2004; Atamer et al., 2007; Şenel et al., 2011; Tahmas Kahyaoğlu, 2014). On the other hand, there has been investigating studies (Tosun, 2016; Haddar, 2017; Ergöz, 2017) examined the physical and chemical properties, fatty acid profiles and aroma compounds of the yayik butter. However, in our knowledge, there is little study investigating the volatile compounds and fatty acids of yayik butter produced from different animal milks. In this study, fatty acid compositions and volatile compounds of goats' and cows' yayik butter were investigated

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Materials and Methods

Material

Eight goats' and cows' yayik butter provided from Adıyaman province of Turkey was used as material.

Determination of fatty acids (FA) profile of yayik butter

Proportional FA compositions of butter were determined in their FA methyl esters (FAME) according to Bannon et al., (1982) and Ackman (1998). The FAME examined by a GC (Agilent 7697A, Agilent Technologies, USA) equiped with a flame ionization detector. The FAME separated with a capillary HP-FFAP column (J&W 19091F – 433, Agilent Technologies, USA; 30 m×0.25 mm i.d; 0.25 µm film thickness). Each sample was injected twice by the GC auto sampler.

Determination of volatile compounds of yayik butter

Injections of the samples into the gas chromatography system and characterization of aroma in the mass spectrometry (GC/MS), identification of the volatile components and determination of retention times were carried out according to Whetstine et al., (2003).

Extraction of volatile components

Extraction of volatile components in butter was performed by Solid Phase Micro Extraction (SPME) method (Stashenko and Martínez, 2007). 10 g samples were weighed into 40 mL amber vials and 1 g of NaCl (10% of the sample amount) and 10 µL internal standard (81 ppm, 2 methyl 3-heptanone + 2-methyl pentanoic acid) were added. The closed vials were stored in -25 ºC deep freezer until analysis. Prior to injection into the gas chromatographic system, the vials were allowed to equilibrate the volatile components for 30 minutes at 40 ºC in the SPME extraction system. After that, 50/30 µm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS, Agilent, USA) was placed on fiber for 30 minutes with fiber for adsorption of volatile components.

Determination, identification and quantification of volatile components

Separation of volatile components was carried out with GC (Agilent 7890A, USA) fitted with flame ionization detector and mass spectrometer. Desorption of the volatile components to injection block was executed with SPME method, manual splitless mode and fiber at 250 ºC kept to desorption for 10 min. The volatile components separated in HP Innowax column (30 m x 0.25 mm x 0.25 µm) were determined by scanning in mass spectrometer (Agilent GC5975 C MSD, USA) at a range of 30-300 eV.

Statistical analysis

Descriptive statistics and independent sample t-test by using SPSS packet program were performed.

Result and Discussion

Fatty acid profiles of yayik butters

Fatty acid (FA) contents of cow and goat samples are shown in Table 1. Saturated fatty acids (SFA) were detected as dominant FAs in cow and goat samples.

It has been previously stated that saturated fatty acids were dominant fatty acids in cows' and goats' milks (Paszczyk et al., 2019). In addition, the total SFA content of goat samples (71.15%) was statistically higher ($P<0.05$) than cow samples (65.29%). Ruminant milks contain higher levels of SFA due to the biohydrogenation of unsaturated fatty acids which is responsible for reducing the toxic effects of unsaturated fatty acids on the development of rumen bacteria (Shingfield et al., 2008). Similarly, Bernard et al., (2018) stated milk of ruminants contains a high percentage of saturated FAs, which accounts for about 70 % of the total FAs. Furthermore, cow samples were characterized by significantly higher ($P<0.05$) contents of unsaturated fatty acids (UNSFA, 34.56%) and monounsaturated fatty acids (MUFA, 29.21%) compared to goat samples (UNSFA, 28.54%; MUFA, 24.11%). Moreover, the ratios of SFA/UNSFA and linoleic/α-linolenic acid (LA/ALA) in cow samples were found to be lower ($P<0.05$) than goat samples. Very high ratio of LA/ALA promotes many diseases such as cardiovascular disease, cancer and inflammatory diseases (Paszczyk et al., 2019) and the optimal ratio of LA/ALA can be ranges from 1:1 to 4:1 depending on the disease considered (Simopoulos, 2002).

Major individual FA in samples was identified as palmitic acid (C16; cow samples, 30.47; goat samples, 30.89%) and this is in agreement with Al-Khalifah and Al-Kahtani (1993) and Paszczyk et al., (2019). The second of the major FAs in cows' and goats' yayik butters was elaidic acid (C18:1n9t), which is monounsaturated trans FA. Trans unsaturated fatty acids in ruminants are synthesized in the rumen by microbial hydrogenation and they have been reported in butter to be between 4-11 % depending on region and season (Sommerfeld, 1983). However, it is seen that the elaidic acid content (cow samples, 24.19; goat samples, 19.91%) in our samples is considerably higher than this value. Furthermore, this can be attributed to the reduction of fiber content and the increase of grain content in animal feeding (Haenlein, 2004). Other FAs, which are determined at the highest amount, were myristic (C14) and stearic (C18) acids. The total amount of palmitic, elaidic, myristic and stearic FAs in the total FAs was 76.82% and 73.16% in cow and goat samples, respectively. The contents of individual dominant FAs were in agreement with to the results reported by Atasoy and Türkoğlu (2010) for Sanlıurfa butter oil. Furthermore, myristic, palmitic and stearic acids were accounted for approximately 53% of total FAs for cow and goat samples.

The tridecylic (tridecanoic; C13), behenic (docosanoic; C22) and α-linolenic acid (C18:3n3) concentrations of cow and goat samples were significantly different ($P<0.05$). Goat samples (0.99%) were characterized by statistically higher ($P<0.05$) contents of tridecylic acid compared to cow samples (0.23%). In addition to these, behenic acid (0.10%) and α-linolenic acid (0.42%) contents in goat samples were found to be lower ($P<0.05$) than cow samples (behenic, 0.29%; α-linolenic, 0.86%). The difference of FAs concentrations may be explained by variations of physiological and anatomical between cow and goat (Haenlein, 2004). Short and medium FAs originated from *de novo* synthesis in the mammary gland, while long FAs arising from either body fat mobilization or dietary fat (Lucas et al., 2008).

In order to better characterize the health effects of fatty acids, indices such as atherogenicity index (AI) and desaturation index (DI) have been proposed (Ulbricht and Southgate, 1991). The low AI is desirable in milk and dairy products to provide protection against coronary heart disease (Paszczyk et al., 2019). AI was found to be 2.24±0.20 and 2.82±0.26 in cow and goat samples, respectively. Similar results were observed by Paszczyk et al., (2019) and Blasko et al., (2010) for butters. Also, the DI expresses the concentration of the unsaturated product proportional to the sum of the unsaturated product and the saturated substrate (Schennink et al., 2008). DI-14, DI-16 and DI-18 were calculated as average 0.08 and 0.078, 0.046 and 0.034, 0.129 and 0.112 in cow and goat samples, respectively.

Volatile Compounds of Yayik Butters

Total concentrations of volatile compounds are shown in Table 2. Total 27 components including alcohols (4), aldehydes (1), acids (9), esters (2), hydrocarbons (6), ketones (3) and terpens (2) were identified in all samples. In a study (Haddar, 2017), 35 aroma components containing acid, alcohol, ketone, aldehyde, ester, alkane, alkene and various compounds were detected in yayik butter produced with different starter cultures. Major components were determined as oxylene, mxylene, valeric, caprylic and butyric acid in cows' yayik butters. In goats' yayik butters, dominant volatile components were quantified as butyric, caprylic, valeric acid, oxylene, mxylene, capric and acetic acid, respectively. Ergöz (2017) reported the major volatile components in buffalo yayik butter were butyric acid, 2-methylbutanoic, 2-nonanone, butyl alcohol, gamma-terpinene, hexanoic acid, n-hexanol, styrene. Furthermore, components such as acetic acid, butyric acid, caprylic acid, 2-methylbutanoic acid, ethylbenzene, styrene, 2-nonanone, 2-undecanone, which are detected by Ergöz (2017) in buffalo yayik butter, were common to our results. Some volatile components identified in our examples such as acetic acid, butyric acid, valeric acid, lauric acid, ethanol, 2-propanol, benzaldehyde, ethylbenzene, limonene were also determined by Senel et al., (2016) for yayik butter.

Alcohols including ethanol, 2-propanol and 3-methyl-2-butanol in yayik butter samples were detected. Furthermore, 2,3 butanediol was only detected in cow samples. Alcohols in dairy products are produced by many metabolic pathways such as lactose metabolism, methyl ketone degradation, amino acids metabolism (Molimard and Spinnler, 1996). Moreover, the native flora of milk plays an important role in the formation of alcohol in dairy products. Despite the ethanol is the precursor of ethyl esters, it has a limited aromatic role in dairy products (Molimard and Spinnler, 1996). In addition, branched-chain alcohols, such as 3-methyl-2-butanol, have been generally accepted as off-flavours components in milk (Centeno et al., 2003). Only benzaldehyde from aldehydes has been identified in cow and goat samples. This can be thought to result from the conversion of reactive aldehydes to alcohol or acid components (Molimard and Spinnler, 1996).

Table 1. Fatty acid acid profiles of cows' and goats' yayik butters ((w/w) %, mean±std error)

Fatty acids	Cows' Yayik Butter			Goats' Yayik Butter			p-value
	Mean	Minimum	Maximum	Mean	Minimum	Maximum	
C4	2.30±0.25	1.45	3.53	2.33±0.24	1.38	3.13	0.93
C6	1.35±0.13	0.57	1.90	1.40±0.28	0.16	2.55	0.88
C8	1.00±0.08	0.52	1.32	1.66±0.44	0.23	3.53	0.18
C10	2.22±0.12	1.60	2.57	5.38±1.55	1.28	12.07	0.08
C11	nd	nd	nd	nd	nd	nd	-
C12	2.71±0.12	2.31	3.32	3.70±0.37	2.50	5.10	0.03
C13	0.23±0.05	nd	0.49	0.99±0.32	nd	2.36	0.05
C14	10.53±0.53	8.47	13.02	10.70±0.55	9.12	14.10	0.83
C14:1	0.96±0.14	0.57	1.54	0.94±0.19	0.18	1.69	0.93
C15	1.35±0.09	0.87	1.80	1.32±0.14	0.79	1.93	0.86
C15:1	nd	nd	nd	nd	nd	nd	-
C16	30.47±1.30	25.56	38.22	30.89±1.40	24.60	36.58	0.83
C16:1	1.51±0.23	0.92	2.99	1.15±0.19	0.44	2.10	0.25
C17	0.75±0.03	0.58	0.91	0.69±0.11	0.16	1.21	0.62
C17:1	0.42±0.04	0.25	0.63	0.38±0.07	0.18	0.73	0.61
C18	11.63±0.86	6.54	13.91	11.66±0.50	8.73	13.32	0.98
C18:1n9c	1.80±0.26	0.65	2.86	1.47±0.18	0.56	2.20	0.32
C18:1n9t	24.19±1.00	19.18	28.91	19.91±1.79	14.73	29.68	0.06
C18:2n6c	0.58±0.18	0.20	1.66	0.47±0.08	0.22	0.90	0.61
C18:2n6t	2.19±0.14	1.41	2.65	1.95±0.14	1.40	2.67	0.26
CLA (C18:2 cis 9, trans 11)	0.28±0.03	0.17	0.44	0.24±0.03	0.11	0.36	0.38

CLA (C18:2 trans 9, cis 11)	0.30±0.03	0.20	0.43	0.32±0.05	0.13	0.51	0.72
C18:3n6	0.41±0.04	0.27	0.64	0.37±0.08	0.16	0.75	0.62
C18:3n3	0.86±0.07	0.59	1.12	0.42±0.06	0.19	0.71	0.001
C20	0.33±0.02	0.25	0.41	0.31±0.03	0.18	0.44	0.56
C20:1	nd	nd	nd	nd	nd	nd	-
C20:2	nd	nd	nd	nd	nd	nd	-
C20:3n6	nd	nd	nd	nd	nd	nd	-
C20:3n3	0.05±0.04	nd	0.29	0.14±0.10	nd	0.80	0.43
C20:4n6	0.32±0.06	0.11	0.53	0.22±0.04	nd	0.35	0.18
C20:5n3	0.32±0.05	nd	.49	0.22±0.06	nd	0.50	0.25
C21	nd	nd	nd	0.02±0.02	nd	0.14	0.35
C22	0.29±0.07	nd	0.61	0.10±0.05	nd	0.32	0.05
C22:1n9	0.35±0.08	nd	0.80	0.27±0.07	nd	0.44	0.51
C22:1	nd	nd	nd	nd	nd	nd	-
C22:2	nd	nd	nd	nd	nd	nd	-
C22:6n3	0.05±0.05	nd	0.37	0.09±0.09	nd	0.73	0.67
C23	nd	nd	nd	nd	nd	nd	-
C23:1	nd	nd	nd	nd	nd	nd	-
C24	nd	nd	nd	nd	nd	nd	-
C24:1	nd	nd	nd	nd	nd	nd	-
SFA	65.29±1.31	59.67	71.59	71.15±2.06	62.54	78.05	0.03
UNSFA	34.56±1.34	28.02	40.25	28.54±2.03	21.82	37.46	0.03
MUFA	29.21±1.03	24.30	33.00	24.11±1.96	18.04	33.57	0.04
PUFA	5.35±0.43	3.72	7.25	4.44±0.27	3.38	5.33	0.10
SFA/UNSFA	1.92±0.12	1.48	2.55	2.62±0.26	1.67	3.58	0.03
LA/ALA	2.33±0.20	1.37	3.16	3.77±0.60	1.40	6.75	0.04
AI	2.24±0.20	1.53	3.34	2.82±0.26	2.09	4.24	0.10
Total ω-3	1.27±0.13	0.92	2.00	0.87±0.16	0.49	1.67	0.07
Total ω-6	2.90±0.31	1.73	4.67	2.71±0.18	2.01	3.51	0.61
Total ω-9	2.14±0.31	0.65	3.35	1.74±0.23	0.56	2.58	0.32
DI-14	0.084±0.013	0.051	0.150	0.078±0.013	0.018	0.126	0.74
DI-16	0.046±0.004	0.032	0.073	0.034±0.004	0.016	0.056	0.12
DI-18	0.129±0.012	0.090	0.179	0.112±0.014	0.047	0.171	0.39

SFA: sum of saturated fatty acids, UNSFA: sum of unsaturated fatty acids, MUFA: sum of monounsaturated fatty acids, PUFA: sum of polyunsaturated fatty acids, CLA: Conjugated linolenic acid, LA: linoleic acid, ALA: α- linolenic acid, AI: atherogenicity index (sum of lauric (C12:0), palmitic (C16:0), and 4 times myristic acid (C14:0) contents divided by the unsaturated fatty acids content), **ω:** omega, DI: desaturation index (DI-14: C14:1/(C14:0 + C14:1); DI-16: C16:1/(C16:0 + C16:1); DI-18:C18:1n9c/(C18:0 + C18:1n9c)), nd: not detected.

Butyric, valeric, caprylic, capric, lauric, benzoic, acetic, propionic and 2-methylbutanoic acids were quantified in all samples. In cow samples, valeric, caprylic, butyric and capric free fatty acids were dominating, respectively. Butyric, caprylic, valeric and capric free fatty acids in goat samples were the major free fatty acids. Most of the detected major free FAs have previously been reported as dominant free fatty acids in butters (Şenel et al., 2011; Méndez-Cid et al., 2017; Iradukunda et al., 2018). Lipase or esterase enzymes are responsible for lipolysis, which is responsible for the formation of free FAs. The source of these enzymes is milk itself (lipoprotein lipase) or psychrotrophic microorganisms (Vagenas and Ioannis, 2012). The short and medium chain free fatty acids are mostly formed by lipoprotein lipase (Collins et al., 2003). Goat samples had a higher amount of short and medium chain free fatty acids than cow samples. This may be related to the higher concentration of lipoprotein lipase in the cream phase in goat milk compared to cow's milk (Chilliard et al., 1984). Similarly, Şenel et al., (2011) reported that level of total free fatty acids was higher in goats' yayik butter. Although the presence of short-chain free fatty acids plays an important role in the characteristic flavor of goats' milk products, the high amount of free fatty acids is associated with hydrolytic rancidity (Chilliard et al., 1984). The long-chain free fatty acids have little effect on flavor due to their high detection thresholds. They also serve as precursors to other aroma components such as esters, ketones, aldehydes (Vagenas and Ioannis, 2012).

Table 2. Volatile compounds of cows' and goats' yayik butters (ppm, mean±std error)

	Cows' Yayik Butter			Goats' Yayik Butter			
	Mean	Minimum	Maximum	Mean	Minimum	Maximum	p-value
Alcohols							
Ethanol	24.92±9.98	nd	75.61	5.08±4.12	0.00	33.18	0.10
2,3 butanediol	1.36±0.89	nd	5.73	nd	nd	nd	0.17
3-methyl-2-butanol	17.22±10.51	nd	78.10	4.21±4.21	nd	33.67	0.28
2-propanol	68.06±36.53	nd	246.81	38.36±29.12	nd	231.42	0.54
Aldehydes							
Benzaldehyde	2.53±1.72	nd	12.56	15.76±12.13	nd	99.99	0.30
Acids							
Acetic acid	78.90±25.27	13.84	230.32	190.62±107.50	10.79	897.19	0.33
Propionic acid	5.34±3.51	nd	23.12	nd	nd	nd	0.17
Butyric acid	106.96±44.95	nd	401.73	527.11±337.50	17.43	2781.340	0.26
Valeric acid	183.73±40.59	48.88	351.23	441.95±292.51	0.00	2393.53	0.41
Caprylic acid	143.89±33.04	57.56	284.98	461.75±238.76	31.15	2000.56	0.23
Capric acid	88.95±18.85	28.100	157.69	202.47±64.16	25.42	481.14	0.13
Lauric acid	10.07±8.47	nd	67.97	69.79±29.83	nd	259.01	0.09
Benzoic acid	8.43±7.20	nd	58.31	37.51±20.86	nd	158.84	0.22
2-methylbutanoic acid	2.71±2.71	nd	21.68	3.67±2.43	nd	16.45	0.80
Esters							
Methyl caproate	nd	nd	nd	7.84±4.90	nd	13.88	0.15
Ethyl oleate	62.99±34.21	nd	256.81	20.30±20.30	nd	162.36	0.30
Hydrocarbons							
Hexane	14.01±12.43	nd	100.40	71.62±62.00	nd	501.14	0.38
Heptane	23.94±13.28	nd	99.03	68.94±26.92	nd	179.11	0.16
Ethylbenzene	19.42±4.80	nd	38.26	21.74±3.31	10.10	40.63	0.70
Mxylene	157.94±29.39	43.05	294.40	238.61±52.78	50.46	515.94	0.20
Oxylene	230.06±66.49	41.55	658.64	244.67±44.36	122.82	500.38	0.86
Styrene	51.13±8.22	12.43	95.56	68.34±9.40	29.80	103.44	0.19
Ketones							
3-hydroxy-2-butanone	2.23±1.46	nd	9.38	5.33±3.02	nd	23.50	0.37
2-Nonanone	6.91±2.96	nd	22.22	13.20±3.58	nd	25.65	0.20
2-undecanone	3.82±1.63	nd	11.48	2.86±2.02	nd	21.68	0.72
Terpens							
Limonene	2.45±1.24	nd	8.35	3.20±1.24	nd	7.67	0.67
Beta-Caryophyllene	1.23±0.81	nd	5.15	3.76±1.70	nd	13.44	0.20

nd: not detected

Ethyl esters of fatty acids contribute to the aroma of dairy products at low levels, while at higher levels it causes a flavor defect called "fruity". The presence of high amounts of ethyl esters of long chain fatty acids (C12 or above) can give the product an undesirable soapy, tallowy odor (Liu et al., 2004). Ethyl oleate which is one of the long chain free fatty acid ethyl esters was detected in cow and goat samples. Particularly high amounts were detected in goat samples. Methyl caproate was found only in goat samples. 3-hydroxy-2-butanone, 2-nonanone and 2-undecanone from ketones in samples were quantified at low amount. Ketones can be perceived at low levels and cause typical odors such as fruity, floral, and musty (Vagenas and Ioannis, 2012). Moreover, ketones such as 2-nonanone and 2-undecanone are known to contribute to the aroma of dairy products (Vagenas and Ioannis, 2012).

Hydrocarbons including hexane, heptane, ethylbenzene, mxylene, oxylene and styrene were identified in the butter samples. The concentrations of hydrocarbons, particularly xylene and mxylene, were quite high in butter samples. Hydrocarbons, the secondary products of lipid autoxidation, do not directly affect the aroma, but lead to the formation of other aroma components (Bintsis and Robinson, 2004). Limonene and beta-caryophyllene were determined at low levels in yayik butter samples. Terpenes, which are found as secondary metabolites in plants, are transferred directly to the milk with feeding and they are important for determining the geographical origin (Bontinis et al., 2012). Particularly, terpenes such as limonene are a known component of citrus essential oils (Condurso et al., 2008).

Conclusion

The fatty acid compositions and volatile compounds of goats' and cows' yayik butters were investigated in this study. About 60-70% of the identified fatty acids in samples were saturated fatty acids and saturated fatty acids in goats' yayik butters were found higher than cows' butters. Myristic, palmitic and stearic acids were major saturated fatty acids in all samples. Furthermore, goats' and cows' yayik butter samples were different in tridecylic, behenic and α-linolenic acid contents. Total 27 volatile components including alcohols, aldehydes, acids, esters, hydrocarbons, ketones and terpens were quantified in samples. In general, acids and hydrocarbons were the most important volatile components in all samples. Furthermore, dominant volatile compounds in samples were detected as oxylene, mxylene, butyric, caprylic and valeric acids.

In addition to these, it has been found that the fatty acids and volatile compounds of the samples changed within a very wide margin. These can be explained by the fact that production is mostly done by local farmers at home and there is no standard production technique. Further studies require to investigation of standard yayik butter production. Moreover, future studies should be focused to investigate the fatty acid profiles and flavor components in yayik butters from a wider region at different time intervals.

Compliance with Ethical Standards

Conflict of interest

The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Author contribution

The contribution of the authors is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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Single pixel scanning based millimeter wave imaging

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Abstract

A food processing line is comparatively vulnerable to contaminants, whether through inattentive workers and employees or from the processing machines made largely out of steel or from raw materials that are contaminated themselves. Any of these risks can affect quality resulting in poor hygiene or even can put customer health in danger. Millimeter-wave imaging plays a significant role in many fields such as security inspection and medical diagnostics. Moreover, millimeter wave instruments are useful in detecting objects behind and inside visibly opaque barriers such as, concrete walls and plastic boxes. We describe a millimeter wave imaging system that uses a one-dimensional detector in combination with a single pixel mask in order to acquire two-dimensional images out of buried objects inside a bread. Our system can be used to detect metals and salty substances for bread quality control and defect detection. The system uses 60 GHz center frequency to image the target in transmission mode which utilizes a heterodyne sub-harmonic receiver placed in a bi-static configuration.

Keywords: Single pixel, Millimeter wave, Non-intrusive quality control, Foreign object defect detection

Introduction

The invention of cameras has made image retrieval an important research topic. Detector arrays have been used in modern digital cameras to retrieve images. Many digital cameras and cellphones use the technology of complementary metal-oxide-semiconductor (CMOS) and charge-coupled-devices (CCDs) which allow them to take pictures consisting of millions of pixels coerced in a microcircuit no larger than a millimetric spot. Modern digital cameras contain over twenty million pixels, in fact integrating more than this seems out of necessity and a waste of data storage.

Conventional imaging in the spectrum region lower than 30 GHz is impractical due to large apertures needed. Since 1940s, it is known that equipment operating in this part of the spectrum (above 30 GHz) provides excellent penetration of the atmosphere and other inhibitors. In this study, we are mainly interested in systems operating at frequencies higher than 30 GHz and having smaller apertures, therefore a frequency above 30 GHz can readily penetrate a bread which allows to image buried objects inside it.

Single pixel (SP) imaging techniques allow to record an object without a direct line of sight with the object. The earliest technique introduced with SP imaging has been named ‘flying-spot’ that is patented in 1884 by Nipkow who offered the use of a perforated disk to modulate a light source for scanning light spots across a scene (Sen, 2005). Many sophisticated flying-spot cameras were presented later, however this technique is not preferable as it suffers from low signal-to-noise ratio (SNR). It is feasible to reconstruct an image with a SP detector (Pittman, 1995; Shapiro, 2008; Duarte, 2008; Bromberg, 2009). The modern technologies use spatial light modulation range from mechanical masks (Heidari, 2009) to micro-opto-electro-mechanical systems (Coltuc, 2015) and metamaterials using SP algorithm to reconstruct the scene (Bai, 2017). SP camera is a distinct structure for imaging systems, so random masks for imaging based on compressive sensing has been offered by (Chan, 2008).

Millimeter wave and THz imaging add significant contributions to visible, IR and X-ray imaging systems. The advantage of millimeter wave propagation is that it can be used in

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low visibility situations in addition to the fact that it is feasible to work both day and night. Millimeter-wave imaging is an emerging field for rapid imaging systems, since millimeter waves can diffuse through materials such as clothes and polymers for security and detection applications.

In this paper, single pixel scanning based millimeter wave images are obtained for buried objects inside a bread in which screw and salt (inside a nylon transparent plastic bag) are buried respectively for defect detection inside the sample bread. For detecting these objects, a 60 GHz center frequency system in addition with a single pixel mask has been used. Printed circuit board (PCB) covered with copper has been utilized as a mask and a hole with the size of 6.25×6.25 mm2 single pixel exists (without copper) to allow the millimeter wave beam pass through it. The object is mechanically scanned by the single pixel mask in a zig-zag pattern to acquire a two-dimensional image.

Theoretical Background

SP masking technique is applied for the data collected by a SP detector. However, the source and detector positions and the millimeter wave beam are static. The SP mask is formed to obtain the original image. Therefore, the original image is scanned through the mask with a SP hole, in order to acquire corresponding parts of the original image with the size of the specified pixel hole on the PCB mask. For each movement of the mask, a part of the target is recorded through the SP hole and these portions are added to obtain the original image depicted in Figure 1.

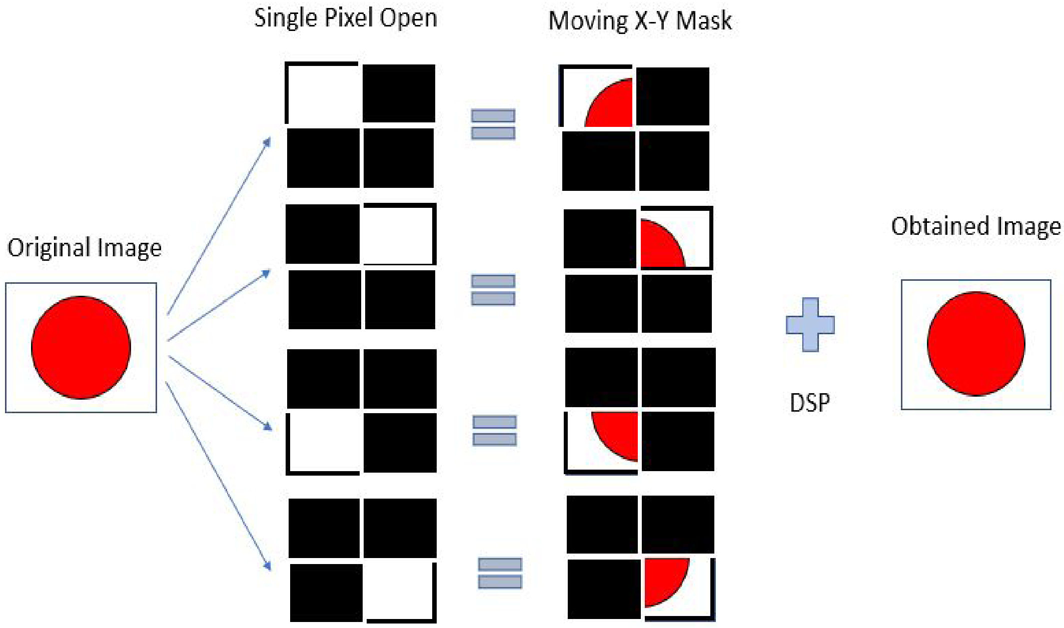

Figure 1. For the original image reconstruction, the single pixel mask is moved in X and Y directions in order to acquire two-dimensional image. The obtained data through single pixel is collected hence, original image is obtained.

Experimental setup

The experimental setup is essentially made of a millimeter wave transmitter, receiver, horn antennas and lenses, where the imaging system operates at a central frequency of 60 GHz. The object is imaged using a frequency-modulated signal. A 10 GHz local oscillator signal is generated using an yttrium iron garnet (YIG) that is amplified with an RF power amplifier. Following that, the amplified signal is multiplied by an RF tripler. The generated 30 GHz signal is modulated through an RF mixer. The modulated signal is then doubled by a sub-hormonic mixer. The 60 GHz signal is transmitted to the collimating lens via the horn antenna. The resulting signal is passed through the single pixel mask filter. Finally, the resulting signal is passed through the single pixel mask which is collected and detected on the receiving antenna by the collecting lens.

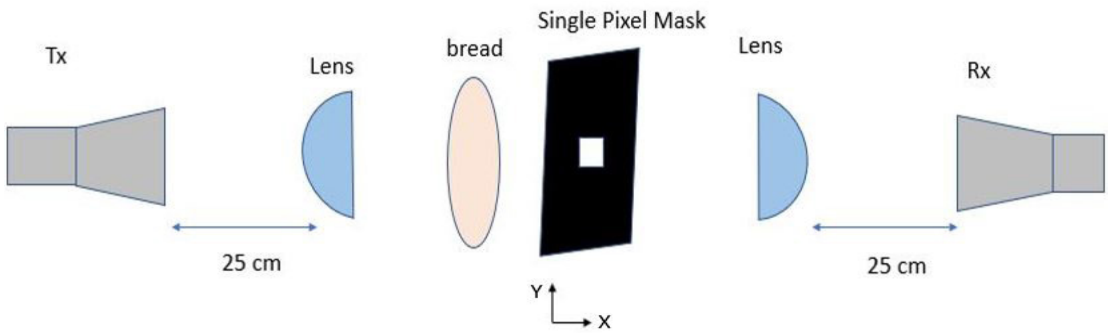

Figure 2. A basic schematic diagram of the millimeter wave system

The signal coming from the transmitting antenna is aligned parallel to the optical axis with the collimating lens located at a distance of 25 cm. The incoming signal illuminates the field of view of the object plane whose image is going to be obtained. The collimated signal is then passed through the single pixel, which is focused to the receiver. However, the collimating lens is made of HDPE material with a diameter of 20.5 cm and a focal length of 25 cm.

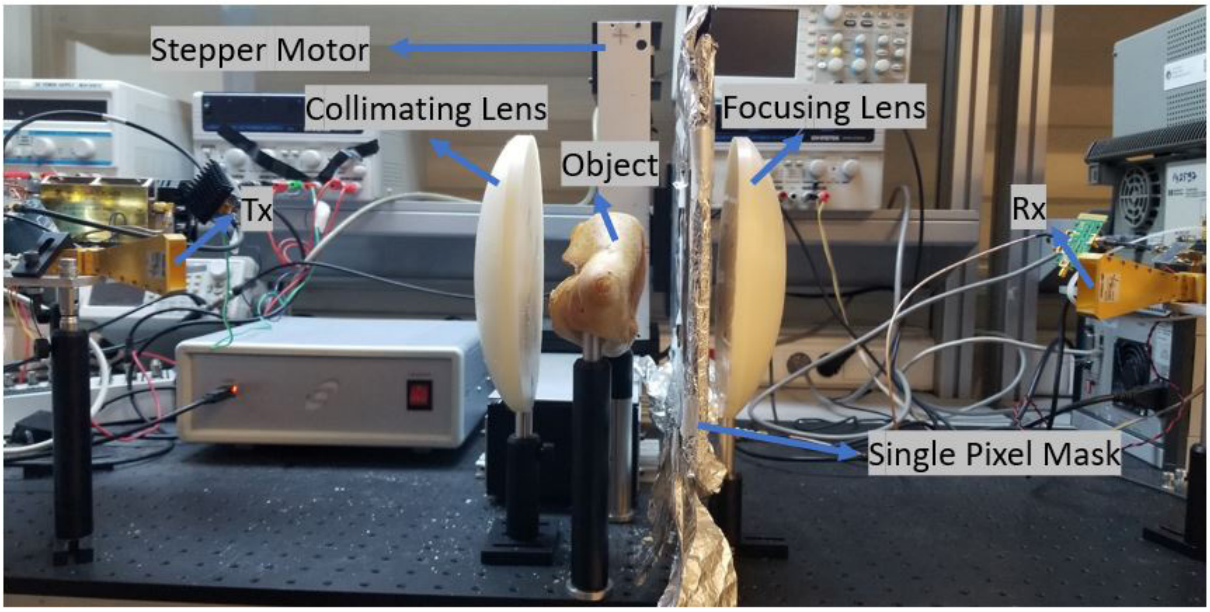

Figure 3. Experimental setup of the system

The SP mask is in the imaging plane and scanned in the x-y direction. However, a two-dimensional movement (x-y) is required in order to form the image in the SP detector. The image passed through the SP is focused by the lens which is located 25 cm away from the receiving antenna. The receiver side lens is made of POM material which directs and focuses the incoming wave to the receiver. The refractive index of the lenses is approximately n = 1.54 and the radius is r = 40 cm. A medium frequency signal power is obtained at the receiver and converted to DC voltage via a logarithmic RF power detector. The pixel size of each mask is selected as 6.25 x 6.25 mm^2 in order to reduce the diffraction effect in the system with 60 GHz center frequency and 5.0 mm wavelength. Due to the 5 x 10 cm^2 viewing area of the sample, the resulting images were formed with a resolution of 8 x 16 pixels.

Results and Discussion

Imagery can be generally categorized in two ways. The first is known as passive imaging that uses the reception of natural radiation where, objects emit electromagnetic radiation at a certain degree of temperature, which is reflected from the scene (Yujiri, 2003). The second is by sending radiation from a source towards the scene and gathering the reflection, which is known as active imaging. In this experiment an active millimeter wave imaging system is adopted.

In the image reconstruction procedure, the single pixel is mechanically scanned by the help of a stepper motor that moves the single pixel PCB 16 pixels in x-direction and moves 8 pixels in y-direction to scan the field of view, with a size of 5 x 10 cm^2 of the sample bread. Therefore, the object is placed in the illuminated area of the bread.

The single pixel mask is made from a 10 x 10 PCB covered with copper and exactly a SP hole left open in the middle of the PCB. The size of the pixel is 6.25 x 6.25 mm2 that is scanned on the surface of the object 128 times in a two-dimensional pattern. The millimeter wave penetrates the bread with little signal attenuation and penetrates metal and dense masses with large signal attenuation. The transmitted beam is scattered through the solid and dense materials hence, results in a low power transfer to the receiver that helps to recover the hidden object inside the sample bread. A target with an approximate length of 4 cm is placed in the middle of the bread horizontally and the SP mask is scanned through the sample bread. Hence, the foreign object is imaged as depicted in Figure 4.

Before placing the object, the signal-to-noise power ratio (SNR) is 23 dB. After placing the bread, the incoming signal experiences 3 dBm attenuation. Locating foreign objects inside the bread further attenuates the transmitted signal by 12 and 19 dBm for salt and screw, respectively. Since the metal atoms are bounded together by a strong chemical bond called metallic bonding, the screw causes higher attenuation than salt. The signal is more attenuated where the foreign object is placed and as a result of this, less power is transferred to the receiver. After analyzing the signals, the foreign objects can readily be seen as depicted in Figure 4 and Figure 5. The dynamic range is 23 dB without placing an object between the transmitter and receiver.

After placing the screw inside the bread, it is imaged inside it and then the screw is removed. Following that, the nylon plastic bag filled with salt is placed inside the bread. The same procedure has been repeated, scanning the field of view with the SP mask and the data is sent to the receiver for each movement of the stepper motor where, each step length is 6.25 mm. The foreign object is detected inside the sample bread as depicted in Figure 5.

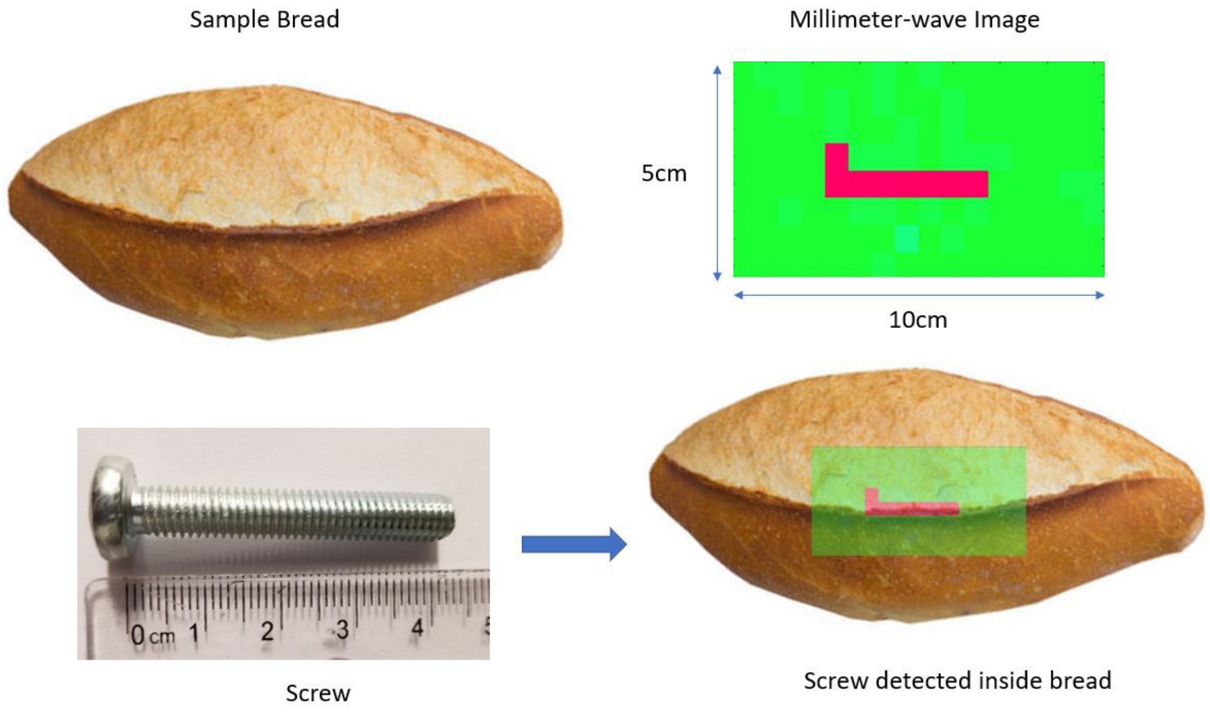

Figure 4. The screw is placed inside the bread considering an image area of 5 x 10 cm^2. However, the image of the foreign object seems sharp due to the pixels not being rounded the pixels.

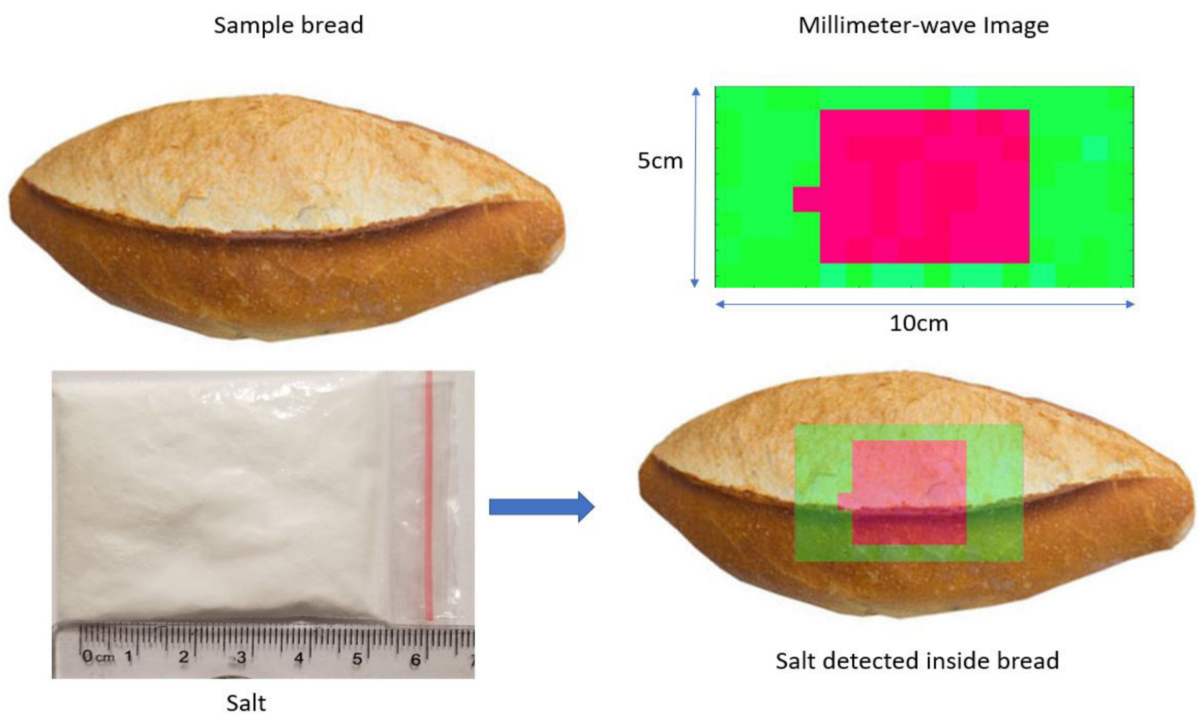

Figure 5. Salt is placed inside the bread and as a dense mass mm-wave can barely pass through it that results in detecting the mass. The minimum detectable object is 2 mm^2

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Conclusion

As a result of all, a SP scanning based millimeter wave system has been demonstrated for detecting foreign objects inside a bread. This method can be used for non-intrusive quality control and foreign objects detection. The application of this method with bread and other food supplements is proposed for various real-life scenarios which threaten the human life. Experimental procedure is done on a sample loaf of bread that weights 250 gr with dimensions of 30 cm and 10 cm length and width, respectively. We have imaged the objects in transmission mode, therefore, there would be no effect on the imaging performance if the sample is rotated or placed in a different way. Since there is no rounding, the boundary pixels look sharper. The minimum detectable object inside the bread is 2 mm2. Studies on imaging plastic materials that cannot be detected due to our operating frequency band and require higher frequency for more sensitive detection are planned in the future.

Compliance with Ethical Standards

Conflict of interest

The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Author contribution

The contribution of the authors is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

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Research Article

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Evaluation of yield and quality performance of some spring bread wheat (*Triticum aestivum L.)* genotypes under rainfall conditions

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Abstract

The study was conducted in randomized complete block design with four replications at two locations (Sanliurfa and Diyarbakir) under rainfall conditions during 2013-2014 growing season. The purpose of the study is to determine the bread wheat genotypes with high yield, large adaptation ability and high grain quality. Twenty advanced bread wheat lines and five check varieties were used as materials. The data were evaluated by using the variance and GGE biplot analysis methods. Significant differences among genotypes were determined for test weight (TW), thousand grain weight (TGW), grain yield (GY) and heading time (HT) at the significant level of 1%, while, less significant levels ($p \leq 5\%$) were found for wet gluten content (WG), zeleny sedimentation (ZS) and protein content (PR). According to GGE biplot analysis results, positive correlations were determined between TW and TGW, and also PR, WG and ZS. It has also been determined that there is a negative relationship between HT and TW, TGW. According to the stability graph, the genotypes of G23 and G8 were found to be highly efficient and moderately stable, and both could be candidate varieties. Additionally, Dinç variety and G11 advanced line can be used as genitors in bread wheat breeding programs for grain yield and quality.

Keywords: Bread wheat, GGE biplot, Stability, Yield

Introduction

Wheat is the first cultivated plant among cereals. Wheat's sowing area production and consumption are high around the world and in our country. In addition, wheat is a strategic cultivation plant with high adaptability. World wheat cultivation is 219 million hectares and production is 758 million tons. Yet, wheat production in the world is not sufficient to solve people's nutritional problems (IGC, 2018). Wheat sowing is 7.7 million hectares in Turkey. However, production is 21.5 million tons and wheat comes first in human nutrition (TUIK, 2017). Bread and other products made from wheat are the most important food sources for humans in Turkey. The annual wheat and wheat products consumed per person is above 200 kg in Turkey. Turkey ranks first in the world in terms of wheat consumption per person (Morgounov et al., 2016). The amount and quality of protein in wheat has been reported to be one of the important criteria in determining wheat quality. When protein ratios were classified; they are evaluated as very high for 14-17%, high for 11- 14% and middle class for 10-12% (Grausgruber et al., 2000; Kizilgeci et al., 2015).

Zeleny sedimentation in bread wheat is one of the methods used to determine protein quality. While quality parameters such as protein content, hectoliter weight and thousand grain weight are affected by environmental conditions, it is reported that zeleny sedimentation value is highly affected by genetic factors rather than environmental effects (Grausgruber et al., 2000; Kizilgeci et al., 2015). In recent years, GGE biplot analysis method has been used in many fields (Yan and Tinker, 2006; Aktas et al., 2017). One of the most important reasons for the intensive use of biplot analysis by researchers is the graphical representation of many features of genotypes in this analysis method.

Furthermore, it can be shown that this analysis method allows the visual comparison and interpretation of the correlation

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between both genotype and traits (Aktas et al., 2017). The aim of this study is to determine the adaptation ability, grain yield and quality characteristics of bread wheat genotypes which can be candidates for cultivar registration under rainfall conditions of Southeast Anatolia Region.

Materials and methods

The study was carried out in two locations (Diyarbakir and Sanliurfa) under rainfall conditions in 2013-2014 growing season. In the research, 20 advanced bread wheat lines with spring nature obtained from CIMMYT were used. In the experiment, Pehlivan with winter nature, Sagittario with alternative nature and Dinç, Cemre and Adana-99 cultivars with spring nature were used as standard. The pedigrees of all genotypes used in the experiment are shown in Table 1. The cultivars used as standard are commonly grown in the region. While the average long term rainfall in Diyarbakir was 495.0 mm, it fell 356.0 mm in the 2013-2014 wheat growing season. The rainfall was lower than the average of long-term (Figure 1). In Diyarbakir, the temperature in December of the 2013-2014 season was much lower than the average of long term (Figure 2). In Diyarbakir for long term the average temperature is 12.2 ^{0}C and the average relative humidity is 52.7% (Anonymous, 2014). In Sanliurfa long terms years, average rainfall is 430 mm, and 312.8 mm rainfall was recorded during the 2013-2014 season (Figure 3).

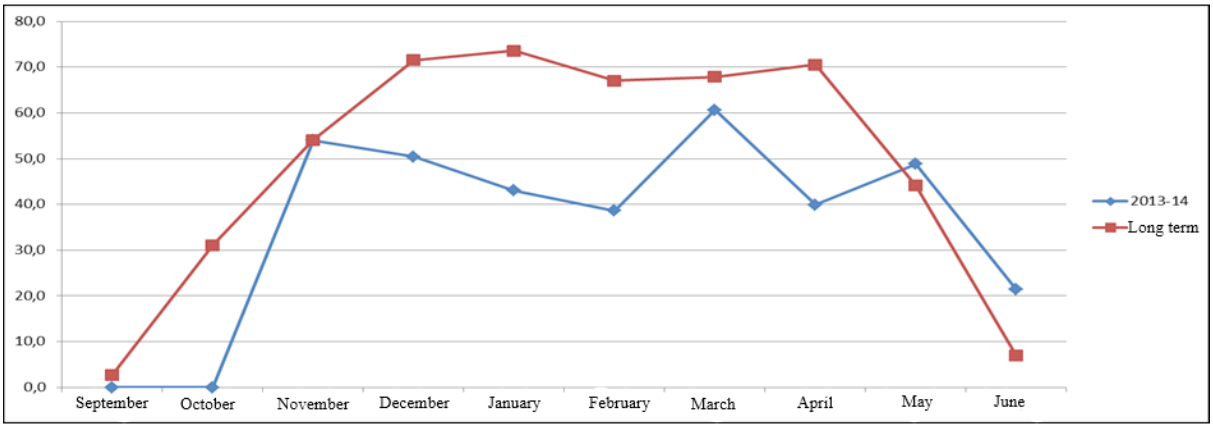

Figure 1. The rainfall graph of Diyarbakir province

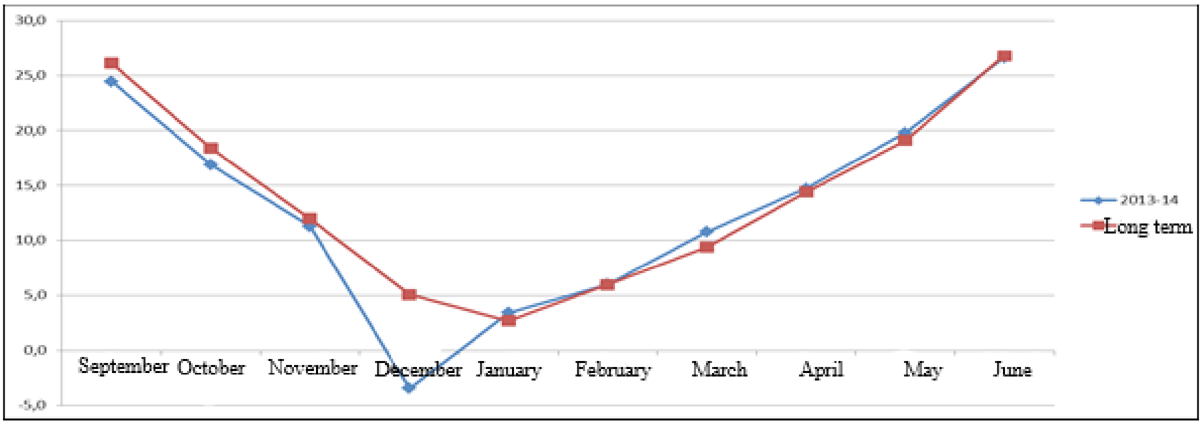

Figure 2. Temperature graph of Diyarbakir province

During the growing season, rainfall was lower than the long term average. In Şanliurfa, for long terms the average temperature was 18°C and the average relative humidity was determined as 58.2% (Anonymous, 2013). In Diyarbakir soil texture of the experiment area is clayey. Determined values were as follows: total salt content (%): 0.246, PH (s): 7.75 (slightly alkaline), lime content (%): 6.26, phosphorus content (kg ha^{-1}): 12.8, organic matter (%): 0.676 (soils poor by organic matter), saturation with water (%): 77. In Sanliurfa, water saturation was 50%, water saturated soil PH was 7.6 phosphorus (P_2O_5) 52 kg ha^{-1}, organic matter content was determined as 1.1% (Anonymous, 2013). Cultivation of the experiments was practiced with parcel seeder. Also, the harvest was made with parcel harvester. In the study, parcel length was set as 5 m and row spacing as 20 cm. In addition, each parcel consisted of 6 row. 450 seeds per square meter were planted with experiment seeder. 60 kg ha^{-1} pure nitrogen (N) and 60 kg ha^{-1} pure phosphorus were applied with sowing in the experiments. In addition, 80 kg ha^{-1} pure nitrogen (N) was given at the end of the tillering period. Experiment cultivation in both locations took place in November. In Sanliurfa location harvest was carried out one week earlier than Diyarbakir location.

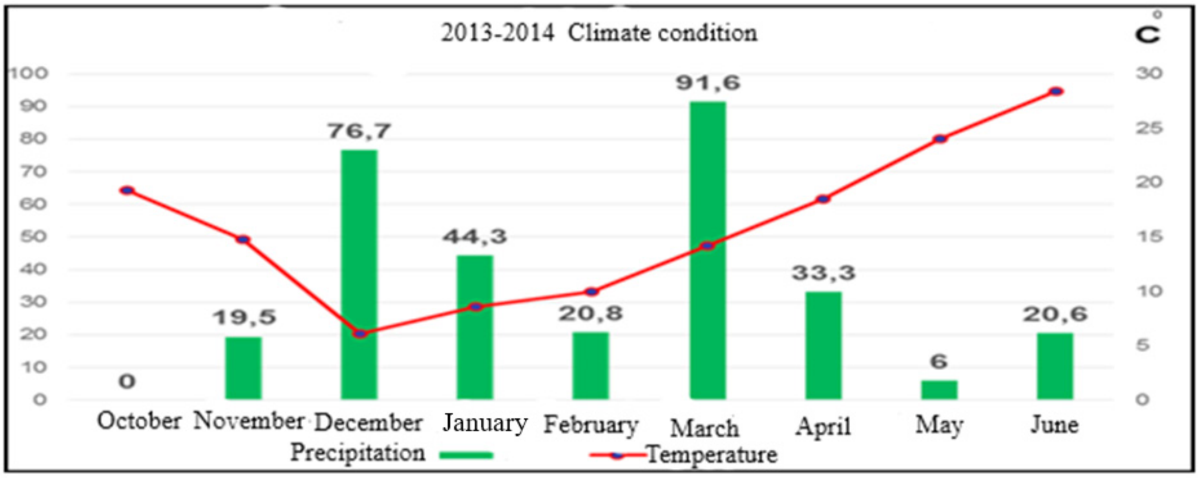

Figure 3. Temperature and precipitation graph of Sanliurfa province

Table 1. Pedigree and origin of bread wheat genotypes used in the study

Genotypes (G)	Pedigree	Breeding Organization or Origin
G1	Qamar-4 Cmss97m03159t-040y-0b-0ap-2ap-…	CIMMYT
G2	D67.2/Parana 66.270//Ae.Squarrosa (320)/3/…(synthetic)	CIMMYT
G3	Cno79//Pf70354/Mus/3/Pastor/4/Bav92/5/Mılan	CIMMYT
G4	Babax/Ks93u76//Babax/3/2*Sokoll Cmsa06m	CIMMYT
G5 (Dinç)	Standard	GAP UTAEM
G6	D67.2/Parana 66.270//Ae.Squarrosa (320)/3/(synthetic)	CIMMYT
G7	Krıchauff/2*Pastor/4/Mılan/Kauz//Prınıa/3/Bav	CIMMYT
G8	Heılo//Sunco/2*Pastor Cmsa06y00492s-040zty-	CIMMYT
G9	Chıh95.7.4//Inqalab 91*2/Kukuna Ptss06ghb..	CIMMYT
G10 (Pehlivan)	Standard	TTAEM
G11	Kachu #1/Kırıtatı//Kachu Cmss06y00778t-099..	CIMMYT
G12	Saual/Yanac//Saual Cmss06y00783t-099topm..	CIMMYT
G13	Prl/2*Pastor*2//Fh6-1-7 Cmss06y00793t-099…	CIMMYT
G14	Frncln/Rolf07cmss06b00013s-0y-099ztm-099y	CIMMYT
G15 (Cemre)	Standard	GAP UTAEM
G16	Becard/Kachu Cmss06b00169s-0y-099ztm-099.	CIMMYT
G17	Becard/Akurı Cmss06b00411s-0y-099ztm-099y	CIMMYT
G18	Rolf07*2/5/Reh/Hare//2*Bcn/3/Croc_1/Ae.…	CIMMYT
G19	Usher-16 Crow's'/Bow's'-1994/95//Asfoor-5…	CIMMYT
G20 (Sagittario)	Standard	TASACO TARM.
G21	Croc_1/Ae.Squarrosa(213)//Pgo/3/Cmh81.38/2 (synthetic)	CIMMYT
G22	Chen/Aegılops Squarrosa (Taus)//Bcn/3/Bav92. (synthetic)	CIMMYT
G23	Mısket-12-Btı735/Achtar//Asfoor-1ıcw01-…	CIMMYT
G24	Rebwah-12/Zemamra-8-Rebwah-12/Zemamra-.	CIMMYT
G25 (Adana-99)	Standard	DATAE

In the study, the homogeneity of the variances was examined for all parameters involved, and locations were subjected to combined analysis because the variances were homogeneous. In addition, statistical analysis was performed on 4 replications for grain yield, and analysis of quality characteristics was performed on 2 replications due to labor and cost high.

The heading time (HT) was recorded as the number of days from the 1st of January. Grain yield (GY) was determined by weighing the product obtained after harvesting the whole parcel with a balance of 0.01 g. A thousand grain weight (TGW) was determined by weighing 1000 seeds of each genotype with a balance of 0.001 g. Test weight (TW) was determined by weighing 1 liter of seed and multiplying the value by 100. Protein content (PR) analysis was determined using Near Infrared model 6500 device in accordance with AACC 39-10 method (Anonymous, 1990). In zeleny sedimentation (ZS) analysis, ICC-No. 115 method was applied (Anonymous, 1982). The amount of wet gluten (WG) was reached by using Glumatik 2200 gluten washing device according to ICC standard 155/1 method (Anonymous, 1994). The research was carried out in randomized block design with 4 replications under rainfall conditions.

The variance analysis of the data obtained from the study was performed by using JMP 13.0 statistical package program. The differences between the means were examined by LSD test ($p \leq 0.01$ and $p \leq 0.05$) (Gomez and Gomez, 1984; Kalayci, 2005). GGE biplot analysis was performed using Genstat 12th (Genstat, 2009) statistical package program in order to evaluate the relationship between traits and genotype-traits, and all genotypes are shown visually in the graphics. In addition, prominent genotypes were evaluated.

Results and discussion

According to variance analysis (ANOVA), differences between the mean values of the characteristics examined were statistically significant ($p \leq 0.01$ or $p \leq 0.05$) (Table 3). In the study, the highest grain yield was obtained from Dinç variety (4070 kg ha^{-1}) and G23 advanced line (4085 kg ha^{-1}).

Table 2. Variance analysis table showing the mean of squares the investigated properties

	Average squares								
Variance resources	DF1	GY	DF2	TW	TGW	PR	ZS	WG	HT
Location	1	723.9	1	176.7*	53.7	405.2**	6021.8*	346.7*	1102.2**
Replication	6	12767.8	2	9.1	9.0	1.5	78.8	16.9	7.8
Genotype	24	7649.7**	24	10.1**	33.5**	1.1*	38.8*	13.1*	109.8**
Location*Genotype	24	9333.8**	24	1.1	7.8**	1.9**	66.3**	22.1**	1.8
CV (%)		11.1		1.2	5.4	4.8	11.7	7.6	3.4

**: statistically significant at the 0.01 level, *: statistically significant at the 0.05 level. DF1: degree of freedom for grain yield, DF2: degree of freedom for other properties other than grain yield, GY: grain yield, TW: test weight, TGW: thousand grain weight, PR: protein content, ZS: zeleny sedimentation, WG: wet gluten, HT: heading time

While the highest grain yield was obtained from Sagittario variety (4430 kg ha^{-1}) in Diyarbakir; in Sanliurfa the highest grain yield was obtained from G23 advanced line (4310 kg ha^{-1}) (Table 3). Because the rainfall in Diyarbakir is higher than Sanliurfa, and the weather conditions are cooler, Sagittario, which has an alternative nature, comes forward in this location. While it was determined that most of the existing materials were affected by the extreme cold weather conditions in Diyarbakir in March, this situation is thought to have a negative effect on grain yield values. Researchers reported that, the effect of wheat heredity on grain yield is great. Same researchers have also emphasized that, the soil structure (clay, loam, sandy), agronomic applications (seed bed preparation, fertilization, etc.) and climate conditions have great impact on grain yield, too (Doğan and Kendal, 2012; Kiliç et al., 2012a; Kendal, 2013; Ali, 2017). In the study, the average test weight ranged from 78.2 to 83.7 kg hl^{-1}. The mean highest test weight was obtained from the G7 forward line (83.7 kg hl^{-1}). The highest test weight was obtained from the G8 advanced line (85.0 kg hl^{-1}) in Diyarbakir, while the G11 advanced line (82.6 kg hl^{-1}) was obtained in Sanliurfa (Table 3). Test weight is significantly affected by the amount of precipitation received during the season and the distribution of precipitation on a monthly basis. As a matter of fact, Diyarbakir location receives more rainfall than Sanliurfa location. This was reflected on the test weight. Test weight has been reported to vary depending on the volume, shape and density of wheat grain (Protic, 2007). Also, in studies conducted in spring wheat, it was reported that test weight ranged from 75.4 to 80.0 kg hl^{-1} (Kilic et al., 2012b). Our findings obtained from the test weight are similar.

In the study, the average thousand grain weight ranged from 28.1 to 39.1 g, while the average of the experiment was found to be 32.8 g. The G12 advanced line (39.1 g) has the highest average value in terms of thousand grain weight. In the location of Diyarbakir, the highest thousand grain weights were obtained from G12 advanced line (39.3 g). In Sanliurfa location, G11 advanced line (39.8 g) had the highest thousand grain weight value (Table 3). Studies conducted in different years under Diyarbakir conditions reported an average of thousand grain weight between 22.6-34.6 g (Kilic et al., 2012a; Ali, 2017). Our findings are similar to those of the studies in terms of average thousand grains weight.

Table 3. Mean values of the investigated features

	GY(kg ha^{-1})			TW (kg hl^{-1})			TGW (g)		
Genotypes	DB	SU	Av.	DB	SU	Av.	DB	SU	Av.
G1	4300	3650	3975	81.7	78.8	80.3	32.1	29.3	30.7
G2	3970	3640	3805	84.6	80.3	82.5	29.9	31.9	30.9
G3	3510	3720	3615	84.7	81.3	83.0	37.0	36.0	36.5
G4	3830	4050	3940	82.4	78.7	80.5	31.4	27.9	29.6
Dinç	4330	3810	4070	84.3	81.5	82.9	31.1	29.5	30.3
G6	3410	3620	3515	81.9	79.4	80.7	34.4	31.3	32.8
G7	3650	3550	3600	84.9	82.5	83.7	34.9	34.6	34.8
G8	3900	4170	4035	85.0	81.1	83.1	31.6	27.8	29.7
G9	4210	3720	3965	82.9	80.6	81.8	33.8	31.3	32.5
Pehlivan	3160	2840	3000	82.0	79.4	80.7	33.8	32.8	33.3
G11	2270	3750	3010	83.0	82.6	82.8	35.4	39.8	37.6
G12	3050	4130	3590	80.8	79.1	80.0	39.3	39.0	39.1
G13	4150	3820	3985	81.3	76.7	79.0	37.3	33.1	35.2
G14	4380	3650	4015	80.5	77.7	79.1	33.0	32.4	32.7
Cemre	3910	2910	3410	82.6	79.6	81.1	35.6	27.5	31.6
G16	3170	4160	3665	84.0	81.8	82.9	37.6	38.6	38.1
G17	3650	3650	3650	81.7	78.2	79.9	32.9	31.3	32.1
G18	3490	3600	3545	80.6	77.2	78.9	30.8	29.6	30.2
G19	4280	3290	3785	79.6	76.9	78.2	29.3	27.0	28.1
Sagittario	4430	3520	3975	82.2	80.2	81.2	35.1	29.8	32.4
G21	3570	3890	3730	83.6	81.3	82.4	33.1	31.6	32.4
G22	2940	4050	3495	79.9	79.1	79.5	32.8	35.9	34.3
G23	3860	4310	4085	81.0	78.8	79.9	34.4	32.8	33.6
G24	3470	2960	3215	80.7	79.9	80.3	31.9	35.0	33.4
Adana-99	3890	3390	3640	84.5	81.2	82.9	30.5	26.6	28.6
Average	3711	3674	3693	82.4	79.8	81.1	33.5	32.1	32.8
LSD $_{(0.05)}$	586**	565**	404**	2.3**	2.4**	1.4**	4.2**	3.2**	2.5**

DB: Diyarbakir, SU: Sanliurfa, Av.: Average

The highest average protein content was obtained from the G11 forward line (17.5%). While the G11 forward line (17.3%) had the highest value in terms of protein ratio in Diyarbakir location, no significant difference was found between genotypes in the location of Sanliurfa (Table 4). In Sanliurfa, rainfall amount is lower than Diyarbakir, while high temperature and drought during the grain filling period is higher. Therefore, the deposition of starch in the grain is proportionally low in the location of Sanliurfa. Due to climatic factors, it is seen that the protein ratios are higher in Sanliurfa. It has been reported that, total precipitation amount, temperature and drought stress and total amount of pure nitrogen in wheat growing season during grain filling and season have an effect on protein content. (Porceddu, 1973; Karaman, 2017). Grain protein ratio is important in determining the quality of wheat, but there is a negative relationship between grain yield and protein ratio (Tekdal et al., 2014; Karaman, 2017).

In the study, mean zeleny sedimentation value ranged from 35.0 to 49.4 ml. In terms of zeleny sedimentation value, the highest mean value was obtained from G11 advanced line (49.4 ml). While the G11 advanced line (52.4 ml) was prominent in Diyarbakir location in terms of zeleny sedimentation, no statistically significant difference was observed between genotypes in Sanliurfa location (Table 4). Sahin et al. (2016), in a study of wheat under Konya conditions, reported that the average zeleny sedimentation value is 39.4 ml. Although the genotypes and environmental conditions used in the study were different, the results obtained were similar to the results obtained from our study.

In the study, the highest mean wet gluten amount was obtained from the G11 advanced line (40.0%), while the average of the experiment was found to be 34.3%.

In terms of wet gluten amount, the highest value was obtained from G11 advanced line (44.2%) in Diyarbakir location, but no significant difference was found between genotypes in the location of Sanliurfa (Table 4). It has been reported that gli-

Table 4. Means of the investigated properties and groups formed

Genotypes	PR (%)			ZS (ml)			WG (%)			HT (day)		
	DB	SU	Av.	DB	SU	Av.	DB	SU	Av.	DB	SU	Av.
G1	13.4	17.1	15.2	29.4	42.6	36.0	31.0	33.6	32.3	101.5	94.0	97.8
G2	13.3	18.2	15.7	28.5	50.0	39.2	30.9	37.4	34.1	107.5	100.5	104.0
G3	13.3	17.9	15.6	28.0	46.8	37.4	30.8	36.4	33.6	102.0	95.0	98.5
G4	12.9	17.3	15.1	26.8	43.1	35.0	29.4	34.1	31.7	104.5	97.5	101.0
Dinç	12.7	17.5	15.1	25.6	45.0	35.3	28.7	34.9	31.8	103.0	96.0	99.5
G6	13.1	17.6	15.4	27.4	45.7	36.6	30.1	35.4	32.7	103.0	96.0	99.5
G7	13.3	17.5	15.4	29.3	45.2	37.2	30.8	35.0	32.9	102.0	95.0	98.5
G8	13.8	18.0	15.9	32.6	48.5	40.5	32.3	36.6	34.4	105.5	103.0	104.3
G9	13.7	17.9	15.8	31.1	47.2	39.1	32.2	36.2	34.2	105.0	98.0	101.5
Pehlivan	13.6	19.4	16.5	30.9	56.5	43.7	31.6	41.6	36.6	115.5	108.5	112.0
G11	17.3	17.7	17.5	52.4	46.5	49.4	44.2	35.8	40.0	93.5	86.5	90.0
G12	14.1	17.0	15.5	32.1	45.9	39.0	33.3	33.2	33.2	98.0	91.0	94.5
G13	13.5	18.5	16.0	30.2	50.1	40.2	31.4	38.3	34.8	101.5	94.5	98.0
G14	14.3	17.7	16.0	34.6	46.6	40.6	33.9	35.8	34.9	102.0	95.0	98.5
Cemre	13.6	19.1	16.4	30.2	55.7	42.9	31.6	40.6	36.1	114.0	107.0	110.5
G16	15.6	16.9	16.2	42.2	40.4	41.3	38.7	32.8	35.7	95.5	88.5	92.0
G17	13.1	18.1	15.6	28.5	48.8	38.7	30.0	37.0	33.5	107.5	100.5	104.0
G18	14.2	18.4	16.3	34.3	50.2	42.2	33.6	38.0	35.8	101.5	94.5	98.0
G19	12.8	19.0	15.9	26.7	55.5	41.1	28.9	40.0	34.4	106.0	104.0	105.0
Sagittario	13.0	18.9	16.0	27.4	53.7	40.5	29.8	39.8	34.8	112.0	105.0	108.5
G21	14.8	17.4	16.1	38.0	44.4	41.2	35.9	34.5	35.2	102.5	95.5	99.0
G22	15.1	17.5	16.3	37.9	44.6	41.2	36.6	35.1	35.9	103.5	96.5	100.0
G23	13.7	17.0	15.3	30.4	42.5	36.5	31.9	33.1	32.5	107.5	100.5	104.0
G24	13.5	17.6	15.5	29.1	46.1	37.6	31.2	35.4	33.3	110.0	103.0	106.5
Adana-99	13.9	17.1	15.5	34.0	43.9	38.9	32.6	33.5	33.1	102.0	95.0	98.5
Average	13.8	17.8	15.8	31.9	47.4	39.6	32.4	36.1	34.3	104.3	97.6	100.9
LSD(0.05)	1.5**	n.s.	1.1*	9.1**	n.s.	6.6*	5.1**	n.s.	3.7*	7.6**	6.3**	4.9**

adin and glutenin proteins come together to form gluten, while gliadin proteins are effective on the fluency of the dough, and glutenin proteins have been reported to play a role in the elasticity of the dough (Kizilgeci et al., 2015). In studies conducted on the amount of gluten in different years and places, Altinbas et al. (2000), obtained a value of average 34.9%, Kızılgeçi et al. (2015), on the other hand, obtained wet gluten values ranging from 31.4-42.6%. Wet gluten content values obtained in our study were similar with the results of these researchers.

In study, in terms of mean heading time, the late heading time was obtained by Pehlivan (112 days) and the earliest heading was of the G11 advance line (90 days) (Table 4). Ali (2017), in his study carried out under Diyarbakir conditions using 20 advanced lines and 5 standard varieties in bread wheat, reported that there was a 8-9 day difference in the heading time between the earliest and latest genotypes. In addition, it has been reported that the environmental conditions that occur at different developmental stages of genotypes are effective on heading time (Araus et al., 2007; Rahman et al., 2009). In terms of heading time, there was a 22 day difference between the earliest genotype and the latest genotype as a differentiation from the previous studies. This may be due to the fact that the varieties included in the trial have a wide variation as a developmental nature, and that the distribution of rainfall on a monthly basis during the production season is irregular. In addition, genotypes may have reacted differently due to lack of total precipitation.

Evaluation of the parameters examined by GGE biplot analysis method

The GGE biplot analysis method provides a visual interpretation of the relationship between the genotypes and the environment, the characteristics studied, and the environment.

Therefore, it has been used extensively by researchers recently (Hagos, 2013). In GGE biplot analysis, PC1(principal component 1-1. main component) shows the efficiency of genotypes and PC2 (principal component 2-2. main component) shows the stability of genotypes (Yan et al., 2000). Therefore, it is desirable that an ideal genotype has a high PC1 value in terms of the studied characters and a PC2 value close to zero (0) (Farshadfar et al., 2013; Aktas, 2017).

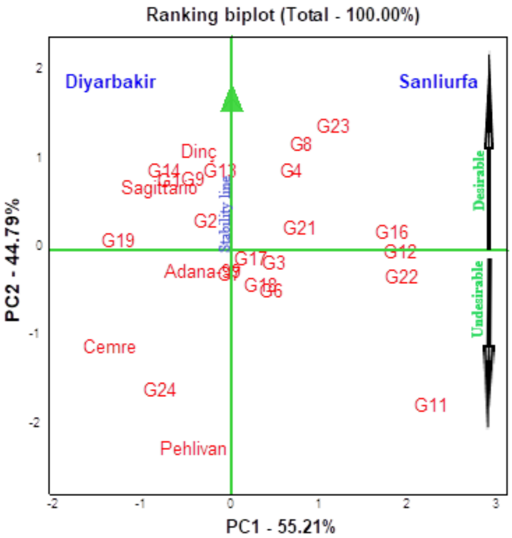

Figure 4. GGE-biplot graph showing the stability of genotypes

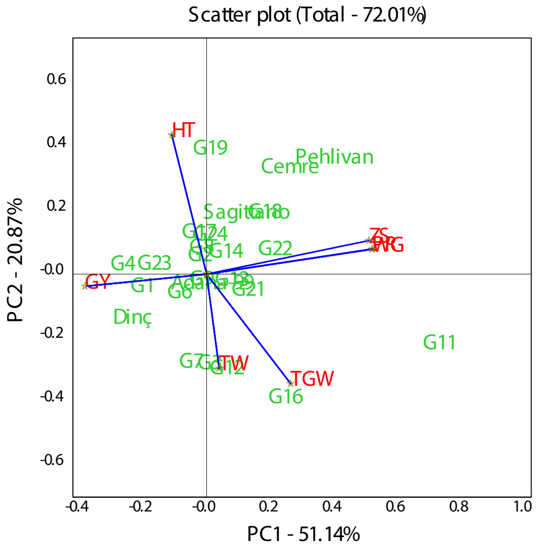

Figure 5. GGE-biplot graph showing genotype-property relationship

In Figure 4, a visual representation of the stability and yield order of genotypes is given, 55.21% of total variation is represented by PC1 and 44.79% by PC2. According to the AEC (average environment coordination) method, genotypes (PC1 value higher than zero), representing the average grain yield and located to the right of the line dividing the axis, had higher grain yield than the average. In addition, the remaining genotypes had lower grain yield values than the average (Figure 4).

The G23 advanced line is a genotype with the highest grain yield (PC1 value). The fact that G23 is close to the stability line indicates that it is moderately stable. Also; G4, Dinc, G8 and G13 can be said to be good in terms of grain yield. Genotypes close to the center of the axis (G17, G3, etc.) showed values close to the experimental average in terms of grain yield. Biplot analysis method, which presents the correlation between the examined parameters visually and enables the evaluation, has been used by many researchers recently. In addition, biplot analysis, which presents the correlation between genotype and traits as a whole, has big superiority over the correlation analysis showing only the relationship between two traits (Yan and Kang, 2002; Yan and Reid, 2008; Akcura, 2011; Kilic et al., 2012b). The relationship between the properties examined in the GGE biplot graph is given in Figure 5. 51.14% of the total variation, was represented by PC1 and 20.87% by PC2. From the features examined; since the vector angle is less than 90 degrees between TW and TGW, ZS and PR and WG, there is a high positive correlation between features. Also, since there is an inverse angle of about 180 degrees between the HT vector and the TW and TGW vectors, it can be said that there is a high negative correlation between HT and TW and TGW under the conditions of the study. It has been reported that, the variation of genotypes increases as the length of the vectors increases, and the length of the vectors representing the features or the distance of the vector from the origin indicates the variation of genotypes in terms of the relevant feature (Abate, 2015). As can be seen in Figure 5; HT, ZS, PR and WG vectors are long, GY and TW vectors are short. This shows that the variation between genotypes in GY and TW is lower than as in other parameters. The prominent genotypes shows in the study are as follows, respectively: Dinç, G8, G14 and G23 for GY; G11 for ZS, PR and WG; G12 and G16 for TGW; G7, G8 and G3 for TW (Figure 5). Genotypes located near the center of the axis showed values close to the trial average for all investigated properties.

Conclusion

According to the results of the research, there was a positive correlation between TW and TGW, ZS with PR and WG. Also, there was a negative relationship between HT and TW and TGW. In terms of GY; Dinç variety, G8, G14 and G23 advanced lines; for TW; Dinç variety, G3, G7, G8, G11, G16 advanced lines and Adana-99 variety; for TGW; G11, G12 and G16 advanced lines; for PR, ZS and WG; Pehlivan variety and G11 advanced line were in the front row. It is determined that G8 and G23 advanced lines have good adaptation ability and high grain yields in rainfed conditions in Southeastern Anatolia Region. Additionally, these lines may be candidate varieties because the quality values of these lines are above or close to the experiment average and are acceptable values. Also, It is concluded that Dinç and G11, which are prominent in terms of many features, should be used as genitors in breeding programs.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

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Artificial neural network application for forecasting the nitrogen oxides in the atmosphere at the microclimate conditions: example of Iğdır city in Turkey

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Abstract

The aim of this research is forecasting the NO_x, NO_2 and NO concentration levels with different artificial neural networks structures (ANNs) and determining the best ANNs structure for forecasting emissions. For this aim, it was used one learning function and, six different transfer function pairs with three different neuron numbers. The MATLAB software helped constructing ANNs models. In addition, the air pollutants and meteorological factors were used as input parameters simultaneously at the ANNs. The end of the research, NO_x, NO and NO_2's concentration levels were modelled with high accurate levels. The R^2 values of the NO_x, NO and NO_2 were calculated as 0.998, 0.995 and 0.997, respectively. The best results were obtained from ANNs structures which used Logarithmic sigmoid - Symmetric sigmoid transfer functions with 20 and 30 neuron number for forecasting of the NO_x and NO concentration levels, respectively. In addition, the forecasting of NO_2 emission rate, the best results were determined from the ANNs structure used Logarithmic sigmoid - Linear transfer function with 30 neuron number. According to sensitivity analyses and correlation tests, it was concluded that O_3, SO_2, wind direction, wind speed, and relative humidity inputs were more effective on the NO_2, NO and NO_x concentrations than the other inputs. Finally, it can be said that with the use of both air pollutants and meteorological factors as input parameters simultaneously the artificial neural network models can be simulated the concentration level of NO, NO_x and NO_2 with high accuracy.

Keywords: Air pollutions, Meteorological factor, ANN, Transfer function, Learning function

Introduction

The atmospheric pollution, which impairs on the respiratory and cardiovascular system, is an important factor both environmental conditions and human health, so it should be continuously controlled and observed (Zhang et al., 2012). Generally, pollutant emissions and meteorological factors affect the air pollution level. Some of the researchers, which aimed determining reasons the air pollutant, stated that the air pollution was influenced negatively by atmospheric pollutants (Gantt et al., 2010; Urbanski et al., 2011; Gao et al., 2014) while the others stated that it was affected by meteorological factors. (Wang et al., 2013; Wang et al., 2014 a; Wu et al., 2014 b; Russo et al., 2015).

NO_x has an important role in terms of the air pollution. NO_x affects both air pollution and human health so causes diseases such as pulmonary edema and damaged central nervous system, tissue, etc. (Lal and Patil, 2001). In addition, NO_x undergoes various complex reactions to generate several secondary pollutants which are known to be even more harmful than their precursor.

The forecasting of the air pollution is an important issue in the terms of both environmental pollution evaluations and precautions for countries' air pollution situation in the future. So, lots of researches have been done for modelling with statistical methods of air pollution levels, and in these researches generally examined the relationship among the air pollutants (Ozel and Cakmakyapan, 2015). In addition, autoregressive integrated moving average model (Samia et al., 2012; Jian et al., 2012), artificial neural network (Chaudhuri and Acharya, 2012; Elangasinghe et al., 2014; Feng et al., 2015; Pauzi and Abdullah, 2015; Zhang et al., 2017), community multi-scale air quality model (Chen et al., 2014;Wu et al., 2014a; Djalalova et

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al., 2015), fuzzy inference system (Domannska and Woktylak, 2012), grey model (Pai et al., 2013), and other hybrid methods (Chen et al., 2013; Corporation, 2013; Russo and Soares, 2014; Yahya et al., 2014) can be listed which using methods for modelling air pollution levels.

Feed forward back propagation (FFBP) generally used in the ANN. An FFBP has the presence of one or more hidden layers, whose computation nodes are correspondingly called hidden neurons of the hidden units. The function of hidden neurons is to intervene between the external input and the network output in some useful manner. By adding one or more hidden layers, the network is able to extract higher order statistics. The ability of hidden neurons to extract higher order statistics is particularly valuable when the size of the input layer is large. The source nodes in the input layer of the network supply respective elements of the activation pattern (input vector), which constitute the input signals applied to the neurons (computation nodes) in the second layer (i.e. the first hidden layer). The output signals of the second layer are used as inputs to the third layer, and so on for the rest of the network. Typically, the neurons in each layer of the network have as their inputs the output signals of the preceding layer only. The set of the output signals of the neurons in the output layer of the network constitutes the overall response of the network to the activation patterns applied by the source nodes in the input (first) layer. The FFBP are trained using the LM optimization technique. This optimization technique is more powerful than the conventional gradient descent techniques (Cigizoglu and Kisi 2005).

The aim of this study is to model atmospheric NO_x, NO and NO_2 emissions with different artificial neural network structures in microclimate atmospheric conditions in Iğdır/Turkey. Iğdır is adjacent to Iran, Nakhichevan and Armenia. In addition, the Metsamor nuclear power plant in Armenia is only 16 km from the border of Iğdır city. The level of air pollution is quite high throughout the year. For this purpose, 18 different artificial neural structures (ANNs) were examined with different transfer functions and neural numbers. Unlike the other studies, in this research, as input parameters not only atmospheric pollutants (SO_2, O_3, NO, NO_2, NO_x and PM_{10}) but also meteorological factors (relative humidity, air pressure, air temperature, wind direction and wind speed) were used at the ANNs models.

Materials and Methods

In the research, it was used the atmospheric and meteorological data which obtained by Turkey Ministry of Environment and Urbanization / National Air Quality Monitoring between the 01/102016 - 06/11/2018 in Iğdır city. The detail data of pollutants and meteorological factors are plotted in figure 1 and figure 2, respectively. Data were divided into three parts as 70% for training, 15% for cross validation, and 15% for testing to prevent overfitting. For prediction the NO_x, NO and NO_2 emissions, 18 different artificial neural network structures were used with different transfer functions and neurons numbers. The input and output parameters which used in the ANNs structures have been illustrated in the table 1 and ANNs structure was illustrated figure 3. In the research one learning function, six different transfer function combinations and 3 different numbers of neurons were used in the artificial neural structures (ANNs). The architecture of the ANNs model was given table 2. The studied network was implemented under MATLAB 7.10 (the MathWorks, Inc. Natick,MA,USA software), with the Neural Network Toolbox 4 (Maltlab, 2015).

Table 1. The input and output parameters for forecasting of NO_x, NO and NO_2

Forecasting NO_x		Forecasting NO		Forecasting NO_2	
Input	Output	Input	Output	Input	Output
S_O2 NO O_3 NO_2 PM_{10} rh ap at wd ws	NO_x	SO_2 NOx O_3 NO_2 PM_{10} rh ap at wd ws	NO	SO_2 NO O_3 NOx PM_{10} rh ap at wd ws	NO_2

rh: Relative humidity (%); ap: Air pressure (mbar); at: Air temperature (°C); wd: Wind direction (degree); ws: Wind speed ($m \cdot s^{-1}$).

Table 2. Functions and neurons numbers used in the ANNs

Learning function	Transfer functions	Neurons
Levenberg-Marquardt (trainlm)	Logsig-logsig Purelin-purelin	10
	Tansig-tansig Logsig-pureline	20
	Logsig-tansig Pureline-tansig	30

logsig: Logarithmic sigmoid transfer function, purelin: linear transfer function; tansig: symmetric sigmoid transfer function

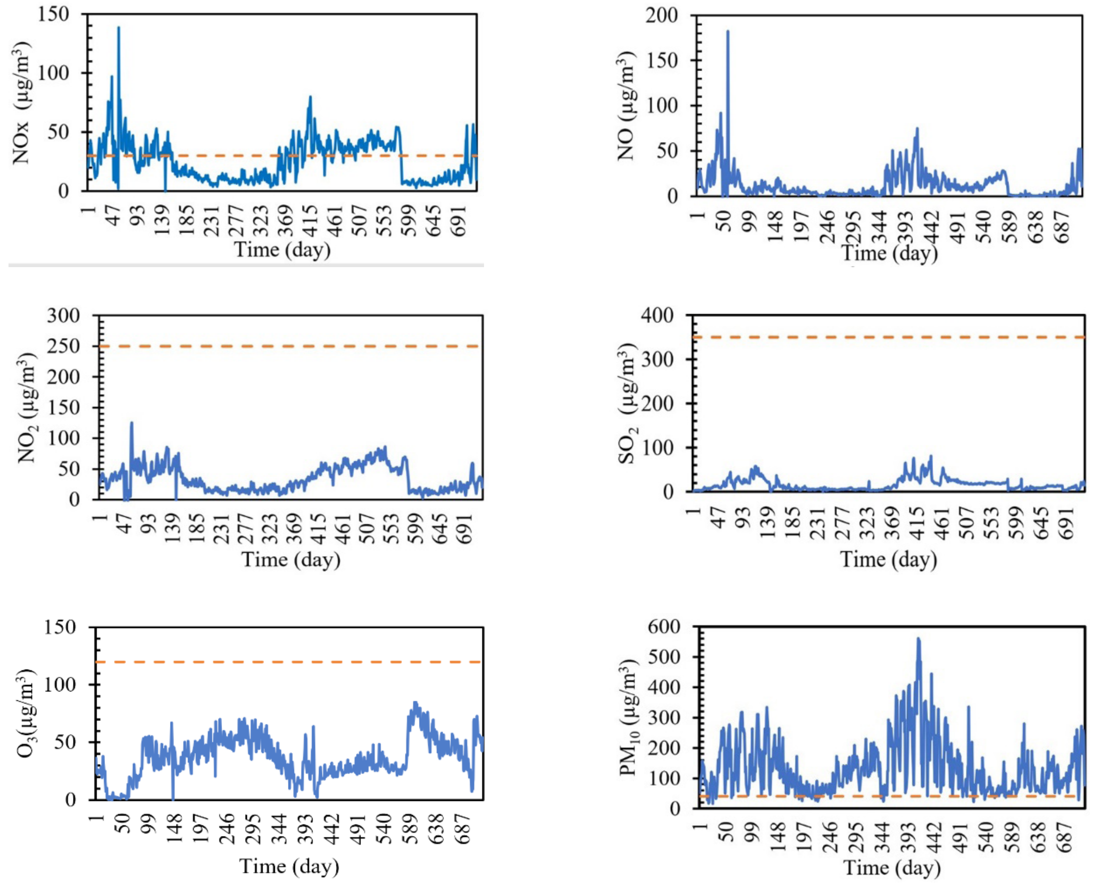

Figure 1. Air pollutant concentrations 1/10/2016-30/10/2018 horizontal line refers to the daily legal limits

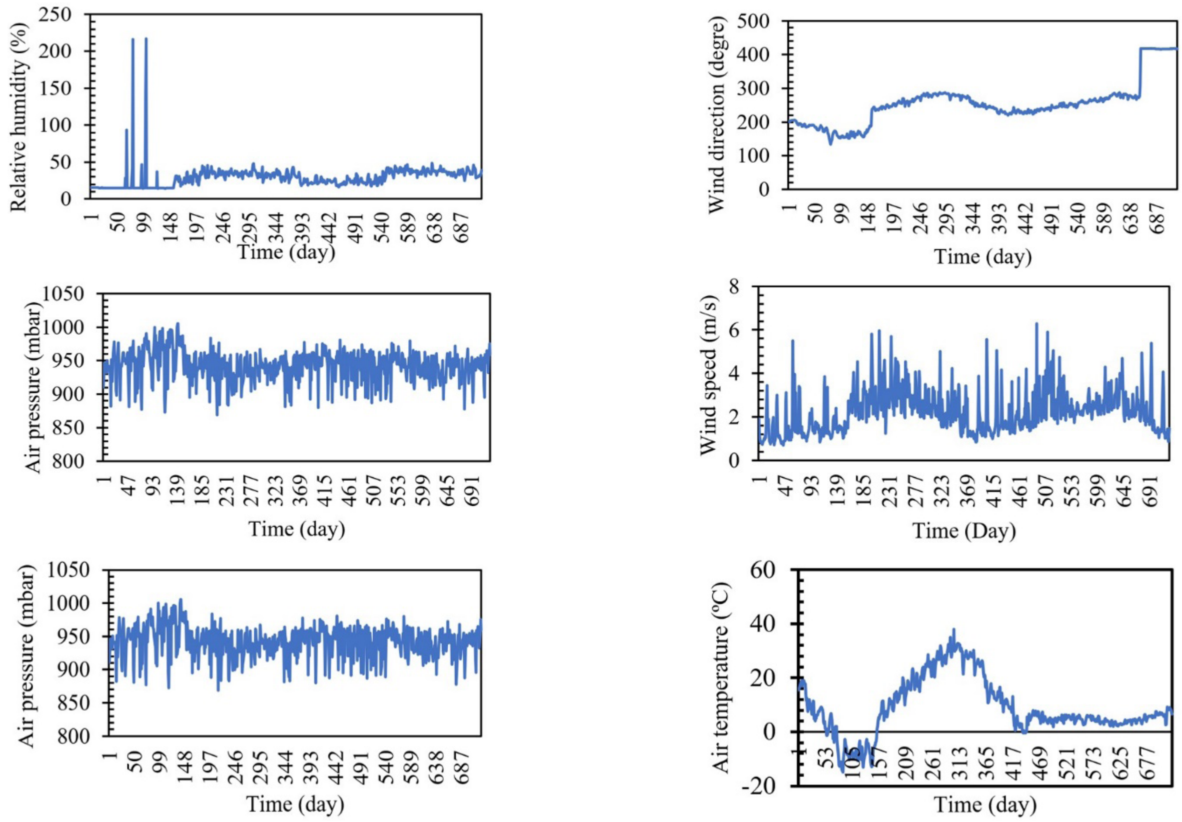

Figure 2. Meteorological factors 1/10/2016-30/10/2018

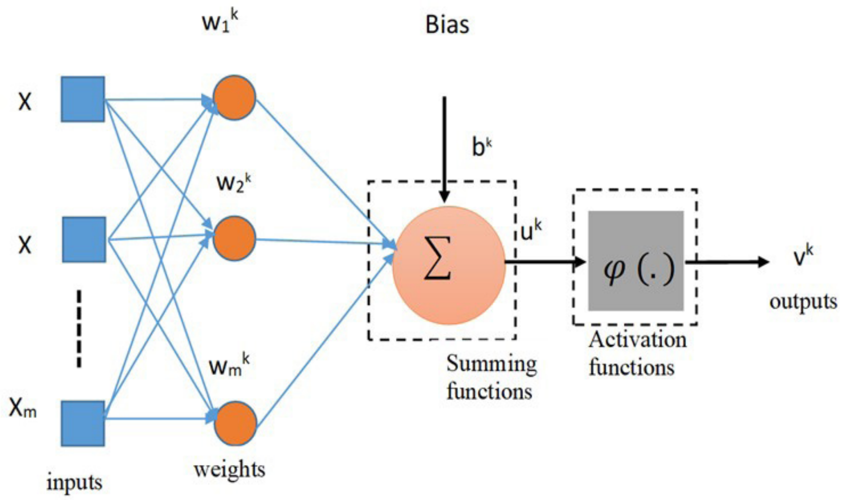

Figure 3. ANNs structure

Correlation tests

In order to determine the relationship between the inputs parameters and NO_x, NO, NO_2, firstly, the distribution of the data was examined. For this purpose, SPSS statistics program was used. As a result of the analyzes, it was determined that both inputs parameters and NO_x, NO and NO_2 concentrations determined a non-parametric distribution. These data were then subjected to correlation tests. The data didn't show a linear distribution, for this reason, Spearman's correlation tests were used. Correlation coefficients were taken into consideration in the interpretation of the test. The relationship between the factors with a correlation coefficient of less than 0.5 was considered as the weak correlation, and if this value was between 0.5 and 0.7, it was concluded that the change between the factors was moderately correlated. If the correlation coefficient is greater than 0.7, it was consideration a high correlation between the factors (Zou et al., 2003).

Sensitivity Analyzing

To appoint how much significant the input parameters are, the weight matrices and Garson equation (eq. 1) were used (Aleboyeh et al., 2008). In the equation Ij is percentage of the relative importance of the jth input variable on the neurons and W^{ih} and W^{ho} are the matrices of weights between input-hidden layer and hidden-output layer respectively, N is the total number of neurons in the corresponding layer, respectively, and subscripts 'k', 'm' and 'n' are indices referring to the neurons in input, hidden and output layers, respectively.

$$I_j = \frac{\sum_{m=1}^{Nh}\left(\left(\left|W_{jm}^{ih}\right| / \sum_{k=1}^{Ni}\left|W_{km}^{ih}\right|\right)\times\left|W_{mn}^{ho}\right|\right)}{\sum_{k=1}^{Ni}\{\sum_{m=1}^{Nh}(\left|W_{km}^{ih}\right| / \sum_{k=1}^{Ni}\left|W_{km}^{ih}\right|)\times\left|W_{mn}^{ho}\right|\}} \quad \ldots\ldots(1)$$

Performance evaluation

The performance of constructed ANNs models was statistically measured, in terms of the mean square error (RMSE) (eq.2), mean absolute error (MAE) (eq.3) and coefficient of determination (R^2) (eq.4). The model is considered accurate when R^2 is close to 1.0, while RMSE must be as small as possible. MAE is a measure used to evaluate how close the estimates are to the observed (real) results. In these equations; where, n is the number of data, Ypi is the predicted value from observation i, Ydi is the real value from observation i, and $\bar{Y}$ is the average of the observed values.

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}\left(Y_{pi} - Y_{di}\right)^2} \quad \ldots\ldots(2)$$

$$MAE = \frac{1}{n}\sum_{i=1}^{n}\left|Y_{pi} - Y_{di}\right| \quad \ldots\ldots(3)$$

$$R^2 = 1 - \left(\frac{\sum_{i=1}^{n}\left(Y_{pi} - Y_{di}\right)^2}{\sum_{i=1}^{n}\left(Y_{pi} - \bar{Y}\right)^2}\right) \quad \ldots\ldots(4)$$

Results and Discussions

Results of correlation tests

In order to determine the relationship between the inputs parameters and NO_x, NO, NO_2 the distribution of the data were given in the table 3. When the correlation coefficients are analyzed, it is seen that the effects of other pollutant gases on NO_x, NO and NO_2 are more effective than atmospheric conditions. In addition to, among the atmospheric conditions, wd, ws and at were determined to be more effective on NO_x, NO and NO_2.

Table 3 The results of Spearman's correlation test

NOx		NO		NO_2	
NO	0.907	NO_X	0.907	NO_X	0.899
NO_2	0.899	O_3	-0.741	NO	0.704
O_3	-0.695	NO_2	0.704	SO_2	0.665
SO_2	0.643	SO_2	-0.597	O_3	-0.571
wd	-0.578	wd	-0.536	wd	-0.563
ws	-0.537	ws	0.528	rh	-0.506
wt	0.501	at	0.514	at	0.416
rh	-0.500	PM_{10}	0.432	ws	-0.386
PM10	0.398	rh	-0.424	ap	0.288
ap	0.333	ap	0.308	PM_{10}	0.268
NO_X	1	NO	1	NO_2	1

rh: Relative humidity; ap: Air pressure; at: Air temperature; wd: Wind direction; ws: Wind speed.

Results of Prediction NO_x emissions

The artificial neural networks structures and their statistical results for prediction of NO_x emissions was given in table 4a. When examined the statistical results, it can be seen the highest R^2 (0.998) and lowest MAE (0.007) values were obtained from the ANN 11 structure. In this structure with 30 neurons it was used Levenberg-Marquardt and Logarithmic sigmoid - Symmetric sigmoid as learning and transfer functions, respectively.

The ANN 11 structure's training, test and validation graphs were illustrated in the figure 4b. As can be seen from figure 4, the training, test and validation's R values are higher than 0.99. According these results it can be said that the network performance has high accuracy level. This high accuracy level can be understood from the figure 5 as well.

Table 4. The statistical results of different ANN structure for NO_x concentration

Model	Learning function	Transfer function	Number of neurons	RMSE	MAE	R^2
ANN 1	trainlm	Logsig-logsig	10	0.680	0.640	0.005
ANN 2	trainlm	Purelin-purelin	10	0.019	0.007	0.994
ANN 3	trainlm	Tansig-tansig	10	0.033	0.022	0.982
ANN 4	trainlm	Logsig-pureline	10	0.017	0.010	0.995
ANN 5	trainlm	Logsig-tansig	10	0.014	0.007	0.997
ANN 6	trainlm	Pureline-tansig	10	0.041	0.032	0.974
ANN 7	trainlm	Logsig-logsig	20	0.680	0.640	0.013
ANN 8	trainlm	Purelin-purelin	20	0.019	0.008	0.994
ANN 9	trainlm	Tansig-tansig	20	0.022	0.013	0.992
ANN 10	trainlm	Logsig-pureline	20	0.012	0.008	0.998
ANN 11	trainlm	Logsig-tansig	20	0.011	0.007	0.998
ANN 12	trainlm	Pureline-tansig	20	0.042	0.032	0.972
ANN 13	trainlm	Logsig-logsig	30	0.680	0.640	0.000
ANN 14	trainlm	Purelin-purelin	30	0.020	0.008	0.994
ANN 15	trainlm	Tansig-tansig	30	0.018	0.010	0.994
ANN 16	trainlm	Logsig-pureline	30	0.016	0.007	0.996
ANN 17	trainlm	Logsig-tansig	30	0.015	0.009	0.996
ANN 18	trainlm	Pureline-tansig	30	0.041	0.031	0.973

trainlm: Levenberg-Marquardt; logsig: Logarithmic sigmoid transfer function; pureline: Linear transfer function; tansig: Symmetric sigmoid transfer function

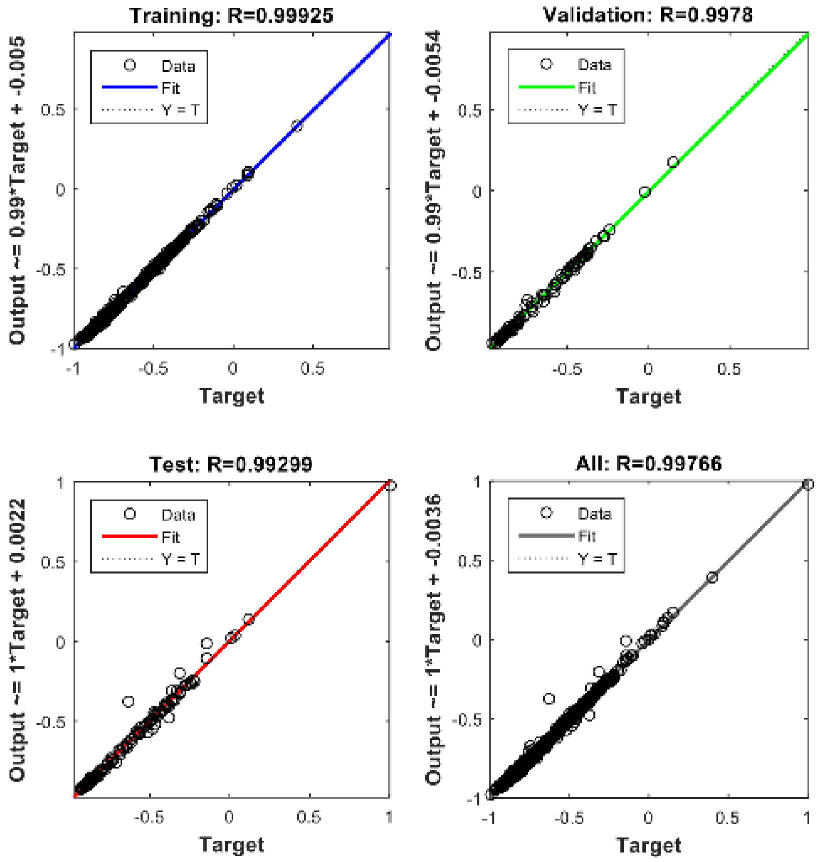

Figure 4a. The ANN 11 structure's network performance for forecasted NO_x emission

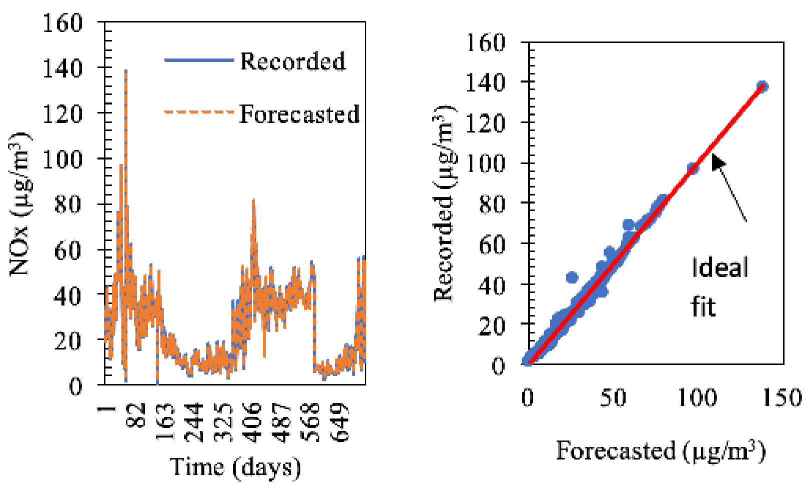

Figure 4b. Recorded and forecasted values for NO_x emission in the ANN 11 structure

Result of Prediction of NO emissions

The statistical results for forecasted of NO concentration can be seen at the table 5. The best statistical results among the ANN structures was obtained at the ANN 17 structure. In the ANN 17 structure the R^2 and MAE value were calculated as 0.995 and 0.006, respectively, and also, this structure used learning and transfer functions as Levenberg-Marquardt, Logarithmic sigmoid- Symmetric sigmoid respectively, with 30 neurons. The ANN 17's network performance can be also seen in the figure 5. The R^2 values of the training, validation and test are 0.998, 0.990 and finally 0.987, respectively. Figure 6 illustrated that, the recorded and forecasted values of NO emission in the ANN 17 structure. As it can be seen at the figure 6, the NO concentration was modelled with high significance level with the ANN 17 structure.

Table 5. The statistical results of different ANN structure NO

Model	Adaption learning function	Transfer function	Number of hidden neurons	RMSE	MAE	R^2
ANN 1	trainlm	Logsig-logsig	10	0.741	0.848	0.189
ANN 2	trainlm	Purelin-purelin	10	0.000	0.007	0.985
ANN 3	trainlm	Tansig-tansig	10	0.001	0.011	0.976
ANN 4	trainlm	Logsig-pureline	10	0.000	0.007	0.986
ANN 5	trainlm	Logsig-tansig	10	0.000	0.012	0.982
ANN 6	trainlm	Pureline-tansig	10	0.001	0.008	0.980
ANN 7	trainlm	Logsig-logsig	20	0.742	0.850	0.223
ANN 8	trainlm	Purelin-purelin	20	0.000	0.008	0.985
ANN 9	trainlm	Tansig-tansig	20	0.000	0.005	0.994
ANN 10	trainlm	Logsig-pureline	20	0.000	0.010	0.988
ANN 11	trainlm	Logsig-tansig	20	0.001	0.016	0.977
ANN 12	trainlm	Pureline-tansig	20	0.001	0.016	0.978
ANN 13	trainlm	Logsig-logsig	30	0.742	0.850	0.009
ANN 14	trainlm	Purelin-purelin	30	0.000	0.008	0.985
ANN 15	trainlm	Tansig-tansig	30	0.000	0.007	0.992
ANN 16	trainlm	Logsig-pureline	30	0.000	0.009	0.989
ANN 17	trainlm	Logsig-tansig	30	0.000	0.006	0.995
ANN 18	trainlm	Pureline-tansig	30	0.000	0.009	0.991

trainlm: levenberg-marquardt; logsig: logarithmic sigmoid transfer function; pureline: Linear transfer function; tansig: Symmetric sigmoid transfer function

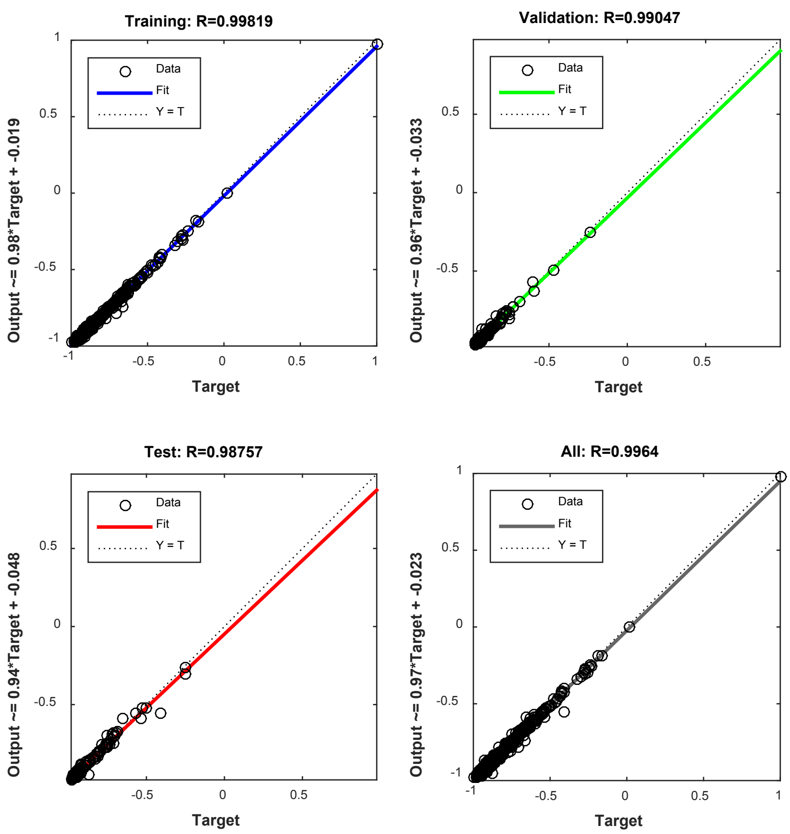

Figure 5. The ANN 17 structure's network performance for prediction NO emission

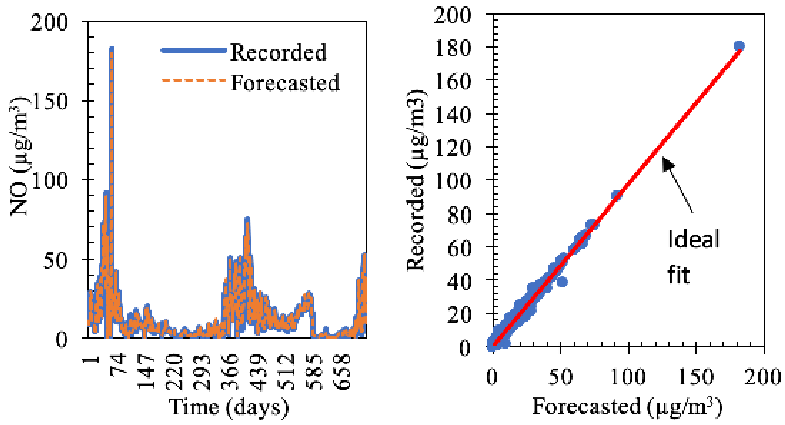

Figure 6. Observed and predicted values for NO emission in the ANN 17 structure

Results of Prediction NO_2 emission

In the research the NO_2 emission values was predicted with high accuracy level (R^2: 0.997, MAE:0.009) at the ANN 16 structure (Table 6). In this structure the learning and transfer functions were used as Levenberg-Marquardt and Logarithmic sigmoid – Linear, respectively with 30 neurons. When examined the ANN 16 structure's network performance it can be seen that high R^2 values such as 0.999, 0.993 and 0.992 for training, validation and test results, respectively (Figure 7). As shown at the figure 8, the NO_2 concentration was modelled with high significance level with the ANN 16 structure.

Table 6. The statistical results of different ANN structure NO_2

Model	Adaption learning function	Transfer function	Number of hidden neurons	RMSE	MAE	R^2
ANN 1	trainlm	Logsig-logsig	10	0.526	0.454	0.003
ANN 2	trainlm	Purelin-purelin	10	0.041	0.018	0.984
ANN 3	trainlm	Tansig-tansig	10	0.037	0.017	0.987
ANN 4	trainlm	Logsig-pureline	10	0.028	0.017	0.992
ANN 5	trainlm	Logsig-tansig	10	0.031	0.019	0.991
ANN 6	trainlm	Pureline-tansig	10	0.042	0.028	0.983
ANN 7	trainlm	Logsig-logsig	20	0.524	0.452	0.040
ANN 8	trainlm	Purelin-purelin	20	0.044	0.022	0.981
ANN 9	trainlm	Tansig-tansig	20	0.030	0.018	0.991
ANN 10	trainlm	Logsig-pureline	20	0.021	0.013	0.996
ANN 11	trainlm	Logsig-tansig	20	0.025	0.015	0.994
ANN 12	trainlm	Pureline-tansig	20	0.042	0.028	0.983
ANN 13	trainlm	Logsig-logsig	30	0.520	0.439	0.296
ANN 14	trainlm	Purelin-purelin	30	0.041	0.016	0.983
ANN 15	trainlm	Tansig-tansig	30	0.025	0.016	0.994
ANN 16	trainlm	Logsig-pureline	30	0.019	0.009	0.997
ANN 17	trainlm	Logsig-tansig	30	0.037	0.020	0.987
ANN 18	trainlm	Pureline-tansig	30	0.042	0.028	0.983

trainlm: Levenberg-Marquardt; logsig: logarithmic sigmoid transfer function; pureline: linear transfer function; tansig: Symmetric sigmoid transfer function.

Results of Sensitivity analyses

The weights used in determining the relative important of the input and output values were given in table 7. In addition, the results of the sensitivity analysis were illustrated at the figure 9, figure 10 and figure 11 for NO_x, NO and NO_2, respectively.

When examined the figures, the effects of pollutant gases for NO_x, NO and NO_2 were determined more effective than atmospheric conditions. The most effective pollutant gases in NO_x modeling were determined as NO, NO_2, O_3 and SO_2. In addition, the most effective atmospheric conditions in the NOx model were calculated as wd, ws, at and rh (Figure 8). Similar results were obtained in NO and NO_2 models (Figure 9, Figure 10). When examined the correlation test it can be seen that similar results.

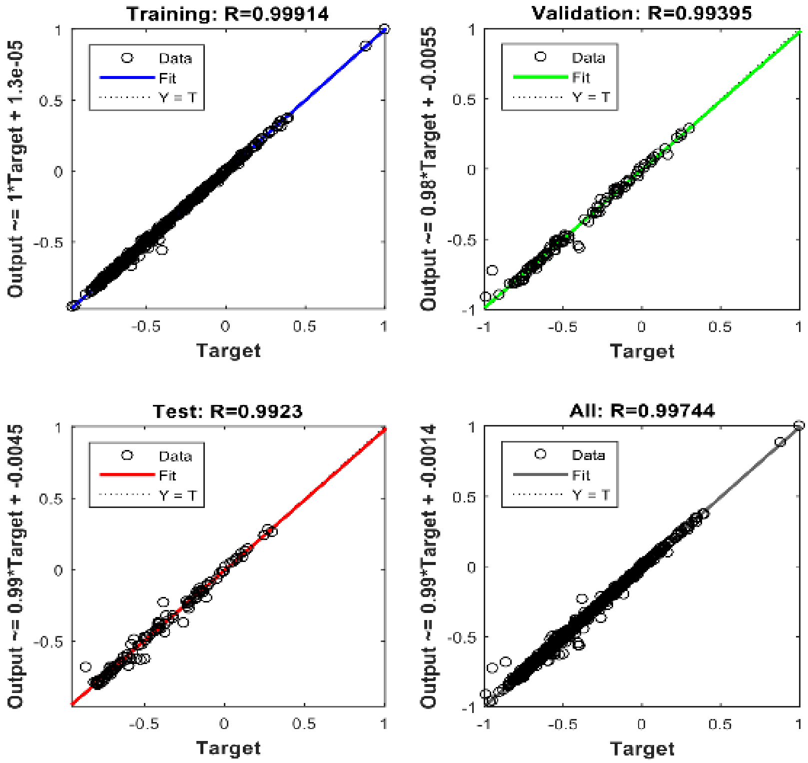

Figure 7. The ANN 16 structure's network performance for prediction NO_2 emission

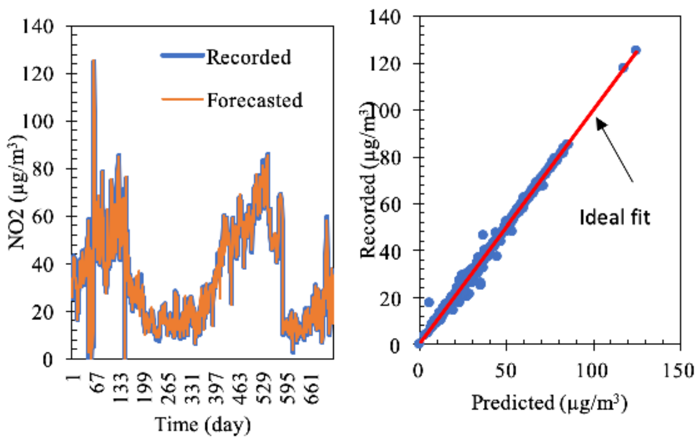

Figure 8. The ANN 16 structure's network performance for prediction NO_2 emission

Table 7. The weights between input and output values for NO_x, NO and NO_2

				W^{ih}						W^{ho}
rh	wd	ap	ws	at	SO_2	NO_2	NO	O_3	PM_{10}	NOx
0.81	2.72	0.46	0.34	0.88	-0.88	-0.19	-4.46	-0.07	1.56	-3.9
				W^{ih}						W^{ho}
rh	wd	ap	ws	at	SO_2	NO_X	O_3	NO_2	PM_{10}	NO
0.36	1.53	-0.22	0.99	0.54	1.55	-1.92	1.68	1.81	0.47	-0.22
				W^{ih}						W^{ho}
rh	wd	ap	ws	at	SO_2	NO_X	NO	O_3	PM_{10}	NO_2
-0.71	0.83	0.53	-0.60	0.63	-1.88	-2.51	-2.01	0.89	0.48	0.48

rh: Relative humidity; ap: Air pressure; at: Air temperature; wd: Wind direction; ws: Wind speed

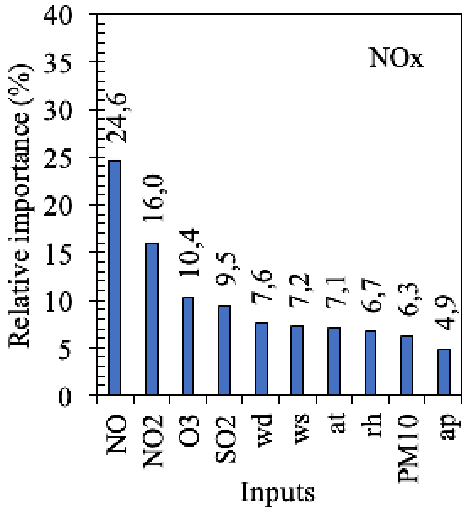

Figure 9 Relative importance values for NOx at the ANN 11 structure

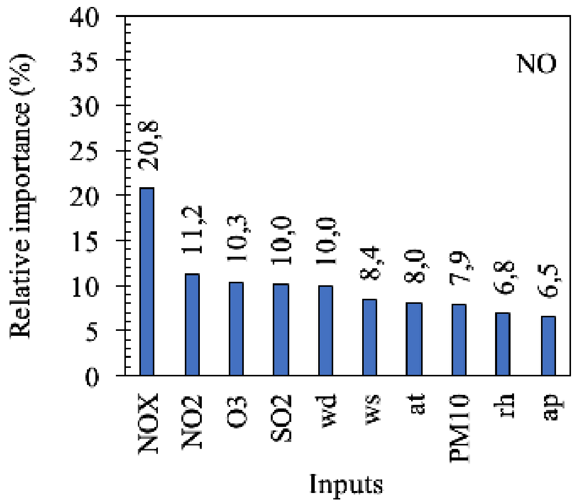

Figure 10 Relative importance values for NO at the ANN 17 structure

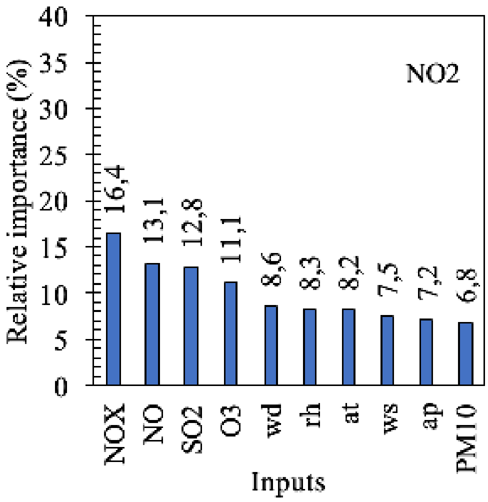

Figure 11 Relative importance values for NO at the ANN 16 structure

The main reason of nitrogen oxides emission (nitric oxide and nitrogen dioxide) is vehicle exhaust. Nitrogen dioxide forms as a result of reactions between the nitric oxide and ozone. (Gardner and Dorling, 1999). The amount of the air pollutant in the atmosphere are influenced by emission rate, chemical transformation and climate-atmospheric condition. Especially winds transport effects dispersion of pollutants. In addition, air temperatures are another important factor for NO_2 emission (Jiang et al., 2005).

The city of Iğdır where the research is conducted, located on the international road route. All the roads to Iran, Azerbaijan and Armenia pass through this city. In addition, Iğdır has microclimate properties. Due to its geographical location, there is not enough air circulation throughout the province. Therefore, the air pollution level throughout the province is quite high in all seasons of the year. The presence of large erosion sites in some districts of the province causes an increase in the level of particulate pollution. For these reasons, modeling of pollution throughout the province is very important in terms of future measures.

Artificial neural network has different learning rules and these effects the forecast accuracy. Chaudhuri and Acharya, (2012) studied effects of different learning rules in the ANN for forecasting concentration of atmospheric pollutants, and at the end of the research they determined non-linear perceptron model was better for forecasting the concentrations SO_2, CO and PM_{10}. However, for forecasting NO_2, delta learning is better than non-linear perceptron. Several researches have focused on the modelling air pollution with used ANN (Nagendra and Kahre,2006; Hrust et al., 2009; Kurt and Oktay, 2010; Cheng et al., 2012; Perez 2012; Wang et al., 2019; Alimissis et al., 2018). However, Elangasinghe et al., (2013) stated that at the forecasting studies the selection of the input parameters is very important for model performance, and different air pollutant used as input parameters is not enough high accuracy model. So, meteorological factors should be used together with air pollutants for high accuracy models (Singh et al, 2012; Yan Chan and Jian, 2013).

In this study, both meteorological factors and other air pollutants were used as input parameters, and obtained the best model performances (R^2>0.99) for NO_x, NO and NO_2 forecasting. Finally, it can be said that the ANN methods are very efficient for forecasting the air pollution concentration when the using appropriate input parameters.

Conclusion

The main objective of this research is to forecast the NO_x, NO and NO_2 concentration level in the atmosphere. For this purpose, 18 different ANNs structures were examined. In these structures, the one learning function (Levenberg-Marquardt) and six different transfer functions (logarithmic sigmoid - logarithmic sigmoid, linear - linear, Symmetric sigmoid - Symmetric sigmoid, logarithmic sigmoid - linear, logarithmic sigmoid - Symmetric sigmoid and Linear - Symmetric sigmoid) with three neuron (10,20,30) numbers were tested. In the networks, both meteorological factors (relative humidity, air pressure, air temperature, wind direction, wind speed) and air pollutants (SO_2, O_3, $PM_{10,}$ NO_x, NO and NO_2) were used as input parameters. At the end of the research, the NO_x, NO and NO_2 were modelled with high accuracy level (R^2>0.99). The best models for NO_x and NO concentration levels have been observed at the logarithmic sigmoid - symmetric sigmoid transfer functions with 20 and 30 neuron number, respectively. In addition, the best results have been determined from the ANNs structure which used Logarithmic sigmoid - Linear transfer function with 30 neuron number at the modelling of NO_2 concentration levels. In the sensitivity tests, it was concluded that O_3 SO_2, wd, ws, and rh inputs were more effective on the NO_2 NO and NO_x concentrations than other inputs. Similar results were obtained in the correlation tests.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Mechanization and agricultural farm structure in the agricultural area of the Dardanelles region

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Abstract

This study was conducted to determine the current mechanization in agricultural farms of the Bayramic-Ezine-Kumkale agricultural plains, in Canakkale (Dardanelles) region of west of Turkey. For this purpose, a questionnaire was carried out for 401 farms capable of growing both field and horticulture crops. Results indicated that each farm having small size characteristic has at least one tractor, but 19.20% of farms had more than one tractor. The status of having one (91.67%) or more (41.67%) tractors in a farm was higher in Bayramic, due to field and horticulture crops, than both others. However, the highest number of tractors was recorded in Kumkale farms (60.00%), followed by Ezine (31.65%) and Bayramic (8.35%). Most of them are young, but 12.00% are older than 24-year, especially Massey Ferguson-135, Universal and Fiat (54C, 480). The most used tractor brand has been New Holland (32.15%), followed by Massey Ferguson (18.99%), Fiat (9.11%), John Deere (8.10%), Case IH (7.85%), Same (5.05%), Deutz (4.05%), Steyr (3.54%), Valtra (2.28%) and others (Ford, Hattat, Erkunt, Basak, Tumosan, Universal, Kubota and Landini). On average three-plain, 77.03% of farms were preferred to purchase the new tractors, 22.97% preferred the second-hand ones. Tractor was 0.99 per farm, but it was the highest in Ezine (1.17). Agricultural area per tractor was 117 decare on average three-plain, the highest for Kumkale (136 decare) and the lowest for Ezine (83 decare tractor^{-1}). Machinery per tractor was 7.67 for all farms, but Kumkale (8.78) had the highest, and then followed by Bayramic (6.58) and Ezine (5.87).

Keywords: Mechanization, Farmer Status, Crop Pattern, Farm Structure

Introduction

Turkey's agricultural economy is among the top ten in the world, with half of the country consisting of agricultural area and nearly a quarter of the population employed in agriculture. The country is a major producer of wheat, sugar beet, cotton, tomatoes, and it is the top producer in the world for apricot and hazelnut. Therefore, the agriculture sector is a raw material that provides the industry with an economic and social contribution to the national income and industrial sectors. In order to meet the needs of rapidly growing human communities, more qualified and quantitative production in agricultural areas is one of the main purposes of agricultural cultivation in nowadays. For this purpose, the use of technological facilities in agriculture such as agricultural mechanization has become inevitable. In recent years, the necessity and tendency of reducing the labour directly affecting cultivation costs increases the importance and development of mechanization in agricultural activities. Tractor is the main important indicator taken into consideration in the activities of agricultural areas for determining the mechanization level. Canakkale region is one of those areas where many annual and perennial crops are grown throughout the country because of many agricultural locations. In the region, agricultural cultivation is carried out on 3320 thousand decare of agricultural area corresponding to 1.39% of the country level by 240 million decare (TUIK, 2018). The current number of agriculture farms is around 49 thousand most of which

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are family farms employing by family labour corresponding to 2.45% of the national (over 2 million). However, 45.13% of them are only under Farm Recording System which significant contributions to farmers, especially information flow and other services such as soil analysis, weather forecasts. The average size of farms is 66 decare in the region which is lower than the country (76 decare). Small size agricultural farms ranging from 0 to 50 decare constitutes 66.76% of the total farms in the region, while several sources define small farms as those with less than 20 decare of cultivated (World Bank, 2003; Hazell et al., 2007). Medium-sized farms vary between 50 and 200 decare and the share of farms in total is 31.23%, while the number of farms of 200 decare and above represents 2.00% and being the larger parcel of farms. Although the presence of small size farms in the region is proportionally high, cereals are generally grown in medium and large sized farm parcels in dry agricultural areas as dry farming. In addition, vegetables, fruits, corn and rice which are usually grown under irrigable conditions are mostly cultivated in small size parcels. Moreover, there has been an increase recently in legume cultivation, due to increasing livestock incentives by government, which is taken into crop rotation with cereals, especially in dry farming under rainfed conditions. On the other hand, 34.15% (~1 million decare) of the total cultivated agricultural area in the region tend to be irrigable when 67.47% of these areas (764 thousand deacre) were recently irrigated by water supplied from irrigation dams (for example, Bayramic), ponds and groundwater wells. In all agricultural area of the region, field crops were grown in 415 thousand decare (53.20%) under the irrigable conditions growing mostly rice, maize, alfalfa, beans, while vegetables (tomato, pepper, melon, cabbage and others) were cultivated in around 196 thousand decare of the area, the remaining of 169 decare were cultivated for horticulture (apple, cherry, peach, pear, plum, etc.).

The potential of agricultural cultivation is popular in the study area when considering the geographical structure and climate characteristics, and also the soil structure, crop pattern, cultivation systems under both dry-farming (rainfed) and irrigable conditions. In recent years, with the being of water resources into dams and ponds, as well there has been an increase in the crop variety, especially under irrigable conditions, and there is also an effort to achieve higher efficiency from unit area. For higher crop yield from unit area, the agricultural mechanization which accounts for almost 40.00% of the total investment of farms, especially tractor, is one of most important factors being effect on crop yield (Ruiyin et al., 1999) because tractor is one of the most important power sources in agriculture (Singh, 2006). For this reason, it is to determine the current-mechanization as tractor (brand, age and other properties) and other social status and farm structure for the agricultural plains of Kumkale, Ezine and Bayramic which are located in basin of Bayramic Dam, the part of which is also known as Karamanderes Basin. A questionnaire survey was conducted in the pre-determined agricultural farms by using the Farmer Registration System under Directorate of Agriculture and Forestry. The questionnaire was completed by interviewing face-to-face with farmers in the villages of the three-plain. The data obtained from the studied farms were evaluated in Excel spreadsheet to achieve the results of the mechanization and farm characteristics.

Material and Method

Study area

The study was conducted in Canakkale (*Dardanelles*) region (39°27'-40°45' N, 25°40'-27°30' E, altitude: 10 m a.s.l.). The region surrounds the southern edge of Ida mountain (1774 m elevation) which is one of the most representative nature water source in the area. Therefore, it has a few agricultural plains due to land fragmentation in the foothill of the mountain in topological view. This region covers an area of 9737 square kilometres, lying in South Marmara Region of west of Turkey which is surrounded by three sides by Mediterranean, Black and Aegean Seas (Figure 1). In the study area, annual rainfall, average humidity, the lowest and highest temperature are 620 mm, 65%, 12 °C and 30 °C, respectively, on average years of 1958-2018 (average, National Meteorological Service) (Figure 2). Many annual and perennial crops are growing under both irrigation and dry farming systems under specific agricultural locations having different micro climatic conditions due to the hills and altitudes created by Ida mountain. The water used in these agricultural locations is generally provided by the transformation of ground-wells, dams (Bayramic Dam) or ponds as surface water collecting by rivers from Ida mountain. Therefore, some of the most important agricultural plains are Bayramic-Ezine-Kumkale where different annual (e.g., tomatoes, pepper, corn,) and perennial (apple, cherry, peach, walnut) crops are grown. Irrigable cultivation systems have recently increased by 98.20% the replacement of the dry farming due to increasing irrigation facilities such as dams or other water resources, especially in the agricultural area of three-plain. The 70.41% of the total irrigable agricultural area are mostly irrigated by dams and groundwater wells in the region, but this is lower at the country level with 65.00%.

Sampling method and data collection

During 2017-2018 growing season, the study was conducted in villages of three-agricultural plain have already irrigated by Bayramic Dam, with a capacity of 96 m³ and completed at the beginning of 2000 years. The size of required sample was determined using Neyman method in order to collect data from the studied area (Yamane, 1967).

$$n = \frac{N^2 \ x \ s^2 \ x \ t^2}{(N-1)\, d^2 + (s^2 \ x \ t^2)} \qquad (1)$$

where n is the required population (sample size), N is the number of farmers in the target population, s is the standard deviation, t is the t-value at 95% confidence limit (1.96), and d is the acceptable error. The permissible error in the sample size was defined to be 5% for 95% confidence. Based on this method of sampling, 401 farms were identified from the study area. 123 villages among 177 villages of three-plain in the Bayramic basin area were recorded, whose main occupation was agriculture. The questionnaire was conducted in only 30 of 123 these villages. Thus, the number of questionnaires were 11 out of 34-village on the Bayramic plain, 11 out of 39-village on Ezine and 8 out of 50-village in centre of region covering only Kumkale (Table 1). Researchers used the questionnaire to conduct personal interviews face to face with farmers or workers known to use machinery intensively. Questions concentrated especially on the farmer social statues, farm structure and the use of mechanization in the agricultural cultivation. Data was analysed to find out the required results of the study. All data obtained from the questionnaire were evaluated in Excel programme.

Figure 1. Location of Canakkale (Dardanelles) region in west of Turkey

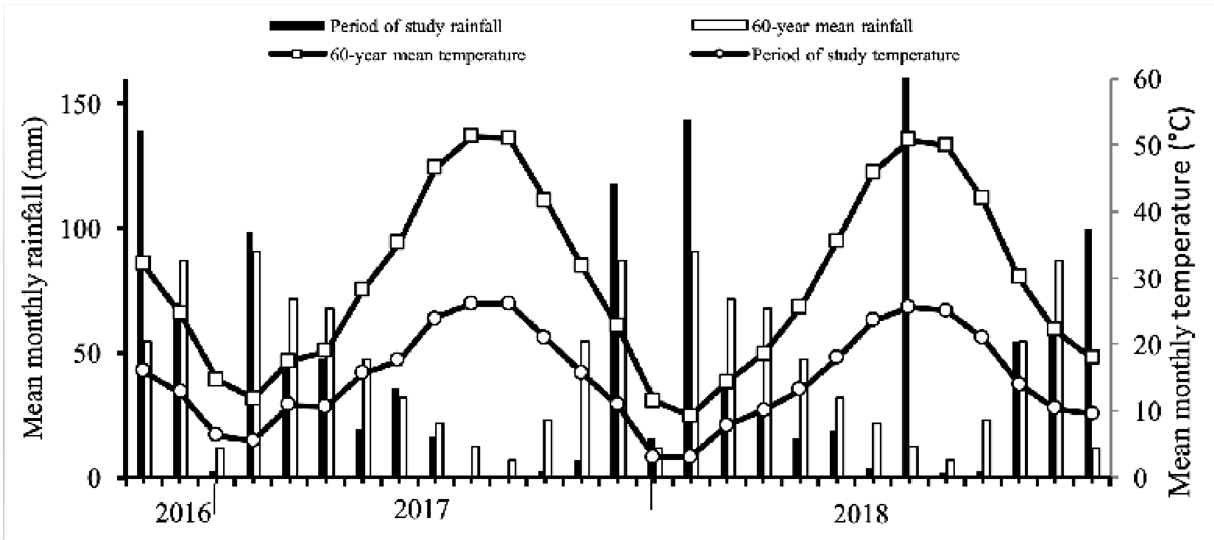

Figure 2. Monthly rainfall and mean temperature from August 2016 to December 2018, and for long term

Table 1. The number of villages and farms in the study area of three-agricultural plain

Plain	Village	Village in basin area		Questioned village		Total farms in districts of plain	Possible farms to be questionnaire of districts	Questioned farms of districts	
	(num)$^{+*}$	(num)$^{++}$	(%)	(num)$^{+++}$	(%)	(num)	(num)	(num)	(%)
Bayramic	75	34	45.33	11	32.25	2433	2157	36	1.67
Ezine	49	39	79.59	11	28.21	1164	926	107	11.56
Kumkale	53	50	94.34	8	16.00	4535	4090	258	6.31
Total	177	123	69.49	30	24.39	8132	7173	401	5.59

*, it refers to the number and used in the same meaning throughout the text; $^{+}$the number of total villages of Bayramic, Ezine and Kumkale distirtcs; $^{++}$ the number of total villages only located in Bayramic Dam Basin area; $^{+++}$ the number of questioned villages within each agricultural plain.

Results and Discussion

Social status of farmers

The socio-economic characteristics of the farmers including age, family population, the role of family person in agriculture and educational status are outlined in the following paragraphs (Table 2, 3, 4). The number of human labour has significant importance to maintain the quality of crops by doing physical labour of agricultural practices and operating an agricultural machinery. Age of farmer employed in each farm is a significant indicator for qualified and conscious cultivation. The age distribution of the farmers is ranging between 20 and 76 years on average three agricultural plains (Table 2). After interviewing farmers, data clearly indicate that the majority of the farmers are belonging to middle-age group (20-50 years) (Table 4). The average age of the farmers in the Ezine agricultural plain is 50.13 years, while it was 46.39 and 41.47 years in Kumakle and Bayramic, respectively (Table 2). It was indicated that farmers occupied with agriculture in the plains of Kumkale and Bayramic are younger than in Ezine. The farmers covered a narrow of age groups with the least under 42 years in villages of Bayramic. In recent years, the agricultural incentives provided by goverment for agricultural cultivation have increased the interest and efforts of the young agricultural engineers in agriculture sector. On the other hand, the increase in the use of mechanization in agriculture and the use of various type machinery that require high technology knowledge which was known more by younger age farmer groups. In contrast, it was found that majority of the farmers were in the age of 41-50 years by 29.80%, while age under 30 years and over 60 years is comparable very low by 11.87% and 6.08%, respectively (Table 4). The average age of the farmers is 44.82 years, and it means that more middle-age group was occupied with agriculture activities in the studied area. This shows that the income of the young people is mainly from non-agricultural sources. At the same time, this can be considered as a sign that young people prefer to live in the city instead of living in the village. Similarly, there were few young farmers in European countries; only about one in ten European countries farmer (10.60%) were under the age of 40 years (EuroState, 2018). In another study carried out in the same area of this study by questionnaire for vineyard farmers (Aydın et al., 2017) found the highest labour rate in 41-50 years range by 31.20%, followed by 51-60 years by 30.90%. In addition, another study conducted in Europe, Asia, Africa where were observed similar results that the labour of age in agriculture was stated to be between 40 and 45 years (Matthews, 2008). Author found that 40-49 age were more popular in European countries when the least farmer age was under 40 years. On the other hand, it was reported that the major of farmers (57.90%) is older than 55 years in a study conducted in European countries, while only 6% are younger than 35 years (EuroState, 2018). It was concluded that younger farmers especially tend to manage the largest farms where many small farms are managed by older farmers, often beyond the normal retirement age.

Table 2. Age and number of the farmers in the farms of three-agricultural plain

Plain	Farms (num)	Age-known farmers (num)	Age-known farmers (%)	Age ranges (year) Max.	Min.	Average
Ezine	107	93	86.92+	72	23	50.13±11.78 (23.50)+
Kumkale	258	187	72.48	76	23	46.39±10.92 (23.55)
Bayramiç	36	36	100.00	62	20	41.47±9.78 (23.59)
General	401	316	78.80	76	20	46.94±11.33 (24.14)

+Mean age± standard deviation (coefficient of variation); + Rate in all farms of each agricultural plain.

Sometimes labour by manpower were used to performed the agricultural activities, for example, such as hand-hoeing, harvesting, etc. (Table 3), but the intensity of use of human labour varies according to the working person in agriculture activities for each family. When considering all of the studied farms, the farming systems remain a predominantly family activity and many farms are family-run with only family members providing help on the farm at different times of the year, and that there are seasonal peaks in labour in harvesting, particularly in the olive for this area. The number of person in the family are changing between 3 and 4 persons, and family size consists of 4 persons on the average of all families (Table 3), and three in every four family members are working regularly in agriculture. Farmers are generally composed of middle-size families (3-4 person per family). In a similar study conducted by Aydın et al. (2017) in the same area for vineyard cultivation concluded that middle-size family is the highest as 44.20% in total while multi person type family is 21.30%.

According to the gender status of the existing family population and the status of working in agriculture, the number of male working per farm is approximately one and a half-person, this was recorded for female as one-person. Agricultural activities were female dominated profession with relatively few male farmers because of many input hand hoeing and hand harvesting practices doing by female in the studied farms (Table 3). 60.16% of the total person were females and 39.84% is males on average three-plain farm, there are results introduced by EuroState (2018) for Netherlands, Latvia and Lithuania where the only one in every twenty farmers was female, corresponding to the 44.90% of farmers. However, the farms manager, who are responsible the normal daily finical and cultivation routines of running a farm, are typically male and relatively old. Only one family male member per farm can be take responsibility as a farm manager who was majority male and 45 years of age and more (Table 4) while this was even lower among female farmers. In contrast, there was a relatively low rate of farmers of 40 years of age or less in many farms of study, and only about one in every twenty-five managers was a young farmer under the age of 40 years. In contrast, in European countries, 71.50% of farmers were male and they are relatively old in EU, 55 years of age or more (EuroState, 2018). As seen in Table 2, although the use of mechanical energy in agriculture is increasing, human labour is still an important resource. Harvesting and other similar practices were still carried out by human in the area the fact that the labour force in agriculture is needed.

Table 3. Family size and gender status in the questioned farms

	Total family person	Gender rate in family		Gender status in agriculture	
Plain	(num)	Male (%)	Female (%)	Male (num)	Female (num)
Ezine	3.64±1.69 (46.51)+	58.12	41.88	1.39±0.87 (62.63)	1.34±0.85 (63.57)
Kumkale	4.17±1.59 (38.15)	61.75	38.25	1.55±0.78 (50.27)	0.96±0.80 (83.22)
Bayramiç	3.75±0.76 (20.12)	53.93	46.07	1.33±0.53 (40.09)	1.14±035 (30.80)
General	4.00±1.57 (39.36)	39.84	60.16	1.49±0.79 (52.81)	1.05±0.79 (75.19)

+Average family person number± standard deviation (coefficient of variation).

In considering different levels of education in three-plain, most of the farmers have basic primary education by 58.98%, followed by seconder and high school by 19.59% and 12.47%, respectively (Table 4). The rate of farmers who graduated from university was found very low by 8.86% compared to other education levels, but this rate was higher than in national level with 6.00% (TUIK, 2018). In general, young farmers had higher levels of educational attainment in terms of full agricultural training, and they had followed up to date professional training courses including those on new or innovative farming practices. Only 0.50% farmers have no-education which was lower than in the education level of the national agriculture by 15.20% (TUIK, 2018). In similar, a study conducted for the different countries resulted that 83.82% of the farmers had different education levels while the rest of them had no-formal education (Matthews, 2008). In another study concluded by Aydın et al. (2017), they found that all farmers have different level of education when the proportion of the university graduation is very low by 0.60%. In other hand, they concluded that the farmers with primary and high school education were higher by 70.61% and 14.52%, respectively, but the proportion of the secondary school was lower by 13.23%.

Table 4. General characteristics of farmers of the studied farms

Age	Age		Education level (%)				
range	(year)	(%)	No-formal	Primer	Secondary	High-school	University
20-30	26.02±3.75	11.87	-	46.97	16.33	18.37	18.33
31-40	36.03±2.91	24.49	-	55.45	18.39	13.63	12.53
41-50	45.33±2.77	29.80	-	59.53	14.37	17.00	9.10
51-60	55.39±2.96	27.78	-	63.00	24.50	8.17	4.33
61-+	66.13±4.84	6.08	0.50	69.95	24.36	5.19	0.00
Ave./Tot.	44.82±11.38	100.00	0.50	58.98	19.59	12.47	8.86

Agricultural Organizations

In the scope of the questionnaire, the farmers are conducted their activities under some agricultural organizations which are usually known at the national level, for example, the farmer registration system (FRS), farmer association, dealers (agrochemical, machinery, seed, etc.), agricultural cooperative (agricultural development, irrigation, fisheries), the agriculture chamber. Farmers often prefer the agricultural organizations in order to be able to carry out their agricultural activities confidently and to benefit from the support given by government since they are small type farms and can't reach to the market by their own opportunities. According to Table 5, the farmers had a high tendency to follow to the relevant agricultural organizations to register their own information and kept their rights. The farmers were mostly registered in the FRS of the Directorate of Agriculture and Forestry by 65.84% among the studied farmers (Table 5). Majority of the farmers in the region have been registered for the FRS, and approximately 33.00% of them were found to be composed of farmers who were settled in Bayramic, Ezine and Kumkale agricultural plains. The FRS of professional association of the farmers and governmental organization has an important role in the arrangement of agricultural policies, in the updating of farmer information in the supervision of agricultural support programs, especially for field crops, followed by the farmer associations (51.87%) and the agriculture chambers (31.92%). The irrigation association, known as water user's association from 2008, is the one of associations to serve agricultural water to farmers and make investments for related substructures such as land-levelling, on-farm irrigation system, and operate and maintain existing systems. The majority of irrigated area by groundwater is more common in the studied farms and managed by this association compared to the areas irrigated with surface water such as dams or ponds. In addition, in recent years with the introduction of irrigation dams (e.g. Bayramic Dam) in the studied area, the main priority of the members of the association is to make new investments and improve water collection rates, especially more efficient of the dripping irrigation systems (Figure 2). The chambers of agriculture are a non-governmental organization and play a significantly role in rural development. They provide services in fields such as farmers' registery, the determination of the product prices, balancing of the input prices in the market, laboratory services, soil analysis, registration of the farmers, and recording of the farming files. However, this organisation has been more active in different countries of the world and played an important role in the modernization of the agriculture (Compagnone et al., 2013) and political decision-making at the local and national level (Spiewak et al., 2016).

Table 5. Registration of agricultural organizations in the agricultural farms

	NSF	FRS		FA		AD		COOPS		KOSGEB		AC	
Plain	(num)	(num)	(%)+	(num)	(%)+	(num)	(%)+	(num)	(%)	(num)	(%)+	(num)	(%)+
Ezine	107	67	62.62	41	38.32	8	7.48	4	3.74			20	18.69
Kumkale	258	180	69.77	152	58.91	4	1.55	12	4.65	11	4.26	104	40.31
Bayramiç	36	17	47.22	15	41.67	3	8.33			1	2.78	4	11.11
General	401	264	65.84	208	51.87	15	3.74	16	3.99	12	2.99	128	31.92

+Percentage in the number of studied farms. NSF, number of studied farms; FRS, farmer registration system; AD, agrochemical dealer; COOPS, agricultural cooperatives; AC, agriculture chamber; FA, farmer association; KOSGEB, small and medium-sized farm development organizations.

Agricultural dealers, cooperatives, and small and medium-sized farm development organizations (KOSGEB) are low and range from 2.99% to 3.99%. However, despite the high rate of agricultural organization systems, farmer cannot effectively arrangement on the marketing of the agricultural products as well as determining current prices. Although the cooperatives have little effect on marketing of fresh fruit and vegetable products in all around the studied areas, the cold-storage facilities have an important role to keep the agricultural products for a long term. There are 5 and 4-cold storage (as company or cooperative) in the area of Kumkale and Bayramic plains, respectively, and these are usually store apple, peach, pear, plum, quince and date which are commonly grown-fruits in the study areas, especially for the post-irrigation period (Table 9). On the other hand, it is revealed that the farmers registered in more than one organization have high reasonable level in the area. For example, a farmer with FRS was also found to have registered at the same time in the dealer, cooperatives (agricultural development, irrigation, fishers, sugar beet growers) and agricultural chamber, etc. Although agricultural cooperatives are more active at the national level with large multi-purpose integrated service organization and provide a range of services such as purchasing inputs and equipment (e.g. seed, seedling, milking machines, etc.), providing specialized services (e.g. veterinary services, cold storage facility for some agriculture products, farmer training and extension) as well as processing (e.g. rice cleaning and grading, olive processing) they are very low level in the study area by 3.99%, especially TARIS (association of agricultural sales cooperatives) olive processing due to quite olive growing area (67.76%) within the fruits (Table 9). There is one TARIS processing olive and olive oil in the study region, but there are 33-cooppeartive as TARIS that are operating only in Aegean region with the same purpose. At the national level, the total of cooperative members is about 8 million that makes 11.00% of the population (Okan and Okan, 2013). For example, Agricultural Development Cooperatives, may be established in rural municipalities, villages and districts, include various cooperatives to improve the agricultural production (e.g. the production of olive and fruits etc.) and assist socio-economic development of the members and reduce economic vulnerability.

Farm structure, crop pattern and agricultural machinery

Farms were conducted their agricultural activities in both owned and rental agricultural areas (Table 6), but the management of practices in the area may vary depending on the annual capacity of agricultural activities of farmers such as crop pattern and crop rotation within the same year. On average three-plain, it was recorded that 15.30% of farms are cultivated their agriculture activities on rental fields which are varying from year to year due to the fluctuations of the rental price, and 84.70% are occupied in doing their owned area (traditional owned). The costing of rental price per unit of agricultural area may also vary starkly between the farms of villages within the same agricultural plains because there are different factors depending on localised productivity factors (soil quality, slope, drainage etc.). For example, renting one-unit of agricultural area was most expensive in the lowlands, with the highest regional average, being almost twice compared with slope or dry farming area.

Table 6. Availability of agriculture area in the farms based on ownership and rental status

	Ezine (d+i) (decare)			Kumkale (d+i) (decare)			Bayramic (d+i) (decare)			Generel (d+i) (decare		
	Own	Rent	Total	Own	Rent	Total	Own	Rent	Total	Own	Rent	Total
Total	9239	1193	10432	35469	7103	42572	3840	-	3840	39309	7103	46412
%	88.56	11.44	100.00	81.61	18.39	100.00	100.00	-	100.00	84.70	15.30	100.00
Ave.	840	239	948	3941	1421	4730	349	-	349	1310	355	1547
Max.	2311	380	2576	10737	2770	10737	1220	-	1220	10737	2770	10737
Min.	200	50	200	430	100	430	110	-	110	110		110
St	661.68	119.08	711.27	3764.31	1006.29	4059.93	321.48	-	321.48	2123.68	744.68	2420.72

d, dry farming area in the studied farms; i, irrigable farming area in the studied farms; St, standard deviation (coefficient of variation)

Canakkale region has high potential of the agricultural cultivation for both crop and livestock by using mechanical energy in almost all agricultural activities with slightly human energy. Machinery per unit agricultural area of the farms are higher than national level (TUIK, 2018), usually using mechanical power, except in fruit harvest operations which were done by human, for example, apple, grape, and olive in some farms. Considering arable agricultural structure and water resources from the study area located in Karamenderes basin, machinery and other agricultural technology, for example; drip-irrigation, has been widely used (Figure 2). Machinery was intensively used in all cultivation practices of the different agriculture branches, especially in Kumkale, followed by Bayramic and Ezine. In three-plain, agricultural cultivation is usually carried out in small size parcels which are irrigated by ground water or streams collected from Ida mountain to dams (e.g. Bayramic Dam) (Table 7). Wheat among the crop patterns was usually cultivated in more farms by 76.56% in regardless of dry or irrigation conditions, followed by pepper (46.13%), tomatoes (45.64%) and maize (33.42%). These were followed by the farms which are cultivated olive (26.68%), and rice, peach and barley by around 20.00%. The size of 73.13% of the farms is below 50 decare among the studied farms due to the high level of land fragmentation. 6.18% of them have the agricultural area of less than 5 decare, whereas only 3.55% are being in 150 decare and above that are known as commercial farms increasing recently in the region despite small size farms. Crop pattern of the post-irrigation was increased compared to the pre-irrigation period by using Bayramic Dam (Table 8, 9). Considering crop pattern for pre-irrigation period (Table 8), it has been identified that there are a limited variety of crops, but agriculture area was found higher than the post-irrigation covering 1996-2018, due to the shift many agriculture areas to facilities such as housing, operation building and agriculture product processing units (e.g. cold storage, olive factory), especially in Kumkale plain decreasing by approximately 86.00%. In pre-irrigation, cereals are commonly grown, especially under dry farming, whereas crop growth under irrigation conditions is more restricted (Table 8). Although wheat is the most important crop among cereals in the area of the current basin for both periods, but its cultivation area is shifted to crops such as maize, rice, and others (cherry, peach, walnut) under irrigable agricultural areas. Although the cultivation area of barley, rye, oats and some legumes, which are generally used to feed the animal, decreased compared to the pre-irrigation period since they continue to be grow in the post-irrigation period (Table 8, 9). However, some crops (e.g. silage maize, clover) that have been cultivated by using water of dams in period covered post-irrigation between 1996 and 2018 (Table 9), and their growing area are increased by using Bayramic Dam for the agricultural irrigation. For example, the growing of grain maize was increased by approximately 5.5 times in only Bayramic plan during the post-irrigation period compared with pre-irrigation when silage maize was grown approximately 17 thousand decare in three-plain agriculture areas (Table 9). This was supported increasing the number of maize harvester and stalk shredder by 75.32% and 98.45%, respectively (Figure 2). The growing of the clover or silage maize are usually under the drip irrigation which has been found to be increased by 77.15% and 100.00%, respectively (Table 9). The practices with irrigation is also increased the using of the water pump in the studied farms by 8.55% (Figure 2). Pre-irrigation agricultural practices of the three-plain agriculture areas, the crops such as silage maize, sorghum, grass, canola, safflower and rice don't have almost growing areas, but they were grown with the irrigation applications (Table 9). By growing such as crops, it has encouraged to grow livestock (Özpınar, 2002), and it also caused to the opening of factories processed milk to produce cheese

Table 7. Number and size of parcels according to crop pattern for both field and horticulture branch in the farms

	<5 decare		5-49 decare		50-149 decare		≥150 decare		Total <5-≥150 decare	
Crop	(num)	(%)	(num)	(%)	(num)	(%)	(num)	(%)	(num)	(%)
Wheat	5	1.63	179	58.31	98	31.92	25	8.14	307	76.56
Pepper	14	7.57	164	88.65	5	2.70	2	1.08	185	46.13
Tomatoes	24	13.11	141	77.05	17	9.29	1	0.55	183	45.64
Maize	1	0.75	109	81.34	19	14.18	5	3.73	134	33.42
Olive	6	5.61	70	65.42	29	27.10	2	1.87	107	26.68
Rice	0	0.00	29	35.37	37	45.12	16	19.51	82	20.45
Peach	2	2.44	66	80.49	14	17.07	0	0.00	82	20.45
Barley	0	0.00	57	69.51	23	28.05	2	2.44	82	20.45
Cherry	9	15.79	48	84.21	-	-	-	-	57	14.21
Oat	2	3.51	40	70.18	14	24.56	1	1.75	57	14.21
Apple	4	7.69	43	82.69	5	9.62	0	0.00	52	12.97
Bean	7	17.07	34	82.93	-	-	0	0.00	41	10.22
Sunflower	-	-	20	50.00	16	40.00	4	10.00	40	9.98
Melon	3	11.11	24	88.89	-	-	-	-	27	6.73
Plum	6	27.27	16	72.73	-	-	-	-	22	5.49
Vineyard	8	42.11	11	57.89	-	-	-	-	19	4.74
Vetch	-	-	17	100.00	-	-	-	-	17	4.24
Field bean	3	20.00	10	66.67	2	13.33	-	-	15	3.74
Watermelon	1	1.19	83	98.81	-	-	-	-	84	20.95
Strawberry	2	16.67	10	83.33	-	-	-	-	12	2.99
Apricot	4	36.36	7	63.64	-	-	-	-	11	2.74
Cotton	-	-	9	90.00	1	10.00	-	-	10	2.49
Trifolium	-	-	8	100.00	0	0.00	-	-	8	2.00
Total	101	6.18	1195	73.13	280	17.14	58	3.55	1634	100.00

or other dairy products. It has been found that silage maize is commonly growing crop by 94.06% in the north of Ezine and Bayramic plains located in the central part of the Karamenderes basin (Table 9); therefore, livestock has become an important agricultural occupation in the same area. This was increased agricultural equipment in the machinery park, especially in number of silage maize harvester, stalk shredder, baler, mover, feed preparation and dairy milking machine and weed tiller (Figure 2). By intensive agriculture system in the current study areas covering Bayramic-Ezine-Kumkale plains, there has also been an increase in surface tillage machinery such as rototiller (89.60%) and rotovator (35.48%) which were usually used in conservation soil tillage systems. Rice started to be grown by the using irrigation application, and it has begun to be cultivated widely in the east of the Ezine plain and partially in the south of Kumkale (Table 9), and this leads to increase the use of combine by 10.04%. On the other hand, there has been an increase in the number of tractor, especially in the last five years by 17.56% in regardless of the tractor power size and brand compared to pre-irrigation period, it means that each farmer has at least one tractor. Moreover, the use of drip irrigation systems instead of sprinkler, which can use water more economically, has become more widely used, especially in the cultivation of vegetables and fruits.

Table 8. Crop pattern in the pre-irrigation period of the farms according to crop branches

Branch	Crop	Bayramic		Ezine		Kumkale		Total		General
		(decare)	(%)	(decare)	(%)	(decare)	(%)	(decare)	(%)	(%)
Field crops	Wheat	69152	28.40	64390	26.44	109984	45.16	243526	42.87	69.02
	Maize	310	40.90	185	24.41	263	34.70	758	0.13	
	Barley	37584	42.31	31870	35.88	19370	21.81	88824	15.64	
	Rye	798	75.57	115	10.89	143	13.54	1056	0.19	
	Oat	17384	83.92	936	4.52	2396	11.57	20716	3.65	
	Vetch	1448	23.85	1638	26.98	2986	49.18	6072	1.07	
	Potatoes	498	87.06	-	-	74	12.94	572	0.10	
	Broad bean	16418	28.62	15994	27.89	24944	43.49	57356	10.10	
	Chickpea	4134	25.56	1932	11.95	10108	62.50	16174	2.85	
	Cotton	-	-	34213	37.58	56836	62.42	91049	16.03	
	Sunflower	246	2.44	458	4.54	9394	93.03	10098	1.78	
	Sesame	16058	75.26	1900	8.90	3380	15.84	21338	3.76	
	Peanut	142	60.17	94	39.83	-	-	236	0.04	
	Clover	690	17.92	2320	60.26	840	21.82	3850	0.68	
	Bean	1046	24.31	1913	44.46	1344	31.23	4303	0.76	
	Kidney bean	158	56.83	100	35.97	20	7.19	278	0.05	
	Animal bean	-	-	1750	97.22	50	2.78	1800	0.32	
	Total	166066	29.24	158058	27.83	242082	42.62	568006	100.00	
Fruits	Apple	23725	90.64	100	0.38	2350	8.98	26175	13.80	23.05
	Peach	1500	43.99	110	3.23	1800	52.79	3410	1.80	
	Strawberry	-	-	20	100.00	-	-	20	0.01	
	Pear	130	100.00	-	-	-	-	130	0.07	
	Cherry	60	100.00	-	-	-	-	60	0.03	
	Apricot	-	0-	-	-	60	100.00	60	0.03	
	Grape	20070	64.99	3560	11.53	7250	23.48	30880	16.28	
	Olive	32600	25.37	88775	69.08	7140	5.56	128515	67.76	
	Almond	200	47.62	-	0.00	220	52.38	420	0.22	
	Total	78285	41.27	92565	48.80	18820	9.92	189670	100.00	
Vegetables	Onion	1383	24.33	2464	43.34	1838	32.33	5685	8.72	7.93
	Garlic	112	21.21	252	47.73	164	31.06	528	0.81	
	Leek	50	9.11	234	42.62	265	48.27	549	0.84	
	Carrot	50	71.43	10	14.29	10	14.29	70	0.11	
	Radish	50	48.08	10	9.62	44	42.31	104	0.16	
	Cauliflower	14	8.67	100	61.92	47.5	29.41	161.5	0.25	
	Cabbage	163	14.78	505	45.78	435	39.44	1103	1.69	
	Lettuce	108	39.56	20	7.33	145	53.11	273	0.42	
	Spinach	105	28.38	65	17.57	200	54.05	370	0.57	
	Purslane	-	-	-	-	10	100.00	10	0.02	
	Parsley	22	52.38	20	47.62	-	-	42	0.06	
	Rocket	10	100.00		0.00	-	-	10	0.02	
	Tomato	2188	5.77	11725	30.93	24000	63.30	37913	58.13	
	Cucumber	325	20.09	963	59.52	330	20.40	1618	2.48	
	Pepper	243	18.74	228	17.58	826	63.69	1297	1.99	
	Okra	110	24.28	253	55.85	90	19.87	453	0.69	
	Eggplant	200	11.03	563	31.05	1050	57.92	1813	2.78	
	Pumpkin	40	17.94	80	35.87	103	46.19	223	0.34	
	Pea	233	10.30	-	-	2030	89.70	2263	3.47	
	Melon	1900	40.88	1298	27.93	1450	31.20	4648	7.13	
	Watermelon	2150	35.29	1493	24.50	2450	40.21	6093	9.34	
	Total	9456	14.50	20283	31.10	35488	54.41	65227	100.00	
General (total)		253807	30.84	270906	32.92	296390	36.02	822903		100.00

Table 9. Crop pattern in the post-irrigation period of the farms according to crop branches

		Bayramic		Ezine		Kumkale		Total		General
Branch	Crop	(decare)	(%)	(decare)	(%)	(decare)	(%)	(decare)	(%)	(%)
	Wheat	93030	63.63	40586	27.76	12579	8.60	146195	44.56	
	Maize	1711	18.62	5494	59.78	1986	21.61	9191	2.80	
	Barley	45479	62.53	22290	30.65	4960	6.82	72729	22.17	
	Rye	125	55.80	60	26.79	39	17.41	224	0.07	
	Rice	0	0.00	6221	86.44	976	13.56	7197	2.19	
	Oat	18700	67.14	7450	26.75	1701	6.11	27851	8.49	
	Vetch	3900	43.42	4400	48.99	682	7.59	8982	2.74	
	Broad bean	1020	31.04	2100	63.91	166	5.05	3286	1.00	
	Chickpea	3500	76.09	680	14.78	420	9.13	4600	1.40	
	Cotton	0	0.00	100	87.72	14	12.28	114	0.03	
Field crops	Sunflower	360	5.26	2992	43.69	3496	51.05	6848	2.09	53.54
	Sesame	2870	91.00	170	5.39	114	3.61	3154	0.96	
	Clover	7500	58.18	4900	38.01	490	3.80	12890	3.93	
	Bean	1100	71.29	320	20.74	123	7.97	1543	0.47	
	Animal bean	1350	47.01	970	33.77	552	19.22	2872	0.88	
	Maize(silage)	9000	53.58	6800	40.48	998	5.94	16798	5.12	
	Sorghum	1590	75.46	450	21.36	67	3.18	2107	0.64	
	Grass	150	38.66	220	56.70	18	4.64	388	0.12	
	Canola	860	100.00		0.00		0.00	860	0.26	
	Safflower	50	100.00		0.00		0.00	50	0.02	
	Total	192295	58.65	106203	32.39	29381	8.96	327879	100.00	
	Apple	29925	96.62	328	1.06	720	2.32	30973	12.74	
	Peach	6760	67.10	660	6.55	2655	26.35	10075	4.14	
	Strawberry	120	88.89	10	7.41	5	3.70	135	0.06	
	Pear	560	84.85	50	7.58	50	7.58	660	0.27	
	Cherry	4940	90.23	265	4.84	270	4.93	5475	2.25	
	Apricot	77	7.93	558	57.47	336	34.60	971	0.40	
	Grape	19720	91.83	1500	6.99	254	1.18	21474	8.83	
	Olive	40320	25.20	116530	72.84	3134	1.96	159984	65.81	
	Almond	690	22.22	2300	74.07	115	3.70	3105	1.28	
Fruits	Date	42	47.73	20	22.73	26	29.55	88	0.04	39.70
	Quince	260	44.22	310	52.72	18	3.06	588	0.24	
	Plum	425	55.19	225	29.22	120	15.58	770	0.32	
	Medlar	3	37.50		0.00	5	62.50	8	0.00	
	Pomegranate	20	4.30	420	90.32	25	5.38	465	0.19	
	Peanuts	26	50.98	18	35.29	7	13.73	51	0.02	
	Hazelnut	17	100.00		0.00		0.00	17	0.01	
	Chestnut	44	100.00		0.00		0.00	44	0.02	
	Walnut	3550	43.28	4300	52.43	352	4.29	8202	3.37	
	Total	107499	44.22	127494	52.45	8092	3.33	243085	100.00	
	Onion	190	39.26	230	47.52	64	13.22	484	1.17	
	Garlic	135	85.99	12	7.64	10	6.37	157	0.38	
	Leek	25	31.25	40	50.00	15	18.75	80	0.19	
	Carrot	2	50.00		0.00	2	50.00	4	0.01	
	Radish	6	42.86	2	14.29	6	42.86	14	0.03	
	Cauliflower	85	18.85	350	77.61	16	3.55	451	1.09	
	Cabbage	90	31.58	160	56.14	35	12.28	285	0.69	
	Lettuce	177	44.25	151	37.75	72	18.00	400	0.97	
	Spinach	116	41.13	140	49.65	26	9.22	282	0.68	
	Parsley	20	62.50	2	6.25	10	31.25	32	0.08	
Vegetables	Tomato	6800	34.26	10600	53.40	2450	12.34	19850	47.96	6.76
	Cucumber	114	32.95	217	62.72	15	4.34	346	0.84	
	Pepper	5180	49.68	4879	46.79	368	3.53	10427	25.19	
	Okra	25	29.41	55	64.71	5	5.88	85	0.21	
	Eggplant	42	23.60	100	56.18	36	20.22	178	0.43	
	Pumpkin	200	79.68	40	15.94	11	4.38	251	0.61	
	Pea	453	29.45	1009	65.60	76	4.94	1538	3.72	
	Melon	950	32.93	1550	53.73	385	13.34	2885	6.97	
	Watermelon	1100	30.29	2400	66.10	131	3.61	3631	8.77	
	Total	15710	37.96	21937	53.01	3733	9.02	41380	100.00	
General (total)		315504	51.52	255634	41.75	41206	6.73	612344		

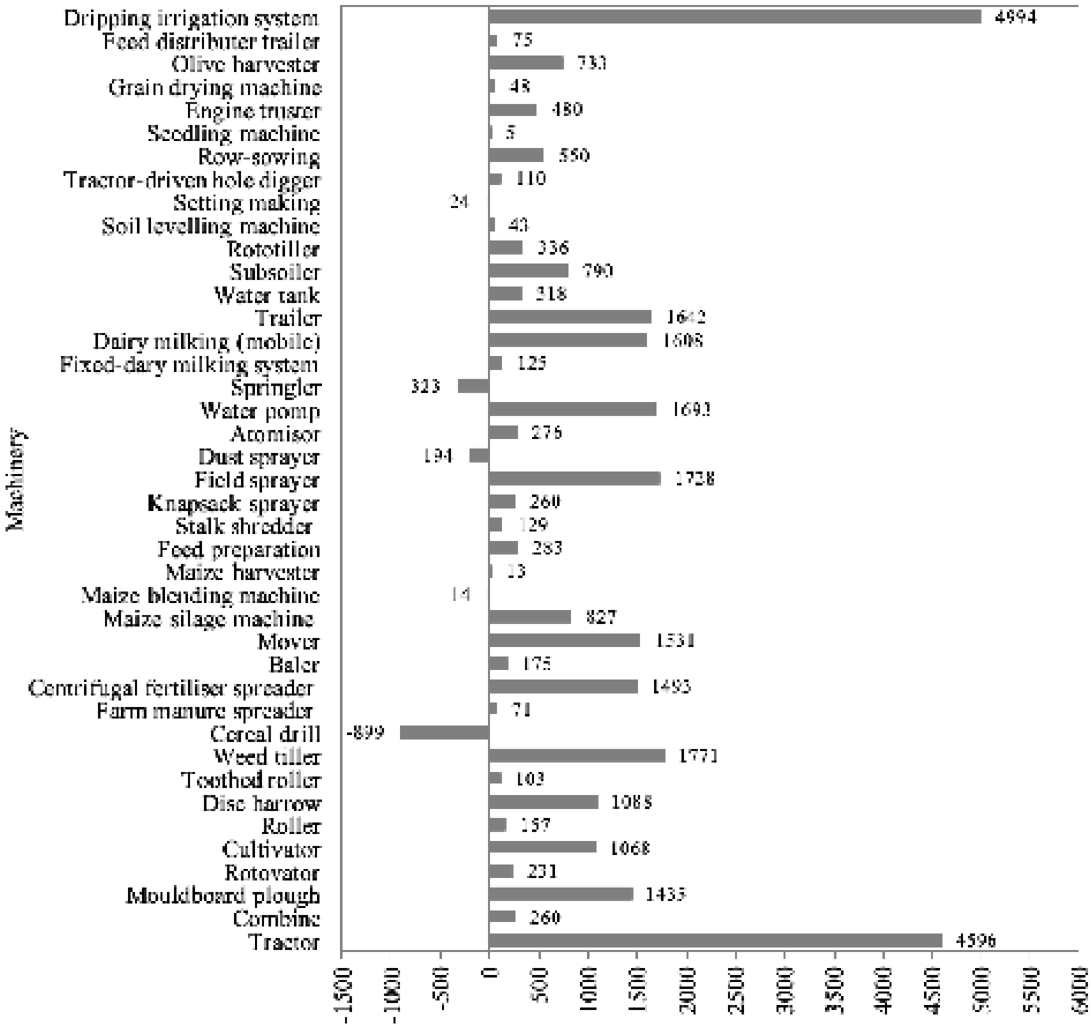

Figure 2. Increase and decrease in the number of agricultural machinery in post-irrigation period (the first quarter of 2018 compared to pre-irrigation period (1996)

Agricultural mechanization and its indicators in the farms

The relationship between agricultural area and tractor

Most farms are family farms and only employ family labour and they are considerably smaller than those in the national, with the size of the average farm in country being 65 decare. Small scale farming is important characteristic of the region agriculture. Region agriculture also suffers from inadequate farm management and technology such as farm tractors and machineries, water shortages and droughts, an inefficient rural credit system to produce the agriculture area, as well as high costs. The farmers for increasing agricultural production of the region, especially for the three-agricultural plain where the study was carried out, are expected to be further productivity growth with irrigation schemes supporting improvements such as Bayramic Dam, and with agriculture tractor and machinery. Tractor has traditionally been used on farms to mechanise several agricultural operations and accessed as mechanization level in terms of number per farm and unit area (Ozmerzi, 1998) (Table 10). A modern tractor is used for ploughing, tilling, planting, landscape maintenance, moving, or spreading fertiliser and cleaning bushes. Tractor offers advantages on small farms as well as horticultural operations, and the various benefits of using tractors to mechanise farming. Effect of tractor power on agricultural cultivation is quite important and varying according to agricultural area. Therefore, tractor power may differ considerably in different area and productivity and it was positively correlated with potential unit farm power. The average agricultural area per tractor was 117 decare on average three-plain, but this was found to be higher for Kumkale with 136 decare tractor-1 which was higher than two other plains, 116 decare tractor-1 for Bayramic and 83 decare tractor-1 for Ezine. When compared with national level, agriculture area per tractor was higher and recorded as 220 decare in 2004, but it was found lower by 178 decare in 2012 (Akdemir, 2013) and 147 decare in 2014 (Civelek, 2016) and 116 decare in 2018 (Yücel, 2019). This means that the tractor number was increased by year. On the other hand, when the current tractors per farm was considered, the average of three plains was 0.99 tractor farm-1. It can be say that there was less than one tractor per farm. The numbers of tractor per farm was determined as 1.17. 0.92 and 0.92 in Ezine, Kumkale and Bayramic, respectively. There are results concluded by Oğuz et al. (2017) who recorded higher tractor per farm for Konya as 1.57 tractor farm-1. They also concluded that tractor number per farm was higher (1.64) in large size parcels than in small size parcels (1.17). On average three-plain, machinery per tractor was found as 7.67 which describes conventional farming systems are still dominant in the area because of using many equipment in tillage, seedbed preparation, protection and other cultural practices. It also means that conservation and direct cultivation systems are not much known or used by many local farmers. In Figure 3, it is clearly shows that the machinery used in all studied farms are more suitable for traditional agriculture. This is especially confirmed by the fact that the number of mouldboard plough is one or more per tractor, while other machinery using for conservation or sustainable management systems were lower, for example rototiller, rotovator, etc. On the other hand, it was concluded that from studied farms during the questionnaire, although farmers are willing to buy more new tractors (Table 17), they are not very conscious of the replacement of existing machinery used in conventional agriculture. However, the largest farms such as commercial have managed to improve their technical equipment thanks to the funds from the national budge. However, in general, the majority of machinery are overworked and fully exploited in the studied farms. In general, the owners of small farms do not invest for the new machinery, but prolong the utilization life of the existing machinery even to 30 or 40 years. This increases the frequency and costs of repair. Machinery per tractor was resulted higher by 7.67 for studied farms than the national level by 7.26, but it was lower than some agricultural areas which were located more close the study area, for example; Edirne (9.13), Kırklareli (8.81) and Tekirdağ (9.67), and Thrace region (9.24) (Abdikoğlu, 2019).

Table 10. Agriculture area (owned+rental), tractors and their indicators in the farms of three-plain

Plain	Agri. area (decare)	Farm (num)	Tractor (num)	Machinery (num)	(decare farm^{-1})	(decare tractor^{-1})	(tractor farm^{-1})	(machinery tractor^{-1})
Ezine	10432	107	103	734	97	83	1.17	5.87
Kumkale	32140	258	239	2080	125	136	0.92	8.78
Bayramic	3840	36	33	217	107	116	0.92	6.58
General	46412	401	395	3031	116	117	0.99	7.67

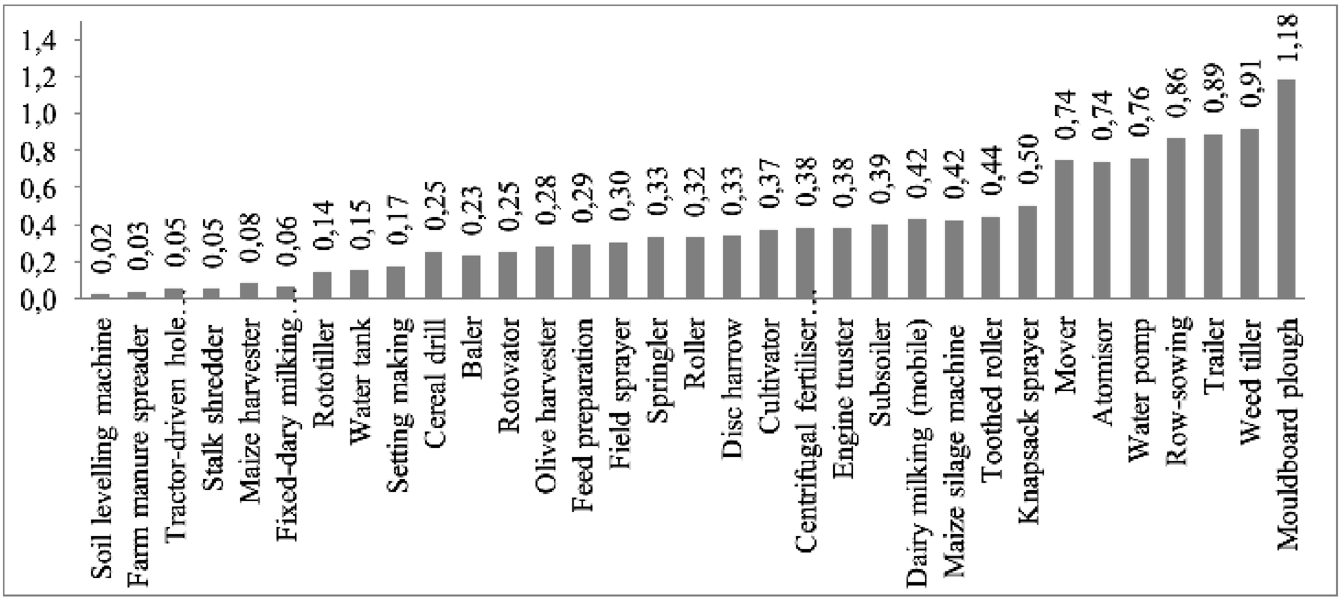

Figure 3. Number of machinery per tractor in each farm depending on average farms of three agricultural plains

Availability tractors and their brands

In questioned agricultural farms, the tractor brands were determined to be more than half of the existing tractor in the country with 30 brands. Thus, 17 tractor brands were identified in the studied agricultural farms and it was determined that they consist of both foreign and domestic brands according to Turkish Association of Agricultural Machinery and Equipment Manufacturers (Tarmakbir, 2018). New Holland was the highest (32.15%) brand within all tractors, followed by Massey Ferguson (18.99%), and Fiat (9.11%), John Deere (8.10%), Case IH (7.85%), Same (5.06%), Deutz (4.05%), Steyr (3.54%), Valtra (2.28%), Tumosan (2.53%), Basak (1.27%), Universal (1.27%), Hattat (0.51%) and Kubota (0.25%), etc. The reason for the higher number of New Holland and Massey Ferguson is the existence of seller dealers and maintenance-repair service facilities in the region. The opportunity of the service and seller dealers for both brands were sometimes found to provide sales to some tractors such as Hattat. Kubota, Valtra and Deutz. It determined that some tractor brands, for example Same, Universal, Stery and Tumosan, were generally sold as second hand at the same seller dealers (Table 11). Similarly, according to the results concluded by Aybek and Sener (2009) for a local agricultural area which are under intensive agriculture, Massey Ferguson has been reported to be the most used tractor brand with a rate of 36.30% in regardless of model and size. Others recorded that the most commonly used tractor at the national level was Massey Ferguson with a ratio of 32.69 (Civelek, 2016). The same researcher recorded that the other most commonly used tractor brands were Fiat and then New Holland sold by Turk Tractor Company. According to a study conducted in another local area of the country in the same period, it was concluded that the farms had more Tumosan by 30.00%, and then Massey Ferguson (18.18%), New Holland (15.46%) and John Deere (10.00%) (Keleş et al., 2016). When considering these studies carried out under the different region conditions, it can be said that the use of different brands of tractor varies according to the region's climate, crop pattern and most importantly the income level of the farmers.

Table 11. Number and rate of tractors by brands in all farms of three-plain

Brand	Ezine	Kumkale	Bayramic	General
MF	24 (19.20)+	38 (16.03)	13 (39.39)	75 (18.99)
NH	35 (28.00)	89 (37.55)	3 (9.09)	127 (32.15)
FR	4 (3.20)	3 (1.27)	0 (0.00)	7 (1.77)
FI	26 (82.16)	10 (16.67)	0 (0.00)	36 (9.11)
SM	7 (5.60)	12 (5.06)	1 (3.03)	20 (5.06)
ER	1 (0.80)	1 (0.42)	0 (0.00)	2 (0.51)
B	1 (0.80)	3 (1.27)	1 (3.03)	5 (1.27)
CS	12 (9.60)	16 (6.75)	3 (9.09)	31 (7.85)
ST	2 (1.60)	10 (4.22)	2 (6.06)	14 (3.54)
TM	3 (2.40)	6 (2.53)	1 (3.03)	10 (2.53)
JD	4 (3.20)	23 (9.70)	5 (15.15)	32 (8.10)
DT	5 (4.00)	10 (4.22)	1 (3.03)	16 (4.05)
UN	0 (0.00)	4 (1.69)	1 (3.03)	5 (1.27)
KB	0 (0.00)	1 (0.42)	0 (0.00)	1 (0.25)
LN	0 (0.00)	3 (1.27)	0 (0.00)	3 (0.76)
VLT	0 (0.00)	7 (2.95)	2 (6.06)	9 (2.28)
HT	1 (0.80)	1 (0.42)	0 (0.00)	2 (0.51)
Total	125 (31.65)	237 (60.00)	33 (8.35)	395 (100.00)

+ Percentage of tractor brand within total tractors of three-plain. MF, Massey Ferguson; NH, New Holland; FR, Ford; HT, Hattat; FI, Fiat; SM, Same; ER, Erkunt; B, Basak; CS, Case IH; ST, Steyr; TM, Tumosan; JD, John Deere; DT, Deutz; UN, Universal; KB, Kubota; LN, Landini; VLT, Valtra.

The status of having different tractor brands of the farms was determined on the basis of each agricultural plain and the results of the farms having one or more than one brands are given in Table 12, respectively. 19.45% of farms have more than one tractor brands (Table 12), while 74.31% of farms have only one tractor brand (Table 12). The single brand used in the farms are usually New Holland (26.68%), Massey Ferguson (11.22%), John Deere (7.48%), Case (5.49%) and Fiat (5.49%), followed by others such as Same, Steyr, Deutz. etc. (Table 12). It was determined that the farms having more than one tractor brands generally use dual tractor such as NH+NH (4.74%), NH+MF (3.49%), NH+JD (2.50%), NH+CS (1.75%) and NH+TM (1.25%), and others. In both cases. the New Holland can be used widely in the farms that it may be result of its service and seller dealers in the region. The largest proportion of the farms having more than one tractor brands was found in Bayramic with 41.67% of 36 farms, followed by Kumkale with 20.93% of 258 farms and Ezine with 8.41% of 107 farms (Table 12). The reason using of the more tractor brands in Bayramic plain may be attributed to the different agricultural branches such as field crops, horticulture as well as animal production. Aybek and Sener (2009) recorded that 89.30% of farms had one tractor, 7.80% had two, 0.50% had three and 2.40% had four tractors, regardless of brands in an area of intensive agriculture located in Çukurova region.

Effective methods and factors to purchase the tractors for farms

The 395 tractors were recorded (Table 11) in the studied farms in regardless of the brand, size and age of them, but the purchase status of only 370 was determined (Table 13) while no-information was obtained about the purchasing of the remaining of 25 tractors. The first-hand buying as new one, and second-hand purchasing of tractors are identified in farms of all villages (Table 13), but they were recorded only one in some farms, and two type purchasing in others. When considering the results, it is concluded that one of the way to have the tractors in farms is to purchase new tractor which corresponds to 77.03% for three-plain. On the other hand, it was determined that the rate of the tractor ownership in the second-hand was 22.97%. It was concluded that with the change in agricultural cultivation branches and crop pattern in the three agricultural plains of the post-irrigation period, the tractors requirements with the different characteristics has increased and it is appropriate to meet purchasing with second-hand tractors to continue without interruption of the farm operations. In addition, it is also say that the standard type tractors are sufficient for the completion of the work in animal production, especially for feeding operations. For this purpose, it is also emphasized that it is more economical to purchase the second-hand type of tractors without active working properties. Considering that the studied farms having tractors, agricultural cultivation branches have been identified as an important factor in combination with the availability of service facility, spare parts, while Özpınar and Çay (2018) concluded similar results for purchasing tractor for farms. They also reported that the tractor power was the more efficiently factor, followed by the service availability, PTO properties and others such as the bank loan, fuel saving, wheel and gear characteristics (Table 14).

Table 12. Number and rate of farms having one or more than one tractor brands in three-plain

	Number of farms having one tractor				Number of farms having more than one tractor				
Brand	Ezine	Kumkale	Bayramic	Total	Brand	Ezine	Kumkale	Bayramic	Total
MF	3 (2.80)+	38 (14.73)	4 (11.11)	45 (11.22)	NH+NH	1 (0.93)	16 (6.20)	2 (5.56)	19 (4.74)
NH	9 (8.41)	86 (33.33)	12 (33.33)	107 (26.68)	NH+FI	1 (0.93)	-	-	1 (0.25)
FR	-	2 (0.78)	-	2 (0.50)	NH+MF	-	7 (2.71)	7 (19.44)	14 (3.49)
HT	1 (0.93)	1 (0.39)	-	2 (0.50)	NH+B	1 (0.93)	-	-	1 (0.25)
FI	12 (11.21)	10 (3.88)	-	22 (5.49)	NH+SM	1 (0.93)	3 (1.169	-	4 (1.00)
SM	2 (1.87)	12 (4.65)	1 (2.78)	15 (3.74)	NH+JD	1 (0.93)	7 (2.719	2 (5.56)	10 (2.50)
ER	1 (0.93)	1 (0.39)	-	2 (0.50)	NH+TM	-	5 (1.94)	-	5 (1.25)
B	-	3 (1.16)	1 (2.78)	4 (1.00)	NH+ER	-	3 (1.16)	1 (2.78)	4 (1.00)
CS	4 (3.74)	15 (5.81)	3 (8.33)	22 (5.49)	NH+CS	-	7 (2.71)	-	7 (1.75)
ST	1 (0.93)	9 (3.49)	2 (5.56)	12 (2.99)	MF+MF	-	1 (0.399	1 (2.78)	2 (0.50)
TM	2 (1.87)	6 (2.33)	1 (2.78)	9 (2.24)	MF+FI	2 (1.87)	3 (1.16)	-	5 (1.25)
JD	2 (1.87)	23 (8.91)	5 (13.89)	30 (7.48)	MF+FR+DT	1 (0.93)	-	-	1 (0.25)
DT	-	10 (3.88)	1 (2.78)	11 (2.74)	JD+JD	-	-	1 (2.78)	1 (0.25)
UN	-	3 (1.16)	1 (2.78)	4 (1.00)	JD+KB	-	1 (0.39)	-	1 (0.25)
KB	-	1 (0.39)	-	1 (0.25)	TM+ST	-	1 (0.39)	1 (2.78)	2 (0.50)
LN	-	3 (1.16)	-	3 (0.75)	CS+CS	1 (0.93)	-	-	1 (0.25)
VLT	-	5 (1.94)	2 (5.56)	7 (1.75)					
Total	37 (34.58)	228 (88.37)	33 (91.67)	298 (74.31)	Total	9 (8.41)	54 (20.93)	15 (41.67)	78 (19.45)
FN	107	258	36	401	FN	107	258	36	401

+ The percentage of farms having tractor brand in each agricultural plain of farms. FN, total number of farms questioned for each agricultural plain.

The role of financial capital as a factor of agricultural cultivation is to facilitate economic growth and development. Credit is an important instrument that enables farmers to obtain requirements with consumption materials and also plays an important role in increasing agricultural productivity. The availability of credit enables farmers to purchase the required inputs and machinery to carry out farm operations on time (Marandi and Rashidpour, 2017). Agricultural cultivation in the studied area needs more agricultural credit availability because of certain structural characteristics, notably its small family farm. Most farms are small-scale family type, fragmented and scattered (Özpınar, 2002). So, agricultural credit has great precaution for their development and they meet their credit requirements from formal funds for loans through public sector, alongside with informal sources. At the national level, however, Ziraat Bank and Agricultural Credit Cooperatives have been the principle supplier of loanable funds in the agriculture sector (Gunes and Movassaghi, 2017) as formal credit sources. They also concluded that Ziraat Bank, private banks (domestic and foreign-owned and operated), agricultural credit and sales cooperatives and other cooperatives (e.g. Pankobirlik) are the major formal suppliers of credit, but wealthy farmers and money lenders are among the informal credit sources which are generally provide short term loans, saddling borrowers with high interest rates. On the other hand, small-scale farms need in order to meet short term requirements such as purchasing fuel and long term purposes; for example, investment in agricultural area, irrigation facilities and machinery. When the financial methods used in the purchasing of tractors for farms were considered, it was found that the highest system was agricultural loan system with 61.63% by private or public (Ziraat) banks, whereas in cash purchasing was lower with 35.28% because loan system gives the farmers enough time opportunity to do their re-payments step-by-step (Table 14). Meanwhile,

Table 13. Number and rates of tractors in the studied farms of three-plain according to the purchase

	Ezine			Kumkale			Bayramic			Total of three-plain		
Brand	FH	SH	TT	FH	SH	TT	FH	SH	TT	FH	SH	TT
MF	11 (68.75)	5 (31.25)	16	35 (89.74)	4 (10.26)	39	1 (33.33)	2 (66.67)	3	47 (81.03)	11 (18.97)	58
NH	24 (75.00)	8 (25.00)	32	83 (96.51)	3 (3.49)	86	11(100.00)	-	11	118 (91.47)	11 (8.53)	129
FR	-	1 (100.00)	1	2 (66.67)	1 (33.33)	3	-	-	-	2 (50.00)	2 (50.00)	4
FI	11 (73.33)	4 (26.67)	15	6 (60.00)	4 (40.00)	10	-	-	-	17 (68.00)	8 (32.00)	25
SM	6 (66.67)	3 (33.33)	9	5 (41.67)	7 (58.33)	12	1 (100.00)	-	1	12 (54.55)	10 (45.45)	22
ER	1 (100.00)	-	1	1 (100.00)	-	1	-	-	-	2 (100.00)	-	2
B	-	-	-	2 (66.67)	1 (33.33)	3	1 (100.00)	-	1	3 (75.00)	1 (25.00)	4
CS	4 (57.14)	3 (42.86)	7	10 (66.67)	5 (33.33)	15	2 (100.00)	-	2	16 (66.67)	8 (33.33)	24
ST	3 (23.08)	10 (76.92)	13	9 (69.23)	4 (30.77)	13	2 (100.00)	-	2	14 (50.00)	14 (50.00)	28
TM	4 (80.00)	1 (20.00)	5	6 (85.71)	1 (14.29)	7	1 (50.00)	1 (50.00)	2	11 (78.57)	3 (21.43)	14
JD	4(100.00)	-	4	9 (42.86)	12 (57.14)	21	5 (100.00)	-	5	18 (60.00)	12 (40.00)	30
DTZ	-	1 (100.00)	1	8 (80.00)	2 (20.00)	10	1 (100.00)	-	1	9 (75.00)	3 (25.00)	12
UN	-	-	-	3 (100.00)	-	3	-	1 (100.00)	1	3 (75.00)	1 (25.00)	4
KB	-	-	-	1 (100.00)	-	1	-	-	-	1 (100.00)	-	1
LN	-	-	-	2 (66.67)	1 (33.33)	3	-	-	-	2 (66.67)	1 (33.33)	3
VLT	-	-	-	7 (100.00)	-	7	1 (100.00)	-	1	8 (100.00)	-	8
HT	1 (100.00)	-	1	1 (100.00)	-	1		-	-	2 (100.00)	-	2
Total	69 (65.71)	36 (34.29)	105	190 (80.85)	45 (19.15)	235	26 (86.67)	4 (13.33)	30	285 (77.03)	85 (22.97)	370

FH, first-hand (new) tractor; SH, second-hand (old) tractor; TT, total tractor (first and second hand) for each agricultural plain.

farmers have more confidence in Ziraat Bank because of giving subsidized credit. It is determined that the loan system is generally pay with five percentage cash in advance and the rest is repaid within next 20 or 60 months as long term. Additionally, the loan system is the most effective system to purchase the tractor for farms because it provides financial facilities for long-time period. In addition to this purchase method, agricultural credit system is not preferred by farmers due to its low advantage compared to the loan system due to higher interest rate. Moreover, it was also emphasized that the loan system facilitates provide to the farmers to purchase different type, varying power size, axle type, tractor brands, and improving the agricultural equipment and mechanization level increasing the yield and ensured food security, but this system is varying depending on bank loan system (Özpınar and Çay, 2018). On the other hand, two factors have been emerged to be important to purchase tractors for farms, one of which is the size of the agricultural area, and other is the appropriate or reasonable price of the tractor (Table 15). Therefore, when the Table 15 is considered, it was said that the size of the area is more effective factor to purchase a tractor when the 67.15% of the farms have preferred this type method. On the other hand, the rest of the farms have encouraged the reasonable price in regardless of tractor brand, power size and axle number or type because they have emphasized that the reasonable price is sometimes the easy way system due to simple access way of tractors.

Table 14. Number and rate of farms in terms of methods of the purchasing tractors

	Cash		Loan		Agricultural credit coop.		Total	
Plain	(num)	(%)	(num)	(%)	(num)	(%)	(num)	(%)
Ezine	32	35.56	48	53.33	10	11.11	90	100.00
Kumkale	75	32.89	153	67.11	-	-	228	100.00
Bayramic	14	53.85	11	42.31	1	3.85	26	100.00
General	121	35.28	212	61.63	11	3.20	344	100.00

Table 15. Number and rate of farms according to effective factors of purchasing tractors

	Area size		Reasonable price		Total	
Plain	(num)	(%)	(num)	(%)	(num)	(%)
Ezine	52	56.52	40	43.48	92	100.00
Kumkale	171	74.03	60	25.97	231	100.00
Bayramic	11	39.29	17	60.71	28	100.00
General	234	67.15	117	32.85	351	100.00

The proficiency level of the current tractors in farms

Considering on average tractor number in three-agricultural plains, 64.62% of farms have been found to be sufficient tractors to carry out their agricultural operations (Table 16). However, it is stated that the current tractors are not sufficient to carry out agricultural operations in 35.38% of farms which were occupied more than one agricultural branches such as field, horticulture and as well as animal production. The common of animal production in the region together crop production has revealed the requirement for tractors with different power sizes and characteristics. On the other hand, in some villages of the Bayramic and Ezine agricultural plains, it was determined that the cultivation of the horticulture together with the field cultivation increased the requirement of the tractors in different power and brands. In addition, the absence of sharing farm machinery or tractor system in the region, it was determined that each farmer have to buy required tractor and machinery to carry out their agricultural operations on time. On the other hand, farmers borrow the machinery or tractors from neighbours that is traditionally sustainable system in the area.

Table 16. Number and rate of farms in terms of the proficiency level of the tractors

	Sufficient		Insufficient		Total	
Plain	(num)	(%)	(num)	(%)	(num)	(%)
Ezine	49	56.32	38	43.68	87	100.00
Kumkale	156	67.53	75	32.47	231	100.00
Bayramic	16	66.67	8	33.33	24	100.00
General	221	64.62	121	35.38	342	100.00

Varying and distribution of age statues of owned tractors by farms based on brands

The age characteristics of the tractor brands in the studied farms have been observed in similar for the country farms (Table 17). Current tractors in the farms were classified according to their age on the basis of brand, and then they were divided into two groups as young (0-20-year) and old (20-year and over) while it has been declared that the different economic life for tractor, for example, 20-24 years at the national level. The age grouping on the brand basis was done by selecting the youngest and oldest age tractors. When considered according to age groups; the age of tractors such as Massey Ferguson, Fiat and John Deere are quite high compared to others. For example, Massey Ferguson and John Deere were found to be in the age group of 44 years which is old age group category. However, although there were found to be very old tractors for both tractor brands, the youngest tractors were also recorded for the same brands because of the reason of its long term using in the agriculture area. The other reason may be the presence of Massey Ferguson brand in the region due to the availability of sale dealers, service facilities and spare-parts which were especially settled in Ezine district. On the other hand, some models of New Holland, Erkunt, Kubota and Valtra were found younger than Massey Ferguson and John Deere, for example, although New Holland brand was used in the country for long time, they were reasonability took place in young group range for the studied farms. It can be said that the availability of New Holland tractors in farms as well in the region are directly depending on the availability of the sale dealers, service facilities which provide an increase in the use of this brand. In the region, New Holland tractors have progressively increased in the use of the agriculture after the sales dealers and service facilities were served in the area from 2010-year. Therefore, New Holland has caused to be in the category of young age as well as in the study farms. Similarly, the same opportunities for young age brands have allowed to increase its sales and used widely in the area. Özpınar and Çay (2018) found similar results about tractors age in respect to brands. On the other hand, many tractor brands found within 15-year economic life age range (Tezer and Sabancı, 1997). In a similar study, it was found that the economic life of 45.71% of total tractors is over than 25-year old at the national level, regardless of brands and sizes (Civelek, 2016) who declared that 9.02% of remaining tractors are in range 20-25 years. 11.03% in 15-20 years, 13.11% in 10-15 years and 21.14% under the age of 10 years. In another intensive agriculture region, it was concluded that 20.80% of tractors were at the age of 16 or more while 79.25% of them were under 16 years (Aybek and Senel, 2009). They explained that 30% of young tractors have 0-5 years, while 36.30% of them are 6-10 years, 12.00% are 11-15 years. When economic life of a tractor is considered to be 20-year (Yılmaz and Sümer, 2018), it can be seen that 12.50% of tractors in the study area have already completed their economic life (Table 17) while 87.00% was in economic life although they have low working hours in year, 500-600 hours compared to developed countries with 1000 hours per year (TAGEM, 2019). Considering tractor age at the national level, 54.00% of tractors varied between 1-24 years, while 46.00% are over 25 years, 50.90% of tractors over 25-years are over 40 years, and remaining take place 25-40 years. It also concluded that very old tractor usage reduces agricultural cultivation activity whereas increases fuel usage costs and greenhouse gas emissions due to old technology engines (Civelek, 2016). Therefore, it needs changing old tractors with new tractors which reduce engine emission levels and time loses on the field with benefits such as electrical control, GPS guidance and ISO-Bus systems. The reason the use of old tractors is due to the low annual working hours with 600-hour in the country compared to 12 thousand hours in development countries. Using of such as old tractors will result in high fuel usage, extend working hours, extra labour costs that means less production and profit.

Table 17. Age of tractors according to their brands in all farms

Brand	Age group (year)		Young		Old		Tot. Trac.
	Young	Old	(num)	(%)	(num)	(%)	(num)
MF	7.29±4.61(63.25)+	44.33±15.63(35.25)	60	89.55	7	10.45	67
NH	4.88±3.52(72.15)	6.75±4.92(72.95)	120	94.49	7	5.51	127
FR	6.75±4.92(72.95)	44.38±19.25(43.38)	3	75.00	1	25.00	4
FI	13.50±16.26(120.47)	27.50±15.31 (55.66)	21	84.00	4	16.00	25
SM	4.60±2.41(52.35)	12.50±10.97(87.73)	17	77.27	5	22.73	22
ER	5.00±1.00(20.00)	6.00±2.00(8.00)	1	100.00		0.00	1
B	5.00±3.83 (76.59)	17.67±4.73(26.75)	2	40.00	3	60.00	5
CS	5.75±3.30(57.46)	10.33±8.04(77.82)	21	87.50	3	12.50	24
ST	18.20±12.85 (70.62)	24.00±3.46(14.43)	20	80.00	5	20.00	25
TM	4.91±3.52(71.59)	8.33±4.93(59.19)	13	86.67	2	13.33	15
JD	4.00±4.24(106.07)	8.33±7.07(84.85)	24	80.00	6	20.00	30
DTZ	5.85±1.30(22.23)	15.50±20.21(130.37)	11	91.67	1	8.33	12
UN	15.50±20.21(130.37)	35.75±6.88(19.25)	3	75.00	1	25.00	4
KB	4.91±3.52(71.59)	5.00±2.94(58.88)	1	100.00		0.00	1
LN	3.00±1.00(33.33)	6.75±4.92(72.95)	3	100.00		0.00	3
VLT	5.00±3.35 (66.93)	7.17±2.47(34.42)	8	100.00		0.00	8
HT	5.33±2.52(47.19)	15.50±20.51(132.30)	2	100.00		0.00	2
			330	88.00	45	12.00	375

+Mean tractor age± standard deviation (coefficient of variation); MF, Massey Ferguson; NH, New Holland; FR, Ford; HT, Hattat; FI, Fiat; SM, Same; ER, Erkunt; B, Basak; CS, Case IH, ST, Steyr; TM, Tumosan; JD, John Deere; DTZ, Deutz; UN, Universal; KB, Kubota; LN, Landini; VLT, Valtra.

Conclusion

The existence of the possibilities for the sustainability of agricultural cultivation have crucial importance. Sometimes the existence of these possibilities is not enough for sustainable agriculture, but also they have to be used correctly. Therefore, it is necessary to know agricultural possibilities in an agricultural area and to determine them for to be planned for next projections. For this purpose, a questionnaire is conducted to make the necessary determinations about agriculture activities which were performed by mechanization possibilities and human sources. The questionnaire survey was focused to determine the agriculture structure and mechanization for some villages of Bayramic-Ezine-Kumkale agricultural plain in Canakkale region. In studied farms, families generally have four persons on average, and the two male and one female person per family are working and occupying in the agriculture activities. Farmers have some organizations to keep their products right, for example; more popular was the farmer recording system followed by agriculture chamber, agrochemical dealers, and others. On the other hand, agricultural activities are performed according to traditional cultivation systems despite having enough tractors. Tractors of different brands have been recorded in the studied farms, they are Massey Ferguson, New Holland, Ford, Valtra, Tumosan, Deutz, Kubota, Erkunt, Hattat, Case. However, Massey Ferguson and New Holland were the highest because of existence of their service facilities in the region. Farms generally have more than one tractor brands due to existence of more than one agriculture occupation branches such as field, horticulture crops, and even animal production. The number of tractors per farm is acceptable level in studied farms with 0.99, but it was less than one tractor. Agricultural area per tractor was found higher by 117 decare tractor-1 in the studied farms. The number of machinery per tractor is approximately 8 on average three-agricultural plain, more suitable for traditional cultivation systems. The agriculture area per farm was 116 decare on average three plains, but it consists of many small parcel size which are small than 50 decare. Farmers were preferred the ways that is to purchase the tractor for farms using terming system (loan), which is ranges between 20 and 60 months compared with cash and agricultural credit systems. 88% of tractors recorded in studied farms were included in young group varying 0 and 20 years, while 12.00% were old, 20 years and over, particularly including old series of Massey Ferguson, Ford, Fiat.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Not applicable.

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Data availability

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First record of the pest and parasitic intermediate host snail *Cochlicella barbara* (Linnaeus, 1758) in the south-eastern Anatolia

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Abstract

The objective of the present study is to inform the last distribution of the invasive and harmful species *Cochlicella barbara* (Linnaeus, 1758) in Turkey, as well as to draw attention to the possible danger of this distribution on regional vegetation, agriculture, and parasitism. *C. barbara* was found for the first time in south-eastern of Anatolia in April 2019 in the campus area of the Dicle University in Diyarbakır, Turkey. *C. barbara* is admitted as a harmful organism and classified as an agricultural pest in most of the countries. Species in this genus have been intercepted in postal packages, soil, and cargo as well as can be easily transported with plants, building materials, household goods, etc. Above all, it is an intermediate host of nematode and fluke parasites of livestock. Knowing the distribution of this species will not only contribute to the registration of a new species in the list of gastropods of the region but also facilitate the control of the species across Turkey.

Keywords: *Cochlicella barbara*, Intermediate host, Pest snail, Diyarbakır

Introduction

Around 30.000 to 35.000 terrestrial gastropod species have been reported worldwide. After Arthropoda, Mollusca is known as the second-largest phylum among all animal phyla and the Gastropoda class constitutes the richest group of the mollusks in diversity. In general, gastropods are divided into three groups according to the presence and location of the respiratory organs: Prosobranchia (gills in front of the heart), Opisthobranchia (gills behind the heart) and Pulmonata (gill deficiency, but use a multi-veined mantle for breathing). Because of these properties, they have been able to live in a wide variety of different habitats for millions of years. It is thought that there are around 20.500 terrestrial pulmonates worldwide (Larbaa and Soltani, 2013). It has been reported that approximately 500 terrestrial gastropod species living in Turkey, nearly 60 of them are slugs (Schutt, 2005). Besides, about 60 species of land snails and slugs inhabit South-eastern Anatolia. Species belonging to the *Ambigolimax, Assyriella, Buliminus, Calaxis, Cecilioides, Cernuella, Chondrula, Cryptomphalus, Eobania, Eopolita, Euchondrus, Granopupa, Helix, Lauria, Limacus, Lindholmia, Monacha, Orculella, Oxyloma, Pene, Pomatias, Pseudochondrula, Turanena, Vitrea, Xeromunda, Xeropicta, Zebrina, Zonitoides, Ambigolimax* genus are dwelling in south-eastern Anatolia, particularly in Mardin, Diyarbakır, Siirt, Batman, Urfa districts (Schutt, 2005; Ekin and Şeşen, 2018). There isn't enough data about the final distribu-

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tion of terrestrial gastropods and their damage to agriculture and human health not only in south-eastern Anatolia but also in Turkey. With this study, it is also aimed to raise awareness of some harmful gastropods such as *Cochlicella barbara* in advance by drawing attention to possible damages of the species.

C. barbara is an invasive species and capable of rapid reproduction. It is known worldwide by different names such as banded conical snail, pot-bellied snail, potbellied helicellid, small pointed snail, small conical snail, tower snail, cornet Mediterranean snail. The geographical distribution of this species is generally the European coastal regions around the Mediterranean. In Turkey, presence of this species previously reported in Marmara Region (İstanbul - Üsküdar, Yeşilköy; Çanakkale - Kilitbahir), Mediterranean Region (Antalya - Lara; Adana - Karataş), Aegean Region (Muğla - Bodrum), eastern Black Sea Region (Trabzon - Esiroğlu, Maçka, Değirmendere) (Figure 2) (Schutt, 2005). However, it is the first time this species has been found in the south-eastern Anatolian region. So far, this species has not been observed in the Central Anatolia Region, Eastern Anatolia Region and Western Black Sea Region of Turkey.

This paper aims to provide information about the presence of *C. barbara*, an invasive and potential parasite carrier species in south-eastern Anatolia, and to draw attention to the potential hazards of the species to regional vegetation, agriculture, and other gastropod species.

Material and Methods

C. barbara was collected from the agricultural lands around Diyarbakır (Geographic coordinates of Diyarbakır are 37°54' 36.00'' N - 40°14' 24.00'' E; Elevation above sea level is 674 m, 2211 ft.) and the settlements on the banks of the Tigris, in April 2019. It was observed that these snails have lived in the center of Diyarbakır and the agricultural areas around the city, in some different localities. Even if a population density calculation was not performed, their numbers were quite high. Species identifications were made based on snail morphology and collected specimens compared to those in shell-library databases formed by Şeşen and Schutt in 2005, also Henk MIENIS opinion was taken for the correctness of the identification of the species. In the collected area, the snails have been observed almost everywhere in damp shaded areas, under the leaves, on walls, trees, shrubs, pavements, and empty shells have been found all over the area. The snails were both photographed in their habitats and the laboratory and some of the shells are kept into the boxes for further studies (Figure 1).

Result and Discussion

Morphological features of the species may sometimes show small differences. However, generally, the shell of the species is whitish, greyish or yellowish with brown spots or bands, 7-8 whorls; umbilicus is very narrow but rarely closed. Juveniles slightly keeled. In some habitats, the species is very light yellowish, dorsum with blackish-brown pigments, two dark lines from the sides to the upper tentacles, one dark medial line on the dorsum. Its size is between 4.5-7 x 7-15 mm, usually 5 x 8-10 mm (Schutt, 2005).

Conical snails are known to activate the post-aestivation cycle later than round snails. Life cycles of *C. barbara* which is conical can be annual or biennial. The small size of the snail, which is capable of rapid reproduction, means that it is widely spread through the transport of infested plants, vegetables, fruits or other commodities. It lives for approximately one year. Mating occurs in the spring with egg-laying continuing through summer and autumn. Eggs hatch soon after in autumn and early winter, or during the following winter (Herbert, 2010).

The geographical distribution of this species is generally the European coastal regions around the Mediterranean (including Algeria and Egypt). The species has recently spread to the inner parts of Europe, for example to Albania, Belgium, Croatia, France, Greece, Italy, Israel, Netherlands, Portugal, Spain, and Great Britain (Kerney and Cameron, 1979; Cook, 1997; Morrondo et al., 1992; Herbert, 2010). *C. barbara* is native to western Europe, particularly the Mediterranean Region. However, it has been reported to be distributed in California, Azores, Bermuda, South Africa, Japan, Australia, New Zealand, and South Africa. The snails can be found in dry areas near the sea, specifically in dunes and sometimes inland. The species prefers areas with relatively dry Mediterranean climates, especially near the coast. It invades cultivated and barren areas in gardens, forests, suburbs, and cultivated areas by creating numerous individuals in favorable conditions and mostly spend more time under grass (Herbert, 2010). Additionally, in a study of *Lauria cylindracea, Anion hortensis, C. barbara, Pupoides albilabris*, and *Xerotricha conspurcata* including their distribution to Los Angeles, Orange, and Riverside counties in California, it was emphasized that *C. barbara* easily adapted to the metropolises and spread fast (Vendetti et al., 2018).

C. barbara is registered as a harmful organism by Australia, Japan, the United States, Chile, and Korea. The agricultural ministries of America, Australia, and South Africa have accepted this creature as an agricultural pest and offered serious control methods. *C. barbara, C. conoidea*, and *C. ventricosa* are considered a hitchhiker pest as having been found in ships, vehicles, and containers. They are intercepted in cars, postal packages, soils, cargos (Godan, 1983) and can be easily transported with plants, building materials, and household goods. The presence of *Cochlicella spp*. can be estimated by chewing or rasping damage to plants, presence of eggs, juveniles and adults, empty snail shells, mucus and slime trails, and feces like a ribbon (Herbert, 2010). These harmful species can cause serious damage to grain and oilseed production and polluted crops during harvesting; this may result in reduced or even rejected consignment quality (Roth and Hertz, 1997; Baker, 2002). They may cause direct feeding damage to canola in early winter, just as they attack pastures and other crops in southern Australia (Baker, 1986; 2002; Gu et al., 2007; Suzanne and Kerrie, 1999). *C. barbara* can cause appreciable agricultural and horticultural damage when population densities are high. Densities of over 1,000 snails/m^2 have been recorded in New Zealand. Damage to legume-based pastures has been reported in south-eastern Australia (Herbert, 2010).

C. acuta and *C. barbara* are known to be intermediate hosts of nematodes and trematodes which infect man and domestic animals and fluke parasites of livestock. Prevention and early intervention are essential. The harmful effects of the species can be best monitored by a combination of cultural and chemical control. Their natural predators are carnivorous beetles (Godan, 1983; Morrondo et al., 2005). *C. acuta* is an intermediate host of both *Müllerius capillaris* (Müller) and *Cystocaulus ocreatus* Davtian, lungworms of sheep (Godan, 1983). *C. barbara* can act as an intermediate host for *Protostrongylus rufescens* (sheep lungworm) (Herbert, 2010). Sheep are naturally infected by bronchopulmonary nematodes on pasture. Adult mollusks are found to be higher than juvenile ones and increased the frequency and intensity of the nematode infection (Morrondo et al., 1992). Larvae development of Nematoda, *Protostrongylidae* in *C. barbari*, were studied in detail and possible impact on infection of infected small ruminants was reported northwest Spain (Morrondo et al., 2005). *Brachylaima cribbi* is a terrestrial trematode of birds and mammals that selects land snails as the first and second intermediate hosts. The presence of *B. cribbi* sporocysts in 6,432 terrestrial snails collected from eight geographic regions of Australia was investigated in a study. Four snail species, *Theba pisana, Cernuella virgata, C. acuta*, and *C. barbara* were found to be the first natural intermediate hosts of this nematode (Andrew and David, 2003).

Figure 1. *Cochlicella barbara* from the center and around Diyarbakır, Turkey.

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Systematic of *Cochlicella barbara*

Phylum: Mollusca
Class: Gastropoda
Subclass: Heterobranchia
Infraclass: Euthyneura
Subterclass: Tectipleura
Superorder: Eupulmonata
Order: Stylommatophora
Suborder: Helicina
Infraorder: Helicoidei
Superfamily: Helicoidea
Family: Geomitridae
Subfamily: Geomitrinae
Genus: Cochlicella
Species: *C. barbara* (Linnaeus, 1758)

Synonyms of *Cochlicella barbara*

Bulimus ventricosus (Draparnaud, 1801)
Cochlicella ventricosa (Draparnaud, 1801)
Helix barbara (Linnaeus, 1758)
Helix bulimoides (Moquin-Tandon, 1855)
Helix ventrosus (A. Férussac, 1821)
Prietocella barbara (Linnaeus, 1758)

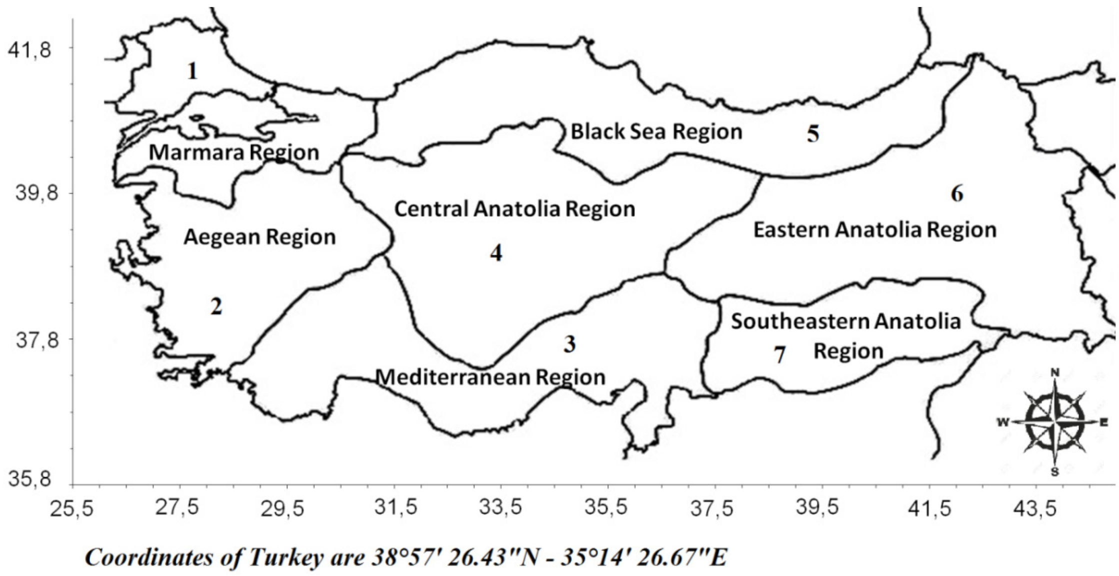

Figure 2. Distribution of *C. barbara* in Turkish regions. The species has been reported to be distributed in regions 1, 2, 3, 5; Marmara Region (İstanbul - Üsküdar, Yeşilköy; Çanakkale - Kilitbahir), Mediterranean Region (Antalya - Lara; Adana - Karataş), Aegean Region (Muğla-Bodrum), Black Sea Region (Trabzon - Esiroğlu, Maçka, Değirmendere) (Schutt 2005). The first time, the distribution of the species is seen in 7 numbered region (Diyarbakır) (Geographic coordinates of Diyarbakır are 37°54’ 36.00” N - 40°14’ 24.00” E) (Elevation above sea level: 674 m, 2211 ft.)

Conclusion

As a result, the distribution of this species is very fast and easy. If the necessary precautions are not taken and the necessary community awareness is not established, it can cause diseases that can be transmitted from animals to humans and can cause serious harm to agriculture. Since 2005, there is no evidence of any studies about the last distribution of the species in Turkey. Unless serious and extensive research is not done, the current distribution of the species and disease spreading potential cannot be learned. As soon as possible, adequate information should be reported about *C. barbara*, which is considered as a risk factor.

Compliance with Ethical Standards

Conflict of interest

The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Author contribution

The contribution of the authors is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethics committee approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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Research Article

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Determination of sugar, total phenol contents and antioxidant activity of various parts 'Uzun' pistachio cultivar (*Pistacia vera* L.)

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Abstract

The aim of this study was conducted to determine the sugar content, total phenol and antioxidant activity of various parts of 'Uzun' pistachio trees during 2018 (high bearing, or "on"-year trees) and 2019 (low bearing, or "off"-year trees) growing season. This research was carried on 35-year old, fruitful or unfruitful (showing alternate bearing) trees that were grafted on *Pistacia vera* rootstock at the Gaziantep provinces of Turkey. Total phenolic content of the samples were determined by the Folin Ciocalteu method by Spectrophotometer. Total antioxidant activitiy of samples were evaluated using the 1, 1-diphenyl-2- picrylhydrazyl (DPPH) radical scavenging method. Radical scavenging activities and total phenolic content of the samples changed depending on the different parts of shoot, leaves, nuts (hull and hard Shell) and kernel. Sugar compounds of Pistachio were detected by using HPLC. The highest antioxidant values were observed in hull (86,02% on-year) and hard Shell (85,37 % on-year) and the lowest were in kernel samples. The highest total phenol amount was recorded hard Shell and the lowest values were in kernel (81,23% off-year) samples. Fructose content (12,10 g/100 g) was found to be higher than the contents of sucrose (3,10 g/100 g) and glucose (5,48 g/100 g) in 'off' year tree and the dominant sugar was found fructose. All tissues that the amount of sugar content in 'off' year was higher than 'on' year. The results suggest at phytochemicals in 'Uzun' pistachio variety has potent antioxidant activities that is important for human nutrition.

Keywords: Free-radicals, Antioxidant, DPPH, Pistachio, Phenol, Sugar compounds

Introduction

Turkey is a significant genetic center of pistachio (Pistacia vera L.), and growing pistachio species has a wide distribution. Pistachio is the most economically important cultivated species of Pistacia genus belonging to Anacardiaceae family, order Sapindales. Like for a lot of fruit species. Turkey is also one of the place of pistachio origin and having an important genetic sources and the center of the generation and evolution of pistachio varieties. Our country is the third largest producer of pistachios after Iran and USA. Especially, the Southeastern Anatolia region having both suitable geographical site and various climatic and soil conditions and pistachios are can be grown economically. In the production of pistachio, Southeast Anatolia Region covers 95% of the country's production area and 91.5% of the total production (Arpaci and Atli., 1996; Gundesli et al., 2018, 2019a). 'Uzun' pistachio is the most important and widely grown cultivar Gaziantep province of Southeastern Anatolia region (Ak and Fidan, 2013). Pistachio shows alternate bearing (also known as biennial bearing), a phenomenon which refers to trees with irregular crop load from year to year and is typically observed in many commercial fruit trees. In other words, a tree's yield alternates between high fruit load (on) and low fruit load (off). Due to having fluctuations in productions between "on" and "off" years, producers and consumers encounter with financial loss (Acar et al., 2006; Gundesli et al., 2019a). Recently, many researchers have made great efforts to find safe and powerful natural antioxidants from various plant species. Antioxidants have protective

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effects on food relations in different diseases such as cardiovascular disease, cancer, aging and cataracts. (Dashpande et al., 1996; Fukuda et al.,2003; Kornsteiner et al., 2006; Rohman et al., 2010; Usanmaz et al., 2018; Gul and Tekeli, 2019; Gundesli et al., 2019b). Many edible fruits as harmless antioxidant sources have been investigated in terms of antioxidant properties. One of the nutritional value of nut fruit species is pistachio. Pistachio has a unique flavor and is rich in biochemical compounds. Pistachio is known as a natural antioxidant source with its rich biochemical compounds including phenolics, vitamin and fatty acids. However, antioxidant properties of pistachio may vary depending on cultivation, planting area, climate and cultural practices (irrigation, fertilization etc.). Recently, studies on the biochemical substances of pistachio, it has been reported that pistachio is a good source of antioxidants and phenolic compounds (Tokusoglu et al., 2005; Arcan and Yemenicioglu, 2008; Tsantili et al., 2010; Taghizadeh et al., 2018). Limited studies are conducted of researches on sugar contents, total phenolic and antioxidant contents of the various parts of 'Uzun' pistachio cultivar to grown in GAP region. Thus, the objectives of this study were to determine the sugar compounds, total phenolic content and antioxidant activities of different tissues such as shoot, leaves and nuts from 'Uzun' pistachio cultivar.

Materials and Methods

Plant materials

The experiment conducted in 2018 (high bearing, or "on"-year trees) and 2019 (low bearing or "off"-year,) growing seasons at the Research and Experimental area of the Pistachio Research Institute in Gaziantep provinces in Turkey. Thirty-three years old trees belong to 'Uzun' cultivar grafted on Pistacia atlantica Desf. rootstock and planted at 10x10 m intervals were used as plant materials. In this study, shoots, leaves, and nuts (hull and hard shell) were sampled from the current year's shoots (fruiting branch) 'on' and 'off' year trees.

Characteristics of "Uzun" cultivar: The tree structure is semi-upright and strong. It is one of the middle flowering cultivars with yellowish-green flowers. Bunch intensity is medium and resistance of split is poor. Fruit splitting rate is 69.34%, and 100-nut weight is 110.69 g. Kernel fruit color is green and it is a variety with a tendency to an absolute alternate bearing. Chilling requirement is 600 days/hours and the need for total temperature is 3797 degree-days. It can be utilized in the food industry and confectionery (Atlı et al., 2003; Afshari et al., 2009; Ak and Fidan 2013).

Plant tissue sampling

In 2018 and 2019, the samplings were collected from shoot (in July) leaves (in August), nuts (harvest time: September) which is 'On' and 'Off' year trees. For total phenolic and total antioxidant capacity analysis, one-year-old branches from different directions of the canopy (north, south, east and west), per replication; young leaves (50 number), shoots (10), nuts (hull and hard Shell-50 number), and fruit kernel (50 number) were excised and immediately transferred to the on dry ice, separated into leaves, shoots, and nuts, frozen in liquid nitrogen, in laboratory. Kernels and endocarp of fruits were separated. The samples were rinsed with sterile distilled water to remove dust and soil and liyophilizied (ilShin Freeze Dryers, FD-8518, Ede, Netherlands), using a lyophilizer and then homogenized using coffee grinder and stored at +4^{o}C.

Sugar analyses

The samples were prepared according to the method described by Kafkas et al. (2007), with minor modifications. Briefly, sugar analysis extraction was performed using acetonitrile: ultra deionized water (50:50). 1 g of sample weighed was solved in the extraction solvent and vortexed for 30 seconds. Then the samples were treated in an ultrasonic water bath for 15 minutes. After the extract was filtered through a 0.25 µm membrane filter (Schleicher and Schuell, Dassel, Germany) prior to injection (20 µl) onto a 7.8 x 100mm HPLC column (CARBOSep COREGEL 87C) at 75 °C. The mobile phase that acetonitrile/deionized water was supplied by an isocratic pumping system (LC-10A, Shimadzu, Kyoto, Japan) at a flow rate of 0.6 ml min1. After separation, the compounds from the mixture were passed through a refractive index detector (RID-10A, Shimadzu, Kyoto, Japan) linked to a chart recorder (CRUA, Shimadzu, Kyoto, Japan). Each sample was assayed triplicated and sugars concentrations were expressed as mg/100g dry weight (D.W.). Mixed external standard solutions containing glucose, fructose, and sucrose, at different concentrations were injected into the column and peaks were used to generate calibration curves for each sugar. The levels of sugars content in different tissues was calculated using peaks and the calibration curves.

Determination of total phenolic content

The content of total phenolic compound was determined by the Folin-Ciocalteu reagent using the modified by Spanos and Wrolstad (1990). 50 µl %80 methanolic extract of dried material was diluted by water followed by adding 250 µl of Folin-Ciocalteu reagent and 750 µl sodium carbonate (20%, w/v). The extracts were centrifuged (centrifuge NF 200, Nuve, Belgium) at 5500 r.p.m. for 15 min and filtered. The solvent was incubated at room temperature in dark for 2 hours. The absorbance of all samples was measured at 760 nm using Multiskan TM GO Microplate Spectrophometer. The results were expressed as gram gallic acid equivalent per grammes of extract (g GAE/ g DW).

Determination of DPPH radicals stevenging activity

The ability of hydrogen donating or radical scavenging of sample extracts was measured bu using the stable free radical (DPPH) (1,1-dipheny 2-picrylhydrazyl) method according to the method of Brand-Williams et al. (1995), with some modifications (Duarte-Almeida et al., 2006). Abaout 1950 µl of the 1mM solution of methanolic DPPH was mixed with 50 µl extract solution in methanol. The absorbance was measured at 517 nm against the corresponding blank solution as 80% methanol and control were prepared bu taking 1950 µl od DPPH by adding 50 µl distilled water instead of sample. Percentange inhibition of radical DPPH was calculated based on control reading by the following equation.

DPPH-Percentage % of reduction power = $((Ac-As/-A_c))$ x100

DPPH % inhibition = $((A_c-(As-A_b))/A_c)$x100

A_c: absorbance of control (standart)

A_s: absorbance of sample

A_b: absorbance of blank

Quantitative and statistical analyses

All the samples were directly injected to the reverse phase chromatography column. The sugar standards, glucose, fructose, sucrose, were dissolved in water at a concentration of 30 mg ml^{-1}. All samples and standards were injected three times each and mean values were used. The data were analyzed by JMP statistical software from SAS (V7) (SAS Institute Inc. Cary, NC, USA) and all the analytical values were average of three replications. The significantdifferences were compared by the least significant differences (LSD) test executed at 5% level of probability. The means ± the standard error (SE) were calculated from three independent experiments.

Results and Discussion

Biochemical compounds and natural antioxidants have become very important in human nutrition in recent years and are increasingly consumed by consumers. For this reason, it attracts the attention of many researchers and a lot of research is being conducted on this subject. Various parts of pistachio are known to have a high content of polyphenols, vitamin and sugar etc., that are all potent antioxidants and that may have protective effects against different diseases. Pistachio shells contain greater amounts of phenolics than the skin and nuts compared to those found in previously recognized phenolic sources (Tomaino et al., 2010;Garavand et al., 2017). When the studies in previous years were examined, it was found that most of these studies were usually done on fruit. In our study, different from the literature, especially shoots and leaves were examined.

Results of antioxidant activities and total phenolic contents of the different pistachio tissues in 'on' and 'off' year trees are presented in Table 1. It was found that the total phenolic (TPC) and antioxidant capacity (TAC) contents were significant in different tissue samples ($P < 0.05$). TPC values according to research results in the 2018 (on-year) and 2019 (off-year) years. It has been found to be change between shoot 120,034 (off-year) to 135,916 (on-year) mg gallic acid /g, leaf 88,101 (off-year) to 95,842 (on-year) mg gallic acid /g, hull 185,063 (off-year) to 272,624 (on-year) mg gallic acid /g, hard shell 268,003 (off-year) to 392,022 (on-year) mg gallic acid /g and kernel 76,379 to 95,608 mg gallic acid /g, respectively (Table 1). Figure 2 depicts individual parts expressed as a percentage of the total phenolic content and contributing over 35% of the total phenolic content of dehulled part. Total phenol contents in 'on' year were higher than in 'off'year trees. The results show that the total phenolic content of the hull and hard shell nuts was higher than that of the shoot and leaf. Goli et al., (2005) reported that shells contain antioxidant substances and can be used as an additive in foods. This variety contains more phenolic substances in their some tissues and it is advisable to consume seed with varieties because of their potential health benefits (Table 2). The data obtained from the present study are in accordance with other studies (Orhan et al., 2012; Kavak et al. 2010; Dogan et al., 2017). TPC values determined in different pistachio species and varieties 122,78 mg gallic acid /g, 120,64 mg gallic acid /g, 81.12 mg gallic acid /g and 43.81 gallic acid /g, Topcu et al. (2007), Tavakoli and Khodaparast (2013), Farhoosh et al., (2008) and Orhan et al. (2012) is lower than the study respectively, Ballistreri ve ark (2009); 184.71 to 349 mg gallic acid /g, Hatamnia et al., (2014): 189 to 330 and Polat (2016); 79.92 to 198.07 mg gallic acid /g compatible with the study, respectively, 452.95 588 mg gallic acid /g, 690.28 mg gallic acid/g, Atmani et al., (2009), Goli et al., (2005) Azadpour et al., (2015) 's were found to be higher than the study, respectively. TAC inhibition values according to research results in the 2018 and 2019 years; It has been found to be change between shoot 84,721 to 85,379 %, leaf 84,212 to 85,315 %, hull 79,839 to 86,062%, hard Shell 81,411 to 84,216% and fruit kernel 81,237 to 84,186%, respectively (Table 1 and Figure 2). The results antioksidant % radical-scavenging that to be change between shoot 69,668 to 70,373 %, leaf 67,011 to 70,063 %, shelled nuts 69,745 to 70,587%, dehulled 65,976 to 68,780% and kernel 65,870 to 68,778%, respectively (Table 1 and Figure 3). The results show that the total antioxidant activity. It was determined that antioxidant activity properties of different parts of 'Uzun' pistachio cultivar were not the same and leaves and shelled nuts had higher. Hosseinzadeh et al., (2012) found similar results in studies showing the antioxidant activity on pistachio. However, in previous studies in different pistachio species that Polat (2016), Durak and Ucak (2015), Azadpour et al., (2015) and Rezai et al., (2015) reported different results from the study that antioxidant activity in the different parts in pistachio 31,42 to 90.96%, 64.4%, 21.1% and 23.02 % respectively.

The soot, leaf and nut sugar concentrations (g/100g) and total sugar of "on" year trees in the year 2018, "off" year trees in year 2019 of "Uzun" pistachio cultivar were given in Table 2 and Figure 4, respectively. We found significant differences ($p < 0.05$) in sugars among different organs between 'on' and 'off' years in 'Uzun' pistachio cultivar. The highest sucrose content was determined of leaf (8,58 g/ 100 g) , shoot (6,54 g/100 g) and nuy (3,12 g/100 g) in 'off' year and the lowest of shoot (1,85 g /100 g) in 'on' year trees. The highest glucose content was determined of nut (5,48 g/ 100 g), leaf (5,99 g/100 g) and shoot (4, 36 g/100 g) in 'off' year and the lowest of shoot (3,20g /100 g) in 'on' year trees. The highest fructose content was determined of nut (12,10 g/ 100 g) , leaf (2,07 g/100 g) and shoot 2,47 g/100 g) in 'off' year and the lowest of leaf (1,72 g /100 g) in 'on' year trees. The results in study indicate all tissues that the amount of sugar content in 'off' year was higher than 'on' year (Table 2, Figure 4). The finding was in agreement with those of several previous researchers (Nzima et al 1997, Vemmos 1999b and Baninasab and Rahimi, 2006) reported that current different organs in 'on' years had higher amount of total sugar than 'off' year. Baninasab and Rahimi (2006) found sucrose, glucose and fructose concentration of nuts in "on" year between 19.64-121,94 mg/g, 2.58-9.23 mg/g and 14.46.3.74 mg/g, respectively. On the other hand, Karacali (1990) that suggested different plant contents of fructose, glucose, sucrose and maltose contents changed depending on species, varieties, genotypes and accessions. Kazankaya et al.

(2008) identified sugar content pistachio kernels belonging to different varieties contained kernels of Siirt variety had the highest fructose content (5.04 g/100 g), followed by Siirt (4.49 g/100 g), E-1 (4.00 g/100 g), Halebi (3.59 g/100 g) and H-1 (2.67 g/100 g), respectively. Glucose content (6.26 g/100 g) of B-1 kernels was detected in the highest amount, followed by Kirmizi (4.25 g/100 g), H-1 (4.13 g/100 g), Halebi (3.96 g/100 g) and Buttum (3.94 g/100 g), respectively. Sucrose content (4.74 g/100 g) of Buttum kernels was determined at the highest level, followed by V-1 (4.17 g/100 g), H-1 (4.17 g/100 g), B-1 (3.93 g/100 g) and Halebi (3.68 g/100 g), respectively. The same researchers also studied walnut genotypes contained 0.35-2.67 g/100 g fructose, 0.13-6.26 g/100 g glucose, 1.76-4.17 g/100 g sucrose and 0.23-0.74 g/100 g maltose. Sugar components of hazelnut and almond were 0.80 and 4.00 g/100 g fructose, 1.52 and 0.86 g/100 g glucose, 2.91 and 3.23 g/100 g sucrose, respectively. In addition, in study our results had higher sugar contents than other some pistachio varieties. The finding was in agreement with those of several previous researchers (Baninasab and Rahimi, 2006; Kazankaya et al., 2008).

Table 1. Total Phenolic content and Antioxidant capacity at different tissues of 'Uzun' pistachio cultivar of 'on' and 'off' years

Tissues	Total phenolic (mg gallik asit/100 g)		Antioxidant activity % inhibition		Antioksidant activity % radical-scavenging	
	'On' year	'Off' year	'On' year	'Off' year	'On' year	'Off' year
Shoot	135,916[c] ±3,640	120,034[c] ±3,206	85,379[b] ±0,515	84,721[a] ±0,463	70,373[a] ±0,298	69,668[a] ±0,391
Leaf	95,842[d] ±2,230	88,101[d] ±2,023	85,315[b] ±0,237	84,212[a] ±0,447	70,063[a] ±0,210	67,011[b] ±0,534
Hull	272,624[b] ±5,353	185,063[a] ±3,748	86,062[a] ±0,071	85,839[d] ±0,378	70,587[a] ±0,319	69,745[a] ±0,802
Hard Shell	392,022[a] ±4,163	268,003[a] ±2,986	84,216[c] ±0,421	81,411[c] ±0,325	68,780[b] ±0,418	65,976[d] ±0,219
Kernel	95,608[d] ±1,346	76,379[e] ±1,207	84,186[c] ±0,421	81,237[b] ±0,325	68,778[b] ±0,418	65,870[c] ±0,219
Total	992,012	737,58				
LSD%5	6,58**	5,04**	0,62**	0,69**	0,57**	0,95**

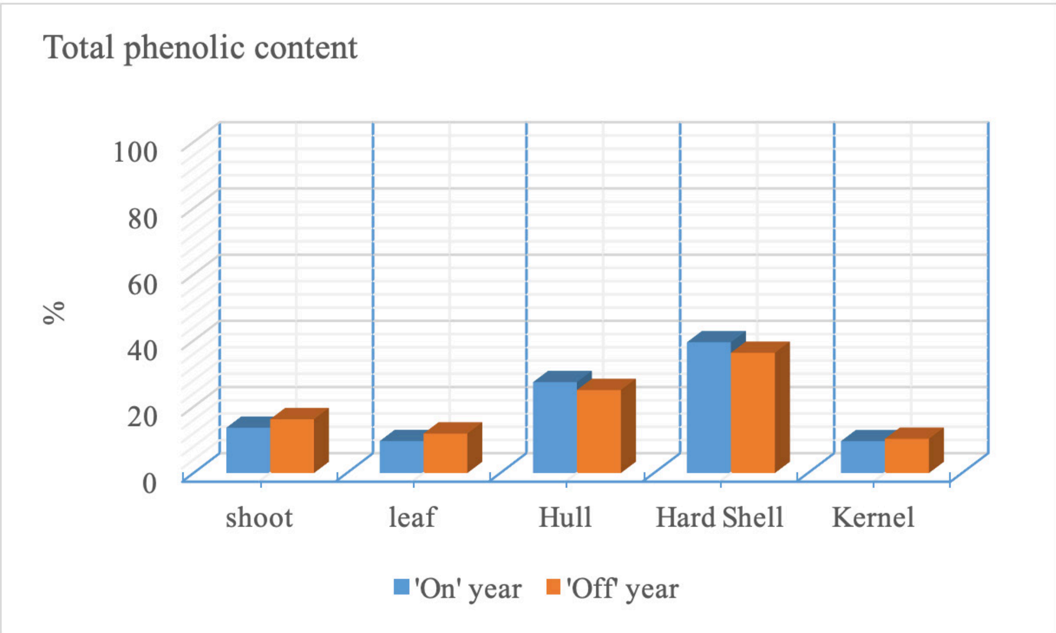

Figure 1. Total phenolic content of pistachio tissues in 'on' and 'off' years. Percentages were calculated based on the each tissue values x 100/total phenol.

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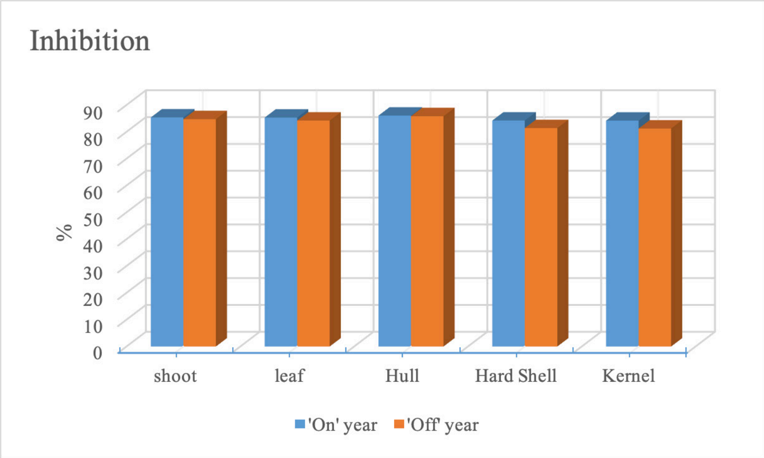

Figure 2. Antioxidant activity % inhibition in pistachio different tissue of ‘on’ and ‘off’ years

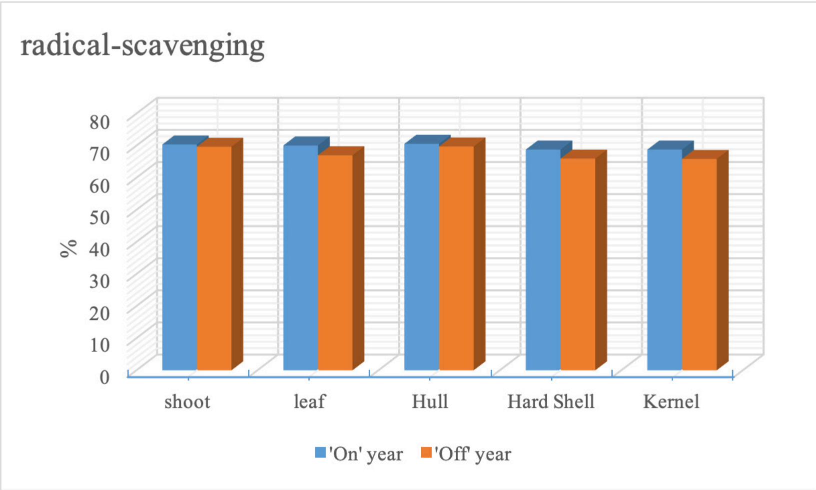

Figure 3. Antioksidant activity % radical-scavenging in pistachio different tissues of ‘on’ and ‘off’ years

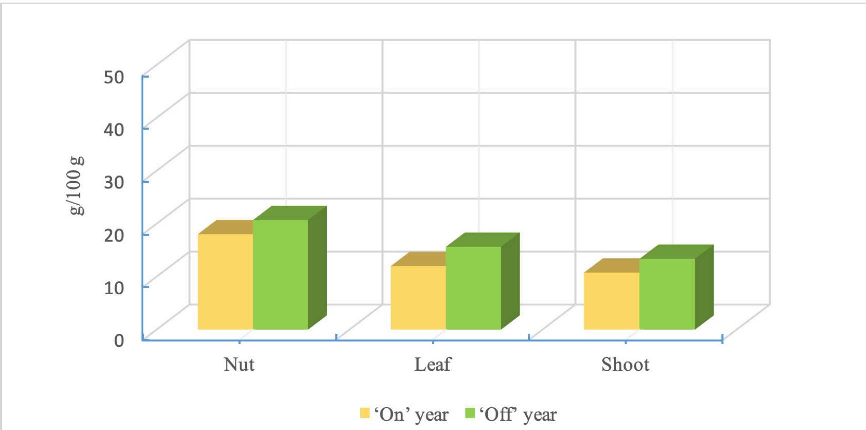

Figure 4. Total sugar concentration of different tissues of “Uzun” pistachio cultivar

Table 2. Soot, leaf and nut sugar compounds concentration (g/100g) of "Uzun" pistachio cultivar of 'on' and 'off' years

Tissues		Sucrose	Glucose	Fructose	Total sugar
Shoot	'On' year	5,71^{b}±0,30	3,20^{b}±0,21	1,85^{b}±0,08	10,76
	'Off' year	6,54^{a}±0,39	4,36^{a}±0,22	2,47^{a}±0,13	13,37
	D%5$_{OnXoff\text{-}year}$	0,77**	0,49**	0,55**	
Leaf	'On' year	6,71^{b}±0,16	3,57^{b}±0,35	1,72^{b}±0,19	12,00
	'Off' year	8,58^{a}±0,13	4,99^{a}±0,21	2,07^{a}±0,10	15,64
	D%5$_{OnXoff\text{-}year}$	0,33**	0,63**	0,35**	
Nut	'On' year	2,67^{a}±0,12	4,30^{b}±0,17	11,07^{b}±0,38	18,04
	'Off' year	3,12^{b}±0,15	5,48^{a}±0,25	12,10^{a}±0,43	20,70
	D%5$_{OnXoff\text{-}year}$	0,30**	0,74**	0,72**	

Conclusion

In the fruit species (including pistachio) are of interest to researchers because of their antioxidant properties and are due to phenolic compounds and composition of sugars influences the taste and it can vary to varieties, ecological conditions, technical and cultural practice. However, this study showed that pistachio has strong radical scavengers and is a fruit that is a good source of natural antioxidants for medical and commercial uses. Thus, pistachio can be eaten as part of a diet to alleviate the symptoms of chronic and degenerative diseases that are reported to increase in the world. In Turkey, pistachio shells are used as industrial waste. However, as a result of the study, it was determined that most of sugar compositions, the total antioxidant activity and total phenolic content of 'Uzun' pistachio cultivar were found to be rich in sugars, phenolic content and antioxidant content in the shelled and dehulled nuts. Therefore we recommend the use in particular of different organs of pistachio in food and other industries (e.g. medicine, cosmetics). In addition, in respect of its antioxidant properties, it can be used as a natural antioxidant in some foods (snack, sweets, icecream etc.).

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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Not applicable.

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Research Article

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Fatty acid profiling in animal feeds and related food matrixes using a fast GC/MS method and *in situ* derivatization

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Abstract

Fatty acid determination is used for the characterization of the lipid fraction in foods, providing essential information regarding feed and food quality. Most edible fats and oils are composed primarily of linear saturated fatty acids, branched, mono-unsaturated, di-unsaturated, and higher unsaturated fatty acids. To attain this information we developed a gas chromatography (GC) method that can separate fatty acids from C_4 to C_{24} using mass spectrometry identification. A simplified sample preparation procedure was applied so it is not time-consuming and short enough to avoid fat degradation. Additionally, one-step derivatization was applied to obtained fatty acid methyl esters in situ in the gas chromatograph injection port, using tetramethylammonium hydroxide and a high polarity polyethylene glycol-based cross-linked microbore chromatographic column was coupled to achieve the separation of 60 compounds in under 15 minutes with extreme sensibility. The versatility of the method allows fatty acid profile (including saturated [SFA], monounsaturated [MUFA], and polyunsaturated fatty acids [PUFA]) information to be gathered in different products of primary production i. raw materials commonly used in the production of animal feed, ii. profiles for balanced feed for laying hens, beef cattle and dairy cattle and iii. products of animal origin intended for human consumption, such as meat, eggs, and milk. Our data (performance parameters and fatty acid profiles) support the validity of the results; the method can be used for quality assurance both in productive species feed and feed ingredients, pet food, and related food matrices. The technique presented herein can be used as a high-throughput routine screening tool to assess fat quality as this data is paramount to improve animal nutrition and health and animal-derived products of human consumption.

Keywords: Animal feeds, Fat content and quality, Food-producing animals, GC/MS

Introduction

High-quality diets must input all necessary nutrients to maintain the animals' physical structure, biological functions, improve their physiological state and health while considering the species which the feed was meant to target (Makkar, 2016). Though, livestock feeding should also consider improvements in production (Thornton, 2010; Makkar, 2016), i.e., guaranteeing efficient growth, a persistent production without affecting the health of the animal or increasing the price of food. Therefore, their costs may limit the use of some feed ingredients. On the other hand, pet nutrition is mostly oriented toward optimizing the companion animal nutrition and health status (Di Cerbo *et al*., 2017). Thus, high-end feeding materials are preferred to formulate the latter type of feeds.

As protein and energy are considered limiting nutrients (NRC, 2001; Rostagno *et al.,* 2017), feed formulation should contemplate these requirements foremost. The use of fats and oils in animal feed, (contribution of dietary fat and fatty acids) is deemed to be essential as an energy concentrated nutrient (e.g., acylglycerols and emulsifiers), a carrier for other hydrophobic compounds (Poorghasemi *et al.,* 2013), and as feed palatability modulator, especially for cats and dogs (NRC, 2006;

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Çentingül and Yardimci, 2008; FEDIAF, 2016). Additionally, fat reservoirs supply essential fatty acid requirements. Therefore, feed materials are commonly classified by their energy input and, after that, by compatibility, digestibility, gastrointestinal functionality (Celi *et al.,* 2017), and accessibility. As such, agro by-products from local productive enterprises are exploited (Wood and Fearon, 2009; Ajila *et al.,* 2012)

However, as corn and soybean meal are versatile staple foods, they can be used in most livestock production systems. Both ingredients can represent as high as 60% of the dietary inclusion, though, corn meal is an adequate source of energy (3 294 kcal metabolizable energy kg^{-1}) whereas soybean meal is considered mostly a protein source (44 – 48 g/100 g on dry matter basis) (Rostagno *et al.,* 2017; Shepon *et al.,* 2016). Moreover, by-products in vegetable oil refining such as lecithin, soapstock, acid oil, and fatty acid distillate may also be included within diets (Kerr *et al.,* 2015).

Fat (e.g., obtained as a by-product of the rendering industry) and vegetable oil, a subgroup of lipids, production has increased as these substances are directly supplemented into livestock and poultry feed and pet foods (Kerr *et al.,* 2015). Fatty acid addition has demonstrated beneficial effects in several species such as horses (Hess and Ross-Jones, 2014), pigs (Rostagno *et al.,* 2017; Liu *et al.,* 2018), dairy cows (NRC, 2001; Harvatine and Allen, 2006), poultry (Poorghasemi *et al.,* 2013; Rostagno *et al.,* 2017) and, especially, management of several diseases and clinical problems in pets (Lenox and Bauer, 2013; Wąsik *et al.,* 2016). Of particular interest are, for example, linoleic (9c12c-$C_{18:2}$), eicosapentaenoic acid (5c8c11c14c17c-$C_{20:5}$), and docosahexaenoic acid (4c7c10c13c16c19c-$C_{22:6}$), ($C_{20:5}$) in puppy and dog food (Ahlstrøm *et al.,* 2004); relevant for cardiovascular health and nervous system development (Biagi *et al.,* 2004; Fraeye *et al.,* 2012).

On the another hand, as feed sits at the beginning of the food chain, knowledge about the fatty acid composition may improve animal nutrition (Baltić *et al.,* 2017) and enhance food products derived from such animals (Moran, 1996) (e.g., conjugated linoleic acid isomers [9c11t and 10c12t-$C_{18:2}$] and *trans* vaccenic acid [11t-$C_{18:1}$] in pasture-fed bovine meat and milk) (Daley *et al.,* 2010). In contrast, lipid and fatty acid deficiencies carry a plethora of health issues both for animals and humans alike (Sardesai, 1992).

After that, the development of fast and accurate analytical methods are necessary as all feed and food stakeholders should be able to assess lipid quality and nutritional value. Analytic approaches have historically made emphasis on food for human consumption, including gas chromatography (GC)-based Official Methods of Analysis^{SM} (e.g., AOAC 991.39, 975.39, 996.06, 994.15, 985.21, 963.22, 985.20, 965.49, 969.33). Several alkylation derivatization reagents have been used to generate the more volatile esters needed to perform the chromatography (Christie, 1993). Recently published research also had the same tendency and even compared transesterification or derivatization methods (Topolewska *et al.,* 2014; Topolewska *et al.,* 2015; Salimon *et al.,* 2017). However, all these methods usually rely on columns of considerable length, which results in long chromatographic runs (i.e., 60 minutes or more) to achieve an analytical separation (especially true for $C_{18:1}$, $C_{18:2}$, and, $C_{18:3}$).

Herein we report a method involving the direct extraction of fat using diethyl ether and the formation of methyl esters in the heated injection port of a GC coupled with mass spectrometry (MS) detector. The non-esterified fatty acids in a methanolic solution are pyrolyzed and suffer oxidative cleavage by the organic base catalyst. We chose this derivative formation technique as is highly practical but still able to render quantitative results. A similar approach has been applied to paint resins (West, 1975), human serum lipids (Haan *et al.,* 1979), and bacterial cells (Dworzanski *et al.,* 1990), to name a few, but, to our knowledge, never to feed or food products. Furthermore, butylated hydroxytoluene is added, during preparative stages of the method, to protect unsaturated fatty acids. Additionally, the chromatograph was equipped with a microbore short column which we applied the method successfully to a diverse group of samples that include animal feed and related matrices such as fats and oils, chicken eggs, bovine milk, and muscle tissue.

Materials and Methods

Reagents

Diethyl ether (309966, $(CH_3CH_2)_2O$, for HPLC, ≥ 99.9%, inhibitor-free), hydrochloric acid (320331, ACS reagent, 37%), 2,6-Di-tert-butyl-4-methylphenol (B1378, ≥ 99.0% purity) and tetramethylammonium hydroxide (TMAH, 334901, 25 wt. % solution in methanol) and trimethylphenyl ammonium hydroxide (TMPAH, 79266, 0.5 mol L^{-1} in methanol for GC derivatization) were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade methanol (MeOH, LiChrosolv®) was acquired from Merck Millipore (Merck KGaA, Darmstadt, Germany).

Analyzed Samples

Profiles were determined for feed ingredients such as corn meal (n = 35), soybean meal (n = 19), and peanut meal (n = 7). Fats and oils samples examined included animal fat (n = 8), palm oil (n = 8), and by-pass fat (n = 8). Feed samples analyzed encompassed layer hen feed (n = 10), beef cattle feed (n = 8), and dairy cattle feed (n = 10). Later, related food commodities tested involved chicken eggs (n = 11), bovine (n = 35), water buffalo (n = 11), and lamb (n = 11) meat tissues, and milk samples (n = 12). Also, our analysis included wet (n = 8) and dry extruded dog food (n = 20), dry extruded puppy food (n = 12), and wet (n = 6) and dry extruded cat food (n = 8). Finally, twelve forage mixtures were collected from Costa Rican northern lowlands cattle farms.

Sample fat extraction

A 100 g sample was milled and sieved to 1 mm (using a ZM 200 ultracentrifuge mill, Retsch GmbH, Haan, Germany), after that a subsample of ca. 1 gram of each feed or feed ingredient sample was set in a 50 mL glass beaker, 5 mL of diethyl ether were added and mixed using an ultrasonic shaker (USC200TH, VWR International, Center Valley, PA, USA) for 5 minutes. In the case of extruded meat samples, 2.5 mL of a 9 mol L^{-1} HCl solution in ethanol and 2.5 mL diethyl ether was added for extraction. Each egg sample was constituted by a dozen units, so four randomly chosen eggs were scrambled and freeze-dried

(LABCONCO, FreeZone 4.5 Liter, Kansas City, MO, USA), a gram of the resulting powder was used for fat extraction. Fresh forage was quartered and cut in bits and also freeze-dried before extraction. Freeze-drying was also applied to cat and dog food wet samples. Meat samples were processed using a knife mill (GM 300, Retsch) before fat extraction, a gram of minced meat was treated. Finally, bovine milk samples fatty acids extraction involved their direct mixing with a dichloromethane–ethanol solution (2:1), as described by Stefanov et al. (2010). Afterward, a 200 µL aliquot was transferred to a GC 2 mL vial (Agilent Technologies, Santa Clara, CA). Then, 800 µL of diethyl ether and 1 000 µL of a, previously prepared, 0.25 g/100 mL TMHA solution in methanol are added to the same vial. Two µL of the resulting mixture is injected into the GC system.

Additional nutritional, quality and functional assays

The total fatty acid content in oils and fats was performed using the AOCS method Ca 3a-46. Method 954.02 was used to assess fat content in by-pass fat as well. Fat content for the majority of feed and feed ingredient samples was determined using method AOAC OMA^{SM} 920.39. On the other hand, crude fat and water activity (a_w) were calculated in extruded pet foods by acid hydrolysis AOAC OMA^{SM} 954.02, and Aqualab chilled mirror methods (measurement performed at 24.50 ± 0.24 °C, Aqualab 4TE, Decagon Devices, Pullman, WA, USA), respectively. AOAC OMA^{SM} 940.28 was used to assess free fatty acids in animal fats, palm oil, and by-pass fats; results expressed as g palmitic acid per 100 g sample. Egg, meat, and raw milk total fat content were determined by AOAC OMA^{SM} 925.32, 960.39, and 989.04, respectively.

Chromatographic conditions

Qualitative analyses of the volatile compounds were carried out using an Agilent gas chromatograph (7820, Agilent Technologies) equipped with an Agilent Technologies J&W DBWAX microbore column of 10 m length, 0.1 mm diameter, 0.1 µm film thickness and Agilent 5977E mass spectrometer (MSD). The carrier gas was helium at a constant flow of 0.3 mL min^{-1}. The GC oven temperature was kept at 50°C for 0.34 minutes and programmed to 200 °C at a rate of 72.51 °C minute^{-1}, this temperature was kept constant 0.17 minutes and then programmed to 230 °C at a rate of 8.7 °C minute^{-1}, held for 7.9 minutes for a total run time of 13.93 min. The split ratio was adjusted at 30:1. The injector, transfer line, ion source, and quadrupole temperatures were set at 250, 250, 230, and 150 °C, respectively. The mass range was 50-450 m/z. Electron energy was set at 70 eV, 150 °C. FAME mixtures GLC-486 (n = 40 analytes) and GLC-860 (n = 60 analytes, Nu-Chek Prep, Inc., Elysian, MN, USA) were used as quality control comparing retention times and mass spectra with those found in the analyzed samples (Figure 1A, B). Several compounds were used to check mass tuning including tetradecanoic (6.16 min; M^+ 227.6 m/z), pentadecanoic (6.72 min; M^+ 243.4 m/z), hexadecanoic (7.58 min; M^+ 256.3 m/z), octadecanoic (9.70 min; M^+ 285.5 m/z), *cis*-13-octadecanoic (10.21min; M^+ 285.7 m/z) and 9*Z*-octadecenoic (7.78 min; M^+ 284.1 m/z), (*Z*,*Z*)-9,12-octadecadienoic (10.86 min; M^+ 280.0 m/z) acids (Figure 1C). Constituents were identified by matching their spectra with those in NIST library 14. Only hits with a match factor above 80% were considered (Figure 1C). Enanthic acid (≥ 99%, 75190, Sigma-Aldrich) was used as an internal standard. 9c11t-$C_{18:2}$, 10c12t-$C_{18:2}$, $C_{12:0}$, 4c7c10c13c16c19c-$C_{22:6}$, 11t-$C_{18:1}$ were concurrently monitored by simultaneous ion monitoring (SIM) mode (total dwell time 100 ms and cycles 8.3 Hz. For compounds with no analytical standard injection, identification should be considered as tentative.

Results

Performance parameters and method peculiarities

From all analytes, $C_{18:0}$ showed a higher limit of detection (lower sensitivity) with 0.16 mg L^{-1}. In contrast, $C_{19:0}$ showed a higher sensitivity with 0.06 mg L^{-1}. Limits of detection determined in an extinction experiment using mix GLC-486. On feed samples, the sensitivity is calculated for corn meal, soybean meal, poultry layer feed, cattle feed, pet foods, and resulted in 0.146, 0.395, 0.066, 0.203, 0.047 g/100 g fat, respectively. On the other hand, values for $C_{18:0}$ expressed in chicken eggs, bovine milk and muscle tissue are determined to be 0.027, 0.041, and 0.011 g/100 g fat, respectively. C_{16} to C_{20} z values for rapeseed oil were found between -2.04 and 2.11, C_{18} compounds were found to be the most variable concerning the robust mean (Table 1). In a second performance test, using dry cat food, in which mostly MUFA and PUFA were assayed, z values, for $C_{16:1}$ to $C_{22:6}$, ranged from -1.34 to 1.56 (Table 1). The sum of SFA, MUFA, and PUFA were also tested. Our data were compared to a reference method (i.e., AOAC OMA^{SM} 996.06), though the reference method showed less deviation from the robust mean z values (1.14 to 1.47) for each fatty acid group (Table 1). C_{16} and 9c-$C_{18:1}$ showed the absolute difference (-37.25 and 20.54) between the methods (i.e., the proposed vs. derivatization using TMPAH vs. reference) making this a robust method (Table 2).

Feed ingredients

Data for three vegetable ingredients and fat and oils are presented. Corn meal presented a higher proportion of PUFA (544.59 ± 54.24 g kg^{-1} fat) than MUFA (282.81 ± 45.12 g kg^{-1} fat), and SFA (172.84 ± 23.78 g kg^{-1} fat). 9c-$C_{18:1}$ and 9c12c-$C_{18:2}$ predominates (i.e., 227.94 ± 123.09 and 444.07 ± 85.09 g kg^{-1} fat) (Table 3). Soybean meal showed average values for SFA, MUFA, and PUFA of 209.09 ± 29.22, 197.71 ± 40.37, and 593.81 ± 34.19 g kg^{-1} fat, respectively, with a predominance of linoleic acid (504.02 ± 122.97 g kg^{-1} fat) (Table 3). Peanut meal has a similar overall profile of SFA, MUFA and PUFA as the above two ingredients (i.e., 222.98 ± 63.93, 113.38 ± 28.81, 663.33 ± 77.56 g kg^{-1} fat) (Table 3). Regarding sample fat content, the 9c12c-$C_{18:2}$ contribution is higher for peanut than corn meal. On the contrary, in the animal fat exhibit a tendency toward SFA and MUFA (465.60 ± 11.50 and 430.90 ± 9.10 g kg^{-1} fat, respectively) which, in turn, reflects on 9c-$C_{18:1}$ concentration (i.e., 415.50 ± 15.30 g kg^{-1} fat) (Table 4 and Figure 2A). A similar profile is found in by-pass fat (i.e., 454.55 ± 55.95 and 483.95 ± 72.25 g kg^{-1} fat for SFA and MUFA, respectively) where $C_{18:0}$ is the most prominent fatty acid (i.e., 450.93 ± 57.83 g kg^{-1} fat). The same is true for palm oil (i.e., 436.83 ± 25.78 and 460.93 ± 29.81 g kg^{-1} for SFA and MUFA) (Table 4).

Compound feeds

Overall, compared to MUFA, layer hen feed have a higher PUFA ratio (i.e., 433.44 ± 43.2 and 327.59 ± 32.94 g kg^{-1} fat, respectively) (Table 5 and Figure 2B). The most significant fatty acids include palmitic, oleic, and linoleic (i.e., 170.28 ± 17.79, 314.83 ± 30.41, and 369.48 ± 55.00 g kg^{-1} fat, respectively) (Table 5). Cattle feed presents a higher concentration of SFA and MUFA (430.26 ± 134.42 and 358.09 ± 140.84 g kg^{-1} fat) with an average oleic acid input of 387.70 ± 56.57 g kg^{-1} fat (Table 5). In contrast, dairy cattle feed has a higher PUFA and MUFA (434.90 ± 34.42 and 335.18 ± 29.47 g kg^{-1} fat) with a considerable linoleic acid input (369.20 ± 26.06 g kg^{-1} fat) (Table 5).

Food samples (Feed-related matrices)

Eggs show an almost equivalent concentration of SFA and MUFA (i.e., 407.26 ± 80.64, and 427.25 ± 79.37 g kg^{-1}). In contrast, PUFA input is moderate (i.e., 165.66 ± 28.29 g kg^{-1} fat) with 9c-$C_{18:1}$ and 9c12c-$C_{18:2}$ as the predominant fatty acids (i.e., 363.20 ± 96.37 and 158.34 ± 57.95 g kg^{-1} fat) (Table 6 and Figure 2C). Interestingly, all meat tissues analyzed possess a similar range of total fat. Additionally, have a tendency toward SFA and MUFA where buffalo meat and lamb meat present the higher concentration of said fatty acids (i.e., 636.26 ± 99.44 and 462.81 ± 55.65 g kg^{-1} fat) (Table 6). Notwithstanding, 9c12c-$C_{18:2}$ levels are higher in bovine meat (405.31 ± 70.22 g kg^{-1} fat). Bovine milk presented a total fat of 32.93 ± 5.30 g kg^{-1} (Table 6). Interestingly, SFA largely predominates (i.e., 792.83 ± 84.52 g kg^{-1}). However, 9c-$C_{18:1}$ and $C_{16:0}$ are both major fatty acids present (i.e., 259.81 ± 58.22 and 268.02 ± 25.10 g kg^{-1} fat, respectively) (Table 6).

Pet foods

In the case of dog food, data are presented in adult and puppy dog food. Dry foods (less than 10 g/100 g moisture) showed, on average, a higher concentration of SFA (526.79 ± 151.31 g kg^{-1} fat) compared to wet foods (> 80 g/100 g moisture, 386.77 ± 85.88 g kg^{-1} fat) (Table 7). On the other hand, MUFA and PUFA remained in the same trend in both wet foods (382.02 ± 91.06 and 175.45 ± 37.78 g kg^{-1} fat, respectively), and dry foods (317.74 ± 84.48 and 167.40 ± 90.98 g kg^{-1} fat, respectively) (Table 7 and Figure 2D). About the presence of omega-3 and omega-6 fatty acids, higher levels of both types of fatty acids were found in wet foods (e.g., 9c12c-$C_{18:2}$ at 372.50 ± 57.03 g kg^{-1} of fat) (Table 7). In wet and dry foods, a higher concentration of 9c-$C_{18:1}$ and 9c12c-$C_{18:2}$ was found (i.e., 376.10 ± 35.48 and 194.88 ± 54.65 g kg^{-1} fat, respectively) (Table 7). Dry puppy foods have lower concentrations of MUFA and PUFA (i.e., 291.30 ± 61.62 and 132.51 ± 80.36 g kg^{-1}, a piece) compared to adult dog foods and trivial levels of 5c8c11c14c17c-$C_{20:5}$ and 4c7c10c13c16c19c-$C_{22:6}$ (Table 7). In the case of cat food, the data show a lower concentration of SFA, in wet foods when compared to dry foods (384.75 ± 105.39 and 560.92 ± 147.65 g kg^{-1} fat, respectively) (Table 7). Later, the concentration of MUFA in the wet foods is higher than in the dry ones (390.15 ± 51.15 and 287.85 ± 119.73 g kg^{-1} fat, respectively), while the PUFA presented a similar trend (225.10 ± 130.89 and 151.35 ± 53.10 g kg^{-1} of fat, respectively) (Table 7 and Figure 2D).

Forage blends fatty acid profiling

Forage mixtures used in the feeding of beef and dairy cattle show a predominance for SFA with ranging from 815.52 to 440.33 g kg^{-1} (Table 8). The concentration of MUFA and PUFA is similar in most samples. In forages with parts of PUFA less than 60.00 g kg^{-1}, there is an absence of 11c14c17c-$C_{20:3}$. On the other hand, all the forage samples have $C_{16:0}$ (Table 8). PUFA for the grass mixtures ranged from 21.25 to 375.47 g kg^{-1} fat (Table 8) with 9c-$C_{18:1}$ and 11c14c17c-$C_{20:3}$ with levels ranging from 136.45 to 262.58 and 94.15 to 164.87 g kg^{-1}, respectively.

Discussion

Performance parameters and method peculiarities

Sensitivity-wise, results are intuitive as meat samples, and pet foods have a more substantial fat content among their respective groups, which in turn, result in a lower limit of detection expressed within the matrix. Performance parameters obtained during the validation procedure (e.g., *z* values) speak toward an accurate, true, and relatively unbiased method (experimental *z* values should be between -2 and 2 to be deemed acceptable (Sykes *et al.,* 2014). The simple precision analysis demonstrated that minor modifications (i.e., using a different mass detector, derivatization agent, and conditions during the assay) do not affect the method performance considerably. A specific advantage that this method presents is the catalyst; under our experimental conditions, the transesterification occurs spontaneously, which, in turn, means a fewer step procedure. Finally, additional performance parameters such as %RSD for retention times and areas, k, α_s, N, and R_s are reported for the proposed method (Table 2) and are deemed adequate for a fit-to-purpose method (US FDA, 2015; Bhardwaj *et al.,* 2016; Borman and Elder, 2018). As a final consideration regarding the method scope, neither the column nor the mass spectra can distinguish among *cis*/*Z* and *trans*/E isomers. Such is the case for elaidic acid/oleic acid, linoelaic/linoleic, palmitoleic/palmitelaic. Fortunately, only *cis* isomerism is naturally occurring (except for ruminal fats), endogenous radical stress is the responsible mechanism *trans* isomerism in non-processed food and feed samples (Chatgilialoglu *et al.,* 2013).

Feed ingredients

Corn meal is used in more than half of animal diets, especially for poultry. However, in laying hen feeds, maximum inclusions of 65 g corn meal/100 g feed are usually achieved mostly due to economic and practical reasons. Mean crude fat contents range from 35.0 to 37.0 g kg^{-1} for this matrix, a value well in line with our experimental results (39.14 g kg^{-1}). Corn meal is considered a source of 9c12c-$C_{18:2}$ (1.78 g/100 g corn meal), a fact which is supported by our data (444.07 g kg^{-1} fat, 1.73 g/ 100 g corn meal). In contrast, 9c12c15c-$C_{18:3}$ acid exhibits higher levels (i.e., 106.61 g kg^{-1} fat, 0.42 g/100 g corn meal) than those reported (0.03 g/100 g corn meal) by other researchers (Sauvant *et al.,* 2004; Rostagno *et al.,* 2017). On the other hand, soybean meal also can be used in higher rates in animal diets. However, in laying hen feeds, inclusions of a maximum 30 g soybean meal/100 g feed are usually achieved. Soybean meal and hulls are utilized during feed formulation both by-products from the soybean oil industry (Kerr *et al.,* 2015).

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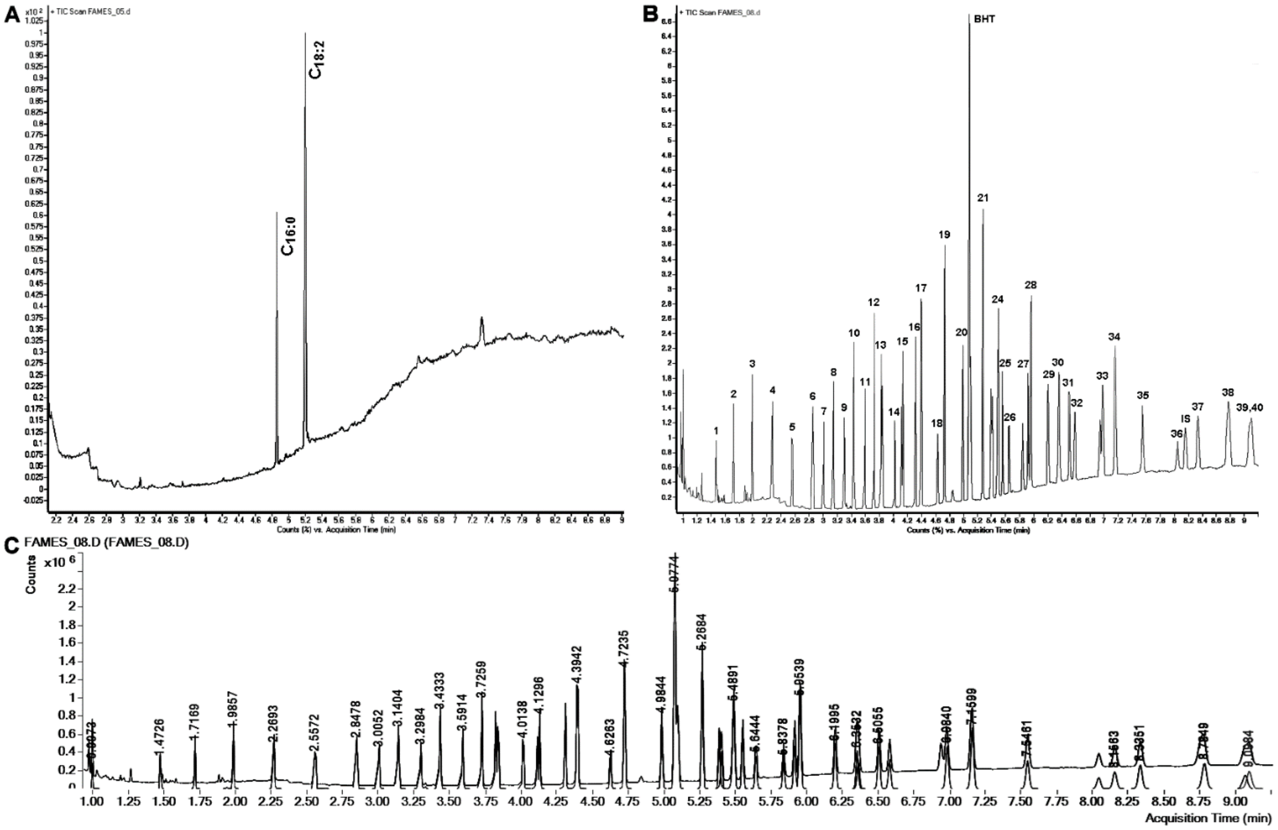

Figure 1. A. Methyl palmitate (hexadecanoic acid methyl ester) and methyl linoleate (9*Z*,12*Z*-octadecadienoic acid methyl ester) at 1.25 mg L^{-1} in diethyl ether. B. chromatographic separation of 40 fatty acid methyl esters mixture (GLC-486, from hexanoate, $C_{6:0}$, to lignocerate, $C_{24:0}$). 1 $C_{6:0,\ tr=1.47}$ 2 $C_{7:0,\ tr=1.72}$ 3 $C_{8:0,\ tr=1.99}$ 4 $C_{9:0,\ tr=2.27}$ 5 $C_{10:0,\ tr=2.56}$ 6 $C_{11:0,\ tr=2.85}$ 7 10e-$C_{11:1\ 10:1,\ tr=3.00}$ 8 $C_{12:0\ 12:0,\ tr=3.14}$ 9 $C_{1\text{-}P\text{-}12:0,\ tr=3.30}$ 10 $C_{13:0,\ tr=3.43}$ 11 7c-$C_{16:1,\ tr=3.59}$; 12 $C_{14:0,\ tr=3.73}$ 13 $C_{15:0,\ tr=4.01}$ 14 $C_{16:0,\ tr=4.31}$ 15 9c-$C_{16:1,\ tr=4.39}$ 16 $C_{17:0,\ tr=4.63}$ 17 10c-$C_{17:1,\ tr=4.72}$ 18 $C_{18:0,\ tr=4.98}$ 19 9c-$C_{18:1,\ tr=5.08}$ 20 9c12c-$C_{18:2,\ tr=5.26}$ 21 $C_{19:0,\ tr=5.39}$ 22 6c9c12c-$C_{18:3,\ tr=5.40}$ 23 10c-$C_{19:1,\ tr=5.49}$ 24 9c12c15c-$C_{18:3,\ tr=5.55}$ 25 9c11t-$C_{18:2,\ tr=5.64}$ 26 $C_{20:0,\ tr=5.84}$ 27 11c-$C_{20:1,\ tr=5.95}$ 28 11c14c-$C_{20:2,\ tr=6.19}$ 29 $C_{21:0,\ tr=6.35}$ 30 8c11c14c-$C_{20:3,\ tr=6.36}$ 31 5c8c11c14c-$C_{20:4,\ tr=6.50}$ 32 11c14c17c-$C_{20:3,\ tr=6.58}$ 33 $C_{22:0,\ tr=6.98}$ 34 11c-$C_{22:1,\ tr=7.16}$ 35 11c14c-$C_{20:2,\ tr=7.55}$ 36 7c10c13c16c-$C_{22:4,\ tr=8.04}$ 37 4c7c10c13c16c-$C_{22:5,\ tr=8.33}$ 38 $C_{24:0,\ tr=8.78}$ 39 15c-$C_{24:1,\ tr=9.07}$ 40 4c7c10c13c16c19c-$C_{22:6,\ tr=9.10}$. All analytes with a relative area sum of 2.70 g/100 g except for 15, 18, 19, 20, and, 27 at 3.8 g/100 g. C. Mass spectrometry identification based library match for mixture (GLC-486).

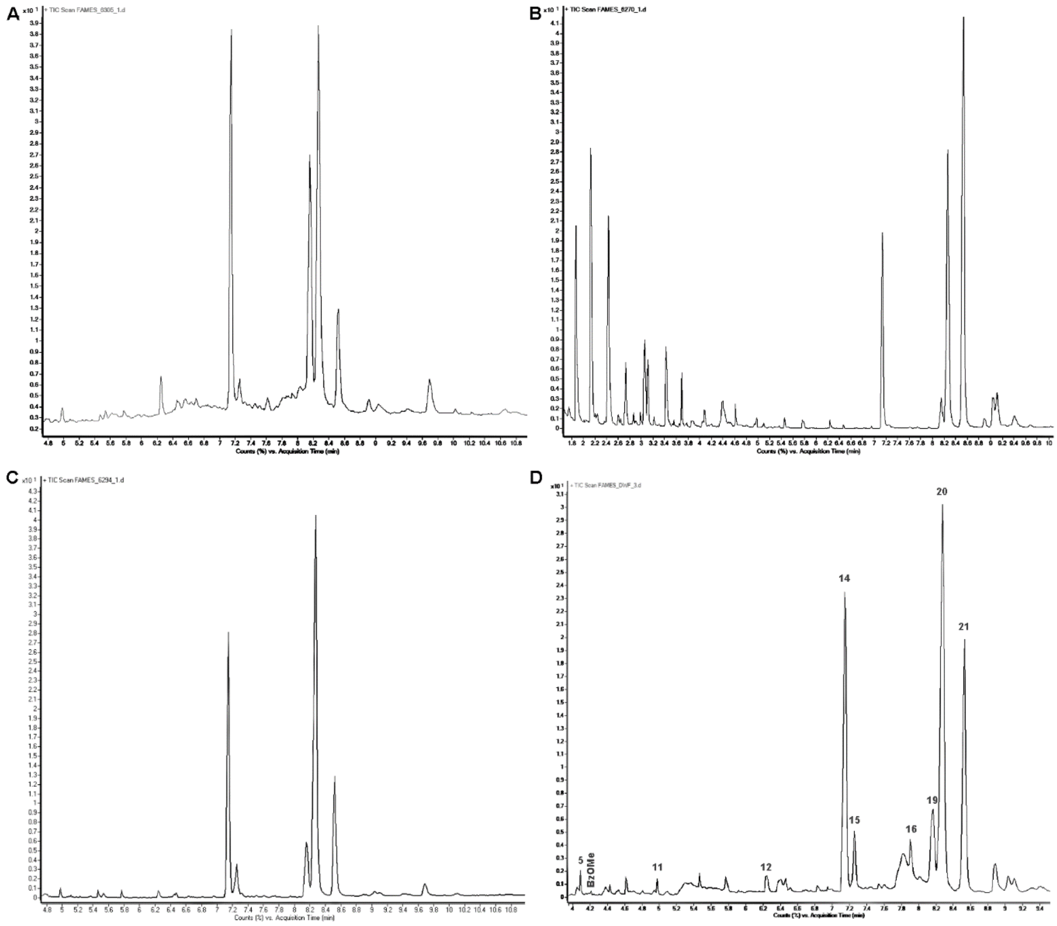

Figure 2. Fatty acid profiles (based on their methyl esters) using the proposed method of A. an animal fat. B. Layer feed. C. Chicken eggs. D. Wet dog food, more relevant acids identified were 5 $C_{10:0,\ tr=2.56}$ Benzoic acid methyl ester, 11 7c-$C_{16:1,\ tr=3.59}$; 12 $C_{14:0,\ tr=3.73}$ 14 $C_{16:0,\ tr=4.31}$ 15 9c-$C_{16:1,\ tr=4.39}$ 16 $C_{17:0,\ tr=4.63}$ 19 9c-$C_{18:1,\ tr=5.08}$ 20 9c12c-$C_{18:2,\ tr=5.26}$ 21 $C_{19:0,\ tr=5.39}$

Table 1. Method performance parameters values obtained for three different food commodities

Rapeseed oil (LGC Standards AFPS 025).			
Fatty acid shorthand	***Mean ± U_x*** [a]	***Assigned value***	***Range or z value***
	Concentration, g fatty acid/kg fat		
$C_{14:0}$	0.78 ± 0.04		0.50 to 0.78 *g fatty acid/kg fat*
$C_{16:0}$	44.20 ± 2.07	44.20	0.00
9c-$C_{16:1}$	1.86 ± 0.09	1.95	-0.35
$C_{18:0}$	15.60 ± 0.73	19.00	-2.04
9c-$C_{18:1}$	647.60 ± 30.39	583.46	2.11
9c12c-$C_{18:2}$	201.30 ± 9.45	205.20	-1.29
9c12c15c-$C_{18:3}$	78.60 ± 3.69	83.19	-1.05
$C_{20:0}$	5.60 ± 0.26	5.41	0.11
9c-$C_{20:1}$	10.01 ± 0.47		9.45 to 11.05 *g fatty acid/kg fat*

Dry Cat Food (AAFCO Check Sample program 2018-25)						
Fatty acid shorthand	***g fatty acid/100 g fat***	**Corrected for fat content** [b]	**Reported range**	**Robust mean**	**Robust standard deviation**	***z* value**
		g fatty acid/100 g sample				
9c-$C_{16:1}$	3.46	0.543877	0.4115 - 0.535	0.45401	0.05751	1.56
9c12c-$C_{18:2}$	10.51	1.652067	1.435 - 2.19	1.8441	0.30459	-0.63
9c12c15c-$C_{18:3}$	0.018	0.282942	0.2545 - 0.37	0.31063	0.0528	-0.52
4c7c10c13c16c19c-$C_{22:6}$	1.11	0.174481	0.1325 - 0.225	0.16461	0.04116	0.24
5c8c11c14c17c-$C_{20:5}$	0.42	0.06602	0.0835 - 0.145	0.10393	0.02817	-1.34

Component	**Sum of saturated fatty acids (SFA)**		**Sum of monounsaturated fatty acids (MUFA)**		**Sum of polyunsaturated fatty acids (PUFA)**	
	Assay, *g fatty acid/100 g fat*	***z* value**	**Assay, *g fatty acid/100 g fat***	***z* value**	**Assay, *g fatty acid/100 g fat***	***z* value**
			Wet cat food (Priida Round 75)			
	Robust mean = 35.83 *g fatty acid/100 g fat* [c]		**Robust mean = 49.11 *g fatty acid/100 g fat*** [c]		**Robust mean = 16.56 *g fatty acid/100 g fat*** [c]	
Proposed method	38.09	1.26	52.71	1.47	17.50	1.14
Reference Laboratory[d]	34.25	-0.88	49.26	0.06	15.89	-0.81
			Canned meat (Priida Round 86)			
	Robust mean = 37.52 *g fatty acid/100 g fat* [c]		**Robust mean = 46.71 *g fatty acid/100 g fat*** [c]		**Robust mean = 16.84 *g fatty acid/100 g fat*** [c]	
Proposed method	40.10	1.40	41.10	-2.40	18.70	0.69
Reference Laboratory[d]	34.60	-1.60	46.50	-0.1	17.30	

[a]U_x calculated as the result of the fatty acid with the most variability for (i.e., $C_{18:1}$) n = 5 replicates, measured on five different days, using a coverage factor of 95% where k = 2. [b]Fat obtained by acid hydrolysis (15.72 ± 0.47) g/100 g [c]Analysis for wet cat food and canned meat-based on results by 16 and 7 laboratories, respectively. [d]Reference laboratory used method AOAC 996.06.

Table 2. Robustness assays for the selected method

	Proposed method		Derivatization using TMPAH		Reference Laboratory	
		g fatty acid/kg fat			*g fatty acid/ kg fat*	
Fatty acid shorthand	***Concentration***	***Assigned value ± U_x***	***Concentration***	***Difference***	***Concentration***	***Difference***
			Sunflower oil LGC Standards QFCS 246 sample 778			
2c-$C_{4:1}$	ND		0.20	-0.20	ND	0
$C_{4:0}$-diacid	0.20		ND	0.20	ND	0.20
2Me-$C_{10:0}$	0.40		ND	0.40	ND	0.40
$C_{9:0}$-diacid	0.61		ND	0.61	ND	0.61
$C_{13:0}$	ND		0.30	-0.30	ND	0
$C_{14:0}$	ND	0.80 ± 0.11	0.91	-0.91	0.73	-0.73
$C_{16:0}$	35.05	64.30 ± 0.90	49.79	-14.74	72.34	-37.29
11c-$C_{16:1}$	0.41	1.11 ± 0.05	0.81	-0.40	0.95	-0.54
$C_{18:0}$	35.96	34.33 ± 0.45	37.67	-1.71	31.97	3.99
9c-$C_{18:1}$	269.27	260.73 ± 3.07	283.31	-14.04	248.73	20.54
9c12c-$C_{18:2}$	620.34	612.95 ± 3.06	615.90	4.44	636.92	-16.58
9c11t-$C_{18:2}$	20.91		ND	20.91	ND	20.91
9c12c15c-$C_{20:3}$	1.31	1.93 ± 0.04	1.41	-0.10	2.03	-0.72
$C_{20:0}$	4.14	2.40 ± 0.07	2.83	1.31	1.91	2.23
11c-$C_{20:1}$	ND		1.72	-1.72	0	0
$C_{22:0}$	12.65	6.70 ± 0.21	11.41	1.24	4.41	8.24
$C_{24:0}$	3.63		3.54	0.09	ND	3.63
Sum of SFA	92.64		106.45	-13.81	111.37	-4.92
Sum of MUFA	269.68		286.04	-16.36	249.68	36.36
Sum of PUFA	642.56		617.31	25.25	638.95	-21.64
Fatty acid shorthand	**5977B[b]**			**5977E[b]**		
	Retention time (min)	**Area**	**Retention time (min)**		**Area**	
$C_{6:0}$	1.4992	87381	1.5055		91982	
$C_{16:0}$	4.0277	701584	4.0716		750947	
$C_{18:1}$	4.9024	733871	4.9467		715453	
$C_{18:2}$	5.1196	450546	5.1606		405774	
4c7c10c13c16c19c-$C_{22:6}$	9.3794	180352	9.4452		169777	
Overall	**Maximum**		**Minimum**			
Retention time (min) %RSD[a]	2.707 for $C_{5:0}$		0.238 for 5c8c11c14c17c-$C_{20:5}$			
Area %RSD[a]	14.101 for 5c8c11c14c-$C_{20:4}$		1.268 for 9c-$C_{18:1}$			
Retention factor (k)	0.62		13.16			
Selectivity (α)	1.84		1.01			
Theoretical plates (N)	77108 $C_{11:0}$		2155 for $C_{24:0}$			
Resolution (R_s)	14.42		0.57 between C21:0 and 8c11c14-$C_{20:3}$			

[a]Analysis based on three individual samples assayed on different days. [b]A sample was analyzed using the same chromatographic conditions indicated above but using a different instrument model, different analyst, and days.

Table 3. Fat analysis and fatty acid profiling of feed ingredients using the proposed method

Fatty acid shorthand	*Mean ± SD*	*Median*	*Maximum*	*Minimum*
	Concentration, g fatty acid/kg fat [a]			
Corn meal (*n* = 35)				
$C_{8:0}$	21.32 ± 44.77	1.85	121.40	0.20
$C_{16:0}$	166.51 ± 45.14	151.00	260.70	87.10
11c-$C_{16:1}$	16.37 ± 22.96	6.00	75.30	3.10
$C_{18:0}$	61.63 ± 48.24	50.30	201.30	5.00
9c-$C_{18:1}$	227.94 ± 123.09	243.10	529.30	5.60
9c12c-$C_{18:2}$	444.07 ± 85.09	463.30	588.30	174.20
11c14c17c-$C_{20:3}$	14.99 ± 13.51	12.00	36.20	1.20
9c12c15c-$C_{18:3}$	106.61 ± 81.70	88.65	343.40	1.10
11c-$C_{18:1}$	156.46 ± 88.41	194.10	253.70	3.00
9t12t-$C_{18:2}$	49.15 ± 46.61	31.90	110.10	4.30
Sum of SFA	172.84 ± 23.78	166.80	222.10	121.70
Sum of MUFA	282.81 ± 45.12	276.00	377.50	196.00
Sum of PUFA	544.59 ± 54.24	546.50	659.30	409.50
Crude fat	39.14 ± 4.16	39.00	45.90	32.90
Soybean meal (*n* = 19)				
$C_{16:0}$	186.82 ± 63.17	169.60	379.20	120.50
$C_{18:0}$	39.37 ± 13.85	37.00	87.10	27.20
9c-$C_{18:1}$	155.94 ± 65.28	157.40	235.90	21.50
9c12c-$C_{18:2}$	504.02 ± 122.97	533.90	643.60	65.80
9c12c15c-$C_{18:3}$	71.66 ± 22.19	75.55	101.30	23.30
11c14c17c-$C_{20:3}$	52.28 ± 23.67	52.60	79.50	10.10
Sum of SFA	209.09 ± 29.22	201.05	269.10	171.90
Sum of MUFA	197.71 ± 40.37	202.45	256.40	130.10
Sum of PUFA	593.81 ± 34.19	588.70	663.30	518.00
Crude fat	16.94 ± 4.18	16.95	23.30	8.40
Inca Peanut meal/*Plukenetia volubilis* L. (*n* = 7)				
$C_{5:0}$	16.25 ± 13.25	16.25	29.50	3.00
$C_{9:0}$	16.20 ± 6.18	15.00	24.30	9.30
$C_{10:0}$	8.93 ± 10.87	1.30	24.30	1.20
$C_{12:0}$	3.00 ± 0.40	3.00	3.40	2.60
$C_{14:0}$	10.46 ± 8.01	12.80	20.40	1.00
$C_{16:0}$	119.78 ± 55.02	117.55	194.40	54.40
$C_{18:0}$	55.70 ± 17.52	60.60	74.10	31.70
9c-$C_{18:1}$	212.15 ± 123.82	187.50	450.00	98.00
9c12c-$C_{18:2}$	242.73 ± 100.26	291.65	326.90	32.20
9c12c15c-$C_{18:3}$	256.57 ± 126.94	271.60	413.80	27.80
9c11t-$C_{18:2}$	7.47 ± 3.25	6.30	11.90	4.20
11c14c-$C_{20:2}$	59.40 ± 14.58	59.90	77.00	41.30
Sum of SFA	222.98 ± 63.93	236.30	303.20	125.70
Sum of MUFA	113.38 ± 28.81	101.40	169.90	81.20
Sum of PUFA	663.33 ± 77.56	672.00	776.40	527.00
Crude fat	451.53 ± 89.90	504.60	524.50	325.50

[a]Only fatty acids with > 1 g/100 g concentration are shown.

Table 4. Fat analysis and fatty acid profiling of fats and oils using the proposed method

Fatty acid shorthand	*Mean ± SD*	*Median*	*Maximum*	*Minimum*
	Concentration, g fatty acid/kg fat[a]			
	Animal fat (*n* = 8)			
$C_{14:0}$	15.55 ± 1.45	15.55	17.00	14.10
$C_{16:0}$	345.85 ± 43.95	345.85	389.80	301.90
$C_{17:0}$	13.75 ± 3.25	13.75	17.00	10.50
$C_{18:0}$	83.35 ± 18.15	83.35	101.50	65.20
9c-$C_{18:1}$	415.50 ± 15.30	415.50	430.80	400.20
9c12c-$C_{18:2}$	100.45 ± 17.65	100.45	118.10	82.80
Sum of SFA	465.60 ± 11.50	465.60	477.10	454.10
Sum of MUFA	430.90 ± 9.10	430.90	440.00	421.80
Sum of PUFA	103.50 ± 20.70	103.50	124.20	82.80
Crude fat	875.47 ± 40.82	846.63	933.27	846.62
Free fatty acids (as oleic acid)	15.55 ± 10.49	11.70	33.20	5.60
	Palm oil (*n* = 8)			
$C_{12:0}$	12.10 ± 1.30	12.10	13.40	10.80
$C_{14:0}$	32.63 ± 18.11	26.60	57.20	14.10
$C_{16:0}$	423.00 ± 39.12	413.55	480.60	384.30
$C_{18:0}$	67.50 ± 13.12	65.20	84.60	52.70
9c-$C_{18:1}$	383.50 ± 66.42	380.55	465.70	307.20
9c12c-$C_{18:2}$	71.85 ± 29.73	84.15	97.80	21.30
9c12c15c-$C_{18:3}$	60.40 ± 9.30	60.40	69.70	51.10
9c11t-$C_{18:2}$	40.60 ± 10.50	40.60	51.10	30.10
Sum of SFA	436.83 ± 25.78	432.10	477.10	406.00
Sum of MUFA	460.93 ± 29.81	452.85	507.50	430.50
Sum of PUFA	102.23 ± 19.87	96.70	132.70	82.80
Crude fat	966.03 ± 26.38	967.12	997.82	933.25
Free fatty acids (as oleic acid)	2.09 ± 0.61	1.97	3.28	1.47
	By-pass fat (*n* = 8)			
$C_{14:0}$	24.40 ± 3.16	25.50	27.60	20.10
$C_{16:0}$	276.60 ± 0.00	276.60	276.60	276.60
11c-$C_{16:0}$	34.30 ± 0.00	34.30	34.30	34.30
$C_{17:0}$	9.50 ± 0.60	9.50	10.10	8.90
$C_{18:0}$	184.27 ± 11.13	189.00	194.90	168.90
9c-$C_{18:0}$	450.93 ± 57.83	456.70	518.70	377.40
9t12t-$C_{18:2}$	14.67 ± 6.19	10.90	23.40	9.70
9c12c-$C_{18:2}$	45.47 ± 8.90	45.60	56.30	34.50
9c12c15c-$C_{18:3}$	14.10 ± 5.00	14.10	19.10	9.10
Sum of SFA	454.55 ± 55.95	454.55	510.50	398.60
Sum of MUFA	483.95 ± 72.25	483.95	556.20	411.70
Sum of PUFA	58.20 ± 21.10	58.20	79.30	37.10
Crude fat	792.27 ± 125.58	845.60	912.30	618.90
Free fatty acids (as oleic acid)	0.48 ± 0.42	0.27	1.20	0.17

[a]Only fatty acids with > 1 g/100 g concentration are shown.

Table 5. Fat analysis and fatty acid profiling of compound feed using the proposed method

Fatty acid shorthand	*Mean ± SD*	*Median*	*Maximum*	*Minimum*
	Concentration, g fatty acid/kg fat[a]			
Layer hen feed (*n* = 10)				
$C_{16:0}$	170.28 ± 17.79	169.80	206.10	143.50
9c-$C_{16:1}$	14.35 ± 8.53	14.55	32.50	2.90
$C_{18:0}$	57.39 ± 18.95	54.00	94.80	29.80
9c-$C_{18:1}$	314.83 ± 30.41	310.30	376.50	273.70
9c12c-$C_{18:2}$	369.48 ± 55.00	348.20	509.20	307.70
9c12c15c-$C_{18:3}$	20.46 ± 6.80	21.15	28.10	9.30
9c11t-$C_{18:2}$	26.39 ± 13.08	27.35	49.90	10.60
10t12c-$C_{18:2}$	28.70 ± 5.06	29.60	37.20	22.20
Sum of SFA	235.41 ± 34.30	233.30	296.30	189.80
Sum of MUFA	327.59 ± 32.94	327.00	386.90	290.10
Sum of PUFA	433.44 ± 43.21	429.00	518.50	371.50
Crude fat	45.99 ± 8.56	44.20	68.00	37.30
Beef cattle feed (*n* = 8)				
$C_{6:0}$	11.37 ± 0.87	11.60	12.30	10.20
$C_{8:0}$	18.40 ± 6.28	16.10	28.90	12.50
$C_{10:0}$	21.35 ± 6.86	22.20	28.10	12.90
$C_{12:0}$	21.93 ± 8.88	19.45	36.00	9.80
$C_{14:0}$	12.72 ± 2.66	12.80	16.20	9.00
$C_{16:0}$	230.36 ± 20.99	234.40	261.10	206.80
9c-$C_{16:1}$	8.13 ± 3.25	8.70	11.80	3.90
$C_{18:0}$	39.97 ± 14.27	45.60	60.90	20.10
9c-$C_{18:1}$	387.70 ± 56.57	407.00	464.50	269.80
9c12c-$C_{18:2}$	98.97 ± 50.17	95.95	162.70	23.90
9c11t-$C_{18:2}$	105.22 ± 12.27	107.10	121.50	88.60
10t12c-$C_{18:2}$	48.28 ± 24.99	49.50	75.90	6.90
Sum of SFA	430.26 ± 134.42	388.10	666.30	274.30
Sum of MUFA	358.09 ± 140.84	389.90	591.70	180.10
Sum of PUFA	211.41 ± 54.37	225.50	282.70	133.80
Crude fat	93.22 ± 30.83	85.60	142.40	59.31
Dairy cattle feed (*n* = 10)				
$C_{8:0}$	19.30 ± 7.06	20.30	27.40	10.20
$C_{12:0}$	23.50 ± 26.16	11.45	67.90	3.20
$C_{14:0}$	13.10 ± 7.17	12.20	22.30	4.80
$C_{16:0}$	166.14 ± 13.17	161.30	191.20	154.50
$C_{18:0}$	30.60 ± 8.33	28.70	46.30	22.20
9c-$C_{18:1}$	335.18 ± 29.47	334.60	372.60	294.80
9c12c-$C_{18:2}$	369.20 ± 26.06	364.80	404.00	333.50
11c14c-$C_{20:2}$	47.72 ± 8.44	43.90	63.70	40.30
9c11t-$C_{18:2}$	24.17 ± 2.94	23.00	28.20	21.30
Sum of SFA	229.94 ± 39.95	212.80	305.60	190.00
Sum of MUFA	335.18 ± 29.47	334.60	372.60	294.80
Sum of PUFA	434.90 ± 34.42	414.60	477.80	399.60
Crude fat	47.21 ± 13.41	48.00	67.30	22.10

[a]Only fatty acids with > 1 g/100 g concentration are shown.

Table 6. Fat analysis and fatty acid profiling of food commodities using the proposed method

Fatty acid shorthand	*Mean ± SD*	*Median*	*Maximum*	*Minimum*
	Concentration, g fatty acid/kg fat[a]			
Chicken eggs (*n* = 11)				
Benzoic acid	15.17 ± 5.82	14.00	22.80	8.70
2-Me-$C_{4:0}$	8.70 ± 2.20	8.70	10.90	6.50
$C_{16:0}$	254.27 ± 19.68	249.10	297.30	228.50
9c-$C_{16:1}$	40.99 ± 6.13	41.90	48.60	33.00
$C_{18:0}$	140.87 ± 79.06	111.75	264.70	57.40
11c-$C_{18:1}$	16.60 ± 0.00	16.60	16.60	16.60
9c-$C_{18:1}$	363.20 ± 96.37	406.00	470.10	194.30
9c12c-$C_{18:2}$	158.34 ± 57.95	141.10	330.00	90.90
9c12c15c-$C_{18:3}$	7.43 ± 4.70	10.40	11.10	0.80
11c14c-$C_{20:2}$	12.86 ± 5.73	14.90	18.00	2.50
11c14c17c-$C_{20:3}$	59.10 ± 0.00	59.10	59.10	59.10
10t12c-$C_{18:2}$	16.55 ± 6.55	16.55	23.10	10.00
$C_{20:0}$	11.15 ± 9.15	11.15	20.30	2.00
9t11c-$C_{18:2}$	11.80 ± 6.80	11.80	18.60	5.00
5c8c11c14c-$C_{20:4}$	3,06 ± 0.49	2.80	3.80	2.50
Sum of SFA	407.26 ± 80.64	379.30	550.50	313.60
Sum of MUFA	427.25 ± 79.37	448.20	517.00	273.50
Sum of PUFA	165.66 ± 28.29	167.50	210.30	120.30
Total fat content	91.73 ± 16.13	83.60	125.60	70.30
Bovine meat tissue (*n* = 35)				
$C_{7:0}$	11.43 ± 9.77	5.20	35.10	1.70
$C_{8:0}$	6.79 ± 3.12	6.10	12.20	1.60
$C_{9:0}$	8.05 ± 3.55	6.45	15.20	3.80
$C_{10:0}$	8.94 ± 5.51	7.60	18.60	2.20
$C_{14:0}$	57.13 ± 35.05	43.85	163.10	12.40
$C_{15:0}$	9.39 ± 6.91	8.05	38.60	3.10
11c-$C_{16:1}$	32.84 ± 16.35	26.60	73.60	15.80
$C_{16:0}$	309.14 ± 75.85	309.65	607.30	48.40
9c-$C_{12:1}$	19.42 ± 27.55	11.40	113.30	5.30
9c-$C_{16:1}$	27.92 ± 19.49	24.70	73.50	3.00
$C_{17:0}$	13.80 ± 8.46	10.10	35.90	5.90
10c-$C_{17:1}$	8.77 ± 2.17	8.80	11.90	4.80
$C_{18:0}$	133.04 ± 41.75	131.70	211.40	45.10
9c-$C_{18:1}$	405.31 ± 70.22	394.10	609.20	269.70
9c11t-$C_{18:2}$	10.45 ± 4.33	9.60	19.50	5.80
9c12c-$C_{18:2}$	27.06 ± 30.14	13.85	116.80	3.00
11c14c-$C_{20:2}$	16.53 ± 6.54	16.00	24.80	8.80
Sum of SFA	526.41 ± 74.06	524.35	678.80	376.10
Sum of MUFA	437.61 ± 78.87	412.60	615.90	275.40
Sum of PUFA	33.43 ± 32.22	24.80	118.60	0.00
Total fat content	308.90 ± 66.07	310.55	486.80	158.00
Water buffalo (*Bubalus bubalis*) meat tissue (*n* = 11)				
$C_{14:0}$	35.58 ± 10.95	33.40	57.70	22.30
$C_{15:0}$	13.58 ± 4.09	12.20	20.80	8.00
15Me-$C_{16:0}$	7.30 ± 5.09	3.80	14.50	3.60
11Me-$C_{16:0}$	27.47 ± 5.41	24.10	35.10	23.20
$C_{16:0}$	270.87 ± 44.58	248.50	355.10	221.70
$C_{17:0}$	27.53 ± 6.27	30.20	35.80	14.10
9c-$C_{16:1}$	27.48 ± 8.29	23.70	44.90	18.60
14Me-$C_{16:0}$	8.68 ± 3.25	7.50	13.90	5.80
$C_{18:0}$	248.55 ± 67.47	246.10	378.10	148.40

9c-$C_{18:1}$	326.78 ± 45.84	337.75	393.90	253.50
14c17c-$C_{18:2}$	7.87 ± 1.86	7.20	10.40	6.00
9c11t-$C_{18:2}$	13.41 ± 2.25	13.50	16.80	10.70
9c12c-$C_{18:2}$	15.74 ± 10.90	14.30	36.10	4.00
Sum of SFA	636.26 ± 99.44	618.70	933.70	554.20
Sum of MUFA	335.81 ± 97.99	359.00	423.60	48.70
Sum of PUFA	27.88 ± 8.47	25.00	46.50	17.30
Total fat content	356.00 ± 130.40	332.10	548.20	106.10
Lamb meat tissue (*n* = 11)				
$C_{14:0}$	30.46 ± 5.76	32.00	3.90	1.82
$C_{15:0}$	6.34 ± 2.78	5.10	1.08	0.36
$C_{16:0}$	250.74 ± 24.62	259.80	26.99	18.3
9c-$C_{16:1}$	17.18 ± 5.70	17.45	2.62	0.48
$C_{17:0}$	16.39 ± 6.30	15.60	3.10	0.42
10c-$C_{17:1}$	8.80 ± 5.59	4.90	1.67	0.48
$C_{18:0}$	336.47 ± 54.99	360.40	39.69	23.09
9c-$C_{18:1}$	291.35 ± 60.70	266.90	40.08	21.97
9c11t-$C_{18:2}$	9.30 ± 3.90	9.50	1.36	0.12
10t12c-$C_{18:2}$	3.00 ± 0.10	3.00	0.31	0.29
Sum of SFA	523.30 ± 53.59	523.30	603.70	386.50
Sum of MUFA	462.81 ± 55.65	471.00	598.40	369.20
Sum of PUFA	16.10 ± 9.52	15.80	30.10	1.40
Total fat content	301.50 ± 110.45	355.60	406.10	131.40
Raw bovine milk (*n* = 12)				
$C_{6:0}$	20.39 ± 4.78	20.25	30.80	12.30
$C_{8:0}$	17.72 ± 7.44	18.00	36.40	7.50
$C_{10:0}$	34.41 ± 13.27	32.40	65.20	15.10
$C_{12:0}$	40.43 ± 14.04	36.95	77.70	25.80
$C_{14:0}$	116.68 ± 27.16	113.80	173.00	75.20
$C_{15:0}$	11.90 ± 1.35	12.70	13.00	10.00
$C_{16:0}$	268.02 ± 25.10	270.50	315.80	218.50
$C_{16:1}$	45.19 ± 78.44	13.30	277.40	5.00
$C_{17:0}$	18.00 ± 0.50	18.00	18.50	17.50
$C_{17:1}$	12.81 ± 6.32	10.80	26.80	3.40
$C_{18:0}$	11.12 ± 7.94	13.10	26.80	1.30
9c-$C_{18:1}$	259.81 ± 58.22	267.80	359.40	158.40
6c-$C_{18:1}$	134.77 ± 75.42	109.90	291.40	55.50
9c12c-$C_{18:2}$	16.10 ± 4.86	16.00	22.10	10.20
Sum of SFA	792.83 ± 84.52	822.70	906.30	626.80
Sum of MUFA	159.77 ± 78.74	133.70	314.60	48.50
Sum of PUFA	47.46 ± 19.92	41.20	81.60	17.70
Total fat content	32.93 ± 5.30	32.70	49.40	23.70

[a]Only fatty acids with > 1 g/100 g concentration are shown.

Table 7. Fatty acid profile and water activity for commercially available pet foods

Fatty acid shorthand	*Mean ± SD*	*Median*	*Maximum*	*Minimum*
	Concentration, g fatty acid/kg fat[a]			
Wet dog food (*n* = 8)				
$C_{14:0}$	14.93 ± 4.60	13.40	22.50	10.40
$C_{16:0}$	259.12 ± 34.90	253.20	327.80	212.40
$C_{16:1}$	45.95 ± 9.44	41.95	62.00	37.90
$C_{18:0}$	124.62 ± 37.36	104.65	188.80	90.30
9c-$C_{18:1}$	376.10 ± 35.48	394.55	403.90	301.90
9c12c-$C_{18:2}$	143.13 ± 31.37	140.00	199.80	103.40
11c14c-$C_{18:2}$	16.50 ± 5.78	15.20	27.50	11.60
5c8c11c14c17c-$C_{20:5}$	4.25 ± 0.72	4.15	5.30	3.40
4c7c10c13c16c19c-$C_{22:6}$	1.85 ± 0.93	1.80	3.20	0.60
Sum of SFA	386.77 ± 85.88	369.35	494.70	246.60
Sum of MUFA	382.02 ± 91.06	413.50	483.30	203.40
Sum of PUFA	175.45 ± 37.78	190.10	216.70	115.70
Dry dog food (*n* = 20)				
$C_{2:0}$-diacid	16.33 ± 10.66	13.10	33.80	1.40
$C_{3:0}$	11.90 ± 12.60	5.50	33.20	0.50
$C_{4:0}$-diacid	15.18 ± 11.31	10.65	36.60	1.80
$C_{5:0}$	3.68 ± 4.52	1.90	15.50	1.10
$C_{12:0}$	20.77 ± 14.82	15.80	46.70	5.90
$C_{14:0}$	15.70 ± 8.61	15.55	29.30	2.20
$C_{16:0}$	359.10 ± 94.31	341.00	547.00	224.90
9c-$C_{16:1}$	12.63 ± 1.62	13.30	14.20	10.40
$C_{18:0}$	76.24 ± 37.49	76.50	169.80	22.40
9c-$C_{18:1}$	283.57 ± 45.81	291.30	352.20	192.70
9c12c-$C_{18:2}$	194.88 ± 54.65	186.70	328.00	122.50
9c12c15c-$C_{18:3}$	7.43 ± 2.19	7.65	10.10	4.30
11c14c17c-$C_{20:3}$	8.98 ± 4.05	10.45	12.80	2.20
Sum of SFA	526.79 ± 151.31	545.90	810.60	290.70
Sum of MUFA	317.74 ± 84.48	315.35	495.30	169.70
Sum of PUFA	167.40 ± 90.98	207.40	345.30	19.60
Crude fat	125.20 ± 15.54	125.90	155.60	106.00
a_w	0.5356 ± 0.0961	0.5492	0.6790	0.3720
Dry puppy food (*n* = 12)				
$C_{2:0}$-diacid	20.27 ± 6.79	20.95	28.70	10.40
$C_{3:0}$	14.46 ± 9.25	12.30	29.30	2.00
$C_{4:0}$-diacid	35.06 ± 33.70	24.20	109.40	7.40
2-Me-$C_{6:0}$	14.23 ± 10.47	10.30	35.50	5.10
2-Me-$C_{5:0}$	45.97 ± 47.34	26.40	111.20	0.30
$C_{8:0}$	10.24 ± 6.35	12.50	18.60	2.80
$C_{11:0}$	16.90 ± 8.07	17.30	27.90	5.10
9c-$C_{12:1}$	28.10 ± 14.60	19.00	48.70	16.60
$C_{14:0}$	19.95 ± 2.79	20.90	22.60	15.40
$C_{16:0}$	378.27 ± 89.09	410.00	511.20	257.00
$C_{18:0}$	78.11 ± 23.37	81.80	105.60	40.00
9c-$C_{18:1}$	212.61 ± 37.89	206.80	309.90	173.70
9c12c-$C_{18:2}$	112.99 ± 32.99	115.10	173.80	48.70
13t-$C_{18:1}$	214.90 ± 46.30	214.90	261.20	168.60
Sum of SFA	590.88 ± 122.96	568.80	813.10	348.90
Sum of MUFA	291.30 ± 61.62	276.70	396.90	183.00
Sum of PUFA	132.51 ± 80.36	116.30	254.10	3.90
Crude fat	132.35 ± 14.14	126.85	153.70	116.80
a_w	0.5837 ± 0.0682	0.5966	0.6694	0.4339

Wet cat food (n = 6)				
6c-$C_{16:1}$	35.18 ± 2.96	34.95	38.70	32.10
2Me-$C_{4:0}$	8.08 ± 2.66	8.00	11.20	5.10
$C_{16:0}$	257.40 ± 81.62	248.50	379.70	152.90
$C_{18:0}$	151.08 ± 25.96	154.20	179.70	116.20
9c-$C_{18:1}$	372.50 ± 57.03	393.90	425.30	276.90
9c12c-$C_{18:2}$	172.88 ± 5.53	172.60	180.80	165.50
9c11t-$C_{18:2}$	58.35 ± 3.65	60.00	61.30	52.10
11c14c- $C_{20:2}$	64.50 ± 6.50	63.60	74.50	56.30
11c14c17c- $C_{20:3}$	44.15 ± 8.24	41.30	57.90	36.10
Sum of SFA	384.75 ± 105.39	359.95	551.80	267.30
Sum of MUFA	390.15 ± 51.15	404.80	441.70	309.30
Sum of PUFA	225.10 ± 130.89	206.55	423.40	63.90
Dry cat food (n = 8)				
$C_{2:0}$-diacid	19.48 ± 18.09	11.70	48.50	0.80
$C_{3:0}$-diacid	12.68 ± 8.51	12.85	24.40	0.80
$C_{4:0}$-diacid	17.51 ± 9.54	19.80	31.70	3.40
$C_{4:0}$	8.58 ± 7.66	8.10	17.90	0.20
2-Me-$C_{10:0}$	13.30 ± 4.36	12.20	19.10	8.60
$C_{11:0}$	8.50 ± 1.90	8.50	10.40	6.60
$C_{14:0}$	48.10 ± 16.78	47.45	71.30	26.20
$C_{16:0}$	368.38 ± 61.73	352.30	460.60	298.00
9c-$C_{16:1}$	29.10 ± 7.35	26.90	39.00	21.40
$C_{18:0}$	97.33 ± 14.70	106.40	109.00	76.60
9c-$C_{16:1}$	192.13 ± 89.01	212.95	289.50	36.40
9c-$C_{16:1}$	128.70 ± 67.41	128.70	227.00	44.70
Sum of SFA	560.92 ± 147.65	559.40	842.90	391.80
Sum of MUFA	287.85 ± 119.73	326.40	387.80	42.40
Sum of PUFA	151.35 ± 53.10	137.30	236.30	78.90
Crude fat	126.02 ± 15.67	129.40	144.30	107.40
a_w	0.5477 ± 0.0505	0.5332	0.6369	0.4987

[a]Only fatty acids with > 1 g/100 g concentration are shown.

Table 8. Fatty acid analysis for multi-species forages found along with dairy cattle farms in Costa Rica

	***Botanical sample*[a] (*Composition. g/100 g*)**			***Major components*[b] (*Concentration. g/kg*)**			
Sample 1	Ratana (57)	Tanner (18)	Brachiaria (14)	$C_{5:0}$ (118.02)	$C_{14:0}$ (145.06)	$C_{16:0}$ (437.44)	13c-$C_{18:1}$ (278.23)
Sample 2	Tanner (53)	Ratana (36)	Tropical kudzu (4)	$C_{14:0}$ (111.21)	$C_{16:0}$ (580.08)	$C_{18:0}$ (124.23)	9c-$C_{18:1}$ (141.65)
Sample 3	Ratana (44)	Aleman (33)	Tanner (19)	9:0-diacid (118.02)	$C_{14:0}$ (125.90)	$C_{16:0}$ (465.73)	13c-$C_{18:1}$ (183.31)
Sample 4	Ratana (42)	Other grasses (31)	Tanner (21)	2-Me-$C_{10:0}$ (13.21)	12-Me-$C_{13:0}$ (199.71)	$C_{16:0}$ (460.61)	9c-$C_{18:1}$ (292.52)
Sample 5	Other grasses (44)	Aleman (27)	Tanner (22)	2-Me-$C_{14:0}$ (113.40)	$C_{16:0}$ (379.41)	9c-$C_{18:1}$ (136.45)	11c14c17c-C20:3 (164.87)
Sample 6	Guinea (50)	Other grasses (32)	Poró (13)	$C_{14:0}$ (133.31)	$C_{16:0}$ (307.02)	9c-$C_{18:1}$ (189.01)	11c14c17c-$C_{20:3}$ (160.06)
Sample 7	Guinea (40)	Ratana (37)	Other grasses (17)	$C_{16:0}$ (418.45)	$C_{18:0}$ (199.71)	13c-$C_{18:1}$ (262.58)	11c14c17c-$C_{20:3}$ (107.81)
Sample 8	Other grasses (53)	Tanner (31)	Guinea (11)	$C_{14:0}$ (154.56)	$C_{16:0}$ (313.55)	$C_{18:0}$ (211.02)	$C_{18:1}$ (222.44)
Sample 9	Tanner (53)	Other grasses (33)	Aleman (9)	$C_{14:0}$ (62.38)	$C_{16:0}$ (452.40)	13c-$C_{18:1}$ (202.12)	11c14c17c-$C_{20:3}$ (114.93)
Sample 10	Other grasses (51)	Tanner (21)	Aleman (10)	$C_{14:0}$ (147.25)	$C_{16:0}$ (478.00)	$C_{18:0}$ (152.37)	14c-$C_{18:1}$ (164.78)
Sample 11	Guinea (69)	Ratana (21)	Other grasses (8)	$C_{16:0}$ (332.66)	$C_{18:0}$ (202.21)	9c-$C_{18:1}$ (173.58)	11c14c17c-$C_{20:3}$ (173.18)
Sample 12	Tanner (44)	Other grasses (33)	Ratana (21)	$C_{16:0}$ (435.36)	$C_{18:0}$ (185.50)	9c-$C_{18:1}$ (194.20)	11c14c17c-$C_{20:3}$ (94.51)

Sample	1	2	3	4	5	6	7	8	9	10	11	12
						Concentration. g/kg)						
Sum SFA	700.52	815.52	709.65	673.53	492.81	440.33	618.16	679.13	514.78	777.62	534.87	620.86
Sum MUFA	278.23	141.65	183.31	292.52	136.45	189.01	262.58	222.44	202.12	164.78	173.58	194.2
Sum PUFA	21.25	42.83	107.04	33.93	370.74	375.47	176.32	98.43	333.04	57.6	283.24	255.3

[a]Para: *Brachiaria mutica* (Forssk.) Stapf. Tanner: *Brachiaria arrecta* (Hack. ex T. Durand & Schinz) Stent. Ratana: *Ischaemum indicum* Houtt.. Tropical kudzu: *Pueraria phaseoloides* (Roxb.) Benth. Guinea: *Megathyrsus maximus* (Jacq.) B.K. Simon & S.W.L. Jacobs. Aleman: *Echinochloa polystachya* (Kunth) Hitchc.
[b]Represented the four most abundant acids found. other fatty acids include 2-Me-$C_{5:0}$. 2-Me-$C_{6:0}$. 2-Me-$C_{7:0}$. 6-Me-$C_{7:0}$. 2-Me-$C_{8:0}$. $C_{10:0}$. $C_{11:0}$

Soybean meal inputs, nutritionally, 1.05 – 1.83 g crude fat, 0.68 g 9c12c-$C_{18:2}$, and 0.09 g 9c12c15c-$C_{18:3}$ per 100 g material (Sauvant *et al.,* 2004). Values consistent with our data (504.02 g kg^{-1} fat, 0.85 g/100 g soybean meal and 71.66 g kg^{-1} fat, 0.12 g/100 g soybean meal, respectively). Additionally, energy-wise, it can still impart 2 120 kcal metabolizable energy kg^{-1}. Notwithstanding, the primary dietary input of soybean meal lies in amino acids (e.g., 2.74 – 2.91 g lysin/100 g) (Sauvant *et al.,* 2004; de Blass *et al.,* 2010; Rostagno *et al.,* 2017). Crude fat and fatty acids obtained for the Inca peanut meal are consistent with those reported elsewhere (Pereira de Souza *et al.,* 2013). Beneficial impacts on supplementing fats and oils are based on their high-energy coefficients, their nitrogen-keeping effect in a body, positive influence on metabolism regulation and accumulation of vitamins in tissues (Janovych and Lagodyuk, 1991). For animal-derived fats, inclusion rates vary from 3 to 7 g/100 g in layer hens and up to 3 g/100 g in ruminants (NRC, 2001; de Blass *et al.,* 2010; Rostagno *et al.,* 2017).

These ingredients have a high SFA and MUFA ratio. As a result, fats usually have compact buildings; several types of fats are available for use in food, pet foods, and feed applications (Sharma *et al.,* 2013). Poultry, swine, and bovine-derived fat inputs 8 681, 8 080, and 7 401 kcal metabolizable energy kg^{-1}. Additionally, contributes 20.5, 9.2 to 9.63 and 3.1 g 9c12c-$C_{18:2}$/100 g fat (Chilliard *et al.,* 2001; de Blass *et al.,* 2010; Rostagno *et al.,* 2017). Our mean values of 9c12c-$C_{18:2}$ round up to 8.79 g/100 g fat. On the other hand, vegetable oils with high diversity in fatty acids are preferred. In this regard, palm oil has a low peroxidizability and is a good source of $C_{16:0}$, 9c-$C_{18:1}$ and 9c12c-$C_{18:2}$ (39.2, 44.0, and 10.0 g/100 g, respectively) (Kerr *et al.,* 2015). Our data concur with values reported elsewhere with 9c-$C_{18:1}$ and 9c12c-$C_{18:2}$ mean values at 37.04 (383.50 g kg^{-1} fat) and 10.86 g/100 g (112.45 g kg^{-1} fat) (Sauvant *et al.,* 2004). Finally, by-pass fat (usually calcium salts from fatty acids) are not retained in the rumen (and even can evade reticulum, omasum, and abomasum) (Chilliard *et al.,* 2001). Saponified fats derived from palm oil and tallow report mean values of 4.1 and 42.7 g $C_{18:0}$/100 g by-pass fat, respectively (NRC, 2001; de Blass *et al.,* 2010). Our data show by-pass fats with a high content of stearic acid (i.e., 35.71 g/100 g by-pass fat, 450.93 g kg^{-1} fat) which hints toward an origin from palm oil.

Incorporation of long-chain fatty acids such as $C_{16:0}$ and $C_{18:0}$, found in palm fats, greatly influence lactation efficiency (Paintoni *et al.*, 2015; Boerman *et al.*, 2017). Also, the application of supplements (as high as 4 g/100 g inclusion) (FEDNA, 2009; de Blass *et al.,* 2010) of protected fats and polyenoic fatty acids of different age and productive groups of cattle demonstrates positive metabolic and productive effects (Pavkovych *et al.,* 2015). Less palatable (e.g., low fat) foods, may result in rejection by animals (an effect mainly observed in pets). Finally, free fatty acids in fats and oils are a measurement of hydrolysis due to storage or processing (Mahesar *et al.,* 2014). These compounds are less stable than neutral oil and, thus, more prone to oxidation and rancidity (Mahesar *et* *al.,* 2014). Data found herein are well in line with maximum thresholds for fats and oils (i.e., 4-8 g/100 g expressed as oleic acid) (Baião and Lara, 2005; Azeman *et al.,* 2015).

Compound feeds

In the case of dairy and beef cattle feeds, dietary input has the primary purpose of providing the substrate for the ruminal microbiota. In turn, the type of substrate modifies the rumen itself and its fermentation characteristics (Duarte *et al.,* 2017). Microorganisms such as bacteria, fungi, and protozoa can degrade complex structures (e.g., forages) and free utilizable nutrients (NRC, 2001; FEDNA, 2009; Duarte *et al.,* 2017). After rumen-mediated lipolysis (where metabolisms of long-chained fatty acids and hydrogenation of unsaturated fatty acids take place), fat biohydrogenation takes place, a process responsible for the concentration and proportion of fatty acids in tissue and milk (Woods and Fearon, 2009; Castillo *et al.,* 2013). As the choice of animal feeding system influences animal products (Schmitt et al., 2018), fatty acid-rich feed ingredients are also included in ruminant diets to ensure biotransference to meat and milk which, in turn, have demonstrated to an extent to improve public health (Givens, 2015; Schmitt *et al.,* 2018). In ruminants, nutritional requirements are based on weight, age, stage of production, physiological stage, as well as physical activity. However, no minimum thresholds have been set for fatty acids (NRC, 2001; FEDNA, 2009). High-throughput dairy production has a high demand for energy (needed net energy for lactation 1.8 Mcal kg^{-1}) (NRC, 2001). Hence, feed (especially in 9c-$C_{18:1}$, 9c12c-$C_{18:2,}$ and 9c12c15c-$C_{18:3}$) are based mostly on vegetable ingredients to obtain a fatty acid-rich balanced formulation. Dairy feed analyzed herein obtained 434.90 ± 34.42 g PUFA kg^{-1} fat and 369.20 ± 26.06 g 9c12c-$C_{18:2}$ kg^{-1} fat.

In poultry breeding, especially the laying hens, their production phase requires a minimum contribution of fat of 2.5 g/100 g, which also includes 1.35 g/100 g of linoleic acid with the primary objective of increasing the egg size and production (NRC, 2006; FEDNA, 2008). In contrast, broilers require a lower contribution of linoleic acid (1.0 g/100 g) (FEDNA, 2008; Rostagno *et al.,* 2017). The above data match that obtained during our trial (i.e., 1.7 g linoleic acid/100 g; 369.48 g/kg of fat).

Pet foods

Dogs are considered facultative carnivores. As their diet is supplemented with carbohydrate sources (up to 50% input), fatty acid biosynthesis in the animal intestine increases (NRC, 2006; FEDIAF, 2016). In contrast, cats, despite being strictly carnivorous, require an essential input of 5c8c11c14c-$C_{20:4}$ (NRC, 2006). Additionally, pets need, mandatorily, feed ingredients which input 9c12c-$C_{18:2}$ (as they are not able to synthesize it from linolenic acid) (Biagi *et al.,* 2004; NRC, 2006). During their development, a minimal input of 9c12c-$C_{18:2,}$ 9c12c15c-$C_{18:3}$, 5c8c11c14c-$C_{20:4}$, 5c8c11c14c17c-$C_{20:5}$, and 4c7c10c13c16c19c-$C_{22:6}$ is required (Biagi *et al.,* 2004; NRC, 2006). Values obtained for the above mentioned fatty acids are below 1.00 g kg^{-1} fat in the dry pet foods analyzed, an expected result as only fish-based pet foods are usually rich in 5c8c11c14c17c-$C_{20:5}$ and 4c7c10c13c16c19c-$C_{22:6}$ (Biagi *et al.,* 2004). Deficiencies in omega-3 and -6 generate sight and learning issues as it concentrates in the brain and retina during gestation and subsequent development (NRC, 2006; Fraeye *et al.,* 2012; Cherian, 2017). After 6 to 8 weeks of birth, pups start feeding on compound feed, which must provide twice as much maintenance energy (FEDIAF, 2016). However, when compared, adult dog food and puppy food show similar mean values in crude fat. Hence, the difference in energy requirement, mentioned above, is not being satisfied with fats.

Water activity in pet foods

AAFCO check sample dry cat food 2018-25 sets a 0.444 ± 0.031 as robust mean and standard deviation. Experimental data for the same sample was 0.4373 and a *z* value of -0.22. Water activity obtained for a dry extruded adult dog, puppy, and cat foods were 0.5356 ± 0.0961, 0.5837 ± 0.0682, and 0.5477 ± 0.0505, respectively. These values are relatively higher than those reported elsewhere for the cat (0.30-0.50) and dog food (0.30-0.54) (Baser and Yalçin, 2017). However, according to international guidelines, these values still rank local feeds as low-moisture animal food (US FDA, 2018). Also, these values are well below the threshold for bacterial pathogen growth (i.e., $a_w \geq 0.92$) (US FDA, 2018). Increased water activity may have a severe impact on pet food shelf life (US FDA, 2018). However, as a cost management strategy, the Costarican feed industry usually maintains moisture contents between 8 and 10 g/100 g. a_w has demonstrated to be a functional alternative to moisture content analysis (Van der Hoeven-Hangoor *et al.,* 2014) and is related to lipid quality and has proved to influence lipid oxidation (Choe and Oh, 2013) lipid modification (Lee and Parkin, 2001) mycoflora and fumonisin B_1 accumulation (Marín *et al.,* 2001).

Food samples (Feed-related matrices)

The composition of the acids can vary concerning the animal's diet, as such, there are differences between the grazing, and strictly stabled animals are observed (Woods and Fearon, 2009; Cabrera and Saadoun, 2014). In Costa Rica, cattle are grazed and, as such, considerable concentrations of 9c11t-$C_{18:2}$ (which has anti-carcinogenic activity) in meat tissue and bovine milk is observed. $C_{18:0}$ inhibits the activity of $C_{14.0}$ and $C_{16:0}$ since they are responsible for the hearts' health (Cabrera and Saadoun, 2014). Besides, the figures reported in Table 6, on lamb and beef, coincide with other published data (Woods and Faeron, 2009). Though meat tissue is not usually considered a good source of linoleic/linolenic acids (Cabrera and Saadoun, 2014), in meat tissue samples tested, said fatty acids are the predominant PUFA; which are not synthesized by humans and are, therefore, essential (Cabrera and Saadoun, 2014). Additional reports indicate that milk, another by-product of bovine production, has on average 3.5 – 4 g/100 g of fat (NRC, 2001; FEDNA, 2006), which is composed approximately from 50% of fatty acids of chains of 4 to 16 carbons (from acetic acid and butyric acid from ruminal fermentation), while the other 50% is composed of fatty acids of 16 to 18 carbons (from intestinal absorption) (FEDNA, 2006; Castillo *et al.,* 2013). On the other hand, bovine milk is usually characterized by providing a more significant proportion of palmitic acid (on average 28 g/100 g), oleic acid (21.2 g/100 g) and myristic acid (10.8 g/100 g) (Woods and Faeron, 2009; Markiewicz-Kęszycka *et al.,* 2013).

The egg, a high-consumption staple food, has relatively low production costs and a high nutritional value (e.g., crude protein 12.5 g/100 g, energy 150 kcal/100 g, also has all the vitamins, except for vitamin C) (Moreiras *et al.,* 2013; Khan *et al.,* 2015). According to other literature, eggs contain 30 - 35 g/100 g of SFA, while the main MUFA is oleic acid (22 - 26 g/100 g), and palmitic acid (8 - 10 g/100 g) (both on average 42 - 46 g/100 g of MUFA). On the other hand, is considered a food rich in oleic acid (42.7 g/100 g), linoleic acid (17.2 g/100 g), and in PUFA such as docosahexaenoic acid and arachidonic acid (Woods and Faeron, 2009; Khan *et al.,* 2015; Cherian, 2017). All of the above coincides with our data. As a point of interest, in poultry farms it is common to supplement fatty acids in poultry feeds, intending to modify the fatty acid profile in the eggs and in the poultry meat, to transfer the benefits towards human health (Woods and Faeron, 2009; Fraeye *et al.,* 2012; Khan *et al.,* 2015).

Forage blends fatty acid profiling

Costa Rican farming systems dairy cattle are grass-fed; forages are incorporated into full rations that are complemented compound feed. Often, forages are nutritional relevant as they can include PUFA to animal diet (Woods and Faeron, 2009; Glasser *et al.,* 2013). 9c12c15c-$C_{18:3}$ was reported elsewhere (Glasser *et al.,* 2013) as a prominent fatty acid was not found in our survey at significant levels. Though the forages assayed here are considered of relatively low nutritional quality, 9c-$C_{18:1}$ and 11c14c17c-$C_{20:3}$ are among the most abundant in the grass blends which depending on their concentration in the diet of ruminants, can furthermore modify the profile of fatty acids in milk and meat (Castillo *et al.,* 2013).

Conclusions

Fatty acid profiles from economically essential feed ingredients, such as soybean and corn meal, can be used for feed formulation and energy balance. In addition, the use of fat and oils in animal feed contributes to an increase in patability (as in the case of pet food), or to increase the energetic density (use of by-pass fat in bovine feeding).

Possible associations can be drawn from the fatty acids composition found in compound feed and the related matrix obtained from the food-producing animal (e.g., poultry feed vs. eggs). Up to some extent, fatty acid profiles can be useful data to trace the source and origin of feed ingredients. Additionally, it can be used as routine quality control to ensure lipid sources meet specifications and the requirements; to this aim, a high-throughput, and accurate methods, like the one used herein, should be developed. In this regard, FAME profiling should be included within national-wide feed/food monitoring programs, especially for those fatty acids considered as essential.

Compliance with Ethical Standards

Conflict of Interest

Astrid Leiva and Fabio Granados-Chinchilla declare that they have no conflict of interest.

Author Contribution

The contribution of the authors is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethics committee approval

This article does not contain any studies with human or animal subjects. Ethics committee approval is not required.

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Data availability

Not applicable.

Informed Consent

Not applicable.

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Multiple response optimization to determine the suitable solvent for the extract production from defatted grape seed powder: A simplex lattice mixture design approach

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Abstract

In this study, effects of different solvents (ethanol, methanol and water) on bioactive performance of defatted grape seed powder (GSP) were investigated using simplex lattice mixture design approach. Also, multiple response optimization process was applied to determine the best solvent type for the high bioactive GSP extract production. For this purpose, the bioactive compound concentrations and their antioxidant and antiradical properties were characterized and the effect of solvent type on the processing variables was modelled. Total phenolic and flavonoid contents of GSP ranged between 0.31-7.29 mg GAE/g and 44.3-537.4 mg CE/kg sample respectively. In addition to that, DPPH and $ABTS^{\cdot+}$ radical scavenging activity of the samples were in the range of 2.11-80.5% and 0.31-4.08 µg Trolox/ g sample respectively. The effect of solvent type showed a significant effect on all studied bioactive parameters and the best solvent mixture was determined as ethanol (33.84%), methanol (20.17%) and water (45.99%) by the considering the all studied parameters.

Keywords: Grape seed powder, Solvent, Optimization, Bioactivity

Introduction

Grape (*Vitis vinifera* L.) is an important fruit especially the most basic raw material for the wine industry and grape pomace including the skin, seed, stalk etc., is the main processing waste of this fruit. The grape pomace is a reasonable and lucrative raw material for the cosmetic, pharmaceutical and food industries because it is rich in some important bioactive compounds namely fatty acids, tocopherols, proanthocyanidins, sterols, etc. (Demirtaş et al., 2013, Barba et al., 2016). Grapes are one of the mostly cultivated fruits by an approximate annual production of 58 million metric tons (FAO, 1997). Teixeira et al. (2014) reported that the grape seeds ratio in the whole pomace is 38-52% on a dry weight basis. In many researches, antioxidant, anti-inflammatory and antimicrobial characteristics of grape seeds were reported (Oliveira et al., 2013; Sofi et al., 2016; Soto et al., 2015). Saito et al. (1998) reported that the grape seeds are rich in monomeric phenolic compounds such as catechin and epicatechin and these compounds are responsible for the antimutagenic and antiviral performance of the sample. Jayaprakasha et al. (2001) reported that the grape seed is accepted as a dietary supplement because of its health benefits of catechins and procyanidins. It was reported that the most of the bioactive compounds (approximately 75%) existed in the skin and seeds of the fruit (Pinelo et al., 2006). Due to these important biological properties of grape pomace especially seeds, there is an increased interest for the extraction of polyphenols to be used as an antioxidant instead of synthetic antioxidants in foods because of the possible undesirable effects on human health (Jayaprakasha et al., 2003). Bioactivity of grape seed powder (GSP) or another food material is affected by some processing factors such as solid/liquid ratio, extraction time, temperature, type and also extraction solvent. The solvent used for the extraction of polyphenols has a significant effect on the extraction yield and also the final product bioactivity. Many solvents such as methanol, ethanol, acetone, diethyl ether, ethyl acetate (Bonilla, Mayen, Merida, & Medina, 1999; Lafka, Sinanoglou, & Lazos, 2007), and their binary or ternary mixtures of each other or combinations with distilled water have

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been widely used for the extraction of phenolics (Lafka et al., 2007). Downey and Hanlin (2010) reported that the tannins yield changed with the type of solvent (acetone or ethanol) or the percentage of water in the mixtures significantly. Similarly, Bosso et al. (2016) studied the effects of some solvents (water, ethanol, acetone, ethyl acetate), as pure or in binary and ternary mixtures and they reported that the different solvents had different effects on the composition of condensed tannins in GSP extracts. Clearly, there are different studies aiming to determine the suitable solvent type for the extraction of food sample.

The main aim of the current study was to determine the best solvent mixture for the extraction of grape seed powder (GSP). For this purpose, multiple response optimization technique was selected to see the simultaneous effect of solvent mixtures to compose the best solvent mixture to provide a highly yielded GSP extract in terms of bioactivity. So, ethanol, methanol and water were the solvent used and simplex lattice mixture design was used for the optimization of processing variables.

Material and Methods

Material

Grape seeds were provided from a local seller in Kayseri, Turkey. The seeds were ground using a grinder and exposed to oil extraction using n-hexane at the ratio of 1:2 sample/n-hexane. The sample was mixed on the magnetic stirrer for 30 min and then n-hexane was removed from the residue in a fume hood. The residue was dried at room temperature for 2 hours and then the defatted GSP was used for further extraction studies.

Extraction of bioactive compounds from GSP

For the extraction of bioactive compounds from grape seed powder (GSP), three different solvents (ethanol, methanol and water) and their mixtures at different levels as shown in Table 1 were used. To determine the best solvent or solvent mixture achieving high bioactivity, simplex lattice mixture design approach was used as shown in Table 1. A 1 g of the GSP sample was weighed and 30 mL of the solvent prepared according to the experimental design points (Table 1) was incorporated into the tubes and then the tubes were mixed by vortex for 1 min and then covered tightly and placed in a shaking water bath at 25 ºC. The samples were extracted for 1 hour and then the samples were centrifuged at 9000 *g* at 5 ºC for 3 min. Finally, the supernatants were filtrated using a filter paper (0.45 μm) and then the extracts were subjected to further bioactive analysis.

Bioactivity tests for the GSP extracts

Determination of total phenolic content (TPC)

Total phenolic content analysis of the samples was determined according to the methodology described by Singleton and Rossi (1965) and Köprü et al. (2020). For this purpose, 200 μL of extract was mixed with 1800 μL of distilled water. Then, 1 mL of Folin Cioceltau reagent (1:10) was added and waited for 1 min. Finally, 2 mL of sodium carbonate (2% w/v) was added and the tubes were vortexed. At the end, the samples were incubated for 2 h at room temperature in dark conditions. After incubation, the absorbance of the samples was recorded at 765 nm using a UV-vis spectrophotometer (Shimazdu, Japan). Total phenolic content of the samples was calculated as mg gallic acid equivalent (mg GAE/g sample) and the measurements were replicated with four repetitions.

Determination of total flavonoid content (TFC)

The total amount of flavonoids in the samples was determined according to the methodology described by Zhishen et al. (1999). For this purpose, 500 μL of sample was mixed with 2 mL of water and 150 μL of $NaNO_2$ (5% w/v) was added to the samples. After 5 min, 150 μL of $AlCl_3$ (10% w/v) was placed into the samples and they were incubated for 6 min and then 1 mL of NaOH and 1.2 mL of water was added to complete the sample volume to 5 mL. After mixing with vortex, the samples were incubated for 10 minutes at room temperature and in dark environment and the absorbance values of the samples were measured by UV-vis spectrophotometer (Shimadzu, Japan) at 510 nm. Total flavonoids were calculated as mg CE (catechin equivalent) / kg sample using calibration curves prepared with catechin standard. The measurements were replicated with four repetitions.

Determination of condensed tannin (CT) level

Condensed tannin contents of the samples were performed by applying the method proposed by Sun et al. (2002). For this purpose, 1 ml of extract was mixed with 2.5 ml of vanillin (prepared with 1% methanol) and 2.5 ml of H_2SO_4 (prepared with 25% methanol) was added. Subsequently, the samples were incubated for 15 minutes in a water bath at 30 ºC and the absorbance values were recorded at 500 nm by UV-vis spectrophotometer (Shimadzu, Japan). The measurements were replicated with four replicates.

Determination of antiradical activity

DPPH radical scavenging activity

Antiradical activity of the samples was determined using DPPH as radical by the method of Köprü et al (2020). For this purpose, 100 μL of nondiluted extract was mixed with 3900 μL of DPPH solution (0.2 mM in methanol). The mixture was vortexed well and the samples were incubated for 30 min at room temperature in dark conditions. At the end of the incubation, the absorbances of the samples were recorded at 517 nm using a UV-vis spectrophotometer (Shimadzu, Japan). Antiradical activity of the samples was calculated as % inhibition as following:

$$\% \text{ Inhibition: } [(Abs_{control}-Abs_{sample})/Abs_{control})]*100 \quad (1)$$

$ABTS^{.+}$ radical scavenging activity

$ABTS^{.+}$ radical scavenging activity of the samples was determined by the method of Wettasinge et al. (2002) and Mathew and Abraham (2006). At the beginning, 7 mmol $ABTS^{.+}$ was dissolved in some amount of water and treated with 2.45 mmol potassium persulfate. The mixture was then allowed to stand at room temperature for 16 hours until a dark blue color appeared and was ready for analysis. This dark blue solution was diluted with buffer solution (pH 7.4) until the absorbance was 0.7 at 734 nm. Then, 2 ml of this diluted solution ($ABTS^{.+}$ solution) was mixed with 100 μl of the diluted sample extract in appropriate proportions (1:30) and after 6 min incubation, the absorbance values were measured at 734 nm by UV-vis spectrophotometer (Shimadzu, Japan). The reduction in ABTS$^{·+}$ radical cation was calculated in percent according to the following equation and the results are given as µg Trolox/g sample. All experiments were carried out in 4 repetitions.

% Inhibition: $[(Abs_{control}-Abs_{sample})/Abs_{control})]*100$ (2)

Determination of antioxidant activity

Iron chelating activity (ICA)

Iron chelating activities of the samples were determined by Rival et al. (2001) by making partial modifications. For this purpose, 1 mL of the sample extract diluted as 1:10 was taken and 3.7 mL of ethanol (95% v/v) was added. Then, 100 µl of $FeCl_2$ was added to the samples and immediately after vortexing the samples 200 µ ferrozine (5 mM) was incorporated. The homogeneously mixed samples were allowed to incubate for 10 min at room temperature in the dark and the absorbance values of the samples were measured by UV-vis spectrophotometer (Shimadzu, Japan) at 562 nm. Iron chelating activity values of the samples were calculated as % inhibition using the following equation. All experiments were carried out in 2 repetitions with 4 replicates.

% Inhibition: $[(Abs_{control}-Abs_{sample})/Abs_{control})]*100$ (3)

Antioxidant activity by phosphomolybdenum (AA)

Antioxidant activity values of the samples were determined using the phosphomolybdenum method proposed by Prieto et al. (1999). In this context, firstly the test solution (0.6 M sulfuric acid (30 mL), 28 mM sodium phosphate (28 mL) and 4 mM ammonium molybdate 40 mL) was prepared freshly by combining, and then 0.4 mL sample was mixed with 4 mL test solution and the mixture was vortexed and the test tubes were allowed to incubate in a water bath at 95 °C for 90 min. At the end of the period, absorbance values of the samples were measured by UV-vis spectrophotometer (Shimadzu, Japan) at 695 nm. Results of antioxidant activity were given in mg ascorbic acid equivalent (mg AAE / kg) using calibration curves plotted with ascorbic acid standard. All experiments were carried out in 2 repetitions with 4 replicates.

Data modeling and optimization

Simplex lattice mixture design (SLMD) was used to determine the effects of different solvents namely ethanol (X_1), methanol (X_2) and water (X_3) on the bioactive performance of the grape seed powder extract. To determine the optimum solvent type to produce high bioactive grape seed extract having high antiradical and antioxidant capacities, multiple response optimization process was followed. As is seen in Table 1, the component proportions were expressed as the fractions of the mixture with a sum (X_1+X_2+X_3) of one. The following polynomial equation of function x_i was fitted for each factor assessed at each experimental point. This polynomial model differs from full polynomial models because it does not contain a constant term (intercept equal to zero). This polynomial model equation was:

$$Y = \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_{12}X_1X_2 + \beta_{13}X_1X_3 + \beta_{23}X_2X_3 \quad (4)$$

where Y is the estimated response; β_1, β_2, β_3, β_{12}, β_{13} and β_{23} are constant coefficients for each linear and nonlinear (interaction) term produced for the prediction models of processing components. The analysis was performed using uncoded units. Square root transformation was applied for the studied parameters except AA.

The computational works, mathematical modeling, preparing the ternary contour graphical presentations of the models, were performed using Design Expert statistical software. JMP statistical package software (Version 5.0.1.a, SAS Institute. Inc. Cary, NC) was used to compute the predicted equations. The correlations between the parameters were determined using XLSTAT for Windows.

Results and Discussion

Bioactive compound levels of the samples

Table 2 shows the change in total phenolic content (TPC) of the samples according to the solvent used. As is seen, the lowest TPC level was determined as 0.33 and 0.31 mg GAE/g for the sample prepared by only ethanol (runs 5 and 12) while the highest TPC (7.29 mg GAE/g) was determined for the sample extracted by using ethanol: water mixture (1:1). The change in the TPC content depending on the solvent used was statistically significant ($p<0.05$, Table 3). The ANOVA results for the studied parameters were tabulated in Table 3. As is seen, the model selected for TPC was found as significant ($p<0.05$) and the linear effects of the studied solvents (ethanol, methanol and water) were determined as to be significant ($p<0.05$). Only the interaction effects between the ethanol and methanol were not significant which means that the mixture of both two organic solvents was not effective on the extraction of bioactive compounds. The fitted second order polynomial equation for total phenolic content is as follows:

$$Y_{TPC}=0.59X_1+2.17X_2+1.32X_3+0.69X_1X_2+6.81X_1X_3+2.09X_1X_3 \quad (R^2=0.982)$$

As could be seen from the equation, the fitting ability of the model is quite good due to high coefficient of determination ($R^2>0.980$). The change in TPC depending on the solvent type was illustrated in Fig.1 as ternary plots. It was observed that TPC of the samples increased significantly ($p<0.05$) toward to the edge of ethanol:water mixture while the lowest values were determined in the vertex of the ethanol. It was concluded that the highest TPC levels could be obtained by using the organic solvent: water mixtures. Similar results were obtained by Mildner-Szkudlarz et al. (2010) for the winemaking waste extracts and they reported that the best solvent for the higher polyphenol extraction was ethanol:water. It is well known that the solvent power depends on its polarity which is related to its dielectric constant and the phenolic substance extractability is correlated by the degree of polarity (Bosso et al., 2016).

Total flavonoid content (TFC) of the samples were given in Table 2 and it was determined that the TFC of the samples ranged between 44.3-537.4 mg CE/kg sample. The highest TFC was determined in the sample obtained by binary mixture of ethanol and water at the same proportion (1:1) while the lowest one was for the sample produced by using sole water as solvent (runs 2 and 15). The effect of solvent type was found as significant and also the model selected for the description of the TFC depending on the solvent type was significant

($p<0.05$). The linear mixture of the solvents affected the TFC of the samples significantly also ($p<0.05$, Table 3). In addition to that, the interaction effect between the solvents of ethanol and water and methanol and water was significant ($p<0.05$, Table 3). The change in TFC of the samples based on the solvent type was illustrated in Fig.1. As is seen clearly, TFC was the lowest in the vertex of the ethanol and water point and it increased significantly towards to the middle edge of both these two solvents (ethanol and water) which means that the binary mixture of ethanol and water is the best solvent to extract the flavonoids from the grape seed powder. The fitted second order polynomial equation for total phenolic content is as follows

$Y_{TFC}=8.10X_1+20.19X_2+6.65X_3+3.98X_1X_2+59.95X_1X_3+16.73X_1X_3$ $(R^2=0.982)$

As could be seen from the equation, the fitting ability of the model is quite good due to high coefficient of determination ($R^2>0.980$). Bosso et al., (2016) stated that the TFC of the grape seed was affected by the solvent type significantly ($p<0.05$) and they concluded that the highest TFC was in the sample extracted by 75% ethanol compared to sole ethanol or water and 50% acetone compared to sole acetone or water.

As other bioactive compounds, condensed tannin (CT) presents in GSP ranged between 23.3-3346.6 mg CE/kg sample. The highest CT levels were determined for the samples extracted by only methanol (runs 6 and 9) while the lowest values were found for the sample treated with only the water (runs 2 and 15). As could seen from the Table 2, the differences between the samples were significant because there was a huge different between the lowest and highest values of CT levels depending on the extraction solvents ($p<0.05$). The linear mixture of the used solvents also affected the CT content of the samples and also the binary interactions except ethanol and methanol showed a significant effect on CT levels of the samples ($p<0.05$, Table 3). Fig 1 shows the change in CT content of the GSP samples depending on the solvent as ternary plots and it is clear from the figure that the highest CT levels were monitored in the samples extracted with only methanol because the highest CT levels placed towards to the vertex of the methanol while the lowest values were on the vertex of the ethanol and water. It could be said that the tannins in GSP are highly soluble in only methanol. To describe the effect of the processing variables on the CT level of the samples, the fitted second order polynomial equation for condensed tannin contents is as follows:

$Y_{CT}=16.12X_1+57.76X_2+3.88X_3+14.33X_1X_2+141.8X_1X_3+24.76X_1X_3$ $(R^2=0.967)$

As is seen from the high determination coefficient, the fitting ability of the samples is quite high. Bosso et al. (2016) investigated the effect of different solvents on the condensed tannins of grape seed powder and they reported that the acetone:water mixture was the best solvent compared to ethanol and ethyl acetate for the extraction of condensed tannins (proanthocyanidins). In another study, Downey and Hanlin (2010) reported that the best solvent for the extraction of condensed tannins from grape skin was the binary mixture of the acetone:water compared to ethanol:water. They also stated that the single solvents of ethanol and water were not effective on the extraction of tannins as similar to the current research results.

Antiradical activities of the samples

Antiradical activities of GSP extract were determined by two different methods namely DPPH and $ABTS^{.+}$ radical scavenging tests. As is known, the antiradical activity of extract or antioxidant substances is related to their ability hydrogen-donating performance and DPPH or $ABTS^{.+}$ radicals convert them a stable molecule by accepting an electron from the extract or other antioxidant substances (Gulcin et al., 2004). So, the most of the bioactive compounds generally are donors of hydrogen atoms (H) and the radicals of DPPH capture the H atoms and convert to the neutral form (Savitri et al., 2019). DPPH results were given as % inhibition of DPPH radical and tabulated in Table 2 for all solvent types. The % inhibitions values were in the range of 2.11-80.5 for the samples and the lowest antiradical performance was recorded for the sample extracted by only sole ethanol (runs 5 and 12) while the highest was for the sample extracted by the binary mixture of ethanol and water at 1:1 ratio (run 7). The results showed that the differences among the DPPH radical scavenging activities were influenced by the solvent type ($p<0.05$). The ANOVA results showed that the linear mixture of the solvents showed a significant effect on the DPPH radical scavenging activities and also the binary mixtures of ethanol and water or methanol and water showed a significant effect on the antiradical performance. The selected model was found as significant and constructed polynomial model to predict the antiradical activities of the sample showed a good fitting capacity with quite high coefficient of determination. The polynomial model for DPPH radical scavenging activity was given in the following;

$Y_{DPPH}=1.70X_1+7.06X_2+3.17X_3+4.35X_1X_2+24.66X_1X_3+9.76X_1X_3$ $(R^2=0.956)$

The change in antiradical performance of the samples was illustrated as ternary plots in Fig.2. Antiradical activity of the samples decreased dramatically towards to the vertex of ethanol while it increased towards to the edge of ethanol: water mixture. It was seen that the antiradical activity of the samples determined by the inhibition of the DPPH radical was well correlated significantly with TPC and TFC with the correlation coefficients as 0.963 and 0.944, respectively ($p<0.05$).

The another test to evaluate the antiradical performance of the samples was $ABTS^{.+}$ radical scavenging test. The $ABTS^{.+}$ radical scavenging values of the samples were in the range of 0.31-4.08 μg Trolox/g sample. The highest $ABTS^{.+}$ radical scavenging activity value was measured for the sample extracted by the binary mixture of ethanol and water and the lowest values were for the sample extracted by only sole ethanol. The linear effects of the solvents used were determined to be significant on the radical scavenging activity and also, the influence of the binary mixtures except ethanol and methanol showed a significant effect on $ABTS^{.+}$ radical scavenging activity. The selected model effect was also determined as significant ($p<0.05$, Table 3). Fig. 2 shows the change in $ABTS^{.+}$ radical scavenging activity of the samples depending on the extraction solvent as ternary plots. As is seen, the lowest values were placed on the vertex of ethanol and the antiradical activity increased significantly towards to the edge of ethanol and water (p<0.05). The constructed polynomial model for the ABTS radical scavenging abilities showed a good fitting ability with high coefficient of determination as following;

$Y_{ABTS^{\cdot+}}=0.65X_1+1.62X_2+1.03X_3+0.44X_1X_2+4.91X_1X_3+2.20X_1X_3$ (R^2=0.949)

The correlation analysis showed that there was a positive and significant correlation between DPPH and $ABTS^{\cdot+}$ radical scavenging test. And also, there was a significant and positive correlation between TPC and DPPH (r=0.964, p<0.05) and TPC and $ABTS^{\cdot+}$ (r=0.966, p<0.05). Baydar et al. (2007) reported that the grape seed extract produced by acetone:water:acetic acid (90:9.5:0.5) mixture showed a strong DPPH radical scavenging activity compared to BHA and BHT synthetic antioxidant substances and their antiradical performances is directly related to polyphenolic content. Guendez et al. (2005) investigated the low molecular weight of polyphenols in GSP extract and they reported that certain constituents of seeds are particularly responsible for strong antiradical effect. They found a lower correlation between the TPC and DPPH radical scavenging activity as r=0.649.

Antioxidant capacities of the samples

Antioxidant characteristics of the GSP extracts were evaluated by two important methods namely iron chelating ability (ICA) and antioxidant activity (AA) by phosphomolybdenum approach. Table 2 shows the ICA values ranged between 2.53-87.80% and the lowest chelating activity was determined for the sample extracted by only ethanol (runs 5 and 12). The highest ICA values were determined for the samples extracted by the methanol. Similar to the other bioactive parameters, the differences among the ICA values depending on the solvent were significant (p<0.05). As could be seen from the Table 3, linear effects of the solvents were also showed a significant effect on the chelating ability values. Also, the interaction effects between the aqueous binary mixtures of the solvents were determined as significant. The change in the ICA values of the GSP extracts was illustrated in Fig. 3 as ternary plot and it is seen clearly from the figure that the ICA values increased towards to the methanol-water edge while the lowest values were recorded towards to the vertex of ethanol and water. The constructed polynomial model for the ICA values showed a good fitting ability with high coefficient of determination as following;

$Y_{ICA}=2.18X_1+9.04X_2+6.56X_3+6.62X_1X_2+8.89X_1X_3+5.00X_1X_3$ (R^2=0.882)

To evaluate the antioxidant performance of the samples, the samples were exposed to phosphomolybdenum assay. The results of the AA values were given in Table 2 and as it is seen, the lowest AA value was determined as 3.65 mg AAE/g for the sample extracted by sole ethanol similar to ICA values. The highest AA value (14.77 mg AAE/g) was recorded for the sample extracted by the binary mixtures of methanol and water. Antioxidant activity determined by phosphomolybdenum approach was affected by the solvent type significantly similar to the other bioactive parameters (p<0.05). As could be seen in Table 3, the selected model was found as significant and the processing variables showed a significant effect on the studied parameter (p<0.05). The change in AA depending on the solvent type was also showed in Fig.3 as ternary plots and it is clear from the figure that the AA values increased towards to the edge of ethanol:water or methanol:water while the lowest values were placed on the vertex of ethanol and then methanol (Fig.3). The constructed polynomial model for the AA values showed a good fitting ability with high coefficient of determination higher than 0.907 as following;

$Y_{AA}=4.16X_1+9.13X_2+8.76X_3-2.12X_1X_2+29.45X_1X_3+20.57X_1X_3$ (R^2=0.907)

Multiple response optimization to determine the best solvent mixture

The best solvent or solvent mixture was studied using the all characterized bioactive parameters and so, multiple response optimization was performed using the desirability functions. Optimization was conducted on squared values of real measurements because of calculating the ratio of max to min was higher than 10. Both minimization and maximization procedure were followed to calculate the limit values considering the all bioactive parameter values and the calculated results were tabulated in Table 4. Minimization process showed that the minimum values for the bioactive parameters would be at 100% ethanol usage for the extraction of GSP (Fig.4). By using this solvent, TPC and TFC would be 0.59 mg GAE/g and 8.09 mg CE/kg. Also, the lowest antiradical activities characterized by DPPH and $ABTS^{\cdot+}$ radical scavenging tests would be at 1.71% and 0.65 µg Trolox/g sample, respectively. It was resulted that the sole ethanol usage as a solvent in the extraction of bioactive compounds from defatted GSP is not suggested due to very low bioactivity. To suggest a good solvent system a maximization process was applied using desirability functions and the calculated results for the studied parameters were tabulated in Table 4. As is seen from the Fig.4, the optimized solvent mixture is not a sole organic solvent, on the contrary the ternary mixture of ethanol (33.84%), methanol (20.17%) and water (45.99%) is suggested. Using that solvent mixture, the maximum TPC and TFC would be 2.54 mg GAE/g and 21.02 mg CE/kg sample, respectively. Also, the maximum antioxidant parameter values would be 8.92% and 13.59 mg AA/kg for ICA and AA, respectively. Desirability function values for both process were also acceptable. It was concluded that by the multiple response optimization for more than one parameter having important effect on the sample bioactivity showed good optimization result. So, by the determination of good solvent mixture determined by multiple response optimization, a GSP extract having bioactivity could be produced.

Conclusions

Grape seed is a food waste of winemaking industry but recently its powder form can be evaluated as a functional food matrix due to its good bioactive properties. It is rich in many phytochemical compounds and shows good antioxidant, antiradical and also antimicrobial activities. To produce an extract from GSP, selection of correct solvent or solvent mixture has a critical role because the extract yield and its bioactivity are affected from the solvent polarity. In this study, an optimized ternary solvent mixture was suggested to provide a high bioactive GSP extract. In this regard, the highest bioactivity based on the concentrations of the total bioactive compounds and their antiradical and antioxidant activities, could be obtained by the solvents of ethanol, water and methanol at the determined ratios using multiple response optimization process. The results of the current work could be evaluated by the industries which process the GSP extracts.

Table 1. Simplex lattice mixture design showing the solvent levels used for the extraction

Runs	Coded levels			Uncoded levels		
	X_1	X_2	X_3	X_1	X_2	X_3
1	0.50	0.50	0.00	50.0	50.0	0.0
2	0.00	0.00	1.00	0.0	0.0	100.0
3	0.50	0.50	0.00	50.0	50.0	0.0
4	0.33	0.33	0.33	33.3	33.3	33.3
5	1.00	0.00	0.00	100.0	0.0	0.0
6	0.00	1.00	0.00	0.0	100.0	0.0
7	0.50	0.00	0.50	50.0	0.0	50.0
8	0.17	0.67	0.17	16.7	66.6	16.7
9	0.00	1.00	0.00	0.0	100.0	0.0
10	0.67	0.17	0.17	66.6	16.7	16.7
11	0.50	0.00	0.50	50.0	0.0	50.0
12	1.00	0.00	0.00	100.0	0.0	0.0
13	0.00	0.50	0.50	0.0	50.0	50.0
14	0.17	0.17	0.67	16.7	16.7	66.6
15	0.00	0.00	1.00	0.0	0.0	100.0

X_1: Ethanol, X_2: Methanol, X_3: Water

Table 2. Bioactive performance of the defatted grape seed powder

Runs	TPC (mg GAE/g)	TFC (mg CE/kg)	CT (mg CE/kg)	DPPH (%)	$ABTS^{\cdot+}$ (µg Trolox/g)	ICA (%)	AA (mg AA/g)
1	2.23	217.4	1558.0	30.01	1.33	39.88	5.39
2	1.82	44.3	25.0	14.11	0.93	47.74	9.28
3	2.33	234.4	1526.7	27.96	1.39	48.24	5.73
4	5.38	365.8	1699.5	64.40	3.94	78.76	12.67
5	0.33	62.0	211.3	2.11	0.31	2.53	3.65
6	4.58	387.1	3163.8	48.17	2.77	83.04	9.45
7	6.88	492.4	2093.5	80.50	4.08	35.60	14.00
8	5.81	451.6	3002.8	58.67	3.21	77.91	11.79
9	4.61	408.3	3346.6	51.19	2.56	87.80	8.63
10	4.26	338.7	2140.0	55.00	3.43	67.59	11.92
11	7.29	537.4	2130.2	71.20	4.00	33.37	13.70
12	0.31	63.3	239.2	2.37	0.43	5.55	3.86
13	5.31	328.3	1508.0	66.60	3.50	71.18	14.77
14	4.72	272.8	786.9	39.64	3.02	68.94	10.72
15	1.85	49.3	23.3	8.63	1.35	41.62	9.23

TPC: Total phenolic content, TFC: Total flavonoid content, CT: Condensed tannin, ICA: Iron chelating activity, DPPH: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, $ABTS^{\cdot+}$: 2,2-Azinobis (3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging activity, AA: Antioxidant activity

Table 3. ANOVA results showing the significance of the studied solvent effect on the bioactive parameters

Source	df	TPC	TFC	CT	DPPH	ABTS$^{\cdot+}$	ICA	AA
Model	5	96.02*	100.9*	53.97*	38.92*	33.74*	13.53*	17.45*
Linear mixture	2	61.80*	55.87*	74.42*	20.72*	16.22*	27.44*	14.42*
X_1X_2	1	3.39	1.63	1.20	4.18	0.94	4.14	0.22
X_1X_3	1	330.5*	369.3*	117.6*	134.6*	117.7*	7.45*	42.12*
X_2X_3	1	21.95*	20.33*	2.53*	14.98*	16.67*	1.67*	14.52*
Residual	9							
Lack of Fit	4	23.92*	10.68	94.36*	7.89*	6.88	16.0	46.3*
Pure Error	5							
Cor Total	14							
R^2		0.982	0.982	0.967	0.956	0.949	0.882	0.907
Adj R^2		0.971	0.973	0.950	0.931	0.921	0.817	0.855

$^{\phi}$ X_1: Ethanol, X_2: Methanol, X_3: Water , TPC: Total phenolic content, TFC: Total flavonoid content, CT: Condensed tannin content, ICA: Iron chelating activity, ABTS$^{\cdot+}$: 2,2-Azinobis (3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging activity, DPPH: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, AA: Antioxidant activity (mg AA/kg) *p<0.05

Table 4. Multiple response optimization values for the extraction of defatted grape seed powder

	Minimization process				Maximization process			
Response parameters	X_1	X_2	X_3	*Desirability*	X_1	X_2	X_3	*Desirability*
	100	0	0		33.84	20.17	45.99	
TPC (mg GAE/g)	0.59				2.54			
TFC (mg CE/kg)	8.09				21.02			
CT (mg CE/kg)	16.12				44.37			
ICA (% Inh.)	2.24			0.921	8.92			0.898
DPPH (% Inh.)	1.71				8.50			
ABTS$^{\cdot+}$ (μg Trolox/g)	0.65				2.01			
AA (mg AA/kg)	4.16				13.59			

$^{\phi}$ X_1: Ethanol, X_2: Methanol, X_3: Water , TPC: Total phenolic content, TFC: Total flavonoid content, CT: Condensed tannin content, ICA: Iron chelating activity, ABTS$^{\cdot+}$: 2,2-Azinobis (3-ethylbenzthiazoline-6-sulphonic acid) radical scavenging activity, DPPH: 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity, AA: Antioxidant activity (mg AA/kg). Square root transformation was applied for the studied parameters except AA.

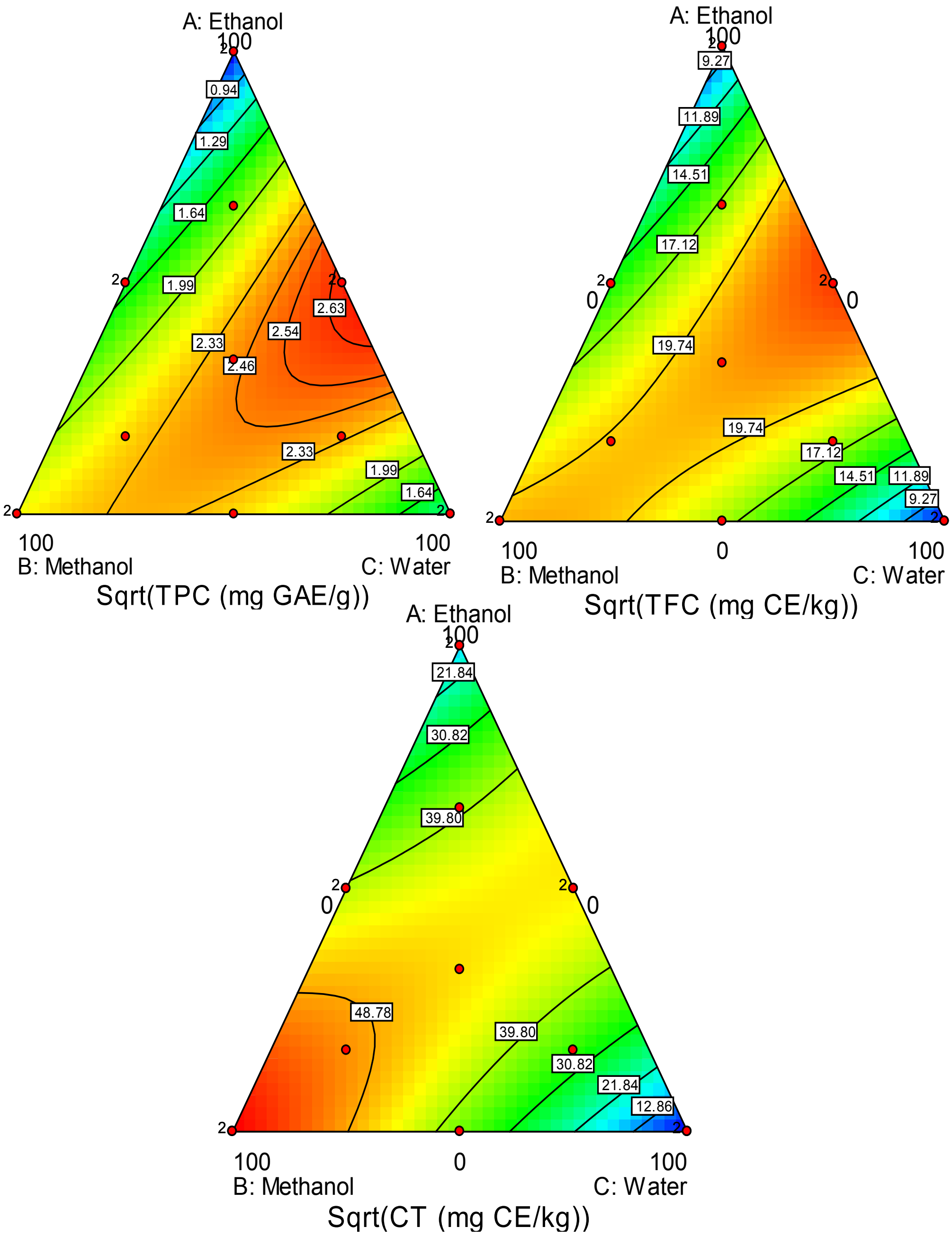

Figure. 1 Ternary contour plots showing the change in total phenolic content (TPC), total flavonoid content (TFC) and condensed tannin (CT) levels of defatted grape seed powder

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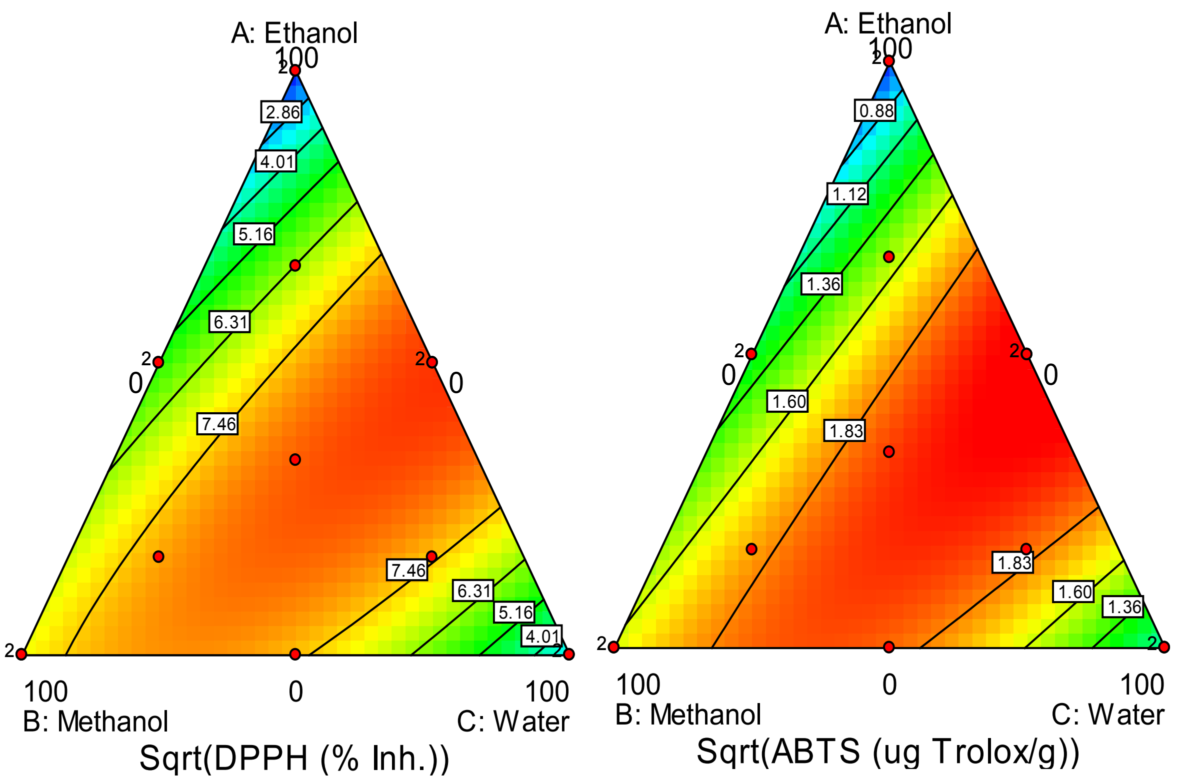

Figure 2. Ternary contour plots showing the change in DPPH radical scavenging activity and ABTS$^{\cdot+}$ radical scavenging activity of defatted grape seed powder

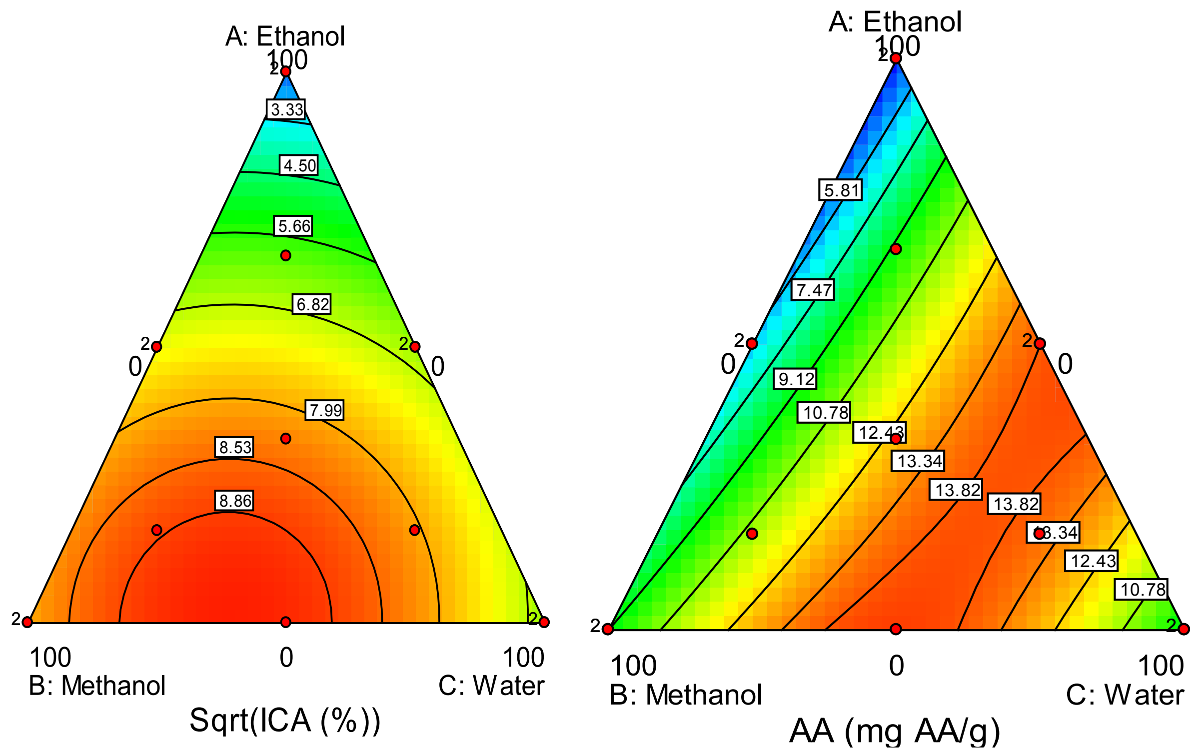

Figure 3. Ternary contour plots showing the change in iron chelating activity (ICA) and antioxidant activity (AA) of defatted grape seed powder

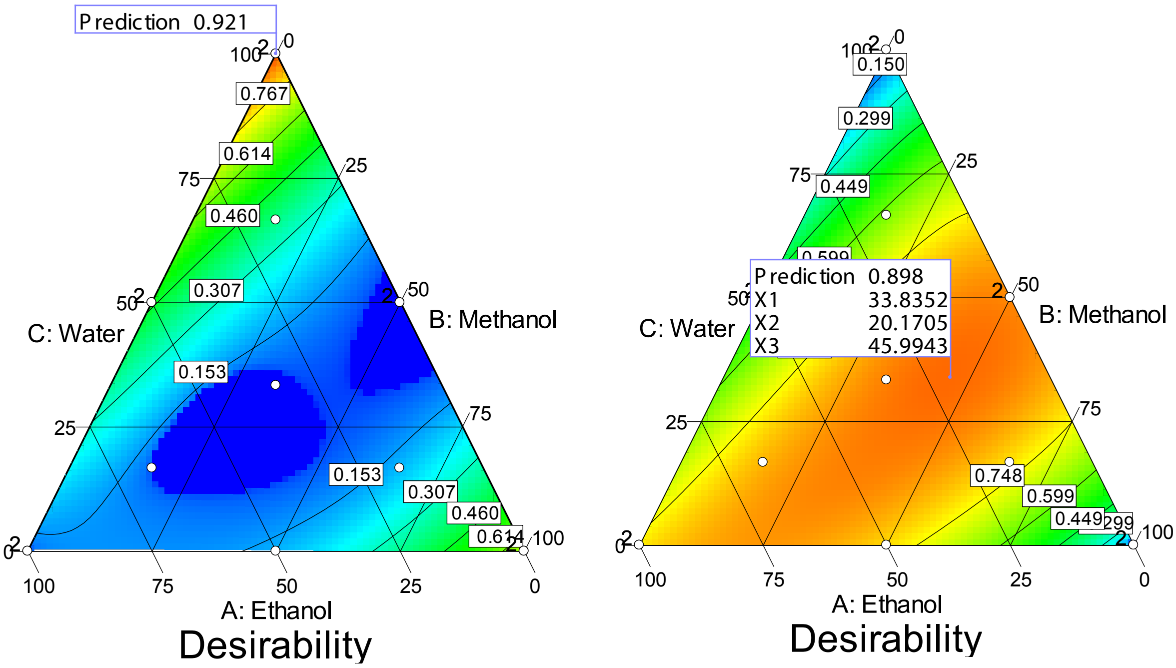

Figure 4. Ternary contour plots showing the desirability function values for the maximum and minimum response values according to the solvent mixture types. Maximum values at the right ternary plots and minimum values at the left ternary plots.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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Data availability

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Consent for publication

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Research Article

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Effects of extra sulfur fertilization on soil pH and grain mineral concentration in wheat

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Abstract

Increases in crop yield and decreases in industrial sulfur emissions reduce the amount of sulfur (S) in the soil. Sufficient S and proper pH play an important role in achieving the targeted yield and quality of wheat. In recent years, composite fertilizers containing sulfur and mineral substances have been produced and used in wheat production as fertilizers. In this study, an investigation was made as to whether the use of such fertilizer is sufficient for sulfur fertilization. Basal fertilizer (13.25.5 + 10 (SO_3) + Zn (0.5)) (250 kg ha^{-1}) was applied to the whole plot, and additional elemental sulfur was added. The amounts of sulfur in the plots at the end of the applications were 10, 300, 600 and 900 kg ha^{-1} S. One year later, the high dose (600 and 900 kg ha^{-1}) S resulted in a decrease in pH of about 0.5 pH units, while two years later, all S doses resulted in a 1 pH unit decrease. However, the changes in the second year were not induced by the S treatments. Accordingly, no significant effect of S doses on grain protein, mineral content or yield was determined. Wheat variety and year had a significant effect on grain nutrient content. The lowest sulfur dose (10 kg ha^{-1} S) used in the experiment can be said to be sufficient for yield and mineral concentration.

Keywords: Wheat mineral concentration, Sulfur fertilization, Soil pH

Introduction

Sulfur has long been known to be an essential element for higher plants (Duke and Reisenauer, 1986). It is used in plant protein, amino acid and enzyme synthesis (Scherer, 2001). Sulfur fertilizer and emissions of S are the main sources of S in the soil. In the last decades, sulfur emissions in Europe (Hicks et al., 2002) and the use of sulfur fertilizer have decreased significantly. Although there was a fluctuation between 1990 and 2015, there has been no significant reduction in sulfur emissions in Turkey (Anonymous, 2017). The amount of sulfur in the soil decreases due to increased yield, low S emission, intensive agriculture, the use of low S content fungicides, stubble burning, etc. (Gupta et al., 1997). When the yield increases threefold, the amount of S removed from the soil approximately doubles (McGrath et al., 1996). In recent years, sulfur deficiencies have emerged in wheat production areas in Europe and many other parts of the world (Zhao et al., 1998; Tisdale et al., 1986; Inal et al., 2003).

The availability of nutrients such as iron and zinc depends on soil pH (Tisdale et al., 1993; Sönmez et al., 2008). Sulfur is applied to the soil in different doses to amend alkaline soil pH and to make some nutrients available (Modaihsh et al., 1989; Kaplan and Osman, 1998; Usta, 1995). There is a positive correlation between the availability of sulfur in the soil and wheat nutrient contents. It also increases bread quality (Randall and Wrigley, 1986; Rendig, 1986; Ryant and Hrivna, 2004). In recent years, basal fertilizers containing sulfur and micronutrients have been used frequently in Turkey. This study was carried out to determine the effect of basal fertilizer containing sulfur and the application of extra granular sulfur on soil pH and wheat nutrient concentration.

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Materials and Methods

The experiment was conducted in the 2015-16 and 2016-17 growing seasons at the experimental farm of the Faculty of Agriculture of Isparta Applied Science University. Two wheat varieties were used, one for bread (Tosunbey) and one for durum (Ç-1252). In the first year, the trial area was divided into four main plots and basal fertilizer (13.25.5 + 10 (SO3) + Zn (0.5)) was applied to all plots at the rate of 250 kg ha^{-1} (32.5 N, 62.5 P, 12.5 K and 1.25 kg ha^{-1} Zn) . In addition to the sulfur from the fertilizer, granular sulfur was applied to the other plots (2, 3, 4) at 300, 600 and 900 kg ha^{-1} S. Ten days before planting, sulfur scattered on the soil surface was mixed (15-20 cm deep) by rotavator. Wheat sowing was performed on 15 October 2015 with a plot seed drill. The plots had six rows arranged with 20 cm between rows, and a row length of 4 m. In addition to the basal fertilizer, Ammonium Nitrate® fertilizer was applied in the spring at a rate of 47.5 kg ha^{-1} N (Feekes 5).

In the second year of the experiment, sowing was performed on the same main plots, and S fertilization was not applied again. The sowing was carried out on 21 October 2016, and 60 kg ha^{-1} P (TSP®) fertilizer and 32.5 kg ha^{-1} N (Ammonium Nitrate®) fertilizer were applied. In the spring (Feekes 5), Ammonium Nitrate® fertilizer was applied at 47.5 kg ha^{-1} N. Soil characteristics determined by analyzing the soil before the experiment are given in Table 1, and the climatic data of the growing periods are given in Table 2.

Table 1. Soil characteristics of the trial field

Clay%	Silt%	Sand%	pH	$CaCO_3$%	OM%	Cu(ppm)	Mn(ppm)	Fe(ppm)	Zn(ppm)	N(%)	P(ppm)	K(ppm)
16.2	44.8	39.0	8.5	29.4	1.60	1.50	5.4	1.70	0.75	0.10	15.0	108

pH measurements were made in soil samples taken from soil depths of 30 cm in the main plots of the experimental field at specific intervals (Figure 1). Yield was determined by harvesting by hand. Wheat grain samples taken for nutrient concentration determination were washed and rinsed with pure water and dried in 65°C ovens for 2 days. The dried grain samples were milled and sieved through a 0.5 mm sieve. Wheat flour samples (0.5 g) were digested with an H_2SO_4 / $HClO_4$ mixture (4:1 by volume), and grain Ca, Mg, K, Fe, Cu, Zn and Mn concentrations were measured by Atomic Absorption Spectroscopy (AAS-Varian, FS 240) (Uygur and Şen, 2018). Phosphorus concentration was determined by a spectrophotometer (T-80) after adding vanadomolybdate coloring reagent. Nitrogen concentration was determined by the Kjeldahl method. The ANOVA procedure was performed with SPSS software. Separation of the main effects was performed by Duncan test at $p < 0.05$.

Table 2. The climate data of experimental years

Climatic factors	Years/ months	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Means /Total
Mean temperature (°C)	2015-16	14.6	9.1	2.5	1.3	7.3	7.6	14.0	14.6	21.6	25.0	11.8
	2016-17	14.8	7.2	0.3	-0.8	3.0	7.3	10.6	14.9	20.1	25.2	10.3
	Long term	12.9	7.4	3.5	1.9	2.9	6.2	10.7	15.6	20.2	23.6	10.4
Total precipitation (mm)	2015-16	23.1	17.5	6.4	101.6	33.3	59.9	47.8	87.6	12.4	25.7	415.3
	2016-17	1.6	48.8	33.5	87.8	3.6	74.4	25.6	149.5	30.9	13.1	468.8
	Long term	38.0	46.3	84.9	72.2	64.7	54.2	56.0	51.4	29.8	14.6	512.1

Results and Discussion

The pH values of the soil samples taken from the plots at various times are given in Figure 1. At the beginning of the experiment, the pH value was 8.52. After five months of sulfur application (after 147 days), the pH of all sulfur doses had decreased by an average of 0.5 and was between 8.01 and 8.11. After application at a dose of 10 and 300 kg ha^{-1} S in the second measurement and at a dose of 10 kg ha^{-1} S in the third measurement, relative increases in pH were observed. At the second year harvest (after 629 days), pH had decreased by 0.20 and 0.32 degrees compared to the previous measurement (after 509 days). There was a 1 degree decrease in pH in all S doses compared to the beginning. During the whole trial period, fluctuations were observed at doses of 10 and 300 kg ha^{-1} S, while a steady decrease was observed at doses of 600 and 900 kg ha^{-1} S. A high sulfur content in soil has the effect of oxidizing S with organic matter, and giving a high pH (> 6.5) (Zhao et al., 2015). The ability of applications of elemental S to reduce soil pH was seen in a previous study (Modaihsh et. al., 1989; Usta, 1995). The decrease of soil pH became more pronounced in the second year, but this decrease is likely to be a seasonal effect, with the basal fertilizer treatment also showing the same pH as the elemental S treatments. However, the difference after the second sampling may be explained in terms of the oxidation of S, which gives off some H ions, reducing soil pH as is evident in Fig 1. Since soil carbonate content is very high, the pH differences are not long lasting, and the treatment effect in the second year is not evident.

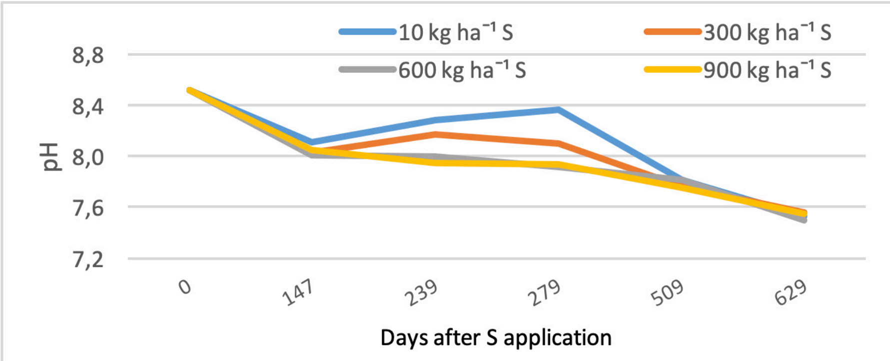

Figure 1. The soil pH values

Wheat yield and nutrient concentrations of grain are given in Table 3. Nitrogen content (N) and protein ratio were found to be different between the years, with the second year (2.76%) higher than the first year (2.00%). The nitrogen content (2.09% and 2.83%) of Tosunbey was higher in both years compared to Ç-1252 (1.92% and 2.69%), and the difference was statistically significant in the second year. Among the sulfur doses, the lowest N content was found at a 600 kg ha^{-1} S dose (1.78% in the first year, 2.64% in the first year), and the highest at a 900 kg ha^{-1} S dose in both years (2.22% in the first year, 2.83% in the second year). Among the interactions, the lowest N rate was determined at the 600 kg ha^{-1} S dose and Ç-1252 cultivars (1.66% in the first year, 2.53% in the second year), while the highest N rate was at the 900 kg ha^{-1} S dose and cultivar Ç.1252 (2.27%) in the first year. In the second year, the highest N concentration was found at the 10 (basal fertilization) and 900 kg ha^{-1} S dose and Tosunbey variety (2.91%). Although the highest protein ratio was found at a dose of 900 kg S, the protein ratio was not proportional to the S doses. Sulfur is a main component of protein, and S deficiency can limit the rate of grain protein (Westermann, 1993). Protein synthesis requires 1 part S per 15 parts N by weight, and lack of sulfur availability affects N assimilation rather than nitrogen uptake (Stewart and Porter, 1969; Freney et al., 1978; Sahota, 2006). Under normal yield conditions, it is necessary to apply one tenth of sulfur to the amount of nitrogen (Flowers et al., 2007). Considering the protein yield per decare in this study, it can be said that even the amount of S in the basal fertilizer (10 kg S ha^{-1}) is able to sustain the protein synthesis without any adverse effect.

It does not seem possible to make a positive or negative interpretation of the effect of sulfur doses on grain nutrient contents. Rasmussen et al. (1975), Zhao et al. (1999), and Ercoli et al. (2012) stated that sulfur uptake and assimilation vary depending on the N/S ratio in the soil, irrigation, and amount and time of S and N fertilization. In both years of our study, the lack of precipitation in the period of germination and the irregular distribution of precipitation during the growing period limited the vegetative development and yield of the wheat. This in fact negatively affected nitrogen efficiency and the yield. S has a synergistic effect with nitrogen (Rossini et al., 2018), and can be expected to have no effect on the content of other nutrients under low nitrogen use efficiency and yield. Zinc can be transported to the grain by forming ligands with S-containing amino acids (Haydon and Cobbett, 2007; Torrance et al., 2008). Concentrations of zinc and other nutrients did not show significant changes with different sulfur doses. This shows that even the lowest sulfur dose used (10 kg ha^{-1}) is sufficient in terms of nutrient content at these yield values. Grain nutrient concentrations (N, P, K, Mg, Cu, Zn and Mn) were significantly higher in the second year. In the first year, only Fe concentration was found to be high, and in fact there may be an effect of S induced pH changes in such behaviour. As the yield increases, the concentration of minerals in the grain decreases (Graham et al., 1999; Garvin et al., 2006) in relation to the dilution effect, and grain quality is affected by climatic conditions (Ducsay and Lozek, 2004). High grain nutrient concentrations in the second year of the experiment can be explained by the decrease in yield. Grain nutrient concentrations of Tosunbey were higher than those of Ç-1252, except for potassium. There are wide variations between varieties and species in terms of nutrient concentrations (Liu et al., 2006; Zhao et al., 2009; Kara, 2013; Uygur and Şen, 2018).

No significant difference in yield was found between cultivars. Although grain yields were quite low in both years, the second year value (1202 kg ha^{-1}) was lower than the first year (1893 kg ha^{-1}). The first trial year (October 2015, November 2015 and December 2015) and second trial year (October 2017) had well below the average amount of rainfall (Table 2). This had a negative effect on the germination rate, and was the main reason for the low yield. Sulfur doses had no effect on yield in the first year, but yield increased in parallel with increasing sulfur doses in the second year. The yield was lowest in the control 10 kg ha^{-1} S (1012 kg ha^{-1}) and was highest at 900 kg ha^{-1} S (1485 kg ha^{-1}). The wheat plant removes 15-25 kg S per hectare and needs 2-3 kg S to produce 1 ton of grain (Zhao et al., 1999). Inal et al. (2003) stated that sulfur fertilization of 20 kg ha^{-1} may be sufficient to reach sufficient yield in bread and durum wheat varieties. However, Zhao et al. (1999) stated that this dose (15-25 kg ha^{-1}) should be increased to increase the yield. This explains S doses not having an effect on the yield in the first year, but contrasts with the yield values in the second year. Environmental factors were likely to have determined the yield in the first year, and it shadowed the main effect of S fertilization on yield.

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Table 3. Wheat grain mineral concentrations and yield at different sulfur doses
(different letters indicate a statistical significance, ns: not significant)

		N %				P mg kg^{-1}				K mg kg^{-1}				Ca mg kg^{-1}				Mg mg kg^{-1}			
Sulfur	Variety	2015-16		2016-17		2015-16		2016-17		2015-16		2016-17		2015-16		2016-17		2015-16		2016-17	
10 kg ha^{-1}	Tosunbey	2.23	ab	2.91	a	2474	f	3499	c	2840	cd	3966	a	347	b	446	ab	916	a	1271	a
	Ç-1252	1.81	ef	2.61	cd	2926	cd	4680	a	2665	de	3896	a	292	c	379	cd	833	bc	1153	bc
300 kg ha^{-1}	Tosunbey	2.05	bcd	2.75	bc	3076	ab	3382	c	2276	f	3554	b	413	a	419	b	953	a	1088	cd
	Ç-1252	1.95	cde	2.86	ab	2995	abc	2643	d	3335	b	3914	a	290	c	353	d	815	cd	1082	cd
600 kg ha^{-1}	Tosunbey	1.90	de	2.75	b	2946	bcd	3208	c	2580	e	3531	b	404	a	377	cd	930	a	1206	ab
	Ç-1252	1.66	f	2.53	d	2654	e	2742	d	2415	ef	3948	a	308	c	370	cd	782	d	1038	d
900 kg ha^{-1}	Tosunbey	2.17	abc	2.91	a	3081	a	4292	b	3841	a	3839	a	367	b	453	a	930	a	1300	a
	Ç-1252	2.27	a	2.74	bc	2858	d	4288	b	3007	c	3938	a	295	c	389	c	871	b	1081	cd
Mean		2.00	B	2.76	A	2876	B	3592	A	2870	B	3823	A	339	B	398	A	879	B	1152	A
St Er.		0.20		0.10		211		733		506		197		49		37		64		105	
Sulfur	10 kg ha^{-1}	2.02	ab	2.76	ab	2700	c	4989	a	2757	b	3930	ns	319	ns	413	ab	874	ns	1212	ns
	300 kg ha^{-1}	2.02	ab	2.80	ab	3035	a	3012	b	2805	b	3734		351		385	ab	884		1085	
	600 kg ha^{-1}	1.78	b	2.65	b	2799	b	2975	b	2497	b	3739		356		374	b	855		1122	
	900 kg ha^{-1}	2.22	a	2.83	a	2969	ab	4290	a	3424	a	3888		331		421	a	900		1190	
Variety	Tosunbey	2.09	ns	2.83	a	2894	ns	3595	ns	2885	ns	3723	b	383	a	424	a	932	a	1216	a
	Ç-1252	1.92		2.69	b	2858		3588		2856		3924	a	296	b	373	b	825	b	1088	b

		Fe mg kg^{-1}				Cu mg kg^{-1}				Zn mg kg^{-1}				Mn mg kg^{-1}				Yield kg ha^{-1}			
Sulfur	Variety	2015-16		2016-17		2015-16		2016-17		2015-16		2016-17		2015-16		2016-17		2015-16		2016-17	
10 kg ha^{-1}	Tosunbey	42.3	a	26.0	bc	4.4	a	6.1	ab	20.0	ab	31.0	a	39.2	a	43.2	a	1481	cd	911	d
	Ç-1252	35.7	bcd	26.0	bc	3.9	bc	5.0	ab	16.1	c	29.2	ab	27.8	b	40.0	b	2162	ab	1114	cd
300 kg ha^{-1}	Tosunbey	37.4	bc	27.1	bc	4.4	a	6.5	a	16.3	c	24.9	d	39.0	a	41.8	ab	2011	abc	1048	cd
	Ç-1252	39.7	ab	25.1	c	4.6	a	4.9	b	18.1	bc	26.0	cd	30.3	b	32.7	e	1368	d	1145	cd
600 kg ha^{-1}	Tosunbey	35.2	cd	35.0	a	4.5	a	6.0	ab	21.6	a	28.1	bc	37.0	a	39.4	bc	1777	bcd	1265	abc
	Ç-1252	33.4	cd	28.3	bc	3.6	c	5.9	ab	15.2	c	29.9	ab	26.5	b	35.6	d	2400	a	1164	bcd
900 kg ha^{-1}	Tosunbey	39.6	ab	29.5	b	4.5	a	6.3	ab	21.7	a	32.1	a	37.4	a	40.3	b	2168	ab	1452	ab
	Ç-1252	32.1	d	29.2	b	4.1	ab	6.1	ab	18.0	bc	27.8	bc	29.7	b	37.0	cd	1777	bcd	1519	a
Mean		36.9	A	28.3	B	4.3	B	5.9	A	18.4	B	28.6	A	33.4	B	38.8	A	1893	A	1202	B
St Er.		3.8		3.4		0.4		0.9		2.8		2.7		5.4		3.5		427		235	
Sulfur	10 kg ha^{-1}	38.9	a	26.0	b	4.1	ns	5.6	ns	18.0	ns	30.1	a	33.5	ns	41.6	a	1821	ns	1012	c
	300 kg ha^{-1}	38.5	ab	26.1	b	4.5		5.7		17.2		25.4	b	34.6		37.2	b	1689		1097	bc
	600 kg ha^{-1}	34.3	b	31.7	a	4.1		5.9		18.3		28.9	a	31.7		37.5	ab	2088		1215	b
	900 kg ha^{-1}	35.9	ab	29.3	ab	4.4		6.2		20.0		29.9	a	33.5		38.7	ab	1972		1485	a
Variety	Tosunbey	38.6	a	29.4	ns	4.5	a	6.3	a	19.9	a	29.0	ns	38.2	a	41.2	a	1859	ns	1169	ns
	Ç-1252	35.2	b	27.2		4.1	b	5.5	b	16.9	b	28.2		28.6	b	36.3	b	1927		1235	

Conclusions

Basal fertilizers containing sulfur and micro nutrients have been widely used in wheat production. It can be said that the amount of sulfur (10 kg ha^{-1} S) in the basal fertilizer (13.25.5 + 10 (SO_3) + Zn) or any other fertilizer containing S are sufficient in terms of yield, protein and nutrient content. There is no need for extra granular sulfur applications in wheat production. However, monitoring of S levels in soil and plants in the coming years would have beneficial features to guarantee sustainable yield and protein.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

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Response of certain tomato (*Solanum lycopersicum*) genotypes to drought stress in terms of yield and quality in Sırnak

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Abstract

In the present study, threelocal tomato genotypes:Yarbasi, Tepekoyu andFereng (*Solanum lycopersicum*); and one commercial tomato variety: Kamenta $F_{1,}$ were subjected to dry stress (100%, 50%, 25%) through controlled irrigation after the first flowering of the tomato plant. The experiment was carried out with four replicates,and with eightplants per replicate. A statistically significant difference was observed between drought stress administered and the methods of administration compared to the control plants only in the drought stress administered in the amount of chlorophyll. The total yield of the Fereng tomato genotype indicated that both stresses were less affected by 50% and 25% drought stress. It carried out to be significantly reduced (50% and 25%) in both dry stresses.

Keywords: Tomato, Drought stress, Yield, Chlorophyll

Introduction

The tomatooriginates in South America, althoughit has become one of the most important fruits all around the worldin terms of production and consumption, and Turkey is no exception in this regard. In recent years, the yield of tomatoes in Turkey has increased as a result ofdevelopments in seed production and technological advances (Ertürk, 2015).

Due to global warming, the duration and severity of ecosystems in the current state (drought and temperature) is increasing worldwide (Trenberth et al., 2014). Drought and salt stress responses have been reported to have decreasedin melonproduction in connection to such parameters as plant height, stem diameter, number of leaves and leaf area (Kuşvuran, 2010). In a study examining the relationship between the morphological, physiological and biochemical responses of different tomato, eggplant and melon genotypes to drought,it was reported that effective criteria exist for the measurement of tolerance to drought stress based on the scale value, plant age weight, leaf area, leaf water potential and stomatal conductance intomato, eggplant and melon genotypes (Kiran et al., 2015).

In a study of 55 tomato genotypes carried out by Daşgan et al., the samples were classified as tolerant, moderately tolerant or sensitive to drought and salinity. In addition, dry weight, plant total leaf area, stomatal conductance, leaf osmotic potential, leaf water potential, different concentrations in the green parts and root, and the membrane injury index were reported to be the best parameters (Daşgan et al., 2018).

In the present study, physiological and morphological measurements were made of the responses of the different tomato genotypes to drought stress under the climate and soil conditions of Şırnak. The aim in this regard was (1) to investigate the reactions of certain physiological and morphological parameters of tomatoes under two different drought conditions, and (2), toidentify the relationshipbetween the physiological and morphological analysisresults and the measuredtomato findings. The present study will not only help provide an under-

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standing of the drought-stressed physiological mechanisms of tomato plants, but will also serve as a guide for future breeding studies involving tomatoes.

Materials and Method

In the present study, three local genotypes (Yarbasi, Tepekoyu, Fereng) and one commercial variety (Kamenta) of tomato were selected for analysis. The experiment was carried out in the research area of the Sırnak University Faculty of Agriculture, Department of Horticulture. Seeds were sown in viols on March 15, 2019 in a 2:1 peat and perlitesoil, and theseedlings were planted on April 15, in 120 cm rows at 50 cm centers. The plants were harvestedon 31 August, with three replications and eightplantsper repetition. The drought stress application was started 30 days after the planting of the seedlings.

For the experiment, the amount of water given to the plants and the time of irrigation were realized 3 times a week in three different irrigation applications. The water was applied to the plants in line with the evaporation values read daily from the evaporation boiler (Class Apan). In the study, 16 mm branded drip irrigation laterals were positioned at 50 cm centers with a flow rate of 2 L h^{-1}forthe irrigation system. Temperature, humidity and precipitation data was obtained from the Şırnak Regional Metrology Directorate (fig. 1,2), and thetotal amount of water per plant per soil during production was determined (Table 1). The amount of irrigation water given to the plants in the experiment was determined through the following formula:

IR =A* E pan *kcp * P

in which:

IR = Amount of Water Spent (m^3) A= Parcel size (da)

E (pan)=Evaporation (mm) kcp = Number of floors of tomato plant (0.80)

P = Flora % P = Crown width of tomato plant (cm) Row spacing $^{-1}$(cm)

Table 1. Total irrigation water applied to tomato plants in different applications (Liter Plant $^{-1}$)

Application	Before stress (15.04.2019–15.05.2019)	Afterstress (16.05.2019–30.08.2019)	Rainfall *	Total amount of water used
100%	43.6 L	265.62 L	151 L	455.22 L
50%	43.6 L	132.81 L	151 L	327.41 L
25%	43.6 L	66.40 L	151 L	261 L

*Rainfall dates: (01.04.2011–30.04.2011) 97 L-(01.05.2011–31.05.2011) 49L(01.06.2011–30.06.2011) 4L-(01.07.2011–31.07.2011) 1 L

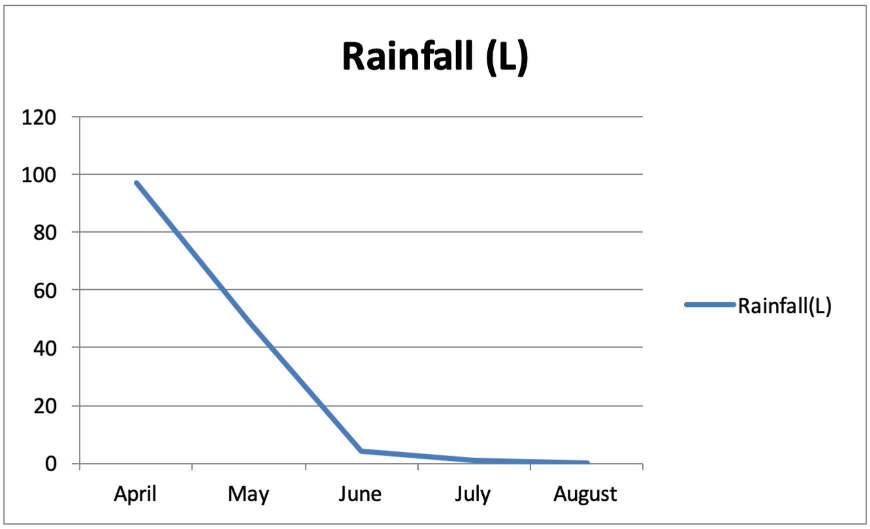

Figure 1. Rainfall values recorded during the experiment

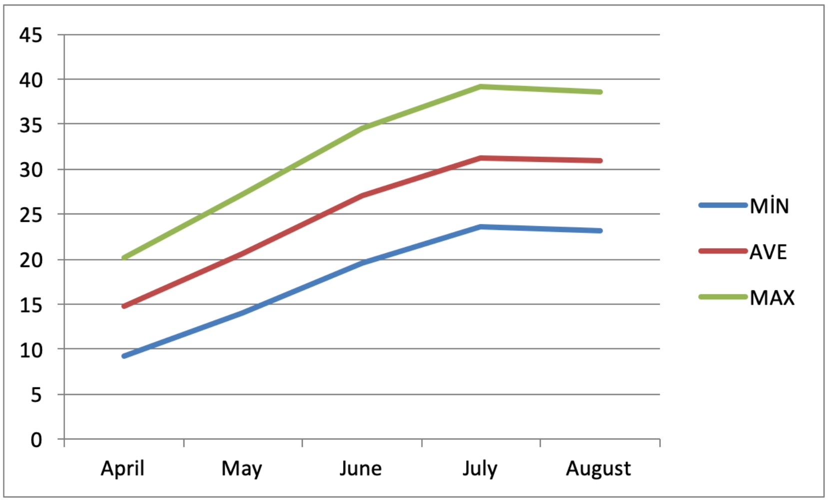

Figure 2. Maximum, medium and minimum temperature values recorded during the experiment

In the present study, the fruit length (mm), fruit diameter (mm), fruit wall thickness (mm), chlorophyll content of tomato leaf, TSS (%), number of fruit (pieces fruit-1), fruit weight (g), total yield (kg HA^{-1}) produced with different irrigation amounts (100% (Control), 50%, 25%) were measured.

The experiment was established carried outin accordance with the experiment design. The data was analyzed using the JMP software package. The differences between the mean values of the investigated properties was determined through an $LSD_{(0.05)}$ test.

Results and discussion

The average values of the different tomato genotypes and the percentage change in values were examined. Thefruit length values corresponding to 100%, 50% and 25% irrigation applicationwere respectively 61.02 (mm) 58.36 (mm) and 56.56 (mm), while theaverage changes were determined as -4.62 at 50% irrigation and -12.13 at 25% irrigation. Table 2 shows that the different drought stress applications are affected according to their own control in tomato genotypes fruit size. Accordingly, when the average values are examined, fruit length is found to decrease by an average of -4.62% in a 50% irrigation application. In the 25% irrigation application, fruit length was found to decreaseby -12.13% as an average among all genotypes (Table 2).An analysis of the data in Table 2 reveals that while fruit length under drought stress was affected most in the Tepekoyu and Fereng genotypes at 50% irrigation, the genotype was the least and most affected genotype at 25% irrigation. The fruit diameter of the studied Tepekoyu tomato genotypewas 67.00 mm in the control sampleand also 67.00 mm in the 50% irrigation application, meaning no-difference between the samples under 50% irrigation and the controls (Table 3). In the study by Daşgan et al. (2009) comparing 50%-restricted PRD open and closed systems and full irrigation open and closed systems in greenhouse hydroponic cucumber cultivation, the effect of the different applications on fruit diameter was reported to be insignificant.Drought and salt stress conditions have been reported to decrease such parameters as plant height, stem diameter, number of leaves and leaf area in melons (Kuşvuran, 2010). The samples most affected by drought stress at 50% and 25% irrigation were the Fereng and Yarbasi genotypes, whereas the most affected genotypes under 50% and 25% irrigation drought stress were the Drape and Kamenta genotypes (Table 4).

Among the studied tomato genotypes, it was found that the mean BRIXvalue was 6.37 in the control, 6.78 in the 50% irrigation and 6.63 in 25% irrigation samples. It can thus be concluded that the ratio of BRIX increases as the level of aridity increases. In an examination of the BRIX values, the highest value was found to be 7.33 in the 25% irrigation sample and the lowest value was 5.53 in the 100% irrigation. Ergun (1994) stated that it is inversely proportional to the water supplied through irrigation (Table 5). In the present study, the tomato fruit weight 50% irrigation% change rate -24.84% genotypes most affecting genotype was Yarbasi and the least affected genotype was Fereng genotype% -11.31% change. For the 25% of the irrigation rate% -36.29% of the most affecting genotype was the Yarbasi, and the least affecting genotype was Ferenge % -24.24% was determined (Table 6). It has been determined that water stress applied to tomato crops causesa drop in both yield and fruit weight (Sanchez Rodriguez et al., 2010; Alp and Kabay, 2017).

Table 2. Tomato genotypes examined under different drought stresses and the effects on fruit length (mm) values and percentages % change according to control

Genotype	100 % irrigation Control (mm)	50% irrigation (mm)	25% irrigation (mm)	50%irrigation %change according to control	25%irrigation %change according to control
Yarbasi	80.91±2.12 a	77.63±2.13 a	74.76±1.54a	-4.05	-7.60
Tepekoyu	64.34±4.79 b	65.26± 0.44 b	52.26±2.65ab	1.43	-18.77
Fereng	52.51± 3.53 c	44.72± 3.18 c	41.43± 2.25 b	-14.83	-21.10
Kamenta	46.33±2.65 c	45.85±2.08 c	57.78±0.57ab	-1.03	-1.03
Mean	61.02	58.36	56.56	-4.62	-12.13
$LSD_{0.05}$	7.29	3.46	4.23	-	-

Table 3. Tomato genotypes examined under different drought stressesand the effects on fruit diameter (mm) values and percentages % change according to control

Genotype	100 % irrigation Control (mm)	50% irrigation (mm)	25% irrigation (mm)	50%irrigation %change according to control	25%irrigation %change according to control
Yarbasi	59.00±1.00b	55.13±0.67b	56.49±1.20b	-6.56	-4.25
Tepekoyu	67.00±1.00a	67.00± 0.69a	61.83±0.80a	0.00	-7.72
Fereng	41.18±0.40c	40.14±0.83c	36.66±0.87c	-2.53	-10.98
Kamenta	35.26±0.58d	33.36±0.71d	33.99±0.62d	-5.39	-3.60
Mean	50.61	48.90	47.16	-3.62	-6.64
$LSD_{0.05}$	1.27	1.26	2.03	-	-

Table 4. Tomato genotypes examined under different drought stressesand the effects on fruit wall thickness (mm)values and percentages % change according to control

Genotype	100 % irrigation Control (mm)	50% irrigation (mm)	25% irrigation (mm)	%50 irrigation %change according to control	%25 irrigation %change according to control
Yarbasi	3.38± 1.00 b	3.06±0.67 c	3.22±1.20b	-9.47	-4.73
Tepekoyu	2.55± 1.00 c	2.38±0.69 d	2.35±0.80 c	-6.67	-7.84
Fereng	3.39± 0.40 b	3.26±0.83 b	3.14±0.87 b	-3.83	-7.37
Kamenta	5.60± 0.58 a	5.23±0.71 a	4.69±0.62 a	-6.61	-16.25
Mean	3.73	3.48	3.35	-6.64	-9.05
$LSD_{0.05}$	0.28	0.19	0.28	-	-

Table 5. Tomato genotypes examined under different drought stressesand the effects on BRIX values and percentages % change according to control

Genotype	100 % irrigation Control	50% irrigation	25% irrigation	50%irrigation %change according to control	25%irrigation %change according to control
Yarbasi	5.53±0.45	6.24±0.08 c	6.64±0.10	12.84	20.07
Tepekoyu	6.66±0.15	6.80±0.005 b	6.62±0.24	2.10	-0.60
Fereng	6.43±0.25	6.91±0.08 b	6.74±0.32	7.47	4.82
Kamenta	6.85±0.27	7.19±0.24 a	7.33±0.10	4.96	7.01
Mean	6.37	6.78	6.63	6.84	7.83
$LSD_{0.05}$	ns	0.22	ns	-	-

ns: not significant

Table 6. Tomato genotypes examined under different drought stressesand the effects on fruit weight (per fruit -1)values and percentages % change according to control

Genotype	w100 % irrigation Control (per fruit $^{-1}$)	50% irrigation (per fruit $^{-1}$)	25% irrigation (per fruit $^{-1}$)	50%irrigation %change according to control	25% irrigation %change according to control
Yarbasi	206.66±3.51a	155.33±1.15a	131.66±9.01a	-24.84	-36.29
Tepekoyu	150.66±5.13b	132.35±10.06b	111.00±3.06b	-12.15	-26.32
Fereng	144.33±6.65b	128.00±5.19 b	109.35±7.76b	-11.31	-24.24
Kamenta	108.66±1.15c	94.00 ±5.29 c	83.66±5.03 c	-13.49	-23.01
Mean	152.57	127.42	108.91	-15.45	-27.46
$LSD_{0.05}$	9.66	13.40	15.46	-	-

An analysis of the average fruit yield values reveals the mean number of fruit in the 50% irrigation application to be 30.25 according to their controls, and 31.00 in 25% irrigation application, as an average of all genotypes (Table 7). Accordingly, the number of tomatoes and fruits increased under both drought stresses as an average of all genotypes when compared to the controls. Generally, as the drought stress of tomato plants is increased, fertilization takes place, continuing the generation of tomatoes, and so the number of fruit increases. It has been found previously in the tomato study of Akhoundnejad (2011),the watermelon study of Karipçin et al. (2008) and the pepper study of Berenyi (1971) that the application of water stress in the cultivation of tomato, pepper and watermelon reduced both the number of flowers and fruit,and that the fruits remain small.

Table 7. Tomato genotypes examined under different drought stressesand the effects on fruit(Plant number-1)values and percentages % change according to control

Genotype	100 % irrigation Control (Plant number^{-1})	50% irrigation (Plant number^{-1})	25% irrigation (Plant number^{-1})	50%irrigation %change according to control	25%irrigation %change according to control
Yarbasi	25±1.00 c	28±1.52 b	24±1.73 b	12.00	-4.00
Tepekoyu	23±1.52 c	27±1.52 b	31±0.5 a	17.39	34.78
Fereng	34±2.51 a	38±0.57 a	34±1.00 a	11.76	0.00
Kamenta	30±2.08 b	28±1.00 b	35±1.00 a	-6.67	16.67
Mean	28	30.25	31	8.62	11.86
$LSD_{0.05}$	2.13	2.30	ns	-	-

ns: not significant

When the average values of the tomato genotypes in the study andthe % change, 100%, 50% and 25% total yield values in irrigation applications, respectively 75,440.25 (plant ha^{-1}) 58,384.25 (plant ha^{-1}) and 48,562.50 (plant ha^{-1}), and considering the% change averages, a decrease of -22.32 was noted in the 50% irrigation samples and of -35.15 in the 25% irrigation samples was determined. It can be understood from Table 9 that the total yield of the different tomato genotypes under different drought stress applications differs from that of the controls. An analysis of the mean values reveals that the total yield from the Fereng genotype subjected to 50% and 25% irrigation reduction was found to be the least affected,at -3.52% and -12.33%, respectively; while the 50% and 25% irrigated Tepekoyu genotype was found to be the most affected, with values of -33.97% and -45.98% (Table 9). Previous studies have found total yield and fruit weight to decrease as drought stress is increased in different melon genotypes (Akhoundnejad and Dasgan, 2019). It has further been reported that a decrease in yield in field crops may be related to the continuation of chlorophyll loss during grain filling, and that different physiological mechanisms in the plant can help determine temperature tolerances under field conditions (Reynolds et al.,2001).

Conclusion

In this study of drought sensitive and tolerant tomato genotypes in the Sırnak province, significant differences were found in fruit size, fruit number, fruit diameter, total yield, chlorophyll content, fruit weight, and tolerant and sensitive varieties. In the present study, the Fereng genotype was found to be stress tolerant in terms of total yield values under 50% and 25% drought stress conditions. It was concluded from the study that the parameters applied to determine the effects of drought stress on tomato genotypes under the conditions in Şırnak are appropriate for the selection of genotypes tolerant to drought stress, and may also be considered in breeding programs in the future for the development of an exclusive line.

Table 8. Tomato genotypes examined under different drought stresses and the effects on chlorophyll (%)values and percentages % change according to control

Genotype	100 % irrigation Control (%)	50% irrigation (%)	25% irrigation (%)	50%irrigation %change according to control	25%irrigation %change according to control
Yarbasi	65.63±2.22b	51.63±0.83c	45.22±2.74b	-21.33	-31.10
Tepekoyu	65.22±1.51b	56.48±0.78c	44.45±1.04b	-13.40	-31.85
Fereng	71.78±0.87a	67.54±0.91a	52.57±0.84a	-5.91	-26.76
Kamenta	68.61±1.29ab	51.48±1.04c	51.78±1.46a	-24.97	-24.53
Mean	67.81	56.78	67.81	-16.40	-28.56
$LSD_{0.05}$	0.45	0.19	2.25	-	-

Table 9. Tomato genotypes examined under different drought stressesand the effects on total yieldvalues (plant ha^{-1}) and percentages % change according to control

Genotype	100 % irrigation Control (plant ha^{-1})	50% irrigation (plant ha^{-1})	25% irrigation (plant ha^{-1})	%50 irrigation %change according to control	%25 irrigation %change according to control
Yarbasi	85630±3356a	64857±2641a	51290±2179b	-24.26	-40.10
Tepekoyu	76568±1208b	50556±856b	41361±1877c	-33.97	-45.98
Fereng	70043±1717c	67580±882 a	61410±1349a	-3.52	-12.33
Kamenta	69520±946c	50380±796 b	40189±1895c	-27.53	-42.19
Mean	75440.25	58384.25	48562.5	-22.32	-35.15
$LSD_{0.05}$	3372	3020	2098	-	-

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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Research Article

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Determination of fatty acid profile and antioxidant activity of Rosehip seeds from Turkey

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Abstract

Rosa canina is a less known horticulture plant that its fruits are the source of phytochemicals and have medicinal properties. However, it can be cultivated commercially like other fruit trees. In this study, fatty acid composition and antioxidant capacity of rosehip seeds were investigated. The results indicated that seeds are rich in Omega-6 fatty acids (51.49%). Linoleic acid, oleic acid, α-linolenic acid, and palmitic acid are the main fatty acids in rosehip seed oils, respectively. Moreover, the ratio of ω-6 fatty acids: ω-3 fatty acid ratio which is in desirable range for qualified oil. The seeds also showed high antioxidant activity (130.64 mgTE/100g).

Keywords: Rosehip, Fatty acids, Omega-6, Antioxidant activity

Introduction

Recently, the interest for plant origin oils has increased due to high nutritious value (Nwosu et al., 2017). Processing of fruits by-products and wastes such as seeds can lead to evaluate the new source of oil for the fulfillment of growing population needs (Kamel et al., 1985).

Rosa canina L. (Rosehip or dog rose) belongs to the Rosaceae family, *Rosa* genus which has around 100 species distributing in North America, Europe, the Middle East, and Asia (Ercisli, et al., 2007). Rosehip species is resistant to environmental stress factors and can be grown at low soil fertility or a harsh climate wherever from valleys to high altitude plateaus, even above 1,500 m altitude (Okatan et al., 2019). Among all, 25 species are spread throughout Turkey (Ercisli, 2004). Turkey has different climatic characteristics, therefore, viticulture activities are distributed to different geographical regions and these differences directly affect biochemical compounds of plants (Gundesli et al., 2018). Two species of *Rosa* are common species in Turkey, *Rosa canina* L. (dog rose, rosehip) and *Rosa damascena* Mill. (damask rose). Rosehip (dog rose) fruits are a rich source of healthy contents such as polyphenols, sugars, organic acids, bioflavonoids, carotenoids, tocopherol, vitamins, minerals, tannins, amino acids, pectin and volatile oils (Cınar and Colakoglu, 2005, Ercisli et al., 2007). Polyphenols as the secondary metabolites of plants promote health. It is proved that these compounds have anti glycemic, antiviral, anticancer and anti-inflammatory activities and also antiallergic and antimicrobial properties (Gundesli et al., 2109). Rosehip fruits have been used in folk medicine and have economic value (Ercisli, 2005). Due to having laxative and diuretic properties, rosehip fruits are useful for regulating the menstrual cycle (Nojavan et al., 2008). The fruits are also used for the treatment of flue, inflammatory disease and infections (Demir et al., 2014). Rosehip fruits are consumed in different forms such as marmalades, tea, jellies and jams and also as dried fruits (Yildiz and Alpaslan, 2012). Rosehip fruits are the rich source of antioxidant compounds such as α-tocopherol, ascorbate, glutathione, β-carotene, anthocyanins and other phenolics (Tumbas et al., 2012). Antioxidant nutrients play an important role in controlling free radicals. Degenerative diseases such as cancer, cardiovascular and nervous diseases are caused by free radicals that are produced by normal metabolism conditions or external factors in the body (Zarifikhosrohahi et al., 2018). Although the seeds of Rosehip have both monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), more than 50% of total lipid is composed of polyunsaturated

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fatty acids. The major fatty acids in the rosehip are linoleic acid, linolenic acid, oleic acid, palmitic acid and stearic acid (Fofana et al., 2013). It is proved that oleic acid from the group of MUFAs decreases triacylglycerol of plasma and cholesterol concentrations (Murathan et al., 2016). PUFAs are also effective in the prevention of cancer, atherosclerosis, diabetes and heart disease (Gogus and Smith, 2010). So, the risk of cardiovascular disease can be reduced if 20% to 30% of daily intake comprised of plant fatty acids (Engelfriet et al., 2010).

In this study, it was aimed to determine the fatty acid profile and also antioxidant activity of rosehip seeds obtained from a commercially producing rosehip oil company to evaluate the oil quality for medicinal besides industrial usage.

Materials and methods

Rosa canina L. seeds provided by the commercial company were used in this study. The analyses were done at 5 replicates and the approximately 450-gram seed was used.

Oil extraction

The extraction of total lipids was done by an automatic soxhlet device. One hundred fifty grams of dried were used for oil extraction. The solvent was hexane and extracted oil was weighted for determination of the oil percent in the samples.

Determination of fatty acids

The fatty acids were analyzed by a GC ((Perkin Elmer, Shelton, USA). Chromatographic separation was done using a (30 m×0.25 mm) column equipped with a flame ionization detector (FID). The oven temperature was 120 ºC for 2 min, raised to 5 ºC /min to 220 ºC, which was held for 10 min, while the injector and the detector temperatures were set at 280 ºC and 260 ºC, respectively. The results were expressed in GC area % as a mean value and ± standard deviation.

Determination of DPPH radical scavenging capacity

The free radical scavenging activity of the seed extracts was analyzed using 2,2-diphenyl-1- picrylhydrazyl (DPPH) assay (Kostiæ et al., 2013). The color loss of DPPH solution was measured at 517 nm scavenging the reaction of DPPH radical with the sample. One hundred microliters of methanolic extract of seed samples were reacted with fresh methanolic DPPH solution and incubated 30 min at room temperature. Then, the absorbance was read at 517 nm against the blank. The ability of the extracts to inhibit DPPH (% RSC) was computed from the decrease in absorbance. The data were expressed as milligram of Trolox equivalent (TE) per 100 g of sample.

Results and Discussion

Total fat, fatty acid composition and antioxidant activity of Rosehip seeds are shown in Table 1. The total lipid of analyzed rosehip seeds was determined as 5.56 %. Kazaz et al., (2009) and Ilyasoglu (2014) reported the total lipid of *Rosa canina* seeds as 7.15 and 6.29%, respectively. Ercisli et al., (2007) determined that the total fat of *Rosa canina* fruit flesh without seed is 1.78%. Murathan et al., (2016) studied the total fat of rosehip species fruit with seeds between 5.38-7.84%., *Rosa canina* as 6.92%. Furthermore, Szentmihalyi et al., (2002) reported the oil content of rosehip from different extraction methods such as Soxhlet extraction, ultrasound water bath, microwave extraction, supercritical fluid extraction and subcritical fluid extraction as 4.85, 3.25, 5.26, 5.72, 6.68%, respectively. As mentioned, the results of this study are confirmed by the findings of previous studies and are at the same range, although this study result is lower than Kazaz et al., (2009), Ilyasoglu (2014) results are higher than Szntimihalyi et al., (2002). Murathan et al., (2016) reported higher total fat of fruit because used both fruit flesh and seeds together. The total fat amount may be affected by the extraction method (Szentmihalyi et al., 2002) and environmental conditions (climate and altitude, etc.) (Ilyasoglu 2014).

The results showed that linoleic acid, oleic acid, and α-linolenic acid and palmitic acid are the main fatty acids in rosehip seed oils, respectively (Figure 1). Rosehip seeds had a high amount of total poly saturated fatty acids comprising Linoleic acid (51.49%), α-Linolenic acid (13.46%) and g-linoleic acid (0.07%). Linoleic acid, the omega-6 fatty acid, is known as an essential fatty acid because the human body cannot synthesize it. Therefore, rosehip seed oils are greatly nutritious oils reducing serum cholesterol and can be used for the remedy of cardiovascular disorders (Nicolosi et al., 2004, Manzoor et al., 2007). Omega-6 fatty acids: omega-3 fatty acid ratio is the scale of oil value for being healthy by controlling blood cholesterol. The accepted range is 1:1 to 4:1 (Yehuda, 2003). According to this study result, the ratio of 3.82 is in the ideal ratio and makes the rosehip seed oil as a valuable candidate for omega fatty acids. While the results of linoleic acid in this study are similar to the results obtained Ilyasoglu (2014) (54.05), the results from Kazaz et al., (2009) (48.84%) and Murathan et al., (2016) (27.97%) were lower. Comparing Ercisli et al., (2007) results showed that that the amount of linoleic acid in fruit flesh (51.18%) and the seeds from this study are (51.49%) compatible. Szentmihalyi et al., (2002) reported the oil content of rosehip from different extraction methods between 35.94% and 54.75%. Comparing the results obtained from the method used in this study showed that the result also is higher than data from Szentmihalyi et al., (2002). However, the amount of α-Linolenic acid in this study is lower than previous studies except Murathan et al., (2016) study.

Ten saturated fatty acids were detected at rosehip seed oil in this study which the main ones were palmitic acid (4.22%) and stearic acid (2.66). The results of saturated fatty acids are in the range of data obtained from other studies (5.031-11.06%). The amount of total saturated fatty acids (8.81%) was lower than poly saturated fatty acids (87.65%) in studied rosehip seed oil. According to previous studies PFA is higher than data obtained from this study Ercisli et al., (2007) (91.27% of fruit flesh), Kazaz et al., (2009) (91.63%), Ilyasoglu (2014) (92.92%). The oleic acid (22.49%) was the predominant monosaturated fatty acid in rosehip seed oil which is in accordance with previous studies. The differences among studies may be due to different cultivar and genotypes used along with different oil extraction methods. DPPH radical scavenging capacity of studied rosehip seed was determined as 130.64 mgTE/100 g. There is limited study on the antioxidant capacity of rosehip seeds. Ilyasoglu (2014) reported that the antioxidant activity of methanolic extract of rosehip seeds was 10.40 µmol TE/g (260.3 mgTE/100 g) with TEAC method. Okatan et al., (2019) studied DPPH

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antioxidant capacity of different genotypes of rosehip fruits. They reported the average DPPH activity of rosehip fruits as 169.1 µg/ml. The results showed that the antioxidant capacity of studied rosehip was lower than Ilyasoglu (2014) which may be due to different genotypes besides different methods. Roman et al., (2013) studied the antioxidant activity of *Rosa canina* biotypes whole fruits from Transylvania. The researchers reported that DPPH antioxidant activity among biotypes varied from 63.35 to 127.8 µM Trolox/100 g. The result of this study is in this range that proves different biotypes and locations affect the antioxidant capacity of fruits.

Table 1. Total fat, fatty acid composition and antioxidant activity of Rosehip seeds

DPPH	130.64 mg TE/100 g
Total fat	5.56 g/100 g
Myristic Acid (C14:0)	0.23±0.001
Palmitic acid (C16:0)	4.22±0.12
Stearic acid (C18:0)	2.66±0.08
Arachidic acid (C20:0)	1.20±0.04
Caprylic acid (C8:0)	0.05±0.002
Behenic acid (C22:0)	0.20±0.01
Tricosanoic acid (C23:0)	0.04±0.001
Lignoseric acid (C24:0)	0.10±0.004
Margaric Acid (C17:0)	0.08±0.003
Pentadecanoic acid (C15:0)	0.03±0.001
ΣSFA	8.81
Palmitoleic acid (C16.1)ω−7	0.05±0.002
Oleic acid (C18:1n9c)ω−9	22.49±0.17
Eicosenoic acid (C20:1n9c)ω−9	0.09±0.003
ΣMUFA	22.63
a-Linolenic acid (C18:3n3) ω−3	13.46±0.002
Linoleic acid (Cl8:2n6c) ω−6	51.49±0.35
g-Linolenic acid (Cl8:3n6c) ω−6	0.07±0.002
Σ PUFA	65.02

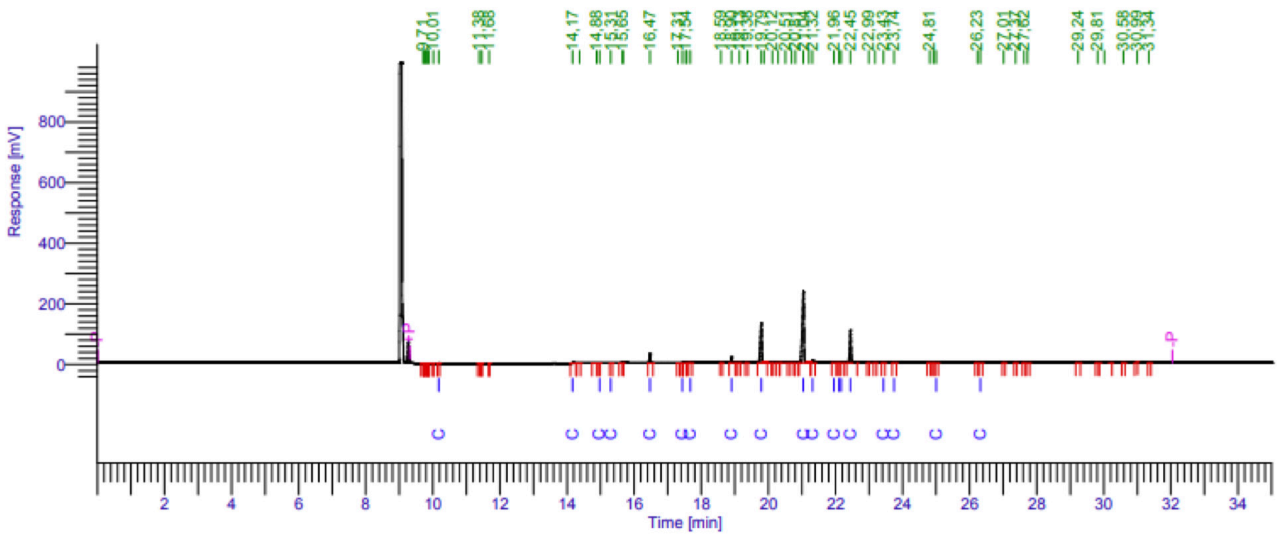

Figure 1. The chromatogram of fatty acid profile of *Rosa canina* L

Conclusion

The results of this study proved that the rosehip seeds from the commercial company producing rosehip oil are a good source of high omega-6 fatty acid. Moreover, the seeds have high antioxidant capacity which is the key to controlling free radicals causing cancers and inflammatory disease. Therefore, seed oil can be evaluated as a highly notorious and healthy source in the human diet and also as bio-fuels.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

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Research Article

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Determination of the factors affecting utilization rate of Eastern Anatolian semiarid public rangelands in Turkey

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Abstract

Rangelands are important natural resources for the nations with a various measurable and immeasurable outputs such as forage for farm animals, biological diversity, soil and water conservation and ecosystem functions. However, unconscious exploitation has resulted in weakening, deterioration and exhaustion of these natural resources in time. In achieving an effective, sustainable use at a minimum environmental cost without foregoing economic and social development, policy measures towards conscious utilization, conservation and restoration of these resources are of vital importance. Furthermore, user-friendly, robust policy measures require correct scientific information on the actual utilization of rangelands. The effects of various natural and human induced factors on the rangeland forage yield and its utilization rate were researched in this study. Data were collected from the rangelands of 11 villages in five districts of Erzurum province, Turkey. Descriptive statistics and mixed effect panel regression models were used in data analysis. According to the results it was concluded that 1) because of heavy grazing pressure versus low forage production, high-altitude sites, east and southwest slopes should specifically be given the priority in rangeland rehabilitation studies, 2) drought resistant species should be preferred for the overseeding practices due to the xeric nature of southerly slopes, 3) to avoid excessive exploitation and to realize balanced utilization in all rangeland sites, grazing plans should be developed and strictly followed by each village authority, and 4) Heavy grazing pressure on rangelands gets even worse in drought seasons. Therefore, rangelands should be relatively lightly utilized in such seasons not to cause herbage yield losses and other unwanted outcomes in subsequent years.

Keywords: Conscious utilization, Sustainable use, Natural and human induced factors, Mixed effect panel regression

Introduction

Eastern Anatolia region in Turkey has favourable conditions for animal production due to its vast meadow and rangeland asset. Rangeland dependent extensive animal production has been a way of livelihood generation in the region for centuries. The grazing farm animals in the region include indigenous breeds and their crosses with commercial ones, which are well adapted to regional geo-climatic conditions and utilize the rangeland more efficiently. The proportion of the purebred animals is low in the region. In Turkey, rangelands are commonly used vegetation covers, whose rights are left to the legal entity of each village with certain demarcation by the laws. Village flocks and herds graze separately under the supervision of herders or shepherds with daily excursions starting with sunrise and ending with the sunset (Kara et al., 2014).

The basis of the developing grazing plans, indicating how long and how many animals to be kept in rangelands, is to determine or estimate quantity of the forage to be grazed. For sustainable use it is of vital importance to know how much of the forage to be grazed without damaging the rangelands. A grazed, trampled or destroyed part of rangeland forage has been reported to be a measure of utilization for given rangeland, and its share in total production is described as rangeland utilization rate. It is suggested that utilization rates of rangelands should be justified according to rangeland condition. For example, utilization rates of 20–30% for alpine tundra, 35–

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45% for western mountainous rangelands, 40–50% for short grass prairies, 45–60% for tallgrass prairies, and 45–55% for cool season grasslands have been recommended (Vallentine, 1990, cited in Gökkuş and Koç, 2001). Accordingly, 25–30% and 30–40% of utilization rates were suggested for poor and moderate condition rangelands and 50–55% of utilization rate was recommended for very good condition rangelands (Gökkuş and Koç, 2001).

Rangeland forage and its utilized proportion, namely the utilization rate or factor are all affected by a number of natural (e.g. geographic aspect) and human induced factors (e.g. stocking rate). In Turkey, a considerable number of invaluable studies were conducted on rangelands. Yet mainly botanical composition was handled and the studies seeking to determine the forage production were limited. In some related studies, production potential and/or utilization degree of the different rangeland sites (e.g. hills, hillsides) was indirectly categorized (i.e. poor, moderate, heavy, excessive, etc.) considering some indicators (e.g. canopy cover, rangeland condition, proportion of some certain plant species). Utilization rate and the factors effective on it were out of their scope and/or not handled in a comprehensive manner. In their study, Kara et al. (2019) estimated the rangeland dry forage yield and utilization rate but they did not mention how they were affected under various human induced and natural rangeland related factors. The present paper aimed to answer these questions using the same study data. To this end, rangeland forage production and its utilization rate were examined under the effect of various rangeland properties, such as altitude, distance to village, stocking rate, rangeland condition, and geographical aspects. Study findings will be expected to provide valuable information for the future rangeland and animal related studies, not only in Turkey but also in countries sharing similar agroecological conditions, cultural and historical backgrounds of rangeland use pattern.

Materials and Methods

Materials

The primary material of this study was obtained from the vegetation surveys and the forage harvested from cages and random quadrats from the 12 permanent representative sites in the rangelands of 11 villages in Erzurum province, Turkey. In addition, the relevant records of the official institutions related to the study were used as secondary material.

Study Area

The study area covers Erzurum province that reflects the main characteristics of the Eastern Anatolia region of Turkey regarding geography, climate, production type, and pattern (Figure 1). This region is known for its suitability for livestock production due to its one-third share in total rangeland asset of Turkey. That is, the rangelands have determined the way of livelihood generation and extensive livestock production system has prevailed for centuries in the region. It has very rugged geography and very harsh terrestrial climate and is located within the 39 54' 31'' northern latitudes and 41 16' 37'' eastern longitudes. Altitude is ranging from 2000 m asl in plateaus to 3000 m asl and higher in the mountains and can be as low as 1000-1100 m asl in valley floors and 1500 – 1800 m asl in plains. Despite the existence of plain areas, the topography is mostly fragmented, and the dominant vegetation is steppe grasses (60%) as woodland is scarce (%6). Winters are long and harsh, and summers are short and hot. In a long term (1975 to 2006), the average number of frozen days and the days with snow cover are 154 and 113 days, respectively, while annual average temperature and total precipitation are 5.5 °C and 453.3, respectively (TÜMAS, 2013). Annual precipitation was 436.6 and 317.8 mm for the study years of 2007 and 2008, and seasonal precipitation for April-October period was 308.5 and 234.1 mm respectively. Thus, the year 2008 was distinctively drought with negative balances of 74.4 and 118.8 mm corresponding annual and seasonal precipitations respectively.

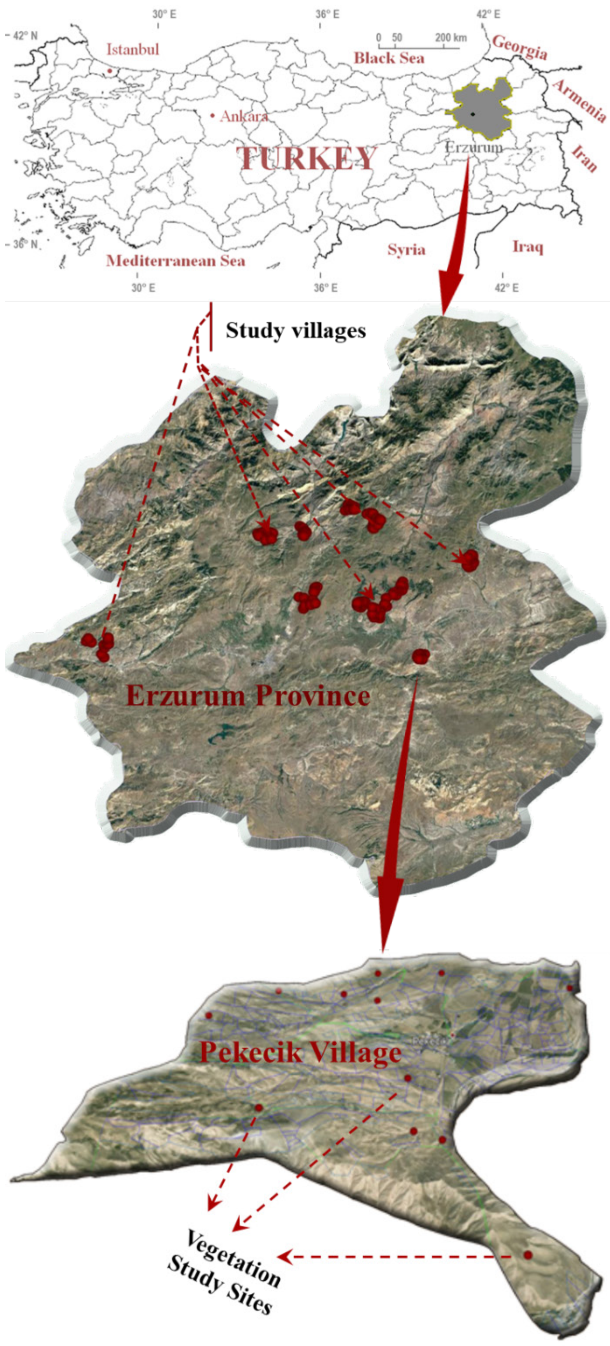

Figure 1. The study area in Turkey
Source: Adapted from Kara et al. (2019)

Selection of the villages

Study villages were selected with a special emphasis to represent over the surrounding area, to ensure that they were free from nomadic movements and boundary problems, and that their rangeland demarcation and allocation studies have been completed. Thus, from Aşkale, Narman, Pasinler, Köprüköy, Horasan, and Tortum districts in a total of 11 villages were selected for the study. The distance among the villages varied from a minimum of 7.9 km to a maximum of 126.5 km. The villages share more or less similar production patterns but differ from each other regarding the acreage of rangelands and the total animal asset. As seen in Table 2, animal asset in villages fluctuates over years which could be explained with the change in number of inhabitants and the policy measures. That is, migration to urban areas especially from mountain villages decrease the number of inhabitants (farmers) and, in turn, number of grazing animals in time. On the other hand, number of animals may increase due to policy measures towards encouraging animal production which conversely cause increased stocking rates in the same villages. So, the stocking rate differences among the villages and within the same village in time are an expected phenomenon.

As mentioned earlier, village rangelands in Turkey are in common use and grazing is not managed according to the herbage production and rainfall. In a private farm with a private rangeland property, farmer considers the optimum stocking rate for better use of his or her rangeland. In common use, however, rangelands are used in an opportunistic manner by ignoring their capacity.

Vegetation studies

The vegetation studies were performed in 2006 to characterize the rangeland vegetation and calculate the condition of the rangelands. Vegetation was studied using modified wheel point method according to Koç and Çakal (2004) at representative 12 permanent sites in each study village with four replications along the 100 m transect lines in main directions (Figure 2 and 3).

Table 1. Rangeland, animal asset (in animal units), and rangeland stocking rates of the study villages

Villages in the Study Area	Rangeland Asset[1] (ha) (a)	Animal Asset (AU)[1] 2007 (b)	Animal Asset (AU)[1] 2008 (c)	Stocking Rate ($AU.ha^{-1}$) 2007 ($d=b\times a^{-1}$)	Stocking Rate ($AU.ha^{-1}$) 2008 ($e=c\times a^{-1}$)
Köşk	7349	1160	1418	0.158	0.193
Taşağıl	1177	518	600	0.440	0.510
Yeniköy	576	674	606	1.170	1.052
Yayladağ	452	538	510	1.191	1.128
Demirdöven	430	1159	832	2.690	1.935
Pekecik	217	111	239	0.512	1.101
Gerek	2138	734	941	0.340	0.440
Şehitler	883	716	718	0.811	0.813
Esendurak	191	79	140	0.412	0.733
Tipili	1548	330	442	0.213	0.286
İncedere	595	245	327	0.412	0.549
Total	15556	6264	6772	0.759	0.795

Figure 2. Vegetation study using modified wheel point method[2]

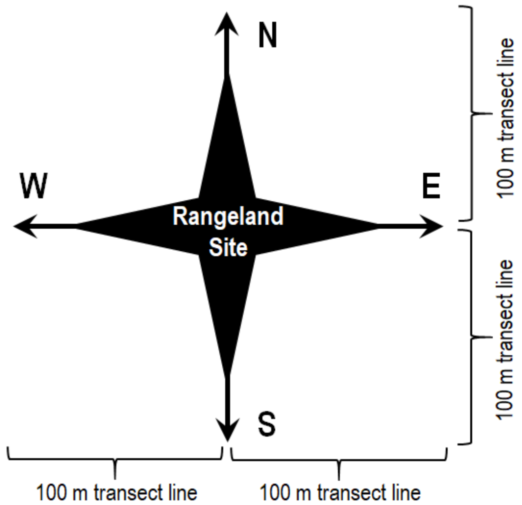

Figure 3. Vegetation reads in rangeland sites

[1]Obtained from the official records of the directorates of agriculture operating in the study area

[2]Wheel-point method (WPM) of Griffin (1989) is based on a rimless wheel apparatus. It is rolled along the transect line on its spokes by the aid of a handle attached to it. A counter on the apparatus counts the number of revolutions the wheel has made. Two opposite spokes are tapered, and the ends of the rest are covered with rubber buffers to make rolling easier over the ground. This method was modified by replacing the original 5 cm diameter measurement unit with the rings holding an area of 3.14 cm2 as in loop method in order to make it efficient in the areas exposed to erosion (Çakal et al., 2012). In modified version of this method, the rimless wheel has a radius of 31.85 cm to make 200 cm perimeter allowing two reads with a-one-meter intervals. Thus, 50-wheel revolutions sum up 100 reads in a 100 m-line transect.

Calculation of rangeland condition

The rangeland condition is described as the comparison of the existing state of the rangeland vegetation cover at a given site with the best possible state under the similar prevailing conditions (Koç et al., 2003). By employing the vegetation study data from each site in every studied village, the rangeland condition was calculated according to the Rangeland

Quality Degrees, explained by De Vries et al. (1951), cited in Koç et al. (2003), using Equation 1.

$$R_{Condition} = \sum P_i \times QS_i \qquad [1]$$

Where P_i denotes the relative abundance of i^{th} species, calculated as the proportion of individuals of i^{th} species to the total number of individuals coincided at the studied site, and QS_i symbolizes the quality rating of the i^{th} species (Kara, 2019). It expresses the values given to each species according to the grazing and productivity attributes of the coincided species such as productivity, post-grazing regeneration ability, and palatability and varies between –1 and 10. In this qualification, a toxic plant receives –1 point and a score between 1 and 10 indicates the degree of other desirable properties (Koç et al., 2003; Altın, 2001; Koç and Gökkuş, 1996). In this method, vegetation cover is accepted as the product of climate and soil, as such that the information on climax vegetation is not needed. In this method, the rangeland condition, i.e., rangeland quality degree, varies between 1 and 10 and is classified as 1–2: very poor, 3–4: poor, 5–6: moderate, 7–8: good, and 9–10: very good condition (Koç et al., 2003).

Calculation of the rangeland utilization rate

Rangeland utilization rate is the percentage of forage grazed or removed by animals out of the total forage produced by rangeland to fulfil the condition not to cause rangeland degradation (Gökkuş and Koç, 2001). 132 cages (one cage at each of 12 permanent rangeland sites in each of 11 study villages) with 1 m height and 1 m × 1 m floor area were placed in the rangelands of study villages before the grazing season of 2007. Lost and disassembled cages were fixed and completed to 132 before the grazing season in 2008. Forage under the cages was clipped to the ground at the end of the grazing seasons to estimate the seasonal rangeland forage production. Unavailable observations due to the lost or disassembled cages were treated as missing data.

In estimating the forage removed by grazing animals, rangeland stubble was sampled by clipping to the ground using four random quadrats (equivalent to cage floor area, e.g. four quadrats = 1.0 m^2) in surrounding areas of each cage at the end of the grazing seasons. The harvest weights of the forage and stubble and their dry weights after dehydration at 70 °C for 48 h in an oven were recorded.

As also reported elsewhere (Kara et al. 2019), rangeland utilization rate was calculated based on the weight of the stubble left after grazing. To this purpose, first, the utilized rangeland forage was calculated by subtracting stubble from the forage yield and converted to per hectare yield. Finally, the utilization rate was calculated by dividing the forage utilized by the total forage yield (Gökkuş and Koç, 2001).

Data analysis

As mean averages rangeland forage production and utilization rate were presented elsewhere. In this paper were examined the effects of some natural and human induced factors on the rangeland dry forage yield and utilization rate. The variables of interest are detailed in Table 3. Of all the variables, stocking rate was calculated using the secondary data obtained from the provincial and district directorates of agriculture operating in the study area while the rest was obtained from the vegetation studies.

It is very easy to clarify the effect of independent variables on dependent variables when linear estimators, i.e., regression coefficients, are used. However, in order to control the heterogeneity due to the entities considered in the study, panel data regression is suggested for unbiased results (Baltagi, 2005). In the present study, the data were collected from a considerably wider area, which covers the rangelands of 11 villages. The distance from the studied rangeland sites to the village varies from 360 m to 6790 m as the villages are apart from each other from a minimum of 7.9 km to a maximum of 126.5 km. Thus, due to the inevitable heterogeneity the panel data regression was suitable for data analysis.

The most prominent techniques used to analyse panel data are fixed effect (FE) and random effect (RE) models.

Table 2. The details of the study variables

Variable	Explanation
DRY Forage Yield	Rangeland dry forage yield (kg.ha^{-1})
Utilization Rate	Utilization rate of the studied rangeland site (%)
Rangeland Condition	Rangeland condition of the rangeland sites (as the fragment of 10)
Altitude	Altitude of the studied rangeland sites (m)
Distance	The distance of the studied rangeland site from the village (m)
Stocking Rate	Stocking rate at village rangelands (AU per hectare)
Bare Ground	Bare ground percentage of the studied rangeland sites (%)
Legumes	Number of legume species in the botanical composition of the rangeland sites (in number)
Grasses	Number of grass species in the botanical composition of the rangeland sites (in number)
Forbs	The number of forb species in the botanical composition of the rangeland sites (in number)
Species Richness	Number of herbaceous species encountered at the studied rangeland sites (in number)
Species Abundance	Number of individuals per species encountered at the studied rangeland sites (in number)
Grazing Season	Grazing season (2007= the first year; 2008=the second year)
Geographic Aspect	Geographical aspect of the studied rangeland sites (1 = Flat; 2 = North; 3=South; 4=East; 5=West; 24= Northeast; 25=Northwest; 34=Southeast; 35=Southwest)

FE explores the relationship between predictor and outcome variables within an entity (country, person, company, etc., herein, permanent rangeland sites). Each entity has its own individual characteristics that may or may not influence the predictor variables. Unlike the FE model, the variation across entities is assumed to be random and uncorrelated with the independent variables in RE model (Torres-Reyna, 2007). Yet, in case RE model is appropriate as in the present study, the mixed linear model is suggested to incorporate both fixed and random variables (Cameron and Trivedi, 2010; Adkins and Hill, 2011). For that reason, the mixed linear model is employed in data analysis as descriptive statistics methods were also employed to summarize the variables.

Table 3. Geographical aspects of the studied rangeland sites

Geographical Aspects	Frequency	%
Flat	18	13.6
North	20	15.2
South	34	25.8
East	13	9.8
West	9	6.8
Northeast	14	10.6
Northwest	7	5.3
Southeast	14	10.6
Southwest	3	2.3
Total	132	100.0

Mixed effect linear regression model can be written in the form of Equation 2 (Torres-Reyna, 2007; Cameron and Trivedi, 2010; Gujarati, 2011).

$$Y_{it} = \alpha + \beta_1 X_{1it} + \beta_2 X_{2it} + \cdots + \beta_k X_{kit} + u_{it} + \varepsilon_{it} \quad [2]$$

Y_{it} represents a dependent variable
X_{it} represents explanatory (independent) variables
α represents intercept
β_1 to β_k represent slope coefficients
k represents k^{th} coefficient
i represents i^{th} individual
t represents the time
u represents between entity/individual error term
ε represents within entity/individual error term

In the regression models, the categorical variables were represented by dummy variables, in number less by one than the classification of the qualitative variable (Gujarati, 2011). In this study, the variable of the geographical aspects had nine categories including flat (zero) aspect. Thus, it was represented by eight dummies and four of them were for main directions and four were for intermediate directions. Since the dummy variable for flat aspect was not included in the regression model, the coefficients of other dummy variables should be interpreted in relation to the reference category (flat aspect) as the coefficients of other continuous independent variables represent the marginal change in the dependent variables as a result of one-unit change in the continuous dependent variable of interest at *ceteris paribus*.

The rangeland dry forage yield and its utilization rates were considered to be the functions of the continuous and discrete variables given in Table 3. Skewness-Kurtosis test and graph methods were used to control the normality of the residuals of the model (Gujarati, 1995; Torres-Reyna, 2007; Park, 2008; Gujarati, 2011; Adkins and Hill, 2011). The data analysis was performed using Stata SE 14.2 software package (StataCorp, 2015).

The normality assumption was failed according to the Skewness-Kurtosis test. However, according to the normal probability plot distribution of the residuals obtained and the fact that the normality test may determine statistically significant yet negligible deviations from normality (Anonymous, 2013), the deviations from the normal line can be omitted since they have no real effect on the linear regression tests. As a matter of fact, it becomes more difficult to meet the normality assumption with a larger sample size, since even small differences are detected. Therefore, it can be accepted that normality may be a problem when the sample size is small (Lumley et al., 2002). Due to using short panel of two years, cross-sectional dependence or contemporaneous correlation and serial correlations were not tested in our study as these need to be addressed only in macro panels with long time series data (Baltagi, 2005; Torres-Reyna, 2007). However, heteroskedasticity in the error term is reported to be one of the commonly encountered problems in cross sectional data and Robust Standard Errors procedure is suggested to cure heteroskedasticity problem (Torres-Reyna, 2007; Gujarati, 2011; Adkins and Hill, 2011). Accordingly, I employed clustered robust standard errors procedure in regression analysis since the studied sites were clustered geographically (Table 3).

Results

Rangeland properties

Some of the rangeland properties based on the vegetation data collected from the 132 sites in the rangelands of 11 villages in the study area are presented in Table 4. Because mentioned elsewhere (Kara et al., 2015), vegetation study results, e.g. the species encountered, and their family groups were not given here to avoid repetition. The rangeland condition of the study area falls within the poor to moderate condition classes, making an average of 3.23 score in fraction of 10 (Table 4).

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Rangeland dry forage yield

According to mixed effect panel data regression model (Table 5), grazing season (year) and stocking rate had significant negative effects (p<0.01) on rangeland dry forage yield. The rangeland condition, distance to village, species richness, number of species for legumes and grasses, bare ground and altitude × distance interaction did not significantly affect the rangeland dry forage yield. The model suggests that shifting from the first to the second year of the study, dry forage yield decreases 459 kg per hectare and a one-unit increment in stocking rate likely causes 354 kg decrease in dry forage yield per hectare. As for geographical directions, northwest slopes yielded very significantly (p<0.01) and east slopes yielded significantly (p<0.05) less dry forage per hectare in contrast to flat (zero) aspect, i.e. northwest slopes yielded 603 kg and east slopes yielded 204 kg less in contrast to reference flat aspect (Table 5). Similarly, southwest slopes also yielded less than the reference aspect, but the difference was only marginally significant (p<0.1). Moreover, northeast and southeast slopes yielded more as north, south and west slopes yielded less dry forage than reference flat aspect (Figure 3a), yet the differences with the reference were not significant (p>0.05).

Table 4. Some of the rangeland properties reported for the studied sites in village rangelands

Variables		Observations	Minimum	Maximum	Average	St. Deviation
Altitude (m)		131	1593	2847	2088.27	269.48
Distance to Village (m)		129	360	6790	2354	1374.10
Bare Ground (%)		132	5.00	49.00	25.61	9.57
Species Richness		132	8	28	19.09	4.12
	Legumes	*132*	*0*	*7*	*3.29*	*1.66*
	Grasses	*132*	*1*	*7*	*3.86*	*1.18*
	Forbs	*132*	*3*	*19*	*11.95*	*3.07*
Species Abundance		132	51	95	74.39	9.57
	Legumes	132	0	43	14.26	8.85
	Grasses	132	6	40	20.93	6.26
	Forbs	132	11	63	39.20	9.78
Rangeland Condition		132	1.89	5.06	3.23	0.66
Stocking Rate		22	0,16	2,69	0,78	0,61
Rangeland dry forage yield		193	130	7200	1012,3	826,1
Rangeland dry stubble yield		193	11	2036	310,8	308,1
Utilized rangeland forage		193	0	6401	701,6	681,1
Rangeland utilization rate		193	0	96	69,1	19,7

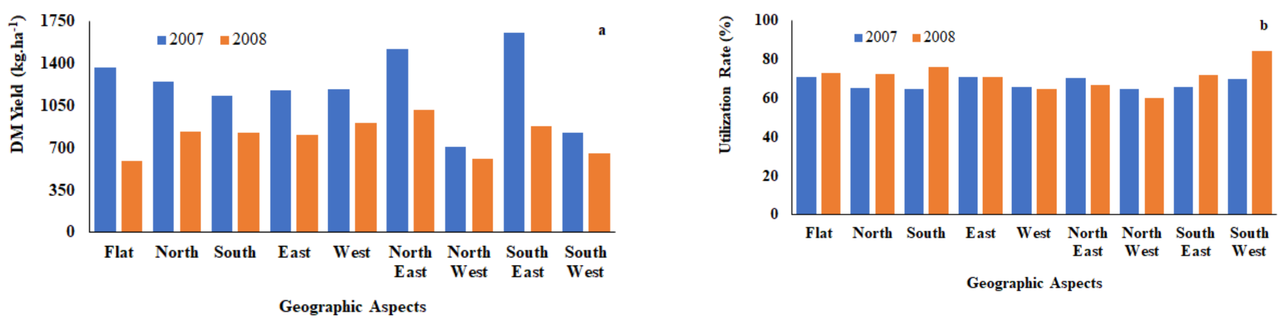

Figure 3. Rangeland forage yields (a) and utilization rates (b) by the aspects of studied site

Table 5. The results of mixed effect regression analysis for the factors affecting rangeland dry forage yield

Independent Variables		Coefficient	Robust Std. Err.	z	P>|z|
Grazing Season (Year)		-458,519	90,426	-5,070	0,000
Altitude		-0,745	0,579	-1,290	0,198
Rangeland Condition		256,453	260,026	0,990	0,324
Distance to Village		-0,254	0,308	-0,830	0,409
Altitude × Distance to Village		0,000	0,000	0,770	0,438
Stocking Rate		-350,089	115,485	-3,030	0,002
Bare Ground		-2,805	8,152	-0,340	0,731
Species Richness		34,552	24,612	1,400	0,160
Legumes		-136,498	121,796	-1,120	0,262
Grasses		14,755	62,108	0,240	0,812
Geographic Aspects					
	North	-18,854	84,716	-0,220	0,824
	South	-44,697	73,967	-0,600	0,546
	East	-238,949	93,796	-2,550	0,011
	West	-17,698	41,146	-0,430	0,667
	Northeast	153,562	117,417	1,310	0,191
	Northwest	-601,069	83,423	-7,210	0,000
	Southeast	142,552	99,852	1,430	0,153
	Southwest	-265,540	156,251	-1,700	0,089
Constant		922352,300	181006,200	5,100	0,000
Random-effects Parameters		Estimate	Robust Std.Err.		
SiteNo: Identity					
	Var (Constant)	301631.4	236570.8		
	Var (Residual)	256724.4	46014.4		

Log pseudo likelihood: -1505.044; Obs.=189; Group variable: SiteNo, Groups:110 (Std. Err. Adjusted for 9 clusters in Geographic Aspects)

Rangeland utilization rate

In mixed effect regression analysis, dry forage yield and its squared form were included into the model as independent variables because of a quadratic relationship detected between dry forage yield and its utilization rate. According to the results, grazing season and altitude had significant positive effects on utilization rate ($p<0.05$) while rangeland condition and stocking rate were positively but only marginally effective ($p<0.1$) on utilization rate. The results suggest that a 1000 m increment in altitude brings about 33% more utilization and a one-unit increments in rangeland condition (one out of 10, e.g. an increase of 10%) and stocking rate (one-unit AU per hectare) causes 2% and 10.6% more utilizations respectively at *ceteris paribus*, considering the two-year averages.

As for the qualitative variable the geographic aspects, east and southwest slopes were utilized very significantly ($p<0.01$) more compared to reference flat aspect. Again, north, south and southeast slopes were utilized more as northwest, west, northeast and northwest slopes were less utilized in respective order compared to reference aspect, but the differences were not meaningful ($p>0.10$).

Discussion

The main focus of this study was the determination of factors affecting rangeland forage production and its utilization rate This was tackled by considering the effects of some natural (e.g. geographic aspects) and human induced factors (i.e. stocking rate). Although type, depth and nutrient content of soils, sloping degree, prevailing wind directions, evapotranspiration are all affecting rangeland biomass and may also directly or indirectly affect rangeland utilization rate, for the ease and simplicity of the study these all factors were not handled, and they were kept beyond the scope of this study and have been left as the subjects for further studies. Moreover, the findings related to rangeland vegetation and condition were not touched in detail in this study because a number of previously conducted studies in the region revealed more or less similar patterns (Erkovan et al., 2003; Öztaş et al. 2003; Dumlu et al., 2011; Avağ et al., 2012; Çomaklı et al. 2012; Çakal, 2016).

In forage production model (Table 5), very significant negative sign of the coefficient of 'grazing season' variable indicated a low yield in the second year. This can be explained with the negative balances of annual (74.4 mm, 27%) and seasonal (118.8 mm, 24%) precipitations in the same year.

Since moisture plays a key role in the composition, structure, and density of the plant communities in the areas with less than 600 mm annual precipitation (Kutiel and Lavee, 1999, cited in Maren et al., 2015), in such places including the study area rangeland forage production is mainly determined by rainfall (Duan et al., 2017). In line with our findings, a significant effect of the precipitation on rangeland forage was also reported by O'Connor and Rouxt (1995), Khumalo and Holechek, (2005), Koç (2001) and Browning et al. (2012).

Table 6. The results of mixed effect regression analysis for the factors affecting rangeland utilization rate

Independent Variables	Coefficient	Robust Std. Err.	z	P>|z|
Grazing Season (Year)	6,247	2,606	2,400	0,017
Altitude	0,033	0,014	2,320	0,020
Rangeland Condition	2,011	1,175	1,710	0,087
Distance to Village	0,008	0,012	0,720	0,475
Altitude × Distance to village	0,000	0,000	-1,210	0,225
Dry Forage Yield	0,003	0,002	1,140	0,255
Dry Forage Yield Squared	0,000	0,000	-0,340	0,732
Stocking Rate	10,636	5,875	1,810	0,070
Bare Ground	0,297	0,185	1,610	0,108
Species Richness	-0,362	0,668	-0,540	0,588
Legumes	0,221	1,347	0,160	0,870
Grasses	0,446	2,163	0,210	0,837
Geographic Aspects				
North	3,085	2,183	1,410	0,158
South	1,845	1,267	1,460	0,145
East	6,708	2,267	2,960	0,003
West	-0,893	2,422	-0,370	0,713
Northeast	-0,557	1,477	-0,380	0,706
Northwest	-4,186	4,283	-0,980	0,328
Southeast	0,152	1,173	0,130	0,897
Southwest	10,986	1,386	7,930	0,000
Constant	-12552,470	5252,536	-2,390	0,017
Random-effects Parameters	Estimate	Robust Std.Err.		
SiteNo: Identity				
Var (Constant)	23.915	29.824		
Var (Residual)	293.532	43.996		

Log pseudo likelihood: -812.30; Obs.=189; Group variable: SiteNo, Groups:110, (Std. Err. Adjusted for 9 clusters in Geographic Aspects)

In utilization rate model, on the other hand (Table 6) significant and positive signed coefficient of the ‘grazing season’ variable means that utilization rate increased significantly in the relatively drought second year of the study, suggesting that heavy grazing problem worsens in the years of low forage yield, which is also obvious in Figure 3b. Whereas, light or moderate grazing is suggested in drought seasons not to cause yield losses in subsequent years (Pieper and Donart, 1975. Ganskopp and Bedell, 1981).

The effect of forage yield and its squared (quadratic) form on utilization rate were not significant ($p>0.05$).As a priori and so reported by Okatan et al. (1999), I would expect less herbage yield at higher altitudes due to shorter vegetation period resulting from low temperatures. In the dry forage yield model, the negative sign of ‘altitude’ variable fulfils this expectation, but it is not significant ($p>0.05$). Özgür et al. (2017), in line with present findings, reported no significant differences between the dry forage yields at different altitudes. However, non-significant less herbage yield at higher altitudes significantly resulted in higher utilization rates ($p<0.05$). According to the utilization rate model (Table 6), 1000 m increment in altitude causes 33% increase in utilization rate at *ceteris paribus*. It may bring one’s mind that the higher the altitude the higher the utilization rate, suggested by the present study findings, contradicts with the low DM intake at higher elevations reported by Christen et al. (1996). However, this is not contradictory and could be explained with lower herbage production at higher elevations due to the reasons explained before e.g. cooler temperature and shorter vegetation period. Accordingly, the negative signed coefficient of ‘altitude’ variable in the dry forage yield model refers to low herbage production at higher altitudes, although not significant (Table 5). Again, remembering higher utilization rates in cases of herbage scarcity may be helpful. Nevertheless, Tamartash (2012) reported no relationship between livestock utilization and elevation.

The effect of distance to village (from the rangeland sites) and distance × altitude interaction were not significant in any of the two models.

Although grazing or carrying capacity calculations were based on the available forage amount (Gökkuş and Koç, 2001), Danckwerts and Aucamp (1984) reported that the range condition has a significant effect on grazing capacity. However, despite the positive signed coefficient, the effect of range condition on dry forage yield was not significant in the present study. In utilization rate model, though only marginally significant ($p<0.1$), rangeland condition had a positive effect on utilization rate, which could be explained with selective grazing of animals.

On the other hand, the effects of stocking rate on rangeland dry forage yield and utilization rate were found very significant ($p<0.01$) and only marginally significant ($p<0.1$) respectively. It may come to mind that there is no sense to include stocking rate variable into the dry forage yield model due to the cages used to save the herbage from grazing animals. However, it would be better to remember that the current stocking rates provide an indication of the past grazing pressure. For that reason, very significant negative effect of stocking rates in the dry forage yield model could be explained by the degradation of the rangelands due to heavy grazing pressure prevailing over decades.

Conversely, the utilization rate model, reflects the effect of current stocking rates on the utilization rate since it considers the readily grazed or removed forage amounts. This model suggests that a one-unit increment in stocking rate (one animal unit increase per hectare) resulted in 10.6% more utilization (consumption) per hectare of the rangeland (Table 6).

On the other hand, mountain relief indirectly affects the rangeland vegetation through affecting climatic factors, e.g., orographic precipitation on windward slopes or rain shadows on leeward slopes, wind speed, net radiation, evapotranspiration, soil moisture tension and soil temperature (Lambert and Roberts, 1976), which, in turn, significantly affect the quantity of soil organic carbon, total nitrogen and enzyme activity by altering the rate of litter decomposition and the activity of soil microorganisms (Nahidan et al., 2015).

For this reason, the northeast and southeast facing slopes yielded the highest herbage as the northwest and southwest slopes yielded the lowest (Figure 3a), though the yield differences among the aspects were not meaningful ($p>0.1$).

Dry forage yield model suggested that when shifted from the reference flat (zero) aspect to the northwest slopes, rangeland dry forage yield decreases in about 600 kg per hectare. This contradicts the observation of Wangchuk et al. (2013), who reported higher dry forage yields on the north-westerly slopes. Similarly, Okatan et al. (1999) reported higher yields for the plots with a northerly aspect compared to those with a southern aspect. However, Pournemati et al. (2017) reported that there was no significant relationship between total production and topographic factors ($p>0.05$). Thus, different researchers reported different results. The reason for this discrepancy might be that southerly aspects receive more sunshine in the northern hemisphere while northerly aspects are cooler and more humid than southerly aspects in general. Therefore, southern slopes, especially in the years with sufficient rainfall, are expected to produce more herbage due to more photosynthesis. However, soil nitrogen is also another important factor limiting plant vegetative growth in a majority of ecosystems (Tisdale et al., 1995) and southern slopes are expected to have less organic matter content and so nitrogen, because of warmer temperature and more xeric nature than northern slopes (Maren et al., 2015), which may also limit plant growth despite availability of sufficient moisture in soils. Thus, according to the project for the National Rangeland Utilization and Management, the organic matter content of the southern slopes was 3.39 ±0.28% and that for northern slopes was 3.93 ±0.31% in Erzurum province (Avağ et al., 2012). Thus, as seen from Figure 3a, the southeast and northeast facing slopes showed the highest yield in both years with different rankings, the southeast facing slopes ranked first in the humid season of 2007 while the northeast facing slopes ranked first in the drought season of 2008.

Regarding the aspect and utilization rate relationship, as in line with our findings, Tamartash (2012) reported that slope and aspect are highly correlated with livestock utilization. As stated earlier, in the years of low forage production, utilization rate gets significantly higher (Table 6). However, as also clearly seen in Figure 3b, the scarcity of forage in the northwest slopes did not result in a higher utilization rate as the southwest slopes had the highest utilization rate (11%) with reference to flat as-pect ($p<0.01$) although the northwest and southwest slopes produced the lowest dry forage yield, when compared the both slopes in terms of their utilization rates. This might be explained with the preference of village herders for southwest facing slopes due to warmer temperature and their aversion to northwest facing slopes due to a cooler temperature. Nevertheless, further studies are needed to prove the present findings.

Conclusion

Rangeland forage production was negatively and significantly affected by grazing season, most likely due to negative precipitation balance in the second year. Again, significantly ($p<0.05$) increased utilization rate against very significantly ($p<0.01$) decreased forage production in the second year suggests worsening grazing pressure on rangelands in the years of low forage production. Moreover, stocking rate was determined to be an important factor causing yield losses and increasing the grazing pressure on rangelands. Thus, the rangeland sites with a heavy grazing pressure history significantly yield less forage. When it comes to rangeland condition, despite its ineffectiveness on forage yield, its positive effect on utilization rate indicates selective grazing of the animals. That is, good condition sites were grazed more heavily. Another noteworthy finding is related to altitude and geographic aspect. Despite its insignificance, high-altitude sites produce less forage ($p>0.05$) but are significantly more utilized ($p<0.05$). Similarly, east ($p<0.05$), northwest ($p<0.01$) and southwest ($p<0.1$) slopes significantly yield less forage but except northwest, east and southwest slopes were significantly ($p<0.01$) utilized more compared to reference flat (zero) aspect. Accordingly, following conclusions and lessons can be drawn from the study;

1. Because of heavy grazing pressure versus low forage production, high-altitude sites, east and southwest slopes should specifically be given the priority in rangeland restoration and rehabilitation.
2. Due to the xeric nature of southerly slopes, drought resistant species should be preferred for the over-seeding practices
3. Overgrazing also brings about or accelerate erosion in rangelands. Rehabilitation of the eroded rangelands is difficult or most of the time impossible. To avoid excessive exploitation and to realize balanced utilization in all rangeland sites, user-friendly grazing plans fitting well to the socio-cultural and socio-economic conditions of the villagers should be developed and strictly followed by each village authority.
4. Heavy grazing pressure on rangelands gets even worse in drought seasons. Therefore, this point must strictly be considered in grazing plans not to cause herbage yield losses in subsequent years.

Compliance with Ethical Standards

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

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Data availability

Not applicable.

Consent for publication

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