e-ISSN: 2458-8377

http://sjafs.selcuk.edu.tr



# Selcuk Journal of Agriculture and Food Sciences

Number: 36

Volume: 1 APRIL

Year: 2022



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# **Product Information**

Publisher	Selçuk University Faculty of Agriculture
Owner (On Behalf of SUAF)	Prof. Dr. Zeki BAYRAMOĞLU Dean
Editor in Chief	Prof. Dr. Ercan CEYHAN, Selcuk University, Turkey
Printing House	Selçuk University
Date of Publication	25.04.2022
Language	English
Frequency	Published three times a year
Type of Publication	Double-blind peer-reviewed, widely distributed periodical
Indexed and Abstracted in	TR DİZİN GOOGLE SCHOLAR SCIENTIFIC INDEXING SERVICES (SIS) ARAŞTIRMAX CAB ABSTRACTS CROSSREF CAB DİRECT MIAR SCILIT ESJİ Dimensions OAJI.net
Web Address	http://sjafs.selcuk.edu.tr/
Address	Selçuk University, Faculty of Agriculture, 42075, Konya, Turkey Telephone : +90 (332) 223 28 56 Fax : +90 (332) 241 01 08 E-mail: eceyhan@selcuk.edu.tr



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Research Article

SJAFS

(2022) 36 (1), 1-7 e-ISSN: 2458-8377 DOI:10.15316/SJAFS.2022.001

# Determination of Forage Yield and Some Quality Characteristics of Silage Sorghum Genotypes at Different Water Stress Levels \*\*

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#### ARTICLE INFO

ABSTRACT

Article history: Received date: 14.09.2021 Accepted date: 21.12.2021	The study was conducted in order to determine the forage yield and some quality characteristics of silage sorghum genotypes at different water stress levels in the Randomized Complete Block Design arranged in split plots under Konya ecological conditions in 2020.
Keywords: Climate Change Drought Water Stress Forage Crops Sorghum	Three irrigation treatments (I <sub>1</sub> : Full irrigation; I <sub>2</sub> : 75% of I <sub>1</sub> ; I <sub>3</sub> : 50% of I <sub>1</sub> ) and 14 silage sorghum genotypes supplied from other countries were used in this study, irrigation subjects formed the main parcels and the genotypes sub-plots. In the study, the lowest and highest values were; 4756 kg da <sup>-1</sup> (I <sub>3</sub> )-6757 kg da <sup>-1</sup> (I <sub>1</sub> ) for green herbage yield; 1149 kg da <sup>-1</sup> (I <sub>3</sub> )-2002 kg da <sup>-1</sup> (I <sub>1</sub> ) for dry matter yield, 8,1 % (I <sub>1</sub> )-9,6% (I <sub>2</sub> ) for crude protein, 33.3% (I <sub>2</sub> )- 34.5% (I <sub>1</sub> ) for ADF ratio, 55% (I <sub>3</sub> )- 58.4% (I <sub>2</sub> ) for NDF ratio and and cellulose ratio was determined as 25.6% (I <sub>3</sub> )-28.2% (I <sub>1</sub> ) respectively. The genotypes used in the study, in which the efficiency of irrigation water use efficiency increased as water stress increa- sed, the lowest and highest dry matter yields were obtained from genotypes G- 11 (4214 kg da <sup>-1</sup> ) and G-4 (6961 kg da <sup>-1</sup> ), respectively, while a total of 8 genoty- pes had higher green herbage yield values than the study average

#### **1. Introduction**

Nowadays, climate change, which threatens agricultural production and access to safe food, especially drought in many parts of the world, emerges as one of the most important problems of human beings. As a result of global climate change, temperature increases and decreasing precipitation in many parts of the world significantly increase the severity of the drought event.

The areas most affected by drought are agricultural areas. It is one of the main problems of agricultural production even in countries that are advanced in agriculture. In the world, 21-22 million km<sup>2</sup>, which corresponds to approximately 16% of the terrestrial area, is considered to be arid and semi-arid regions.

Solving the drought problem caused by water scarcity and lack of precipitation and making agriculture more sustainable requires the application of more drought-tolerant agricultural products and technologies (Kapluhan, 2013). The water required for the production of food and other agricultural products corresponds to 3.100 billion m<sup>3</sup>, which corresponds to approximately 70% of the water withdrawn from rivers and groundwater. It is stated that if water resources cannot be increased and used efficiently, this amount will increase to 4.500 billion m<sup>3</sup> by 2030 (Anonymous, 2011). Unless there is an increase in the amount of land and water, it is expected that the water consumption used in agriculture will increase by 70-90% until 2050.

Negative effects of climate change around the world, it is also likely to be seen in the Mediterranean basin, where our country is located, negative effects are expected especially on water resources and precipitation regime.

The annual precipitation average of our country is 574 mm, this value shows significant differences according to regions, It is much lower especially in Central Anatolia (320 mm) and S. Eastern Anatolia regions (532 mm), where field agriculture is intense and which contains a significant part of the country's agricultural lands (Anonim, 2020).

The Konya basin, which is foreseen as one of the regions that will be adversely affected in climate change scenarios, is a region that is not very rich in terms of water resources and where groundwater is used significantly in agriculture. Although the amount of water withdrawn in the region per year is approximately 2.6

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<sup>\*\*</sup>This study has been produced from a part of the first author's doctorate thesis prepared under the supervision of the first author.

billion m<sup>3</sup>, the amount of safe water is foreseen as 1.8 billion m<sup>3</sup>. This situation, together with the decrease in precipitation, causes a water deficit every year (Anonymous, 2009).

According to TUIK (2017) data, Turkey has 16.6 million cattle and 44.3 million sheep and goat. Approximately 60 million tons of quality roughage is needed annually for this animal availability. However, our quality roughage production remains at the level of 40 million tons.

Konya closed basin is an area where plant and animal production is intensively carried out and has an important place in the formation of agricultural policies of our country. Animal husbandry has increased significantly in the region with support policies carried out in the last 20 years.

The region ranks first in the country in terms of the presence of cattle and small animals (the number of small animals is 13% of the country, and the number of cattle is 5%), the need for roughage has also increased significantly. Although there is a significant range of pastures in the region, most of these pastures have lost their qualifications and due to its very low herbage yield roughage shortages occur and roughage transfer occurs to the region at high cost from other regions within the country. However, a significant part of the roughage produced in the region is produced in irrigated areas, this puts pressure on water resources.

The animal potential of the region, using water effectively for a sustainable agricultural production that will be planned considering the soil and climate structure and water resources, resistant to water stress and in this case, it can produce more and quality biomass compared to similar purpose plants and it is important to reproduce and adapt new plants in the region, which reduce pressure on water resources. In this context, sorghum is an important plant, and has an important place for arid semi-arid regions, ts adaptability is quite good in areas with high temperature, limited rainfall and low soil fertility. At the same time, sorghum stands out as a plant that can respond positively to additional irrigation in arid areas with low and irregular rainfall (Wani ve ark., 2012)

Sorghum is a C4 plant that has high and quality herbage yield and has the opportunity to grow in different ecologies. It is mostly grown for fodder, food and industrial use. Sorghum produces twice as many roots as Table 1 corn (House, 1985) It produces more biomass by using water and plant nutrients (N,P,K) more effectively (House, 1985; Sanderson ve ark., 1992; Howell ve ark., 2008) than corn and other plants (Kimbrough, 1990; Bean ve ark., 2002).

Silage sorghum is a plant that can be used as an alternative to corn in animal feeding, At the same time, it can be stable in dry periods, more resistant to high temperatures and droughts, able to grow back rapidly after harvest, it stands out as a plant that can be used as an alternative to corn because it is more resistant to pests and diseases.

With this study, Konya closed basin with an important agricultural area in our country and similar regions can produce biomass in high quantity and quality by using water resources more effectively, able to drought tolerant, it was aimed to determine the green and dry matter yield, some quality parameters and irrigation water use efficiency of sorghum silage genotypes obtained from abroad in sorghum plant at different water stress levels.

#### 2. Materials and Methods

The study was carried out in the experimental field of Konya Bahri Dağdaş International Agricultural Research Institute in 2020 growing season (370 86' 03.41" N ve 320 55' 63.64" E). The place where the research is conducted has characteristics of a continental climate, summers are hot and dry, winters are cold and barely snowy. The average precipitation of the region for many years is 320 mm and the precipitation regime is irregular. A large amount of precipitation falls during the winter months. While the total precipitation in the study period was 321.4 mm, the precipitation between May and August, which is the growing period of sorghum, was 74.3 mm. While the total precipitation of the region for many years was around 329.2 mm, the precipitation amount between May and August was 82.4 mm (Table 1).

The area where the study was carried out has a clayey-loam structure, its organic matter is 2.3%, pH 7.4, and the amount of lime is determined as 23.5%.

The experimenatl area don't have any salt problem and some physical and chemical analysis results of the study area given in Table 2.

Precipitations and tempereature throughout the growing season of the experimental are

Months (May August.)									
Years	Climatic data	May	June	July	August	Annual total precipitation (mm)			
1020 2020	Mean Temp. (°C)	15.9	20.1	23.5	23.3				
1929-2020	Precipitation (mm)	43.4	25.7	7.0	6.3	329.2			
2020 Vaara	Mean Temp. (°C)	16.2	20.3	25.5	23.3				
2020 Years	Precipitation (mm)	23.4	35.8	0.5	12.8	204.2			

bonne son	some son properties of the experimental area												
Deepth (cm)	Sand (%)	Silt (%)	Clay (%)	Structure	Field capa- city (%)	Villing point (%)	Volume we- ight(g cm <sup>-3</sup> )	Hq	EC (dSm <sup>-1</sup> )	Lime (%)	Drganic Mat- ter (%)	P2O5 (kg da <sup>-</sup> 1)	ζ2Ο (kg da <sup>-1</sup> )
0-30	7.4	30.8	61.8	CL	26.9	17.2	1.26	7.6	0.80	34.4	2.4	14.5	113
30-60	8.6	29.4	62.0	CL	27.8	18.9	1.39	8.2	0.48	30.7	2.1	11.9	65
60-90	6.1	27.8	66.1	CL	26.4	17.1	1.32	8.3	0.41	29.9	1.9	13.2	54

Table 2 Some soil properties of the experimental area

A total of 14 genotypes were used in the study, including 12 silage sorghum genotypes obtained from the Table 3

USDA (U.S. Department of Agriculture) and 2 standart genotypies (Table 3).

Genotypes used in the study

Variety no	Pedigri	Orjin
G-1	PI 155519	South Africa
G-2	PI 181081	Sudan
G-3	PI 501106	Turkey
G-4	PI 552851	Taiwan
G-5	PI 560351	Mexico
G-6	PI 560355	İndia
G-7	PI 591005	Mexico
G-8	PI 599917	Ethiopia
G-9	PI 601925	Ethiopia
G-10	PI 602844	Ethiopia
G-11	PI 602851	Ethiopia
G-12	PI 602852	Ethiopia
G-13	Rox	BATEM
G-14	Sumac	BATEM

After the first soil preparation was done with a plow, duplication was done before planting and the field was made ready for planting. Considering the results of the analysis together with the soil preparation, 10 kg of phosphorus and 4 kg of nitrogen were given per decare. The remaining part of the nitrogen fertilizer was given in parts by drip irrigation system and completed to 15 kg da-1. The sowing was carried out in six rows with a seeder on May 06 2020, with 45 cm row spacing and 5 cm row sowing norm. The plot area is designed as 5 m long x 2.7 m wide, a total of 13.5  $m^2$ . Harvesting was done by removing the edge effects from the sides and the ends of the rows, and mowing the middle rows with a motor scythe during the dough formation period. Weed control was done mechanically and chemically.

In the study, a pressure regulated drip irrigation system was used, and irrigation was applied with pipes with a flow rate of 2 l/h and a dripper pitch of 30 cm, one lateral to each row. In the study, the amount of irrigation water to be applied to the gravimetric method was calculated by taking soil moisture at a depth of 0-90 cm, which is the effective root depth of sorghum.

Three irrigation applications were studied in the study, for full irrigation (I1), after determining the volume weight of the trial area, field capacity, wilting point, When 50% of the useful water at 0-90 cm effective root depth is consumed, the reduced water according to Equation-1 is completed to the field capacity (Kara, 2011).

$$dn = (FC - CM) \times D$$
(1)  
100

Equation-1;

IV

dn = The net amount of irrigation water to be applied in each irrigation (mm),

FC = Field Capacity (% Volume),

CM = Current moisture (% Volume),

D = Effective root depth (mm)

In 75% irrigation ( $I_2$ ), 75% of the irrigation water to be applied to the  $I_1$  subject, and 50% of the irrigation water to be applied to the  $I_1$  subject in the 50% irrigation (I<sub>3</sub>) irrigation water was given. In this study, 50 mm of irrigation water was applied to all plots in order to bring a uniform plant emercing and soil moisture to field capacity after planting. In the study in which the green herbage yield, dry matter yield, protein ratio, acid detergent fiber (ADF) ratio, neutral detergent fiber (NDF) ratio, cellulose ratio and irrigation water utilization efficiency (IWUE) properties were researched, the observations of vield and vield components were determined by Mülavim et al. (2009) ADF and NDF, crude cellulose measurements according to Van Soest et al. (1991) and protein analysis was determined according to Soylu et al. (2010). Irrigation water use efficiency was determined by Howell et al. (1990) using by the Equation-2.

$$IWUE = Y/I$$
(2)  
Equation-2;

IWUE: Irrigation water use efficiency (kg da<sup>-1</sup> mm<sup>-1</sup>),

#### Y: Dry matter yield (kg da<sup>-1</sup>),

I: Amount of irrigation water (mm).

The results obtained from the study, which was carried out in three replications according to the split-plots trial design in random blocks, variance analysis was performed with the JMP 11.1 statistical package, and the groupings between the subjects were made according to the LSD test.

#### 3. Results and Discussion

#### 3.1. Green and Dry Herbage Yield (kg da<sup>-1</sup>)

In the study, there was a decrease in green and dry herbage yield with the decreasing amount of irrigation water. While the highest green and dry herbage yield (6757 kg da<sup>-1</sup> – 4756 kg da<sup>-1</sup>) was obtained in full irrigation (I<sub>1</sub>), the lowest value was obtained from I<sub>3</sub> (2002 kg da<sup>-1</sup> – 1449 kg da<sup>-1</sup>), (Table 4).

Considering the average of varieties, the highest green herbage yield is from genotype G-4 (6961 kg da<sup>-1</sup>), the lowest yield was obtained from genotype G-18 (4214 kg da<sup>-1</sup>) and seven genotypes (1, 3, 4, 5, 6, 9 and 10) were given higher values than the trial average.

While the highest and lowest values in terms of dry matter yield were obtained from genotypes G-10 (2108 kg da<sup>-1</sup>) and G-11 (1156 kg da<sup>-1</sup>) respectively, six genotypes (3, 4, 5, 6, 9 and 10) had higher values than the trial average. When the water stress x genotype interaction is examined in terms of green herbage yield, the highest and lowest values for I<sub>1</sub> are from genotypes G-4 (8833 kg da<sup>-1</sup>) and G-12 (4509 kg da<sup>-1</sup>), As for I<sub>2</sub>, it is obtained from genotypes G-10 (7434 kg da<sup>-1</sup>) and genotype G-11 (4061 kg da<sup>-1</sup>), In I<sub>3</sub> subject where water stress is the highest, the highest value was obtained from genotype G-4 (5868 kg da<sup>-1</sup>), and the lowest value was obtained from genotype G-2 (3751 kg da<sup>-1</sup>).

In terms of hay yield, the highest and lowest values are from genotypes G-4 (2525 kg da<sup>-1</sup>) and G-12 (1267 kg da<sup>-1</sup>) subject I<sub>1</sub>, regarding to I<sub>2</sub>, genotype G-10 (2344 kg da<sup>-1</sup>) and genotype G-12 (1152 kg da<sup>-1</sup>), and as for I<sub>3</sub>, were obtained from genotype G-4 (1961 kg da<sup>-1</sup>) and genotype G-2 (998 kg da<sup>-1</sup>) (Table 4). Dahmardeh et al., (2015) reported that water stress decreased the green and dry herbage yield and increased the water use efficiency in their study where they irrigated the silage sorghum plant at the rate of 80%, 60% and 40% of their water consumption in semi-arid climate conditions.

Table 4

The values of green and dry matter yield obtained from sorghum genotypes at different water stresses from the study (kg da<sup>-1</sup>)

		Green herb	age yield (kg da		Dry matte	er yield (kg da <sup>-1</sup> )		
Genotype	I <sub>1</sub>	$I_2$	$I_3$	Mean	$I_1$	$I_2$	$I_3$	Mean
G-1	7465 bc	5561 g-j	4599 l-p	5875 DE	2211 b-d	1551 l-o	1250 p-r	1671 CD
G-2	5802 f-1	4719 k-o	3751 q	4757 G	1769 g-l	1388 n-q	998 r	1385 F
G-3	6341 ef	6094 e-h	5416 h-k	5950 CD	1902 e-1	1766 g-1	1595 j-n	1754 C
G-4	8833 a	6183 e-g	5868 e-h	6961 A	2525 a	1833 f-k	1961 c-g	2106 A
G-5	7804 bc	6041 e-h	4769 k-n	6205 CD	2233 bc	1683 1-m	1392 n-q	1769 C
G-6	7967 b	5758 f-1	4952 j-m	6226 CD	2521 ab	1698 h-m	1577 k-n	1932 B
G-7	6202 e-g	5595 g-j	4463 l-p	5420 F	1924 e-1	1677 1-m	1330 n-q	1644 C-H
G-8	6563 de	5611 g-j	4264 m-q	5480 EF	2030 c-g	1685 1-m	1370 n-q	1695 CD
G-9	7141 cd	6095 e-h	5828 f-h	6354 BC	2220 b-d	2054 c-f	1929 e-1	2068 AB
G-10	7700 bc	7437 bc	4924 j-m	6687 AB	2057 c-f	2344 ab	1925 e-1	2108 A
G-11	4643 l-p	4061 n-q	3938 pq	4214 H	1278 p-r	1170 q-r	1020 r	1156 G
G-12	4509 l-p	4411 l-q	4056 o-q	4325 H	1267 p-r	1152 q-r	1182 q-r	1200 G
G-13	5861 e-h	5096 1-l	4310 m-q	5089 FG	1852 e-1	1600 j-n	1296 o-q	1583 DE
G-14	7767 bc	7344 bc	5094 1-l	6735 AB	2236 bc	2118 b-е	1464 m-p	1939 B
Mean	6757 A	5715 B	4756 C	5734	2002 A	1694 B	1449 C	1715
CV (0.01)	7.6				CV (0.01)	9.6		
SD Genotype	411		LSD G*I	711	LSD Genot	ype157	LSD G*I	272
SD Irrigation	141				LSD İrrigatio	on 115		

In previous studies, Aydinşakir et al. (2018) reported the fresh grass yield of 4442 - 9926 kg da<sup>-1</sup>, dry herbage yield in the range of 744 - 2107 kg da<sup>-1</sup> in 5 different irrigation subjects (100% - 75% - 50% - 25% - 0%) in sorghum, Keten and Değirmenci (2020) reported green herbage yield as 3200-6000 kg da<sup>-1</sup>, Özmen (2017) reported green herbage yield as 4003-11812 kg da<sup>-1</sup>, dry herbage yield between 534-2560 kg da<sup>-1</sup>.

The green and hay yields obtained from this study are compatible with previous studies, and the differences are thought to be due to the environment and genotype. When the protein ratio of the genotypes was examined in the study, it was found that the protein ratio was lower in full irrigation, the highest and lowest protein ratio values were obtained from genotypes G-9 and G-7 in I<sub>1</sub> irrigation, from genotypes G-12 and G-7 in I<sub>2</sub>, and from genotypes G-2 and G-1 in I<sub>3</sub>. In terms of variety averages, the highest value was 11.5% (genotype G-2) and the lowest value was 6.8% (genotype G-7) (Table 5).

ADF ratios also had lower values with decreasing irrigation water. When the genotype averages are examined, it is seen that the highest value was obtained from genotype G-11 (40.1%) and the lowest value was obtained from genotype G-9 (30.4%). The highest and lowest ADF values are among the genotypes G-11 (43.9%) and G-9 (26.7%) in full irrigation, G-2 (36.2%) and G-9 (30.9%) genotypes (I<sub>2</sub>) in 75% irrigation, and for I<sub>3</sub>, G-11 (39.8%) and G-8 (29.1%) genotypes were obtained (Table 5).

While  $I_1$  and  $I_2$  subjects had similar values in terms of NDF ratios,  $I_3$  had the lowest value. In terms of varieties, it is seen that the highest NDF value was obtained from genotype G-11 (%) 68.2, and the lowest value was obtained from genotype G-7 (51.5%). The highest and lowest NDF values are from genotypes G-11 (70%) and G-9 (49.0%) for  $I_1$ , G-11 (67.9%) and G-7 (51.2%) for  $I_2$ , and for  $I_3$ , It was obtained from genotypes (66.5%) and 8 (50.4%) (Table 6). It was observed that cellulose ratios decreased with decreasing water, similar to ADF values in terms of cellulose ratios, when the average of the genotypes is examined, it is seen that the highest cellulose ratio value is obtained from genotype G-11 (34.0%) and the lowest value is obtained from genotype G-9 (22.9%). When examined in terms of water x genotype interaction, the highest and lowest cellulose values are from genotypes G-11 (36%) and G-9 (20.7%) in I<sub>1</sub>, from genotypes G-11 (33.1%) and G-10 (22.8%) in I<sub>2</sub>, As for I<sub>3</sub>, it is seen that it was obtained from genotypes G-11 (32.9%) and G-8 (22.5%) (Table 6).

#### Table 5

The values of protein and ADF ratios obtained from sorghum genotypes at different water stresses from the study (%)

	Protein ratio	(%)	ADF (%)	)				
Genotype	$I_1$	$I_2$	$I_3$	Mean	I <sub>1</sub>	$I_2$	$I_3$	Mean
1	8.4 m-o	9.5 jk	5.0 x	7.7 F	37.6 cd	33.0 k-q	35.1 g-j	35.2 C
2	10.1 hı	12.0 bc	12.4 ab	11.5 A	37.8 cd	36.6 d-g	35.1 g-j	36.5 B
5	9.7 1-k	10 h-j	8.8 lm	9.5 C	34.7 h-k	33.6 1-р	32.1 o-r	33.4 D
4	8.5 mn	8.8 lm	9.3 kl	8.9 D	38.7 bc	34.3 h-1	32.6 l-r	35.2 C
5	7.4 r-t	7.9 o-r	7.5 q-s	7.6 F	37.2 с-е	34.0 h-n	33.5 ј-р	34.9 C
6	6.9 tu	6.9 t-v	7.7 p-r	7.2 G	35.6 e-h	35.2 e-j	34.3 h-l	35.0 C
7	6.0 w	6.3 vw	8.1 n-p	6.8 H	32.9 k-q	30.1 st	31.5 q-s	31.5 EF
8	6.9 s-u	10.3 g-1	8.1 n-p	8.4 E	32.1 n-r	31.6 q-r	29.1 t	30.9 FG
9	10.9 ef	10.8 e-g	9.2 kl	10.3 B	26.7 u	30.9 r-t	33.5 j-p	30.4 G
10	6.0 w	11.8 cd	8.9 lm	8.9 D	31.0 r-t	31.3 q-s	32.7 l-r	31.6 EF
11	7.0 s-u	7.7 p-r	6.7 uv	7.1 GH	43.4 a	37.1 c-f	39.8 b	40.1 A
12	7.7 p-r	7.9 o-r	8.9 lm	8.2 E	34.1 h-m	35.6 e-h	35.5 e-1	35.0 C
13	7.5 q-s	11.3 de	10 h-j	9.6 C	32.3 m-r	32.3 m-r	32.2 n-r	32.3 E
14	10.5 f-h	12.6 a	10.7 fg	11.3 A	33.8 h-o	30.9 r-t	31.9 p-s	32.2 E
Mean	8.1 C	9.6 A	8.7 B	8.8	34.9	33.3	33.5	33.9
CV (0.01)	4.0				CV (0.01)	3.4		
SD Genotyp	e0.33		LSD G*I	0.56	LSD Genoty	/pe1.08	LSD G*I	1.87
SD Irrigatio	n 0.24				LSD Irrigation ns			

Table 6

The values of NDF and cellulose ratios obtained from sorghum genotypes at different water stresses from the study (%)

		NDF	F (%)		Cellulose ratio (%)				
Genotype	$I_1$	$I_2$	$I_3$	Mean	I <sub>1</sub>	$I_2$	$I_3$	Mean	
G-1	64.2 d	60.0 g-1	52.8 s-v	59.0 C	29.9 d	26.3 l-n	27.8 h-j	28.0 C	
G-2	63.8 de	64.8 cd	59.1 h-j	62.6 B	30.1 d	28.2 g-1	26.1 l-o	28.1 C	
G-5	58.6 h-k	58.3 1-k	54.4 p-s	57.1 D	26.5 l-n	25.5 o-q	24.4 rs	25.4 D	
G-6	62.3 ef	55.9 m-p	53.8 q-s	57.4 D	31.3 c	27.7 ц	25.9 m-o	28.3 C	
G-7	58.7 h-k	57.9 j-k	53.3 r-t	56.6 DE	29.4 d-f	28.6 f-h	25.7 n-p	27.9 C	
G-8	55.8 m-p	61.0 fg	55.6 n-p	57.5 D	29.7 de	29.0 e-g	27.9 h-j	28.9 B	
G-9	51.5 u-w	51.2 vw	51.9 t-w	51.5 I	26.0 l-o	24.3 r-t	23.2 u-w	24.5 F	
G-10	51.8 t-w	54.7 o-r	50.4 wx	52.3 I	24.7 q-s	24.0 s-u	22.5 w	23.7 G	
G-15	49.0 x	56.3 l-o	54.8 o-r	53.4 H	20,7 x	23.9 s-u	24.2 r-t	22.9 H	
G-17	53.2 r-u	57.7 j-l	53.7 q-s	54.9 G	26,7 k-m	22.8 vw	24.0 s-u	24.5 F	
G-18	70.0 a	67.9 b	66.5 bc	68.2 A	36.0 a	33.1 b	32.9 b	34.0 A	
G-19	61.2 fg	60.3 gh	55.7 n-p	59.0 C	29.8 de	30.0 d	24.9 p-r	28.2 C	
G-21	57.5 j-m	56.3 l-o	52.6 s-v	55.5 FG	27.3 jk	23.9 s-u	23.2 u-w	24.8 EF	
G-22	57.1 k-n	55.4 n-q	55.7 n-p	56.1 EF	26.8 kl	23.5 t-v	25.0 p-r	25.1 DE	
Mean	58.2 A	58.4 A	55.0 B	57,.2	28.2 A	26.5 B	25.6 C	26.8	
CV (0.01)	1.9				CV (0.01)	1.95			
LSD Genotype(G)	1.0		LSD G*I	1.76	LSD Genotype(G)	0.49	LSD G*I	0.85	
LSD Irrigation (I)	1.17				LSD Irrigation(I)	0.27			

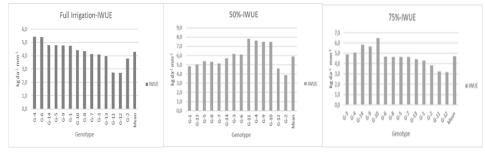
Since the digestion level of ADF and NDF is very slow in animal nutrition, a low ratio in the ration (Van Soest, 1994) is desirable features, and an increase in insoluble fibers in plants experiencing water stress, It is one of the physiological responses of plants to prevent moisture loss (Jahansouz et al., 2014), With the increase in the amount of irrigation, the amount of ADF, NDF and crude fiber and ash increased, similar results Uzun et al. (2017), Cotton et al. (2013) reported by. This situation is thought to be due to the decrease in the leaf/stem ratio with the increase in plant height under irrigated conditions (Pedersen et al., 2005). Contrary to these aspects, Kızıloglu et al. (2009) found that ADF and NDF values increased with water stress, Islam et al. (2012) reported that the amount of irrigation water did not affect the ADF values. From previous studies, Jahansouz et al. (2014) NDF ratios in 50-75-100% irrigation in sorghum, respectively; reported as 59.4% - 55.8% - 55.6%, on the other hand, Keten and Değirmenci (2020) determined the ADF rate as 25.7%-26.1%; NDF rate of 50.8%-52%; reported in the range.

In another study, Cotton et al. (2013) found the ratio of ADF to be 36.5% - 31.5% and NDF ratio to be 59.2 -54.4% in sorghum in irrigated and water stress conditions, respectively. As a result of the study, it was seen that decreasing irrigation decreased the rates of ADF, NDF and cellulose. There are different opinions about the effects of water stress on protein ratio. Similar to the results of this study, Keten (2020), and Khaton et al. (2016) reported that the protein value increased with increasing water (Jahansouz et al., 2014), Uzun et al. (2017) and Liu et al. (2013) reported higher protein content in water stress. Islam et al, (2012) reported that water stress had no effect on protein in maize plant. In previous studies on sorghum, Liu et al. (2013) 10.14% to 14.86; Saghafi et al. (2013) 8.11% to 10.48; Chakravarthi et al. (2017) 5.29% to 21.24; Canyiğit and Okant (2018) 9.1% to 13.1; Kır and Şahan (2019) reported that it was between 7.3% and 10.4%.

In this study, it is thought that the differences in the protein, NDF and ADF ratio values obtained under different water treatment conditions and the differences in their results are due to the characteristics of the environment and especially the genotypes.

Irrigation water use efficiency (IWUE), which is an important parameter for the regions where the negative effects of climate change are increasing and for the regions where water is limited, is a feature that determines the dry matter ratio obtained with unit water and should be considered in product planning in regions with water scarcity.

In the study, the IWUE value increased in water stress issues, and when the IWUE values obtained at different irrigation levels from the genotypes were examined, In I<sub>1</sub> irrigation, G-4 and G-6 have high IWUE values, in I<sub>2</sub> genotypes G-9, G-10 and G-14 came to the fore, and in I<sub>3</sub> where the most severe stress is applied, genotypes G-4, G-9, G-10 and G-11 have high IWUE values and observed that they used water more effectively under stress conditions (Figure 1).



#### Figure 1

The values of NDF and cellulose ratios (kg da<sup>-1</sup> mm) obtained from sorghum genotypes at different water stresses from the study.

In previous studies, Keten and Değirmenci (2020) reported the range of 7.8-6.3 kg da<sup>-1</sup>mm<sup>-1</sup>, Gönülal (2020), on the other hand, found the irrigation water usage efficiency (IWUE) in the range of 4.9 - 7.1 kg da<sup>-1</sup> mm<sup>-1</sup> in his study (100% - 50%) in which he applied two different water issues.

#### 4. Acknowledgements

In the study carried out to determine the responses of different silage sorghum genotypes to water stress in Konya ecological conditions, green forage yields varied between 4756-6757 kg da<sup>-1</sup> according to the genotype. In the study, it was observed that some genotypes gave better yields than other varieties in moderate and severe water stresses. It is thought that sorghum genotypes with high irrigation water use efficiency are suitable for arid and semi-arid regions and can be used as genetic resources in breeding studies.

#### 5. Acknowledgements

The manuscript was produced from Ramazan Çağatay ARICI's PhD thesis.

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http://sjafs.selcuk.edu.tr/sjafs/index

Research Article

SJAFS

(2022) 36 (1), 8-12 e-ISSN: 2458-8377 DOI:10.15316/SJAFS.2022.002

### Fleece Yield and Some Characteristics of Wool in Anatolian Merino Sheep

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ARTICLE INFO	ABSTRACT
Article history: Received date: 21.09.2021 Accepted date: 21.12.2021	In this study, greasy fleece yield and some wool characteristics and the effects of gender, age and live weight on these characteristics were investigated in 60 heads of Anatolian Merino sheep reared in Babayakup neighborhood of Polatlı District of Ankara. Least squares means of live weight before shearing, greasy
<b>Keywords:</b> Anatolian Merino Live Weight, Greasy Fleece Yield, Wool Characteristics.	fleece yield, fiber diameter, fiber length, breaking strength, fiber elasticity and clean fleece percentage were found to be $68\pm0.89$ kg, $2.97\pm0.19$ kg, $24.42\pm0.49$ µm, $7.92\pm0.36$ cm, $19.15\pm1.25$ Cn/tex, $25.38\pm1.83$ %, $48.16\pm2.17$ %, respectively. The effects of gender (p<0.001) and age (p<0.05) on live weight before shearing, the effects of body weight group (p<0.05) on fiber diameter and the effects of gender (p<0.05) on fiber length were found to be significant. According to the findings, it was concluded that the fiber diameter value of the research material sheep is 60 sortiman, therefore it will be evaluated in the class of fine wool sheep and it is suitable for worsted fabric production.
	desired properties in technical applications (Dellal et al.,

#### 1. Introduction

Merinoization studies were started for the first time in Turkey in 1841, and domestic Kıvırcık sheep and German Fleece-Meat Merino were crossbred in 1934 and merino conversion studies were carried out (Halıcı, 2009). Later, in the 1950s, as a result of crossbreeding of German fleece-meat merino (GFMM) and Akkaraman in Malya and Ulaş State breeding farms, a thinlong-tailed Anatolian Merino sheep with 75-80% Merino genotype was developed (Ertuğrul, 2012).

When the wool characteristics of Anatolian Merino sheep, which is a Merino crossbred, are examined, it is evaluated in the fabric industry by producing yarn from wool yarn or from wool-synthetic mixture due to the thin and moderately uniform nature of its fleeces.

Although the share of fleece in the product obtained from sheep has decreased today, wool yield and quality should be emphasized due to organic agriculture, protection and development of rural economy and significant changes in industrial production and consumer tendency in recent years. The healthier structure of natural fibers, and because animal fibers are 100 % biodegradable in nature, they are renewable. They have some properties that are not found in other animal and vegetable fibers such as coloration, curl, flexibility, felting, insulating, heat resistance, antimicrobial, dirt repellency, high comfort, and they are rare fiber that can meet many desired properties in technical applications (Dellal et al., 2010; Tüfekçi and Olfaz, 2014; Dellal et al., 2020).

In this study, some of the fleece yield and quality criteria of Anatolian Merino sheep were evaluated, upto-date data on fleece were obtained, and it is aimed to contribute to the literature in a way that can guide breeding studies in this way, and to offer suggestions on improving the quality of wool by reviewing its suitability for fabric yarn production.

#### 2. Materials and Methods

The animal material of the research consisted of 60 heads of Anatolian Merino sheep and their fleeces, which were raised in the Babayakup District of Ankara province, Polath county.

Anatolian Merino is a sheep with 75-80% GFMM genotype, which was developed as a result of crossbreeding the GFMM and the domestic sheep breed Akkaraman. Fleece-Meat is yield-oriented and generally spreads in the western parts of the Central Anatolia Region. They are generally white in color, have a large body, full thighs and meaty legs. The tail is lean and thin, and the fleece is of 60-64 sortiman quality.

Sheep were kept in barns under the conditions of peasant sheep management on days when the weather was not suitable in winter, and approximately one kg of concentrated feed and one kg of roughage were given

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per animal. They were grazed on the pasture under favorable weather conditions. Supplementary feeding was ensured at the return of pasture in the early spring and autumn periods. The sheep were kept in the pasture for about eight months and in the barn for four months, and stubble grazing was also done in the summer.

Shearing was done with a shearing machine at the end of May. During the shearing and the night before shearing, the animals were not fed and their live weights were determined by weighing with 100 g precision before shearing, and their ages were determined by looking at their teeth at the same time (as there were no records). Ethical approval was given by the Faculty of Veterinary Medicine ethical committee in Selcuk University (No:2021/133). The determined weight, age and gender were recorded at the same time and placed in a small nylon bag together with the fleece samples and kept under protection. As Ertuğrul (1996) reported, approximately 30 g of fleece samples were taken from the flank (rib) region of each animal. After shearing, the amount of fleece obtained by shearing from each animal was determined by weighing it with 10 g precision scale.

Analysis of physical properties such as Diameter (fiber/fiber diameter,  $\mu$ m), Strength (cN/tex), Elasticity (%), Clean fleece percentage (%) in fleece samples was performed at International Center for Livestock Research and Training General Directorate-the Department of Breeding and Genetics - Wool and Mohair Laboratory (Ankara) according to ASTM and IWTO standards. Fiber lenght was determined by taking the average lenght of 50 hairs randomly taken from each fleece sample. Each fiber (hair) was stretched from both ends and measured in mm, the average lengths were converted to cm.

Assuming that there is no significant interaction between the factors whose effects on wool yield characteristics were examined in statistical analysis, a computer package program developed by Harvey (1987) was used in the analysis of environmental factors. "Least Squares Variance Analysis " was used for fleece yield and properties, and the following mathematical model was used.

 $Y_{ijkl} = \mu + a_i + b_j + c_{k+}e_{ijkl}$ 

 $Y_{ijkl}$ : *i.* age, *j.* the live weight group *k.* gender *l.* sheep's fleece yield and characteristics (greasy fleece yield, yield, diameter, length, elasticity and strength)

 $\mu$  : overall mean,

 $a_{i:i}$  effect of sheep age, (  $i=\leq 2, 3, 4, \geq 5$ ),

 $b_{j:j}$ . effect of live weight [1.(35.00-55.50 kg), 2.(55.51-64.00 kg) and 3.(64.01-103.00 kg) group)],

 $c_k$ : *k*. the effect of gender, k=1, 2 (Erkek, Dişi),

eijkl: random error.

The significance test of the differences between the groups was done with Duncan test (Düzgüneş et al., 1993).

#### 3. Results and Discussion

In Babayakup District, the average live weight of 60 Anatolian Merino sheep in the hands of the breeder before shearing was found to be  $68.54\pm0.895$  kg. While the live weights of the sheep vary between 35 and 103 kg, the average live weight is  $79.49\pm1.843$  for males and  $57.60\pm0.649$  kg for females. The effects of gender (p<0.001) and age (p<0.05) on live weight before shearing were found to be significant. Four-year-old sheep had the highest body weight ( $71.53\pm13$  kg), while the average body weight of one-aged sheep ( $65.30\pm1.91$  kg) was the lowest. The means of the least squares of the greasy fleece yield, the thinness (fiber diameter), length (fiber length), strength, elasticity and clean fleece percentage properties of the fleece are given in Table 1.

As can be seen from Table 1, the mean of the least squares of the greasy fleece yield calculated in the herd is 2.972±0.199 kg. The 2.97 kg greasy fleece yield value obtained is similar to the value reported as 2.912 kg by Özsoy (1974) for the Merino sheep, and similar to the values reported as 2.84 and 2.87 kg by Dellal et al. (2000a) and Arık et al. (2002) for the Anatolian Merino. The greasy fleece weight is considerably higher than 2.398 kg reported by Tellioğlu (1975) for Merino, 2.7 and 2.4 kg reported by Koyuncu et al. (1996) for Kıvırcık and Türkgeldi sheep; higher than 1.91 and 2.42 kg reported by Tekin et al. (1999) for German Black-Headed and Hampshire Down sheep; and higher than 1.58 kg reported by Peşmen and Yardimci (2012) for the Menemen sheep, respectively. On the other hand, 3.7 and 3.5 kg reported by Yalçın et al. (1972) for Konya Merino yearling lamb and dams, 3.20 and 5.01 kg for German Meat Merino ewe and rams and 3.35 kg for Karacabey Merino reported by Akçapınar (1983), 3.35 kg reported by Oğan (1994) for Karacabey Merino, 3.2 kg reported by Koyuncu et al. (1996) for Merino sheep, 3.29 kg reported by Tekin et al. (1999) for Turkish Merino, 3.35 kg ve 3.79 kg reported by Ünal and Akçapınar (2001) for Central Anatolian Merino sheep and yearling lamb, 5.65 kg reported by Uzun (2008) for Karacabey Merino, 3.57 and 3.15 kg reported by Tuncer and Cengiz (2018) for Anatolian Merino and Ile de France x Anatolian Merino crossbred (F1) sheep are less than fleece yield value of all meat breeds x merino hybrid (F1) sheep studied by Şahan et al. (1995). Differences in greasy fleece yield value result from genetic and environmental factors.

The effect of gender, age and live weight group on greasy fleece yield was insignificant. In terms of greasy fleece yield, Özsoy (1974) reported the effect of age in Atatürk University Merino flock, Ünal and Akçapınar (2001) reported the effect of age in Central Anatolian Merino ewes sheep in yearling lambs, and Bağkesen and Koçak (2018) reported the effect of age on Ramlıç and Dağlıç sheep, significantly on the other hand, the effect of age was found to be insignificant by Yılmaz and Altın (2004) in Kıvırcık sheep and Dellal et al. (2000b) in various crossbreds. Şahan et al. (1995) found the effect of gender on meat breeds and Merino crossbred sheep (F1) significant; and Tekin et al. (1999) found the effect of age, gender and live weight on greasy fleece yield to be significant in German Black-Headed meat sheep. The insignificant effect of age, gender and weight group in this study can be explained by the low variation due to the low number of sheep.

The diameter of the fibers and the uniformity are in the first place in determining the quality class of the fleece. The quality of the products made from quality yarn and quality fabric obtained from these yarns is directly related to the fineness of the fleece hair. In the study, the fineness value of fleece hair was determined as 24.423±0.495 µm. When this diameter is evaluated on the basis of the British Bradford system, the corresponding sortiman value is 60. In other words, the fleece of the sheep in the study is classified as thin fleece. The values of 24.1 µm reported by Koyuncu et al. (1996) for Türkgeldi sheep and 24.97 µm by Uzun (2008) for Karacabey Merino sheep are quite close to the values calculated in this study. On the other hand, this study fineness value is less than 25.77 µm reported by Tellioğlu (1975) for Merino sheep, 28.73 µm reported by Dellal et al. (2000a) for Anatolian Merino, 28.34 and 30.91 µm reported by Uzun (2008) and Peşmen and Yardimci (2018) for Menemen sheep, and again lower than 25.16 and 25.47 reported by Tuncer and Cengiz (2018) for Anatolian Merino and Ile de France x Anatolian Merino sheep. However, average diameter value of 24.423 µm calculated in this study is higher than fiber fineness averages of 22.3 and 22.4 µm reported by Yalçın et al. (1972) for Konya Merino female yearling lambs and dams, 23.19 µm reported by Özsoy (1974) for Merino sheep, 20.27 µm reported by Oğan (1994) for Karacabey Merino sheep, 20.4 µm reported by Koyuncu et al. (1996) for merino sheep, 21.32 µm reported by Tekin et al. (1999) for Turkish Merino, 22.19 and 22.37 µm reported by Ünal and Akçapınar (2001) for Central Anatolian merino sheep and yearling lambs, and again higher than 23.46 and 23.97 µm reported by Arık et al. (2003) for Anatolian Merino and Ile de France x Anatolian Merino crossbred sheep.

In this study, the effect of the live weight group, which was one of the factors whose effect on fleece diameter was examined, was found to be significant at p<0.05, while the effect of gender and age was insignificant. The finest fiber diameter average of 22.84  $\mu$ m belongs to the 3rd age group (64.01-103.00 kg), and the highest fiber diameter average belongs to the 1st age group (35.00-55.50 kg) with 25.75  $\mu$ m. Peşmen and Yardimci (2018) reported that the effect of age on the diameter was significant in Menemen sheep, while Tekin et al. (1999) reported that the effects of age, gender, and live weight were insignificant in Turkish Merino and German Black-Headed Meat sheep.

Fiber length affects draft, twist, varn structure, smoothness and yarn production method in yarn manufacturing (Sönmez, 1963). In industry, the length of the curl is taken as a basis, which is an important factor when evaluated according to the way of use. In this study, the fiber length was calculated as 7.923±0.362 cm. The 7.45 cm length value reported by Unal and Akçapınar (2001) for the Central Anatolian Merino sheep in Konya Livestock Research Institute is close to the value calculated in this study. It is lower than the values of 9.96 cm and 9.22 cm (Dellal et al., 2000a, Ünal and Akçapınar 2001) reported for Anatolian Merino and Central Anatolian Merino yearling lambs, and than 10.49 and 9.83 cm, respectively, determined by Uzun (2008) in Karacabey Merino and Menemen dams. It is higher than the mean lengths of 4.78 and 4.79 cm, respectively, reported by Arık et al. (2003) for Anatolian Merino and Ile de France x Anatolian Merino (F1).

In this study, the effect of gender on fiber length was found to be significant (p<0.01), while the effect of age and live weight group was found to be insignificant. Peşmen and Yardimci (2012) and Bağkesen and Koçak (2018) reported opposite results regarding the effect of age on length.

Strength and durability in fleece are expressed as grams of the weight that the hairs that make up the fleece can withstand until they break. Durability is one of the important quality indicators of fleece. Raw materials with high strength are preferred in the textile industry (Kaymakçı, 2016). In the study, the average strength value of Anatolian Merino sheep was 19.153±1.252 cN/tex. This value is lower than 20.69 cN/tex reported by Peşmen and Yardimci (2012) for Menemen sheep. However, it is higher than the strength value reported by Dellal et al. (2000a) and Arık et al. (2003) for Anatolian Merino sheep. The fleece strength value was reported as 7.5, 8.2, 5.2, 10, 7.6, 15.2, 18.3, 4.05 and 5.53 g, respectively, by Yalçın (1972) for Konya Merino female yearling lambs and dams, by Şahan et al. (1995) for Merino female and male yearling lambs, by Koyuncu et al. (1996) for Merino, Kıvırcık and Türkgeldi sheep, and by Uzun (2008) for Karacabey Merino and Menemen sheep.

In this study, it was determined that the effects of the factors (gender, age and live weight group) whose effects on strength were examined were insignificant. Peşmen and Yardımcı (2012) found the effect of age on strength to be significant in Menemen sheep. Yalçın et al. (1972) reported that fluctuations in strength were not related to age.

	Greasy Fleece Yield	Fiber Diameter	Fiber Length	Breaking Strength	Elasticity	Clean Fleece Percentage
	(kg)	(µm)	(cm)	(cN/tex)	(%)	(%)
N	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE
50	2.97±0.199	24.42±0.495	$7.92{\pm}0.362$	19.15±1.252	25.38±1.833	48.16±2.175
	NS	NS	*	NS	NS	NS
50	$3.45 \pm 0.130$	25.12±0.324	9.43±0.239ª	$17.93 {\pm} 0.818$	22.82±1.198	52.58±1.418
10	$2.49{\pm}0.471$	23.73±1.174	$6.42{\pm}0.847^{b}$	$20.37 \pm 2.969$	$27.95 \pm 4.342$	43.75±5.139
	NS	NS	NS	NS	NS	NS
10	$3.47 \pm 0.298$	24.00±0.723	$8.45 \pm 0.567$	$19.02 \pm 1.845$	23.72±2.714	49.27±3.239
11	$2.91 \pm 0.252$	24.79±0.629	$8.45 \pm 0.485$	17.94±1.582	22.95±2.324	51.49±2.781
12	3.13±0.269	24.73±0.661	$8.05 \pm 0.499$	18.52±1.663	26.71±2.408	50.56±2.923
15	$2.55 \pm 0.277$	24.07±0.716	$7.53 \pm 0.528$	$20.03 \pm 1.811$	$26.95 \pm 2.645$	$44.58 \pm 3.078$
12	$2.79 \pm 0.307$	24.52±0.767	$7.14 \pm 0.540$	20.25±1.916	$26.60 \pm 2.814$	44.92±3.289
	NS	*	NS	NS	NS	NS
19	$3.09 \pm 0.242$	$25.75{\pm}0.580^{a}$	$8.83 \pm 0.462$	$15.83 \pm 1.445$	$24.48 \pm 2.197$	52.87±2.611
21	$3.04 \pm 0.280$	$24.69 \pm 0.691^{b}$	$7.63 \pm 0.505$	19.47±1.732	24.85±2.551	47.16±3.010
20	$2.79{\pm}0.363$	$22.84{\pm}0.854^{\circ}$	$7.31 \pm 0.664$	$22.15 \pm 2.208$	26.82±3.314	$44.46 \pm 3.889$
	50 50 0 1 2 5 2 9 21	Fleece Yield (kg) N LSM $\pm$ SE 0 2.97 $\pm$ 0.199 NS 0 3.45 $\pm$ 0.130 0 2.49 $\pm$ 0.471 NS 0 3.47 $\pm$ 0.298 1 2.91 $\pm$ 0.252 2 3.13 $\pm$ 0.269 5 2.55 $\pm$ 0.277 2 2.79 $\pm$ 0.307 NS 9 3.09 $\pm$ 0.242 1 3.04 $\pm$ 0.280	$\begin{array}{c cccc} Fleece \\ Yield \\ (kg) \\ (\mu m) \\ \hline \\ N \\ LSM\pmSE \\ LSM\pmSE \\ \hline \\ 10 \\ 2.97\pm0.199 \\ 24.42\pm0.495 \\ \hline \\ NS \\ 0 \\ 3.45\pm0.130 \\ 25.12\pm0.324 \\ 0 \\ 2.49\pm0.471 \\ 23.73\pm1.174 \\ \hline \\ NS \\ 0 \\ 3.47\pm0.298 \\ 24.00\pm0.723 \\ 1 \\ 2.91\pm0.252 \\ 24.79\pm0.629 \\ 2 \\ 3.13\pm0.269 \\ 24.73\pm0.661 \\ 5 \\ 2.55\pm0.277 \\ 24.07\pm0.716 \\ 2 \\ 2.79\pm0.307 \\ 24.52\pm0.767 \\ \hline \\ NS \\ * \\ 9 \\ 3.09\pm0.242 \\ 25.75\pm0.580^a \\ 1 \\ 3.04\pm0.280 \\ 24.69\pm0.691^b \\ \hline \end{array}$	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $

Least squares means (LSM) and standard errors (SE) of fleece yield and characteristics in Anatolian Merino sheep

\*a,b,c: The differences between the averages shown with different letters in the same column are significant. \*P<0.05; NS: Insignificant.

The fleece hairs can be extended a little by holding from both ends and stretching, and can regain their original form when released. However, if more tension is applied, the cortex layer is well stretched and the cells are separated from each other and the hair is broken (Sönmez, 1963). This property is called elasticity. When the extension ability of the hairs that make up the fleece shirt is bad, the fleece diminishes while being processed in the factory and thus the fleece yield decreases (Dellal et al, 2000a). The elasticity value was calculated as 25.384±1.833% for the research herd. This value is lower than the values 30.2% - 36.1% reported by Sahan et al. (1995) for meat sheep x Merino crossbred (F1) sheep, 32.5%, 39.3%, 41.5% reported by Koyuncu et al. (1996) for Merino, Kıvırcık and Türkgeldi sheep, 31.48%, 27.35 % reported by Dellal et al. (2000a) and Arik et al. (2003) for Anatolian Merino. On the other hand, it is higher than elasticity value of 21.1% and 23.3% reported by Yalçın (1972) for Konya Merino yearling lamb and dams, 23.69% reported by Arık et al. (2003) for Anatolian Merino x Ile de France crossbred, and than 24.45% reported by Uzun for Karacabey Merino.

Table 1

The effects of gender, age and live weight group factors on elasticity were found to be statistically insignificant. Uzun (2008) found the effect of age on the elasticity of Karacabey and Menemen sheep to be statistically insignificant. On the other hand, Peşmen and Yardımcı (2012) reported the effect of age on elasticity in Menemen sheep to be significant.

The physical property that has the greatest effect on price is Clean Fleece Percentage. The Clean Fleece Percentage value calculated in this study is 48.165±2.175%. This result supports the knowledge that Anatolian Merino sheep are classified as fine wool sheep. This per-

centage value is lower than 53.72%, 51.91%, 59.13% reported by Dellal et al. (2000a), Arık et al. (2003), Tuncer and Cengiz (2018) for Anatolian Merino sheep, 60.92%, 51.3% reported by Tellioğlu (1975), Koyuncu et al. (1996) for Merino sheep, and 54-58%, 51.13% reported by Yalçın (1972) and Arık et al. (2003) for Konya Merino, Ile de France x Anatolian Merino. On the other hand, it is close to 47.9% and higher than 43.1%, calculated by Şahan et al. (1995) for Merino female yearling lambs and male yearling lambs, respectively. Wool grease is found more in fine wool sheep breeds than coarse wool sheep breeds, and because the grease of fabric type fleeces.

It was determined that the effect of the environmental factors (gender, age, body weight group) examined on the Clean Fleece Percentage was insignificant. The effect of age on yield was found to be statistically significant in Menemen sheep (Peşmen and Yardımcı, 2012). Bağkesen and Koçak (2018), on the other hand, stated that the effect of age on the yield in Dağlıç sheep was significant, while it was insignificant in Ramlıç sheep. Uzun (2008) reported that when the values of different genders were examined in his study, the difference between gender groups was not statistically significant.

In this study, fleece yield and properties were investigated on a small sample of Anatolian Merino sheep raised by the public, and according to the results obtained, it was determined that Anatolian Merino wool had significant superior characteristics compared to the wool of domestic sheep. For this reason, it can be said that Anatolian Merino sheep can be an important source in the domestic supply of the fleece that the textile industry needs. To further improve the fleece yield and quality of Anatolian Merino sheep and to produce wool suitable for the needs of the fabric industry, there is a need for increasing the current numbers and serious and planned selection to be applied in this direction.

#### 5. Acknowledgements

This research was supported by a master research project from the Coordinatory of Scientific Research Projects of Selcuk University, Turkey (Project No: 21201021). Thank you for your financial support.

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http://sjafs.selcuk.edu.tr/sjafs/index

**Research Article** 

SJAFS

(2022) 36 (1), 13-19 e-ISSN: 2458-8377 DOI:10.15316/SJAFS.2022.003

## A Research of Affecting Factors on R&D Management in Food

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ARTICLE INFO	ABSTRACT
Article history: Received date: 22.09.2021 Accepted date: 24.12.2021	The purpose of this study was to analyze the R&D approaches of business man- agers and the factors that affect R&D activities in the central districts of the province of Konya in Turkey. The main data of the study were collected by ques- tionnaires that were applied with managers of 67 businesses in the food industry
<b>Keywords:</b> Food Industry R&D Factor Analysis Regression Analysis Mann Whitney U	who were determined by the method of quota sampling. Ac-cording to the re- sults, the effects of the educational status of the business owner, the personnel structure of the business, the date when the business was established, having a plan for R&D activities for the next 5 years, whether or not they had knowledge on the institutions that provide support for R&D projects and the level of collab- oration with universities on the R&D activities of businesses were significantly effective on the level of 5%. The variables that were related to the R&D percep- tions of the business managers were determined by factor analysis, and it was found that there were 5 factors regarding the R&D perceptions of the managers. Regression analysis was used to determine whether or not the factors related to R&D perceptions were effective on conducting R&D activities. As a result of the regression analysis, it was determined that R&D had contribution in the op- erations of the business (Factor I), and the two factors related to universi-ty- industry collaboration (Factor V) had significant effects on businesses' R&D activities on the level of 5%.

#### 1. Introduction

In today's conditions, in order for countries to take a prominent position in the international arena in the sectoral sense, they need to pay importance to innovation activities in their production processes. To achieve this power, they must conduct R&D activities (Dahlman 2007). With the help of R&D and innovation, businesses operate more effectively and efficiently, and consumers are more satisfied (Rajapathirana & Hui 2018). The advanced technology practices of a country contribute to the increase in the prosperity in the country, and therefore, higher living standards (Samimi & Jenatabadi 2014).

R&D activities have an important place in the agriculture and food industry and in increasing the competitive power of countries like Turkey, which have a high potential for agriculture. By applying R&D activities on the production models in the food industry, it will be possible to achieve an economic model where the continuity of employment and efficiency are increased, revenue is raised bilaterally between the producers and industrialists and the state, and social prosperity is achieved (Tan et al. 2017). Businesses may operate transformation activities by conducting R&D activities (Barro 1990). The information which is accessed by the R&D activities in economic sectors allows higher levels of production without additional costs (Silva & de Carvalho 2015). The importance that is paid to R&D is higher in new economic systems (Bozkurt 2015). This is why investments on R&D are considered to be an indicator of the effort of the economy to bring innovation (Pandit et al. 2009).

The food sector, which has developed a lot in recent years, is among the most important economic sectors in Turkey (Ozden & Senkayas 2012; Gursakal et al. 2015). The food sector is a branch of the manufacturing sector which converts plant- and animal-based raw materials that are obtained as a result of agricultural activities into products with long shelf life that are ready for consumption by using multiple different processes (Bulu et al. 2007). In the food sector, Turkey is mostly involved in production that is dependent on imported technology. Additionally, there are significant shortcomings in adapting the imported technology to the conditions in the country (Kuşat 2012). Several practices were carried out in Turkey after 2010 within the scope of the National Science, Technology and Innovation Strategy for the Period of 2011-2016 to determine the priorities of various sectors in science, technology and innovation. The ne-

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cessity of need-based R&D was emphasized in six sectors that require accelerated development as the defense, space, health, energy, water and food sectors. Additionally, in these fields, strategies were adopted to improve human resources for innovation in science technologies, convert research outcomes into goods and services, spread the culture of multidisciplinary R&D collaboration, increase the role of SMEs in the national system of innovation, raise the contribution of the R&D infrastructure in the field of Turkish research and make the innovation in international science technologies effective.

In order to improve R&D and innovation policies and strategies in both the food industry and other industries, it is needed to determine the approaches of businesses in the nationwide sectors on this field. Therefore, it is important in this study to determine the R&D approaches, attitudes and behaviors of business managers of food industry businesses in the province of Konya in Turkey which has a high potential for agriculture and thus a developed food industry, in terms of revealing the existing situation regarding the R&D activities in the food industry.

#### 2. Materials and Methods

The main material of the study consisted of the primary data that were collected with questionnaires used in 67 businesses in the food industry in the central districts of the province of Konya (Karatay, Meram, Selçuklu). Additionally, studies carried out by various organizations and institutions on the topic and statistics were utilized.

The quota sampling method was used to determine the number of the businesses to be included in the study (Acharya et al. 2013). Accordingly, this number was determined as 67. This number represents 24% of all food industry businesses in the region.

The attitudes, behaviors and perceptions of the business managers of the businesses in the food industry in the central districts of Konya were analyzed using a 5point Likert-type scale. A Likert-type scale is the most practical method for measuring attitudes. This is why this method is used prevalently for attitude scales (Hoyle et al. 2002; Yilmaz et al. 2019; Türker 2007)

A factor analysis was carried out to categorize the factors that affect the R&D perceptions of the business managers of the food industry. Kaiser-Meyer-Olkin (KMO) test was used to determine whether or not the data were suitable for factor analysis (Keleş 2019). High rates of variance as a result of factor analysis show that the factorial structure of the scale is strong (Yong & Pearce 2013).

A logistic regression analysis was conducted to determine the factors that affect R&D activities. The logit model is a statistical model that allows a categorization that is appropriate for the rules of probability by probabilistically calculating the estimated values of the dependent variable and analyzes tabulated or raw datasets (Gujarati 2009). Based on studies in the literature, it was determined that the logistic regression model was suitable for this study, and it is frequently used to analyze similar data (Powers & Xie 2008; Unlüer & Günes 2013; Gençdal et al. 2015; Christoforou et al. 2018; Öz & Saner 2021)

The general functional representation of the Logit models is as follows (Gujarati 2009);

$$P(Y) = \frac{e^{(\beta_0 + \beta_1 \beta_1)}}{(1 + e^{(\beta_0 + \beta_1 X_1)})}$$

Dependent variable is whether or not to do R&D.

P(Y) = Probability of being dependent variable

e= The natural logarithm of 10 base and approximately 2.30

X\_i = Number of explanatory variables

 $beta_0 \ and \ beta_1 = Typically estimated by the maximum likelihood (ML) method (Berenson et al. 2012).$ 

The odds rate (OR) used to interpret the logistic regression model is the value obtained by dividing the probability of observing the event by the probability of not observing the event. It is calculated as;

$$OR = \frac{P(Y)}{1 - P(Y)}$$

Mann Whitney U test was used to determine the factors that affected R&D activities among the businesses in the food industry in the central districts of Konya that carried out R&D activities and those that did not conduct R&D activities. Mann Whitney U test is used to determine the differences between two independent variables that are measured continuously. It is the non-parametric equivalent of t-test, which is a parametric test used to determine whether or not the difference between two independent variables such as an X and a Y is significant (it compares the median values of the variables) (MacFarland & Yates 2016).

#### 3. Findings and Discussion

# 3.1. Some Socioeconomic Characteristics of the Businesses in the Food Industry

As some of the socioeconomic characteristics of the food industry businesses that were examined, the study collected data on the business managers' educational status, personnel status, the date of establishing the business, annual revenue, library situation and whether they advertised or not. It was found that most food industry business managers in the region (59.70%) had undergraduate degrees. The educational levels of the business managers in the region were higher than those of the agriculture-based industry business managers in the province of Samsun (Mazgal 2005). 46.27% of the studied businesses had 10-49 employees, 19.40% had 1-9 employees, 17.91% had 50-99 employees, and 16.42% had 100 or more employees. The personnel status of the businesses in the studied region was similar to those in the food industry in the province of Tokat and the industry sector in (Yalçın & Esengün 2008; Üçler & Karaçor

15

2014). The studied businesses had different dates of establishment. The highest rate of the businesses (29.85%) were established in the period of 1971-1990. 25.37% of the businesses were established in 1991-2000, 20.90% were established in 2001-2010, 11.94% were established in 1950-1970, and another 11.94% were established in 2011 or later. It was determined that the food industry businesses in the studied region were usually established after 1998 in similarity to the dates of establishment among the agriculture-based industry businesses in the province of Van (Ulas & Cakir 2006). 58.21% of the studied businesses had annual revenues of higher than 1,000,000 TL, 16.42% had annual revenues in the range of 751,000-1,000,000 TL, 8.96% had annual revenues in the range of 101,000-250,000 TL, 7.46% had annual revenues in the range of 251,000-500,000 TL, 4.48% had annual revenues of 501,000-750,000 TL, and another 4.48% had annual revenues of lower than 100,000 TL. It was reported that the annual revenues of the studied businesses were similar to those of the food industry businesses nationwide, and their annual revenues usually exceeded 1,000,000 TL (Bakkaloglu & Günes 2018).

#### 3.2. The State of R&D in the Studied Food Industry **Businesses**

While 56.72% of the food industry businesses that were studied conducted R&D activities, 43.28% did not. It was determined that the rate of conducting R&D activities among the studied food industry businesses was higher than those of the agriculture-based industry businesses in the provinces of Çanakkale and Samsun and the food industry businesses nationwide (Mazgal 2005; Tan et al. 2017; Bakkaloglu & Günes 2018). It was observed that the vast majority (61.19%) of the studied Table 1

food industry businesses planned to conduct R&D activities in the next 5 years. Among the studied businesses, 61.19% had knowledge about the institutions that provide support for the R&D projects and activities of the food industry businesses in the studied region. In terms of the sources of financial R&D support that were utilized in the food industry businesses, it was determined that 19.40% used support by KOSGEB (the Small and Medium Enterprises Development Organization of Turkey), and 11.94% used support by TÜBİTAK (the Scientific and Technological Research Council of Turkey).

Mann Whitney U test was conducted to determine whether or not some of the socioeconomic characteristics of the businesses were effective on their usage of R&D activities (Table 1). The findings that were obtained as a result of the analysis are described below.

In terms of conducting and not conducting R&D activities, the effects of the educational status of the business owner, the personnel structure of the business, the date when the business was established, having a plan for R&D activities for the next 5 years, whether or not they had knowledge on the institutions that provide support for R&D projects and the level of collaboration with universities on the R&D activities of businesses were significantly effective on the level of 5%.

No significant relationship could be found between whether or not the studied businesses conducted R&D activities and their annual revenues, library situations, levels of advertising, state of following the R&D activities of competitor firms and needs for receiving counselling support within the scope of university-industry collaboration.

Determination of properties of businesses conducted R&D activities and non-R&D activities by Mann Withney U test

				-	-
	R&D Situations	Ν	Mean Rank	Sum of Ranks	Asymp. Sig. (p)
The effects of the educational status	R&D	38	38,09	1447,50	,030
of the business owner	Non-R&D	29	28,64	830,50	,050
Personnel structure of the businesses —	R&D	38	38,53	1464,00	020
Personnel structure of the businesses —	Non-R&D	29	28,07	814,00	,029
The date when the business was es-	R&D	38	29,68	1128,00	024
tablished	Non-R&D	29	39,66	1150,00	,034
A	R&D	38	31,53	1198,00	102
Annual revenues —	Non-R&D	29	37,24	1080,00	,183
I :h	R&D	38	35,88	1363,50	225
Library situations —	Non-R&D	29	31,53	914,50	,225
I and of a decention of	R&D	38	36,42	1384,00	169
Level of advertising —	Non-R&D	29	30,83	894,00	,168
Having a plan for R&D activities for	R&D	38	38,18	1451,00	017
the next 5 years	Non-R&D	29	28,52	827,00	,017
State of following the R&D activities	R&D	38	36,18	1375,00	107
of competitor firms	Non-R&D	29	31,14	903,00	,197
Whether or not they had knowledge	R&D	38	39,95	1518,00	
on the institutions that provide sup- port for R&D projects	Non-R&D	29	26,21	760,00	,001
The needs for receiving counselling	R&D	38	35,08	1333,00	
support within the scope of univer- sity-industry collaboration	Non-R&D	29	32,59	945,00	,506
The level of collaboration with uni-	R&D	38	37,08	1409,00	
versities on the R&D activities of businesses	Non-R&D	29	29,97	869,00	,040

The perceptions, attitudes and behaviors of the business managers on R&D were investigated, and the results are presented in Table 2.

#### Table 2

R&D perceptions, attitudes and behaviors of the food industry enterprises examined

Opinions about R&D management	Mean*	Standard Deviation	Variance
In general, Turkey has done enough in R&D.	1,99	0,913	0,833
Public institutions in Turkey are allocating sufficient resources to R&D activities.	2,21	0,897	0,804
Private sector in Turkey are allocating sufficient resources for R&D activities	2,33	0,975	0,951
In Turkey, R&D activities are in the development stage.	2,88	0,930	0,864
In Turkey, the share allocated to R & D is sufficient.	2,28	0,831	0,691
In Turkey, not given required importance to R&D	3,63	0,935	0,874
The knowledge of the country is not sufficient for R&D.	3,75	0,766	0,586
Technology development activities in the country are in good condition.	2,66	0,880	0,774
Konya has an R&D potential that you work.	2,85	0,973	0,947
Original products are only possible with R&D.	3,88	0,808	0,652
R&D activities are important for businesses.	3,84	0,828	0,685
There is a need to collaborate with universities in Food Industry.	3,09	1,125	1,265
Businesses should allocate at least 10% of their budgets for R&D activities.	3,66	0,930	0,865
R&D is the primary precaution for product development.	3,88	0,789	0,622
There is a direct correlation between companies' R&D investments and their competitiveness.	4,01	0,663	0,439
Technology developed with R&D studies will increase the competitive power of the company.	4,00	0,674	0,455
The activities of competitors must also be considered in R&D studies.	3,97	0,717	0,514
Investors believe that the success and development of the company will be more successful with	3,85	0,875	0,765
innovation.	2.07	0.521	0.070
Businesses have knowledge about technological developments with R & D.	3,97	0,521	0,272
Businesses have information about businesses in the sector with R & D.	3,94	0,600	0,360
Businesses have information about new equipment with R & D.	3,93	0,635	0,403
Businesses have knowledge about R & D, Cost and sales strategies.	3,73	0,709	0,502
Businesses Have information about the market with R & D.	3,69	0,783	0,612
Your organization is systematically monitoring technology and markets to maximize develop- ment.	3,22	1,012	1,025
R & D expenses in your company are considered as investment rather than cost.	3,06	1,099	1,209
If cooperation with the university is requested, our business will participate	3,16	1,298	1,685
Our business does benefit from public / private R & D support.	2,66	1,136	1,289
The importance of R & D activities in business is given.	3,31	1,117	1,249
Technological developments and innovations are constantly monitored in our business.	3,40	1,102	1,214
Our business is updating its strategy according to R & D activities.	3,34	1,038	1,077
R & D activities / investments / expenditures carried out in our business are contributing as a competitive advantage	3,37	1,139	1,298
R & D activities / investments / expenditures carried out in our business contribute to growth and efficiency output.	3,36	1,151	1,324
R & D activities / investments / expenditures carried out in our business contribute to production	3,37	1,153	1,328
output. R & D activities / investments / expenditures carried out in our business contribute as a product	3,33	1,160	1,345
variety output. R & D activities / investments / expenditures carried out in our business contribute to the origi-		,	
nal design / innovation output.	3,30	1,168	1,364
R & D activities / investments / expenditures carried out in our business contribute to patent out- put.	3,13	1,192	1,421
*Strongly agree 5 agree 4 neutral 3 disagree 2 strongly disagree 1			

\*Strongly agree:5, agree:4, neutral:3, disagree:2, strongly disagree:1

The Kaiser-Meyer-Olkin (KMO) test statistic of the attitudes and behaviors of the business managers on R&D was 0.808. This result showed that the distribution of the data was suitable for factor analysis. The significant Bartlett's test result of 2762.022 (p<0.001) showed

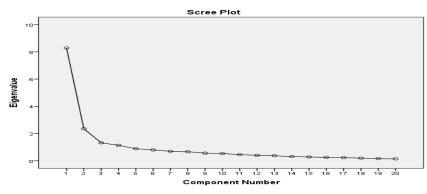
that the data had a multivariable normal distribution. As a result of the analysis, it was determined that the related variables were gathered under 5 factors. 70.04% of the total variance was explained cumulatively by these 5 factors (Table 3).

		Initial Eigenv	alues	r	Total Factor I	Loads	Turne	ed Totals of Fact	or Loads
Factor	Total	Variance %	Cumulative %	Total	Variance %	Cumulative %	Total	Variance %	Cumulative %
1	15,080	41,890	41,890	15,080	41,890	41,890	10,510	29,195	29,195
2	4,005	11,125	53,015	4,005	11,125	53,015	4,524	12,567	41,762
3	3,040	8,443	61,458	3,040	8,443	61,458	3,997	11,102	52,864
4	1,792	4,977	66,435	1,792	4,977	66,435	3,261	9,059	61,923
5	1,297	3,601	70,036	1,297	3,601	70,036	1,873	5,203	67,127
6									
36	0,002	0,006	100,000						
Kaiser-	Meyer-Olk	in Sampling (	Competence					0,808	
	-		-		Khi Square V	alue		2762,022	
Bartlett	's Test of S	phericity		I	Degree of Fre	edom		630	
		- •			n			0.000	

Table 3 R&D perceptions, attitudes and behaviors of the foodindustry enterprises examined

While conducting the factor analysis with the purpose of categorizing the factors that affected the R&D perceptions of the business managers, principal components analysis was conducted with the varimax rotation technique to gather information on the construct validity

of the scale. As seen in Figure 1, when the initial eigenvalue was taken as 1.26 and as a result of the repeated factor analysis, 5 factors were determined. The high values of variance as a result of the factor analysis showed that the factorial structure of the scale was strong (Yong & Pearce 2013).



#### Figure 1

Aggregation graph for the items in the survey

As a result of the factor analysis on the 36 items that were examined to measure the perceptions, attitudes and behaviors of the business managers on R&D (Table 2), the related issues were gathered under 5 factors (Table 4). The factors where related variables gathered were described as; the contribution of R&D on the operations of the business (Factor I), the contribution of R&D on the

competitive power of operations (Factor II), the contribution of R&D on technological development (Factor III), the contribution of the national R&D structure on businesses in the food industry (Factor IV) and the contribution of university-industry collaboration on the R&D activities of businesses in the food industry (Factor V).

#### Table 4

Factor Analysis of Perception, Attitude and Behaviors of Food Industry Management Managers Relate	
Tuetor Thangsis of Teleoption, Thindde and Denaviors of Tood medistry Management Managers Relate	d to R&D

Factor Groups	Scale Items and Factor Loads	Number of İtems
the contribution of R&D on the operations of the business	Item 35 (0,935), Item 33 (0,928), Item 34 (0,912), Item 32 (0,907), Item 31 (0,900), Item 28 (0,840), Item 36 (0,837), Item 29 (0,828), Item 30 (0,825), Item 25 (0,795), Item 27 (0,633), Item 24 (0,629), Item 9 (0,416)	13
the contribution of R&D on the competitive power of operations	Item 15 (0,869), Item 16 (0,854), Item 17(0,711), Item 14 (0,645), Item 18 (0,599), Item 10 (0,577), Item 11 (0,457)	7
the contribution of R&D on technological de- velopment	İtem 21 (0,789), İtem 20 (0,775), İtem 19 (0,756), İtem 22 (0,756), İtem 23(0,702)	5
the contribution of the national R&D structure on businesses in the food industry	İtem 1 (0,887), İtem 2 (0,800), İtem 3 (0,774), İtem 8 (0,615), İtem 7 (-0,605), İtem 5 (0,585), İtem 6 (-0,473), İtem (0,458)	8
the contribution of university-industry collab- oration on the R&D activities of businesses in the food industry	İtem 12 (0,667), İtem 13 (0,590), İtem 26 (0,491)	3

Logistic regression analysis was used to test the effects of the factors related to the perceptions, attitudes and behaviors of the business managers on whether or not they conducted R&D activities (Table 5). 2 of the 5 factors that were investigated in the model had significant effects. The dimensions that had significant effects were Factor 1 and Factor 5. The results showed that, when all the other conditions are kept constant, R&D contributed to the operations of businesses in the food industry, and the business managers that had a tendency to stated that university-industry collaboration contributes to improvement of R&D by businesses also had a higher tendency to conduct R&D activities.

Table 5

Logistic regression model

Name of Variable	В	S.E.	Wald	df	Sig.	Exp(B)
Factor I.	1,729	0,408	17,994	1	0,000	5,633
Factor II.	0,467	0,382	1,495	1	0,221	1,595
Factor III.	0,121	0,366	0,109	1	0,741	1,129
Factor IV.	0,254	0,339	0,563	1	0,453	1,289
Factor V.	0,681	0,353	3,726	1	0,044	1,976
Invariant	0,337	0,330	1,047	1	0,306	1,401

#### 4. Conclusions and Recommendations

This study investigated the R&D approaches of business managers of businesses in the food industry in the central districts of Konya in Turkey and the factors that affect the status of conducting R&D activities.

The personnel status of the businesses was significantly effective on the level of 5% on whether or not they conducted R&D activities. Accordingly, the number of employees and the experiences of the personnel regarding R&D should be increased at businesses that do not conduct R&D activities. Businesses should start systematic plans that would improve R&D activities and employ qualified personnel.

There was a significant relationship on the level of 5% between the businesses' dates of establishment and whether or not they conducted R&D activities. However, considering the effect of R&D on business success, without regard to their establishment dates, awareness about R&D should be raised in businesses for their continuity and sustainable competitive power.

Whether or not the business manager had knowledge on institutions that provide support for R&D projects was significantly effective on the level of 5% on whether or not they conducted R&D activities. It was seen that, although the majority of the business managers (61.19%) had knowledge about the institutions that fund R&D projects and provide assistance, they did not sufficiently utilize the available assistance. Regarding this issue, institutions that provide R&D projects and assistance should inform food industry businesses and establish collaboration with them.

There was a significant relationship on the level of 5% between the businesses' levels of collaboration with

universities and whether or not they conducted R&D activities. Industry-university collaboration should be more prevalent for increasing the R&D activities of businesses in the food industry.

Considering the factors that are related to the perceptions, attitudes and behaviors of the business managers on R&D, it was found that the managers who had positive attitudes towards the idea that R&D contributes to the operation of the business in the food industry and the idea that university-industry collaboration contributes to improvement of R&D by businesses in the food industry had higher rates of conducting R&D activities in comparison to the other managers. In the light of these results, seminars and introduction activities should be planned for managers of businesses in the food industry regarding the importance and advantages of conducting R&D activities. It is needed to fill the gaps in the knowledge of business owners and personnel on R&D with workshops and similar meetings that involve experts from universities and different sectors.

Consequently, state-industry-university collaboration has great importance for businesses in the food industry. These organizations and institutions should perform the responsibility that falls upon them and work in coordination.

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**Research Article** 

#### **SJAFS**

(2022) 36 (1), 20-26 e-ISSN: 2458-8377 DOI:10.15316/SJAFS.2022.004

# **Determination of The Seedling Reactions of Some Two-Rowed Barley Landraces** Maintained at Osman Tosun Gene Bank to Pyrenophora Teres F. Teres

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#### ARTICLE INFO

Article history: Received date: 12.10.2021 Accepted date: 24.12.2021

**Keywords:** Barley Barley landraces Drechslera teres f. teres Disease resistance Net blotch

#### ABSTRACT

Seedling stage reactions of thirty-eight 2-rowed barley landraces representing different areas of Turkey obtained from Osman Tosun Gene Bank to two Pyrenophora teres f. teres isolates were evaluated. In addition, barley cultivars Bülbül 89 and Avci 2002 were included. Landraces exhibited different reactions to the disease and their reactions ranged from resistant- moderately resistant to susceptible. Landrace number 33 obtained from Diyarbakır was found the most resistant to the disease compared to all other landraces whereas landrace number 10 obtained from Bilecik-Söğüt, and cultivar Bülbül 89 were the most susceptible. The majority of the landraces were classified between the Moderately Resistant-Moderately Susceptible to Moderately Susceptible-Susceptible. Landrace number 33 exhibited Resistant-Moderately Resistant reactions to both isolates. This landrace from Diyarbakır could be used as the seed source and in the breeding studies for obtaining barley cultivars resistant to the net form of net blotch disease.

#### 1. Introduction

Barley is one of the oldest domesticated crops and an important cereal (FAO, 2015). It is ranked as the second most important cereal in Turkey (Geçit, 2016; TUIK, 2016). Today barley is used commercially as the main source of food in livestock production, healthful diets, and in the malt industry (Kün, 1996). In Turkey, the average production of barley is estimated at 8.300.000 tonnes per year (TUIK, 2020).

Landraces are recognized for their importance as a germplasm source for barley breeding programs and for improving the genetic diversity of barley and they are adapted to stress factors (Brush, 1995; Attene et al., 1996). Also, barley landraces are used as the main seed source in many of the traditional barley fields by the farmers (Ceccarelli and Grando, 2000).

Landraces are well adapted to the different agroclimatic conditions, and they have emerged as a result of many years of selection. However, with the emergence of genetically uniform, high-yielding, and high-quality commercial varieties, farmers preferred to use commercial varieties. Over time, the replacement of the landraces with a high degree of variation by commercial varieties has resulted in a loss of genetic diversity (Ceccarelli et al., 2000; Ceccarelli and Grando, 2000). Genetic stock studies were started in Turkey in 1938 by Osman

Tosun. Barley landraces have been collected by Osman Tosun and his colleagues from different parts of Turkey. Barley germplasm from several countries is also maintained in Osman Tosun Gene Bank, Ankara, Turkey (Çelik Oğuz et al., 2019).

Pyrenophora teres (anamorphic stage: Drechslera teres) is an important pathogen of barley. Two forms of the disease, spot, and net forms exist. Drechslera teres f. teres incites the net form of the disease (Liu et al., 2011). The disease affects badly the quantity and quality of barley crops worldwide. In places where very susceptible varieties are cultivated, destruction of the crops is expected (Mathre, 1982).

Both forms of the pathogen is common in Turkey (Karakaya et al., 2014; Damgacı, 2014; Celik and Karakaya 2015; İlgen et al., 2017; Özdemir et al., 2017; Ertürk et al., 2018; Saraç et al., 2019; Sivrikaya et al., 2020). Both forms of the pathogen cause numerous races and this may complicate the control efforts. In a study conducted in Turkey, 24 Pyrenophora teres f. teres pathotypes and 26 Pyrenophora teres f. maculata pathotypes were found (Celik Oğuz and Karakaya 2017). Morphological, pathological, and genetic variation was observed among the Turkish isolates of P. teres (Celik Oğuz et al., 2014; Celik Oğuz et al 2019a). Both mating types of fungus were found in Turkey (Celik Oğuz et al.,

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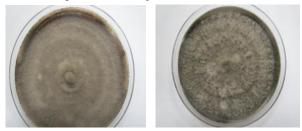
2018). In Ankara, Turkey, on the leaves, left on the ground, and buried, conidia, conidiophores, and pseudothecia of the pathogen were observed. These propagules were more common on the leaves left on the ground. In cooled incubator studies, pycnidia were observed. Cooled incubator studies revealed that fungus in diseased leaves and fungal cultures survived -10°C. Apparently, the fungus can survive during the winter months under Ankara, Turkey conditions (Karakaya *et al.*, 2004).

If much focus is not taken on controlling this disease, great economic losses are expectable in areas where it forms a real threat to barley production. Various measures such as stubble destruction and the application of fungicides can control the disease. However, since plant residues are still capable of producing numerous infectious spores, a complete elimination of stubble inoculum to prevent significant infection for the next season crop is a quite difficult task (Jordan and Allen, 1984). Cultural practices alone seem ineffective for the management of the disease. Chemical fungicides are highly expensive and they are unaffordable for most farmers, reduced sensitivity of the pathogen is expectable, and a fungal population can develop resistant biotypes (Olvång, 1988). Therefore, the application of other methods such as crop rotation and using of disease-resistant barley genotypes has become necessary for the control of the pathogen (Mathre, 1982; McLean et al., 2012). The use of disease-resistant barley genotypes is the most profitable and eco-friendly means of controlling the disease.

The growth of resistant cultivars is the preferred control method (Mathre, 1982; Yazıcı et al., 2015). Landraces are genetic variation sources in plant breeding programs (Yitbarek *et al.*, 1998). Elucidation of the resistance status of barley landraces will be useful in breeding programs. Also, some high-yielding landraces could be used by farmers. Barley landraces can be planted either directly in the field or utilized in breeding programs for developing new resistant varieties. In Turkey, limited work exists on the net type resistance status of barley genotypes (Yazıcı *et al.*, 2015; Çelik Oğuz *et al.*, 2016; 2017, 2019b). In this work, under greenhouse conditions, 38 Turkish barley landraces obtained from Osman Tosun Gene Bank to two *Pyrenophora teres* f. *teres* isolates have been evaluated at the seedling stage. In addition, two barley cultivars Bülbül 89 and Avci 2002 were included in this study.

#### 2. Materials and Methods

This study was accomplished in the laboratory and the greenhouse of the Ankara University, Faculty of Agriculture, Department of Plant Protection, Turkey. All trials in the greenhouse were executed as three replications. A total of 38 barley landraces were used in the study. The landraces used in this study were obtained from Osman Tosun Gene Bank, Ankara, Turkey. In addition, barley cultivars Bülbül 89 and Avci 2002 were included in this study. The locations of the barley landraces were presented in Figure 2.





Single spore culture of Ankara isolate in Potato Dextrose Agar medium (PDA) (left), single spore culture of Sivas isolate in PDA medium (right).

Inoculation was accomplished using 2 *Pyrenophora teres* f. *teres* isolates obtained from the Ankara and Sivas provinces of Turkey (Figure 1). Isolates were obtained from Aziz Karakaya and Arzu Çelik Oğuz, Ankara University, Turkey.

The Gene Bank Registration numbers of the thirtyeight landraces used in the study, the locations from which they were obtained, and the color of their kernels are shown in Table 1



Figure 2 Provinces that barley landraces are obtained.

 Table 1

 Some characteristics of the landraces used in this study

No	Osman Tosun Gene Bank Registration No	Туре	Location Kerne	el color
1	667	2-rowed	Eskişehir-Çifteler	Black
2	885	2-rowed	Diyarbakır-Bismil	White
3	930	2-rowed	Diyarbakır-Karabaş	White
4	900	2-rowed	Diyarbakır-Nusaybin	Black
5	933	2-rowed	Erzincan-Refahiye	White
6	715	2-rowed	Kayseri-Pınarbaşı	White
7	884	2-rowed	Urfa	White
8	684	2-rowed	Ağrı-Tutak	White
9	692	2-rowed	Ankara	White
10	659	2-rowed	Bilecik-Söğüt	White
11	638	2-rowed	Rize-Pazar	White
12	868	2-rowed	Konya-Zıvarak	White
13	671	2-rowed	Çorum-Mecitözü	White
14	709	2-rowed	Sivas-Zara	White
15	707	2-rowed	Niğde-Aksaray	White
16	702	2-rowed	Kırşehir-Kaman	White
17	799	2-rowed	Adana-Kadirli	White
18	854	2-rowed	Nevşehir	White
19	931	2-rowed	Malatya-Pötürge	White
20	914	2-rowed	Erzurum-H.Kale	White
21	825	2-rowed	Kayseri-Pınarbaşı	White
22	707	2-rowed	Malazgirt	White
23	660	2-rowed	Sinop-Gerze	White
24	685	2-rowed	Hatay-Kırıkhan	White
25	644	2-rowed	Gaziantep-Nur.	White
26	664	2-rowed	Isparta	White
27	844	2-rowed	Yozgat-Akdağmadeni	Black
28	771	2-rowed	Konya	Black
29	926	2-rowed	Urfa-Siverek	White
30	915	2-rowed	Sivas-Divriği	Black
31	640	2-rowed	Amasya-Taşova	White
32	673	2-rowed	Isparta-Gelendost	White
33	941	2-rowed	Diyarbakır	White
34	786	2-rowed	Hatay-Korkuteli	White
35	906	2-rowed	Konya-Ereğli	White
36	888	2-rowed	Eskişehir-Ağapınar	White
37	752	2-rowed	Niğde-Bor	White
38	689	2-rowed	Erzurum	White

The inoculation procedure was similar as described by several other investigators (Çelik Oğuz et al., 2019b; Yazıcı et al., 2015; Douiyssi et al., 1998). Fifteen seeds from each landrace were seeded in 7 cm diameter plastic pots containing soil. Plants were watered as necessary. The temperature of the greenhouse was  $18-23\pm2$  °C for night and day with a 14h/10h light/dark regime. For inoculum production, mycelia were scraped from Petri plates using a paintbrush. Inoculum concentration was

adjusted using a hemocytometer to 15-20x10<sup>4</sup> mycelial parts per ml. One drop of Tween 20 was added for every 100 ml of the inoculum (Aktaş, 1995). After inoculation, plants were placed in metal boxes and a plastic cover was placed on top of each box. In addition, boxes and plastic covers were wrapped with nylon sheets. Plants were inoculated at growth stages 12-13 (Zadoks et al., 1974). After four days nylon sheets and plastic covers were removed. Seven days after inoculation, plants were evaluated with a 1-10 scale developed for *D. teres*  f. *teres* by Tekauz (1985). The description of the scale is as follows:

1: R (Resistant),

2: R-MR (Resistant-Moderately Resistant),

3: MR (Moderately Resistant),

4:<u>MR</u>-MS: (Moderately Resistant-Moderately Susceptible),

5:MR-MS (Moderately Resistant-Moderately Susceptible),

6:MR-<u>MS</u> (Moderately Resistant-Moderately Susceptible),

7: MS (Moderately Susceptible),

8: MS-S (Moderately Susceptible-Susceptible),

9: S (Susceptible),

10: VS (Very Susceptible).

A visual scale is presented in Figure 3.

#### 3. Results and Discussion

Ankara and Sivas isolates differed in the nature of their growth on the PDA medium. The single spore culture of Sivas isolate appeared smooth whereas Ankara isolate had fluffy growth (Figure 1). The first symptoms of the disease were observed on leaves of some landraces three days after inoculation. Leaf symptoms characteristic of the net form of the disease appeared first as narrow chlorotic lesions which gradually increased in size and length. Eventually, severely infected leaves showed dark brown longitudinal and transverse striations (Figure 4).

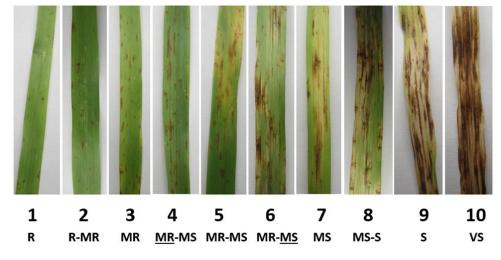


Figure 3

Visual scales used in the experiment according to Tekauz (1985) (Photographs: Aziz Karakaya).



#### Figure 4

Leaves of barley landraces showing different levels of resistance to *Pyrenophora teres* f. *teres* Ankara isolate. a) Şanlıurfa landrace, scale value: 6 (MR-MS), b) Çorum-Mecitözü landrace, scale value: 7 (MS), c)Kayseri-Pınarbaşı landrace, scale value: 6 (MR-MS), d) Diyarbakır landrace, scale value: 2 (R-MR) (Photographs: Hatice Sevde Yüceler)

Evaluations were performed 7 days following inoculation. Results are presented in Table 2 in addition to some information concerning the 2- rowed barley landraces used in this study. Data on disease severity for each landrace are the mean scale values of the three replicates. Table 2

Some characteristics of the barley landraces used in this study and the response of these landraces to 2 *P. teres* f. *teres* isolates at the seedling stage

			Sivas isolate	Ankara isolate
No	Osman Tosun Gene	Location	scale value and reac-	scale value and reac-
110	Bank Registration No	Location	tion type (Tekauz	tion type
			1985)	(Tekauz 1985)
1	667	Eskişehir-Çifteler	5 MR-MS	5 MR-MS
2	885	Diyarbakır-Bismil	8 MS-S	8 MS-S
3	930	Diyarbakır-Karabaş	6 MR- <u>MS</u>	6 MR- <u>MS</u>
4	900	Diyarbakır-Nusaybin	6 MR- <u>MS</u>	5 MR-MS
5	933	Erzincan-Refahiye	7 MS	6 MR- <u>MS</u>
6	715	Kayseri-Pınarbaşı	7 MS	6 MR- <u>MS</u>
7	884	Şanlıurfa	7 MS	6 MR- <u>MS</u>
8	684	Ağrı-Tutak	6 MR- <u>MS</u>	6 MR- <u>MS</u>
9	692	Ankara	6 MR- <u>MS</u>	5 MR-MS
10	659	Bilecik-Söğüt	9 S	8 MS-S
11	638	Rize-Pazar	7 MS	6 MR- <u>MS</u>
12	868	Konya-Zıvarak	7 MS	7 MS
13	671	Çorum-Mecitözü	7 MS	7 MS
14	709	Sivas-Zara	8 MS-S	7 MS
15	707	Niğde-Aksaray	5 MR-MS	6 MR- <u>MS</u>
16	702	Kırşehir-Kaman	7 MS	6 MR- <u>MS</u>
17	799	Adana-Kadirli	7 MS	7 MS
18	854	Nevşehir	8 MS-S	7 MS
19	931	Malatya-Pötürge	7 MS	7 MS
20	914	Erzurum-H.Kale	6 MR- <u>MS</u>	6 MR- <u>MS</u>
21	825	Kayseri-Pınarbaşı	6 MR- <u>MS</u>	6 MR- <u>MS</u>
22	707	Muş-Malazgirt	5 MR-MS	5 MR-MS
23	660	Sinop-Gerze	5 MR-MS	5 MR-MS
24	685	Hatay-Kırıkhan	5 MR-MS	6 MR- <u>MS</u>
25	644	Gaziantep-Nur	7 MS	7 MS
26	664	Isparta	7 MS	7 MS
27	844	Yozgat-Akdağmadeni	7 MS	7 MS
28	771	Konya	7 MS	7 MS
29	926	Urfa-Siverek	7 MS	6 MR- <u>MS</u>
30	915	Sivas-Divriği	7 MS	7 MS
31	640	Amasya-Taşova	5 MR-MS	5 MR-MS
32	673	Isparta-Gelendost	7 MS	6 MR- <u>MS</u>
33	941	Diyarbakır	2 R-MR	2 R-MR
34	786	Hatay-Korkuteli	8 MS-S	7 MS
35	906	Konya-Ereğli	6 MR- <u>MS</u>	6 MR- <u>MS</u>
36	888	Eskişehir-Ağapınar	6 MR- <u>MS</u>	6 MR- <u>MS</u>
37	752	Niğde-Bor	7 MS	6 MR- <u>MS</u>
38	689	Erzurum	8 MS-S	8 MS-S
38 39	89	Bülbül 89	8 MS-S 9 S	8 MS-S
39 40	07	Avci 2002	9 S 4 <u>MR</u> -MS	5 MR-MS
40 Isolate means	_	Avci 2002	<u>4 MR</u> -MS 6.525	<u> </u>

Landrace reactions to Sivas isolate ranged between Resistant-Moderately Resistant and Susceptible. Landrace number 33 obtained from Diyarbakır had a 2 scale value and this landrace was rated Resistant-Moderately Resistant to the disease. Fourteen other landraces exhibited Moderately Resistant-Moderately Susceptible disease reaction. These include landraces with the numbers 1,3,4,8,9,15,20,21,22,23,24,31,35, and 36. The rest of the landraces showed moderately susceptible (MS) to moderately susceptible-susceptible (MS-S) disease responses except for the landrace obtained from Bilecik-Söğüt. This landrace showed a susceptible (S) reaction (scale value 9). Cultivar Bülbül 89 showed a scale value of 9 and cultivar Avcı 2002 showed a scale value of 4. Responses of the landraces to Ankara isolate ranged between Resistant-Moderately Resistant and Moderately Susceptible-Susceptible. Again the landrace number 33 from Diyarbakır was found Resistant-Moderately Resistant (R-MR). Landraces 1, 3, 4, 5, 6, 7, 8, 9, 11, 15, 16, 20, 21, 22, 23, 24, 29, 31, 32, 35, 36, 37 exhibited Moderately Resistant-Moderately Susceptible (MR-MS) reaction to Ankara isolate. Other landraces showed Moderately Susceptible (MS) to Moderately Susceptible-Susceptible (MS-S) reactions. Cultivar Bülbül 89 showed a scale value of 8 and cultivar Avci 2002 showed a scale value of 5. Sivas isolate appeared to be slightly more virulent (Isolate means: Sivas isolate 6.525, Ankara isolate 6.225).

#### 4. Discussion And Conclusion

Results of the present study indicated that reactions of the landraces tested against two *P. teres* f. *teres* isolate ranged between R-MR to S. Among the thirty-eight landraces screened in this study, only one landrace from Diyarbakır exhibited R-MR response to the isolates (scale value: 2). Fourteen and 22 landraces exhibited MR-MS responses to Sivas and Ankara isolates, respectively. The other landraces exhibited susceptible group reactions to the isolates.

Çelik Oğuz et al. (2017), using three *P. teres* f. *teres* isolates, tested 198 landraces of barley. The researchers reported that among the total number of landraces, seven landraces were resistant.

In another study conducted in 2019 by Çelik Oğuz *et al.*, responses of seedlings of 25 barley landraces obtained from Iran against 3 isolates of *P. teres* f. *teres* under a controlled environment were determined. Different degrees of virulence were found among the isolates. Two landraces exhibited MR reactions to one of the *Ptt* isolates.

Legge *et al.*, (1996) evaluated the disease reactions of 176 Turkish barley accessions to barley pathogens prevalent in Canada. A small number of accessions with resistance to *P. teres* f. *teres* were identified.

Barley landraces represent a wide genetic variation for desirable agronomic characteristics (Ergün *et al.*, 2017), and successful transfer of desired agronomical traits with specific genes to new varieties of crops is possible (Newton *et al.*, 2010). In addition, emphasis on the collection of those genetic resources from their natural range or habitat and also their conservation should be undertaken (Frankel and Hawkes, 1975).

In addition to the barley landraces, wild barley (*Hordeum spontaneum*) is also a valuable resistance source. Çelik Oğuz *et al.*, (2019c) screened 104 *H. spontaneum* genotypes using virulent *Pyrenophora teres* f. *teres* isolates. Eight *H. spontaneum* genotypes showed resistant group reactions to 3 virulent isolates of *P. teres* f. *teres*.

Mutation breeding is also used to obtain disease-resistant lines. In a study conducted by Çelik Oğuz *et al.*, (2016), barley cultivar Tokak 157/37 was subjected to gamma irradiation and mutant lines were obtained. Twenty-five mutant barley lines obtained by gamma irradiation were These lines were tested for their seedling resistance status under greenhouse conditions using *Drechslera teres* f. *teres* isolates. Isolate differences were evident. The reactions of the mutant lines to the most virulent isolate differed between moderately resistant-moderately susceptible and susceptible.

This current study showed the variation in barley landraces obtained from Osman Tosun Gene Bank in

Turkey to *P. teres* f. *teres*. Landraces with good resistance such as the one obtained from Diyarbakır (Osman Tosun Gene Bank Registration Number 941) could be employed as a source of resistance genes in barley breeding programs.

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SJAFS

(2022) 36 (1), 27-30 e-ISSN: 2458-8377 DOI:10.15316/SJAFS.2022.005

### Research Article

# Melatonin Differences Between Day and Night Milk in Primiparous Holstein Friesian and Jersey Dairy Cattle

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### ARTICLE INFO

ABSTRACT

Article history: Received date: 13.12.2021 Accepted date: 18.01.2022

Keywords: Holstein Friesian Jersey Daytime Milk Nighttime Milk Melatonin This study was conducted to determine the levels of melatonin in the day and night milk of Holstein and Jersey cows. In the study, samples of daytime milk produced from 27 head of Holstein and 27 head of Jersey cows in the first lactation, which were raised in a private dairy cattle enterprise in the Kaşınhanı neighborhood of Meram district of Konya city Turkey, and night milk samples taken from the same cows that were blackened for one (1) week were used. Melatonin levels in milk samples taken from day and night milk were determined separately for Holstein and Jersey cows with the help of Bovine Melatonin (MLT) Elisa Kit.

In the study, it was determined that the ratio of melatonin in day and night milk in Holstein cows was 2.912 pg/ml and 11.314 pg/ml, respectively, and the ratio of melatonin in Jersey cows was 2.924 pg/ml and 6.954 pg/ml in the same order. The difference between the melatonin levels of the day and night milk of Holstein and Jersey cows was found to be statistically significant (p<0.01). At the end of the study, it can be stated that night milk can be used for medical

purposes and a new production source may arise for producers since there is a significant difference in melatonin between day and night milk.

#### 1. Introduction

The presence of a higher amount of melatonin in milk produced in the dark than in day milk and its relationship with human health has recently attracted the attention of researchers and has led to a remarkable increase in the number of publications on the subject.

Özçelik et al. (2013) reported from studies conducted in humans that while the blood concentration of melatonin is around 0-20 pg/dl during the daytime, it rises to 50-200 pg/dl at night. An average of 30 mg of melatonin is synthesized overnight. There are many factors that affect melatonin synthesis. Light is one of these factors. This hormone, which is secreted during sleep, is secreted intensively, especially at night (between 23:00 and 05:00). For this intense secretion to occur, the environment must be dark. This mechanism is similar in other mammals (Jainudeen et al. 2000; Balch 2010).

Romanini et al. (2019) determined the highest concentration of melatonin in individual cow's milk as 41.94 pg/ml. They reported that there are seasonal changes and that the highest concentration of melatonin is reached at night in winter. There are also studies revealing the relationship between health and melatonin secretion in night milk (Valtonen et al. 2005). Related to melatonin helping to improve symptoms of jetlag (a problem more commonly experienced by people who travel a lot and flight crews in general, with interrupted sleep, early waking, excessive sleepiness, difficulty falling asleep, and reduced sleep quality being the most prominent effects of jetlag) are reports (Liu and Borjigin 2006; Srinivasan et al. 2008). At the same time, melatonin is a very powerful antioxidant, stronger than vitamin C, E or beta carotene, preventing harmful oxidation. In this way, it can reduce the risk of hypertension, heart attack and some types of cancer. In addition, it has been stated that it can be useful in the treatment of Alzheimer's and cancer, preventing the regulation of the immune system, memory loss, vascular occlusion and stroke. Also, there is no toxic level of melatonin consumption (Balch 2010). Romanini et al. (2019) reported that melatonin-

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rich night milk produced and marketed has benefits for public health and business people.

Photoperiod, which is defined as the duration of daylight that the animal is exposed to for 24 hours, also significantly affects milk yield. Photoperiod is applied in two ways as long day photoperiod (16-18 hours of continuous daylight exposure and 6-8 hours of dark exposure) and short day photoperiod (8 hours of day-light and 16 hours of dark exposure). Changes in the exposure time in the photoperiod significantly affect the physiology of many species. Photoperiod has more gained importance in recent years to increase animal health and productivity level in dairy cattle herd management. Considering the climate changes, this management factor will be more important in the future.

This study was carried out to reveal the differences between the melatonin levels in the daytime (10 hours of light) and nightly (14 hours of darkness) synthesized milk of Holstein and Jersey dairy cows and in addition to determining whether the production possibilities of night milk rich in melatonin were and whether there is a difference between these.

#### 2. Materials and Methods

#### Material

The research was carried out in January and the photoperiod is 10 hours of light and 14 hours of darkness in Konya, where the research was conducted during this period. The average daily milk yield and days in milk of the cows in the first lactation, which constitutes the research material, were determined as 30.63±0.72 kg-75.91±4.21 day and 17.16±1.44 kg-73.25±4.01 day for Holsteins and Jerseys, respectively. Milk samples to be analyzed were obtained by milking the milk produced by 27 Holstein and 27 Jersey cows lighted 150 lux at eye level in night time in the first lactation in the afternoon (at 15:00-17:00 milking time) during the day. Then, Holstein and Jersey cows were kept in the barn, which was completely dark at night, for a week, and the nightly synthesized milk was obtained by milking before sunrise (at 3:00-5:00 milking time). Samples of both day and night milk for both breeds were taken into 50 ml tubes homogeneously to represent the total milk with the help of the milk sampling apparatus that can be mounted on the milking system.

#### Method

The milk samples taken were brought to the laboratory in the cold chain (+ 4  $^{\circ}$ C). Milk samples were homogenized at +4  $^{\circ}$ C, taken into 8 ml polypropylene tubes and transferred to the laboratory in a cold chain and dark environment.

After all samples were obtained, the analysis phase was started. Centrifugation was performed twice at 4500

rpm for 15 minutes at +4 °C in the laboratory. After each centrifugation, the fat layer accumulated in the upper part was separated, and the milk samples, which were completely separated from the fat, were kept at -80 degrees until the working day. On the working day, the samples were thawed first at -20 °C, then at +4 °C and finally at room temperature. After dissolution, homogenization was achieved by vortexing. Homogenized samples were analyzed using IBL brand Melatonin direct Saliva ELISA (Non-Extraction) and in accordance with the kit procedure, Rayto RT-2600 Microplate Washer (India) and BMG LABTECH (German) Enzyme-Linked Immuno Sorbent Assay in Selcuk University Faculty of Medicine Biochemistry Laboratory. (ELISA) Melatonin levels were determined in pg/ml via ELISA reader. The quantitation limits of the melatonin kit used are between 0.5 - 50 pg/ml and the samples were not diluted.

#### Statistical analysis

Analysis of the differences between melatonin levels in day and night milk in Holstein and Jersey cows was performed according to the repeated measurements experiment design (Two Factors Experiments with Repeated Measurements On One Factor Levels) in order to prevent dependence since the samples were taken from the same animals before and after darkening (Gürbüz et al. 2003). Statistical analyzes were made with the help of the RStudio statistical package program.

#### 3. Results and Discussion

At the end of the study, it was determined that the level of melatonin in Holstein cows was 2.912 pg/ml in milk synthesized during the day and 11.314 pg/ml in milk synthesized at night.

In the samples examined in Holstein cows, it was observed that the lowest melatonin value in day milk was 1.168, the highest melatonin value was 5.887, and the melatonin ratio in night milk was between 3.520 and 21.510 (Table 1). In Jersey cows, on the other hand, melatonin level was determined as 2.924 pg/ml in milk synthesized during the day and as 6.954 pg/ml in night milk. The melatonin ratios in day milk of Jersey cows ranged from 1.085-4.577, and in night milk from 3.422 to 15.722 (Table 1). The melatonin level in night milk of Holstein cows was approximately 3.9 times the milk synthesized during the day and this difference was statistically significant (p<0.01). In Jersey cows, the melatonin level in night milk was approximately 2.4 times the milk synthesized during the day, and this difference was statistically significant (p<0.01). While the difference between the melatonin levels of milk synthesized during the day in Holstein and Jersey cows was statistically insignificant (p>0.05), the difference in milk synthesized at night was statistically significant (p<0.01).

Table 1 Melatonin lev	els and standard errors in day and night milk		
Breed	Samples	n	

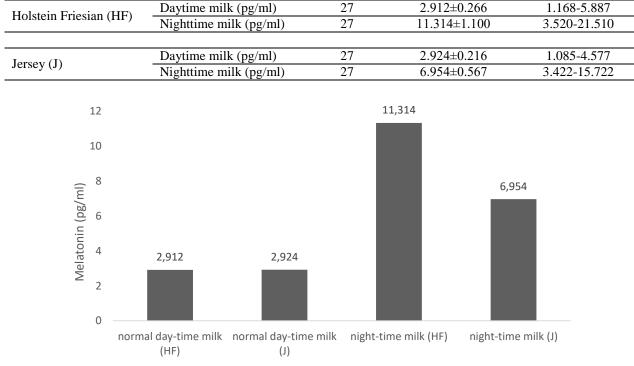


Figure 1 Melatonin levels in day and night milk in Holstein Friesian (HF) and Jersey (J) cows

The differences between melatonin levels in night and day milk of Holstein and Jersey cows can also be seen in Figure 1. According to Holzmann et al. (2019), the ration supplemented with vitamins (C) did not affect the concentration of melatonin in total milk. However, in the night milk, the melatonin level was 6.57 pg/ml in the cows fed the unsupplemented ration, while it was 11.06 pg/ml in the fortified ration group. Milk expressed at night showed melatonin concentrations 1.43 to 2.38 times higher than total milk per day. According to Romanini et al. (2019), in their study investigating melatonin levels and sources in cow's milk, reported differences in levels of melatonin in night milk and total milk according to winter and summer months. In the low yield group, the level of melatonin was found to be around 22 pg/ml in night milk in winter and around 10 pg/ml in summer, while it was around 8 pg/ml in winter and 6 pg/ml in summer in total milk. In the high yield group, it was found as 16 pg/ml in night milk in winter and 11 pg/ml in summer, while it was determined as 9 pg/ml in winter and 4 pg/ml in total milk. The values found in the current study were similar to those reported by Romanini et al. (2019). While Asher et al. (2015) found lower levels of melatonin levels in milk under dark and limited lighting conditions at night, researchers reported values between 15-20 pg/ml in the group with limited lighting, while melatonin level was around 30 pg/ml in night

milk. Sahin et al. (2021), it was determined that the melatonin level in day milk of first lactation cows of Holstein breed was  $103.70\pm6.61$  pg/ml and  $163.13\pm8.96$ pg/ml in night milk. The difference between melatonin levels of day and night milk was statistically significant (P<0.01). The probable reason for this difference is that the kits used to determine the melatonin level are different (In some kits, the reading range for melatonin level varies between 0 and 1000 ((MYBIOSOURCE brand Bovine Melatonin (MLT) ELISA Kit (Competitive ELISA)), in some kits this reading is between 0.5 and 50. (IBL, Melatonin direct Saliva ELISA (Non-Extraction) varies) It may also be that the season and yield level of milk samples are different (high or low yield) (Asher et al. 2015).

#### 4. Conclusion

Melatonin has a vital role for humans and is necessary for a healthy life. Sources rich in melatonin, especially milk, should be evaluated for a healthy and happy life. In Finland and Germany, melatonin-rich milk produced at night from cattle under the name of "night milk" has begun to be produced commercially (Valtonen et al. 2005; Mullins 2010). Valtonen et al. (2005) reported that even the lowest melatonin dose of 0.1 mg was 10 times higher than the total melatonin secreted at

Min-Max

 $\bar{X} \pm S_{\bar{X}}$ 

night. In other words, it is not possible to meet this amount by consuming milk rich in melatonin. Accordingly, "night milk" produced from cows should be consumed at least half a liter based on the reports of Valtonen et al. (2005) in adults, especially children and the elderly, as a supplement to the melatonin secretion of patients. Regarding this issue, Valtonen et al. (2005) reported that melatonin secretion decreases with age and that they use night milk as a material for the elimination of sleep disorders in elderly people, and that they obtain positive results when the patients are given about 0.61 liters/day. Bae et al. (2016) compared the effects of consuming regular milk containing 100 pg melatonin and 47.5 mg tryptophan amino acids and a glass of overnight milk containing 1000 pg melatonin and 58.24 mg tryptophan amino acids. As a result, it has been reported that a glass of night milk containing 1000 pg melatonin provides an increase in sleep comfort and a decrease in insomnia throughout the day.

In some countries, milk rich in melatonin is marketed as "night time milk" separately from normal milk. In Turkey, it is necessary to make legal regulations in this regard and to produce and market this milk rich in melatonin. In this way, milk producers will be able to provide an additional income.

#### 5. Acknowledgements

We thank to Laranda Dairy Cattle Farm for providing the material used in this study.

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http://sjafs.selcuk.edu.tr/sjafs/index
Research Article

SJAFS

(2022) 36 (1), 31-38 e-ISSN: 2458-8377 DOI:10.15316/SJAFS.2022.006

# Determination of Yield, Quality and Morphological Characteristics of Different Hybrid Pepper Cultivar Candidates in Konya Ecological Conditions

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#### **ARTICLE INFO**

### ABSTRACT

Article history: Received date: 15.12.2021 Accepted date: 18.01.2022

Keywords: Agronomy *Capsicum annuum* F1 cultivar Open field PCA Pepper is an important variety of vegetable that has economic value in human nutrition in Turkey and in the world. Continuous changes in producer and consumer demands also create a competitive environment in pepper breeding. Green pepper cultivation is generally carried out in greenhouse cultivation, and its cultivation has become widespread in open field conditions. In the study, 8 F1 (G12, G11, K42, B25, L9, Z22, G14 and L10) pepper varieties with superior characteristics were used as plant material. Some plant, leaf and fruit characteristics as well as yield and quality parameters were examined. As a result of the principal components analysis (PCA) made using theese measurements and observations, the study was explained variations in 6 components at a high rate of 97.94%. G11 and G12 cultivar candidates are located in the positive region of both components in the Score plot graph drawn from the first two components which means that these two candidates showed the highest performance among those evaluated ones. These cultivar candidates showed superior characteristics in terms of yield, fruit weight, fruit width, TTS, pH, L and b parameters. It is thought that these cultivar candidates can be grown in open land conditions having semi-arid climates such as Konya and will contribute to the country's agriculture production.

#### 1. Introduction

Pepper belongs to the Solanaceae family and is a variety of vegetable that is widely consumed in the world and has high economic value. It has been reported that the homeland of pepper, which has been cultivated since the 15th century, is Central and South America (Pickersgill, 1997). According to 2019 data, 38.027.167 tons of pepper was produced in an area of 1.990.926 hectares in the world. In the world pepper production amount, China ranks first with 18.978.027 tons, Mexico ranks second with 3.238.245 tons, and Turkey ranks third with 2.625.669 tons (FAO, 2019). Considering 2020 data of Turkey, 1.291.091 tons of capia (oil-salt paste), 389.957 tons of bell peppers, 838.890 tons of green peppers, 116.967 tons of charliston peppers are produced, and it constitutes 8.4% of our country's vegetable production (TUIK, 2020).

Pepper is used in human nutrition, fresh, cooked, pickled, canned, dried, etc. It is a type of vegetable that is widely consumed in the form of a vegetable, and it has been reported that 100 grams of pepper contains 22 calories, 1 g protein, 0.1 g fat, 4.4 g carbohydrates, 1 g calcium, 1 g phosphorus and 2 g iron. Red, fresh and green peppers are among the vegetables with the highest amount of vitamin C (Sevgican, 1999; Dobón-Suárez et al., 2021).

Biologically, it is known that the flower structure of pepper is hermaphrodite. Although pepper is a self-pollinated species, it is found in foreign pollination at varying rates (3-30%). For this reason, pepper populations with very different characteristics have spread to different parts of Turkey and created rich genetic variation. Within this rich genetic diversity, Bozokalfa et al. (2009) revealed that fruit types and plant characteristics are the main determinants of a genotype that can be integrated into breeding studies.

In addition to the traditional breeding characteristics in the cultivation and consumption of pepper worldwide, market demands and the competition created by this have led producers to use higher yielding and quality varieties (Kartal, 2021). This had a positive effect on the development of pepper breeding. Purpose of pepper

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breeding; is to develop hybrid varieties suitable for open and greenhouse conditions, productive, high quality, resistant to diseases and pests, and able to set fruit in cold conditions. In addition, one of the features that breeders and producers emphasize most in pepper breeding is uniformity (Gülcan, 2020).

The aim of agricultural production is to obtain the highest yield by providing the necessary inputs for the plant to reach its yield potential. For this purpose, the use of hybrid varieties in peppers has increased in recent years. In hybrid varieties, significant success has been achieved in the development of demanded varieties and seed production in terms of yield, quality, durability, adaptation, resistance to diseases and pests (Kaloo, 1988; Etienne et al., 2018). The degree of kinship of the parents plays an important role in the F<sub>1</sub> generation being superior to the parents in the pepper hybrid cultivar breeding program. While the heterosis effect is low in hybrids of genetically very close or very distant lines, heterosis is higher in hybrids of lines with medium level of genetic relatedness (Geleta et al., 2004). Heterosis breeding has an important effect on the development of high yielding varieties in pepper.

Hybrid cultivar breeding in pepper is one of the issues of economic and strategic importance both at national and international level. Pepper breeding studies in Turkey started in the 1980s and many standard varieties have emerged in the light of these studies (Sürmeli and Erdogan, 1985; İnan, 1988; Sürmeli and Şimşek, 1991; Ekiz and Kemer, 1995). Although the usage rate of hybrid pepper varieties has increased in our country in recent years, the use of varieties developed through selection is still available. Some of the hybrid pepper varieties developed in Turkey are the varieties developed by companies operating in our country but of foreign origin.

Thanks to its high production both in the world and in our country, pepper has an important place in economic terms and also for human nutrition; There is a tendency towards the use of hybrid varieties on the basis of parameters such as yield, quality, environmental compatibility, color and aroma. Although  $F_1$  cultivars have been developed in pepper cultivation, there is still a need for new  $F_1$  cultivars with high adaptability in different ecological conditions. In this study, it was aimed to determine some yield, quality and plant characteristics of 8  $F_1$  pepper cultivars under Konya ecological conditions.

#### 2. Materials and Methods

The research was carried out between May-August 2021 in Selcuk University Faculty of Agriculture, in the research field. Climatic data of the trial year belonging 2021 were taken from the climate station located in the trial area, and some climate data were recorded during the trial season. When Table 1 is examined, the highest temperature was 40.3 °C in June. The lowest temperature was 4.9 °C in September, and the average temperature was between 17.3-23.8 °C. The highest precipitation occurred in August and September. As a

result of the soil analysis, it has a pH 7.8 organic matter 1.2% and a clay-loam structure without salinity problem.

#### Table 1

Variations of meteorological parameters of region during experimental years

Mon.	Max. Temp (°C)	Min. Temp (°C)	Mean Temp (°C)	Mean wind speed (m s <sup>-1</sup> )	Precipita- tion (mm)
May	35.9	6.7	18.4	2.4	2.5
June	40.3	11.2	21.6	0.8	1.4
July	36.2	13.1	23.8	2.8	5.8
Aug.	35.2	12.8	23.7	2.4	13.4
Sep.	30.2	4.9	17.3	2.3	14.4

In the experiment, eight hybrid cultivar candidates with having heterosis characteristics, defined by the codes G12, G11, K42, B25, L9, Z22, G14 and L10, developed by the Selko-Agriculture company, which carries out Ar-Ge studies on different vegetable species in Antalya, were used as plant material.

After the drip irrigation pipes were laid on the land cultivated in early spring, seedlings were planted on the row on May 5, 2021. Seedlings were planted in 20 plots of each genotype, with 80 cm row spacing and 50 cm row spacing. Irrigation was done with drip irrigation system at intervals of 5-7 days according to the needs of the plant. When the plants reach a certain height, throat filling process was done and hoeing was done 3 times according to weed growth. 15 days after planting the seedlings, 3.5 kg da-1 of MAP (monoammonium phosphate) and 400 ml of humic acid per decare were given by drip irrigation system. The second fertilization was applied on June 11 with the same amount of humic acid and 200 g potasiyum+magnesium (Potasmag). Approximately ten days after the seedling planting, the increase in soil temperature and the "Luna Tranquility" application, which is effective against root rot, was applied with drip irrigation based on the soil temperature rate. Fruits at harvest size in cultivar candidates were harvested separately from each plot. The first harvest was made on July 18, and the trial was terminated at the end of a total of 6 harvests. In order to make measurements and observations on the fruit, 10 fruits representing the genotype were sampled and necessary measurements and observations were made.

In the experiment, the characteristics taken from the plant, leaf and fruit were determined according to the International Union for Conservation of New Plant Varieties (UPOV) feature document. In plants; plant height (cm), internode length (cm), anthocyanin coloration at level of nodes, color intensity at the nodes, hairiness at the nodes, in the leaves; leaf length and width, leaf color values (L, a\*, b\*) and fruits; yield per plant (g), number of fruits (plant), fruit weight (g), fruit length (cm), fruit width (mm), fruit flesh thickness (mm), fruit stalk length (mm), fruit stalk thickness (mm), TTS, pH, fruit anthocyanin coloration, fruit texture of surface measurements and observations were taken. In the experiment, the standard deviations of the numerical measurements observed taken from different cultivar candidates were taken and interpreted. Observational parameters were tried to be interpreted as percentages. Yield, quality and plant characteristics were subjected to PCA in the JMP-14 computer package program, and the parameters revealing the important differences between F1 cultivar candidates were determined. With the Loading Plot and Score Plot plots drawn from the PC1 and PC2 components from the analysis, the relationships between the parameters and the distinctions between the genotypes were revealed.

# 3. Results and Discussion

In the study, it was observed that the leaf characteristics of eight different F1 pepper cultivar candidates were different from each other (Table 2). When the table was examined, the average leaf length was found to be 77.2 mm. The longest leaf was found to be 86.82 mm from the K42 cultivar candidate. When the leaf width is examined, the average leaf width of the cultivars is 37.29 mm. The highest leaf width was determined as 49.87 mm from the K42 cultivar candidate. The average leaf color L value was found to be 40.24, and the varietal candidate with the brightest value was determined as G12 with 42.59 and and the lowest brightness value was determined as B25 with 38.61 with the lowest brightness. The average leaf color a\* value is -13.98, the cultivar candidate with the highest value is G12 with -15.01, and the cultivar candidate with the lowest value is B25 with -12.90. Leaf color b\* value average was 21.27, the highest value was determined to belong to the G12 variety candidate with 24.53 and the lowest value was determined to belong to the L10 variety candidate with 18.43. A high value of "L" indicates a high brightness, a negative value of "a\*" indicates an excess of green color, a positive increase indicates an increase in redness, a negative increase of "b\*" indicates an increase in yellow color, and a positive value of blue indicates an increase in intensity (Bosland, 1993). Başak (2019) determined the averages of leaf length and width (cm) as 6.20-3.63 cm, respectively, in his study. In another study, leaf widths of 8 types and 129 pepper cultivars were determined as narrow in most of the cultivars (47.3%), wide cultivars were mostly block and stuffed types (14%), and very narrow cultivars were hairy/ornamental types (10.9%). has been observed. In another study, 67 pepper genotypes; They Table 2

found that the leaf width was 2.5 cm and the leaf length was 4.8 cm (Kanal and Balkaya, 2021). The fact that the genotypes in the gene pools have different characteristics indicates the richness of the gene pool. Although the leaf characteristics of the cultivar candidates differ, similar results were obtained in the studies.

When Table 3 was examined, differences were observed between plant heights. The average plant height is 69.08 cm, and it was stated that the cultivar candidate with the longest plant height was K42. When the internode length is examined, the average length is 5.23 cm and the F1 cultivar candidate with the longest internode length was K42 with 6.7 cm. In a study conducted in Samsun with 67 pepper genotypes of C. baccatum; The highest plant height values were measured in CB-68 (47.1 cm), CB-85 (47.0 cm), CB-21 (46.7 cm), CB-49 (46.6 cm) and CB-28 (46.6 cm) respectively (Kanal and Balkaya, 2021). Capsicum species show significant differences in terms of plant heights. Padilha et al. (2016) The plant heights of pepper genotypes of C. annuum were 23.12- 48.72 cm and Sreenivas et al. (2019) stated that it varies between 37.6-110.6 cm. Başak (2019) determined in his study that the average plant height and internode length were 60.24 and 5.63 cm, respectively. Although the results of the research vary according to the genotypes, they generally support the mentioned literature. Considering the anthocyanin coloration at level of nodes, it was observed that there was coloration in all cultivar candidates. It was determined that the color intensity at the nodes was medium in five cultivar candidates, weak in two, and very low in one. No hairiness at the nodes was observed when looking at all cultivar candidates. Başak (2019) and Mutlu et al. (2009) found the hairiness rates of the nodes to be 98% and 85% weak in their study. The results of the research showed that the hairiness at the nodes produced similar results. Kanal and Balkaya (2021), in their examination in terms of stem anthocyanin coloration; determined that 16.5% of genotypes did not have anthocyanin coloration. It was determined that 34.3% of C. baccatum pepper genotypes had low intensity, 32.8% very intense and 16.4% medium intensity anthocyanin coloration. Mutlu et al. (2009) used 185 pepper materials and determined anthocyanin as 1.08% green, 20% light purple, 52.43% purple and 26.49% dark purple at the nodes of the cultivars.

Some leaf measurements and observations of different F1	pepper cultivar candidates in Kor	nya ecological conditions

F1 Variety	Leaf Length (mm)	Leaf Width (mm)	Leaf Color L Value	Leaf Color a* Value	Leaf Color b* Value
G12	84.25±10.20	39.42±4.07	42.59±2.64	-15.01±1.29	24.53±3.78
G11	82.04±8.67	$38.45 \pm 8.35$	41.46±1.29	-14.58±2.52	23.70±0.52
K42	86.82±11.51	49.87±6.46	40.30±1.98	$-14.03 \pm 1.16$	21.47±2.76
B25	78.72±10.83	35.59±5.11	38.61±4.36	-12.90±3.24	19.04±7.56
L9	74.06±14.54	36.27±6.99	39.37±3.17	-13.42±2.43	19.41±4.71
Z22	68.15±7.58	32.72±3.57	40.28±3.53	$-14.10\pm2.21$	22.23±4.89
G14	58.61±8.53	27.77±5.73	40.26±1.57	$-14.29 \pm 1.34$	21.29±2.12
L10	85.02±12.39	38.24±2.30	38.99±1.92	-13.50±1.28	$18.43 \pm 4.02$
Average	77.21	37.29	40.24	-13.98	21.27

F1 Variety	Plant Height (cm)	Internode Lenght (cm)	Anthocyanin Coloration at Level of Nodes	Color Intensity at the Nodes	Hairiness at the Nodes
G12	66.4±4.97	4.7±0.97	current	medium	absent
G11	$73 \pm 9.08$	$5.3 \pm 0.83$	current	weak	absent
K42	79.4±6.58	$6.7{\pm}0.97$	current	very low	absent
B25	62.8±5.38	5±0.93	current	medium	absent
L9	71.8±3.03	5.2±1.15	current	medium	absent
Z22	$62.8 \pm 2.58$	5±0.61	current	medium	absent
G14	64.4±6.10	$5.3 \pm 0.90$	current	weak	absent
L10	71±2.64	4.7±0.75	current	medium	absent
Average	69.08	5.23	-	-	-

 Table 3

 Some plant measurements and observations of different F1 pepper cultivar candidates in Konya ecological conditions.

When Table 4 is examined, the average yield per plant is 905.86 g, the highest with 1103.40 g and G11 variety candidate and the lowest with 526.85 g G14 variety candidate. The average number of fruits per plant was determined as 64.22, the highest number of fruits was determined as 86.29 with K42 and the least number of fruits with 42.69 as G14 variety candidate. Average fruit weight was observed as 16.60 g, and it was determined that the cultivar candidate with the heaviest fruit was G11 with 22.52 g. Cherian and Indira (2003), in their study of 25 different C. chinense Jacq species, determined the number of fruits per plant as 4.0-63.5 and yield per plant as 12.0-185.0 g. In a study conducted in another Capsicum species, the average fruit width values in C. baccatum pepper genotypes were measured between 6.3-26.3 mm (Kanal and Balkaya, 2021). Capsicum species show significant differences in terms of fruit number and yield per plant. In the study conducted with domestic, hybrid and standard pepper varieties; Yield averages per plant varied between 541.4 g/plant (Bozdoğan population) and 203.3 g/plant (Yenipazar population). It has been stated that the hybrid variety gives an average value of 344.4 g/plant (Gülcan, 2020). Padilha et al. (2016) in their studies; the highest fruit number per plant was P138 genotype with an average of 890, and P143 genotype with an average yield per plant of 510. They reported that the genotype with the highest fruit weight emerged as P202 with 17.33 g per fruit. It is seen that there are differences between yield and yield components among the various studies. These differences are thought to be caused by cultivars, ecological factors and growing conditions. When the fruit lengths are examined, the average is 17.17 cm, the variety candidate with the longest fruit length is G14 with 18.3 cm, and the variety candidate with the shortest fruit length is G12 with 16.1 cm. The average fruit width is 16.55 mm, the largest fruit width is L10 with 18.07 mm, and the lowest fruit width is B25 with 15.45 mm. The average fruit flesh thickness was 2.34 mm, and the cultivar candidate with the thickest flesh was determined as Z22 with 2.89 mm. Başak (2019) determined the average fruit length, fruit diameter, and fruit flesh thickness to be 15.97 cm, 15.98 mm, and 1.89 mm, respectively. In the study conducted on 45 chilli pepper genotypes, they found fruit length of 13.92-95.26 mm and fruit diameter of 5.26-15.92 mm (Sreenivas et al., 2019). In his study, Gülcan (2020) determined fruit length of 10.6-13.3 cm, fruit diameter of 18.8-25.1 mm and fruit flesh thickness in the range of 2.2-2.8 mm. Binbir (2010), in their study on 26 different pepper populations and three different standard pepper varieties, found the average fruit length of 12.34 cm, width of 3.5 cm and weight of 42.20 g. Fruit lengths and widths in pepper are directly related to harvest time and genetic structure.

Table 4

Yield and some fruit measurements of d	lifferent F1 pepper	r cultivar candidates	s in Konva ecol	ogical conditions

		1	11		e	
F <sub>1</sub> Variety	Yield per Plant	Number of Fruits	Fruit Weight	Fruit Length	Fruit Width	Fruit Flesh
1 vallety	(g)	(plant)	(g)	(cm)	(mm)	Thickness (mm)
G12	1073.20	74.00	17.68	16.1±0.916	17.096±1.52	2.34±0.47
G11	1103.40	61.40	22.52	16.8±1.12	$17.14 \pm 1.38$	$2.04{\pm}0.44$
K42	1006.14	86.29	13.82	$16.3 \pm 1.98$	15.67±3.32	2.13±0.22
B25	816.47	57.53	15.18	$18.2 \pm 0.67$	$15.45 \pm 0.81$	2.05±0.31
L9	902.93	67.07	15.77	$16.5 \pm 1.37$	$16.91 \pm 3.08$	2.47±0.11
Z22	842.07	55.60	16.47	$17.3 \pm 0.44$	16.64±1.102	$2.89{\pm}0.38$
G14	526.85	42.69	14.60	18.3±1.432	15.5±1.75	2.76±0.49
L10	975.85	69.15	16.78	$17.9 \pm 2.45$	$18.07 \pm 2.00$	$2.06 \pm 0.18$
Average	905.86	64.22	16.60	17.17	16.55	2.34

			• 1	11	5	U
F1 Variety	Fruit Stalk Length (mm)	Fruit Stalk Thickness (mm)	TTS	pH	Fruit Anthocyanin Coloration	Fruit Texture of Surface
G12	32.17±4.13	4.51±1.08	4.9	5.96	absent	wrinkled
G11	30.54±1.48	3.69±0.43	5	5.52	absent	straight
K42	30.28±2.45	3.59±0.31	4.6	5.68	absent	straight
B25	29.47±5.11	3.70±0.47	4.1	5.62	absent	wrinkled
L9	32.44±1.45	$4.46 \pm 0.79$	5.1	5.79	absent	S. wrinkled
Z22	43.07±11.64	3.80±0.32	4.8	5.92	absent	straight
G14	31.66±4.44	4.33±0.40	4.5	5.42	absent	S. wrinkled
L10	35.23±3.15	3.65±0.43	5.1	5.91	absent	wrinkled
Average	33.11	3 97	476	5 73		-

Some fruit measurements and observations of different F1 pepper cultivar candidates in Konya ecological conditions

When Table 5 is examined, it has been observed that the average fruit stalk length is 33.11 mm, the highest stalk length is Z22 with 43.07 mm and the lowest stalk length is 29.47 mm with B25 variety candidate. The average fruit stalk thickness was 3.97 mm, the highest stalk thickness was obtained from the G12 variety candidate with 4.51 mm and the lowest fruit stalk thickness was obtained from the K42 variety candidate with 3.59 mm. It has been determined that fruit stalk lengths vary between 18.5-71.1 mm in pepper genotypes of C. baccatum (Kanal and Balkaya, 2021). Taş (2020) reported that fruit stalk lengths in pepper genotypes of the C. chinense species ranged between 19.9-61.9 mm. Başak (2019) found the fruit stalk length as 4.15 cm and stalk thickness as 4.14 mm in their study. In another study, it was reported that the length of the fruit stalk varied between 25.5-32.7 mm (Gülcan, 2020). Ermis et al. (2019) measured the fruit stalk thickness between 2.4-10 mm. Considering the studies, fruit stalk length and fruit stalk thickness are approximately similar. The mean TTS

value was 4.76, the highest TTS value was determined as 5.1 in L9 and L10 cultivar candidates, and the lowest TTS value was determined in B25 cultivar candidates with 4.1. Average pH value was 5.73, G12 variety candidate with the highest pH value of 5.96 and G14 variety candidate with the lowest pH value of 5.42. Although the high amount of TTS is a desirable feature especially in the tomato paste industry, this is also true for sharp peppers used for drying and industrial purposes. Başak (2019) found the amount of TTS in the range of 2.7-6.3% in her study. Similarly, Karaağaç and Balkaya (2010) reported that the amounts of TTS varied between 5.0-7.6%. Öntürk (2018) found in his study that the pH values of pepper populations varied between 4.83 and 5.59. Fruit anthocyanin coloration was not observed in any F1 cultivar candidate. Fruit surface structure was observed as 3 straight, 3 wrinkled and 2 slightly wrinkled in 8 cultivar candidates. Mutlu et al. (2009) reported that most peppers did not have anthocyanin coloration in their study.

Table 6

Table 5

Principal component analysis of yield, quality and morphological characteristics of different  $F_1$  pepper cultivar candidates under Konya ecological conditions

Items	PC1	PC2	PC3	PC4	PC5	PC6
Eigenvalue	6.41	4.26	3.42	1.81	1.63	1.03
Percentage of variance	33.77	22.46	18.04	9.57	8.62	5.46
Cumulative variance	33.77	56.24	74.28	83.85	92.48	97.94
Eigenvectors						
YL	0.355	0.114	0.142	-0.068	-0.089	-0.139
NF	0.341	-0.079	0.145	0.124	0.264	-0.086
FW	0.153	0.248	-0.049	-0.303	-0.476	0.072
FL	-0.320	-0.115	0.110	-0.097	-0.280	-0.026
FWi	0.149	0.331	0.256	0.027	-0.179	0.276
FFT	-0.221	0.180	-0.265	0.382	0.141	0.059
FSL	-0.092	0.258	0.023	0.556	-0.186	-0.180
FST	-0.094	0.237	-0.139	-0.151	0.538	0.305
TTS	0.183	0.305	0.087	0.155	-0.074	0.541
pH	0.104	0.278	0.267	0.320	0.226	-0.273
FTS	-0.156	0.077	0.290	-0.362	0.383	0.044
PH	0.317	-0.175	0.003	0.108	0.002	0.436
IL	0.167	-0.360	-0.216	0.219	0.067	0.093
CIN	-0.163	0.312	0.309	-0.043	0.066	-0.160
LL	0.327	-0.048	0.252	-0.135	0.007	-0.215
LW	0.347	-0.182	0.079	0.083	0.123	-0.138
L	0.191	0.248	-0.338	-0.172	0.094	-0.156
а	-0.157	-0.243	0.378	0.096	-0.033	0.044
b	0.164	0.215	-0.391	-0.119	-0.016	-0.272

\*YL (Yield per plant), NF (Number of Fruits), FW (Fruit Weight), FL (Fruit Length), FW (Fruit Width), (FFT) Fruit Flesh Thickness, (FSL) Fruit Stalk Length, (FST) Fruit Stalk Thickness, FTS (Fruit Texture of Surface), (PH) Plant Height, (IL) Internode Length, (CIN) Color Intensity at the Nodes, (LL) Leaf Length, (LW) Leaf Width, (L) Leaf Color L Value, (a) Leaf Color a\* Value, (b) Leaf Color b\* Value

Leaf, plant and fruit characteristics measurements obtained from eight different F<sub>1</sub> pepper cultivar candidates were subjected to PCA in the study (Table 6). As a result of the analysis, 6 independent principal component axes were obtained regarding the 19 identification features examined. These axes represented 97.94% of the total variation. The eigen values of the first 6 components were found to be between 1.03 and 6.41. The fact that the Eigen values are greater than 1 indicates that the weight values of the component are reliable (Mohammadi and Prasanna, 2003; Seymen et al., 2019; Seymen, 2020; Yavuz et al., 2020; Yavuz et al., 2021). In studies, it is reported that it is reported that when more than 25% of total variation explains in the first two components, it could be used for PCA (Mohammadi and Prasanna, 2003; Seymen et al., 2019; Yavuz et al., 2021). It is obvious that the strong explanation of PCA will give important results about the usability of this analysis and the parameters looked at.

As a result of PCA, the first component (PC1) explained 33.77% of the study, while yield per plant, number of fruits, pH, leaf length and leaf width explained positively, in this component, whereas fruit length was the parameters described in the negative direction in this component. The second component (PC2) explained 22.46% of the study; fruit width, TTS and color intensity at the nodes explained positively in this component, whereas internode length explained negatively in this component. The third component (PC3) explained 18.04% of the study; color intensity at the nodes and leaf color a\* value was the parameters that explained it positively inthis componentwhereas leaf color L value and leaf color b\* value was places in the negative direction of component. The fourth component (PC4) explained 9.57% of the study; fruit flesh thickness, fruit stalk length and pH were positively described in this component whereas fruit weight and fruit texture of surface were negatively described parameters in this component. Fifth component (PC5) explained 8.62% of the study; fruit stalk thickness and fruit texture of surface explained positively in this component, fruit weight negatively explained variable inthis component. The sixth component explained 5.46% of the study; fruit stalk thickness, TTS and plant height were the parameters that explained positively inthis component. In studies on pepper characterization, Zewdie and Zeven (1997) reported that the first six PC factors represented 58% of the total variation, Rivera Martinez et al. (2004) reported that the first three basic components accounted for 72% of the total variation. Keles (2009) performed PCA analysis in terms of 25 traits that he addressed in his study on the characterization of pepper genotypes, and as a result of the analysis, Keleş (2009) determined that the first three PC axes covered 50% of the cumulative variation. In another study, as a result of PCA, which included 34 morphological features of pepper samples, it was determined that PC with 9 factors explained 85.35% of the total variation (Binbir, 2010). PCA is used as an important and descriptive analysis method for comparing multiple data and defining genetic pools.

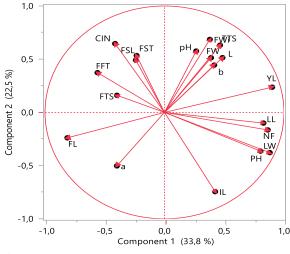
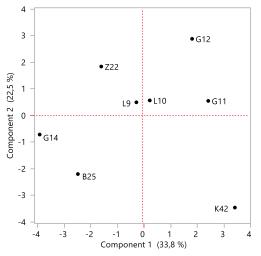


Figure 1

Loading Plot drawn from PC1 and PC2 as a result of the principal component analysis made from the yield, quality and morphological characteristics of different  $F_1$  pepper cultivar candidates in Konya ecological conditions





Score Plot plot drawn from PC1 and PC2 as a result of the principal components analysis of yield, quality and morphological characteristics of different F<sub>1</sub> pepper cultivar candidates under Konya ecological conditions

Using PC1 and PC2 components, a loading plot was created to examine the interrelationship between plant, leaf and fruit characteristics (Figure 1). It has been reported that if the angle between the vectors in the figure is  $<90^{\circ}$ , there is a positive relationship, if it is  $>90^{\circ}$ , there is a negative relationship, and if the angle between the vectors is 90°, there is no significant relationship (Yan and Kang, 2002; Yavuz et al., 2020; Seymen, 2021). When the figure is examined, a strong positive correlation was found between the parameters FW, FWi, TTS, pH, L and b\*. In addition, there was a strong positive correlation between FST, FSL, CIN, FFT, and FTS, while these parameters were negatively correlated with IL.

A score plot was created for the evaluation of eight different  $F_1$  pepper cultivar candidates using PC1 and

PC2 components (Figure 2). Considering the positive region of both components, G11, G12 and L10 cultivar candidates showed significant results in terms of yield and quality. G11 and G12 cultivar candidates YL, FWi, FW, pH, L, and b\* parameters were the parameters that obtained high values and differentiated G11 and G12 cultivar candidates from the other groups.

#### 4. Conclusion

The differences in yield, fruit, leaf and plant characteristics of eight different F1 pepper cultivar candidates were evaluated in Konya ecological conditions. As a result of PCA, the study was explained at a high rate of 97.94% in 6 components. G11 and G12 cultivar candidates located in the positive region of both components in the Score plot graph drawn from the first two components that explained the study the most were determined as superior cultivar candidates. These F1 cultivar candidates showed superior characteristics in terms of yield, fruit weight, fruit width, TTS, pH, L and b\* parameters. It is thought that these F1 cultivar candidates can be grown in open land conditions in regions such as Konya with semi-arid climates and will contribute to the country's agriculture production.

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Selcuk Journal of Agriculture and Food Sciences

http://sjafs.selcuk.edu.tr/sjafs/index

**Research Article** 

SJAFS

(2022) 36 (1), 39-47 e-ISSN: 2458-8377 DOI:10.15316/SJAFS.2022.007

# **Evaluation of Growing Some Legume Forage Crops as Second Crop**

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## **ARTICLE INFO**

# ABSTRACT

Article history: Received date: 14.12.2021 Accepted date: 27.01.2022

Keywords: Legumes Second Crop Common Vecth Field Pea Green Yiled Aim of the present research was evaluation of growing some legume forage crops as second crop following to cereal harvest under irrigated conditions. Field trial was realized under Seydişehir Town - Konya City / Turkey ecological conditions for 2 years during the both vegetation periods of 2019-2020 years by 4 replications according to randomized blocks design. As material; forage pea (*Pisum sativum L.*), soybean (*Gly*cine max. L.), hairy vetch (Vicia villosa Roth.), common vetch (Vicia sativum L.), and fenugreek (Trigonella foenum-graecum L.) were used. According to the results of the research, statistically significant differences were found for plant height and green herbage yield as mean of the years. The obtained data also showed that the highest plant height and green herbage yield were taken from forage pea and common vetch. Additionally, plant height was between 126.76-117.94 cm values for pea and common vetch, while green herbage yield was 3085.50<sup>-1</sup> and 2788.63 kg da<sup>-1</sup> for pea and common vetch, respectively. Consequently, legume forage crops as second crop following to the harvest of cereals may be successfully grown under irrigated conditions.

# 1. Introduction

Legume forage crop growing is essential to decrease the feeding costs that approximately 70% in livestock enterprises (Parlak and Sevimay 2007; Alçiçek et al 2010; Sabancı et al 2010, Özkan and Demirbağ 2016). In some cases, roughage is indispensable for livestock enterprises which is provided from pastures that has low yield, the hay of main crops, straw, and stem (Açıkgöz et al 2005; Özkan and Demirbağ 2016). According to various reports, the productivity potential of livestock consuming these kind of forage crops is low (Alçiçek et al 2010; Göçmen and Parlak 2017) and excessive usage of concentrated feed is increased the feeding costs (Açıkgöz et al 2005). In recent years, there is a need for farmers to produce their feed in closed system livestock (Sabancı et al 2010).

Previous studies that forage crops can be successfully grown as the main crop and second crop as well under several ecological conditions (Açıkgöz et al 2005; Acar et al 2007). Nevertheless, non-competitive forage crops comparing to other crops are more grown as a byproduct, second crop or intercropping (Açıkgöz et al 2005; Alçiçek et al 2010). Deep root system and legumes performing high biomass that is quite important by view of agriculture welded by their benefits like protecting and improving the soil and increasing organic matter of the soil, in addition to production of forage (Anonymous 2000; Çeçen et al 2005; Zai et al 2008; Özyazıcı et al 2009; Ceyhan et al 2014; Kahraman 2017). Therefore, the ratio of forage crops should be increased to provide the need of desired quality roughage and also protect the health of soil. These crops should be concentrated on their cultivation opportunities as a by-product, second crop, or intercropping to increase the cultivation area of non-competitive forage crops compared to other crops (Açıkgöz et al 2005). In particular, agricultural lands that have the opportunity to irrigate remains fallowing lands for 3-4 months after the barley harvest in Konya (Özer 1992; Acar 1995; Acar et al 2007; Parlak and Sevimay 2007; Kahraman and Onder 2018). In this period, pastures are the inefficient period in similar ecologies (Özer 1992; Acar 1995), when it is hard to find out green fodder (Sabancı et al 2010).

Growing legume forage crops as the second crop after harvesting of cereals have been extensively examined b by many researchers as Özer (1992), Acar (1995), Kerimbek and Mülayim (2003), Aşıcı (2006), Taşpınar

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et al (2009), Dereli (2015), İleri et al (2020). For instance, Özer (1992) suggested that legumes can be successfully grown by direct sowing in the July-October period after wheat harvest in Konya ecological conditions. Another similar study by Acar (1995) recommended fenugreek, common vetch, and mixtures of field pea+oat in irrigable fields in the July-October period after barley harvest under conditions of Konya and similar ecologies in an attempt to produce high-quality roughage. Report by Kerimbek and Mülayim (2003) implicated that maize alone or a mixture of maize, common vetch and peas can be grown after grain harvest in Konya conditions to obtain green herbage yield and silage. Additionally, Asıcı (2006) implied that growing of pea can be arranged after wheat harvest in Seydişehir Town - Konya City ecological conditions in an attempt to produce high-quality roughage. The study of Taspinar et al (2009) reported 400 kg da<sup>-1</sup> green yield of common vetch after cereal harvest under conditions of Eskişehir. In a recent study, Dereli (2015) emphasized that annual forage legumes can be grown in the July-October period after barley harvest in Eskişehir conditions. A recently study by İleri et al (2020) reported the possibility of the cultivation of annual forage legumes after wheat harvest under conditions of Eskişehir. In light of the mentioned studies, it is possible to grow short-vegetation forage legumes under irrigated conditions in this period (Acar 1995; Kerimbek and Mülayim 2003; Çeçen et al 2005; Acar et al 2007; Parlak and Sevimay 2007).

For the mentioned reasons above, aim of the present research is to determine the most suitable legume forage crop that can be grown for roughage as a second product in the period after the grain harvest in irrigated agricultural lands in Konya - Turley ecological conditions. Therefore, the results of the present research will contribute to the scientific literature by providing the roughage demand for the livestock enterprises. Furthermore, evaluation of legume forage crops as second crop will add new knowledge to the literature and new insights to the relative researchers.

#### 2. Materials and Methods

Present research was conducted to determine the best legume forage crops as the second crop after harvesting of cereal (barley) under irrigated conditions, between July and September for 2 years during both 2019 and 2020 vegetation periods. Field experiment was realized in a farmer's field under the ecological conditions of Seydişehir Town of Konya City in Turkey. Field trial was set up according to randomized blocks design with 4 replications, by each experimental plot covering 8 m<sup>2</sup> (4m x 2m) total area. Each plot consisted from 10 rows by 20 cm of spaces.

According to long-term (1964-2021) climate data (data collected from: Konya Meteorology 8th Regional Directorate), the average of total annual rainfall of Seydişehir is 742.9 mm, the average temperature is 10.8°C and the average relative humidity is 62.1%. Similar with the long term climatic period, 279.4 mm, 15.6°C, and 56.9 % were detected respectively during the period of experiment in 2019 year. Total rainfall, relative temperature, and relative humidity were 585.6 mm, 12.4°C, and 58.7 % respectively during the period of experiment in 2020 year. Experiment soil was characterized as follows: loamy structure, neutral reaction (pH 7.27), lower level of organic matter (0.88 %), enough level of phosphorus (17.07 kg da<sup>-1</sup>), higher content of potassium (109.71 kg da<sup>-1</sup>), and a higher level of lime (2.34%). The aim of present research is the determination of the most suitable legume forage crop that can be grown for desired roughage qualifications as a second product in the period after the grain harvest in irrigated agricultural lands in Konya conditions.

As material of the study, Özkaynak cultivar of forage pea (Pisum sativum L.), Yemsoy cultivar of soybean (Glycine max. L.), Munzur-98 cultivar of hairy vetch (Vicia villosa Roth.), Kubilay-82 cultivar of common vetch (Vicia sativum L.), and population of fenugreek (Trigonella foenum-graecum L.) were used. Sowing of the seeds were made by hand in the first week of July in each experimental year (2019 and 2020). Seeding rates were applied that 15 kg da<sup>-1</sup> for forage pea (Turgut et al 2005), 8 kg da<sup>-1</sup> for fenugreek (Acar 1995), 12 kg da<sup>-1</sup> for common vetch (Ay and Mut 2017), 12 kg da<sup>-1</sup> for hairy vetch, 10 kg da<sup>-1</sup> for soybean (Bilgili et al 2005). Fertilizer was applied that before sowing at the rate of 4 kg da<sup>-1</sup> N with 20.20.0 fertilizer (Ülger et al 1999; Polat and Almaca 2006). Depending on soil and plants conditions, irrigations were done 5 times in total for both of the experiment years. Weed control was done by hand. Legume forage crops were harvested by hand in mid-September in each experimental year.

In the research, plant height (cm) was calculated by measuring and get average the heights from the soil surface to the plant top point of 10 plants in total (Doğan and Terzioğlu 2019). In experimental plots, 50 cm sides from the two rows and the two ends of the rows were taken as side factor and ignored for all the measurements, observations and analysis. Harvesting was performed on a remaining area of 1 m<sup>2</sup> and samples from each plot were weighed to get green forage yields. Plot yields were converted into yields per decare (Acar 1995; Çeri and Acar 2019). Green forage samples (1 kg from each plot) were dried at 70°C for 48 hours and weighed to get hay yields. Then, yields were converted into hay yields per decare (Anonymous 2019).

The investigated data were subjected to variance analysis by computed based statistical program "MSTAT-C" by randomized blocks design with 4 replications. According to the analysis of variance results, statistically significant factor means were compared by the LSD test (Çeri and Acar, 2019). Grouping test was realized according to significance level.

#### 3. Results and Discussion

#### 3.1. Plant Height (cm)

Analysis of variance results related to plant height is given in Table 2. As it is shown in Table 2, according to legume plant species there were statistically significant differences at 1% level for plant height. Statistically insignificant differences were found for year and year x plant interactions.

#### Table 2

Analysis Of Variance Regarding Plant Height Values In Legumes

Source of variation	Degrees of freedom	Sum of squares	F value
Replication	3	75.089	1.2384
Year (A)	1	104.763	1.7277
Plant (B)	3	4246.772	70.0377 **
Year x Plant	3	77.994	1.2863
Error	21	60.636	-
General	31		-

CV %: 7.54, (\*\*) shows that the difference between treatments is significant at the 1% level.

According to the year factor of present research, average values and LSD groups found for plant height are given in Table 3. According to these results, the highest value was obtained with 126.76 cm and 117.94 cm from forage pea and common vetch respectively while the lowest plant height was obtained with 90.90 cm and 77.34 cm from soybean and fenugreek respectively.

# Table 3

Plant Height Values in Legumes (cm)

Plants	Ŷ	Years		
Plants	I. Year	II. Year Avera	Average	
Common vetch	119.93	115.95	117.94 a	
Fenugreek	75.63	79.05	77.34 c	
Field pea	127.88	125.65	126.76 a	
Soybean	96.75	85.05	90.90 b	
Average	105.04	101.43	103.23	

LSD<sub>Plants</sub>: 11.02, Lettering was done according to the significance in the analysis of variance.

The plant height of common vetch was measured 115.95-119.93 cm respectively in 2019 and 2020 research years. The average plant height of common vetch also was measured 117.94 cm in our study (Table 3). Özer (1992), Acar (1995), and Kerimbek and Mülayim (2003) reported the plant height of common vetch that cultivated after cereal harvest, as 58.00 cm, 116.44 cm, 63.24 cm respectively under the conditions of Konya. Dereli (2015) detected that the plant height of common vetch cultivated after cereal harvest ranged between 72.21-83.00 cm under the conditions of Eskişehir. According to these results, the results of Acar (1995) are similar to the findings in our investigation. On the other hand, our research findings are higher than those of Özer (1992), Kerimbek and Mülayim (2003), Dereli (2015).

According to Table 3, the plant height of fenugreek was measured as 75.63-79.05 cm in 2019 and 2020, respectively. The average plant height of fenugreek also was measured as 77.34 cm in our study (Table 3). Acar (1995) reported the plant height of fenugreek that cultivated after cereal harvest, as 75.55 cm under the conditions of Konya. Boran (2011) detected that the plant height of fenugreek was 36.58 cm under the conditions of Ankara. Hosamath and Hedge (2018) reported the plant height of fenugreek ranged between 72.21-83.00 cm under the conditions of India. In another study conducted by Alp (2019), the plant height of fenugreek was determined that range between 20.47-

38.63 cm conditions of Şanlıurfa. Our results related to a plant height of fenugreek were similar to values reported by Acar (1995), Hosamath, and Hedge (2018). But it is higher than those reported by Boran (2011), Alp (2019).

Present research showed the plant height values of forage pea as 127.88 cm in the first research year, as 125.65 cm in the second research year. The average plant height of forage pea also was measured at 126.76 cm in our study (Table 3). Özer (1992), Acar (1995), and Kerimbek and Mülayim (2003) reported the plant height of forage pea cultivated after cereal harvest, as 53.00 cm, 109.44 cm, 81.27 cm respectively under the conditions of Konya. In the similar study conducted under conditions of Seydişehir, Aşıcı (2006) reported the plant height of forage pea ranged between 72.21-83.00 cm. Also, the plant height of forage pea cultivated as the second crop in conditions of Konya was measured by Özdemir (2019) between 43.3-105.0 cm. On the other hand, Dereli (2015) and İleri et al (2020) reported the plant height of forage pea cultivated after cereal harvest, as 119.8 cm and 114.78 cm, respectively under the conditions of Eskişehir. According to these results, the results of Acar (1995), Aşıcı (2006), Dereli (2015), Özdemir (2019), and İleri et al (2020) are similar to the findings in our investigation. But it is higher than those reported by Özer (1992), Kerimbek, and Mülayim (2003).

Table 3 presents that, the plant height of soybean was measured as 96.75-85.05 cm in 2019 and 2020, respectively. The average plant height of fenugreek also was detected as 90.90 cm in our study (Table 3). Ada et al (2009) reported that the plant height of soybean was 76.8 cm in their study under the conditions of Konya. In the study that carried out on soybean in conditions of Bursa, plant height was measured as 98.3 cm by Sincik et al (2009), while Şenbek and Açıkgöz (2019) reported plant height as 81.2 cm under similar conditions. On the other hand, Erdoğdu et al (2013) determined 52 cm plant height from soybean under the conditions of Ankara, while Şahar (2017) reported that plant height soybean that cultivated as a second crop under the conditions of Adana, ranged from 110.5-158.0 cm. Plant height of soybean was reported that ranged from 91.40-114.97 cm by Boydak et al (2018) under the conditions of Bingöl. According to the results obtained in these studies, our research findings are higher than those of Ada et al (2009) and Erdoğdu et al (2013), while being lower than those of Şahar (2017), and also Boydak et al (2018) as well. On the other hand, the results of Sincik et al (2009), Senbek and Açıkgöz (2019) are similar to the findings in our investigation. It was indicated that; in general, plant height of forage type soybeans cultivars is much higher than soybean genotypes (Senbek and Açıkgöz 2019; Açıkgöz et al 2020).

Table 4

Analysis Of Variance Regarding Green Yield Values In Legumes

Data of the present research showed that higher value of plant height from forage pea and common vetch compared with other plants in our study. In general for forage crops, due to the close relationship between green herbage yield and plant height, high plant height is a desirable characteristic (Özköse 2017).

As a comparison of present data, it can be seen that different results have been obtained. It is thought that the differences between our research findings and the findings in the literature are due to the ecological conditions in which the experiments were carried out, the genetic structure of the varieties, agricultural practices, and the purpose of cultivation.

# 3.2. Green Herbage Yield (kg da<sup>-1</sup>)

Variance analysis results related to green herbage yields of legumes is given in Table 4. As it is appeared in Table 4, according to legume plant species there were statistically significant differences at 5 % level between green herbage yields, while according to year x plant interactions there were statistically significant differences at 1 % level between green herbage yields. Statistically significant differences were not observed between green herbage yields of legumes, in terms of years.

-		-	
Source of variation	Degrees of freedom	Sum of squares	F value
Replication	3	529110.417	3.0429
Year (A)	1	499500.125	2.8726
Plant (B)	3	1356779.417	7.8027 **
Year x Plant	3	651517.042	3.7468 *
Error	21	173885.012	
General	31		

CV %: 16.04 (\*) while showing that the difference between treatments is significant at the 1% probability limit. (\*\*) shows that the difference between treatments is significant at the 5% probability limit.

Data of the present research showed that, average values of green herbage yields and LSD groups found for green herbage yields are given in Table 5. According to these results, the highest green herbage yield of the legumes was obtained with 3085.50 kg da<sup>-1</sup> from forage

pea and it was followed by common vetch by 2788.63 kg da<sup>-1</sup>. The lowest green herbage yield was obtained from soybean by 2199.38 kg da<sup>-1</sup>, while the green herbage yield of fenugreek was observed at 2322.50 kg da<sup>-1</sup> (Table 5).

#### Table 5

Green Herbage Yields Of Legumes (kg da<sup>-1</sup>)

Dianta	Ye	A	
Plants	I.Year	II. Year	- Average
Common vetch	2762.00 ab	2815.25 ab	2788.63 ab
Fenugreek	2138.50 cd	2506.50 bc	2322.50 b
Field pea	3341.00 a	2830.00 ab	3085.50 a
Soybean	2654.25 bc	1744.50 d	2199.38 b
Average	2723.94	2474.06	2599.00

LSD<sub>Plants</sub>:11.02, LSD<sub>Int</sub>: 613.2, Lettering was done according to the significance in the analysis of variance.

As the first and second years of the present study, the green herbage yield of common vetch was obtained that 2762.00 kg/ In our research in the first and second years, the green herbage yield of common vetch was obtained

that 2762.00 kg da<sup>-1</sup> and 2815.25 kg da<sup>-1</sup> respectively. The average green herbage yield of common vetch also was measured at 2788.63 kg da<sup>-1</sup> in our study (Table 5). Some researchers have determined different values for

green herbage yield of common vetch that cultivated after cereal harvest. For example, Özer (1992), Acar (1995), Kerimbek and Mülayim (2003) reported that the green herbage yields of common vetch that cultivated after cereal harvest, as 2297.3 kg da-1, 2128.55 kg da-1, 1204.0 kg da<sup>-1</sup> respectively under the conditions of Konya. Also, in the studies conducted after cereal harvest under the conditions of Eskişehir, green herbage yield of common vetch was detected by Dereli (2015) and Taşpınar et al (2009), between 352.8-552.3 kg da<sup>-1</sup> and 400 kg da<sup>-1</sup>, respectively. According to these results, while the average green herbage yield of common vetch was similar to the values found by Özer (1992) and Acar (1995) it was higher than the values reported by the researchers of Kerimbek and Mülayim (2003), Dereli (2015), Taşpınar et al (2009).

As it seen on Table 5, green herbage yield of fenugreek were measured as 2138.50-2506.50 kg da-1 in 2019 and 2020, respectively. The average green herbage yield of fenugreek also was detected as 2322.50 kg da<sup>-1</sup> in our study (Table 5). Acar (1995) reported the green forage yield of fenugreek that cultivated after cereal harvest, 2871.97 kg da<sup>-1</sup> under the conditions of Konya. Karadağ and Büyükburç (1999) were determined the herbage yield as 1006.77 kg da<sup>-1</sup> from fenugreek that is grown as a spring crop in Tokat. In other research, Alp (2019) obtained ranging from 60.04-2156.50 kg da<sup>-1</sup> green herbage yield from fenugreek under the conditions of Şanlıurfa. In different studies that related to the herbage yield of fenugreek were obtained different results. For example, in Western Canada, between 795.7-1644 kg da<sup>-1</sup> green herbage yield was obtained from fenugreek (Basu et al 2009), while in Iraq, 1483-2040 kg da<sup>-1</sup> green herbage yield was obtained from fenugreek (Said et al 2019). it is seen that in the other studies, there was a significant variation in the green herbage yield of fenugreek. According to these results, our findings that for green herbage yield of fenugreek were higher than the values reported by the researchers Basu et al (2009), Alp (2019), Said et al (2019), while our findings were lower than those of Acar (1995).

Present research showed that, the green herbage yield of forage pea was determined as 3341.00 kg da<sup>-1</sup> in the first research year, as 2830.00 kg da<sup>-1</sup> cm in the second research year. The average green herbage yield of forage pea also was measured at 3085.50 kg da<sup>-1</sup> in our study (Table 5). Özer (1992), Acar (1995), and Kerimbek and Mülayim (2003) reported the green herbage yield of forage pea that cultivated after cereal harvest, as 1503.50 kg da<sup>-1</sup>, 2031.51 kg da<sup>-1</sup>, 1416.50 kg da<sup>-1</sup> respectively under the conditions of Konya. In the similar study conducted under conditions of Seydişehir, Aşıcı (2006) reported the green herbage yield of forage pea ranged between 2191.80-5191.20 kg da<sup>-1</sup>. On the other hand, Dereli (2015) and İleri et al (2020) reported the green herbage yield of forage pea cultivated after ce-

real harvest, as 1606.60 kg da<sup>-1</sup> and 850.14 kg da<sup>-1</sup>, respectively under the conditions of Eskişehir. Also, the green herbage yield of forage pea cultivated as the second crop in conditions of Antalya was measured by Çeçen et al (2005) as 1219 kg da<sup>-1</sup>. According to these results, the results of Aşıcı (2006) are similar to the findings in our investigation, while our research findings are higher than those of Özer (1992), Acar (1995), Kerimbek and Mülayim (2003), Çeçen et al (2005), Dereli (2015) and İleri et al (2020).

According to Table 5, the green herbage yield of soybean was measured as 2654.25-1744.50 kg da<sup>-1</sup> in 2019 and 2020, respectively. The average green herbage vield of soybean also was detected as 2199.38 kg da-1 in our study (Table 5). Erdoğdu et al (2013) was determined the herbage yield as 2101 kg da<sup>-1</sup> from soybean that grown on irrigable lands in Ankara. Kökten et al (2014) obtained ranging from between 1204.7-1652.7 kg da<sup>-1</sup> green herbage yield from soybean under the conditions of Bingöl. In different studies that related to the herbage yield of soybean were obtained different results. For example, in Bursa, ranged between 1204.7-1652.7 kg da<sup>-1</sup> green herbage yield was obtained from soybean that grown as a second crop (Açıkgöz et al 2015), while in Adana, ranged between 1904.2-4529.5 kg da<sup>-1</sup> green herbage yield was obtained from soybean that grown as a second crop (Şahar 2017). On the other hand, Akıncı (2019) was determined the herbage yield of soybean between 826.39-1199.17 kg da<sup>-1</sup> from soybean under the conditions of Kayseri, while Senbek ve Açıkgöz (2019) was obtained as 4177.8 under the conditions of Kayseri. According to these results, our findings that for green herbage yield of soybean were higher than the values reported by the researchers Kökten et al (2014) Akıncı (2019), while our findings were lower than those of Açıkgöz et al (2015), Şenbek and Açıkgöz (2019). Also, the results of Erdoğdu et al (2013) with Sahar (2017) are similar to the findings in our investigation.

By view of the green herbage yield of legume forage crops, there are differences between the findings in our investigation and the results of previous studies in the literature. This can be attributed to the different cultivars used in this study, the different ecological conditions in which the experiments were carried out, and possibly to the different agricultural, practices as compared with the other studies.

# *3.3. Hay Yield (kg da<sup>-1</sup>)*

Variance analyze results related with hay yields of legumes is given in Table 6. As it is appeared in Table 6, according to year x plant interactions there were statistically significant differences at 1 % level between hay yields. On the other hand, statistically significant differences were not observed between the hay yield of plants, in terms of year and legume plant species.

Source of variation	Degrees of freedom	Sum of squares	F value
Replication	3	22220.769	1.8304
Year (A)	1	10.465	0.0009
Plant (B)	3	5710.915	0.4704
Year x Plant	3	64380.551	5.3033 **
Error	21	12139.606	
General	31		

 Table 6

 Analysis Of Variance Regarding Hay Yield Values In Legumes

CV %: 17.01, (\*\*)shows that the difference between treatments is significant at the 1% probability limit.

According to the two-year average results of our research, although legume plant species hadn't a significant statistically effect on hay yield production, while the highest hay yield was obtained from common vetch (678.68 kg da<sup>-1</sup>), this was followed by field pea (660.74 kg da<sup>-1</sup>), soybean (629.40 kg da<sup>-1</sup>) and fenugreek (621.68 kg da<sup>-1</sup>) respectively (Table 7).

Table 7 Hay Yield Values Of Legumes (kg da-1)

Plants	Ye	ears	A
Flains	I.Year	II. Year	Average
Common vetch	625.63 ab	731.73 a	678.68
Fenugreek	538.68 ab	704.68 ab	621.68
Field pea	680.55 ab	640.93 ab	660.74
Soybean	747.93 a	510.88 b	629.40
Average	648.19	647.05	647.62

LSD<sub>int</sub>: 220.6, Lettering was done according to the significance in the analysis of variance.

Common vetch produced hay 625.63-731.73 kg da-<sup>1</sup>, respectively period of experiment in 2019 and 2020. Also, according to the two-year results of our research, Common vetch produced hay average of 678.68 kg da<sup>-1</sup> (Table 7). In previous studies that were conducted on irrigable lands after cereal harvest under the conditions of Konya, the values hay yield of common vetch were determined ranged between 291.6-494.8 kg da<sup>-1</sup> (Özer 1992; Acar 1995; Kerimbek and Mülayim 2003). On the other hand, the hay yield of common vetch that cultivated as the second crop in conditions of Antalya was measured by Çeçen et al (2005) 561 kg da<sup>-1</sup>. Our research findings are higher than those of this study. In addition to these, Açıkgöz and Çelik (1986) reported as 803.2 kg da<sup>-1</sup> the hay yield of common vetch that cultivated on drylands under the conditions of Bursa. While Eğritaş (2014) determined ranged between 362.70-667.13 kg da<sup>-1</sup> the hay yield of common vetch under the conditions of Ordu, Kavut (2016) reported that as 875 kg da-1 under the conditions of İzmir. According to these results, our research findings are lower than those of Açıkgöz and Çelik (1986) with Kavut (2016), while being similar to the finding of Eğritaş (2014).

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Previous research implied that, hay yield values of forage pea were determined as 680.55 kg da<sup>-1</sup> in the first research year, as 640.93 kg da<sup>-1</sup> cm in the second research year. The average hay yield of forage pea also was measured as 660.74 kg da<sup>-1</sup> in our study (Table 7). In previous studies that were conducted on irrigable lands after cereal harvest under the conditions of Konya, the values hay yield of forage pea were determined ranged between 297.2-321.08 kg da<sup>-1</sup> (Özer 1992; Acar 1995; Kerimbek and Mülayim 2003). On the other hand, the hay yield of forage pea cultivated as the second crop in conditions of Antalya was measured by Çeçen et al (2005) 317 kg da<sup>-1</sup>. Present research findings are higher than those of this study. In addition to these, Açıkgöz and Celik (1986) reported as 764.0 kg da<sup>-1</sup> the hay yield of forage pea that cultivated on drylands under the conditions of Bursa. While Uzun et al (2011) determined

ranged between 653.3-794.7 kg da<sup>-1</sup> the hay yield of forage pea under the conditions of Bursa, Koçer (2011) reported that as 642 kg da<sup>-1</sup> under the conditions of Isparta. Doğan (2013) reported as 944.93 kg da<sup>-1</sup> the hay yield of forage pea under the conditions of Kırklareli. According to these results, while our findings were lower than those of Açıkgöz and Çelik (1986), Doğan (2013), are similar to the findings of Koçer (2011).

In Table 7, the hay yield of soybean was measured as 747.93-510.88 kg da<sup>-1</sup> in 2019 and 2020, respectively. The average hay yield of soybean also was detected as 629.40 kg da<sup>-1</sup> in our study (Table 5). In different studies that related to the herbage yield of soybean were obtained different results. For example, in Gümüşhane, ranged between 356-555.60 kg da<sup>-1</sup> hay yield was obtained from soybean (Okcu 2015), while in Adana, ranged between 442.9-1523.3 kg da<sup>-1</sup> hay yield was obtained from soybean that grown as a second crop (Şahar 2017). Kökten et al (2014) obtained ranging from between 524.6-703.1 kg da<sup>-1</sup> hay yield from soybean under the conditions of Bingöl. On the other hand, Başaran et al (2019) was determined the herbage yield of Yemsoy cultivar ranged between 255-284 kg da<sup>-1</sup> under the conditions of Yozgat, while Akıncı (2019) was obtained from soybean ranged between 247.71-357.90 kg da<sup>-1</sup> under the conditions of Kayseri. According to these results, our findings that for hay yield of soybean were higher than the values reported by the researchers Okcu (2015), Başaran ve ark. (2017), Akıncı (2019). Also, the results of Kökten et al (2014) and Sahar (2017) are similar to the findings in our investigation.

Green hay yield of legume forage crops, there are differences between findings in our investigation and the results of previous studies in the literature. This can be attributed to the different cultivars used in this study, the different ecological conditions in which the experiments were carried out, and possibly to the different agricultural, practices as compared with the other studies.

# 4. Conclusion

Present research was realized to the aim of determination the possibilities of growing some legume forage crops as second crop after harvesting of cereal harvest under the conditions of Seydişehir Town of Konya City in Turkey during both vegetation periods of 2019 and 2020 year.

According to the statistical analysis, significant differences were found for green herbage yield and plant height of legume forage crops. Additionally, the forage pea and common vetch presented higher green herbage yield and hay yield compared to soybean and fenugreek. The highest green herbage yield was obtained from forage pea (3085.50 kg da<sup>-1</sup>), which was followed by common vetch by (2788.63 kg da<sup>-1</sup>). Similarly, highest plants height value was detected as 126.76 cm and 117.94 cm on the forage pea and common vetch, respectively. Although statistically insignificant differences, the highest hay yield was found in the common vetch by 678.68 kg da<sup>-1</sup> value, while it was followed forage pea by 660.74 kg da<sup>-1</sup> value. Based on these results, the forage pea and common vetch can be recommended to grow in similar ecological conditions due to their high green herbage and hay yield.

In the light of present findings, it may be concluded that the fodder pea and common vetch may be recommended for purpose of producing roughage after cereal (barley) harvest. Therefore, these crops can be considered to satisfy the forage demand in livestock farming. Future studies should focus on investigation the possibility of more productive legume forage crops after cereal harvest in different ecological conditions. Consequently, researches about the other suitable forage crops in the same or different regions during similar periods will add new knowledge to present knowledge to provide the forage demand for livestock farming.

#### 5. Acknowledgements

This study is a part of Ali ÖZEL's PhD thesis. Also, Ali ÖZEL was supported by Scientific Research Projects (BAP) Coordinatorship with their projects no 19201003.

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Selcuk Journal of Agriculture and Food Sciences

http://sjafs.selcuk.edu.tr/sjafs/index

SJAFS

(2022) 36 (1), 48-57 e-ISSN: 2458-8377 DOI:10.15316/SJAFS.2022.008

# **Research Article**

# Studies on Determination of Strawberry Cultivars Suitable for Ereğli-Konya Ecological Conditions\*\*

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# ARTICLE INFO<sup>1</sup>

# ABSTRACT

Article history: Received date: 06.12.2021 Accepted date: 19.01.2022

Keywords: Strawberry Cultivars, Yield, Quality, Ereğli-Konya In order for strawberry varieties to be recommended in any region, the ecological demands of the varieties must be determined by making adaptation studies. For a successful strawberry production, variety adaptation plays an important role in the growing region. In strawberry cultivation, one of the most important purposes of breeding programs is to regulate yield and fruit quality. This study was carried out in order to determine the yield and quality characteristics of four strawberry cultivars (Albion, Monterey, San Andreas and Portola) using frigo seedlings in the Ereğli district of Konya province in 2019-2020. In the study, the earliest flowering and the highest yield were observed in Portola variety in both years. The highest fruit weight was obtained from Monterey (8.19 g) in the first year and Portola (7.72 g) in the second year. The highest number of fruits per plant was obtained from Portola (11.85-8.20 units/plant) in both years. The highest fruit firmness was determined in Monterey (1.47-1.49 kg/cm<sup>2</sup>) cultivar in both years. The highest TSS content was determined in Albion (12.40-15.10%) in both years. In the experiment, the highest L (brightness) values were found in San Andreas (35.77-36.82) variety in both years. The highest C (color intensity) value was determined in San Andreas (41.25-43.53) cultivar in both years. The darkest red fruits were determined in Monterey (h°=30.33) and Portola (h°=32.06) in the first year, and in Portola (h°=31.46) and Monterey (C°=32.15) cultivars in the second year. Titratable acidity was found to vary between 1.09% and 1.15% in the first year and between 1.23% and 1.69% in the second year. As a result, it was concluded that the cultivation of Portola variety is appropriate in terms of yield and quality characteristics in Konya province Ereğli conditions.

#### 1. Introduction

Strawberry is a berry fruit belonging to the Fragaria genus of the Rosaceae family. The fleshy fruit of the strawberry is classified as a bulk fruit. Today, cultivated cultivars are included in the Fragaria X ananassa species and constitute a regular part of the diet of millions of people (Martinelli, 1992; Hummer and Hancock, 2009). M.S. in Europe strawberry culture was started in 1300 BC. In the 13th century, the strawberry fruit was used for medicinal purposes by Greek doctors. In the 14th century, strawberry cultivation became widespread among the nobility and the flowers of the strawberry were used more than the fruit. By the 1500s, strawberry began to be studied and classified by botanists. They formed the origin of the cultivated strawberries, Fragaria virginia, which was brought to Europe from North America in the 1600s, and Fragaria chiloensis,

which was brought to Europe from South America in the 1700s (Darrow, 1966).

Strawberry is an important member of the berry group, which is grown in very different ecological conditions due to its high adaptability. Strawberry has become an increasingly important fruit in the world and in our country in recent years. According to 2019 FAO data, the world strawberry production area is 396,401 hectares and the amount of strawberries obtained from this area is 8,885,028 tons. China, which is the most important and top producer country in strawberry production, meets about 36% of the total production. Most of the remaining production is carried out in the USA, Mexico, Turkey, Egypt and Spain. Turkey, which ranked fifth in world strawberry production in 2018 with 440,968 tons, increased its strawberry production by 45,737 tons in 2019 and ranked fourth with 486,705 tons (FAO, 2021).

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<sup>\*\*</sup>This work is part of first author's MSc thesis

Strawberry production in Turkey was 130,000 tons on an area of 9,465 hectares in 2000, while it reached 486,075 tons on an area of 16,089 hectares in 2019. Strawberry production in Turkey is mostly realized in the Mediterranean, Aegean, Marmara and Central Anatolia regions, respectively. The provinces where the production is intense are Mersin, Aydın, Bursa, Antalya and Konya, respectively (TUIK, 2021).

Many epidemiological studies have shown that a diet rich in fruits and vegetables is often associated with a lower incidence of various chronic pathologies, including obesity, infections, neurological diseases and cancer. Strawberry has an important role among fruits due to its high phytochemical content (Halvorsen et al., 2006). Strawberries are an important source of bioactive compounds due to their high levels of vitamin C, folate and phenolic compounds. Moreover, strawberries are economically and commercially important and are commonly consumed fresh or in processed forms such as jams, juices and jellies. Therefore, it is among the most studied fruits in terms of agronomic, genomic and nutritional aspects (Proteggente et al., 2002).

According to its nutrient profile, strawberries are an extremely healthy food choice. First of all, dietary fiber and fructose content contribute to the regulation of blood sugar levels by slowing down digestion. The fiber content also contributes to controlling calorie intake with its satiating effect. Also, no other fat-soluble vitamins such as tocotrienols have been reported in strawberries. Strawberries have attracted great attention due to their high vitamin C content and have become one of the important sources of this vitamin in human nutrition (Giampieri et al., 2012). Considering that folate, along with vitamin C, is one of the richest natural sources of this essential micronutrient among fruits, it plays a crucial role in highlighting the micronutrient content of strawberries. In addition, strawberries are a source of many other vitamins, including, to a lesser extent, thiamine, riboflavin, niacin, vitamin B6, vitamin K, vitamin A, and vitamin E (Tulipani et al., 2008).

Strawberry is one of the most studied fruit species. For this reason, the number of varieties offered to breeders is increasing rapidly. Besides the use of modern techniques; high-yielding, and disease resistant, largefruited varieties should be tested under different ecological conditions (Paydaş and Kaşka, 1992). Thanks to the breeding studies carried out in California and Florida in the USA, varieties with superior characteristics in terms of adaptation to various criteria and different ecologies are obtained and offered to breeders. Since the first years of strawberry production in Turkey, strawberry varieties, mostly short-day varieties, were also tested in other regions, especially in the Mediterranean Region. However, as a result of intensive breeding studies in strawberry, the number of varieties increases a lot and the varieties change rapidly. Adaptation studies should be carried out in order to recommend these varieties to producers in a region. It is known that variety adaptation plays a key role for successful strawberry cultivation in any region, and the necessity of growing different varieties of different ecologies with different growing systems (Özbahçali, 2014).

It has been reported in literature studies that yield and quality characteristics of fruits are affected by genetic or environmental conditions. Many successful studies have been carried out in the field of strawberry adaptation in Turkey. As a result of literature research, studies on the adaptation of strawberry cultivars in Ereğli district of Konya province were not found. For this reason, the study was carried out to determine the performance of different strawberry cultivars in Ereğli-Konya ecological conditions.

# 2. Materials and Methods

This research was carried out in the Alhan neighborhood of the Ereğli district of Konya in the years 2019-2020. Frigo seedlings of Albion, Monterey, Portola and San Andreas strawberry cultivars were used as plant material. Frigo seedlings were obtained from Çiltar A.Ş.

Albion: It was obtained from crossing between Diamante X Cal 94.16-1. It is a moderately neutral day variety. It adapts well to cool and temperate regions. It has good fruit quality and shows this performance all season long. It is an early variety and maintains its fruit size all season (Ateş and Türemiş, 2018).

Monterey: The variety is a medium day neutral variety. It is a hybrid of Albion x Cal 97.85-6. This variety, which is very sweet due to its low acidity, has large and soft fruits. It has a strong plant structure and is sensitive to mildew (Ateş and Türemiş, 2018).

Portola: This variety Cal. 97.93-7 X Cal. it was obtained as a result of hybridization of 97.209-1. In arid and subtropical climates, it shows medium-strong neutral day character under suitable conditions. Performs well in spring and summer plantings. It is moderately resistant to powdery mildew, *Verticillium* wilt and anthracnose (Demirsoy, 2016).

San Andreas: This variety Albion X Cal. it was obtained by crossing 97.86-1. It is a moderately neutral day variety. Its fruits have a good appearance and taste. This variety, which has a nice aroma, is quite early and is resistant to the road. It gives products uninterruptedly and without disturbing the fruit size throughout the season (Erdem and Çekiç, 2017).

Konya province Ereğli district has an area of 2,260 km<sup>2</sup>. It is located on the Ereğli Plain, which is the eastward continuation of the Konya Plain, one of the widest plains in Turkey. The district is surrounded by high mountains. The middle part of the Taurus Mountain range in the south, Hasandağı, Karacadağ and Karadağ volcanic masses, which are volcanic mountains in the north, are located. Between these mountain masses is the wide plains of the Ereğli Plain. The slope of the Ereğli Plain, which has a generally flat topography, varies between 0-10% (Allı, 2019).

In Ereğli, between 1990 and 2020, the hottest month is July and the highest average temperature is 24.1 °C. The annual average temperature of 2019, in which the research was conducted, was 13.1 °C, the highest average temperature was determined in August (24.3 °C) and the lowest average temperature was determined in January (0.6 °C). In 2020, the annual average temperature is 13.5 °C, the highest average temperature is in July (25.7 °C) and the lowest average temperature is in January (0.8 °C) (Table 1).

Table 1 Some meteorological data of Ereğli (Anonymous, 2021)

Year/Month	1	2	3	4	5	6	7	8	9	10	11	12	Average
					Minim	um Temp	erature (°	C)					
1990-2020	-13.9	-13.2	-6.4	-1.3	4	8.2	11.8	11	5.5	0.1	-6.3	-11.5	-1.9
2019	-16.6	-3.7	-5.1	-0.3	4.3	12.9	11.7	12	4	3	-7	-4.1	0.9
2020	-8.4	-12.1	-4.8	-1	3	7.7	13.1	11.7	11.5	6.6	-5.7	-6.1	1.2
					Maksin	um Temp	erature (°	C)					
1990-2020	14.1	17.2	22.5	27.0	30.9	34.4	37.0	36.6	33.6	29.2	21.4	16.6	26.7
2019	14	15.2	19.9	24.3	35	36.3	37.4	37.1	32.9	29.4	24	18.5	27.0
2020	13.8	17.4	23.9	25	33.9	34.4	37.3	36.6	39.6	31.8	19.1	15.4	27.3
					Avera	ge Tempe	rature (°C	C)					
1990-2020	0.1	1.9	6.9	11.8	16.5	20.8	24.1	23.7	19.3	13.6	6.7	2.1	12.2
2019	0.6	4.6	6.8	10.0	18.6	22.3	23.3	23.4	19.4	15.7	9.1	3.8	13.1
2020	0.8	3.2	7.8	11.5	17.4	21.3	25.7	23.9	22.8	17.9	5.9	4.8	13.5
					Ave	rage Hum	idity (%)						
1990-2020	77.0	71.5	62.2	58.2	57.2	52.0	45.4	47.3	52.1	61.1	70.1	77.1	60.9
2019	78.2	73.1	61.7	66.7	44.3	51.2	41.3	46.7	46.1	57.1	65.7	84.1	59.7
2020	82.3	74.2	66.8	60.6	48.6	43.5	37.1	33.5	42.7	33.9	73.7	73	55.8
					Total Pr	ecipition (	mm=kg÷	m²)					
1990-2020	33.2	27.8	33.2	36.0	34.2	27.1	8.8	7.5	14.7	21.7	29.9	38.9	313
2019	66.8	36.0	20.0	47.8	3.4	23.2	0	6.4	4.6	15.0	15.4	51.6	290.2
2020	50.4	21.3	40.2	33.3	18.8	9.5	0.4	0	3.0	1.6	30.0	14.0	222.5

# 3. Results and Discussion

Between 1990 and 2020, the average humidity value in Ereğli is 60.9%. In the year 2019, when the research was conducted, the average humidity is 59.7%, the lowest humidity is July (41.3%) and May (44.3%), and the highest is December (84.1%), January (78.2%) and February (73.1%) were determined as months. In 2020, the average humidity is 55.8%, the lowest humidity is August (33.5%) and September (33.9%), and the highest is January (82.3%), February (74.2%) and November (73.7%) months (Table 1).

The total amount of precipitation in Ereğli between 1990 and 2020 is 313 kg/m<sup>2</sup>. The highest total precipitation was seen in December (38.9 kg/m<sup>2</sup>) and April (36 kg/m<sup>2</sup>), the least in August (7.5 kg/m<sup>2</sup>) and July (8.8 kg/m<sup>2</sup>). The total amount of precipitation in 2019, when the research was conducted, was 290.2 kg/m<sup>2</sup>. The least total precipitation was in July (0 kg/m<sup>2</sup>) and May (3.4 kg/m<sup>2</sup>), and the highest total precipitation was in January (66.8 kg/m<sup>2</sup>) and December (51.6 kg/m<sup>2</sup>). In 2020, the total amount of precipitation is 222.5 kg/m<sup>2</sup>. The least total precipitation was in August (0 kg/m<sup>2</sup>) and July (0.4 kg/m<sup>2</sup>), and the highest total precipitation was in January (50.4 kg/m<sup>2</sup>) and March (40.2 kg/m<sup>2</sup>) (Table 1).

When the data of 2019, in which the research was carried out, are examined, it is seen that the average temperature value (13.1 °C) has increased compared to the long-term average, the average humidity level (59.7%) and the amount of precipitation per square meter (290.2 kg) decreased. Similarly, in 2020, it is seen that the average temperature value (13.5 °C) increased compared to the long-term average, while the average humidity level (55.8%) and the amount of precipitation per square meter (222.5 kg) decreased. Compared to the long-term

average, 2019 and 2020 were hotter in Ereğli and especially 2020 was drier in terms of precipitation.

The analysis of the soil sample taken from a depth of 0-30 cm to represent the trial area was carried out in the Şemsi Bayraktar Analysis Laboratory within the Karaman Chamber of Agriculture. Analysis results are given in Table 2. Accordingly, the soil of the trial area is clayey loam textured, slightly alkaline (pH 7.31), low EC, very calcareous (30.84%), moderately organic matter (4%), rich in P and K. According to the results of the analysis, burnt farm manure and base manure were applied to the trial area before planting the strawberry seedlings.

Ta	ble	2

Soil analysis results of the research area

Analysis Name	Unit	Result	Comment
Constituency	%	53.10	Clay loam
pH (Saturation)		7.31	Slightly alkaline
EC (Saturation)	μS/cm	961	Low
CaCO <sub>3</sub> (Loam)	%	30.84	Too much
Organic matter	%	4	Medium
Phosphorus (P)	mg/kg	190	Very high
Potassium (K)	mg/kg	827	Very high

Strawberry seedlings were planted on the prepared bobbins 60 cm wide and 16 m long, with 30 cm row spacing and 30 cm row spacing. The experiment was established in 3 replications according to the randomized blocks design and 20 seedlings were used in each replication. Before planting the seedlings, the tops of the bobbins are covered with black plastic mulch. The water required during the development period of the plants in the plots was given by drip irrigation system. In the first year of the research, refrigerated seedlings were planted in open field on 14.04.2019. All of the inflorescences and branches that occurred in 2019 were plucked. The first flowering of these plants in 2020 occurred on 8 May. Strawberries started to ripen on 29.05.2019. In the second year of the research, refrigerated seedlings were planted in open field on 10.03.2020. The first flowering was seen on 01.05.2020. The date when the strawberries start to ripen is 25.05.2020.

Flowering onset dates in cultivars were determined by observation. The first flowering date was taken as the date when 5% of the flower petals opened and 70% of the flower petals were opened as the full bloom date. During the growing period, the fruits harvested from each plant were weighed on a scale with an accuracy of 0.1 to determine the yields and average fruit weights per plant. The number of fruits per plant was determined by counting the ripe fruits harvested from each plant (İpek et al., 2009). The hardness of ten randomly selected fruits for pulp firmness (kg/cm<sup>2</sup>) was measured with a penetrometer, and a 5 mm (0.2 cm2) probe was used for the measurement (Agar et al., 1991). Fruit color of ten fruits from each replication was determined by Minolta Konica CR-400 cromometer (Pérez-Sánchez et al., 2010). It was determined as % by refractometer from the fruit juice of the fruits selected from each application (İpek et al., 2009). The titratable acid content of the fruit juice (%) was determined by the titrimetric method. The H+ concentration in the juice of the sampled fruits was determined using a Hanna brand table-type pH meter (İpek et al., 2009). The ascorbic acid content in the samples was determined by the spectrophotometric dichlorophenol indophenol method (mg/100 g) defined by Pearson (Pearson, 1976).

The effects of the applications were examined with three replications. The data obtained were evaluated with the 5% Duncan test using the SPSS statistical program.

#### 3. Results and Discussion

## Flowering dates

In the study, the first flowering showed changes according to the cultivars. The earliest flowering in 2019 was observed on 8 May in Portola cultivar. This variety was followed by Monterey (May 9), Albion (May 10) and San Andreas (May 11). In 2020, the earliest flowering started on May 1 in Portola variety, followed by Albion and Monterey (May 3), and then San Andreas (May 4) (Table 3).

# Table 3

Flowering dates of strawberry cultivars

Cultivars	Flowering Dates		
-	2019	2020	
Albion	10 May	3 May	
Monterey	9 May	3 May	
Portola	8 May	1 May	
San Andreas	11 May	4 May	

## Yield per plant (g/plant)

Yield values per plant (g/plant) and yield per decare (kg/da) of strawberry cultivars for the years 2019 and

2020, in which the research was conducted, are given in Table 4. In 2019, the highest yield per plant was obtained from Portola (94.14 g/plant). This cultivar was followed by San Andreas (55.01 g/plant), Albion (54.21 g/plant) and Monterey (54.03 g/plant), respectively, and the difference between the cultivars was found to be statistically significant.

Yield per plant in 2020 showed a slight decrease compared to the first year. The most productive variety this year was Portola (63.27 g/plant), as in 2019, followed by Monterey (51.35 g/plant), San Andreas (46.11 g/plant) and Albion (43.80 g/plant). When the varieties were evaluated statistically, it was determined that the difference between them was significant (Table 4).

# Table 4

Yield per plant	(g/plant)	of strawberry c	ultivars
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Cultivars	Yield per plant (g/bitki)			
Cultivals	2019	2020		
Albion	54.21 b*	43.80 c		
Monterey	54.03 b	51.35 b		
Portola	94.14 a	63.27 a		
San Andreas	55.01 b	46.11 c		

\*: There is no difference between the averages shown with the same letter in the same column

Many studies have been carried out on strawberry cultivation in different regions of our country. In a study conducted in Kayseri, the highest yield values per plant were found in Crystal cultivar in 2011 with 70.1 g, and the highest in Fern cultivar in 2012 with 914.2 g (Alan, 2013). Cekic et al. (2003), the yield per plant in Tokat was determined as 273.8 g in Maraline cultivar in 2002, 382.3 g in Tudla cultivar in 2003, 392.8 g in Muir and 405.6 g in Maraline cultivar. Again, in a study conducted in Erzurum conditions, the highest yield was obtained from Kabarla (296.2 g), and the lowest yield was obtained from Rubygem (98.6 g) cultivar (Özbahçali, 2014). In the study carried out in Merzifon district of Amasya, the highest yield per plant was found in Monterey (307.8 g), followed by Albion (283.7 g) and San Andreas (243.7 g), respectively. Oğuz (2019) obtained the highest yield per plant from Kabarla (635.88 g) and the lowest yield from Redlans Hope (362.71 g) in Eskişehir. In another study conducted in Kayseri, the highest yield was obtained from the Fern with 843.85 g. followed by Kabarla (660.25 g), Albion (629.85 g) and Sweet Ann (514.5 g), respectively (Çolak et al., 2019). It is seen that the yield values per plant obtained from the strawberry cultivars used in the research in 2019 and 2020 differ from the previous studies. It is thought that the differences in yield value per plant between the studies also differ according to the climate and soil characteristics, ecological factors and cultural measures applied. In addition, very high lime-induced plant growth problems in the research area also negatively affected the yield.

#### Fruit weight

The highest average fruit weight in 2019 was Monterey (8.19 g), followed by Portola (7.94 g), San Andreas (7.39 g) and Albion (5.84 g), respectively. It was determined that the difference between the cultivars was statistically significant. In the second year of the study, while the average fruit weight of Albion and San Andreas cultivars increased slightly compared to the first year, it was determined that it decreased slightly in Monterey and Portola cultivars. When the average fruit weights of the cultivars were examined in 2020, it was determined that it varied between 6.33-7.72 g. Average fruit weights were obtained from the lowest Albion and highest Portola cultivars (Table 5).

#### Table 5

Average fruit weight of strawberry cultivars

Cultivars	Average fruit weight (g)			
Cultivars	2019	2020		
Albion	5.84 d	6.33		
Monterey	8.19 a	6.43		
Portola	7.94 b	7.72		
San Andreas	7.39 с	7.56		
		N.S.		

N.S.: Non-significant.

Although fruit weight is considered a genetic factor due to the characteristic of the strawberry variety, it can be affected by climatic conditions and growing techniques. Among similar studies on fruit weights giving different values in different climatic conditions, in a study conducted in Adana, the largest fruit was 36.0 g and belonged to the H-1 cultivar (Türemiş, 2000), and in a study in Hatay Yayladağı, the highest average fruit weight was 16.0 g. It has been determined that they belong to Muir and Tudla with 15.7 g (Özdemir et al., 2003). In the study conducted under Tokat conditions, it was observed that Rubygem produced the largest fruits with 19.67 g in the first yield year, and Camarosa cultivar 12.47 g in the second year (Saraçoğlu, 2013). In a study conducted in Erzurum, fruit size was determined as 9.0 g in Sweet Ann, 8.3 g in Rubygem, 7.8 g in Crystal, 7.7 g in Kabarla, and 6.5 g in Redlans Hope cultivar (Özbahçali, 2014). Macit et al. (2011) obtained the largest fruits from Redlans Hope (10.09 g) and Kabarla (9.27 g) cultivars in their study in Samsun. In a study conducted in Kayseri, the highest fruit weight was found in the Fern cultivar (8.86 g) (Alan, 2013). In another study conducted in Latvia, the highest fruit weight was obtained from the San Andreas cultivar with 13.3 g (Laugale et al., 2014). Oguz et al. (2017) reported that the average fruit weight of strawberry cultivars ranged from 5.31-7.67 g (Monterey-San Anderas) in their study conducted in Nevşehir conditions. In the study carried out in the Merzifon district of Amasya province, it was observed that the largest fruits were in the Albion (12.8 g) and the smallest fruits were in the Sweet Charlie (7.39 g) cultivar (Geçer et al., 2018). In a study conducted in Eskişehir, it was determined that average fruit weights ranged between 15.0-19.51 g (Kabarla-San Andreas) (Oğuz, 2019). When the results obtained and the results of the literature studies are compared, it is seen

that the fruit size of the strawberry cultivars grown in Konya-Ereğli conditions is at an average level.

# Number of Fruits per Plant

The highest number of fruits per plant in 2019 was Portola (11.85 units/plant), followed by Albion (9.28 units/plant), San Andreas (7.45 units/plant) and Monterey (6.60 units/plant). It was determined that the statistical difference between the number of fruits per plant of the cultivars used in the study was significant. When the fruit numbers per plant obtained in the second-year research were examined, the highest variety was Portola (8.20 units/plant), followed by Monterey (7.98 units/plant), Albion (6.92 units/plant) and San Andreas (6.10 units/plant). It has been determined that the number of fruits obtained from Monterey and Portola varieties is higher than Albion and San Andreas cultivars and they are in the same group statistically (Table 6).

#### Table 6

Number of fruits per plant of strawberry cultivars

Cultivars	Number of fruits per plant			
Cultivals	2019	2020		
Albion	9.28 b	6.92 b		
Monterey	6.60 d	7.98 a		
Portola	11.85 a	8.20 a		
San Andreas	7.45 с	6.10 c		

It can be said that the number of fruits per plant in strawberry depends on the eco-physiological characteristics of the place of study and the characteristics of the cultivated cultivar. Güleryüz et al. (1991) in their study conducted in Erzurum determined that the lowest number of fruits per plant was in Pocahontas (9.38 units/plant) and the highest in Aliso (28.61 units/plant). Gülsoy (2003) found the number of fruits per plant in open cultivation as 8.73 pieces/plant in Sweet Charlie in Van. In the study conducted in Tekirdağ, Gül (2011) reported that the highest number of fruits per plant was Fern with 9.19 units/plant and the lowest number of fruits per plant was Camarosa with 4.46 units/plant. In another study conducted in Van, Aromas (19.49 pieces/plant) and Sweet Charlie (20.10 pieces/plant) cultivars had higher fruit numbers (Geçer and Yılmaz, 2011).

#### Fruit Firmness

In 2019, it was determined that the fruit firmness values of the cultivars were statistically in 3 groups. It was determined that the statistical differences of Portola and San Andreas cultivars were not significant. The lowest fruit firmness was obtained in Albion with 1.20 kg/cm<sup>2</sup> and the highest fruit firmness in Monterey with 1.47 kg/cm<sup>2</sup>. In the second year of the study, fruit firmness values were found to vary between 1.21-1.49 kg/cm<sup>2</sup>. The lowest fruit firmness values were obtained from Albion and the highest Monterey. Portola and San Andreas were found to be in the same group statistically (Table 7).

Table 7 Fruit firmness of strawberry cultivars

Cultivars	Fruit firmness (kg/cm <sup>2</sup> )		
Cultivals	2019	2020	
Albion	1.20 c	1.21 c	
Monterey	1.47 a	1.49 a	
Portola	1.34 b	1.35 b	
San Andreas	1.30 b	1.32 b	

Fruit flesh firmness is one of the most important quality criteria in strawberries. Due to its advantages in harvest, transportation and post-harvest applications, hard fruit varieties are preferred by producers. Fruit firmness is affected by many factors such as ecological factors, fertilization, genotype, growing conditions, fruit size, fruit composition, storage and fruit temperature. Fruits grown in very hot, humid and long-day conditions are soft, while those grown in short-day conditions are hard-fleshed (M1s1r, 2016). Gündüz and Özdemir (2012), in their study in Hatay, determined that the hardest fleshy fruits were from Camarosa, Carmine and Kabarla, and the softest fleshy fruits were from the Ottoman. Alan (2013), in Kayseri, the lowest fruit firmness values are with Kabarla (0.50 kg/cm<sup>2</sup>) with Redlanshope (0.76 kg/cm<sup>2</sup>) and the highest Fern (1.61 kg/cm<sup>2</sup>) with Crystal (1.38 kg/cm<sup>2</sup>) were detected in cultivars. Aguero et al. (2015), in their study in Argentina, the lowest fruit firmness was obtained from Ventana (88.6 g/mm<sup>2</sup>) and the highest from Candonga (99.6 g/mm<sup>2</sup>). M1s1r (2016) determined that among the varieties used in Samsun, Fortuna (0.54 kg/cm<sup>2</sup>) had the highest fruit firmness value. Kandemir (2016) found that the hardest fruits were obtained from the Amiga (0.61 kg/cm<sup>2</sup>), Fortuna (0.41 kg/cm<sup>2</sup>), Monterey (0.43 kg/cm<sup>2</sup>), Benicia (0.43 kg/cm<sup>2</sup>), in the study he conducted using refrigerated seedlings under plastic greenhouse conditions reported that the San Andreas (0.44 kg/cm<sup>2</sup>) cultivars had the lowest hardness value.

# Fruit Color (L, C, h°)

The L value, which indicates the brightness of the upper colors of the fruits, is given in Table 8. The differences between the cultivars were found to be statistically significant. In 2019, the highest brightness (L) among cultivars were determined in San Andreas (L=35.77). This cultivar was followed by Portola (L=34.34), Albion (L=33.21) and Monterey (L=31.51), respectively. In the second year of the study, as in the first year, the highest brightness (L) was determined in San Andreas (L=36.82). This cultivar was followed by Portola (L=31.46), respectively (Table 8).

Table 8

Fruit color L (brightness) values of strawberry cultivars

Cultivars	Fruit color L (brightness)		
Cultivars	2019	2020	
Albion	33.21 bc	33.54 c	
Monterey	31.51 c	31.46 d	
Portola	34.34 ab	34.91 b	
San Andreas	35.77 a	36.82 a	

One of the most important quality factors in strawberries is fruit color. In addition to other quality features, fruit appeal is also very important in the supply of fresh strawberries to the market, increasing the demand for fruit. The outer color of strawberry fruits is accepted as an indicator of maturity and quality in the market. For this reason, studies have been carried out on the subject. In the study conducted in Kayseri, Alan (2013) determined the average fruit L value with the lowest 28.51 in Crystal and the highest 33.90 in Fern cultivars. In a study in Korea, the highest 'L' value was found in San Andreas and Rubygem cultivars (Ruan et al., 2013). M1sir (2016) found the highest L value in Rubygem with 75.1 and the lowest value in Sweet Ann with 52.7 in his study. Oğuz (2019) determined that Sweet Ann (33.44) and San Andreas (32.27) were the cultivars with the highest brightness (L) values among the varieties in his study in Eskişehir conditions.

Differences in fruit color C (color intensity) values were determined between cultivars (Table 9). In 2019, the cultivar with the highest fruit color 'C' was determined as San Andreas (C=41.25). This variety was followed by Portola (C=36.98), Albion (C=35.64) and Monterey (C=32.38), respectively. In the second year of the study, the cultivar with the highest fruit color 'C' was determined as San Andreas (C=43.53). This cultivar was followed by Portola (C=37.89), Albion (C=37.15) and Monterey (C=34.13), respectively (Table 9).

# Table 9

Fruit color C (color intensity) values of strawberry cultivars

Cultivars	Fruit color C (color intensity)		
Cultivars	2019	2020	
Albion	35.64 b	37.15 b	
Monterey	32.38 c	34.13 c	
Portola	36.98 b	37.89 b	
San Andreas	41.25 a	43.53 a	

Misir (2016) found the highest C value in Sweet Ann (31.8), Fortuna (31.1) and Amiga (30.0) cultivars. The lowest C value was followed by Festival (17.0) and Rubygem (18.6). The values of Albion (C=22.5), Monterey (C=25.1) and San Andreas (C=27.9) cultivars used in this study were found to be lower than our study. In another study conducted in Samsun, the highest C value was found in Amiga (38.6) and the lowest in Benicia (29.8). In other cultivars used, C values were determined as Fortuna (36.5), Monterey (34.9), Camarosa (34.0), Rubygem (33.0), Albion (32.4), Festival (32.0), San Andreas (31.9) and Sweet Ann (31.0) respectively (Kandemir, 2016). Oğuz (2019) determined the varieties with the highest fruit color "C" in Eskişehir conditions as San Andreas (35.80) and Sweet Ann (35.76).

The  $h^{\circ}$  value, also known as the color angle value, which indicates the lightness or darkness of the fruit colors, is given in Table 10. It is known that the smaller the color angle value ( $h^{\circ}$ ), the darker the fruit color, and the larger it is, the lighter the fruit color.

In the first year of the study, the cultivar with the darkest fruit among the varieties was Monterey, and the

lightest colored cultivar was San Andreas. In other cultivars, it was determined as Portola ( $h^\circ=32.06$ ) and Albion ( $h^\circ=32.92$ ) from dark to light, respectively. In the second year, the variety with the darkest fruit among the varieties was determined as Portola, and the lightest colored variety was determined as San Andreas, as in the first year. Monterey ( $h^\circ=32.15$ ) and Albion ( $h^\circ=32.98$ ) were determined in other cultivars from dark to light, respectively (Table 10).

#### Table 10

Fruit color h° (color angle value) values of strawberry cultivars

Cultivars	Fruit color h° (color angle value)		
Cultivals	2019	2020	
Albion	32.92 a	32.98 ab	
Monterey	30.33 b	32.15 ab	
Portola	32.06 ab	31.46 c	
San Andreas	33.57 a	34.76 a	

Misir (2016), the darkest red fruits in Sweet Ann (h°=55.4) and Amiga (h°65.5); the lightest red fruits were determined in Festival (h°=127.7), Rubygem (h°=119.9), Camarosa (h°=115.9) and Benicia (h°=109.2) cultivars, respectively. Kandemirli (2016), in his study, determined that Sweet Ann (h°=59.1) had the lightest colored fruit, while Camarosa (h°=39.2) had the darkest fruit. In addition to these cultivars, he determined other measurements as h°=40.3 in Monterey, h°=49.7 in Albion and h°=56.9 in San Andreas. In the study conducted by Oğuz (2019), in Eskişehir conditions, it was determined that the cultivars with the darkest fruit were Redlans (h°=23.30) and Albion (h°=23.41), and the cultivar with the lightest fruit was Sweet Ann (h°=32.22) has done.

#### Total soluble solids (TSS) (%)

In 2019, it was determined that the amount of TSS in cultivars varied between 8.60-12.40%. The lowest amount of water-soluble dry matter was found in Monterey with a value of 8.60%, and the highest amount of water-soluble dry matter was determined in Albion with a value of 12.40%. It was determined that Monterey and Portola cultivars were in the same group statistically (Table 11). TSS value of cultivars in 2020 varied between 13.00-15.10%. The lowest amount of water-soluble dry matter was found in Monterey with 13.00% and the highest in Albion with 15.10%. It was determined that the TSS values obtained in the second year of the study were above the values obtained in the first year. It was determined that the statistical differences in the amount of soluble dry matter between the varieties in the second year were significant (Table 11).

Table 11

TSS amounts of strawberry varieties (%)

Cultinum	TSS val	ues (%)
Cultivars	2019	2020
Albion	12.40 a	15.10 b
Monterey	8.60 c	13.00 a
Portola	9.20 c	13.10 a
San Andreas	10.40 b	14.80 b

TSS ratios are important in terms of affecting the taste of fruits. Therefore, it has been studied in many studies. Alan (2013), in his study in Kayseri, determined the amounts of TSS in the range of 8.46% (Redlanshope) and 10.13% (Fern). Özbahçali (2014), in his study in Erzurum, reported that TSS values ranged between 7.3% (Kabarla) and 9.5% (Rubygem). MISIT (2016) determined the highest TSS content in Albion with 6.8% in the study conducted in Samsun. In another study conducted in Samsun conditions, the highest TSS content among the cultivars was determined in Festival (6.8%), Rubygem (6.7%), Monterey (6.5%) and Albion (6.5%) (Kandemir, 2016). Oğuz (2019), in his study in Eskişehir, found the highest TSS content in Sweet Ann (7.98%) and the lowest in San Andreas (6.26%). Zanin et al. (2019) reported in a study conducted in Brazil that the contents of TSS varied between 7.50 and 9.50%. In this study, Albion was determined as 8.53%, Monterey 8.00% and Portola 7.63%. Özok (2021), in his study conducted in Bursa, determined that the amounts of TSS varied between 6.4% and 9.9%. When the results we have obtained are compared with the results of the studies on the subject, it can be said that the rates of TSS are generally high.

# Titratable Acidity (%)

In 2019, the titratable acidity values of the cultivars were determined to vary between 1.09% and 1.15%. The highest titratable acidity value of the cultivars was found in San Andreas (1.15%), followed by Albion and Portola (1.14%), and the lowest (1.09%) in Monterey. According to the statistical evaluation, only Monterey variety was in a different group from other varieties. According to 2020, the differences between the titratable acidity values of the cultivars were found to be statistically significant. In the second year, it was determined that the titratable acidity of the cultivars varied between 1.23% and 1.69%. The lowest titratable acidity value was determined in Portola (1.23%) and the highest in Albion (1.69%) (Table 12).

#### Table 12

Titratable acidity values of strawberry cultivars

Cultivars	Titretable acidity (%)		
Cultivals	2019	2020	
Albion	1.14 a	1.69 a	
Monterey	1.09 b	1.47 a	
Portola	1.14 a	1.23 b	
San Andreas	1.15 a	1.36 b	

Titratable acidity values of strawberry juices are between 0.05% (Redlanshope) and 0.09% (Fern) in the conditions of Alan (2013), Kayseri conditions, between 0.48% (Fortuna) and 0.75% (Albion) in Misir (2016), Samsun, Oğuz (2019) reported that it varies between 0.38% (Redlans Hope) and 0.43% (Sweet Ann) in Eskişehir. In another study conducted in Samsun conditions, they found the titratable acidity value to be 0.70% in San Adreas and 0.69% in Albion and Monterey cultivars (Soysal et al., 2019). Özok (2021), in his study under Bursa conditions, determined the highest titratable acidity value in Pineberry (0.91%), while the lowest value was found in Monterey (0.53%). The values obtained in our study are generally compatible with the literature data.

pH

In 2019, the pH values of the cultivars changed between 3.75-3.83, the lowest pH was 3.75 in San Andreas and the highest pH was 3.83 in Portola cultivars. When the pH values of the cultivars were examined in 2020, it was determined that they ranged between 3.81-3.85. The lowest pH value was determined in Monterey (3.81) and the highest in San Andreas (3.85) cultivars. The pH values between the cultivars were statistically found in the same group (Table 13).

#### Table 13

pH values of strawberry varieties

Cultivars	pH va	lues
Cultivars	2019	2020
Albion	3.78 ab	3.83
Monterey	3.79 ab	3.81
Portola	3.83 a	3.83
San Andreas	3.75 b	3.85
		N.S.

N.S.: Non-significant

Gidemen (2003) found the highest pH content in Sweet Charlie as 3.59 in the study conducted by the summer planting method in the high tunnel in the Amik Plain. Alan (2013) reported that the highest pH amount in Kayseri conditions was in Redlans Hope (3.60). Özbahçali (2014) determined that pH values varied between 2.3 (Kabarla) and 2.9 (Rubygem) in Erzurum conditions. Oguz et al. (2017) determined that the pH was in the range of 3.61-3.85 (San Andreas–Monterey) under Nevşehir conditions. Kılıç and Yılmaz (2017) determined that pH values varied between 2.69 and 3.75 in Kayseri ecological conditions. Colak et al. (2019) observed that the highest pH value was found in Sweet Ann (3.06) cultivar in Kayseri conditions.

#### Vitamin C

In 2019, the vitamin C values in the cultivars varied between 54.42-68.22 mg/100 g, the lowest vitamin C value was found in San Andreas with 54.42 mg/100 g and the highest vitamin C value was found in Portola cultivars with 68.22 mg/100 g. When the vitamin C values in varieties were examined in 2020, it was determined that they ranged between 43.06-54.28 mg/100 g. The lowest vitamin C value was determined in San Andreas (43.06) and the highest in Monterey (54.28). Vitamin C values between the varieties were found to be statistically significant (Table 14).

# Table 14

Vitamin C values of strawberry varieties

Cultivars	Vitamin C	(mg/100 g)
Cultivals	2019	2020
Albion	54.77 c	50.91 b
Monterey	58.26 b	54.28 a
Portola	68.22 a	50.91 b
San Andreas	54.42 c	43.06 c

Fresh fruits are an important source of vitamin C, which is important for human health. Strawberry fruit is known to contain high vitamin C in general. In the studies, Kavnas and Günay (2003), 16.5-68.9 mg/100g; Gidemen (2003), 45.6-48.9mg/100g; Özbahçali (2014) found it between 38.0-56.0 mg/100g. Kandemir (2016) reported that in the study conducted in Samsun conditions, the content of vitamin C varied between 12.4-33.6 mg/100g, the highest vitamin C value was obtained from Rubygem with 33.6 mg/100g, followed by Benicia with 24.4 mg/100g. In the study conducted in Giresun province, the highest value in terms of vitamin C was obtained from Fortuna (101 mg/100g) (Islam et al., 2019). Temocico et al. (2019) reported that they obtained the highest vitamin C value from Garda (96.80 mg/100 g) in Romanian conditions. Our findings on vitamin C content within the current literature are around the averages given for strawberries.

#### 4. Conclusions

Strawberry has an important share among berry fruits in the world. Turkey is an important country in world strawberry production. The interaction of environment and genotype is particularly important in strawberry. In order for a cultivar to be recommended to a region, adaptation studies must be done. In this study, the characteristics of some strawberry cultivars in Ereğli district of Konya province were investigated.

The highest yield was obtained from Portola in two years in the cultivars included in the experiment. The difference in the yields obtained in the experimental area from the previous studies was due to the very high limeinduced plant growth problems in the area. Chlorosis is seen in calcareous soils because calcium prevents iron intake. It was determined that although the plants were given Iron (Fe) during the growing period, the deaths continued and significantly affected the yield.

In the experiment, the highest fruit weight was obtained from Monterey (8.19 g) in the first year and from Portola (7.72 g) in the second year. The highest number of fruits per plant was obtained from Portola (11.85-8.20 units/plant) in both years. The highest fruit firmness was determined in Monterey (1.47-1.49 kg/cm<sup>2</sup>) in both years. The highest SSCM content was determined in Albion (12.40-15.10%) in both years.

Color is one of the important quality criteria in terms of marketability in strawberries. In the experiment, the highest L (brightness) values were observed in San Andreas (35.77-36.82) in both years. The highest C (color intensity) value was determined in San Andreas (41.25-43.53) in both years. The darkest red fruits were observed in Monterey (h°=30.33) and Portola (h°=32.06) in the first year, and in Portola (h°=31.46) and Monterey (h°=32.15) in the second year.

When the data obtained in the study were evaluated in general, it was concluded that Portola can be recommended among the cultivars examined for strawberry cultivation in the open under the conditions of Konya province Ereğli. Considering that new strawberry cultivars are introduced to the market almost every year, we can say that adaptation studies of varieties for the region should be continued.

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Selcuk Journal of Agriculture and Food Sciences

http://sjafs.selcuk.edu.tr/sjafs/index

# SJAFS

(2022) 36 (1), 58-62 e-ISSN: 2458-8377 DOI:10.15316/SJAFS.2022.009

# Research Article

# **Usage Opportunities of Pomegranate (Punica Granatum) Peel Dried with Different Methods in Whole Wheat Flour Chips**

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# ARTICLE INFO

Article history: Received date: 14.11.2021 Accepted date: 04.03.2022

Keywords:

Pomegranate peel Vacuum drying Convection drying Microwave drying Chips

# ABSTRACT

In this study, pomegranate peels were dried using three different drying methods, vacuum, microwave and convective. Dried peels were ground into powder and replaced (0, 5 and 10%) with whole wheat flour in chips formulation to enhance functional properties of chips. Some physical (diameter, thickness, spread ratio and weight), chemical (total phenolic content and antioxidant activity), color and texture properties of chips samples were determined. While the chips obtained with addition of microwave dried peels were brighter, higher values of yellowness were determined with vacuum dried peels. The increased ratio of pomegranate peel powder caused a decrease in diameter and spread ratio values of chips. The utilization of 10% pomegranate peel powder decreased the hardness of the chips samples. The addition of pomegranate peel powder was increased in total phenolic content and antioxidant activity of chips. As a result, pomegranate peel powder can be used as a functional ingredient in snacks product formulation.

## 1. Introduction

Health consciousness and a healthy lifestyle increase consumers' demand for functional snacks to provide more nutritional sources in the daily diet. Today, many snack products such as chips, crackers, biscuits and cakes are offered for consumption. Many attempts and studies on the improvement of the functional and nutritional properties of snack products have been found in the food industry. For this purpose, there are studies carried out using Moldavian dragonhead leaves in corn snacks (Wójtowicz et al., 2017), tomato in functional snacks (Wójtowicz et al., 2018) and Dracocephalum moldavica L. seeds in snacks (Oniszczuk et al., 2021) in the literature.

Pomegranate peel, which is an important waste product, constitutes approximately 40% of the fruit. Pomegranate peel has a high dietary fiber content (72.68% / 100g) (Gullon et al. 2015), and the majority of dietary fiber (42.53% / 100g) is water-insoluble dietary fiber (Viuda-Martos et al., 2013). Compared to the pulp part of the pomegranate peel, the total phenolic content, total flavonoid, proanthocyanidin and ascorbic acid amounts are approximately 10-fold, 4-fold, 2-fold and 1.2-fold higher, respectively (Negi et al., 2003). Various phenolic substances taken into the body with the consumption of pomegranate peel can have a reducing effect on cardiovascular diseases, cancer varieties, hypoglycemic, apoptotic, anti-inflammatory, anti-parasitic (Sikora et al., 2008; Anderson et al., 2009; Abdel-Rahim et al., 2013). Pomegranate peel powder and extract are included in many product formulations such as bread (Altunkaya et al., 2013), biscuit (Ismail et al., 2014), sponge cake (Zhang et al., 2017) and bio-yogurt (Ibrahim et al., 2020).

Drying is one of the oldest methods of removing water that causes product deterioration due to microbial growth and chemical reactions. Convective hot air, microwave, infrared and vacuum are common in drying techniques. Drying type and condition have an important effect on the quality of end-products (Süfer et al., 2017).

Convective hot air drying is the most common method used for drying food. This drying method has high drying temperature, long drying time and negative effects on nutritional and sensorial properties. Vacuum and microwave drying has several advantages such as lower drying temperature, higher drying rate, uniform energy transmission on the material and suitable endproduct properties (Incedayi et al., 2016; Demiray et al., 2017).

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Consequently, by-products that are obtained during the processing of pomegranate cause environmental pollution and economic losses. Evaluation of nutritionally and functionally valuable these by-products are important in terms of both food enrichment and economic gains. The objective of this study was to investigate the effect of using pomegranate peel powder in the formulation of chips.

# 2. Materials and Methods

#### 2.1. Materials

The ingredients used in chips production, whole wheat flour, red pepper powder, garlic powder, cumin and salt were purchased from local markets. Pomegranate was purchased from the local market and washed and the peel was separated by manually removing the seeds. Peels were sliced with the size of 2 mm.

# 2.2. Drying methods

Fresh pomegranate peels were dried until a constant weight by various drying methods as follows. The peel was dried using three different methods; hot-air drying, microwave drying and the vacuum drying. i) convectional hot-air drying; fresh pomegranate peels were kept in the pre-heated hot air oven (model KD 200, Nüve, Turkey) at 60 °C for 12h; ii) a microwave drying was performed at 360 W for 25 min; (LG SolarDOM, MP-9485, Seul, South Korea) iii) vacuum drying was performed at 60 °C for 7 h, (JSR, JSVO-60T, Gongju, South Korea) respectively. The dried peels were ground with a grinder (Alveo, AHE.OG.01, Konya, Turkey) and sieved with a 500 µm sieve to obtain pomegranate peel powder. Pomegranate peel powders stored at room temperature (25 °C) in polyethylene bags until further analyses.

# 2.3. Chips production

The basic formulation of chips production was formed 100 g whole wheat flour, 2.5 g salt, 2 g garlic powder, 1 g red pepper powder, 1 g cumin, and water. Other samples were produced by replacing 0, 5 and 10 % flour with pomegranate peel powder dried with different methods. All ingredients were mixed with a Hobart mixer (Hobart UK, London). The water in the dough formulation was gradually added to the mixture and mixed. The obtained chips dough is wrapped in cling film. The hydration was provided by wrapping it and keeping it in the dark for 30 minutes. The obtained chips dough was divided into 2.5 g pieces and the dough pieces were compressed in between two Teflon plates

#### 2.6. Statistical analysis

All measurements were performed in duplicate for each sample. The results were expressed as mean  $\pm$ standard deviation. Statistical analyses were performed using the Statistical software JMP 5.0.1 (SAS Institute). The averages of the main variation sources were compared at p < .05 level. for 2.5 min at 300 °C. Chips samples were stored at room temperature until used for further analysis.

# 2.4. Physical analysis

Color analysis of chips samples was carried out using Hunter Lab Chroma Meter (Minolta CR-400, Osaka, Japan). Color values of the chips were recorded as L\* indicted the lightness, which varies from 0 to 100 (black to white), a\* varies from negative to positive (green to red), and b\* from negative to positive (blue to yellow). a\* and b\* values were used to calculate the saturation index (SI) ( $[a^2 + b^2]^{1/2}$ ) and hue angle (arctan [b\*/a\*]). Firstly, the instrument was calibrated with a white reference tile. Then, the color measurement values were determined three times and at five different points for each sample

The diameter and thickness of the chips samples were measured with a digital micrometer (0.001mm, Mitutoyo, Minoto-Ku, Tokyo, Japan). Diameter and thickness measurement values were determined at five different points for each sample. The spread ratio was found using the following equation:

# Spread ratio = Diameter / Thickness

The hardness and fracturability values of the chips samples were measured using a texture analyzer (TA-XT plus, Stable Microsystems, England). The test conditions in this study were as follows: Pretest speed: 1.0 mm/s, test speed: 3.0 mm/s, posttest speed: 10 mm/s, distance: 5 mm.

#### 2.5. Chemical analysis

Extracts of chips samples for total phenolic content (TPC) and antioxidant activity analyses were carried out by agitating 2 g sample with 10 ml solvent (methanol:HCl:water, 8:1:1, v/v/v) in a shaking water bath at room temperature ( $25 \,^{\circ}$ C) and by centrifugation at 3.000 rpm for 10 min. TPC was determined using Folin-Ciocalteu method. TPC content was measured with a Biochrom spectrophotometer (Biochrom, Libra S22, England) at 760 nm. Results were expressed as milligrams of gallic acid equivalents (GAE) per 100 g of dry weight (Gao et al., 2002; Beta et al., 2005).

Antioxidant activity was carried out using the DPPH (2-2-Diphenyl-2-picrylhydrazyl) method (Gyamfi et al., 1999; Beta et al., 2005). The DPPH scavenging capacity was determined as spectrophotometric (Biochrom, Libra S22, England) by measuring the decrease in absorbance at 517 nm. Antioxidant activity value as inhibition percentage was calculated according to equation:

Inhibition%=[(Abscontrol-Abssample)/Abscontrol]× 100

# 3. Results and Discussion

Product color plays an important role in food selection by influencing taste thresholds, sweetness perception, acceptability, food preference, and pleasantness (Clydesdale,1993). Color values of chips samples are given in Table 1. According to pomegranate peel drying methods, the highest L\* values, brightness of chips samples were obtained with microwave, followed by vacuum and convection dried samples (p<0.05). Pomegranate peel drying methods found no significant (p>0.05) on chips a\* values. Similar to the brightness, the drying method caused a significant change in b\* value of the chips samples. Among pomegranate peel drying methods, only vacuum drying had a significanly higher yellowness values of the chips, but convection and microwave drying methods were determined as similar according to b\* values.

When the results were compared in terms of substitute ratio, high usage rates significantly caused a significant decrease from 61.16 to 55.54 in L\* values, from 8.49 to 5.97 in a\* values and from 24.62 to 18.83 in b\* values. This decrease in L\* and b\* values can be explained by decomposition of chlorophyll and other pigments and the increase in enzymatic and non-enzymatic

#### Table 1

Color properties of chips samples.

browning reactions during baking with high utilization ratio of pomegranate peel powder in the formulation (Maskan, 2001; Bölek, 2020). A similar result was also reported a decrease in L\* and b\* values of cookies with the incorporation of pomegranate peel (Ismail et al., 2014). According to the pomegranate peel drying methods, the highest saturation index values were determined in chips samples containing vacuum drying powder. The saturation index values of the chips samples in the present study are given in Table 1, saturation index value (23.72) and of chips produced by vacuum dried pomegranate peel was significantly higher than that of convection dried pomegranate peel (22.73) and microwave dried pomegranate peel (22.31). It can be seen that the higher the substitute ratio of pomegranate peel powder, the greater the decrease (p < 0.05) in saturation index (from 26.04 to 19.76). Differences in hue angle values were found no significant in terms of pomegranate peel drying methods and substitute ratio (p>0.05).

Factor		$L^*$	$a^*$	$b^*$	Saturation Index	Hue Angle
Pomegranate peel methods	drying					
Vacuum		$58.06 \pm 2.55^{b}$	$7.54{\pm}0.89^{a}$	22.48±2.16 <sup>a</sup>	23.72±2.30ª	$71.47{\pm}1.09^{a}$
Convection		55.62±4.74°	7.22±1.31ª	21.55±3.03b	22.73±3.27 <sup>b</sup>	71.56±1.06 <sup>a</sup>
Microwave		$60.68{\pm}0.49^{a}$	6.82±1.41ª	21.23±2.78b	22.31±3.05 <sup>b</sup>	72.31±1.60 <sup>a</sup>
Substitute ratio (%)						
0		$61.16{\pm}0.04^{a}$	$8.49{\pm}0.19^{a}$	24.62±0.25 <sup>a</sup>	$26.04{\pm}0.18^{a}$	$70.98{\pm}0.58^{a}$
5		$57.66 \pm 2.40^{b}$	$7.12 \pm 0.76^{b}$	$21.81 \pm 1.08^{b}$	22.95±1.21 <sup>b</sup>	71.95±1.33ª
10		55.54±4.41°	5.97±0.61°	18.83±1.11°	19.76±1.17°	72.41±1.41ª

Means followed by the same letter within a column are not significantly different from each other (p > 0.05).

The effect of the pomegranate peel drying methods and substitute ratio on the diameter, thickness and spread ratio values of chips samples are shown in Table 2. In terms of the pomegranate peel drying methods, the diameter, thickness and spread ratio values of chip samples dried vacuum, convection and microwave were changed between 42.35 mm and 43.10 mm for diameter; 1.28 mm and 1.45 mm for thickness; and 30.03 and 33.85 for spread ratio values, respectively. However, these value differences were found to be insignificant (p>0.05). When diameter and thickness values were evaluated in terms of substitute ratio, it was observed that as pomegranate peel powder substitute ratio increased, a significant decrease in the diameter values (p<0.05) and a slight increase in the thickness values of chips samples were determined (Table 2). The chips samples without pomegranate peel powder had a higher spread ratio than chips samples containing 5% and 10% pomegranate peel powder. These results are consistent with Ranjitha et al. (2018) reported that a decrease in the diameter in the cookies samples containing a different ratio of pomegranate peels and defatted soybean flour combination (Ranjitha et al., 2018) and pomegranate peel (Jandal and Naji, 2021).

Table	2
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Diameter, thickness and spread ratio properties of chips samples.

Factor	Diameter (mm)	Thickness (mm)	Spread ratio
Pomegranate peel drying			
methods			
Vacuum	$42.35 \pm 2.79^{a}$	$1.28{\pm}0.19^{a}$	33.85±6.92ª
Convection	42.95±2.11 <sup>a</sup>	$1.45{\pm}0.15^{a}$	30.03±4.71ª
Microwave	$43.10{\pm}2.37^{a}$	$1.35{\pm}0.15^{a}$	32.29±4.19ª
Substitute ratio (%)			
0	$45.50{\pm}0.00^{a}$	1.23±0.14 <sup>b</sup>	37.26±4.00ª
5	$42.68 \pm 0.87^{b}$	$1.35{\pm}0.10^{ab}$	31.78±2.65 <sup>b</sup>
10	$40.22 \pm 0.84^{\circ}$	$1.50{\pm}0.17^{a}$	27.12±3.42 <sup>b</sup>

Means followed by the same letter within a column are not significantly different from each other (p>0.05).

Weight, hardness and fractuability properties of chips samples are presented in Table 3. Drying methods used in pomegranate peel and substitute ratio demonstrated no significant effect on weight values of chips samples. When the results are examined in terms of pomegranate peel drying methods, the hardness values of chip samples were shown a difference between 1019.77 g and 1202.57 g. Moreover, according to the substitute ratio, the lowest hardness value was found in the chips samples containing pomegranate peel powder at 10% ratio, while the hardness value of chips samples without pomegranate peel powder and containing 5% pomegranate peel powder were found a similar (p>0.05). The hardness value associated with the gluten

network structure refers to the force necessary to break the end-product. The hardness value associated with the gluten network structure refers to the force necessary to break the end-product (Urgancı and Işık, 2021). The decrease in the gluten network leads to be weaker and fragile structure of the product (Silva et al., 2013). Therefore, the decreased gluten structure with the high substitute ratio of pomegranate peel powder in the chips formulation decreased the hardness values in this study. In the chips, samples containing vacuum dried pomegranate peel powder was lower fractuability values than that of others. As seen in Table 3, the substitution ratio had no significant effect on the fractuability values of the chips samples.

Table 3

Weight, hardness and	fractuability properties	of chips samples.
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Factor	Weight (g)	Hardness (g)	Fractuability (mm)
Pomegranate peel drying methods			
Vacuum	$1.20{\pm}0.09^{a}$	$1019.77 \pm 250.54^{b}$	31.76±0.26 <sup>b</sup>
Convection	1.20±0.11ª	1119.59±129.15 <sup>ab</sup>	31.96±0.13ª
Microwave	$1.18{\pm}0.10^{a}$	1202.57±72.75 <sup>a</sup>	32.01±0.04ª
Substitute ratio (%)			
0	$1.30{\pm}0.00^{a}$	1245.96±33.78 <sup>a</sup>	31.90±0.18 <sup>a</sup>
5	$1.18{\pm}0.08^{a}$	1143.76±115.78ª	31.86±0.28ª
10	$1.10{\pm}0.00^{a}$	952.21±192.18 <sup>b</sup>	31.98±0.10 <sup>a</sup>

Means followed by the same letter within a column are not significantly different from each other (p > 0.05).

Total phenolic content and antioxidant activity values of chips samples are given in Table 4. When the results were compared in terms of pomegranate peel drying methods; it was seen that the TPC values of the chips samples incorporation with different drying pomegranate peel powder was found significantly similar (p>0.05). TPC values of chips samples with vacuum, convection and microwave drying pomegranate peel powder were determined 36.98 mg GAE g<sup>-1</sup>, 35.67 mg GAE  $g^{-1}$  and 36.66 mg GAE  $g^{-1}$ , respectively (p>0.05). As shown in Table 4, an increase in the substitution ratio of pomegranate powder used in the chips formulation was significantly increased in TPC values (p < 0.05). The use of the highest substitution ratio provided a 1.33-fold increase in TPC values in chips samples. Salem et al. (2020) reported that pomegranate peel powder increased Table 4

the TPC of biscuit samples in parallel with an increased substitution ratio.

The antioxidant activity values of chips samples containing vacuum, convection and microwave drying pomegranate peel powder were found as 80.59%, 79.47% and 80.88%, respectively, but this difference was found to be insignificant (p>0.05). When the antioxidant activity content is evaluated in terms of the pomegranate peel powder substitute ratio, antioxidant activity values increased from 71.91% to 86.55 with an increasing ratio (from 0 to 10%). A similar trend was recorded by Urgancı and Işık (2021) reporting a significant increase in both TPC and antioxidant activity values of biscuits fortified with pomegranate peel powder.

Total phenolic content and antioxidant activity properties of chips samples.

Factor	TPC	Antioxidant activity
1 actor	(mg GAE g <sup>-1</sup> )	(%)
Pomegranate peel drying methods		
Vacuum	36.98±13.33ª	80.59±6.85ª
Convection	35.67±12.52ª	$79.47 \pm 6.77^{a}$
Microwave	36.66±12.90ª	$80.88 \pm 7.22^{a}$
Substitute ratio (%)		
0	20.06±0.91°	71.91±7.22°
5	42.41±1.60 <sup>b</sup>	82.47±2.33 <sup>b</sup>
10	46.85±1.93ª	86.55±1.63ª

Means followed by the same letter within a column are not significantly different from each other (p> 0.05). Chemical analysis results are given on dry matter. TPC: Total Phenolic Content.

#### 4. Conclusion

This research was supported by the TUBITAK PublicFoundationsResearch-Development ProjectsSupportingProgramme (1007) project, number 106G053. The authors would like to acknowledge the financial support of TUBITAK and to thank the Director of the Project, Prof. Dr. İsmail ÇAKMAK.

In this study, the effect of pomegranate peel dried with three different methods on chemical, physical, color and texture properties of chips samples was studied. In terms of the pomegranate peel drying methods, a protective effect was shown microwave drying on the L\* value and vacuum drying on the b\* value. The increase of pomegranate peel powder content showed a decreasing effect on L\*, a\* and b\* values. Pomegranate peel powder caused a decrease in diameter and spread ratio, and an increase in thickness values. Hardness values of chips samples decreased as the high used ratio of pomegranate peel powder in the formulation increases. Although the drying method did not have a significant effect on the TPC and antioxidant activity values, the high substitute ratio increased the TPC and antioxidant activity values. In conclusion, a new functional chips production was developed by pomegranate peel powder instead of whole wheat flour.

#### 5. Acknowledgements

Author Mine ASLAN is a 100/2000 The Council of Higher Education PhD Scholar.

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Selcuk Journal of Agriculture and Food Sciences

http://sjafs.selcuk.edu.tr/sjafs/index
Research Article

# SJAFS

(2022) 36 (1), 63-74 e-ISSN: 2458-8377 DOI:10.15316/SJAFS.2022.010

# **Exploring the Rural Poverty Prevalence and Eradication Strategies for Rural Development: The case of Kenya**

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# ARTICLE INFO

ABSTRACT

Article history: Received date: 14.01.2022 Accepted date: 15.03.2022

Keywords: Agriculture Community Participation Poverty Eradication Rural Rural Poverty The World Bank estimates that about 689 million people live on less than \$1.90 a day globally. Sub-Saharan Africa and South Asia collectively account for 85% of this number. In Kenya, 36.1% of the total population live below poverty line, 40.1% in rural and 29.4% in urban areas. This study seeks to determine the contributing factors to rural poverty in Kenya, identify the eradication strategies, and reveal the gaps in the strategies. The study relies on secondary sources of data, including government reports, research articles, theses, international organizations' reports etc. It applies correlation and regression methods of data analysis to test the hypotheses. The study established that the lack of, and inaccessibility of water and food are aggravating factors of rural poverty, while poverty levels do not drop with an increase in the household land size. It also revealed that increasing the income levels of individuals in rural areas reduces poverty. Finally, the study identifies inadequate community participation, political interference, embezzlement of funds, underfunding, resistance to devolution, less transparency and accountability, and duplication of roles as gaps in the strategies. The study proposes sealing the gaps to strengthen the strategies and inform future policies formulation efforts for successful poverty eradication.

# 1. Introduction

Poverty reduction is a priority nationally, regionally, and globally. However, the efforts have been impeded due to the use of conflicting definitions and measurement methods of poverty by different countries. Some have adopted a multidimensional approach to poverty measurement that addresses wider aspects of wellbeing including access to health, housing, and other social aspects aspects (Haveman & Wolff, 2004; Kan et al. 2018). On the other hand, economists tend to define poverty based on hardship, which reflects how much resources a family or an individual can access or their economic well-being and position. Studies have shown that poverty is mainly a rural phenomenon. The World Bank (2020) reported that 80% of the world poor live in rural areas despite rural population accounting for just 48% of the world population. The proportion grows to 83.5% when multidimensional aspects of deprivation are considered (Suttie, 2019). This disparity between urban and rural areas could be attributed to 'urban bias', which has resulted in the insufficient allocation of development resources to agriculture and rural economy (Bezemer & Headey, 2008). Therefore, the probability of suffering poverty and other deprivation in the rural areas where

the global poverty rate of 17.2% is three times that in urban areas (5.3%) (Suttie, 2019).

The World Bank (2021) estimated that 9.2% of the world population (689 million people) lived on less than \$1.90 a day by 2017. The numbers rise to 24.1% and 43.6% when the poverty line is \$3.20 and \$5.50 a day, respectively. Sub-Saharan Africa and South Asia regions collectively account for 85% of the total world poor, with more than a half of poor living in just the five most populous countries i.e., India, Nigeria, Democratic Republic of Congo, Ethiopia, and Bangladesh (Roy & Divyanshi, 2019). In South Asia the numbers declined by half between 1993 and 2015, while Sub-Saharan Africa saw an increase from 276 million in 1990 to 413 million in 2015 (De-La-O-Campos, Villani, Davis, & Takagi, 2018). Many Sub-Saharan Countries have since prioritized agriculture-oriented programs to eradicate poverty, with the sector being the main economic life for most of the rural population, employing about 80% of the active population and contributing between 30% and 50% to their Gross Domestic Product (GDP) (Akouegnonhou & Demirbaş, 2021). Kenya emphasizes the provision of basic social services, economic growth, and the creation of employment in its development goals (Radeny, van den Berg, & Schipper, 2012). The focus

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on economic growth has yielded significant results in Kenyan. The economy grew at an average of 5.3% between 2005 and 2016, a figure higher than the average of 4.9% observed for Sub-Saharan Africa in the same period (World Bank, 2018). However, the entrenchment of poverty in rural parts of Kenya proves that the growth has been skewed towards urban areas; a pointer to 'urban bias'. The Kenya Integrated Household Budget Survey (KIBHS) report of the year 2005/06 found that 47% of the Kenya population lived in poverty, with a majority (85%) in the rural areas (World Bank, 2009). A decade later, the number had dropped to 36.1%. Nevertheless, the rural poor were still more than the urban poor at 40.1% and 29.4% respectively (KNBS, 2018). The global Multidimensional Poverty Index (MPI) method developed Alkire-Foster provides higher figures. According to Oxford Poverty & Human Development Initiative, OPHI (2021), the MPI, covers 100 developing countries and measures acute multidimensional poverty by going beyond the traditional method of poverty measurement that relies on monetary value. It captures an individual's deprivation in health, education, and living standards. The paper therefore seeks to determine the contributing factors to rural poverty in Kenya, identify the eradication strategies, and reveal the gaps in the strategies. Addressing the gaps in the strategies would be important in strengthening the existing policies and inform future policy formulations for successful poverty eradication.

#### 2. Measurement of Poverty

Development progress in the world increasingly is measured based on the number of people living in extreme poverty. High numbers are indicative of low development progress. Countries have therefore adopted different strategies to alleviate poverty, which continues to be a pressing issue globally. Extreme poverty eradication has been listed as the first goal of the United Nations in its Agenda 2030 programs. This would be realised by ensuring access to basic services, promoting equal rights to economic resources, ownership of lands, and natural resources (UN, 2015). These efforts are not new though. As Ferreira et al. (2016) indicate, the global efforts have been implemented through a) the Millennium Development Goals (MDGs), which intended to reduce by half the number of people living in extreme poverty between 1990 and 2015, b) the World bank, that in 2013 set a goal of ending poverty by 2030 and c) the goal to eradicate extreme poverty in all its forms by 2030 through the Sustainable Development Goals (SDGs).

The meaning and measurement of poverty continue to evolve with time to encompass all life dimensions as opposed to the traditional view that focused on economic capability. Economists for instance based their definition on income levels and would determine the poor according to the total headcounts of those below a globally defined poverty line/income level or consumption levels. This is the income poverty which is based on the international poverty lines of US\$1.90, US\$3.20,

and US\$5.50 per day, using the 2011 purchasing power parity prices (Sumner, Hoy, & Ortiz-Juarez, 2020). In its definition of the poor, the European Union goes beyond the income boundary to include any person, family, or a group of people with limited access to resources i.e., cultural, material, and social resources, and therefore not able to afford the minimum acceptable way of life in member states (Gordon, 2006). To delink poverty from just income measurement, the United Nations in 1995 included lack of access to basic services like education, food water, and health, inadequate housing, social exclusion and discrimination, high morbidity and mortality, and the unsafe environment as important poverty enabling factors (UN, 1996). Additionally, a more comprehensive and inclusive measurement of poverty known as global MPI has been used in 100 least developed countries and complements the monetary measurement by including other deprivations like education, health and living standards (Alkire & Foster, 2011). Anyone deprived of at least one-third of the weighted MPI indicators could therefore be classified as multidimensionally poor (OPHI, 2018).

# 3. Comparison of Rural and Urban Areas Poverty in Kenya

Kenya has two levels of government: the national and the 47 county governments. The constitution of Kenya provides that the two levels are distinct and interdependent, and would, therefore, work based on consultation and cooperation (GOK, 2010). Most of these counties are predominantly rural based on the classification of the Urban Areas and Cities Act, which uses population threshold to designate urban areas and cities. The Act defines the minimum population thresholds for a city, a municipality, a town, and a market centre as 250,000, 50,000, 10,000, and 2,000 respectively (ROK, 2019). Therefore, most Kenyan regions that do not meet the threshold have been categorized as urban. The 2019 Kenya Population and Housing Census Report established that majority of the Kenyan population i.e., 32,732,596 (68.82%) lived in rural areas while 14,831,700 (31.18%) lived in urban areas (KNBS, 2019b). Similarly, poverty is more widespread in the Kenyan rural than urban areas. The World Bank (2016) revealed that 90% of those in the bottom 40% of the income distribution in Kenya, live in rural areas. The rural areas also have high levels of illiteracy, child mortality and poverty rates, poor access to basic services like sanitation and electricity, and have approximately 8.3 million people who depend on farming as the main source of livelihood (Kai & David, 2020). However, with increased urbanization which results in urban sprawl, the agricultural areas are quickly transformed into other urban uses, particularly the residential land-uses, which might affect the overall agricultural yield in the urban areas (Caner & Engindeniz, 2021). Insufficient agricultural capacity in these areas could lead to high poverty

rates. Kenya classifies the poor into food poor, extremely poor and overall poor for effective analysis and understanding of the poverty situation.

First, the government uses the extreme poverty measure to determine poverty rates in the country. The World Bank, which is the main source of global information on extreme poverty revised the 'International Poverty Line' to \$1.90 in 2015. Individuals living on less than 1.90 international dollars a day are classified as extremely poor (Cruz, Foster, Quillin, & Schellekens, 2015; Roser & Ortiz-Ospina, 2013). The line is calculated based on the monetary value of an individual's consumption. Likewise, the national poverty line in Kenya is determined based on an individual's or household's consumption. There are different lines for rural and urban areas due to differences in the prices of goods which affect the cost of living. The monthly national extreme poverty lines for urban and rural areas in Kenya have been set at Kenyan shilling (KSh) 1,954 (equivalent to US\$ 0.6 per day) and KSh 2,551(US\$ 0.8 per day) respectively. If the monthly adult equivalent total consumption expenditure per individual is less than the stipulated amount, an individual or household is considered to live in extreme poverty. The 2015/16 KIHBS determined 8.6%, (nationally), 11.2% (rural areas), 6% (periurban) and 3.4% in core-urban live in extreme poverty (KNBS, 2018). The global MPI shows that there are 13.3% (nationally), 4.3% (urban) and 18.0% (rural) who are categorized as 'severe poor' (OPHI, 2020). Both measurements indicate that more people in the rural areas are living in extreme poverty.

Food consumption is the second measurement of poverty in Kenya. The Food and Agriculture Organization (FAO) estimates that over 820 million people are undernourished due to lack of food, (FAO, IFAD, UNICEF, WFP, & WHO, 2019). The United Nations aims to eradicate poverty and achieve food security by ensuring zero hunger. This could be realized by addressing key areas of food security i.e., availability, access, stability, and utilization as identified in the World Food Summit of 1996 (Caraher & Coveney, 2016). The FAO recommended per capita food consumption per person per day is 2800 kcal, subject to regional variations (FAO, 2020). The 2015/16 KIHBS report revealed that 32% of Kenyans (35.8% in rural areas, 29.4% in periurban and 23.8% in urban areas) are unable to consume the minimum daily calorific requirement of 2,250 Kcal determined by the government (KNBS, 2018). The food poverty line in Kenya is thus the average expenditure (excluding the non-food expenditures) needed to attain the daily intake of 2,250 kilocalories, calculated as KSh 1,954 (US\$ 0.6 per day) and KSh 2,551(US\$ 0.8 per day) for rural and urban areas, respectively (KNBS, 2018). Again, it has been determined that more people in rural areas than urban areas live in food poverty.

Finally, 'absolute' or 'overall' poverty is a measurement used to determine the proportion of the population that cannot meet the minimum overall basic needs for the consumption needs. The number of people living in 'overall poverty' may vary in a country depending on

the measurement used. Gentilini and Sumner (2012) explain that the national poverty lines (NPL) set by respective governments may yield different results from international poverty line (IPL) due to difference in methodologies and technical reasons. For instance, the overall poverty rate in Kenya computed based on World Bank revised 2011 PPPs of \$1.90 per person per day was 37.08% in 2015 (Atamanov, Lakner, Mahler, Tetteh Baah, & Yang, 2020). Developing countries prefer to use the food-energy-intake (FEI) and the cost-of-basic needs (CBN) methods when calculating their absolute NPL (Ravallion, 2010). This may give a different result from the IPL. The CBN method specifies a consumption bundle that is thought to be adequate for basic consumption needs and calculates its cost. The cost varies depending on the targeted sub-groups like rural areas, urban areas, age, and gender. Individuals and households whose monthly adult equivalent total consumption expenditure per person is less than the determined poverty lines are said to be 'living in overall poverty'. The overall poverty line for the rural and urban areas have been set at KSh 3,252 (equivalent to US\$ 1.0 per day) and KSh 5,995 (US\$ 1.8 per day) respectively, therefore, 36.1% of Kenyan lived in overall poverty in 2015 (KNBS, 2018). The MPI gives a higher figure of 38.7% (OPHI, 2020). Table 1 shows the overall poverty in urban and rural areas, as well as the national dimension in Kenya using three different measurements comparatively.

#### Table 1

Overall poverty in Kenya

-								
	National Pov-	Global	International					
Scale	erty Line	MPI	Poverty Line					
	(KNBS)	(OPHI)	(IPL)					
National	36.1%	38.7%	36.8%					
Urban	29.4%	41.8%	-					
Rural	40.1%	48.3%	-					
Courses (VND	C 2018) (ODIII 20	Sources (KNDS 2018) (ODLI 2020) and (Atomorphy et al. 2020)						

Source: (KNBS, 2018), (OPHI, 2020) and (Atamanov et al., 2020)

The three measurements indicate that there are more people living in poverty in the rural areas of Kenya than the urban areas. Therefore, the probability of suffering from poverty or deprivation in Kenya increases when one lives in rural areas.

## 4. Materials and Methods

This study primarily relied on secondary data including government reports, international organizations' research, academic articles, theses, conference papers, among others. The bulk of quantitative data used in the study analysis were derived from the different volumes of Kenya Population and Housing Census Report of 2019, the Kenya Integrated Household Budget Survey reports, the 2018 Basic Report on Well-being in Kenya, and the 2019 Gross County Product report. The reports have data on employment, income levels, economic activities, housing, education, health and so forth, which are crucial to understanding the wellbeing of the population and cover the 47 counties. The 2019 Kenya Population and Housing Census Nairobi and Mombasa

Counties classifies Nairobi as 100% urban, while majority of the remaining 45 counties are predominantly rural (KNBS, 2019c). As a result, data from Mombasa and Nairobi Counties were excluded from this study whose focus is rural poverty. The study sample size was 45, representing the 45 counties with rural population. These data in the reports are in government data sources such as official reports, and were collected through primary data collection methods like survey, interviews,

Table 2

focus group discussions, administration of questionnaires etc. To understand the government strategies to combat poverty in the rural areas, the study examined government policies, legislations, and research articles. These data were mainly qualitative that were analysed using the content and interpretive analysis methods. Seven hypotheses shown in table 2 were formulated to examine the existence, the strength, and the direction of the relationship between overall poverty and the contributing factors.

Study hypotheses					
Variables	Hypotheses				
Rural food poverty	H <sub>1</sub> : Access to food in the rural areas could cause a significant drop in overall rural poverty.				
Rural population	H <sub>2</sub> : A decline in rural population results in a fall in the number of people living below the overall poverty line in rural areas.				
Rural agricultural land ownership	H <sub>3</sub> : Rural households with bigger agricultural lands experience less overall rural poverty.				
Natural water	H <sub>4</sub> : A reduction in the number of people in rural areas relying on natural water sources signi- fies a drop in overall rural poverty in rural areas.				
Rambling water	H <sub>5</sub> : The higher number of rural populations using rambling water, the higher overall rural poverty.				
Commercial water	H <sub>6</sub> : The higher use of commercial water sources, the lower overall rural poverty.				
Rural income	H <sub>7</sub> : The higher income levels among the rural population causes a decrease of overall rural poverty.				

#### Source: Authors

The regression method of statistical data analysis was relied upon to test the hypotheses and determine the existence of a relationship between the independent variables and overall rural poverty. Based on the outcome, the hypotheses were either rejected or accepted. The choice of multiple regression analysis technique for this study was due to its ability to simultaneously predict and explain the direct effects of multiple independent variables (not a single explanatory variable) on an outcome variable while accounting for the direct effects of each explanatory variables included in the model (Morrissey & Ruxton, 2018).

Both quantitative and qualitative methods of data analysis were employed in the study. The study focused on two broad areas; the prevalence of rural poverty in Kenya, and the eradication strategies employed by the government. First, the study examined eight variables to understand the rural poverty prevalence. They include a) overall rural poverty, b) rural food poverty, c) rural population, d) rural household agricultural land ownership, e) the use of natural sources of water, f) the use of rambling water sources, g) the use commercial water sources, and h) rural income. Parametric tests methods i.e., correlation and regression analysis were employed to test the hypotheses. Correlation analysis was used to examine the direction and the strength of the relationship between the variables. On the other hand, regression analysis helped in predicting the response of rural poverty (dependent variable) from the seven contributing factors (predictors). Second, to understand the efficiency, and identify the gaps in rural poverty eradication strategies, a qualitative data analysis method was applied. This involved the identification, interpretation, and content analysis of the strategies adopted by the government to realise rural development and achieve a balanced regional growth.

#### 5. Evaluation and Discussion

The forty-seven devolved units and the national government are distinct but interdependent, and have specific roles assigned to them in the constitution (GOK, 2010). Nairobi and Mombasa Counties are 100% urban, while majority of the remaining counties are predominantly rural (KNBS, 2019c). The findings of regression and correlation analysis, and the qualitative analysis of strategies are deliberated in this section.

#### 5.1. Correlation Analysis

The study sought to understand the strength of the relationship between the rural poverty variables for the determination of their statistical significance to the study and their importance in aggravating or eradicating rural poverty. The relationships were classified as strong, moderate, and low at 10%, 5% and 1% significance levels. The correlation table also helped in checking the absence of multicollinearity between the independent variables and how strong the predictables correlated with the outcome variable. The findings are summarized in Table 3.

To determine the absence of multicollinearity between independent variables, a correlation value more than would confirm the existence of multicollinearity, rendering such variables redundant. In the study, all the independent variables' correlation values were less than 0.7, confirming that none of the predictors are multicollinear and therefore valid to be used for the purpose of multiple regression. Additionally, the corre-lation table was relied upon to test if the independent variables showed at least some correlation with the outcome variable. The correlation values greater than 0.3 or more signify a correlation. All the predictors in the study met this assumption except agricultural land (-0.2), which was subsequently eliminated in the multi-ple regression analysis as shown in table 4.

#### Table 3

# Correlation analysis findings

Pearson Correlation	Overall rural	l Rural food poverty	Rural popula-	Agricultural		Rambling wa-		Rural in-
	poverty	FJ	tion	land	ter	ter	water	come
Overall rural poverty (%)	1.000.	.922.000**						
Rural food poverty (%)	.922.000**	1.000.						
Rural Population (%)	.392.004**	.465.001**	1.000.					
Agricultural land (ha)	.201.092*	.080.301	053.366	1.000.				
Natural water (%)	.396.004**	.430.002**	.570.000**	177.122	1.000.			
Rambling water (%)	.411.003**	.265.039**	303.022**	.246.051*	167.137	1.000.		
Commercial water (%)	280.031**	307.020**	626.000**	.164.141	395.004**	.398.003**	1.000.	
Rural income (KSh)	635.000**	543.000**	264.040**	221.072**	343.011**	328.014**	.133.191	1.000.
N	45			Dependent Variable: Overall Rural Poverty				

\*, \*\*, \*\*\* indicate that correlation is significant at the 0.10, 0.05 and 0.01 level, respectively.

Source: Authors

# 5.1.1. Strong Relationships

At p 0.1, 0.05, and 0.01 levels of significance, the following variables were statistically significant and related. Strong positive correlation between the overall rural poverty and rural food poverty, strong positive correlation between the use of natural sources of water and rural population, strong negative correlation between the use of commercial water and rural population, strong negative correlation between income and rural poverty, and strong negative correlation between income and rural food poverty.

# 5.1.2. Moderate Relationships

The following variables statistically significant and related at p 0.1, 0.05, and 0.01 levels of significance: Moderate positive correlation between the rural population and rural food poverty, moderate positive correlation between the use of natural water and rural poverty, moderate positive correlation between the use of natural sources of water and rural food poverty, moderate positive correlation between the use of rambling water and rural poverty, moderate negative correlation between the use of rambling water and rural population, moderate positive correlation between the use of commercial water and the use of rambling water, moderate negative correlation between rural income and the use of natural water, and moderate negative correlation between rural income and the use rambling water.

#### 5.1.3. Low Relationships

At p 0.1, 0.05, and 0.01 levels of significance, the following variables were determined as statistically significant and related: Low positive correlation between the size of rural household agricultural land ownership and rural population, low positive correlation between the use of rambling water and rural food poverty, low positive correlation between the use of rambling water and the rural household agricultural land, low negative correlation between the use of commercial water and rural poverty, low negative correlation between the use of commercial water and rural poverty, low negative correlation between the use of commercial sources of water and rural food poverty, and low negative correlation between rural income and size of agricultural land owned by a rural household.

#### 5.2. Regression Analysis

The study also examined the impact of the contributing factors to overall rural poverty (independent variables) on overall rural poverty (dependent variable). The hypotheses stated in table 2 were tested in the regression analysis. The findings in table 4 show that both the direction of impact and what amount of its

Dependent Variable: Overall Rural Poverty	Unstandardized Coefficients		Standardized Coeffi- cients	t	Sig.	Collinearity Statistics	
	В	Std. Error	Beta			Tolerance	VIF
Constant:	8.138	7.334		1.110	.274		
Rural food poverty	.896	.082	.754	10.904	.000***	.497	2.012
Rural population	029	.071	031	413	.682	.423	2.363
Natural water	.034	.043	.050	.775	.443	.573	1.744
Rambling water	.367	.119	.201	3.080	.004**	.559	1.789
Commercial water	267	.139	128	-1.914	.063*	.533	1.877
Rural Income	-7.382E-5	.000	113	-1.784	.083*	.597	1.674

\* *p* < 0.10, \*\**p* < 0.05, \*\*\**p* < 0.01

Regression analysis findings

Table 4

Source: Authors

First, the findings demonstrate the existence of a relationship between rural food deprivation and the overall poverty rates in rural areas. The B-value for the Rural Food Poverty is 0.896, indicating that an average rise by a single percentage of rural food poverty causes the overall rural poverty to rise by 0.896%, with other factors held constant. This implies that addressing the food access problem in the rural areas would reduce overall rural poverty. The action also complements the United Nation's goal of ending hunger in the world by achieving food security and improving nutrition by the year 2030 (UN, 2015). It is estimated that 35.8% of the Kenyan rural population cannot meet the average daily minimum calorific requirement of 2250 Kcal (KNBS, 2018). Rural food poverty is worsened by harsh climatic conditions affecting a large part of the country that is considered arid and semi-arid (ASAL). The ASAL regions are characterized by low and erratic rainfall which affects agricultural production in the absence of good technology for irrigation and water harvesting. Over 80% of Kenya's landmass is ASAL and home to a third of Kenya's population as well as 70% of livestock in the country (Fitzgibbon, 2012). However, agricultural production in high potential areas of Kenya is also impeded by a lack of assets among farmers, forcing them to cultivate small pieces of land inadequate to sustain living (GOK, 2009). Out of 6,354,211 farming households in Kenya, 5,637,450 households (88.7%) still engage in

subsistence farming, while just 506,687 households (8%) do commercial farming (KNBS, 2019a).

Land sub-division is also practice common in rural areas affecting agricultural land productivity. A study by the Catholic Church's Jesuit Hakimani Centre (JHC) found the cultural practice of sub diving lands among siblings to be a major threat to food security since small land are not agriculturally productive (Jamah & Oduor, 2014). The practice is widespread due to land inheritance practices, individualization of titles, agricultural land value, and high housing demand (Museleku, 2018). Subdivision also reduces the scales required for commercial production on the land by limiting the potential for mechanization, increased use of technology, and expanded infrastructure on the lands. Additionally, despite rural households owning huge lands, drought and less application of modern technology have limited farm utilization resulting in food insecurity. The ASAL regions for instance are home to 36% of Kenyans, and occupy about 89% of Kenyan land (GOK, 2019). The households in the ASAL areas have more lands available for agriculture than those in the non-ASAL areas. However, the use of land for agriculture in ASAL areas is hampered by drought. By contrast, more households in non-ASAL areas practise agriculture despite lower average land ownership. A comparison of land ownership and utilization in the ASAL and Non-ASAL regions is shown in tables 5. and 6

Table 5

Average household agricultural land ownership and utilization in ASAL counties.

ASAL County	Average household Agricultural Land (ha)	Proportion of farming households (%)
Garissa	1.09	34.292
Wajir	4.047	49.995
Mandera	4.419	50.534
Marsabit	1.128	51.073
Isiolo	2.204	45.608
Average per household (ha)	2.578	46.30

Source: Author's computation from (KNBS, 2019a)

Table 6

Average household agricultural land ownership and utilization in Non-ASAL Counties

Non-ASAL Counties	Average household agricultural land (ha)	Proportion of farming households (%)
Non-ASAL Counties		
Bungoma	0.624	78.399
Siaya	0.551	77.95
Kakamega	0.508	77.558
Kisii	0.537	71.554
Murang'a	0.442	72.882
Average total per household (ha)	0.533	75.669

Source: Author's computation from (KNBS, 2019a)

Secondly, the results prove the existence of a relationship between the use of boreholes and wells as their main sources of water and the overall rural poverty. The B-value for the use of rambling water is 0.367, meaning that for every rise in the use of boreholes and wells by a percentage, the overall rural poverty increases by 0.367% holding the other independent variables fixed. Most of the dug wells in the rural areas are unprotected and vulnerable to contamination. The WHO (2020) classifies unprotected dug wells, unprotected springs and surface waters as unimproved water sources that are easily contaminated. In the 45 Counties studied, 11.2% of the population were found to rely on boreholes/tube wells for drinking water (KNBS, 2019a). The boreholes in rural areas are mostly manually drilled. As Danert (2015) explains, manually drilled boreholes have been widely used in the marginalized rural areas of Kenya for irrigation and domestic purposes since the 1970s but raises questions about their safety and water quality considering a lack of understanding of hydrology among the manual drillers. In Kakamega County for instance, it was established that the concentration of mercury, lead, and arsenic in most boreholes was more than World Health Organization recommended levels, and therefore, would pose serious health problems to the residents if used for domestic purposes (Christine, Kibet, Kiprop, & Were, 2018). Martínez-Santos, Martín-Loeches, Díaz-Alcaide, and Danert (2020) point out that the main advantage of manually dug boreholes is their affordability. This explains why among the rural poor, the use of manually dug boreholes is very high as they cannot afford alternative improved sources.

Thirdly, there is evidence linking the use of commercial water and the overall rural poverty levels. The Bvalue for the use of commercial water is -0.267, implying that for every percentage rise in the number of rural populations using commercial water, there is a drop in overall rural poverty by 0.267% holding other independent variables constant. The assumption is that a household that can afford commercial water is not poor. The high poverty levels in rural areas have therefore made the use of commercial water to be significantly low. A study conducted among 450 rural households in Guangxi province, provides a clear relationship between the use of purchased water and wellbeing. In the study, Alasdair et al. (2017) establish that the purchase of bottled waters was common among high-income households with younger, literate, and male heads. In Kenya,

Table 7 The hypothesis test result

consumption of commercial water is more common in urban than rural areas due to higher poverty rates in rural areas. Commercially water is consumed more in schools, restaurants, markets, on streets, mass gatherings, places of work, spotting, and wedding activities among others (Williams et al., 2015).

Finally, it is established that rural income plays a significant role in determining overall rural poverty. The Bvalue for the income is -7.382E-5, suggesting that for every additional earning (KSh) on average income, the rates of rural poverty would reduce by 7.382E-5% holding other independent variables constant. The main source of income in rural areas is agriculture. However, factors like land-subdivision, climatic shocks, declining soil qualities, constantly fluctuating markets for agricultural products, plant diseases and pests' infestations, and low public and private investment in agriculture have affected the performance of the sector (Njeru, 2018). This has affected the sources of income leading to high poverty rates in rural areas.

Overall, access to food, rural income, and access to water have a direct bearing on the well-being of a rural population. Actions aimed at improving their ease of accessibility will undoubtedly lead to a drop in overall rural poverty levels. Effective utilization of the land for agricultural is also important to raise household income, and food availability hence lowering rural poverty levels. This require that impeding factors like drought, land-subdivision, plant diseases, among others are addressed. Table 7 shows a summary of the accepted and rejected hypotheses from the study.

Variables	Hypotheses	Accepted/Rejected hypothe- sis	B-coefficients
Food poverty	H <sub>1</sub> : Access to food in the rural areas could cause a significant drop in overall rural poverty in the rural areas.	Accepted (p< 0.01)	0.896
Rural population Pro- portion	$H_2$ : A decline in rural population result into a dec- reasing level of number of people living below the overall poverty line in rural areas.	Statistically insignificant	
Agricultural land ownership	H <sub>3</sub> : Households with bigger lands available for ag- riculture experiences less poverty in the rural areas.	Statistically insignificant (a correlation value of 0.2 is too weak)	
Use of natural water	H4: A reduction of number of people in the rural areas relying on natural water sources signifies a drop of overall rural poverty in the rural areas.	Statistically insignificant	
Use of rambling water	H <sub>5</sub> : A higher number of rural populations using rambling water means that the overall poverty rates are high.	Accepted (p< 0.05)	0.367
Use of commercial water	H <sub>6</sub> : The use of commercial water sources in rural areas represents a significant decline of rural poverty.	Accepted (p< 0.10)	-0.267
Rural income	H <sub>7</sub> : Higher income levels among the rural popula- tion leads to a decrease of overall rural poverty.	Accepted (p< 0.10)	-7.382E-5

# 5.3. Poverty Eradication Strategies through Decentralization

Decentralization is a governance concept with different dimensions but a common understanding that central entities play lesser roles in the decision-making process by letting entities at lower levels have much bigger roles. It involves "the transfer of power and resources away from central government" and not the other way round (Schneider, 2003). At independence, the founders of the Republic of Kenya declared the fight against poverty, ignorance, and diseases as the main pillar of governance that needed immediate and long-term attention (Nyamboga, Nyamweya, Sisia, & George, 2014). However, the efforts to achieve development were affected by the strongly centralized and vertically integrated regional development planning regime inherited from the colonial government, which encouraged skewed development in the country (Laji, 2019; Rutten, 1990). To achieve balanced growth and rural development, the various governments have attempted to devolve political, administrative, and fiscal aspects through several decentralization strategies like Sessional Paper No. 10 of 1965, Special Rural Development Program, District Focus for Rural Development, Regional Development Authorities, Fiscal Decentralization, and Policy on Devolution. The success and gaps of the strategies are discussed in this section.

#### 5.3.1. African Socialism and Its Applications to Planning in Kenya.

Formulation of Sessional Paper No. 10 of 1965 marked the initial step by the independent government of Kenya to address inequality of development. The policy ensured decentralization of planning functions from the national levels to provinces, districts, and municipalities, and that development implemented by local administrative units was based on local inputs (GOK, 1965). The policy envisaged public investments in high potential areas with an abundance of natural resources, rainfall, transport networks, and fertile lands to yield faster returns for economic growth in the country. However, it can be said that priority projects implementation couldn't be done equitably but favoured some regions over the others resulting in a huge regional development discrepancy (Stiftung, 2012). Public investment in areas that had the highest absorptive capacity meant that the areas that had been ignored during the colonial period got neglected further due to low their low potential, while resources were concentrated in other regions (CRA, 2012). According to Oyugi, J., and Kaara (2018), the policy objective of ensuring that development could trickle down from high to the low potential areas failed due to lack of a framework to redistribute revenues and benefits accrued from the highly productive areas to the areas with low potential. This encouraged unbalanced regional growth even further.

#### 5.3.2. Special Rural Development Programme

The Special Rural Development Programme (SRDP) policy was initiated by the government in 1971 to address plights of the unemployed youth, and poor and

landless farmers in rural areas. The SRDP was born out in regards of concerns that the previous agriculture and rural development policies were inadequate, and new strategies that would create more jobs in rural areas and raise income from agriculture were needed (Ergas, 1982). The pilot projects were tried in six rural administrative divisions and a special fund (S.R.D.P. Funds) that included external funding from donors was established. Project implementation was done by a local committee, assisted by a team of foreign advisors. The policy's goal was to create employment, generate income in the rural areas, develop skills and techniques necessary for Kenya, and to have a domino effect in other rural parts of the country (Cohen & Hook, 1987). Poor coordination between line ministries, lack of technical and administrative capacity at the district level, and inadequate involvement of community contributed to the failure of the policy (Kirori, 2003). The SRDP was finally phased out in 1975.

#### 5.3.3. The District Focus for Rural Development.

The 'District Focus Policy for Rural Development' was formulated in 1983 policy as a response to failure by the SRDP and previous development policies to resolve most of the rural development problems. It distributed government powers, functions, and authorities two levels down from the presidency and provincial headquarters to the officials at the district level who were closer to the people. Rutten (1990) explains that the policy adopted both a 'top-down sectoral approach' at the central government and the 'integrated, horizontal bottom-up approach' at the district level, whereby the ministries at the central government retained the responsibilities of overall policy formulation and planning of both national and multi-district programs, while projects were implemented at the district level. It bestowed the authority to identify and implement district-based projects at the district level instead of the provincial and national levels (Opata, 2004). The aim was to hasten decision making and encourage citizens' participation. Lack of transparency and accountability by government officials, inadequate consultations with the locals, little budget allocation, and political interference were identified as main barriers to the policy success (Omiti, Owino, Otieno, & Odundo, 2002).

#### 5.3.4. Regional Development Authorities

The government's intention to achieve balanced spatial development within and between regions of Kenya has seen it embrace a regional-based development model. In 1974, the government delineated six regions based on rivers and large water bodies in the country as the basis for national development (UN-Habitat, 2016). This would help achieve equitable national development by sustainably using the basin-based resources to create employment and ensure equitable resource distribution to achieve rural-urban balance (GOK, 2020). The policy faces various challenges including lack of a framework for effective community participation, wastage of resources due to duplication of functions, and inadequate funding (KHRC & SPAN, 2010).

#### 5.3.5. Fiscal Decentralization

Inadequate funding and the hesitance by the central government to loosen bureaucratic capture are some of the reasons for the failure of efforts to attain development, resource distribution and, equity in the country, especially in rural areas (Bagaka, 2008). While functions were decentralized, the funds did not follow. According to Mitullah (2012), the parliament created Constituency Development Fund (CDF) and Local Authorities Transfer Fund (LATF) in 2003 and 1999 respectively to reinforce decentralization and overcome regional imbalances. The CDF was allocated 2.5% of the national revenue, while LATF had 5%. Unlike CDF that directed funds directly to constituencies that are political units meant for electoral purposes, LATF was meant for local authorities, which were administrative units. The new constitution 2010 abolished the local authorities, and instead created the 47 counties that are responsible for development in their areas of jurisdiction. The counties are entitled to annual financial allocation from the national government for development. CDF on the other hand is still active but faces challenges like political interference in the management, embezzlement of funds, inadequate citizen participation, lack of oversight on the projects and fund utilization, and inadequate funding (Wanyande & Wanyande, 2016).

#### 5.3.6. Devolution

Kenya constitution has created 47 sub-national governments with a degree of functional autonomy for the purpose of governance and development. Devolution aims to achieve development through decentralization of state organs, functions, and services away from the central government, and enabling communities to manage their affairs by involving them in decision making (GOK, 2010). The county governments are allocated at least 15% of the national revenue to execute the functions assigned to them by the constitution (D'Arcy & Cornell, 2016). Significant developments have been realized in rural areas because of devolution. In the Northern Counties that have traditionally been marginalized, the level of infrastructural development laid down since the onset of devolution is estimated to have surpassed accomplishments achieved in more than fifty years (Kanyinga, 2016). However, its implementation has encountered challenges including the unwillingness of employees in some sectors to work in the devolved units (Kobia & Bagaka, 2014), embezzlement of funds (D'Arcy & Cornell, 2016), conflict within and between county governments for control of county resources (Lind, 2018), delay of financial disbursement and weak public participation (Kimathi, 2017).

#### 6. Conclusion

The study finds that the strategies aimed at realizing rural development and bring about balanced growth in the country have had some degree of success despite challenges. Widely regarded as a failure, the S.R.D.P. played an important role in raising the level of awareness about the issues affecting the poor farmers in

Kenya. On the other hand, the District Focus for Rural Development strategy promoted local communities' participation in development processes, streamlined development projects to the local needs and ensured effective use of local resources. The establishment of the Regional Development Authorities has also accelerated rural development and safeguarded equity in resource distribution and utilization. Through fiscal decentralization, the local communities have been granted a wider role in determining projects that reflects their needs and promoted accountability in projects implementation. Devolution of funds to the counties has allowed for more participation of citizens at the local levels in development prioritization, implementation as well as bridging regional development gaps in the country. The study in the case for Kenya ALSO focused on two areas: the contributing factors of poverty in the rural areas, and the rural poverty eradication strategies adopted by the government

Firstly, the study has established that access to food, rural household land size, access to water, and rural income are important rural poverty variables. Whereas the inaccessibility of water and food have been identified as aggravating factors of rural poverty, the study reveals that the huge landownership by a rural household does not lead to a drop in overall rural poverty. Both land availability and utilization are important elements. Despite large land ownership, agricultural practices are hampered by harsh climate, old agricultural technologies, and land -subdivisions, that have undermined effective land utilization. Contrarily, a rise in rural income resulted in a decline in overall rural poverty. Hence, investing in agriculture would increase access to food and income, thus lowering overall rural poverty.

Secondly, the study has identified the gaps in rural poverty eradication strategies that have impeded the rural development and balanced growth in rural. While the decentralization has had some positive rural development outcomes and reduced regional development discrepancies, the cross-cutting challenges have led to the collapse of strategies or underachievement.

Key negative determinants in the strategies included inadequate community participation, political interference, embezzlement of funds, underfunding of projects, bureaucratic capture, lack of transparency and accountability, duplication of roles, and poor coordination among the ministries and development bodies. Additionally, a weak implementation framework for redistribution of benefits has been identified as a main factor that led to the failure of the sessional paper no 10 of 1965. On the other hand, it is considered that fixing the gaps in the strategies will strengthen decentralization and boost the efforts directed at achieving rural development and a balanced regional growth in the country.

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Selcuk Journal of Agriculture and Food Sciences SJAFS

http://sjafs.selcuk.edu.tr/sjafs/index

**Research Article** 

(202?) 36 (1), 75-81 e-ISSN: 2458-8377 DOI:10.15316/SJAFS.2022.011

# **Role of Biostimulant Priming Applications on Germination, Growth and Chlorophyll Content of Sunflower** (*Helianthus annuus* L.) Cultivars under Salinity Stress

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#### **ARTICLE INFO**

#### ABSTRACT

Article history: Received date: 14.02.2022 Accepted date: 26.03.3022	Salinity, which is one of the abiotic stresses, has become an important obstacle in agricultural areas. The use of humic acids (HA) as a biostimulant is increasing day by day and it is tried to increase the resistance of plants against stress. In this study, the effects of HA application of 0-15ml $L^{-1}$ (4 concentrations) on the re-
Keywords: Abiotic stress Biostimulant Humic acid Seedling growth Sunflower	sistance to salt (S) 0-150 mM L <sup>-1</sup> (4 concentrations) stress in 3 sunflower cultivars (Maximus (C1), Sirena (C2), Reyna (C3) were investigated under laboratory conditions. In the study; germination percentage (GP), mean germination time (MGT), salt tolerance percentage (STP), seeding length (SL), root length (RL), relative water content (RWC), real water content (GSI), total chlorophyll (Chl), chlorophyll stability index (CSI) parameters were examined. As a result of the study, HA applications played a role in reducing the negative effects of salt stress on the examined parameters. It was concluded that HA can be evaluated as an effective material that can be used to increase resistance and tolerance of plants against salt stress.

#### 1. Introduction

Plants are exposed to biotic and abiotic stresses throughout their lives. The most important of these are cold, drought, salinity, flooding and heavy metals (Gontia-Mishra et al. 2014). Today, salinity has become a major problem in agricultural areas all over the world (Gürsoy 2020; 2022). Salt stress is considered one of the most widespread abiotic stresses and very important hampers crop production, especially in arid and semi-arid areas (Hernández 2019). Salinity stress limitations plant growth and development, can induce drastic yield reduction (Alharby et al. 2021; Shahzad et al. 2021). Growth and development in plants begins with germination and plants need to adapt to environmental conditions in order to survive. However, germination is the most sensitive period in the life of plants and is especially important for seedling development.

The priming of seeds allows the acceleration of germination as well as a better growth a greater tolerance to abiotic stress and higher yields (Boucelha et al. 2019). Today, the use of biostimulants has become widespread in order to reduce the effects of stress factors, to ensure sustainability in agriculture and to ensure plant growth (Frioni et al. 2021). With the use of biostimulants, the resistance of plants to abiotic stress was increased and the quality of agricultural production (Bell et al. 2022). Du Jardin (2015), identified seven categories of biostimulants: (i)humic and fulvic acids, (ii)protein hydrolysates and other N-containing compounds, (iii)seaweed extracts and botanicals, (iv)chitosan and other biopolymers, (v) )inorganic compounds, (vi)beneficial fungi and (vii)beneficial bacteria. Humic acid is believed to have an important role in plant growth regulator as a biostimulant (Saidimoradi et al. 2019).

Sunflower (*Helianthus annuus* L.) has a very important place in the production of oil crops in the world and in our country (Beyaz et al. 2018).

The aim of this study is to determine the effects of humic acid and salt applications on germination, seedling growth, chlorophyll content, chlorophyll stability index and salt tolerance index of sunflower varieties.

#### 2.Material and Method

The research was carried out at the Aksaray University Guzelyurt Vocational School and some analyzes were done at Aksaray University Scientific and Technological Research Laboratory (ASÜBTAM). In this study sunflower cultivars (Maximus (C1), Sirena (C2), Reyna (C3) were used. Seeds of sunflower for sterilization, they were kept in 5% sodium hypochlorite solution for 10 minutes and then rinsed several times in distilled water then they were dried at room temperature to their initial weight. Seeds were primed for 24 hours and each

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HA [0 (control) (HA1), 5ml L<sup>-1</sup> (HA2), 10 ml L<sup>-1</sup> (HA3), 15 ml L<sup>-1</sup> (HA4)] doses. For each HA dose, 50 seeds were placed in sterile petri dishes on Whatman No:1 blotting papers and 10 ml of different doses of salt [0 (control) (S1), 50 mM L<sup>-1</sup> (S2), 100mM L<sup>-1</sup> (S3), 150mM L-1 (S4)] concentrations were added. Only water was added to the control petri dish. Filter papers were changed every 2 days and 10 ml of salt containing solutions were added. In order to prevent evaporation the petri dishes are wrapped with parafilm. The research randomized plots experimental design were made with 3 replication according to the trial pattern. Seeds were counted daily and those with a root length of 2mm were considered germinated (ISTA 2003). In the study; germination percentage (GP), mean germination time (MGT), salt tolerance percentage (STP), seedling length (SL), root length (RL), relative water content (RWC), real water content (GSI), total chlorophyll (Chl), chlorophyll stability index (CSI) parameters were examined.

#### Measurements

#### Germination percentage (%)

Germination percentage was calculated using the formula below.

#### *Mean germination time (day)*

MGT=  $\Sigma(Dn)/\Sigma n$ ,

where, n is the seed number germinated on day D, and D is the number of days from the beginning of the germination test (Orchard 1977).

Percentage of salt tolerance (%)

Salt tolerance (%) =  $(DWSP / DWCP) \times 100$ 

DWSP: Dry weight of plant in salt application

DWCP: Dry weight of plant in control application (Uzun Kayıs and Ceyhan 2015)

Determination of relative and real water contents

In order to determine the relative and real water content in the leaf tissues, leaf samples taken from plants belonging to the sunflower cultivars in the control and stress groups were weighed and their fresh weight was determined, then they were placed in glass tubes containing 5 ml of distilled water and kept in the light for 24 hours. At the end of this period, the hydrated leaf samples were weighed again and their weight in turgor condition was determined. Later, these leaf samples will be dried in the oven at 80°C for 48 hours and their dry weight will be determined again. Finally, the relative and actual water contents will be found according to the formulas below (Ritchie et al. 1990; Arslan 2018).

RWC(%) = (FW - DW)/(TW - DW) x 100 (Relative water content)

 $GSI(\%) = (FW - DW)/FW \times 100$  (Real water content)

FW: fresh weight, TW: turgor weight, DW: dry weight *Chlorophyll (mg g*<sup>-1</sup>)

Samples taken from seedlings (0.25 g) of sunflower cultivars grown in the laboratory with HA and S appli-

cation were homogenized with 80% acetone, then filtered and made up to 25 ml with acetone. Then the samples were read in the spectrophotometer at 663 and 645 nm and chlorophll was calculated with the following formula (Lichtenthaler and Welburn 1983).

Chlorophyll a (mg g<sup>-1</sup>) = (12.7\*663 nm)-(2.69\*645 nm)\*V/W\*10000

Chlorophyll b (mg g<sup>-1</sup>) = (22.91\*645 nm)-(4.68\*663 nm)\*V/W\*10000

Total Chlorophyll = Chlorophyll a + Chlorophyll b

Determination of chlorophyll stability index

Determination of the chlorophyll stability index is important in terms of showing the tolerance capacity of the plant against stress. (Mohan et al. 2000)

Leaf sample from the treated plant = 1.000 mg leaf sample was kept in a test tube with water at 55 °C for 1 hour.

Leaf sample from the plant in the control plot = 1.000 mg leaf sample was kept in a test tube containing water at room temperature for 1 hour.

The chlorophyll stability index will be calculated with the help of the following equation by reading both samples in a spectrophotometer at 652 nm.

 $CSI = Absorbance value of the treated sample \times 100$ 

Absorbance value of the control

Statistical analysis

The experimental data obtained at the end of the research, was subjected to analysis of variance using MSTAT-C computer software. Duncan test was applied to determine the significance levels of the differences between means of applications.

#### **3.Results and Discussion**

The variance analysis results of this study, which was conducted to determine the effects of biostimulant applications on the germination parameters, seedling growth, salt tolerance percentage, chlorophyll, chlorophyll stability index, relative and actual water content of sunflower varieties under salt stress, are given in Table 1. When Table 1 is examined, it is seen that the interaction of Cultivars × HA Doses × S Doses is significant at the 1% level in the others, except for the MGT feature. In the MGT parameter, the triple interaction was significant at the 5% level. The applied biostimulant doses had a significant effect on the germination parameters and seedling growth of the cultivars under salt stress. However cultivars, HA doses and Salt doses are important at the 1% level, except for the GP feature. On the other hand, it was determined that the bilateral interactions were statistically significant at the level of 1% in the GP feature, except for the interaction (HA Doses  $\times$  S Doses). In the triple (Cultivars  $\times$  HA Doses  $\times$  S Doses) interaction, significant results were determined at the level of 5% for MGT and 1% for all other properties.

Table 1									
Analysis of varia	nce on the in	vestigate	d parameters	in sunflo	wer cultivars	s humic acid	and salt tre	eatments	
V.C	DE	CD	MOT	CT.	DI	DIVC	COL	C1.1	

V.S.	D.F.	GP	MGT	SL	RL	RWC	GSI	Chl	CSI	STP
						F Value				
Cultivars	2	20.69**	27.93**	13.51**	331.04**	64.68**	40.75**	35.31**	225.03**	68.54**
HA Doses	3	2.01	20.65**	394.78**	79.50**	84.92**	26.08**	17.13**	295.92**	19.86**
Cultivars × HA Doses	6	9.57**	4.61**	51.82**	176.95**	61.88**	27.27**	7.73**	121.81**	17.11**
S Doses	3	272.79**	234.93**	550.57**	69.84**	470.47**	122.17**	156.18**	47.23**	137.62**
Cultivars × S Doses	6	12.42**	13.27**	8.06**	10.88**	5.42**	3.42**	6.84**	13.51**	5.27**
HA Doses × S Doses	9	1.71	2.78**	13.01**	12.04**	9.89**	4.89**	2.86**	49.78**	3.06**
Cultivars × HA Doses ×										
S doses	18	3.60**	1.99*	6.62**	13.70**	9.04**	2.40**	3.49**	8.43**	2.97**
Error	96	1.56	0.028	0.111	0.025	0.561	1.76	0.051	0.471	2.03
CV%		1.30	9.22	5.80	8.45	1.07	1.88	8.69	1.02	1.86

\*\*:significance level at *p*<0.01, \*:significance level at *p*<0.05. VS: Variation source, DF: Degrees of freedom, GP: Germination Percentage, MGT: Mean Germination Time, SL: Shoot Length, RL: Root Length, RWC: Relative Water Content, GSI: Real Water Content, ChI: Total Chlorophyll, CSI: Chlorophyll Stability Index, STP: Salt Tolerance Percentage

#### Table 2

Average values the effect of HA at different concentrations applied to sunflower cultivars under salt stress on GP (%)

						G	P (%)						Mean
$C \times HA$		Maxii	nus			Sirena			Reyn	a			
S Doses	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	
	99.67	100.0	100.0	99.67	99.67		97.33		99.33	99.67		100.0	99.61
S1	а	а	а	а	а	100.0 a	a-e	100.0 a	ab	а	100.0 a	а	Α
	96.33	98.00	98.67	97.00	97.67	97.67	98.00	99.00	98.00	98.00	97.33	98.00	97.81
S2	b-f	a-d	abc	a-f	a-d	a-d	a-d	ab	a-d	a-d	a-e	a-d	В
	95.00	96.33	97.00	96.33	95.00	97.67	97.67	97.33	97.33	94.33	95.00	94.00	96.08
<b>S</b> 3	d-g	b-f	a-f	b-f	d-g	a-d	a-d	a-e	a-e	e-h	d-g	fgh	С
	89.00	92.67	91.67	89.00	91.67	95.67 c-	95.00	95.67	93.00	87.00	91.67	87.00	91.58
S4	ıj	gh	hı	ıj	hı	g	d-g	c-g	gh	j	hı	j	D
Mean	95.00	96.75	96.83	95.50	96.00	97.75	97.00	98.00	96.92	94.75	96.00	94.75*	
	С	AB	AB	BC	BC	А	AB	Α	AB	С	BC	С	
LSD%1	2.680												

\* Dissimilar letters in the column show different groups

When the Duncan test results of the examined traits are examined, it is seen that the lowest germination in terms of GP trait (Table 2) was obtained with 87.0% from S4 salt dose C3 variety and HA2 and HA4 humic acid doses. However, the highest germination was obtained in all 3 cultivars in S1 application and in all other applications (HA2, HA3, HA4) except the control dose (H1) of HA. It was observed that HA application increased the germination rate at all S doses. Gürsoy et al. (2016) applied 4 doses of HA in 3 different growth periods in their study with the winter rapeseed variety Bristol. As a result of the study, they reported that HA applications had a positive effect on plant growth. Kahraman (2017) reported that HA caused an increase in many parameters examined as a result of his study in the form of a 2 year field trial with HA application in cowpea cultivars. Sofi et al. (2018) applied HA and S to alfalfa seeds. As a result of the study, they reported that HA application under salt stress increased seed germination.

Table 3

Average values the effect of HA at different concentrations applied to sunflower cultivars under salt stress on MGT (day)

						MG	T (day)						Mean
$\mathbf{C} \times \mathbf{H}\mathbf{A}$		Maxi	mus			Sirena			]	Reyna			
S Doses	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	
	1.33	1.28	1.22	1.20	1.60	1.50	1.52	1.29	1.44	1.75	1.75	1.73	1.46
S1	1-m	klm	1m	m	e-k	f-m	e-m	klm	g-m	d-g	d-g	d-g	С
	1.55	1.52	1.43	1.43	1.63	1.55	1.60	1.38	1.84	1.64	1.43	1.30	1.53
S2	e-k	e-m	g-m	g-m	e-j	e-k	e-k	h-m	de	e-1	g-m	j-m	С
	1.83	1.66	1.54	1.50	2.19	2.26	2.30	1.75	2.04	1.85	1.63	1.57	1.84
S3	def	e-h	e-l	g-m	bc	bc	bc	d-g	cd	de	e-j	e-k	В
		2.49		2.19	2.43		2.97	2.17	2.36	2.37	1.99	2.02	2.40
S4	2.43 b	b	2.42 b	bc	b	2.93 a	а	bc	b	b	cd	cd	Α
Mean	1.78	1.74	1.65	1.58	1.96	2.06	2.09	1.65	1.92	1.90	1.70	1.66*	
	CDE	DEF	EF	F	ABC	AB	А	EF	A-D	BCD	EF	EF	
LSD%1	0.2712												

\* Dissimilar letters in the column show different groups

When Table 3, which includes the averages of the MGT parameter, is examined, the highest average germination time was determined as 2.97 days at the S4 dose. The lowest MGT was obtained from S1 HA4 application as 1.20 days. It was determined that MGT decreased as the doses of HA applications increased, however, the HA4 dose was effective in decreasing MGT. Ebrahimi and Miri (2016) applied 3 HA doses (0, 15 and 30 g L<sup>-1</sup>) in their study in which they investigated the effects of HA on the germination properties of *Borago officinalis* and *Cichorium intybus* plants. As a result of the study, the results showed that application of 30 g l<sup>-1</sup> humic acid was effective in germination of the plant species and stimulated the plants germination. Gürsoy and Kolsarıcı (2017) determined that the application had a positive effect on yield and yield elements as a result of their study in which they applied HA to the summer rapeseed plant in a leonardite environment. Bulut (2020) reported that humic acid can be applied as an organic supplement against salt stress in his study in which he applied HA to reduce the effect of salt stress on corn seeds.

A 1 (1 CC / CTTA / 1°CC		CI 1.1	
Average values the effect of HA at differ	ent concentrations applied to	o sunflower cultivars i	inder salt stress on SL (cm)

							SL (cm)						Me	an
$\mathbf{C}  imes \mathbf{H} \mathbf{A}$		Maxii	mus			Sirena	1		R	eyna				
S Doses	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4		
	6.23	6.80	7.57	8.69	5.25	6.20	6.95	9.00	5.16	6.85	7.44		7.08	
S1	g-j	e-h	b-e	а	l-o	g-k	d-g	а	1-o	e-h	cde	8.85 a	Α	
	5.15	5.95	6.33	7.67	4.88	5.61	5.88	8.55	4.60	7.14	6.55	7.77		
S2	1-o	1-l	g-j	bcd	m-p	j-m	1-1	а	n-q	c-f	f-1	bc	6.34	В
	4.55	5.00	5.17	6.59	3.92	4.60	4.84	7.43	4.00		5.25	6.10		
S3	o-r	mno	l-o	f-1	qrs	n-q	m-p	cde	qrs	8.27 ab	1-o	h-k	5.48	С
	3.37	3.63	4.10	3.46	2.93	3.89	3.90	6.25	2.94	5.42	3.77	4.97		
S4	st	st	p-s	st	t	qrs	qrs	g-j	t	k-n	rs	mno	4.05	D
Mean	4.83	5.35	5.79	6.60	4.25	5.07	5.39	7.81	4.18		5.75	6.92*		
	Е	D	С	В	F	DE	D	Α	F	6.92 B	С	В		
LSD%1	0 7149													

\* Dissimilar letters in the column show different groups

It was observed that salt stress shortened the seedling length, whereas humic acid doses increased the seedling length in all cultivars. Depending on the salt and humic acid doses, the longest seedling length was obtained in Sirena variety (Table 4). Gürsoy et al. (2016) reported that HA caused the lengthening of the plant in their study where they applied HA to the winter rapeseed plant. Berekati et al. (2019) reported that HA significantly increased plant height in their study in the form of foliar application of HA in rapeseed plants.

Table 5

Table 4

Average values the effect of HA at different concentrations applied to sunflower cultivars under salt stress on RL (cm)

					RL (cm)								
$C \times HA$		Maxii	nus			Sirena			Reyna				
S Doses	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	
	1.20	1.56	1.38	2.00	3.07	3.00	1.58		2.29	2.27	2.34	2.43	2.19
S1	p-s	1-p	n-r	g-j	bc	bcd	l-p	3.22 b	fg	fgh	efg	ef	А
	1.01	1.66	1.37	2.00	2.67	2.88	1.40	1.45	1.90	1.88	1.99	1.64	1.82
S2	rst	j-o	n-r	g-j	de	bcd	n-r	m-q	h-l	h-l	g-k	j-o	В
	0.91	1.20	1.38	2.19	2.81	3.08	1.39	1.26	1.85	1.66	1.80	1.59	1.76
S3	st	p-s	n-r	f-1	cd	bc	n-r	O-S	1-1	j-o	1-m	l-p	BC
	0.71	1.14	1.39	2.09	2.44		1.30	1.12	1.64	1.70	1.60	1.59	1.71
S4	t	qrs	n-r	f-1	ef	3.80 a	n-s	qrs	j-o	j-n	k-o	l-p	С
Mean	0.96	1.39	1.38	2.07	2.75	3.19	1.41	1.76	1.92	1.88	1.94	1.81*	
	F	E	E	С	В	Α	E	D	CD	D	CD	D	
LSD%1	0.3393												

\* Dissimilar letters in the column show different groups

Table 5 shows the effects of humic acid on sunflower cultivars under different salt stress on root lenght. Root length varied statistically, depending on humic acid doses, salt stress and cultivars (Table 1). Even though salt stress increased root length increased with HA applications. Therefore, even if the S dose is the highest, it

is clearly seen that the root length increases with the effect of HA. In general, it was determined that the root length increased with the effect of HA in other applications. Tunçtürk et al. (2020) applied humic acid to the broad bean plant under salt stress conditions. As a result of the study, they reported that they determined that humic acid had positive effects on root development and length.

Table 6

Average va	lues the effect of	of HA a	different	concentrations a	applied	to sunfl	lower cul	tivars und	ler sa	lt stress on	RWC	C(%)	
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						RW	/C (%)						Mean
$\mathbf{C} \times \mathbf{H}\mathbf{A}$		Maxim	18			Sirena			Reyna	l			
S Doses	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	
	71.31	72.20	72.67	74.63	71.90	73.03	72.90	74.30	71.93	71.63	72.49	70.50	72.46
S1	C-1	cde	bcd	а	cde	abc	bcd	ab	cde	c-g	cd	e-j	А
	69.60	71.00	71.40	72.67	69.83	72.13	72.00	72.00	69.43	70.44	71.87	70.37	71.06
S2	h-k	d-1	c-h	bcd	g-k	cde	cde	cde	ıjk	e-j	c-f	e-k	В
	67.43	70.42	69.10	71.90	66.33	68.90	70.00	71.07	67.60	69.53	71.22	63.27	68.90
<b>S</b> 3	lm	e-j	jkl	cde	mn	jkl	f-k	d-1	lm	h-k	C-1	0	С
	65.10	66.23	69.93	68.53	63.30	65.97	67.53	70.00	63.27	66.20	69.00	59.97	66.25
S4	n	mn	g-k	kl	0	mn	lm	f-k	0	mn	jkl	р	D
Mean	68.36	69.96	70.78	71.93	67.84	70.01	70.61	71.84	68.06	69.45	71.14	66.03*	
	E	CD	BC	Α	E	CD	BC	Α	E	D	AB	F	
LSD%1	1.607												

\* Dissimilar letters in the column show different groups

In the RWC feature (Table 6) the lowest RWC was determined as 59.97% in S4HA4. The highest RWC was

determined as 74.63% in S1HA4. On the other hand, when looking at the general average values, the highest

RWC was obtained from HA4. It is seen that RWC also increases with the increase of HA doses. Therefore, it was determined that HA application against salt stress increased RWC. Akladious and Mohamed (2018) applied calcium nitrate and humic acid to pepper plants grown under salt stress. As a result of the study, they reported that the applications caused an increase in Table 7 RWC. Compared to the control, they reported that the highest RWC was obtained from (Ca1+HA2) (Ca1 (control) + HA2 (humic acid 750mg kg<sup>-1</sup>) application. Karimian et al. (2019) S stress in their study on Salvia splendens plant as a greenhouse experiment. They applied HA application in the form of foliar spraying, and reported that RWC increased as a result of the study.

Average values the effect of HA at different concentrations applied to sunflower cultivars under salt stress on GSI (%)

a uu							GSI (%)						Mea
C × HA S Doses		Max	imus				Sirena			Reyr	าล		n
	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	
			73.3										
	72.12	72.70	0 a-	74.60	72.33	73.20	73.67	74.83	73.37	72.27	73.43	71.10	73.08
S1	a-f	a-e	e	а	a-e	a-e	abc	а	a-d	a-e	a-d	b-h	А
	70.73	72.13	72.0	73.83	70.33	73.63	72.67	73.00	70.30	71.07	72.57	68.63	71.75
S2	b-h	a-f	7 a-f	ab	b-1	abc	a-e	a-e	C-1	b-h	a-e	g-k	В
			70.9										
	68.77	72.10	3 b-	72.43	68.27	72.63	70.73	72.17	68.30	69.97	71.43	62.37	70.01
<b>S</b> 3	f-k	a-f	h	a-e	h-k	a-e	b-h	a-f	h-k	d-j	a-h	mn	С
			71.7										
	66.03	68.23	0 a-	70.00	64.07	66.90	68.60	71.77	64.17	67.17	69.80	60.47	67.41
S4	kl	h-k	g	d-j	lm	jkl	g-k	a-g	lm	1-l	e-j	n	D
Mean			72.0										
	69.41	71.29	0	72.72	68.75	71.59	71.42	72.94	69.03	70.12	71.81	65.64*	
	D	BC	AB	AB	D	ABC	ABC	А	D	CD	AB	E	
LSD%1	2.852												

\* Dissimilar letters in the column show different groups

When the GSI parameter is examined (Table 7), the lowest GSI was determined as 60.47% in the S4HA4 application, while the highest 74.83% was determined in the S1HA4 application. Although GSI decreased as salt stress increased, it increased slightly with HA applications. Arslan (2018) investigated the photosynthetic activities of C3 and C4 plants under water constraint conditions and reported that stress conditions cause a decrease in the actual water content of the plants. Similarly, in this study with salt stress, GSI decreased and increased slightly with HA applications.

Table 8

Average values the effect of HA at different concentrations applied to sunflower cultivars under salt stress on Chl (mg g<sup>-1</sup>)

						Chl	$(mg g^{-1})$						Mean
$C \times HA$		Ma	ximus				Sirena				Reyna		
S Doses	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	
	3.00	3.19	3.24	3.31	3.09	3.19	3.29	3.70	3.06	2.96	2.57	3.70	3.19
<b>S</b> 1	b-e	a-d	abc	ab	b-e	a-d	ab	а	b-e	b-f	e-k	а	А
	2.04	2.35	2.34	2.40	2.93	2.59	2.95	3.13	3.00	3.18	2.26	3.19	2.70
S2	jkl	g-l	g-l	f-l	b-f	e-j	b-f	b-e	b-e	a-d	g-l	a-d	В
	1.86	2.19	2.29	2.40	2.66	2.17	2.25	2.58	2.64	2.69	2.15	2.60	2.38
<b>S</b> 3	lm	h-l	g-l	f-l	d-1	h-l	g-l	e-k	d-1	c-h	h-l	e-j	С
	1.45	1.90	1.93	1.98	1.91	2.00	2.10	2.79	2.11	2.36	2.64	2.04	2.10
<b>S</b> 4	m	lm	lm	lm	lm	kl	1-l	b-g	h-l	g-l	d-1	jkl	D
Mean	2.09	2.41	2.45	2.52	2.65	2.49	2.65	3.05	2.70	2.80	2.40	2.88*	
	E	D	CD	CD	BCD	CD	BCD	А	BC	AB	D	AB	
LSD%1	0.4846	i .											

\* Dissimilar letters in the column show different groups

In terms of Total Chlorophyll (Table 8), the lowest Chl was determined as 1.45 mg g<sup>-1</sup> in S4HA1 and the highest in S1HA4 with 3.70 mg g<sup>-1</sup>. Although it was S1 in each application, it was determined that chlorophyll increased with HA application. It was determined that Chl increased with the increase of HA at other S doses. El-Ghamry et al. (2009) showed that application of humic acid and amino acids increased chlorophyll a and b.

Akladious and Mohamed (2018) applied calcium nitrate and humic acid to pepper plants grown under salt stress. As a result of the study, they reported that salt stress applications decreased Chl a and Chl b, but the applications caused an increase in photosynthetic pigments. They reported that the most effective application was obtained from (Ca1+HA2) (Ca1 (control) + HA2 (humic acid 750mg kg<sup>-1</sup>) application.

							CSI (%)						Mea
$C \times HA$		Maxir	nus			Sirena			Rey	na			
S Doses	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA4	
	68.70	69.03	70.97	70.10	66.42	64.40	69.47	72.97	68.83	64.37	66.33	63.43	67.92
S1	e-1	e-h	bcd	c-f	j-m	n-s	d-g	a	e-1	n-s	klm	qrs	А
	68.14	68.59	70.13	69.65	65.17	64.34	68.40	72.43	67.67	64.00	65.85	63.83	67.35
S2	ghı	e-1	cde	d-g	m-p	n-s	f-1	ab	h-k	O-S	lmn	p-s	В
	66.00	67.99	69.42	68.05	63.05	65.77	69.67	71.60	63.00	63.50	68.92	64.43	66.78
<b>S</b> 3	1mn	g-j	d-g	ghı	s	lmn	d-g	abc	s	p-s	e-1	n-s	С
	63.57	67.32	69.32	65.55	60.34	63.26	70.80	70.73	60.70	65.00	71.40	64.97	66.08
S4	p-s	1-l	d-h	mno	t	rs	cd	cd	t	m-q	abc	m-r	D
Mean	66.60	68.23	69.96	68.34	63.74	64.44	69.58	71.93	65.05	64.22	68.13	64.17*	
	D	С	В	С	F	EF	В	А	Е	F	С	F	
LSD%1	1.473												

Average values the effect of HA at different concentrations applied to sunflower cultivars under salt stress on CSI (%)

\* Dissimilar letters in the column show different groups

Table 9

When the averages are examined in terms of chlorophyll stability index (CSI) feature (Table 9), the lowest CSI was determined as 60.34% in S4HA1 application, and the highest in S1HA4 application as 72.97%. Although there were decreases in CSI with salt applications, an increase in CSI was determined as the doses of HA applications increased. Mohan et al. (2000) reported that chlorophyll stability index (CSI) is an indicator of the stress capacity of plants and that high CSI value indicates that stress has no effect on chlorophyll. At the same time, they reported that high CSI is an indicator that the plants resistance to stress, dry matter production and productivity will be high. In this study, the resistance of plants to salt stress increased with the increase of CSI with HA applications.

Table 10 Average values the effect of HA at different concentrations applied to sunflower cultivars under salt stress on STP (%)

						S	TP (%)						Mean
$C \times HA$		Max	imus				Sirena			F	Reyna		
S Doses	HA1	HA2	HA3	HA4	HA1	HA2	HA3	HA	HA1	HA2	HA3	HA4	
								4					
		80.8							80.7				
	79.02	3 a-	80.97	81.47	78.57	77.00	78.53	78.97	7 a-	79.30	81.50	78.17	79.59
S1	a-g	d	bc	ab	a-1	e-j	a-1	a-g	d	a-e	ab	a-1	А
		79.0											
	78.66	3 a-	78.87	79.30	76.90	75.07	76.83	77.87	81.6	77.67	79.93	76.30	78.17
S2	a-h	g	a-g	a-e	e-j	h-n	e-j	b-1	0 a	C-1	a-e	e-l	В
		76.3											
	77.03	3	77.67	75.33	72.87	72.30	75.57	73.30	79.5	73.13	76.97	72.03	75.18
<b>S</b> 3	e-j	e-l	C-1	g-m	l-o	mno	f-m	k-o	7 a-e	k-o	e-j	mno	С
		76.6							74.9				
	73.57	0	77.27	79.07	72.23	71.10	71.77	68.00	7	71.33	77.97	67.73	73.47
S4	j-o	e-k	d-1	a-f	mno	0	no	р	1-n	0	a-1	р	D
Mean		78.2											
	77.07	0	78.69	78.79	75.14	73.87	75.68	74.53	79.2	75.36	79.09	73.56*	
	BC	AB	А	А	DEF	EF	CD	DEF	2 A	DE	А	F	
LSD%1	3.055												

\* Dissimilar letters in the column show different groups

When the salt tolerance percentage (STP) parameter (Table 10) is analyzed, the lowest STP was determined in S4HA4 with 67.73%, and the highest was determined in S1HA3 application as 81.50%. Although STP decreased with salt applications, it was determined that S tolerance increased with HA applications, especially in HA3 and HA4. Uzun Kayis and Ceyhan (2015) reported that percentage of salt tolerance of lentil varieties ranged from 29.79% to 58.87% and this depending on salt levels, salt tolerance of varieties different from each other.

#### 4.Conclusion

In this study, the effects of different doses of HA applications on sunflower seeds under salt stress were determined. As a result of the study, it was determined that HA applications had positive effects on the germination parameters, early seedling growth, chlorophyll content, chlorophyll stability index, and salt tolerance percentage of sunflower seeds under stress conditions. It was determined that HA4 (15ml  $L^{-1}$ ) application gave better results against salt stress, and C2 (Sirena) from the cultivars used in the experiment gave the best results. Besides, applications should be made in other plants and under various stress conditions and their results should be evaluated.

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Selcuk Journal of Agriculture and Food Sciences

http://sjafs.selcuk.edu.tr/sjafs/index

#### SJAFS

(2022) 36 (1), 82-90 e-ISSN: 2458-8377 DOI:10.15316/SJAFS.2022.012

## Research Article

# Seasonal and Annual Changes of Some Climate Factors in Different Areas of Loose Dairy Cattle Barns

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#### ARTICLE INFO

#### ABSTRACT

Article history: Received date: 22.02.2022 Accepted date: 01.04.2022

#### **Keywords:**

Animal welfare Climatic stress Cold stress Critical temperatures Heat stress Dairy cattle barn This study was conducted to determine the changes of some climatic factors throughout the year in different barn areas in a loose system dairy cattle shelter. For this, digital temperature-humidity meters were placed on different shelter areas, and measurements were made for a year. By developing a different and new model as well as the maximum, minimum and average values in a certain time period in five different areas of the shelter, the temperature and humidity values were categorized into specific groups (stressful, slightly stressful, suitable, etc.) and it was determined how long the animals were exposed to what temperature and humidity values. According to the results, the animals were exposed to temperatures between 5-25 °C for approximately 80% of their time in the spring and autumn seasons, 40% in the winter season, and 50-55% in the summer season. At optimum temperatures (10-20 °C), the animals spent approximately 50% of their total time in spring and autumn, 20% in summer, and 15% in winter. Animals were exposed to heat stress (ti≥32 °C) for only 5-7% of their total time in summer and to cold stress (ti <-5 °C) for only 6-14% of the time in winter. Dairy cattle were found to spend 60% of their annual total time in the appropriate temperature range and approximately 33% of the annual time in the optimum temperature range. Animals were exposed to heat stress and cold stress for about 6-7% and 2-3%, respectively of their total time per year. Animals were exposed to the relative humidity in the range of 40-90%, for approximately 50-60% of their total time throughout the year. According to the results of the research, it was determined that open system shelters planned to protect animals from cold in winter and heat in summer, not create a significant climatic stress on animals.

#### 1. Introduction

The main purpose in planning dairy cattle shelters having a very important place in milk production, is to protect animals from poor environmental conditions or to create a suitable and comfortable habitat. To increase the yield per animal, to start with, stress factors in the living environment must be controlled. Sahin & Ugurlu (2017) reported the stress factors causing low productivity in dairy cattle were as structural, climatic and social. Climatic environmental conditions having an important effect on the metabolic and physiological activities of animals can turn into stress factors due to the tension on the organism. Environmental conditions include temperature, air speed, relative humidity, solar radiation and light (Sahin et al 2019). Undesirable temperature is an important factor causing low milk production by dairy cattle, particularly of high genetic value (Nardone et al 2010).

The air surrounding the animal has an important effect on the regulation of body temperature as it affects the heat dissipation and heat gain between the animal and its environment. For example, dairy cattle lose more heat from their bodies in the winter months and try to balance their body temperature by converting some of consumed feed to heat energy to maintain the heat balance in their bodies. However, they convert more nutrients into heat to maintain their body temperature at low temperatures, and this case is defined as cold stress. High yield losses may be observed depending on the food intake of the animal. In hot summers, sensible heat dissipation decreases in animals. The animal tries to dissipate latent heat to expel excess heat accumulated in its body. As a result of these events, the animal to enters the heat stress. During this period, the animal's feed consumption decreases, and consequently, there is notable

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yield loss. The optimum temperature range for adult dairy cattle's is 10-20 °C (Sainsbury & Sainsbury 1988; Webster 1994). Temperatures in the range -6 °C and 25 °C (Sainsbury & Sainsbury 1988), -5 °C to 25 °C (Knížková et al 2002), and -0.5 °C to 20 °C (Herbut & Angrecka 2012) exerts little effect on the performance of dairy cattle.

The range of suitable temperature is wider than the optimum temperature, and at the appropriate temperature below and above optimum temperatures, animals do not face any stress factors as they can easily achieve body heat balance by increasing heat production or heat dissipation. There is generally a negative and significant correlation between milk production and climatic factors and for maximum milk yield, the appropriate temperature range needs to be between 7 °C and 25 °C (Shinde & Taneja, 1986). The suitable temperature range for dairy cattle in lactation is 5-25 °C (Roenfeldt 1998). Temperatures in the range of 10-22 °C and relative humidity values between 50-90% were found to be suitable for animals and had no negative effects on animals (Vtoryi et al. 2018).

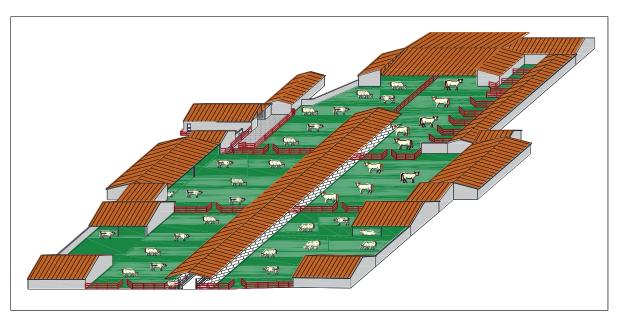
With the reduction in temperature difference between the animal and its environment, the temperature at, which sensible heat emission from the body decreases and becomes difficult can be defined as the maximum temperature. When the issue of excessive heat starts to occur in the living body, the animal enters the live heat stress, and its efficiency reduces as the degree of heat stress increases. According to Kadzere et al (2002), if the animals are unable to discharge excess heat accumulated in their bodies through latent heat dissipation, their body temperature increases, and uncontrolled situation, they may die from hyperthermia. The upper critical temperature for dairy cattle is 25-26 °C regardless of milk production or previous climate adaptation, and a slight decrease in feed intake and milk yield can be observed at these temperatures (Berman et al 1985; NRC 1989; Keown & Grant 1997; West 2003), while milk yield decreases at temperatures above 30 °C (NRC 1981; NRC 1989). Kume et al (1998) reported that 25 °C is a high temperature for lactating dairy cattle, and when the relative humidity is greater than 80%, it affects the temperature. Brouček et al (2009) observed that the critical maximum temperature for cows is 24-27 °C. When the environmental temperature rises above 26 °C, dairy cattle reach a point where they cannot cool themselves sufficiently for a long time, and heat stress begins (Kadzere et al 2002). If the temperature exceeds 27 °C, the optimum production range is exceeded (Brouček 1997; Novák et al 2000). When the environmental temperature rises to the upper critical temperature (27-28 °C), the feeding status and energy balance of the animals

deteriorate (Gaafar et al 2011). Heat stress causes a decrease in milk yield in dairy cattle. The yield decreases by 10% at a temperature of 27-32 °C and relative humidity of 50-90%, and decreases by more 25% at a temperature of 32-38 °C and relative humidity of 50-90% (OACC 2014). The level of heat stress strongly depends on daily fluctuations in average temperature, and if the temperature drops below 21 °C for 3-6 hours at night, the animal has sufficient opportunity to lose the excess heat gained from the previous day (Igono et al 1992; Muller & Botha 1994). The temperature at, which the lost heat from the body begins to increase, the animal performs intensive metabolic activities to maintain body heat balance, and at the minum temperature, the yield losses are inevitable due to cold stress. According to WMO (1989), the lowest critical temperature for dairy cattle is -15 °C, while according to FAO (2016), it is -10 °C unless there are very sudden temperature fluctuations.

In this study, seasonal temperature and humidity changes were analyzed in a free dairy shelter planned as an alternative design. Especially for the open system shelter, it has been tried to determine how the temperature and relative humidity change in different building areas and how much of their total time annual the animals spend in which temperature-humidity range. The climatic comfort performance of open-free system shelters was also researched.

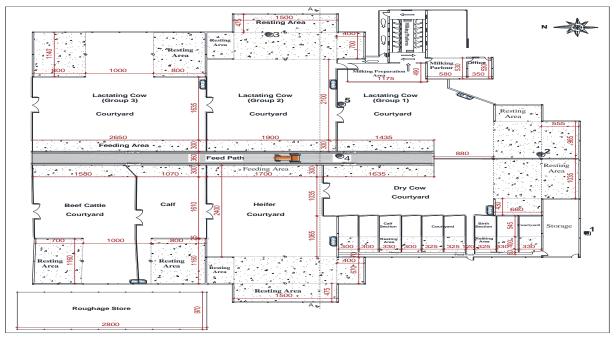
#### 2. Materials and Methods

This research was carried out in the commercial dairy cattle barn planned as a combination of microstructure systems in Konya, Turkey (Ugurlu & Uzal, 2010). The shelter has a total capacity of 140 heads, including 54 dairy cows, 15 dry period, 20 heifers, 15 calves and 14 beef cattle (Table 1). In the semi-open free shelter as an alternative design, from 1 September 2015 to 1 September 2016 to analyze the monthly, seasonal and annual temperature and humidity changes, in certain shelter sections (two in the resting areas, one in the courtyard, one in the feeding area and one in the outdoor area) electronic temperature-humidity measurement devices with hourly measuring were installed 2m above ground. Properties of the devices used for measurements; included 1. Temperature: measurement range was between -40 and 100 °C, with a resolutin of 0.03 °C and sensitivity of 0.33 °C. 2. Relative humidity: the measurement range was 0-100%, with a resolution of 0.4% and sensitivity of  $\pm$  3%. The perspective view and floor plan of the shelter where the research was conducted, and the locations of temperature-humidity meters are presented in Figure 1 and Figure 2.



#### Figure 1

Perspective view of the free system dairy cattle shelter



#### Figure 2

Floor plan of the shelter and layout of devices of loose housing system (•; Climatic measurement points inside the shelter, ■; Out of shelter climatic measurement point

In this study, apart from the maximum, minimum, average, etc. criteria where climatic data are classically evaluated, it has been determined how long the creature was exposed to that climate parameter in a certain time period (day, month, season, and year). Thus, an alternate point of view was developed to evaluate the climatic comfort of an animal and the effects of climate. For this purpose, a different and new model was developed after a detailed literature review to understand the effect of temperature and relative humidity, particularly on milk production and the comfort of animals. A total of 175 680 climate parameters (temperature, and relative humidity) were categorized in certain groups (Table 1 and

Table 2). Eight categories were created by making use of previous results in NRC (1981), Berman et al (1985), Shinde & Taneja (1986), Sainsbury & Sainsbury (1988), NRC (1989), WMO (1989), Igono et al (1992), Muller & Botha (1994), Webster (1994), Brouček (1997), Keown & Grant (1997), Kume et al (1998), Roenfeldt (1998), Novák et al (2000), Kadzere et al (2002), Knížková et al (2002), West (2003), Brouček et al (2009), Gaafar et al (2011), Herbut & Angrecka (2012), OACC (2014), FAO (2016), and Vtoryi et al (2018) considering the temperatures to which animals are exposed (Table 1). It was tried to determine how much of their one-year total times of dairy cattle spent at which temperature and humidity values. In addition, it were classified seven categories by making use of Yüksel (1984), Maton et al (1985), Olgun (1988), Ekmekyapar (1991), Okuroğlu & Yağanoğlu (1993), Ekmekyapar (2001), OACC (2014) ve Vtoryi et al (2018) vb. publications regarding the relative humidity values to which animals are exposed (Table 2).

#### Table 1

Temperature ranges to which animals are exposed and their effect on animals

Temperature ranges exposed by animals (°C)	Effect on animals
<-5 °C	Cold Stress
-5 °C - 0 °C	Mild Cold Stress
$0 \ ^{0}C - 5 \ ^{\circ}C$	Cold Tension
5 <sup>0</sup> C − 25 <sup>o</sup> C	Suitable Temperature
$10 {}^{0}\text{C} - 20 {}^{\circ}\text{C}$	Optimum Temperature
25 °C – 28 °C	Heat Tension
28 °C – 32 °C	Heat Stress
> 32 °C	Excessive Heat Stress

#### Table 2

Relative humidity ranges to which animals are exposed and their effect on animals

Relative humidity ranges exposed by animals (%)	Effect on animals
< %20	Extremely Dry
% 20-40	Dry
% 40-60	Low humidity
% 65-75	Optimum
% 60-80	Suitable
% 80-90	Humid
>%90	Excessively Humid

Monthly, seasonal, and annual temperatures and humidity changes were analyzed in the free system structure developed as an alternative design. Especially for the open system shelter, the effects of temperatures in different building areas on animal welfare and the formation and results of climate criteria on the open system were evaluated.

#### 3. Results and Discussion

The dairy cattle spent 80% of their total time in the autumn season at 5-25 °C, which is a suitable temperature range. During this period, the animals spent about 50% of their time at the optimum temperature range of 10-20 °C. In the autumn season, dairy cattle were at temperatures between 28-32 °C which is heat stress range in only 5% of their total time, especially in resting areas. In this period, animals were exposed to temperatures in the 0-5 °C range, which is defined as the cold tension range, in only 10% of their time (Table 3). According to Figure 3, there was no significant difference between building areas. Since only the number 2 resting area was located to the south, the temperature was 2-3 °C higher than that in the outside environment and the courtyard. According to Shinde & Taneja (1986), Sainsbury & Sainsbury (1988), Roenfeldt (1998), Webster (1994), Novák et al. (2000), West (2003), Herbut & Angrecka (2012), OACC (2014), FAO (2016) and Vtoryi et al (2018), a

comfortable shelter environment was provided for dairy cattle. In the shelter where the research was conducted, semi-closed resting areas were designed to protect animals from cold, air currents and rain, especially in winter. In such a season, temperatures measured in resting areas were higher than in all other areas (Figure 3).

In winter, animals spent about 40% of their total time at 5-25 °C (suitable temperature range) in resting areas that they used extensively to protect from cold. In this period, dairy cattle spent approximately 15% of their time at 10-20 °C (optimum temperature range) (Table 4). In this season, while dairy cattle were exposed to temperatures lower than -5 °C, which causes cold stress, in 11% of their total time in the feeding area and in 13% of their total time in the walking yard, this rate decreased up to 6% in the rest area no 2 (Table 4). In this season, it has been observed that the resting area no 2, which is protected from weather drafts and rains, is used intensely by animals. It has been determined that temperatures (<-5°C) that cause cold stress occurring in a very short time period do not create a climatic stress on animals. According to WMO (1989), Knížková et al (2002), Herbut & Angrecka (2012) and FAO (2016), this tempereture is compatible with the lower limit of the effective temperatures for dairy cattle, in this period the resting areas, which were planned as stagnant areas sheltered from air currents, protected the animals against cold to a large extent. The negative impact of low temperatures on animals can be significantly reduced by keeping the bedding ground as dry as possible and feeding the animals with a high energy diet.

The graphical distribution of temperatures to which the animals were exposed in the spring season is presented in Figure 5. In the spring season, dairy cattle spent 80% of their total time at 5-25 °C being the appropriate temperature range, and 50% of their total time at 10-20 °C being the optimum temperature range. In this season, although the animals were exposed to temperatures between 25-28 °C, which are categorized as heat tension range, while in 6-7% of their total time in resting areas (areas 2 and 3), this rate reduced up to 3-4% in the open shelter area (courtyard and feeding area). In the same season, animals were exposed to temperatures between 28-32 °C, which is the heat stress range, for only 4% of their total time in resting areas, while these temperatures were almost never experienced in the open areas of the shelter (Table 5, Figure 5). Associated with the temperatures rise animals prefer open and draft areas, temperatures in the heat stress range seen over a very short period of time did not exert a negative effect on animals. According to NRC (1981), Shinde & Taneja (1986), NRC (1989), Blackshaw & Blackshaw (1994), Brouček (1997), Novák et al (2000), Kadzere et al (2002), West (2003), Brouček et al (2009), Gaafar et al (2011), OACC (2014), FAO (2016) and Vtoryi et al (2018), a comfortable sheltering environment, which doesn't cause heat stress on dairy cattle, was provided for animal welfare.

Table 3	
Distribution of Temperatures Exposed by Animals in the autumn season	i

			Per	rcent	age I	Distri	butio	on of	Tem	pera	tures	Exp	osed	by A	nima	uls (%	6)							
MONTHS		SI	EPTI	EMB	ER			00	CTO	BER			Ν	IOVE	EMB	ER			Aut	umn S	leasor	n Ave	rage	
Temperature Ranges (°C)	(0-2)	(5-25)	(10-20)	(25-28)	(28-32)	(>32)	(0-2)	(5-25)	(10-20)	(25-28)	(28-32)	(-2-0)	(0-5)	(5-25)	(10-20)	(25-28)	(28-32)	(-2-0)	(0-5)	(5-25)	(10-20)	(25-28)	(28-32)	(>32)
1.Outdoor Area	0	84	55	9	6	0	5	91	61	4	0	7	27	66	36	0	0	2	11	80	51	4	2	0
2. Resting Area	0	71	48	7	10	11	1	85	57	7	6	0	17	82	42	0	0	0	6	80	49	5	5	4
3. Resting Area	0	74	50	8	10	8	1	86	56	7	6	1	19	79	42	0	0	0	7	80	49	5	5	3
4. Feeding Area	0	80	52	9	9	1	4	89	59	6	1	6	26	68	37	0	0	2	10	79	50	5	3	1
5. Courtyard	0	83	55	10	7	0	5	91	61	4	0	9	26	65	37	0	0	3	10	80	51	5	2	0

#### Table 4

Temperatures Exposed by animals in the winter season

		Per	centag	ge Dis	tributi	ion of	Temp	peratu	res Ex	posed	l by A	nimal	ls (%)							
MONTHS		DE	CEMI	BER			JA	NUA	RY			FEE	BRUA	ΛRΥ		Wi	inter S	eason	Avera	ige
Temperature Ranges (°C)	(<-5)	(-2-0)	(0-5)	(5-25)	(10-20)	(<-5)	(-2-0)	(0-5)	(5-25)	(10-20)	(5->)	(-2-0)	(0-5)	(5-25)	(10-20)	(<->)	(-2-0)	(0-5)	(5-25)	(10-20)
1.Outdoor Area	15	55	22	8	0	23	23	30	24	8	3	12	23	61	28	14	30	25	31	12
2. Resting Area	3	33	49	15	5	15	19	31	34	10	0	8	21	71	32	6	20	34	40	16
3. Resting Area	6	41	41	13	3	18	21	29	32	8	0	10	20	70	31	8	24	30	38	14
4. Feeding Area	11	49	31	9	2	21	23	29	27	8	2	12	23	63	28	11	28	28	33	13
5. Courtyard	14	55	23	8	1	22	22	30	25	9	3	13	23	61	28	13	30	26	31	12

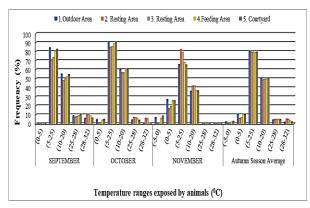


Figure 3

Distribution of temperatures exposed by animals in the autumn season

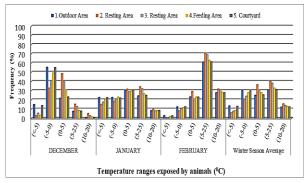
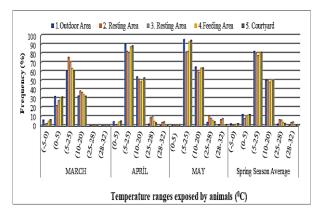


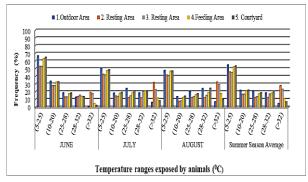
Figure 4

Distribution of temperatures exposed by animals in the winter season



#### Figure 5

Graphical distribution of temperatures exposed by animals in the spring season





Graphical distribution of temperatures exposed by animals in the summer season In the summer season, dairy cattle were exposed to temperatures in the range of 5-25 °C for 55% of their total time, especially in the feeding area and the courtyard. During this period, the animals spent about 20% of their time at 10-20 °C, the optimum temperature range (Table 6). As shown in Figure 6, although the ratio of the optimum temperatures to which the animals were exposed in the summer decreased up to 20% in the total time, the animals spent nearly 75% of their total time in suitable (5-25 °C) and optimum (10-20 °C) temperature ranges. In the summer season, dairy cattle were exposed to temperatures between 28-32 °C, which is the heat stress range, for about 17% of their total time. During this period, animals were exposed to temperatures higher than 32 °C (extreme stress range) for only 5-7% of their total time in open shelter areas (Table 6). In addition, it has been observed that, animals preferred open areas with air flow in the summer season. According to NRC (1989), Blackshaw & Blackshaw (1994), Brouček (1997), Novák et al (2000), Kadzere et al (2002), West (2003), Brouček et al (2009), Gaafar et al (2011), OACC (2014), FAO (2016) and Vtoryi et al (2018), the open shelter system provided a comfortable shelter environment that did not create climatic heat stress on animals in the summer season.

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Percentage Distribution of Temperatures Exposed by animals in the spring season

				Perc	entag	ge Di	strib	ution	of To	empe	rature	es Ex	pose	d by	Anim	als (	%)								
MONTHS			MA	RCH					AP	RİL					MA	٩Y				Spri	ng Se	eason	Ave	rage	
Temperature Ranges (°C)	(-2-0)	(0-5)	(5-25)	(10-20)	(25-28)	(28-32)	(0-2)	(5-25)	(10-20)	(25-28)	(28-32)	(>32)	(0-5)	(5-25)	(10-20)	(25-28)	(28-32)	(>32)	(-2-0)	(0-5)	(5-25)	(10-20)	(25-28)	(28-32)	(>32)
1.Outdoor Area	6	32	61	33	0	0	5	94	54	2	0	0	0	96	65	3	1	0	2	12	83	51	2	0	0
2. Resting Area	2	22	76	38	0	0	1	85	52	9	4	1	0	82	61	11	7	1	1	8	81	50	7	4	0
3. Resting Area	2	28	70	37	0	0	1	84	49	10	4	0	0	82	59	8	8	2	1	10	79	49	6	4	0
4. Feeding Area	5	31	64	34	0	0	4	90	51	5	0	0	0	93	64	6	1	0	2	12	82	50	4	1	0
5. Courtyard	7	32	61	33	0	0	5	91	53	3	0	0	0	95	64	4	1	0	2	12	82	50	3	0	0

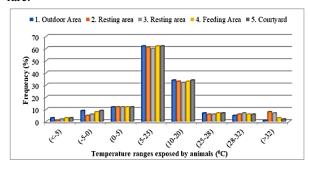
#### Table 6.

Percentage Distribution of Temperatures Exposed by animals in the summer season

		Pe	ercenta	age Di	stribu	ution o	of Ten	iperat	ures E	Expose	ed by	Anim	als (%	)						
MONTHS			JUNE					JULY				A	UGUS	ST		Su	mmer	Seasor	h Avera	age
Temperature Ranges (°C)	(5-25)	(10-20)	(25-28)	(28-32)	(>32)	(5-25)	(10-20)	(25-28)	(28-32)	(>32)	(5-25)	(10-20)	(25-28)	(28-32)	(>32)	(5-25)	(10-20)	(25-28)	(28-32)	(>32)
1.Outdoor Area	67	34	19	13	1	50	18	25	19	6	48	14	21	24	7	55	22	21	19	5
2. Resting Area	53	28	13	14	19	43	15	12	12	32	42	8	13	12	33	46	17	13	13	28
3. Resting Area	53	28	13	16	18	42	14	15	20	23	41	9	14	15	30	45	17	14	17	23
4. Feeding Area	63	33	18	14	5	48	18	19	22	12	47	13	16	19	18	53	21	17	18	12
5. Courtyard	65	33	19	14	2	49	19	21	21	9	47	14	18	25	10	54	22	19	20	7

The temperatures to which dairy cattle are exposed changed considerably in terms of months and seasons in different shelter areas. However, when the average temperatures throughout the year were examined, there was no significant difference between the building areas (Figure 7). Dairy cattle spent about 60% of their total time at 5-25 °C being the appropriate temperature range throughout the year. During the year, the animals were exposed to temperatures in the range of 10-20 °C, which is the optimum temperature range, for approximately 33% of their time. Animals are estimated to be exposed to heat stress in approximately 6-7% and cold stress in 2-3% of their annual total time. To understand exactly the effects of climatic environmental conditions on animal welfare in dairy cattle shelters, this study revealed that annual distribution is important rather than distribution within a period. According to studies by Shinde & Taneja (1986), Sainsbury & Sainsbury (1988), Webster (1994), Roenfeldt (1998), Novák et al (2000), Kadzere

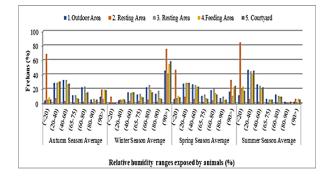
et al (2002), Knížková et al. (2002) Broucek et al (2009), Gaafar et al (2011), Herbut & Angrecka (2012), FAO (2016) and Vtoryi et al (2018), the year-round open shelter system provides a suitable shelter environment that does not create climatic stress in terms of animal welfare.





% Distributions of temperatures exposed by animals throughout the year

Graphical and proportional distributions of relative humidity values in autumn, winter, spring and summer seasons in the shelter are given in Figure 8 and Table 7. According to this study, dairy cattle spent about 7% of their total time in the optimum humidity range (65-75%)and 15% of the total time in the appropriate humidity range (60-80%) in the autumn season. During this season, animals were exposed to the relative humidity range of 40-60% for about 24% of their total time and to the relative humidity in the range of 80-90% for about 4% of their total time. In the autumn season, animals were exposed to relative humidity lower than 20% categorized as extremely dry, for 68% of their total time, especially in the resting area number 2 (Table 7). Generally, the moisture content of the air (excluding high humidity at high temperatures) is often not a problem in assessing climatic comfort for animals. Only low moisture content can cause some breathing problems as it increases the dust content of the air in closed buildings. However, such a problem is not observed in open shelters due to continuous air circulation. In the winter season, dairy cattle were exposed to the optimum humidity range of 65-75% for nearly 9% of their total time. In this period, the animals spent about 17% of their time in the appropriate humidity range of 60-80%. The moisture holding capacity decreases as the air cools down in the winter season and consequently approaches its saturation capacity. For 75% of their total time, the animals are exposed to relative humidity greater than 90%, which is categorized as excessively humid, especially in the resting area numbere 2 (Table 7). Cause of the relative humidity is higher in this area than in other areas, it can be explained as creating a stagnant housing environment protected from winds which has a significant effect on the moisture content of air; this may be due to the increase in the intensity of use by animals in cold periods. This can explain the increase in the moisture content of the environment by increasing evaporation in wet areas created by manure. The shelter environment is suitable for animals in terms of humidity, if very small measures are taken for certain periods of this season. Generally, the negative effect of moisture is enhanced with increasing temperatures.



#### Figure 8

Graphical distribution of relative humidity values categorized in autumn, winter, spring and summer

Dairy cattle were exposed to optimum (65-75%) and suitable range of humidity (60-80%), respectively, in approximately 7% and 17% of their total time in the spring season. In this season, animals were exposed to relative humidity values lower than 20%, which is categorized as extremely dry, for 46% of the total time, especially in the resting area number 2 (Table 7). In summer, dairy cattle wereexposed to 65-75% humidity, which is the optimum humidity range, for about 5% of their total time. In this period, the animals spent about 10% of their time in the appropriate humidity range of 60-80%. In addition, relative humidity values of less than 20% observed for more than 80% of the total time in the resting area no 2 and this season fell to 15-20% in other areas as animals began to prefer open areas with more wind flow and the effects of these values on the physiological activities of the animals were greatly reduced. Low humidity at high temperatures has no adverse effects on animals in the open shelter system. Several researchers (Yüksel, 1984; Maton et al., 1985; Olgun, 1988; Ekmekyapar, 1991; Okuroğlu & Yağanoğlu, 1993; Ekmekyapar, 2001; OACC, 2014; Vtoryi et al., 2018) opine that the relative humidity values determined throughout the year provide a suitable shelter for dairy cattle. During the year, dairy cattle spent approximately 15-20% of their total time in the appropriate humidity range (60-80%), and were exposed to a humidity range of 65-75%, which is categorized as the optimum humidity range for approximately 6-10% of their time (Table 8).

Table 7

D . D'. 1 . C1	1 1 . 1 1 . 1 . 1 . 1 . 1 .		•	1
Percentage Distributions of h	ourly relative humidif	ty values measured in autumn	winter spring an	d summer seasons
i ciccinage Distributions of in	ourly relative number	ly varues measured in autumn	, white, spring an	a summer seasons

											Fre	quen	cy (	%)														
SEASONS	1	Autui	nn S	easor	n Ave	erage	e		Win	ter So	easoi	n Ave	erage	•		Sprii	ng Se	easor	n Ave	erage	•	S	Sumr	ner S	easo	n Av	erag	е
Relative Humidity Ranges (%)	(<20)	(20-40)	(40-60)	(65-75)	(08-09)	(06-08)	(<06)	(<20)	(20-40)	(40-60)	(65-75)	(08-09)	(06-08)	(<06)	(<20)	(20-40)	(40-60)	(65-75)	(08-09)	(80-90)	(<06)	(<20)	(20-40)	(40-60)	(65-75)	(08-09)	(80-90)	(<06)
1.Outdoor Area	4	28	32	11	22	5	9	0	4	15	12	22	13	45	6	27	26	10	18	7	16	11	46	26	6	12	2	2
2. Resting Area	68	7	3	1	2	1	19	9	5	3	3	5	2	75	46	9	6	2	4	2	32	84	6	2	0	1	0	6
3. Resting Area	5	28	32	11	23	6	6	0	4	14	13	25	17	40	7	28	25	12	21	9	10	20	44	24	5	10	1	1
4.Feeding Area	8	29	26	7	14	3	19	0	6	15	9	18	7	54	10	28	22	7	14	4	22	23	41	20	5	9	1	6
5. Courtyard	5	30	27	6	15	5	18	0	5	15	8	15	6	58	8	28	23	6	12	5	24	17	45	22	5	9	2	5

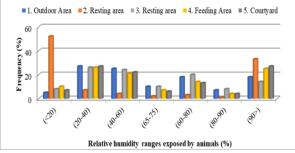
Table 8Percentage distributions of hourly relative humidityvalues measured throughout the year

Percentage Distribu	tion of	Relativ	e Humi	dity Ex	posed	by Ani	mals
		(%	)				
Relative Humidity	20)	-0	(40- 60)	5- 5)	-0	-0	(<(
Ranges (%)	$\mathbb{Z}$	$\overline{O}$ 4	6 (	(6 7:	9) <b>8</b>	8 8	(<06)
1.Outdoor Area	5.2	26.7	24.7	9.7	18.4	6.9	18.2
<ol><li>Resting Area</li></ol>	51.7	6.7	3.8	1.6	3.2	1.3	33.3
<ol><li>Resting Area</li></ol>	7.9	26.0	23.8	10.3	19.8	8.3	14.3
<ol><li>Feeding Area</li></ol>	10.3	26.1	20.7	7.1	13.7	3.8	25.4
5. Courtyard	7.4	27.1	21.9	6.3	12.7	4.4	26.5

In Figure 9, the resting area no 2 had quite dry environments for animals to continue their physiological activities in terms of moisture content throughout the year. During the year, dairy cattle were exposed to less than 20% relative humidity for approximately 52% of their total time in the resting area numbere 2 (Table 8). The reason why this area, which was designed to protect animals from cold, is drier than other shelter areas, may be because over-drying manure absorbs moisture from the air as both are warm (relative humidity decreases as temperature increases; known as a psychometric law) and due to the less use by animals during hot periods. In addition, dairy cattle were exposed to greater than 90% humidity, especially in the resting area numbere 2, for approximately 33% of their total time (Table 8). We also observed that the animals are exposed to relative humidity values in the range of 40-90% at 50-60% of their total time throughout the year. Several studies (Wathes et al., 1983; Yüksel, 1984; Ekmekyapar, 1991; OACC, 2014; Vtoryi, 2018) have reported that the shelter environment is suitable for animal welfare as long as the animals are not permanently exposed to environments with 40-90% relative humidity.

#### 4. Conclusion and Suggestions

When designing shelters, environmental conditions, which have an impact on animal welfare throughout the year rather are important rather than instantaneous or over a specific period. If resting areas planned to create warm and stagnant areas in winter in open shelter systems are planned so that the ground is soft and dry, the effect of cold stress seen in a short period in the winter months can be significantly alleviated or completely eliminated. With the rise in temperatures, unlike resting areas, breezy and covered shadow areas should be created for the animals to have sufficient climatic comfort. In fact, it can be concluded that to build no shelter may be more comfortable for animals in terms of climate than poorly planned shelters. In the climatic conditions of Konya region, it would be appropriate to prefer semiopen barn type for animal welfare for dairy cattle. To conclude, the idea that temperature, which is the main factor for avoiding open systems in animal breeding, especially during cold periods, creates unfavorable environments for animals and that animals are harmed by winter chills has been refuted with this study.





The graphical distribution of relative humidity values categorized throughout the year

#### 5. Acknowledgement

This study was prepared by utilizing the MS Thesis of Elif ŞAHİN supported by the Selcuk University Teaching Staff Training Program Research Projects (ÖYP) under grant number 2015-ÖYP-079.

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Selcuk Journal of Agriculture and Food Sciences

http://sjafs.selcuk.edu.tr/sjafs/index

es SJAFS

(2022) 36 (1), 91-97 e-ISSN: 2458-8377 DOI:10.15316/SJAFS.2022.013

#### Research Article

# Effects of Dephytinized Wheat Bran on Rheological Properties of Dough and Sourdough Fermentation

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#### ARTICLE INFO

Article history: Received date: 02.04.2022 Accepted date: 13.04.2022

Keywords: Dephytinization Wheat bran Sourdough Lactic acid bacteria

#### ABSTRACT

In this study, the rheological properties of flours containing wheat bran or dephytinized wheat bran at different rates (0, 5, 10, 15%) and some physicochemical and microbiological properties of bread doughs produced with sourdough by using these flour mixes were investigated. Four different sourdoughs, which were spontaneous (SS), Vakfikebir (VS), containing *Lactobacillus fermentum* as a starter (LFS) and containing *Lactococcus lactis* as a starter (LCS), were used. The water absorption, softening degree, resistance to extension values of dough increased while the stability, energy and extensibility values decreased as the rate of bran increased for both bran types. The pH and total acidity (TA) values of the bread dough samples generally increased with the addition of bran. The lowest moisture content, TA and LAB count, and the highest pH and yeast count were obtained in VS. The lowest pH and the highest TA values belonged to the bread dough samples containing SS. The number of LAB and yeast counts in bread dough samples increased with addition of bran compared to control sample.

#### 1. Introduction

Wheat bran is a major by-product of milling and a good source of dietary fiber. The consumption of it has several benefits on human health. It reduces the risk of certain cancer types, has positive effects on the digestive system, shortens the intestinal transit time, increases the fecal mass, prevents constipation, cures diverticulosis and irritable bowel syndrome, reduces the risk of obesity, helps weight control, protects against gallstone formation, improves glycemic control, reduces the need for insulin or hypoglycemic substances (Almeida et al., 2013).

The addition of dietary fiber into bread formulation affects the technological properties of dough and product quality. Studies have shown that the water absorption, extensibility and textural properties of flours containing dietary fiber change. Some dietary fiber components such as arabinoxylan,  $\beta$ -glucan have possitive effects on the dough. They increase the dough viscosity and stabilize the gas cells (Rieder et al., 2012).

The bran could be added to flours as a dietary fiber source and used in the production of fiber enriched products. However, the phytic acid content of bran limits the usage. Phytic acid forms insoluble complexes with mineral cations and proteins and reduces their bioavailability and solubility. Phytic acid must be destroyed by an appropriate method before use (Baumgartner et al., 2018). The effects of different biological methods and processes (such as soaking, germination, fermentation, boiling, baking etc.) on phytic acid were investigated in the studies. However, it is stated that these procedures can not completely eliminate phytic acid (Servi et al., 2008). Özkaya et al. (2017) reported that the phytic acid content of wheat bran decreased at the rate of 95.2% by autoclaving for 1.5 h at pH 4.0.

Sourdough, used in bread production, is known to have positive effects on health directly or indirectly. Exopolysaccharides produced by lactic acid bacteria in the sourdough improve the viscoelastic properties of bread dough. It prevents the adverse effects of bran particles on the gluten network and gas cells (Pejcz et al., 2017). The decrease in pH with fermentation increases the endogenous phytase activity and provides a reduction of phytate content by more than 50% (Gobbetti et al., 2019).

There are studies on production and use of dephytinized bran (Baumgartner et al., 2018; Majzoobi et al.,

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This article is taken from Hümeyra ÇETİN BABAOĞLU's PhD thesis supported by The Scientific Research Projects Coordination Unit of Selçuk University with the grant number of 18101022.

2014; Mosharraf et al., 2009; Özkaya et al., 2017; Özkaya et al., 2018; Servi et al., 2008). However, the study about usage of dephytinized wheat bran in sourdough bread dough has not been reported. In this study the effects of wheat bran and dephytinized wheat bran on rheological properties of dough and on some physicochemical and microbiological properties of fermented sourdough were investigated. It was aimed to determine the potential of using dephytinized wheat bran in the production of sourdough bread.

#### 2. Materials and Methods

#### 2.1. Materials

Wheat flour and wheat bran were purchased from commercial companies in Konya, Turkey. Vakfikebir sourdough was obtained from a local bakery in Vakfikebir, Trabzon, Turkey. Wheat bran was milled by a labscale disc miller (Laboratory Mill 3303, Perten) and particle size was reduced to less than 300  $\mu$ m. Lactic acid bacteria used as starter culture in the production of Type 2 sourdoughs were obtained from Cereal and Cereal Products Laboratory of Selçuk University Agriculture Faculty.

#### 2.2. Dephytinization of wheat bran

Wheat bran was mixed with distilled water at a ratio of 1:15 (w/v) and pH of bran slurry was adjusted to 4.0 with acetic acid. After keeping for 30 minutes at 121°C in autoclave, pH of bran slurry was increased to inception pH value with 6 N NaOH. The slurry was filtered thorough by a sieve (with an opening of 200  $\mu$ m), rinsed five times with water and dried at 60°C in an oven to a maximum of 10% moisture content (Özkaya et al., 2017).

#### 2.3. Sourdough production

For the production of spontaneous sourdough, the wheat flour and water were mixed at a ratio of 1:1 (w/v) so that the dough yield (DY = [(weight of flour + weight of water)/weight of flour]\*100) was 200 and left to fermentation at 30°C. In every 24 hours, 10% of the mixture was taken and back-slopping performed not to the way that deteriorate dough yield. Fermentation of sourdough continued until pH of sourdough dropped to 3.6-3.8 and TA reached 0.72-0.90%.

For the production of sourdoughs produced by using starter culture, wheat flour and water were blended in a steril jar at a ratio of 1:1 (w/v). The starter culture was inoculated at least in an amount of  $10^6$  kob/g of lactic acid bacteria in mixture and left to fermentation at  $30^{\circ}$ C for 24 hours. For the preparation of starter cultures, bacteria, kept at -20°C, were inoculated at a rate of 2% into MRS broth medium for reactivation and incubated at  $30^{\circ}$ C for 24 hours by providing the proper incubation conditions with Anaerocult C (Merck, Germany). After the incubation, cells were harvested by centrifugation at 6000 rpm for 10 minutes. The pellet was washed twice with sterile <sup>1</sup>/<sub>4</sub> Ringer solution (Merck, Germany) and the number of LAB was determined by spread plate

technique. The liquid phase was removed by last centrifugation, cell pellet was resuspended in sterile 20% glycerol solution and kept at -20°C until usage as a starter for sourdough production.

#### 2.4. Bread dough-making

For bread dough making, wheat flour was blended with wheat bran or dephytinized wheat bran at the different rates (0, 5, 10, 15%). 1 g sugar, 1.5 g salt, 1 g yeast, 30 g sourdough and water based on water absorption determined in farinograph (the amount of flour and water from sourdough were taken into account) were added to 100 g of flour mixture and kneaded for 10 minutes in a kitchen-type dough kneader (KitchenAid, 5KSM45, ABD) at slow speed. The dough was fermented for 120:35 min (punching, proofing) at 30°C and 80±5% relative humidity.

#### 2.5. Rheological analyses

The rheological characteristics of flour and bran mixes were tested with the farinograph (Brabender GmbH & Co KG, Germany) using Approved Method 54-21 and extensograph (Brabender GmbH & Co KG, Germany) using Approved Method 54-10 (AACC, 2000).

#### 2.6. Physicochemical analyses

Ash, protein and fat contents of flour, wheat bran and dephytinized wheat bran were determined according to AACC Standard Method No: 08-01.01, 46-12.01 (AACC, 2010) and ICC Method No: 136 (ICC, 2002), respectively. Moisture contents, titratable acidity and pH values of flour, wheat bran, dephytinized wheat bran, sourdoughs and bread doughs were determined according to AACC Standard Method No: 44-01.01, 02-31.01 (AACC, 2010) and AOAC Standard Method No: 943.02 (AOAC, 2012), respectively. Falling number and sedimentation test of flour were performed by using AACC Standard Method No: 56-81.04 and 56-61.02 (AACC, 2010), respectively. The phytic acid contents of bran samples were calculated according to Haug and Lantzsch (1983) by measuring the phytate phosphorus spectrophotometrically.

#### 2.7. Microbiological analyses

For microbiological analyses, 10 g of sourdough or bread dough was weighed into sterile stomacher bag and homogenized in 90 ml of 0.1% peptone water. After homogenization, appropriate serial decimal dilutions, prepared with 0.1% peptone water, were used for inoculation by spread plate technique and the results were expressed as log<sub>10</sub> colony forming units per gram sample (log<sub>10</sub> CFU/g). Total lactic acid bacteria were cultured on MRS agar containing 0.05 g/l of cycloheximide to prevent yeast growth and incubated anaerobically at 30°C for 48 h. Yeast was counted on Potato-Dextrose Agar (PDA, Merck, Germany) acidified by sterile tartaric acid (1.4 g/l) after incubation at 27°C for 5 days.

#### 2.8. Statistical analysis

The results were expressed as the mean of two independent replicates with at least triplicate measurements. MINITAB release 18.0 was used to analyse data by performing one-way analysis of variance (ANOVA), followed by Tukey Multiple Comparison Test to verify any significant differences among the means at a 5% significance level (p<0.05).

#### 3. Results and Discussion

#### 3.1. Rheological properties

The changes in the rheological properties (stability, development time, water absorption, softening degree, energy (A), extensibility (E), resistance to extension at constant deformation ( $R_5$ ) and dough maximum resistance ( $R_m$ ) values) of flour added with wheat bran or dephytinized wheat bran are shown in Figure 1.

The water absorption and softening degree values of flour increased while the stability value of flour decreased as the bran rate increased for both bran types. However, considering the development time values, it was seen that the both type of wheat bran utilisation lead to reduce the development time of dough. Additionally, increasing bran addition levels caused decreasing the development time.

The reason for the increase in water absorption is that there are more hydroxyl groups in bran compared to flour, and these groups allow more hydrogen bonds to be established with water molecules (Rosell et al., 2006). The porosity of the insoluble fiber fraction of bran is higher than the soluble fiber fraction. As porosity increases, the number of hydrogen bonds made with water molecules increases, so water absorption increases too (Kethireddipalli et al., 2002). The increase in insoluble fiber concentration of bran by dephytinization process could had caused higher increase in water absorption of flour samples containing dephytinized wheat bran than flour samples containing wheat bran.

The number and strength of the bonds between gluten proteins affects dough stability value. Some physical and chemical interactions during long-term kneading and the weakening of the gluten network with bran addition can lead to a decrease in dough stability value. The flours with high amount and quality of gluten have low softening degree and long development time (Özkaya et al., 2018). It is thought that the dilution of gluten concentration with the addition of bran caused increase in the softening degree values and decrease in the development time values of samples. However, the addition of dephytinized wheat bran compared to control group increased development time values. It has been reported that the long development time in whole wheat flour is due to the interaction between gluten and bran particles and preventing of the protein hydration by bran particles, therefore the kneading process is needed to be applied for a longer time in order to reach maximum consistency (Penella et al., 2006). The high water absorption capacity of bran particles can also be effective in prolonging of the development time. In addition some chemical bonds can not form because of intervention of bran particles between gluten molecules and a decrease in intermolecular attraction force. So it is delayed for the dough to reach the appropriate consistency (Özkaya et al., 2017). The number of disulfide bonds in dough can increase as a result of the washing away of reducing agents by dephytinization process. Therefore, the flour containing dephytinized wheat bran has longer development time, higher stability value and lower softening degree than wheat bran added flour. The heat treatment applied during the dephytinization process increases lipoxygenase activity by inactivating the lipase enzyme, thus disulfide bond formation increases and dough rheology improves (Mosharraf et al., 2009).

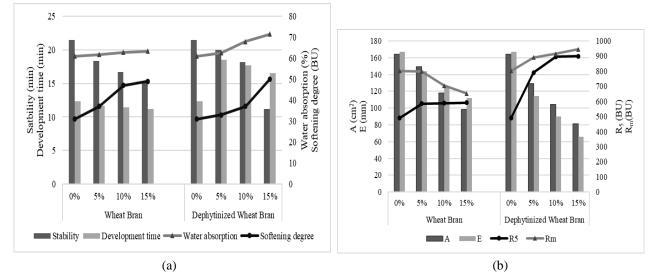


Figure 1

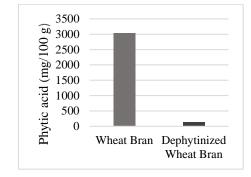
The stability, development time, water absorption and softening degree values (a), and the energy (A), extensibility (E), resistance to extension at constant deformation ( $R_5$ ) and dough maximum resistance ( $R_m$ ) values (b) of flour mixtures containing wheat bran or dephytinized wheat bran at different rates

It can be seen in the extensogram values (Figure 1) that the  $R_5$  value increased along with an increase in addition rate for both bran types and this rise was higher in the flour containing dephytinized wheat bran. The  $R_m$  value increased with an increase in dephytinized wheat bran rate, but it decreased as wheat bran rate increased. As the amount of bran added increased, the A and E values decreased for both bran types and this decline was higher in dephytinized wheat bran added flours compared to wheat bran added flours.

It has been reported that hemicelluloses being in the structure of bran particles, compete with gluten proteins and starch for water, which prevents the complete formation of gluten structure (Hoseney, 1986). In addition, the release of ferulic acid from arabinoxylanes being in the bran fraction and the formation of cross-links between arabinoxylanes and gluten proteins also affects the gluten network. As a result of this effect, the gluten network becomes more resistant to extension (Le Bleis et al., 2015). Therefore, dough resistance to extension value increased while extensibility of dough decreased with the bran additive.

#### 3.2. Physicochemical properties

Moisture, ash, protein, fat, pH and TA values of the flour sample were determined as 12.17%, 0.63%, 13.99%, 1.66%, 6.11 and 0.22%, respectively. These values were 11.39%, 6.14%, 15.53%, 3.76%, 6.48 and 1.21% for wheat bran, while were 5.77%, 6.22%, 14.60%, 3.98%, 6.82 and 1.14% for dephytinized wheat bran, respectively. The sedimentation and falling number values of the flour sample were found as 45 ml and 585 s. The dephytinization process decreased the phytic acid content of wheat bran from 3043.55 mg/100 g to 145.64 mg/100 g (Figure 2). The reason for the lower moisture content of dephytinized wheat bran than wheat bran is the drying process applied after autoclaving. As a result of dephytinization process, while the amount of ash and fat of wheat bran increased, the amount of protein decreased. The decline in protein content may be resulted from the high temperature applied during dephytinization (Khatun et al., 2007), and the washing and filtration processes applied after autoclaving. It has been reported that while the pH values of samples were high, the high titratable acidity values may be due to the buffer effect of the bran proteins (Seiuml et al., 2011). Van Bockstaele et al. (2008) found that the ash, sedimentation and protein values (in dry matter) of wheat flour samples were ranged from 0.56-0.90%, 34-70 ml and 11.6-16.9%, respectively. Prückler et al. (2015) established the ash, fat and protein contents of wheat bran as 5.8%, 5.7% and 15.5%, and these of wheat flour as 0.8%, 1.3% and 13.9%, respectively. Özkaya et al. (2018) determined the ash and protein contents of wheat flour as 0.50%, 13.25% and these of wheat bran as 5.6%, 14.2%, respectively. The differences between data obtained in the studies may arise from the climate, soil and variety and vary in a wide range (Peterson et al., 1992).



#### Figure 2

The phytic acid contents of wheat bran and dephytinized wheat bran

In spontaneous sourdough production, while pH decreased by the fermentation progress, TA value increased. pH and TA values, being 6.16 and 0.14% at the beginning, reached 3.62 and 0.99%, respectively, at the end of fermentation. The changes over time of pH and TA values determined before each back-slopping during fermentation is shown in Table 1. It has been reported that the pH and TA values of sourdough, growing proper ripe, were ranged between 3.6-3.8 and 0.72-0.90% (lactic acid), respectively (Gobbetti and Gänzle, 2012). When the pH and TA values of the sourdough sample are examined at the end of the 5<sup>th</sup> day, it is seen that it is compatible with data in the literature. Wehrle and Arendt (1998) determined that while the initial pH value of spontaneous sourdough was 6.4, it dropped to 3.7 at the end of 40 hours of fermentation. The pH and TA values of VS, LFS and LCS were 4.97-0.23%, 3.66-1.03% and 3.63-1.01%, respectively.

#### Table 1

The changes over time of pH, TA and LAB count during spontaneous sourdough fermentation (mean  $\pm$  std. dev.)

Days	pН	TA (%) (lactic acid)	LAB count (log10 cfu/g)
0	$6.16\pm0.05$	$0.14\pm0.01$	$0.00\pm0.00$
1	$4.64\pm0.05$	$0.61\pm0.08$	$6.66\pm0.10$
2	$3.73\pm 0.01$	$0.94\pm0.00$	$8.58\pm0.05$
3	$3.64\pm0.01$	$0.97\pm0.02$	$8.63\pm0.05$
4	$3.63\pm0.02$	$0.98\pm0.02$	$8.68\pm0.10$
5	$3.62\pm0.00$	$0.99\pm0.01$	$8.77\pm0.01$

The moisture contents, pH and TA values of sourdough bread dough samples containing wheat bran or dephytinized wheat bran are given in Table 2. The addition of bran increased the moisture content of bread samples, but the effect of the bran addition rate was generally insignificant (p>0.05). When the effect of sourdough type on the bread dough moisture content was examined for both bran types, it is seen that the highest results belonged to samples produced with LFS and the lowest results belonged to samples containing VS (p<0.05). Although the moisture contents of the bread doughs containing dephytinized wheat bran were higher than the wheat bran added samples, the effect of the dephytinization was generally insignificant (p>0.05), except for the samples produced with LCS. The pH and TA values of the bread dough samples generally increased with the addition of bran (p<0.05). It was determined that the highest pH and the lowest TA values belonged to the samples produced with VS, the lowest pH and the highest TA values belonged to the bread dough samples containing SS (p<0.05). While the pH values of the samples containing wheat bran were higher than the samples containing dephytinized wheat bran, the effect of the dephytinization on the TA value was generally found to be insignificant (p>0.05).

Desmazeaud (1983) reported that the acid production ability of *Lactococcus* species is higher than that of *Lactobacillus* species. When the TA values of bread dough samples are examined, it is seen that the TA values of samples containing LCS were higher than that of bread dough samples produced with LFS. It is thought that the high TA values of bread dough samples containing SS may had been due to the activity of homofermentative lactic acid bacteria which were present in sourdough microflora, but not dominantly and could not be obtained as pure.

Aplevicz et al. (2013) determined that the initial pH values of bread doughs containing sourdoughs produced with two different *Lactobacillus paracasei* strains and two different *Saccharomyces cerevisiae* strains were between 4.51-4.73, and the lowest pH value at the end of 10 hours of fermentation was 3.44. It was reported that the highest TA values (6.42-6.51 ml 0.1 N NaOH/10 g of sample) belonged to bread doughs containing sourdough produced with yeast.

#### 3.3. Microbial counts of sourdoughs and bread doughs

In spontaneous sourdough production, while the number of lactic acid bacteria increased by the fermentation progress (Table 1), no yeast growth was observed. It is considered that the number of yeast was below the limit that can be determined at the beginning of fermentation period and the process conditions negatively affected the yeast growth (Vogelmann et al., 2009). The pH decrease occurred rapidly in the sourdough sample, the increase in the number of yeast did not occur due to Table 2

5

10

15

0

5

10

15

Wheat bran

Dephytinized

wheat bran

VS

the predominance of lactic acid bacteria in the following days and the effect of the acid formed. Gobbetti and Gänzle (2012) stated that the number of lactic acid bacteria in sourdough varied from 7 to 9  $\log_{10}$  cfu/g. In this study, it is seen that the number of lactic acid bacteria in the spontaneous sourdough sample reached 8.77  $\log_{10}$  cfu/g and was within the range given in the literature. The LAB counts of VS, LFS and LCS were 5.48, 9.00 and 8.96  $\log_{10}$  cfu/g, respectively. The yeast count of VS was 4.92 log10 cfu/g while no yeast detected in other sourdoughs.

The LAB and yeast counts of bread dough samples are given in Table 2. It was determined that the addition of bran in dough increased the number of LAB, but the effect of the addition rate and type of bran on the LAB number was statistically insignificant (p>0.05). It was seen by considering the effect of sourdough type on LAB number that, the sample group with the lowest LAB count was the bread dough samples containing VS (p<0.05). The yeast number of bread dough samples increased with the addition of bran. It is seen that the highest yeast number in general belonged to the samples containing LFS and LCS, while the lowest values belonged to the samples produced with VS (p<0.05).

The organic acids formed as a result of the action of lactic acid bacteria stimulate the metabolic activity of yeasts. When Table 2 was examined, the number of yeast was also high in samples with a high number of lactic acid bacteria. In addition, although there was no yeast in other sourdough samples, the yeast count in bread dough produced with these sourdoughs, increased to 6.33-7.64 log<sub>10</sub> cfu/g, but the yeast number of samples containing VS of which the initial yeast number was 4.92 log<sub>10</sub> cfu/g, reached 7.03 log<sub>10</sub> cfu/g and this rise was lower than that of other samples. Aplevicz et al. (2013) determined that the LAB and yeast counts of bread doughs containing sourdoughs produced with two different *Lactobacillus paracasei* strains ranged from 8.66-8.91 log<sub>10</sub> cfu/g and 7.08-7.18 log<sub>10</sub> cfu/g, respectively.

 $5.41\pm0.02^{\mathrm{aBP}}$ 

 $5.45 \pm 0.04^{aBP}$ 

 $\underline{5.55\pm0.05^{aBP}}$ 

 $5.24\pm0.00^{\rm bcBR}$ 

 $5.35\pm0.04^{\rm abBP}$ 

 $\underline{5.45\pm0.01^{\mathrm{aBP}}}$ 

 $5.17 \pm 0.03^{cC}$ 

 $6.78\pm0.04^{aBP}$ 

 $6.85\pm0.00^{abBR}$ 

 $\underline{6.98\pm0.08^{aBP}}$ 

 $6.72\pm0.01^{\text{bB}}$ 

 $6.80\pm0.03^{\text{bBP}}$ 

 $7.01\pm0.02^{\mathrm{aBP}}$ 

 $\underline{7.03\pm0.04^{aBP}}$ 

Sourdough	Bran type	Rate of bran	Moisture (%)	pН	TA (%)	LAB count	Yeast count
type	Bran type	(%)	Wolsture (70)	pm	(lactic acid)	(log10 cfu/g)	(log10 cfu/g)
		0	$44.51\pm0.04^{\text{bAB}}$	$4.07\pm0.02^{\rm cD}$	$0.73\pm0.01^{bA}$	$8.26\pm0.00^{\text{bB}}$	$6.33\pm0.09^{\text{bC}}$
	XX71 ( 1	5	$44.52\pm0.15^{bBP}$	$4.17\pm0.03^{bcCP}$	$0.80\pm0.01^{abAP}$	$8.56\pm0.03^{\mathrm{aAP}}$	$6.37\pm0.07^{\text{bCR}}$
	Wheat bran	10	$45.39\pm0.04^{\mathrm{aBP}}$	$4.26\pm0.02^{bCP}$	$0.83\pm0.01^{\mathrm{aAP}}$	$8.65\pm0.00^{\mathrm{aAP}}$	$6.57\pm0.09^{\text{bBR}}$
SS		15	$45.47\pm\!\!0.08^{\mathrm{aABR}}$	$4.58\pm0.02^{\mathrm{aDP}}$	$0.87\pm0.03^{\mathrm{aAP}}$	$8.69\pm0.07^{\mathrm{aAP}}$	$7.08\pm0.02^{aBR}$
33		0	$44.51\pm0.04^{\text{bAB}}$	$4.07\pm0.02^{\rm bD}$	$0.73\pm0.01^{\mathrm{aA}}$	$8.26\pm0.00^{\text{bB}}$	$6.33\pm0.09^{\rm cC}$
	Dephytinized	5	$45.28\pm0.11^{abAP}$	$4.12\pm0.03^{abCP}$	$0.74\pm0.00^{\mathrm{aAR}}$	$8.55\pm0.09^{\mathrm{aAP}}$	$6.78\pm0.00^{\text{bBP}}$
	wheat bran	10	$45.67\pm0.46^{abBCP}$	$4.20\pm0.01^{\mathrm{aCP}}$	$0.75\pm0.02^{\mathrm{aAP}}$	$8.64\pm0.01^{\mathrm{aAP}}$	$7.42\pm0.00^{\mathrm{aAP}}$
		15	$46.48\pm0.16^{\mathrm{aBCP}}$	$4.22\pm0.02^{\mathrm{aDR}}$	$0.78\pm0.01^{\mathrm{aAP}}$	$8.67\pm0.00^{\mathrm{aAP}}$	$7.49\pm0.04^{\mathrm{aAP}}$
		0	$43.39\pm0.37^{\mathrm{aB}}$	$5.52\pm0.02^{\rm cA}$	$0.36\pm0.02^{\text{bC}}$	$5.17\pm0.03^{\text{bC}}$	$6.72\pm0.01^{aB}$

 $5.67\pm0.01^{\rm bAP}$ 

 $5.72 \pm 0.02^{abAP}$ 

 $5.77 \pm 0.01^{\mathrm{aAP}}$ 

 $\overline{5.52\pm0.02^{\mathrm{cA}}}$ 

 $5.54\pm0.00^{\rm cAR}$ 

 $5.62\pm0.00^{\text{bAR}}$ 

 $5.75 \pm 0.00^{\mathrm{aAP}}$ 

 $0.39\pm0.01^{\text{bCP}}$ 

 $0.42 \pm 0.01^{\text{bDP}}$ 

 $\underline{0.50\pm0.00^{aCP}}$ 

 $0.3\overline{6\pm0.02^{aC}}$ 

 $0.36\pm0.00^{aDP}$ 

 $0.38\pm0.00^{\mathrm{aDP}}$ 

 $\underline{0.41\pm0.01^{aDR}}$ 

The moisture content, pH and TA values, LAB and yeast counts of bread dough samples (mean  $\pm$  std. error)

 $43.65\pm0.03^{\mathrm{aCP}}$ 

 $43.94 \pm 0.32^{aCP}$ 

 $\underline{44.43\pm0.42^{aBP}}$ 

 $43.39\pm0.37^{\text{bB}}$ 

 $43.71\pm0.19^{\text{bBP}}$ 

 $45.46\pm0.21^{\mathrm{aCP}}$ 

 $\underline{45.67} \pm 0.05^{\mathrm{aCP}}$ 

ne moist	ure content, pH	and IA	values, LAB and ye	east counts of bro	ead dough sampl	es (mean ± std. e	error
		0	$45.37\pm0.03^{bA}$	$4.74\pm0.01^{\text{dC}}$	$0.52\pm0.02^{\text{bB}}$	$8.49\pm0.01^{bA}$	$7.30\pm0.03^{bA}$
	W/h a set la usar	5	$46.22\pm0.09^{abAP}$	$4.95\pm0.01^{\rm cBP}$	$0.56\pm0.03^{abBP}$	$8.63\pm0.03^{\mathrm{aAP}}$	$7.31\pm0.03^{\text{bAP}}$
	Wheat bran	10	$46.43\pm0.00^{abAR}$	$5.10\pm0.01^{bBP}$	$0.58\pm0.01^{abCP}$	$8.69\pm0.02^{\mathrm{aAP}}$	$7.38\pm0.04^{abAP}$
LFS		15	$46.97\pm0.54^{\mathrm{aAP}}$	$5.22\pm0.00^{aBP}$	$0.64\pm0.01^{aBP}$	$8.73\pm0.02^{\mathrm{aAP}}$	$7.50\pm0.02^{\mathrm{aAP}}$
LLP		0	$45.37\pm0.03^{bA}$	$4.74\pm0.01^{\rm cC}$	$0.52\pm0.02^{aB}$	$8.49\pm0.01^{\mathrm{aA}}$	$7.30\pm0.03^{\rm cA}$
	Dephytinized	5	$46.27\pm0.27^{bAP}$	$4.84\pm0.01^{\text{bBR}}$	$0.53\pm0.01^{\mathrm{aCP}}$	$8.50\pm0.03^{\mathrm{aAP}}$	$7.34\pm0.01^{bcAP}$
	wheat bran	10	$47.87\pm0.25^{\mathrm{aAP}}$	$4.87\pm0.01^{\text{bBR}}$	$0.55\pm0.01^{\mathrm{aCP}}$	$8.66\pm0.07^{\mathrm{aAP}}$	$7.49\pm0.05^{abAP}$
		15	$48.26\pm0.25^{\mathrm{aAP}}$	$5.01\pm0.02^{aBR}$	$0.58\pm0.01^{\mathrm{aCP}}$	$8.70\pm0.06^{\mathrm{aAP}}$	$7.53\pm0.00^{\mathrm{aAP}}$
		0	$44.43\pm0.31^{bAB}$	$4.85\pm0.00^{\rm cB}$	$0.56\pm0.03^{\rm cB}$	$8.52\pm0.03^{\rm cA}$	$7.20\pm0.00^{\text{bA}}$
	Wheat hear	5	$44.85\pm0.01^{abBR}$	$4.94\pm0.00^{\rm bBP}$	$0.62\pm0.01^{bcBP}$	$8.62\pm0.01^{bcAP}$	$7.44\pm0.03^{\mathrm{aAP}}$
	Wheat bran	10	$45.53\pm0.14^{abABR}$	$5.10\pm0.00^{aBP}$	$0.71\pm0.01^{abBP}$	$8.65\pm0.02^{abAP}$	$7.48\pm0.01^{\mathrm{aAP}}$
LCS		15	$45.87\pm0.23^{\mathrm{aABR}}$	$5.12\pm0.01^{\mathrm{aCP}}$	$0.77\pm0.02^{\mathrm{aAP}}$	$8.77\pm0.02^{\mathrm{aAP}}$	$7.54\pm0.04^{\mathrm{aAP}}$
LCS		0	$44.43\pm0.31^{bAB}$	$4.85\pm0.00^{\mathrm{aB}}$	$0.56 \pm 0.03^{bB}$	$8.52\pm0.03^{\text{bA}}$	$7.20\pm0.00^{\rm cA}$
	Dephytinized	5	$45.57\pm0.00^{bAP}$	$4.90\pm0.03^{\mathrm{aBP}}$	$0.57\pm0.01^{bBP}$	$8.53 \pm 0.02^{bAR}$	$7.46\pm0.03^{\text{bAP}}$
	wheat bran	10	$47.30\pm0.23^{\mathrm{aABP}}$	$4.91\pm0.01^{aBR}$	$0.62\pm0.01^{abBR}$	$8.58\pm0.02^{\text{bAP}}$	$7.58\pm0.03^{abAP}$
		15	$47.46\pm0.20^{\mathrm{aABP}}$	$4.92\pm0.01^{\mathrm{aCR}}$	$0.69\pm0.00^{aBP}$	$8.73\pm0.02^{\mathrm{aAP}}$	$7.64\pm0.01^{\mathrm{aAP}}$

Table 2 (Continue) The moisture content, pH and TA values, LAB and yeast counts of bread dough samples (mean  $\pm$  std, error

Values followed by different superscript letters (series "a-d") within each column (indicating differences among average of bread dough samples at same sourdough type with same bran type and with different addition rate) by different uppercase letter series "A-D" within each column (indicating differences among average of bread dough samples at different sourdough type with same bran type and with same addition rate) and series "P-R" within each column (indicating differences among average of bread dough samples at same sourdough type with different bran type and with same addition rate) are significantly different at p<0.05.

#### 4. Acknowledgement

This study was supported by The Scientific Research Projects Coordination Unit of Selçuk University with the grant number of 18101022. The authors would like to thank Talha Demirci, Edibe Rabia Özkan and Sümeyye Demirci for their assistance in the isolation and identification of lactic acid bacteria.

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SJAFS

(2022) 36 (1), 98-105 e-ISSN: 2458-8377 DOI:10.15316/SJAFS.2022.014

#### **Research Article**

# Investigation of the Quality Characteristics of Naturally Cured Sucuks with Dill, Spinach and Swiss Chard Powders during Refrigerated Storage

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#### ARTICLE INFO

Received date: 02.04.2022

Accepted date: 13.04.2022

Article history:

**Keywords:** 

Curing agent

Nitrate

Nitrite

Sucuk

#### ABSTRACT

The purpose of this study was to analyze the R&D approaches of business The current study investigated the effects of dill, spinach and Swiss chard powders on the physicochemical (pH, TBARS, colour, residual nitrate and nitrite), microbiological (TMAB, LAB and total yeast-mould) and textural properties (TPA) of sucuks during refrigerated storage for 90 days. Five different groups of sucuk were prepared containing T1: 100 mg/kg sodium nitrite; T2: 100 mg/kg sodium nitrate; T3: dill powder 0.71%; T4: spinach powder 0.29% and T5: Swiss chard powder 0.26%. Swiss chard powder decreased the pH values of samples (P < 0.05). It was determined that the most effective curing agent in terms of TBARS numbers was spinach powder (T4). The residual nitrate was not detected in the groups of T4 and T5 all the refrigerated storage (P < 0.05). Curing with different vegetable powders did not affect the microbiological counts of sample (P > 0.05). Natural curing agents decreased the redness values of samples (P < 0.05). 0.05). The highest chewiness value was determined in the group of T5 (P < 0.05). These results suggest that Swiss chard and spinach powders could be recommended as a natural curing agent in the sucuks.

#### 1. Introduction

Curing in meat technology is defined as the addition of salt, nitrate and / or nitrite and various spices depending on the type of meat product (Sindelar et al., 2007). Nitrate and nitrite have been widely used in cured meat products as essential additives that inhibit pathogens (particularly against *Clostridium botulinum* and its spore germination), slow down the growth of other microorganisms, exhibit antioxidant effects, develop typical red curing color and flavor (Choi et al., 2017; Honikel, 2008; Majou and Christieans, 2018; Skibsted, 2011). Nitrate must be reduced to nitrite in order to have the stated effects (Sebranek and Bacus, 2007; Sindelar and Milkowski, 2012). However, when nitrate and nitrite are used in high concentrations in the production of cured meat products, N-nitrosamines some of which are toxic and carcinogenic compounds, can form in certain conditions (Honikel, 2014; Zarringhalami et. al., 2009). Thus, the meat processing industry searches for alternatives to solve this health risk associated with usage of nitrate and nitrite (Riel et. al., 2017). On the other hand, consumers interest in natural additives instead of synthetic additives in meat products. With the awareness of consumers, the demand for natural / organic products is increasing. In line with this demand, researches on the production and

development of natural/organic products are increasing day by day (Alahakoon et. al., 2015; Jayasena and Jo, 2013). Several studies have been conducted to meet this demand of consumers. Some of the studies are on the usage of natural antioxidants, essential oils, bacteriocins and spices as a substitute to nitrite. Nonetheless, since nitrite is a multifunctional additive, it is difficult to completely substitute with simple substances (Flores and Toldrá, 2021). Due to nitrate content of some plants at considerable amount (Gassara et. al., 2016), the use of nitrite from vegetables in processed meats as a curing agent without synthetic preservatives is the most promising method. A natural nitrate source and nitrate-reducing starter culture must be used in combination to produce typical cured meat properties (Sebranek and Bacus, 2007).

The among plant-derived nitrate sources, celery, spinach, radishes and lettuce have high nitrate content with more than 2500 mg /kg (Gassara et al., 2016; Schullehner et. al., 2018). There are many studies about the usage of especially celery products as curing agent in meat products (Horsch et al., 2014; Magrinya et al., 2009; Myers et al., 2013; Riyad et. al., 2018). However, it was reported that it has allergic compounds (Ballmer-Weber et al., 2002). Therefore, the potential use of different vegetable nitrate sources as curing agent in

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This study was part of the pHD thesis of Ali Samet BABAOĞLU.

meat products need to be investigated. Spinach (Spinacia oleracea), Swiss chard (Beta vulgaris var. cicla) and dill (Anethum graveolens) contain high level nitrate together with antimicrobial compounds and antioxidant components (Jiraungkoorskul, 2016; Pyo et. al., 2004; Riel et al., 2017). In the literature, there is no study regarding the use of dill as a natural nitrate source. Considering the investigations about spinach and Swiss chard, although the studies are present on their usage as a nitrate source (Kim et al., 2017; Nasonova and Tunieva, 2017; Riyad et al., 2018; Shin et al., 2017), there is no report related with usage of them as a curing agent in sucuk. Therefore, the objective of this study was to investigate the effects of dill, spinach, and Swiss chard powders on the quality characteristics of naturally cured sucuks and evaluated their effects by comparing them with sucuks containing synthetic sodium nitrite and sodium nitrate during refrigerated storage for 90 days.

#### 2. Materials and Methods

#### 2.1. Production of dill, spinach and Swiss chard powders

Fresh dill (Anethum graveolens L.), spinach (Spinacia oleracea) and Swiss chard (Beta vulgaris L. var. cicla) were purchased from a local market in Konya, Turkey. After the vegetables were washed, they were dried under natural laboratory conditions (at  $24\pm1$  °C for 84 hours). The dried vegetable powders were ground using a grinder (Arzum, Mulino, AR 151, Turkey) to obtain dill ( $5.74\pm0.01$  for pH), spinach ( $5.85\pm0.01$  for pH) and Swiss chard ( $5.10\pm0.01$  for pH) powders. The powders were sterilized for 2.5 hours at 115 °C in a dry heat sterilizer in order not to affect the microbial quality of the sucuks.

#### 2.2. Manufacture of sucuks and experimental design

Fresh boneless beef (Biceps femoris, Semitendinosus and Semimembranosus muscles) and beef fat were obtained from a local meat plant (Panagro Meat Plant) in Konya, Turkey. Beef meat and fat were initially ground through a 9-mm plate. The sucuk production was conducted in Panagro Meat Plant in Konya, Turkey.

Five different groups of sucuk were produced depending on the curing agents: Treatment 1 (T1), 100 mg/kg sodium nitrite (traditionally nitrite cured); Treatment 2 (T2), 100 mg/kg sodium nitrate; Treatment 3 (T3), dill powder 0.71%; Treatment 4 (T4), spinach powder 0.29% and Treatment 5 (T5), Swiss chard powder 0.26%. The formulations of the sucuks are given in Table 1. According to initially determined nitrate level in dill, spinach and Swiss chard powders used in this study, the addition levels of vegetable powders to sucuk formulations were corresponded to an amount of 100 mg/kg nitrate.

#### Table 1

Formulaton of sucuks showing five different treatments

Formulation		Т	reatments	5	
(g)	T1	T2	T3	T4	T5
Beef meat	100.00	100.00	100.00	100.00	100.00
Beef fat	34.60	34.60	34.60	34.60	34.60
Garlic	3.90	3.90	3.90	3.90	3.90
Spice mixture	4.40	4.40	4.40	4.40	4.40
NaCl	2.00	2.00	2.00	2.00	2.00
Dextrose	0.15	0.15	0.15	0.15	0.15
Ascorbic acid	0.03	0.03	0.03	0.03	0.03
Starter cul- ture*	0.05	0.05	0.05	0.05	0.05
<u>Curing</u> agents**					
Sodium nitrite (NaNO <sub>2</sub> )	0.01	-	-	-	-
Sodium nitrate (NaNO <sub>3</sub> )	-	0.01	-	-	-
Dill powder	-	-	0.71	-	-
Spinach pow- der	-	-	-	0.29	-
Swiss chard powder	-	-	-	-	0.26

\*Starter culture was added to sucuk batter at the level of 10<sup>7</sup> cfu/g. \*\*100 ppm sodium nitrite and sodium nitrate were added to T1 and T2 groups, respectively. Natural curing agents (dill, spinach, and Swiss chard powders) were added to sucuk batter at the level of 100 ppm nitrate equivalent.

T1: 100 ppm sodium nitrite (traditionally nitrite cured); T2: 100 ppm sodium nitrate; T3: dill powder 0.71%; T4: spinach powder 0.29%; T5: Swiss chard powder 0.26%.

For the preparation of sucuk batter, beef meat, beef fat, spice mixture, garlic, dextrose, salt and ascorbic acid were mixed in a grinder (Arı Machine, Turkey) and then selected starter cultures having nitrate reductase activity (mixture of Pediococcus pentosaceus and Staphylococcus carnosus; BFL-T03, Christian Hansen, Hoers Holm, Denmark) were added at a level of 10<sup>7</sup> CFU/kg of sucuk batter.

Each sucuk batter was stuffed into 38 mm collagen casings using a stuffer (Vemag, Maschinenbau, Germany). Sucuks were placed in climatic room for ripening under the following conditions: (1) at 24 °C and 90% relative humidity (RH) for 12 hours, (2) at 20 °C and 85% RH for 12 hours, (3) at 18 °C and 80% RH until the pH reached 5.2-5.3, (4) at 14 °C and 70% RH for 12 hours, (5) at 14 °C and 50% RH for 12 hours, (6) at 11 °C and 50% RH until the water content of sucuks reached 33-34% (end product). The air flow velocity was 0.5 m/s in all stages of the ripening period. Ready to eat sucuk samples were modified atmosphere packaged (MAP) and stored at 4 °C for 90 days. For MAP, sucuk samples were put into gas impermeable trays. Packages were evacuated, filled with a modified atmosphere containing 29.7% carbon dioxide, 0.3% oxygen and 70.0% nitrogen and automatically heat-sealed with a barrier film. The trays had a water vapor transmission rate 10 g/m<sup>2</sup>/24 h/at 38 °C/, 90% RH, 1 atm and oxygen transmission rate 2 cm<sup>3</sup>/m<sup>2</sup>/24 h/at 23 °C, 50% RH, 1 atm. The film had an oxygen transmission rate of 2 cm<sup>3</sup>/m<sup>2</sup>/24 h/bar at 23°C and 50% RH and a water vapor permeability of 10 g/m<sup>2</sup>/24 h at 23 °C and 90% RH.

In this study, all treatments were replicated independently twice. For each replicate, 50 sucuks were produced per treatment. Analyses of pH, TBARS, residual nitrate and nitrite were performed on days 0, 15, 30, 45, 60, 75 and 90. Color analyses were conducted on days 0, 30, 60 and 90. Microbiological analyses were performed on days 0, 45 and 90. Additionally, texture profile analyses (TPA) were conducted on day 0.

#### 2.3. pH measurements

The pH values of samples were determined throughout the refrigerated storage. The pH measurements were conducted with a portable pH meter (WTW Series pH 720, Weilheim, Germany) according to AOAC (2000).

#### 2.4. Determination of lipid oxidation

Thiobarbituric acid (TBARS) method described by Ockerman (1985) was used to determine the lipid oxidation of the sucuk samples. The absorbance of samples was read at 538 nm (UV-160 A, UV-Visible Recording Spectrophotometer, Shimadzu, Tokyo, Japan) against a reagent blank. The TBARS numbers were expressed as milligrams malonaldehyde per kilogram samples (mg MA/ kg sample).

#### 2.5. Residual nitrate and nitrite analyses

The residual nitrate and nitrite contents of the samples were determined according to Cortesi et. al. (2015). For determination of nitrate contents of samples, nitrate was reduced to nitrite by means of cadmium sulphate. Afterwards, nitrite was reacted with sulphanilamide with N-1-naphthylethylenediamine dihydrochloride (NED) and the resulting pinkish dye was measured with a spectrophotometer (UV-160 A, UV-Visible Recording Spectrophotometer, Shimadzu, Tokyo, Japan) at 540 nm. The residual nitrate and nitrite contents were calculated using standard curves of sodium nitrate and sodium nitrite solutions. The residual nitrate and nitrite contents were expressed as mg nitrate per kg sample (mg/kg) and mg nitrite per kg sample (mg/kg), respectively.

#### 2.6. Microbiological analyses

The microbiological analysis of samples was performed by following the procedure of Zhang et al. (2016) with minor modifications. 10 g of sucuk samples were hygienically transferred to the stomacher bags. Then, 90 mL of Ringer's solution (Ringer Tablet, Merck, Germany) was added and blended until a homogeneous mixture was obtained. For each sample, serial decimal dilutions were prepared with sterile Ringer's solution and 1 ml sample of the appropriate dilutions was transferred into selective agar plates. The enumeration of microorganisms was done on the plates, which contain the colonies between 30 and 300 after incubation for specific storage conditions (time, temperature, oxygen etc.). The results were expressed as log10 colony forming units per gram sucuk (log10 CFU/g). Total mesophilic aerobic bacteria (TMAB) were calculated by using Plate Count Agar (PCA, Merck, Germany) after incubation at 37°C for 48 h and then enumerated (Babuskin et al., 2014). The lactic acid bacteria (LAB) were cultured on Man-Rogosa-Sharpe (MRS) agar anaerobically incubated at 37°C for 72 h and then enumerated (Zhang et al., 2016). Yeast-mold were counted on Potato Dextrose Agar acidified by sterile tartaric acid (10 %) (Merck, Germany) incubated at 25°C for 5 days (Gökalp et. al., 1999). The total coliform bacteria medium Violet Red Bile agar (VRBA; Merck, Germany) on the plates was incubated at 37 °C for 24 h and then enumerated (Sagdic et. al., 2011).

#### 2.7. Texture profile analysis

Texture profile analyses of sucuks were conducted using the method of Crehan et. al. (2000) and Herrero et al. (2007). TPA was conducted in accordance with the two-compression method using a texture analyzer (TA-HD Plus Texture Analyser, UK). A cylindrical plate which has diameter of 20 cm and 50 kg load cell were used. The sample was compressed twice, with a 0.1-sec delay between the descents, pre-test speed of 1 mm/sec, test speed of 5 mm/sec, post-test speed of 5 mm/sec and compression of 50%. The following texture profile parameters were determined: hardness (N), adhesiveness (N.s), cohesiveness, springiness and chewiness (N). Sucuk samples were sliced at 1.5 cm height for texture analysis and analyses were performed as 3 parallel slices for each group.

#### 2.8. Colour measurements

Colour properties of sucuks were measured according to Hunt et al. (1991). Chroma meter CR-400 (Konica Minolta, Inc., Osaka, Japan) with illuminant D65, 2° observers, Diffuse/O mode was used for color determination.  $L^*$  (lightness),  $a^*$  (redness) and  $b^*$  (yellowness) parameters of the samples were determined. The measurements were carried out on the outer surfaces of the sucuk samples. Three readings were taken on different parts of outer surfaces for each sample.

#### 2.9. Statistical analysis

This study was conducted in two independent replicates with triplicate sampling and a completely randomized design was employed. A one-way analysis of variance (ANOVA) was performed for all variables (pH, residual nitrate, residual nitrite, TBARS, microbial counts, TPA and colour) by using MINITAB release 18.0 programme. The interaction between curing agent treatment and storage was also analyzed with two-way Anova using the GLM procedure.

The curing agent treatments (T1, T2, T3, T4 and T5) and storage days were analyzed as a fixed factor while the replicate was considered as a random factor. Tukey Multiple Comparison Tests were used to determine the statistical significance among the means at a 5% significance level.

#### 3. Results and Discussion

#### 3.1. pH values

Table 2 indicates the pH values of the sucuks during the refrigerated storage for 90 days. As the refrigerated storage progressed, the pH values of all samples decreased (P < 0.05). The lowest pH values of samples were determined on days 75 and 90 (P < 0.05). It is thought that this decrease in pH values during the refrigerated storage may be due to the activities of lactic acid bacteria in the sucuks (Rubio et al., 2007). While the pH value of the sucuks cured with spinach powder (T4) was higher than the other groups during the storage period, the pH values of the T5 group were the lowest (P < 0.05). The lower pH value ( $5.10\pm0.01$ ) of Swiss chard powder compared to other vegetable powders is thought to may be the reason for this situation. These results are in accordance with Shin et al. (2017) who describe that the use of Swiss chard powder decreases the pH values of pork patties. Similarly, red beet in meat emulsion (Choi et al., 2017) and fermented red beet extracts in frankfurters (Hwang et al., 2017) decreased the pH values of samples.

Table 2.

pH values, residual nitrate and nitrite contents of sucuks during refrigerated storage (Mean ± standard error)

Analyses	Storage periods (Day)			Treatments		
Analyses	Storage periods (Day)	T1	T2	T3	T4	T5
	0	5.25±0.01Aa	5.22±0.02 <sup>Ba</sup>	5.25±0.00 <sup>Aa</sup>	5.25±0.01 <sup>Aa</sup>	5.21±0.02 <sup>Ba</sup>
	15	$5.23{\pm}0.00^{Ba}$	5.19±0.01 <sup>Ba</sup>	5.23±0.01 <sup>Ba</sup>	$5.24{\pm}0.00^{Aa}$	5.20±0.01 <sup>Ba</sup>
	30	5.15±0.01 <sup>Bb</sup>	5.13±0.01 <sup>Bb</sup>	5.16±0.01 <sup>Bb</sup>	5.23±0.00 <sup>Aa</sup>	$5.18 \pm 0.00^{Bab}$
pH	45	$5.14 \pm 0.00^{Cb}$	5.14±0.01 <sup>Cb</sup>	5.13±0.01 <sup>Cb</sup>	5.23±0.01 <sup>Aa</sup>	$5.15 \pm 0.01^{Bab}$
<b>^</b>	60	5.13±0.02 <sup>Bc</sup>	5.13±0.01 <sup>Bb</sup>	5.13±0.00 <sup>Bb</sup>	5.22±0.01Aa	5.12±0.03 <sup>Bbc</sup>
	75	5.07±0.01 <sup>Bc</sup>	5.11±0.01 <sup>ABb</sup>	5.06±0.00 <sup>Bc</sup>	5.17±0.00 <sup>Ab</sup>	5.05±0.02 <sup>Bc</sup>
	90	$5.06 \pm 0.00^{Bc}$	$5.10{\pm}0.01^{ABb}$	$5.06 \pm 0.01^{Bc}$	$5.14{\pm}0.01^{Ab}$	$5.03 \pm 0.00^{Cc}$
	0	23.34±0.55 <sup>Aa</sup>	1.51±1.12 <sup>C</sup>	14.50±0.97 <sup>B</sup>	nd	nd
	15	22.22±0.48 <sup>Aa</sup>	nd	nd	nd	nd
	30	$8.27 \pm 0.45^{Ab}$	nd	nd	nd	nd
Residual nitrate (ppm)	45	8.19±0.51 <sup>Abc</sup>	nd	nd	nd	nd
	60	6.87±0.82 <sup>Abc</sup>	nd	nd	nd	nd
	75	4.44±1.15 <sup>Acd</sup>	nd	nd	nd	nd
	90	$1.30{\pm}0.51^{\rm Ad}$	nd	nd	nd	nd
	0	2.30±0.00 <sup>C</sup>	2.85±0.03 <sup>Ba</sup>	3.64±0.16 <sup>Aa</sup>	2.95±0.06 <sup>Ba</sup>	3.08±0.13 <sup>Ba</sup>
	15	2.23±0.06 <sup>B</sup>	2.59±0.04 <sup>Bab</sup>	3.59±0.24 <sup>Aa</sup>	$2.49 \pm 0.07^{Bb}$	2.26±0.03 <sup>Bb</sup>
	30	$2.49 \pm 0.07^{BC}$	2.39±0.03 <sup>BCbc</sup>	3.25±0.03 <sup>Aab</sup>	2.49±0.06 <sup>Bb</sup>	2.23±0.00 <sup>Cb</sup>
Residual nitrite (ppm)	45	2.43±0.13 <sup>B</sup>	2.36±0.00 <sup>Bbc</sup>	3.02±0.14 <sup>Aab</sup>	2.33±0.03 <sup>Bbc</sup>	2.23±0.00 <sup>Bb</sup>
*** /	60	2.65±0.23	2.36±0.00bc	2.88±0.13 <sup>ab</sup>	2.33±0.04bc	2.22±0.00b
	75	2.23±0.00 <sup>AB</sup>	2.20±0.10 <sup>ABc</sup>	2.62±0.13 <sup>Ab</sup>	2.06±0.03 <sup>Bc</sup>	$2.10\pm0.07^{Bb}$
	90	$2.49 \pm 0.07^{A}$	2.23±0.06 <sup>ABc</sup>	$2.59 \pm 0.10^{Ab}$	2.06±0.03 <sup>Bc</sup>	$2.10\pm0.07^{Bb}$

Within the same row, values with different uppercase superscript letters (<sup>A-C</sup>) indicate significant differences (P < 0.05). Within the same column, values with different lowercase superscript letters (<sup>a-c</sup>) indicate significant differences (P < 0.05).

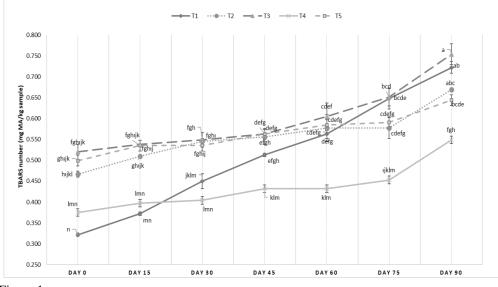
T1: 100 ppm sodium nitrite (traditionally nitrite cured); T2: 100 ppm sodium nitrate; T3: dill powder 0.71%; T4: spinach powder 0.29%; T5: Swiss chard powder 0.26 %.

#### 3.2. Lipid oxidation

Figure 1 shows the effects of different curing agents and refrigerated storage on TBARS numbers of sucuks. The curing with vegetable powders and refrigerated storage significantly affected the TBARS numbers of samples (P < 0.05). As expected, TBARS numbers increased as the refrigerated storage process progressed (P < 0.05) and the highest TBARS number were determined on day 90. The samples cured with spinach powder had the lowest the TBARS numbers, while the group of T3 had the highest lipid oxidation level compared to the other groups (P < 0.05). This situation is probably due to incomplete the reduction of nitrate to nitrite in the group of T3 group. Similar findings have been reported that Swiss chard powder inhibited lipid oxidation in the pork patties (Shin et al., 2017).3.3. Residual nitrate and nitrite contents of fermented sucuks

The residual nitrate and nitrite contents of sucuks are given in Table 2. In the production of sucuks (sucuk batter), 100 ppm nitrate was added to T2 T3, T4 and T5 groups and 100 ppm nitrite was added to T1. In ready-

to-eat samples, in other words, at the beginning of the refrigerated storage, the nitrate contents of T1, T2 and T3 were determined as 23.34, 1.51 and 14.50 ppm, respectively. Interestingly, although T1 cured with sodium nitrite (no addition of nitrate), the highest nitrate content was determined in this group on day 0 (P < 0.05). A possible explanation for this might be that nitrogen dioxide, which is formed as a result of the reduction of nitrate or nitrite, react with the water in the medium and it cause nitrate formation again (Pegg and Shahidi, 2008; Sebranek, 2009). Due to the completely reduction of nitrate in T4 and T5 groups, nitrate was not detected in these groups during refrigerated storage. In T2 and T3, nitrate was not detected on day 15 and after. In the T1 group, the nitrate amount decreased over time (P < 0.05) and the nitrate content was determined as 1.30 ppm on day 90. The results of the current study are consistent with those of Riel et al. (2017) who determined that Mortadella type sausages cured with sodium nitrite had the higher nitrate contents than samples cured with vegetable extract.



#### Figure 1

TBARS numbers of sucuks during refrigerated storage. T1: 100 ppm sodium nitrite (traditionally nitrite cured); T2: 100 ppm sodium nitrate; T3: dill powder 0.71%; T4: spinach powder 0.29%; T5: Swiss chard powder 0.26%.

Curing with different vegetable powders and refrigerated storage significantly affected the residual nitrite contents of sucuks (P < 0.05). As the refrigerated storage progressed, nitrite contents of samples (except for T1) were generally decreased (P < 0.05). T3 had the highest nitrite content at the beginning of the storage while the groups of T1 and T3 had higher nitrite contents than other group on day 90 (P < 0.05). It is thought that as a result of the reduction of nitrate in the group of T3 at the beginning of storage, the nitrite content is higher than the other groups (P < 0.05). The reason for the fluctuations in the nitrite contents of the T1 group during the refrigerated storage is the reduction of nitrate, which is present in high amounts at the beginning of storage, to nitrite over time. Curing with spinach and Swiss chard powders (T4 and T5) had the lowest residual nitrite contents on days 75 and 90 (P < 0.05). These observations are in accordance with Sindelar (2014) and Riel et al. (2017) who describe that residual nitrite contents are

lower in cured meat products with natural agents than in synthetic nitrite cured samples.

#### 3.4. Microbiological enumeration

Microbiological counts (log CFU/g) of sucuks during refrigerated storage are given in Table 3. Curing with different vegetable powders did not affect the TMAB, LAB, yeast and mould and total coliform counts of samples compared to control groups (T1 and T2) (P > 0.05). The refrigerated storage affected the TMAB and LAB counts of the sucuks (P < 0.05). The differences between the TMAB counts of samples were insignificant on days 0 and 45 (P > 0.05) while an increase was determined after 45 days in all groups (P < 0.05). It is thought that the progress of the storage period and the change of gas concentrations in the modified atmosphere package over time may be the reasons for the increase in the TMAB counts of sucuks

#### Table 3.

Microbiological counts (Log CFU/g) of sucuks during refrigerated storage (Mean ± standard error)

Microbiological analyses	Storage periods			Treatments		
(Log CFU/g)	(Day)	T1	T2	T3	T4	T5
	0	5.57±0.04 <sup>b</sup>	5.52±0.04 <sup>b</sup>	5.38±0.05 <sup>b</sup>	5.51±0.11 <sup>b</sup>	5.26±0.05°
Total mesophilic aerobic bacteria	45	5.41±0.01 <sup>b</sup>	5.50±0.01 <sup>b</sup>	5.44±0.04 <sup>b</sup>	5.44±0.03 <sup>b</sup>	5.50±0.01 <sup>b</sup>
-	90	$8.03{\pm}0.01^{a}$	$8.07{\pm}0.09^{a}$	$8.07{\pm}0.02^{\rm a}$	7.99±0.01ª	$8.03{\pm}0.03^{\rm a}$
	0	$7.97{\pm}0.02^{\rm b}$	7.73±0.09 <sup>b</sup>	7.84±0.02 <sup>b</sup>	7.96±0.05 <sup>b</sup>	7.99±0.04 <sup>b</sup>
Lactic acid bacteria	45	8.15±0.03ª	$7.97{\pm}0.03^{a}$	7.87±0.03 <sup>b</sup>	$7.90{\pm}0.15^{b}$	$8.09{\pm}0.02^{a}$
	90	$8.17 \pm 0.00^{a}$	$7.97{\pm}0.02^{a}$	$8.05{\pm}0.00^{\rm a}$	$8.06{\pm}0.02^{a}$	8.09±0.01ª
	0	ndg	ndg	ndg	ndg	ndg
Yeast-mold	45	ndg	ndg	ndg	ndg	ndg
	90	ndg	ndg	ndg	ndg	ndg
	0	ndg	ndg	ndg	ndg	ndg
Total coliform	45	ndg	ndg	ndg	ndg	ndg
	90	ndg	ndg	ndg	ndg	ndg

Within the same column, values with different lowercase superscript letters ( $^{a-c}$ ) indicate significant differences (P < 0.05) for each different microbial criteria. ndg: No detectable growth

T1: 100 ppm sodium nitrite (traditionally nitrite cured); T2: 100 ppm sodium nitrate; T3: dill powder 0.71%; T4: spinach powder 0.29%; T5: Swiss chard powder 0.26%.

It was determined that the LAB counts of sucuks increased with the progress of the refrigerated storage and the highest results were determined on the 90th day (P < 0.05). The yeast-mould and total coliform group bacteria growth were not detected in the samples during the refrigerated storage. It has been reported that the metabolites formed as a result of the activities of lactic acid bacteria and the decrease in pH play an important role in the inhibition of coliform bacteria (de Oliveira Mendonca, et. al., 2004).

On the other hand, it has been stated that nitrate/nitrite inhibits some microorganisms and pathogens that

Table 4.

Textural properties of the sucuk	s during refrigerated storage	(Mean $\pm$ standard error)
i enturui properties of the sucui		(1110411 - 50411441 - 61101)

cause deterioration in meat and meat products (Weiss et. al., 2010). Similarly, Bağdatli and Kundakci (2016) stated that there was no growth of coliform group bacteria in sucuks.

#### 3.5. Textural characteristics

Table 4 shows the textural characteristics of the sucuks. Curing treatment affected the hardness, cohesiveness and chewiness values of samples (P < 0.05) whereas the springiness and the adhesiveness were not (P > 0.05).

Taxtura paramatara			Treatments		
Texture parameters	T1	T2	T3	T4	T5
Hardness (N)	148.70±0.49 <sup>D</sup>	172.90±0.46 <sup>C</sup>	184.30±0.31 <sup>A</sup>	181.50±0.23 <sup>B</sup>	183.80±0.14 <sup>A</sup>
Adhesiveness (N.s)	4.23±0.52	$2.90\pm0.04$	$2.94{\pm}0.42$	$2.65 \pm 0.03$	3.14±0.22
Cohesiveness	0.398±0.01 <sup>AB</sup>	$0.401 \pm 0.00^{AB}$	$0.385 \pm 0.00^{B}$	$0.397 \pm 0.00^{AB}$	$0.406 \pm 0.00^{A}$
Springiness	$0.471 \pm 0.01$	$0.446 \pm 0.01$	$0.424{\pm}0.01$	0.431±0.02	0.463±0.02
Chewiness (N)	$27.93 \pm 0.75^{B}$	30.97±0.37 <sup>AB</sup>	30.04±0.56 <sup>AB</sup>	31.07±2.03 <sup>AB</sup>	34.64±1.29 <sup>A</sup>

T1: 100 ppm sodium nitrite (traditionally nitrite cured); T2: 100 ppm sodium nitrate; T3: dill powder 0.71%; T4: spinach powder 0.29%; T5: Swiss chard powder 0.26%.

Curing with vegetable powder increased the hardness values of the samples and the highest values were determined in T3 and T5 groups (P < 0.05). On the contrary, some studies indicated that the use of red beet powder in emulsified pork sausage (Jin et. al., 2014), the parsley extract powder in mortadella-type sausages (Riel et al., 2017) and the beetroot powder in Turkish fermented beef sausage (Sucu and Turp, 2018) did not affect the textural properties of samples.

A possible explanation for this might be the differences in the treatments (production conditions, addition level of the additives, the form of additive etc.) and sausage compositions (fat and water content) in different studies (Barbieri et. al., 2013). In addition, since the results are directly related to the texture analyser used and the device settings are not given in detail in the studies, it may not be very accurate to compare the differences between the studies (Riel et al., 2017).

#### 3.6. Colour properties

The effects of different curing agents on  $L^*$ ,  $a^*$  and  $b^*$  values of sucuks are presented in Table 5. Curing treatment did not affect the  $L^*$  values of samples (P > 0.05), except the day 60. The groups of T1 and T2 had the highest  $L^*$  values on day 60 (P < 0.05). In terms of refrigerated storage, the lowest lightness values were determined on day 0, and the  $L^*$  value of the samples increased with the progress of storage (P < 0.05).

#### Table 5.

	- f 1	1		(Manual and and a man)
Color characteristics	OI SUCUKS	auring reiri	gerated storage	(Mean $\pm$ standard error)

Analyses	Storage periods (Day)	Treatments					
		T1	T2	T3	T4	T5	
<i>L</i> *	0	27.90±0.72 <sup>b</sup>	25.50±0.61b	25.88±0.29°	27.56±0.10 <sup>b</sup>	29.33±0.74 <sup>b</sup>	
	30	39.23±0.67ª	40.14±0.31ª	38.09±0.19 <sup>ab</sup>	38.41±0.18 <sup>a</sup>	38.64±0.43ª	
	60	$40.25{\pm}0.72^{Aa}$	39.91±0.70 <sup>Aa</sup>	35.53±0.72 <sup>Bb</sup>	$38.25 \pm 0.08^{ABa}$	39.18±0.76 <sup>ABa</sup>	
	90	$40.09 \pm 0.44^{a}$	39.03±0.18ª	38.93±0.70ª	38.72±0.55ª	39.03±0.04ª	
<i>a</i> *	0	16.51±0.17 <sup>A</sup>	15.30±0.28 <sup>AB</sup>	12.53±0.23 <sup>B</sup>	12.86±0.01 <sup>B</sup>	12.13±1.19 <sup>B</sup>	
	30	14.22±0.02 <sup>A</sup>	12.15±0.03 <sup>AB</sup>	10.90±0.28 <sup>B</sup>	11.16±0.24 <sup>B</sup>	12.06±0.91 <sup>AB</sup>	
	60	14.25±0.31 <sup>A</sup>	12.75±0.06 <sup>B</sup>	11.83±0.20 <sup>B</sup>	11.96±0.09 <sup>B</sup>	11.91±0.19 <sup>B</sup>	
	90	$14.01 \pm 0.87^{A}$	13.30±0.49 <sup>AB</sup>	$10.79 \pm 0.45^{B}$	11.62±0.39 <sup>AB</sup>	$12.47 \pm 0.25^{AB}$	
<i>b</i> *	0	9.99±0.18	7.85±0.04 <sup>b</sup>	$8.01{\pm}0.04^{b}$	7.50±0.35 <sup>b</sup>	5.99±0.74	
	30	10.27±0.24	$8.16{\pm}0.06^{ab}$	$8.57{\pm}0.31^{ab}$	8.66±0.22 <sup>ab</sup>	8.26±0.73	
	60	11.26±0.91	$9.04{\pm}0.17^{ab}$	9.92±0.41ª	$9.82{\pm}0.00^{a}$	8.93±0.28	
	90	$10.63 \pm 1.50$	$9.81{\pm}0.36^{a}$	9.26±0.21 <sup>ab</sup>	$9.94{\pm}0.52^{a}$	8.33±0.70	

Within the same row, values with different uppercase superscript letters (<sup>A-B</sup>) indicate significant differences (P < 0.05). Within the same column, values with different lowercase superscript letters (<sup>a-c</sup>) indicate significant differences (P < 0.05). T1: 100 ppm sodium nitrite (traditionally nitrite cured); T2: 100 ppm sodium nitrate; T3: dill powder 0.71%; T4: spinach powder 0.29%; T5: Swiss chard powder 0.26%.

Similarly, Sucu and Turp (2018) reported that  $L^*$  values of the fermented beef sausages increased during refrigerated storage (P < 0.05). Natural curing treatment affected the redness values of samples (P < 0.05), but the effect of storage was not significant (P > 0.05). The

highest  $a^*$  values were determined in the group of T1 whereas T3 had the lowest. The curing with vegetable powders decreased the  $a^*$  values of the samples compared to the control groups. It is thought that this is probably due to the lower  $a^*$  values of the vegetablepowders.

In agreement with our results, Ko et. al. (2017) indicated that the use of young radish and vegetable powder caused a decrease in sausages. Different curing agents did not change the  $b^*$  values of the sucuks, while progress of refrigerated storage increased the yellowness of T2, T3 and T4. This result was in accordance with that Sucu and Turp (2018) of who put forth that beetroot powder did not change the  $b^*$  values of fermented beef sausages after day 0.

# 4. Acknowledgements

This study was funded by The Council of Higher Education's (YÖK) Academic Staff Training Program (OYP) Coordination Unit (Project Number 2016 OYP– 044). This study was part of the pHD thesis of Ali Samet BABAOĞLU.

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Selcuk Journal of Agriculture and Food Sciences

**Research Article** 

http://sjafs.selcuk.edu.tr/sjafs/index

SJAFS

(2022) 36 (1). 106-113 e-ISSN: 2458-8377 DOI:10.15316/SJAFS.2022.015

# **Determination of Morphological Characteristics of Some Prominent Tomato Genotypes**

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# ARTICLE INFO

# ABSTRACT

Article history: Received date: 05.11.2021 Accepted date: 19.04.2022

Keywords: Tomato Genetic resources PCA Morphological characterization This study was carried out to determine some morphological characteristics of 94 tomato genotypes at the S4 level and to reveal the relationships between these materials. In the study, leaf attitude, leaf length, leaf width, number of flowers, fruit color, fruit weight, fruit width, fruit length, the thickness of pericarp, fruit shape, fruit diameter, number of locules, and total soluble solid content (TTSC) were measured and observed in these genotypes. As a result of the phenotypic assessment, the maximum fruit weight values of the genotypes were observed as in G9 (317.59 g), G54 (310 g), G92 (292.85 g), G70 (287.01 g), and G110 (276.66 g); and the lowest fruit weight values were observed in G26 (18.302 g) and G8 (14.48 g). Average fruit length, fruit width, pericarp thickness, and the number of carpels were recorded (69.09 mm, 56.90 mm, 6.37 mm, and 4 carpels respectively). Tomato genotypes were also investigated using Cluster and Principal Component Analysis (PCA) method based on these measurements and observations. As a result of this analysis, five independent principal component axes were obtained. While these axes represent 69.28% of the total variation, the eigen values were ranged between 1.06 and 4.02. According to the PCA analysis results, genotypes G7, G81, G93, and G103 were prominent in terms of leaf length, fruit width, fruit weight, and carpel number parameters. Based on TSSC results, the G65 genotype was found to be the most prominent one, and the genotypes G12 and G114 exhibit promising results for fruit color. A high degree of morphological variation was detected among tomato genotypes.

# 1. Introduction

Tomato is one of the most important vegetable species with a high economic value which is a member of the Solanum genus of the Solanaceae family (Jenkins, 1948; Peralta et al., 2008). Today about 180 million tons of tomatoes are produced in an area of about 5 millions ha in the world. Turkey is among the three biggest tomato producers in the world with a 12.5 million tons production quantity (FAO, 2019). It also has an indispensable position in many countries' kitchens with its various usages. Tomato is a type of vegetable that is consumed fresh as well as frozen canned, tomato paste, ketchup, pickles, sauce, dried tomatoes, tomato juice, puree, and chopped (Günay, 2005). The high economic value of tomatoes has made it the subject of many researchers from cultivation to breeding. It is known that there is a constantly changing market in tomato breeding in Turkey and in the world. The main purpose of tomato production is yield and quality. For this, high genetic

performance is required together with appropriate ecology and appropriate techniques. This is only possible with hybrid varieties having superior qualities and performance. Factors such as yield, quality, durability, and adaptability also provide advantages in hybrid varieties (Kaloo, 1988). In breeding studies, it is important to know the variation among the parental materials in the studied gene pool in terms of hybrid performance (Gözen, 2008; Keskin, 2014). The traditional markers used to determine the relationships between plants are morphological markers. Considering tomato's morphological characterizations; Major traits such as fruit shape, fruit size, green ridge formation in fruit, or intensity of fruit color are in the foreground (Altıntaş et al., 2016; UPOV, 2013). Researchers carry out their studies by making some modifications to the UPOV criteria for their purposes (Kurt, 2019). The fact that the parameters examined in morphological studies are under the influence of many factors and that the properties of the objects subject to observation are related to each other

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causes many variables to be encountered. To find a solution to this problem, multivariate analysis methods have been developed by examining more than one feature at the same time (Tahtalı, 2005). In characterization studies, the cluster and principal component analysis are being done commonly using similarity and differences (Karaağaç and Balkaya, 2010).

Bhattarai et al. (2018), examined 21 plant and fruit characteristics in 91 tomato genotypes. A collection of 123 genotypes, which are characteristiced of the main fruit, has been evaluated over eighteen morphological properties. Morphological traits were subjected to principal component analysis and as a result of the analysis,18 morphological traits explained 46% of the total variation (Sacco et al., 2015). Singh and Aakansha (2015), found that the average fruit weight of 24 tomato genotypes was 47.16 to 112.50 g, fruit length was 30.8 to 60.6 mm, fruit width was 40.9 to 67.1 mm, the number of seed cavity in fruits varied from 2 to 11, and the amount of TSSC (Total Soluble Solid Content) ranged from 4.00 to 5.60% and they found that the differences among the genotypes were significant at the 0.05 level (Terzopoulos and Bebeli, 2010). In another study, a total of 61 local genotypes collected from Eskişehir and Bilecik locations were examined in terms of some morphological and phenological traits. The first three principal component vectors explained 62.8% of the total variation in the Eskişehir region and 55.66% in the Bilecik region (Sönmez et al., 2015). Kal et al. (2020) stated that as a result of principal component analysis with 77 cherry tomatoes, the total variance explained 16.8% in PC<sub>1</sub>, 12.6% in PC<sub>2</sub>, and 10.2% in PC3. Kıymacı (2021) examined the morphological characteristics of 240 tomato lines and the results were subjected to principal component analysis. Finally, in terms of 11 traits examined, the three components explained approximately 48.39 of the studies, the first component explained 24.1% of the total variance. In the present study, it was aimed to perform the morphological characterization of 94 tomato half-way breeding material in the S4 level and to reveal the existing variability in detail with multivariate analyzes.

# 2. Materials and Methods

In the experiment 94 tomato genotypes obtained as a result of crosses made with genotypes showing superior characteristics from a large genetic pool, were used by Selko-Tarim company, which carries out Ar-Ge studies on different vegetable species in Antalya. In the experiment, the seeds were sown on February 10<sup>th</sup>. 2020, and on March 15<sup>th</sup>, the five seedlings from each genotype were planted in the greenhouse in Antalya Aksu at (90x50)x50 cm intervals.

From seed sowing to greenhouse planting and harvesting, all cultural operations have been carried out regularly. The measurements and observations were taken at appropriate times and this morphological measurements and observations were given in Table 1 (UPOV, 2013). In the experiment, yield and fruit measurements taken from 94 tomato cultivar candidates were subjected to principal components analysis (PCA) in the JMP-14 computer package program. The distinctions between genotypes were determined by examining the Score Plot graph created in line with the components obtained because of the analysis.

#### Table 1

Measurements and observations made in tomato genotypes (UPOV, 2013)

Features	Value. ranges. measurement and ob- servations
Leaf attitude	Semi-erect(3), horizontal(5), semi- dropping(7)
Leaf length	Short(3), Medium(5), Long(7)
Leaf width	Short(3), Medium(5), Long(7)
Flower number of cluster	3-5(3), 6-10(5), more than 10 (7)
Fruit color	Light Pink(1), Pink(3), Light Red(5), Red(7), Dark Red(9)
Fruit weight (g) Fruit width (mm) Fruit length (mm) Thickness of peri- carp fruit shape	
Fruit shape	Slight Flattened(1), Round(3). Flat- tened(5), Vertical(7), Heart-shaped(9)
Fruit diameter	Slight Flattened(1), Round(3), Flat- tened(5), Vertical(7), Nonround(9)
Number of locules	
Total soluble solid	
content (TTSC)	

### 3. Results and Discussion

The measurements and observations made on 94 tomato genotypes at S4 level and the leaf and fruit results in these parameters are given below.

Leaf Traits: As a result of the evaluations it was determined that 43.6% of the genotypes were semi-drooping, 47.8% of the were horizontal and 8.5% of them were semi-erect in terms of leaf position. Leaf length values of the genotypes were determined as 19.1% short, 20.2% medium, and 61.70% long; in terms of leaf width, 32.9% of the genotypes had narrow leaves, 41.4% of them had medium leaves, and 26.5% of them had wide leaves. Although these different values are thought to be related to genetic diversity, ecological conditions and cultural practices are considered to be the partly affecting factors. Many studies have found different values in terms of leaf properties in tomatoes; Terzopoulos and Bebeli (2010) reported that 60% of the tomato genotypes had semi-erect leaves; Salim et al. (2020) observed 63.6% horizontal, 27.2% semi-upright and 13.6% semidrooping leaves; Çukadar and Dursun (2012) determined 12.5% short, 50% medium and 43.7% long leaves and 8.3% narrow, 22.9% medium and 68.7% wide leaves.

*Fruit Characteristics:* The maturity time of the fruits was determined as 55.3% medium, 10.6% late, and 34.0% early. In the genotypes evaluated, the color

of the fruit was 1.06% light pink, 39.36% pink, 22.3% light red, 35.1% red and 2.1% dark red. When fruit color is evaluated in different studies; Bhattarai et al. (2018) reported 89.5% red, 5.8% pink, and 4.7% yellow fruit color in tomato genotypes; Jin et al. (2019), observed 57.72% red, and 36.42% pink color; Çukadar and Dursun (2012) determined 2.08% pink, 97.92% red color; Mutlu et al. (2007) stated 1.12% yellow, 50.28% orange, 5.58% pink and 43.02% red color; Altıntaş et al. (2016) reported 1.6% orange, 25% pink, 73.4% red color. Tomato is a rich species for color diversity. On the other hand, easy hybridization with wild tomato species increases this color variance (Ayyıldız, 2017). In this context, different reports are seen in different literature.

Fruit shape and area are important both for the consumer and for transportation. In the present study, it was determined that there was a wide variation in fruit shape and area. When in longitudinal section of fruit shape was examined, the genotypes were classified slightly flattened as 37.2% round as 36.1%, flattened as 19.1%, vertical as 2.1%. and heart-shaped as 5.3%. When the fruit cross-section is examined; the genotypes were classified slightly flattened and flattened as 3.1%, rounded as 61.7%, not round as 31.9%. It has been determined that the number of flowers in the cluster is mostly from three to five. Salim et al. (2020) determined that the fruit shape was found 50% round, 9.10% heart, 31.82% flat, 4.54% elliptical and cylindrical in their tomato breeding lines. In another characterization study, Bota et al. (2014) reported that 50% of the tomato fruit shape was flat. 31% was round, and 19% was others in 171 local tomato genotypes. Bhattarai et al. (2018) reported that the tomato fruit shape was 60% flat, 6% slightly flattened, 1% very round, 8% round, 4% heart-shaped and 21% cylindrical. In the study of Keskin (2014), 11 of the parental tomatoes were round and 6 were not round, while in the hybrids 97 of them were not round, 39 of them were round. Ayyıldız (2017) determined that fruit cross-sectional shape od tomatoes was 80.55% round and 19.44% angular in 36 genotypes. In the study of Çukadar and Dursun (2012), the tomatoes fruit crosssection was determined as 77.08% round, 8.33% angular, 14.59% irregular. Since fruit shape trait is not affected by abiotic and biotic stress conditions, these different results are thought to be caused by the geneticially inherited variability among the genotypes.

The genotypes having the maximum fruit weight were determined as G9 (317.59 g), G54 (310 g), G92 (292.85 g), G70 (287.01 g), and G110 (276.66 g), while the genotypes having the lowest fruit weight were G26 (18.302 g), and G8 (14. 48 g). Oğuz (2010) found that the fruit weight values of 10 genotypes were 30 grams or less, 29 genotypes ranged from 30 to 100 grams, and 47 genotypes varied from 100 to 300 grams.

Ayyıldız (2017) determined the average fruit size as 30-100 g in 17 genotypes, 100-200 g in 8 genotypes, and 200-350 g in 11 genotypes. Our different findings from previously published results related to the fruit weight do not mean negative consequences. Because it is natural that there are differences in the genetic sources of

genotypes. It is also thought that these differences may be caused by differences such as cultivation conditions, variety and climate conditions.

Average fruit length, fruit width and pericarp thickness were measured as 69.09 mm, 56.90 mm and 6.37 mm, respectively. The number of carpels between genotypes varies. The average number of carpels was found in 4. Salim et al. (2020) reported that fruit length and diameter varied from 3.91 to 6.57 cm and from 3.63 to 8.15 cm, respectively, among the genotypes in their tomato characterization study. Kouam et al. (2018) and Yesmin et al. (2014) found those values as from 3.74 to 5.34 and from 3.64 to 5.71, respectively in their tomato characterization studies. In Figàs et al. (2014) tomato characterization study; they foud fruit weight as 2.7-511.6 g, fruit length as 1.88- 9.57 cm, fruit width as 2.15-11.40 cm, number of carpels as 2.00-18.33 and yield per plant as 292-2.851 g. Ayyıldız (2017) determined that 55.55% of the genotypes had usually 2 and sometimes 3 carpels, 41.66% of the genotypes had generally more than 4 carpels, and 2.77% of the genotypes had generally 3 carpels in the S6 level. Keskin (2014) observed in his research that the number of carpel ranged from 2 to 9. In the study of Keskin (2014), the wide range of carpels in tomato is proof that the number of carpels exhibits great variation within genotypes. The TTSC values in tomatoes is 5% on average, and can reach up to 6.5% at most. In the present study, the average TTSC in fruit was measured as 4.3%. Kavitha et al. (2014) determined that the TSSC values ranged from 3.5% to 14.5% in 54 tomato genotypes. In another study, Kathayat et al. (2015) reported that the TSSC values varied from 3.25% to 6.32%.

Principal Components Analysis: The principal component (PC) axes, eigenvalues, variation, and cumulative variation ratios were obtained as a result of Principal Component Analysis (PCA) and factor coefficients indicated the weight values of principal components based on features are presented in detail in Table 2. It has been stated that PCA analysis can be used effectively when the first two components explain more than 25% of the variation in the studies. (Mohammadi and Prasanna. 2003; Seymen et al.. 2019). As a result of the PCA analysis, five independent principal component axes were extracted concerning the 13 morphological characters. These axes represent 69.28% of the total variation. The eigen values of the first 5 basic components were found from 1.06 to 4.02. The eigenvalue 1 or greater means that the weight values of the principal component are reliable (Mohammadi and Prasanna, 2003). Özdamar (2004) reported that for factor coefficients to be reliable in principal component analysis, principal component axes should explain 2/3 of the total variation. When the analysis results are examined, it is seen that 2/3 of the total variation is more than explained by the first six principal component axes (69.28%). Therefore, these axes were taken into account in the evaluation of the analysis (Table 2). The first principal component axis accounts for 30.97 % of the total variation. The second and third principal

components cover 11.31% and 10.31% of the total variation, respectively. In other studies on tomato, 71% (Bernousi et al., 2011), 71.6% (Henareh et al., 2015), 74.63% (Bhattarai et al., 2016), 78.54% (Zhou et al., 2015) observation accounted for the total variation.

#### Table 2

Eigen value, variation and principal component axes of the properties examined as a result of principal component analysis

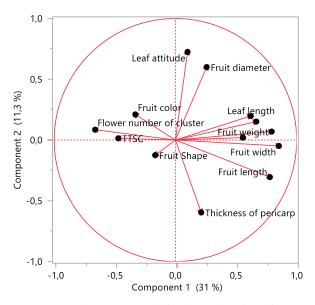
Eigen value         40.26         14.703         13.411         11.058         10.637           Variance%         30.97         11.31         10.316         8.506         8.182           Total         vari-         30.97         42.28         52.596         61.103         69.285           ance %             711.31         10.316         8.506         8.182           Total         vari-         30.97         42.28         52.596         61.103         69.285           ance %             711.31
Total         vari-         30.97         42.28         52.596         61.103         69.285           ance %
ance %         Traits         Prin1         Prin2         Prin3         Prin4         Prin5           Leaf         atti-         0.049         0.597         -0.014         0.396         -0.166
TraitsPrin1Prin2Prin3Prin4Prin5Leafatti-0.0490.597-0.0140.396-0.16
Leaf atti- 0.049 0.597 -0.014 0.396 -0.16
4
tude
Leaf length 0.309 0.163 0.322 -0.358 -0.204
Leaf width 0.277 0.016 0.301 -0.504 -0.21
Flower -0.33 0.070 0.466 0.153 0.085
number of
cluster
Fruit color -0.16 0.173 0.157 -0.291 0.723
Fruit weight 0.395 0.057 0.105 0.205 0.278
Fruit width 0.425 -0.040 0.021 0.123 0.212
Fruit length 0.388 -0.251 -0.029 0.215 0.034
Thickness 0.106 -0.491 0.243 -0.048 -0.05
of pericarp
Fruit Shape -0.08 -0.103 0.622 0.422 -0.19
Fruit diame- 0.128 0.494 0.089 -0.168 -0.17
ter
Number of 0.332 0.125 0.116 0.170 0.354
locules
TTSC -0.23 0.011 0.284 -0.093 0.180

Evgenidis et al. (2011), evaluated three hybrid and four standard tomato cultivars and their morphological characteristics by using cluster and principal component analyses; these cultivars explained 49.15% of the total variance in PC1, in PC2 and PC3 29.63% and 21.23%, respectively; and the hybrid cultivars strongly explained 62.93% of the total variance and 49.15% of the total variance in PC1 associated with yield-related traits such as yield components and yield stability. In another study; the principal component analysis was performed in 71 tomato genotypes, 5 independent principal component axes were obtained regarding the properties examined; and the researchers stated that these axes explained more than 92% of the total variation. Based on this analysis, it is reported that certain fruit characteristics may be important for breeding programs according to consumer demands (Krishna et al., 2016). In a study of tomato breeding lines, they obtained six independent principal component axes for 17 identification traits. They reported that these axes explained 63.35% of the total variation (Jin et al., 2019), Kal et al. (2020) worked with 77 cherry tomatoes, they reported that the total variance explained 16.8% in PC1, 12.6% in PC2, and 10.2% in PC3. Using PC1 and PC2 components, a loading plot was created to examine the interrelationship among the traits. It has been reported that if the angle between the vectors in the figure is  $<90^{\circ}$ , there is a positive relationship, if it is  $>90^\circ$ , there is a negative relationship, and if the angle between the vectors is 90°, there is no significant relationship (Danin-Poleg and Reis, 2001; Seymen et al.,

2019). When the figure 3 is examined, the highest correlation was found between leaf length, fruit width, fruit weight, and carpel number. On the other hand, the highest negative correlation was found between leaf attitude and thickness of pericarp.

# Figure 3

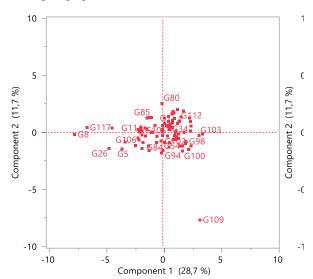
Loading plot graph obtained from PC1 and PC2 as a result of PCA



A score plot was created for the evaluation of 94 tomato lines using PC1 and PC2 components (Figure 4). A score plot was created to evaluate 94 tomato genotypes using PC1 and PC2 components (Figure 4). When the Figure 4 is examined, the genotypes G7, G81, G93 and G103 emerged as the genotypes revealing the best performance associated with leaf length, fruit width, fruit weight, carpel number parameters, which were important in PC1. G65 genotype was found to be significant in terms of the TSSC parameter, while G12 and G114 genotypes were found to be significant in terms of fruit color parameters.

#### Figure 4

Score plot graph obtained from PCA result PC1 and PC2



#### 4. Conclusion

The morphological and agronomic properties of the 94 tomato genotypes in the S4 stage have been evaluated and the relations between these characteristics have been interpreted in this study. As a result of the study, it was revealed that there are some differences in the morphological features obtained from plants and fruits. As a result of the evaluations, the genotypes having the highest fruit weight were G9 (317.59 g), G54 (310 g), G92 (292.85 g), G70 (287.01 g) and G110 (276.66 g), respectively; while the genotypes having the lowest fruit weight were G26 (18.302 g) and G8 (14.48 g), respectively. Average fruit length, fruit width and pericarp thickness, number of carpels were measured as 69.09 mm, 56.90 mm, 6.37 mm and 4 carpels, respectively. Tomato genotypes were investigated using Cluster and Principal Component Analysis (PCA) method based on these measurements and observations. As a result of the analysis, 5 independent principal component axes were obtained. While these axes represent 66.53% of the total variation, the eigen values were ranged from 1.02 to 3.73. According to the PCA analysis results, genotypes G7, G81, G93 and G103 were prominent in terms of leaf length, fruit width, fruit weight and carpel number parameters, respectively. When the TTSC parameter was examined, the G65 genotype came to the fore; and the G12 and G114 genotypes gave the best results in terms of fruit color. Morphological variability was determined to be high among the studied tomato genotypes.

## 5. Acknowledgements

The authors declare that they have no known competing financial interest that could have appeared to influence the work reported in the paper. Thank you Selko Arge Biotechnology LTD, whose greenhouses we use in Antalya. Autor Necibe Kayak is a 100/2000 the Council of higher education PhD Scholar in the Sustainable Agriculture subdivision.

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# 7. Appendices

Table 3 Leaf and fruit characteristics of tomato genotypes

Genotip adı	Leaf at- titude	Leaf width	Leaf length	Flower of clus- ter	Fruit maturity	Fruit color	Genotip adı	Leaf at- titude	Leaf width	Leaf length	Flower of clus- ter	Fruit maturity	Fruit color
G1	7	3	3	3	5	3	G66	3	7	7	3	7	5
G2	7	3	3	3	5	3	G67	7	3	7	3	7	3
G3	5	3	5	3	5	7	G69	5	3	5	3	7	3
G4	5	3	3	3	5	3	G70	7	7	7	3	3	3
G5	3	3	3	3	3	7	G72	5	7	7	3	3	3
G6	5	3	7	3	5	3	G74	3	7	7	3	3	5
G7	5	5	5	3	5	3	G76	7	3	7	3	5	5
G8	5	3	3	7	3	9	G77	7	7	7	3	3	3
G9	5	7	7	3	3	7	G79	7	3	3	7	3	7
G12	5	3	3	3	3	7	G80	7	3	7	3	7	7
G14	7	5	7	3	3	5	G81	5	7	7	3	5	3
G15	7	7	7	3	5	3	G82	5	3	3	3	3	7
G19	7	5	7	3	5	5	G83	5	7	7	3	7	7
G20	7	5	5	3	5	3	G84	5	7	7	5	5	3
G21	7	3	3	5	5	3	G85	7	5	5	3	5	7
G22	5	5	7	3	5	3	G87	5	5	7	3	3	3
G23	7	5	5	3	5	5	G88	5	5	7	3	5	3
G24	5	3	3	3	5	5	G90	7	7	7	3	5	1
G25	7	5	7	3	3	5	G91	5	5	5	3	3	7
G26	5	3	3	7	3	7	G92	7	3	7	3	3	5
G27	7	7	7	3	5	3	G93	5	7	7	3	5	3
G28	7	5	7	3	7	3	G94	5	5	5	3	3	5
G29	5	5	7	3	3	7	G95	5	5	7	3	5	3
G30	3	5	7	3	3	7	G97	5	7	7	3	5	7
G32	7	3	7	3	5	3	G98	5	5	7	3	3	7
G33	5	5	5	3	5	7	G99	5	7	7	3	5	7
G34	5	5	7	3	5	5	G100	5	7	7	3	3	7
G35	7	5	7	3	5	5	G101	5	7	7	3	5	7
G36	5	3	3	3	3	7	G102	7	7	7	3	3	3
G40	5	5	5	3	5	7	G103	7	7	7	3	5	3
G42	5	5	5	3	5	3	G104	7	7	7	3	5	5
G46	7	3	7	5	5	7	G105	7	3	7	5	5	5
G50	5	7	7	3	3	3	G106	5	5	5	3	3	3
G51	7	5	5	3	3	3	G107	5	5	7	5	7	7
G52	5	5	7	3	3	3	G108	7	5	7	3	5	5
G53	7	5	5	3	5	7	G109	3	7	7	3	3	3
G54	3	5	7	3	5	7	G110	7	3	7	3	5	7
G55	7	5	5	3	5	7	G111	5	5	5	3	3	7
G56	5	3	7	3	5	3	G112	7	5	5	3	3	7
G57	5	3	3	3	5	3	G113	5	5	7	5	5	5
G58	3	5	7	3	5	7	G114	5	3	3	3	7	5
G59	7	5	5	3	3	7	G115	5	3	7	3	3	5
G60	5	3	3	3	5	3	G117	7	3	3	7	3	9
G62	5	3	3	3	5	5	G119	7	5	7	3	5	7
G63	7	5	7	3	5	7	G120	, 7	5	5	3	3	3
G64	7	7	, 7	3	5	, 7	G120	, 7	7	7	3	5	7
G65	3	3	3	3	5	5	G124	, 7	5	, 7	3	5	5

# Table 4

Fruit characteristics in tomato genotypes

Genotype Name	Fruit Weight (gr)	Fruit Width (mm)	Fruit Legth (mm)	Thickness Of Pericarp (mm)	Fruit Longitudinal Section	Fruit Cross Section	Number Of Lo- cules	TTSC
G1	197.29	76.8±4.32	60.5±4.02	4.7±0.30	1	3	5	4.1
G2	227	77.4±1.69	64.3±1.17	6.9±0.03	3	3	5	4.2
G3	152.02	65.3±0.08	50.0±2.11	5.6±0.29	1	3	5	6.3
G4	181.96	68.1±2.86	62.6±0.76	5.5±0.24	3	3	4	3.6
G5	109.8	54.7±1.52	50.0±1.71	$6.4{\pm}0.60$	3	3	2	4
G6	134.28	61.4±2.11	$57.6 \pm 4.08$	4.2±0.71	3	5	4	5.81
G7	282.25	81.3±11.62	62.3±6.20	$5.4{\pm}1.08$	5	9	7	4.1
G8	14.49	27.8±1.93	25.7±1.27	3.0±0.26	7	3	2	7
G9	317.6	77.6±5.69	64.1±0.45	6.7±0.76	5	3	6	4.2
G12	168.71	65.9±3.61	53.8±2.20	$4.8 \pm 0.47$	1	9	4	5.5
G14	182.22	70.0±7.04	52.1±3.20	6.6±1.99	5	9	6	5.1
G15	140.99	63.2±5.32	58.3±2.91	5.2±1.35	3	9	4	2.7
G19	139.18	64.9±4.83	63.5±2.29	5.5±0.21	3	3	4	4.9
G20	172.99	63.4±3.71	57.1±4.6	$5.6 \pm 0.56$	1	3	5	4.2
G21	145.28	70.6±4.30	61.2±1.02	5.5±1.57	3	3	3	5.9

G22	168.03	71.60±2.99	63.9±1.71	5.4±1.13	3	3	4	4
G23	177.97	66.42±10.23	63.1±6.02	5.3±0.27	3	3	6	3.6
G24	154.13	$60.4 \pm$	56.6±	$6.4\pm$	3	3	3	3.8
G25	297	75.95±2.41	51.5±1.39	$4.0\pm0.02$	1	9	6	4.4
G26	18.3	57.1±0.81	51.9±0.91	$4.8 \pm 0.50$	9	3	2	6.8
G27	161.5	$65.5 \pm 8.05$	55.2±2.71	$5.0\pm0.43$	3	5	4	3.9
G28	279	65.3±1.97	55.4±2.41	5.1±2.03	5	9	5	3.1
G29	78.31	65.0±3.09	50.3±3.57	4.5±1.54	3	9	2	4.9
C20		71.0 2.2.25	52 0 1 21	4 (10.24	2	2		
G30	104.71	71.8±3.25	53.9±1.31	4.6±0.24	3	3	3	4.1
G32	166.3	72.7±3.90	55.0±2.76	$4.8 \pm 0.30$	1	3	7	3.8
G33	214.79	70.6±0.33	54.0±1.76	$4.8\pm0.22$	1	3	5	4.1
G34	111.5	72.2±7.73	58.0±6.03	5.8±0.28	1	9	5	3.4
054	111.5	12.2±1.13	38.0±0.03		1			5.4
G35	248.73	70.6±3.27	57.0±0.14	6.3±1.18	1	3	5	3.2
					-			
G36	118.58	69.3±5.27	56.8±3.71	6.3±0.79	3	1	3	4.7
40-1	204.46	74.9±5.04	58.5±1.19	5.9±0.29	5	9	6	4.9
42-1	255	74.1±	64.3±	$6.5\pm$	1	3	4	3.6
46.1	197	$64.8 \pm 0.68$	52.6±0.51	5.7±0.62	1	3	6	
46-1	197	04.8±0.08	52.0±0.51	$3.7\pm0.02$	1			3.1
G50	106.34	55.2±6.53	47.8±0.19	6.3±0.14	1	3	2	4.6
G51	94.18	55.9±4.83	50.0±3.53	5.2±0.26	1	3	3	3.8
G52	150.74	63.0±2.29	52.3±2.39	5.8±1.24	3	3	5	3.1
G53	200	70.5±4.92	55.2±1.52	$5.9\pm0.55$	1	9	6	3
G54	310	79.6±5.70	62.6±3.46	5.6±1.42	1	3	6	5.5
G55	187.25	71.9±0.63	56.2±3.23	8.1±1.17	1	3	4	3.8
		71.2+6.00			9	9	4	
G56	204.73	71.3±6.90	63.8±2.99	$6.8 \pm 0.55$				2.8
G57	138.5	63.7±3.07	55.3±1.48	6.6±1.57	3	3	4	4.2
G58	110.62	63.7±7.14	55.9±3.26	6.2±1.12	3	3	2	5.9
G59	81.85	56.1±3.89	49.8±2.06	5.2±0.25	3	3	4	3.8
G60	136.91	63.9±13.49	51.2±4.67	$5.6 \pm 0.46$	1	3	3	4.4
G62	128.33	62.4±7.84	49.6±4.89	4.5±0.98	1	9	5	3.4
G63	167.79	71.6±71.56	60.0±1.47	$4.6\pm0.40$	1	3	6	4
G64	246	71.1±	59.5±	$6.4\pm$	1	9	4	3.8
G65	90.45	52.6±1.20	47.5±1.22	7.0±0.34	3	3	2	3.3
G66	158.46	68.8±5.16	56.7±1.40	7.1±1.14	3	3	3	2.9
G67	105.76	59.1±1.21	51.2±3.00	$6.2 \pm 0.08$	3	3	3	4.1
G69	243.3	88.1±	$65.2\pm$	$4.8\pm$	5	3	4	4.4
070		77.4±	70.1	(7)	3	1	6	
G70	287.01	//.4±	70.1±	6.7±	3	1	6	5.5
G72	186.57	69.7±4.45	58.6±3.22	5.5±0.46	3	3	4	4.3
G74	162.26	65.2±4.97	55.1±5.02	5.8±1.23	1	3	5	4.9
G76	190.76	69.6±7.36	61.9±2.98	6.3±0.65	1	9	5	4.3
					-			
G77	167.75	71.9±3.20	$50.9 \pm 2.00$	$4.2\pm0.84$	1	9	4	4.4
G79	86.33	51.3±0.72	42 1 1 5 1	5.1±0.38	3	3	3	6.5
			43.1±1.51					
G80	128.4	69.6±3.10	53.2±3.01	$5.2 \pm 1.00$	1	9	5	4.5
G81	232.75	77.2±6.76	56.7±4.87	5.7±1.09	5	9	5	4.3
G82	136.46	62.4±4.26	53.2±3.01	5.3±1.48	3	3	4	3.5
G83	259.69	85.0±0.17	61.6±2.95	$5.3 \pm 0.65$	5	9	5	4.7
G84	188.83	73.4±2.49	56.3±4.37	5.3±0.47	3	3	3	6.3
G85	264.74	57.6±2.22	$41.8 \pm 4.06$	$6.2 \pm 0.29$	3	5	4	3.8
				$5.4 \pm 1.16$	1	9	2	
G87	176.89	67.7±4.15	62.7±3.34	5.4±1.16	1		3	5.9
G88	232.27	77.7±7.27	64.0±1.39	$5.8 \pm 0.57$	1	3	4	4.6
					-			
G90	195.11	78.6±10.20	52.0±1.53	4.2±0.79	5	9	5	5.2
G91	184.5	$71.0\pm 5.40$	$58.9 \pm 1.14$	$6.8 \pm 0.86$	3	3	2	5.3
					5		2	
G92	292.86	70.9±17.47	58.1±8.31	7.1±0.82	5	3	5	5.5
G93	219.81	88.2±8.70	77.1±3.26	6.0±0.29	1	9	4	3.4
G94	214.94	76.2±2.60	64.4±4.73	$6.4 \pm 1.08$	3	1	3	4.4
C05	165.92	75 7 11 52	60 8 2 17		5	3	5	2.4
G95	165.83	75.7±11.53	60.8±2.17	5.1±0.74				3.4
G97	206.29	$74.9 \pm 2.04$	60.1±2.92	5.0±0.43	1	3	6	5.4
					F			
G98	258.26	83.4±7.29	56.9±5.43	$3.8 \pm 0.58$	5	3	9	4.1
G99	186.72	69.7±3.32	55.2±0.49	6.8±0.17	1	9	3	4.1
					-			
G100	274.31	86.0±3.26	60.2±1.55	8.0±0.59	5	3	5	4.8
G101	144.94	70.5±2.84	52.4±2.93	6.1±0.99	1	9	6	5.4
G102	213.22	$72.9 \pm 4.00$	55.4±2.29	3.6±0.45	5	3	5	4.01
G103	276.75	$86.0\pm$	64.3±	6.3±	5	3	7	4.8
G104	226.83	68.7±8.72	56.0±1.57	6.1±0.96	5	3	5	3.9
	176	62.5±3.44	60.7±2.64	7.5±0.14	9	9	2	4.7
G105								
G106	96.8	56.0±4.90	$47.0 \pm 4.57$	$4.8 \pm 0.30$	3	3	4	4.1
G107	196	72.9±11.68	57.2±0.31	6.8±0.19	3	3	3	2.8
G108	174	74.1±9.50	58.7±0.81	3.9±0.56	1	9	4	3.6
G109	219	$70.2 \pm$	$56.42 \pm$	$70.2 \pm$	5	3	7	3.7
G110	276.67	78.6±0.40	53.9±0.57	$4.8 \pm 0.48$	1	9	6	4.7
G111	242	76.5±2.35	68.7±2.42	7.3±2.57	3	3	6	2.9
					1	9		
G112	261.67	79.2±3.25	63.4±1.77	5.8±0.42			4	2.7
G113	227.67	78.7±4.34	63.7±2.58	5.4±0.39	9	3	6	2.6
G114	158	$66.2\pm$	$58.4\pm$	$5.6\pm$	1	3	3	5.5
G115	156.75	67.1±6.85	59.1±4.93	7.1±0.39	3	3	4	3.9
G117	22.44	31.8±1.70	34.8±2.02	$3.8 \pm 0.58$	7	3	2	6.2
G119	264	87.5±1.09	58.9±4.6	9.2±0.07	5	9	6	3.9
G120	152.75	63.0±0.42	57.1±2.49	5.6±0.47	9	9	3	4.5
G122	159.67	$80.8\pm$	65.7±	$6.0\pm$	1	9	6	3.5
G124	214.5	72.6±3.17	59.7±2.02	6.7±0.09	3	3	3	3.8

Selcuk Journal of Agriculture and Food Sciences

http://sjafs.selcuk.edu.tr/sjafs/index

**Research Article** 

SJAFS

(2022) 36 (1), 114-119 e-ISSN: 2458-8377 DOI:10.15316/SJAFS.2022.016

# The Effect of Dried Peach Leaves Powders with Different Methods on Lipid Oxidation, Textural and Sensory Properties of Patties

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# ARTICLE INFO<sup>1</sup>

# ABSTRACT

Article history: Received date: 31.12.2021 Accepted date: 17.04.2022

Keywords: Peach leave Beef Oxidation Microwave The aim of this study was to determine the effects of the dried peach leaves powders with different methods on pH and Thiobarbituric acid reactive substances (TBARS) values, cooking, textural and sensory properties of beef patties. Samples were divided into six treatment groups; control (without peach leaf powder/ Butylhydroxytoluene (BHT)), patties with BHT (0.01%), patties containing peach leaf powders (PLP) dried in air (AP) (%1) and in microwave oven (MwP) in three different concentrations (0.5%, 1%, 2%). Sample were stored at +4 °C for 7 days. The pH of raw beef patties containing various levels of PLPs decreased slightly (P < 0.01). The MwP addition significantly decreased (P < 0.01) the TBARS value compared to the without peach leaf powder. At the end of the storage period, the TBARS value of MwP3 was 0.67 mg MDA/kg.

# 1. Introduction

Meat products can be spoiled in two different ways with chemical and microbiological deterioration. The most common chemical degradation in meat products is oxidative rancidity (Kanner 1994). The occurrence of oxidation in meat fat causes quality to deteriorate in meat products, resulting in reduced shelf life and changing in meat quality properties (Fernandez et al. 1997). Owing to their high contents of protein, fat and free water and large surface area (Tamkutė et al. 2021), beef patties are prone to oxidative deterioration.

Using antioxidants in meat and meat products have inhibited or reduced the lipid oxidation. There are many compounds with antioxidant effects, but only a few can be used in food products. The antioxidants may be of synthetic origin and /or of natural origin (Powell et al. 1986). However, since synthetic antioxidants cause toxic effects, the idea of natural antioxidants has increased (Shah et al. 2014). For this reason, studies related to identifying new antioxidants from plant sources because of the high content of phenolic compounds is carried out and provide alternative to currently used conventional antioxidants (Karre et al. 2013). Natural sources of antioxidants may be classified as plant and spice-derived antioxidants, fruit and vegetable-derived antioxidants, and others (Rather et al. 2016).

Peach (*Prunus persica* L.) is a garden plant belonging to the family Rosaceae, grown for its fruits (Gur 2011). Besides the nutritional and pharmacological value of peach fruits, peach leaf is traditionally used as sedative and laxative. It is also reported that peach leaves contain some phenolic compounds such as flavonols, hydroxycinnamates, caffeic acid, quercetin, isoquercetin, cynic acid, tannin, kaempferol, pruzic acid and ursolic acid (Upyr & Komissarenko 2002, Mokrani et al. 2019). Peach leaves have also antibacterial effect against to many pathogens, such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *Listeria monocytogenes*. (Özpınar et al. 2013).

Drying is one of the most important technique and different drying methods such as freeze, microwave, vacuum has been commonly practiced in food technology. Nowadays, hybrid drying techniques have been used to combined with different non thermal methods, air drying method is still preferred due to its low cost and wide applicability (Srikanth et al. 2018).

However, microwave drying can be an advantageous method for food technology. This method promotes short drying time and enhances quality (Carvalho et al. 2021). This study was aimed to determine the influence of beef patties with peach leaf powders dried in air (AP) (%1) in microwave oven (MwP) on the lipid oxidation, color stability, cooking and sensory properties.

### 2. Materials and Methods

## Materials

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Peach leaves (*Prunus persica L.*) were taken from a farmer (Konya, Turkey). The leaves were washed with distilled water and dried with two drying methods. In air drying procedure, the peach leaves were dried on filter papers at 23 °C approximately 7 days. Natural current of air was used for drying the leaves (Mbah et al, 2012). Second method was microwave process which were carried out in 650 W output power and 210-270 s power density (Alibaş 2012; Yaldız Cabi and Sarıçoban, 2019). To make powder, dried peach leaves were separately passed through a blender (Waring Commercial Blendor<sup>®</sup>, USA) for 45 s. Then, all peach leaf powders were placed in separate opaque glass containers and stored at +4 °C until use.

# Preparation of patty

Fresh beef was obtained from a local market at 48 h post-mortem. All subcutaneous and connective tissue were removed from the meat. The meat and fat were minced through a 3 mm plate grinder and mixed with salt 1%). Peach leaf powders (PLPs) dried in microwave oven were added at 0%, 0.5%, 1%, 2% (Control, MwP1, MwP2, MwP3, respectively) and ground peach leaves dried in air (AP) at 1%. As the positive control group, the synthetic antioxidant Butylhydroxytoluene (BHT) was used at a rate of 0.01%. All ingredients were mixed as seperataly, shaped into approximately 4 cm diameters and 1.5 cm thick with a weight of 17–18 g. The patties were placed on plastic container and stored at  $4\pm0.5$  °C. The analyses were carried out on 1., 3., 5. and 7. days of storage. Sensory and, texture analysis and cooking properties were performed on the 3<sup>rd</sup> day.

#### pH value

pH values were measured by a pH meter (pH 3110/SET WTW, Germany). pH value was measured three times for each sample (Ockerman 1985).

## Phenolic compounds in AP and MwP

The individual phenolic compounds in AP and MwP samples were detected using Shimadzu-HPLC (Shimadzu Corporation, Kyoto, Japan) equipped with a PDA detector (set at 280 and 330 nm). In brief, 20 µl of samples and authentic external standards of phenolic compounds were separately injected into the column. The injection volume was 20 µl and the flow rate of the mobile phase was 1 ml/min at 30 °C. The total run time per sample was 60 min. A mixture of mobile phase was consisted of 0.05% acetic acid in water (A) and acetonitrile (B). The gradient programme was employed: 0-0.10 min 8% B; 0.10-2 min 10% B; 2-27 min 30% B; 27-37 min 56% B; 37-37.10 min 8% B; 37.10-45 min 8% B. The chromatograms were recorded at 280 nm. The standard external method was used for the quantitative analysis (Babiker et al. 2021).

# Determination of cooking yields and dimensional shrinkage

Cooking yields and dimensional shrinkage were determined according to the method specified by Jones et al. (1992) and Murphy et al. (1975) as in the following:

$$cooking \ yield(\%) = \frac{weight \ of \ cooked \ patties}{weight \ of \ raw \ patties} x100$$
$$(RT - CT) + (RD - CD)$$

$$DS(\%) = \frac{(RT - CT) + (RD - CD)}{RT + RD} X100$$

- DS : Dimensional shrinkage
- RT : Raw thinckness
- RD : Raw diameter
- CT : Cooked thickness
- CD : Cooked diameter

#### Thiobarbituric acid reactive substances (TBARS) value

The oxidative rancidity of the samples was measured as thiobarbituric acid reactive substances (TBARS) according to Tarladgis et al. (1960). The results were read in a Spectrophotometer (UV- 160 A, UV- Visible Recorder) and calculated as mg malondialdehyde/kg patties.

#### Texture profile analysis (TPA)

Patties were shaped into (36 mm diameter, 13 mm height, 20 g weight). The textural properties (chewiness, hardness, cohesiveness, adhesiveness and springiness) the samples were measured by using a texture analyser with 50 kg load cell and 36 mm diameter probe (Stable Micro Systems TA.HD Plus, TA, Stable Microsystems Godalming, Surrey, UK) (Palamutoğlu and Sarıçoban, 2016).

#### Sensory properties

The samples were evaluated for colour, odour, flavour and taste, texture, appearance, and general acceptability. The patties were cooked in a pre-heated grill for 6 min each side (to give an internal temperature of  $72 \pm 2$  °C), then cooled to  $30\pm1$  °C and served to the panellist on a white plate. Samples were tasted in one session per day and by 10 people per session. Samples were served randomly to the panellists. All semi-trained panellists were between 20 and 30 years old. Panellists evaluated the sensory analyze using a 9-point hedonic scale (1 = dislike extremely, 9 = like extremely) (Yıldız Turp & Serdaroğlu 2010).

#### Statistical analysis

Each parameter was performed two replication and the measures were performed in triplicate. One-way analysis of variance was performed for all variables, using the MINITAB for Windows Release 18. Tukey Multiple Comparison Tests were used to determine the differences among the means at a 5% significance level. The results were presented as the mean values  $\pm$  standard error.

### 3. Results and Discussion

#### Phenolic compounds of peach leaves

The phenolic compounds of AP and MwP are given in Table 1. (+)-Catechin (2.84 mg/100 g), 1,2-Dihydroxybenzene (1.35 mg/100 g), Quercetin (1.02 mg/100 g) were the most abundant phenolics found in AP.(+)-Catechin (2.42 mg/100 g), 3,4-Dihydroxybenzoic Acid (2.06 mg/100 g), Gallic Acid (1.04 mg/100 g) were the major phenolic compounds found in MwP. Table 1 Phenolic compounds of peach leaves dried in the air and in the microwave oven

Phenolic compounds	Concentration (mg/100 g)			
_	AP	MwP		
Gallic Acid	$0.48 \pm 0.11$	$1.04 \pm 0.22$		
3,4-Dihydroxybenzoic Acid	$0.73 \pm 0.06$	$2.06{\pm}1.02$		
(+)-Catechin	$2.84{\pm}0.76$	$2.42 \pm 0.73$		
1,2-Dihydroxybenzene	$1.35 \pm 0.89$	$1.06 \pm 0.39$		
Syringic Acid	$0.40{\pm}0.14$	$0.55 \pm 0.27$		
Caffeic Acid	$0.22 \pm 0.09$	$0.53 \pm 0.18$		
Rutin trihydrate	$0.19{\pm}0.08$	$0.70 \pm 0.27$		
<i>p</i> -Coumaric Acid	$0.22 \pm 0.14$	$0.50 \pm 0.13$		
trans-Ferulic Acid	0.58±0.13	$0.07 {\pm} 0.05$		
Apigenin 7 glucoside	$0.29{\pm}0.09$	$0.26 \pm 0.20$		
Resveratrol	$0.55 \pm 0.18$	$0.58 \pm 0.13$		
Quercetin	$1.02\pm0.13$	$0.72 \pm 0.20$		
trans-Cinnamic Acid	$0.08 \pm 0.01$	$0.29{\pm}0.08$		
Naringenin	ND	$0.09{\pm}0.01$		
Kaempferol	$0.70 \pm 0.14$	$0.82 \pm 0.00$		
Isorhamnetin	$0.78 \pm 0.35$	$0.38 \pm 0.13$		

AP: Peach leaves dried in the air, MwP: Peach leaves dried in the microwave oven. ND: Not determined

Phenolic content of the peach leaf has been influenced by drying method. Lian Sen et al. (1994) reported the presence of 5,7- dimethoxycoumarin, gallic acid, kaempferol and kaempferol derivatives (3,7-dirhamnoside, 3- rutinoside), quercetin, quercetin derivatives (3rhamnoside, 3- rutinoside, 3-galactoside, 3-glucoside, 3sophoriside) in peach leaves. Similar to our study results, Aouidi et al. (2016) dried olive leaves by lyophilized, convection and microwave drying and the highest antioxidant quality was seen in the microwave drying at 600 W.

## Physicochemical properties of beef patties

There were no differences in cooking yield (%) of patties (P > 0.05). As seen in Table 2, percentage cooking yields were ranged from 62.50 to 67.65 %. Al-Juhaimi et al. (2016) stated that using Moringa seed powder increased the cooking yield of samples compared to control group. On the other hand, dimensional shrinkage of the control group was higher than those other patties, the least dimensional shrinkage (7.48%) occurred in MwP3. The dimensional shrinkage decreased with the increasing concentration of MwP (Table 2). Similarly, dimensional shrinkage decreased in patties to which destoned olive cake powder was added and it has been stated that the olive pulp allows to preserve size and shape of the samples throughout the cooking process (Aouidi et al 2016). Alakali et al., (2010) stated that muscle protein denaturation, removal of melted fat and evaporation of some water during heat treatment resulted in shrinkage in patties. In our study, shapes of the beef patties effected by changes in the protein structure because of cooking process of the samples.

Table 2

Cooking yield and dimensional shrinkage values of raw
beef patties containing different levels of peach leaf
powders

Treat-	Cooking yield (%)	Dimensional shrinkage (%)
ments		
Control	67.30±2.28 <sup>A</sup>	$12.31 \pm 0.55^{AB}$
BHT	$64.12 \pm 1.58^{A}$	$14.47 \pm 0.45^{A}$
AP	67.65±1.66 <sup>A</sup>	$7.48 \pm 0.81^{B}$
MwP1	62.59±0.69 <sup>A</sup>	$9.32{\pm}0.75^{AB}$
MwP2	65.52±1.00 <sup>A</sup>	$12.80 \pm 0.20^{AB}$
MwP3	$65.76 \pm 0.82^{A}$	$11.88 \pm 0.49^{AB}$
Signifi-	NS	*
cant		

Values are means of triplicate samples ( $\pm$ SE). NS – not significant (P > 0.05) <sup>A-B</sup> Means within columns with different superscript letters are significantly different (\**P*<0.05).

Control: Raw patties without peach leaf powders (PLP) and BHT. BHT: Raw patties with 0.01% butylhydroxytoluene (BHT). AP: Raw patties 1% peach leaf powder dried in air. MwP1: Raw patties with 0.5% peach leaf powder dried in microwave oven. MwP2: Raw patties with 1% peach leaf powder dried in microwave. MwP3: Raw patties with 2% peach leaf powder dried in microwave.

The pH values of the samples are given in Table 3. The pH of uncooked samples containing various concentrations of peach leaf powder decreased (P < 0.01) (Table 3). pH values of control and BHT were determined as 6.29, 6.16, respectively; the pH values of MwP1, MwP2, MwP3 and AP samples were found as 5.75,5.70,5.67 and 6.06, respectively. Among all treatment groups for storage days, the lowest pH value (5.49) was 2% MwP on day 5 day, the highest pH (6.71) was seen in control group on day 7.

## Lipid oxidation value

The peach leaf powders (PLPs) showed an important inhibitory influence on the TBARS values (Table 3). TBARS values were found between 0.66 and 4.15 MDA mg/kg for 7 days. The addition of PLPs effectively decreased the lipid oxidation values. TBARS values of control, BHT and MwP3 were 2.64, 1.31, 0.75 mg of MDA/kg meat at first day of storage respectively, were 4.13, 1.94, 0.58 mg of MDA/kg meat at the end of the storage day, respectively. Means of TBARS values were higher in the without PLP group than in those containing AP, MwP and BHT. Among all treatment groups, the average lowest TBARS value was MwP3 over the storage period (Table 3). Phenolic compounds and flavonoids in the structure of the PLP are thought to be effective in delaying oxidation. Similarly, Zahid et al. (2019) stated that beef patties treated with BHT, ascorbic acid, and clove extract manifested substantially lower than the control group. Juntachote et al. (2007) also determined that the dried galangal powder decreased the lipid oxidation values of cooked pork meat (P < 0.05) than the control during storage period. They also indicated that TBARS values increased with progressing storage time In a study, using leaf extracts as a natural preservative in meat products, polyphenolic extracts of black currant and cherry leaves were added to the pork sausages. In the sausages stored for 28 days, leaf extracts showed a significant inhibitory effect on TBARS formation (Nowak et al. 2016). In a study using olive leaf powder Table 3

and extract, olive leaves reduced lipid oxidation by 20-25% (Aouidi et al. 2016).

The pH and TBARS values of raw beef patties treated with peach leaf powders at different levels during storage at 4 °C for 7 days

Treatments	Storage t	time (days)		
-	1	3	5	7
рН				
Control	$5.89{\pm}0.04^{Ba}$	$6.18 \pm 0.25^{Aba}$	$6.39{\pm}0.04^{Aba}$	$6.71 \pm 0.09^{Aa}$
BHT	$5.90{\pm}0.05^{Ba}$	5.97±0.19 <sup>Aba</sup>	$6.36{\pm}0.10^{ABa}$	6.42±0.11 <sup>Aa</sup>
AP	$6.00{\pm}0.04^{Aa}$	$5.85{\pm}0.18^{Aa}$	$6.18{\pm}0.19^{Aab}$	$6.20{\pm}0.22^{Aab}$
MwP1	$5.95{\pm}0.05^{Aa}$	$5.53{\pm}0.07^{Aa}$	$5.73 \pm 0.20^{Abc}$	$5.80 \pm 0.15^{Abc}$
MwP2	$5.92{\pm}0.06^{Aa}$	$5.79 \pm 0.03^{Aba}$	5.57±0.11 <sup>Bc</sup>	$5.53 \pm 0.05^{Bc}$
MwP3	$6.02{\pm}0.04^{Aa}$	$5.68{\pm}0.06^{Ba}$	$5.50 \pm 0.09^{Bc}$	$5.49 \pm 0.04^{Bc}$
TBARS (mg MDA/kg)				
Control	$2.64{\pm}0.29^{Ba}$	$3.76{\pm}0.38^{Aa}$	4.15±0.13 <sup>Aa</sup>	$4.14{\pm}0.17^{Aa}$
BHT	$1.31 \pm 0.31^{Abc}$	$1.87 \pm 0.35^{Abc}$	$2.04{\pm}0.33^{Ab}$	$1.94{\pm}0.27^{\rm Ab}$
AP	$1.17 \pm 0.08^{Abc}$	$0.66 {\pm} 0.06^{Cd}$	$0.92{\pm}0.02^{Bc}$	$0.87{\pm}0.04^{ m BCcd}$
MwP1	$2.02{\pm}0.24^{Aab}$	$2.24{\pm}0.41^{Ab}$	$1.89 \pm 0.31^{Ab}$	$2.67 \pm 0.62^{Ab}$
MwP2	$1.41 \pm 0.23^{Abc}$	$1.27 \pm 0.17^{Abcd}$	$1.28 \pm 0.18^{Abc}$	$2.16 \pm 0.52^{Abc}$
MwP3	$0.75 \pm 0.04^{Ac}$	$1.06 \pm 0.19^{Acd}$	0.77±0.11 <sup>Ac</sup>	$0.67{\pm}0.07^{\rm Ad}$

Values are means of triplicate samples (±SE)

<sup>a-d</sup> Means within columns with different superscript letters are significantly different (P < 0.01).

<sup>A-C</sup> Means within rows with different superscript letters are significantly different (P<0.01).

Control: Raw patties without peach leaf powders (PLP) and BHT. BHT: Raw patties with 0.01% butylhydroxytoluene (BHT). AP: Raw patties 1% peach leaf powder dried in air. MwP1: Raw patties with 0.5% peach leaf powder dried in microwave oven. MwP2: Raw patties with 1% peach leaf powder dried in microwave. MwP3: Raw patties with 2% peach leaf powder dried in microwave

#### Texture profile analysis (TPA)

Table 4 indicates the influence of PLPs on the hardness, gumminess, chewiness parameters of uncooked beef patties. Hardness, gumminess (N), chewiness of the samples increased (P < 0.01) using the MwP concentration. The highest hardness (32.84 N), gumminess (11.62 N), chewiness (7.08) values were observed in MwP3 while the lowest values were seen in control group. Sharma and Yadav (2020) stated that the hardness value of chicken meat patties incorporating pomegranate peel and pomegranate aril bagasse flour increased. In our previous study, adding grape leaf powder affected the hardness, chewiness characteristics of meatball samples (Yaldız Cabi and Sarıçoban, 2019). However, Modi et al. (2009) found that some vegetable material caused to decrease chewiness and hardness (N) parameters of meat samples. Springiness (mm) and cohesiveness values of cooked meat were affected by the addition of PLPs. Springiness (mm) values changed from 0.86 (AP) to 0.90 (MwP1 and MwP3). Cohesiveness values are between 0.39 (control) - 0.59 (BHT and MwP2).

Table 4

Textural parameters (TPA) of raw and cooked beef patties treated with different levels of peach leaf powders

Treatments	Hardness (N)	Gumminess (N)	Springiness (mm)	Cohesiveness	Chewiness
Raw					
Control	19.79±0.85 <sup>C</sup>	$7.55 \pm 0.53^{B}$	$0.56{\pm}0.01^{A}$	$0.38 \pm 0.01^{A}$	$4.26 \pm 0.38^{B}$
BHT	$29.32 \pm 0.79^{AB}$	$10.46 \pm 0.24^{A}$	$0.66 \pm 0.02^{A}$	$0.35 \pm 0.01^{A}$	6.95±0.24 <sup>A</sup>
AP	24.11±1.11 <sup>BC</sup>	$9.02 \pm 0.21^{AB}$	$0.62{\pm}0.02^{\rm A}$	$0.37 \pm 0.02^{A}$	$5.69 \pm 0.34^{AB}$
MwP1	$26.37 \pm 0.57^{B}$	$10.66 \pm 0.60^{A}$	$0.64 \pm 0.05^{A}$	$0.37 \pm 0.00^{A}$	$6.84{\pm}0.50^{\rm AB}$
MwP2	$24.36 \pm 0.28^{BC}$	$8.94{\pm}0.31^{AB}$	$0.56 \pm 0.00^{A}$	$0.38 \pm 0.00^{A}$	$5.07{\pm}0.24^{AB}$
MwP3	$32.84 \pm 2.04^{A}$	$11.62 \pm 0.77^{A}$	$0.60{\pm}0.03^{\rm A}$	$0.35{\pm}0.00^{\rm A}$	$7.08 \pm 0.84^{A}$
Significant	**	**	NS	NS	*
Cooked					
Control	205.13±1.61 <sup>A</sup>	$121.40{\pm}10.17^{A}$	$0.89{\pm}0.00^{ m AB}$	$0.39 \pm 0.02^{B}$	109.30±9.26
BHT	250.00±20.21 <sup>A</sup>	146.38±7.83 <sup>A</sup>	$0.87{\pm}0.00^{ m AB}$	$0.59 \pm 0.02^{A}$	127.78±7.54
AP	205.60±11.80 <sup>A</sup>	116.16±4.99 <sup>A</sup>	$0.86{\pm}0.00^{B}$	$0.56 \pm 0.01^{A}$	$100.54 \pm 3.79$
MwP1	199.00±8.86 <sup>A</sup>	$119.04 \pm 4.98^{A}$	$0.90{\pm}0.00^{ m AB}$	$0.54{\pm}0.02^{\text{A}}$	$107.12 \pm 4.16$
MwP2	203.40±15.96 <sup>A</sup>	116.75±5.24 <sup>A</sup>	$0.88{\pm}0.00^{ m AB}$	$0.59 \pm 0.02^{A}$	$104.79 \pm 5.46$
MwP3	240.90±23.11 <sup>A</sup>	112.90±8.74 <sup>A</sup>	$0.90{\pm}0.01^{\text{A}}$	$0.51{\pm}0.04^{AB}$	$101.98 \pm 7.18$
Significant	NS	NS	**	**	NS

Values are means of triplicate samples ( $\pm$ SE). NS – not significant (P > 0.05). <sup>A-C</sup> Means within columns with different superscript letters are significantly different (\*P < 0.05; \*\*P < 0.01).

Control: Raw patties without peach leaf powders (PLP) and BHT. BHT: Raw patties with 0.01% butylhydroxytoluene (BHT). AP: Raw patties 1% peach leaf powder dried in air. MwP1: Raw patties with 0.5% peach leaf powder dried in microwave oven. MwP2: Raw patties with 1% peach leaf powder dried in microwave. MwP3: Raw patties with 2% peach leaf powder dried in microwave.

#### Sensory scores

No significant differences (P > 0.05) were observed in the flavour and taste, odour, structure and texture properties. However, the addition of PLPs affected (P < 0.05) the colour, appearance, general acceptability of beef patties. The panellists preferred the colour of the control patties and patties with BHT (Table 5). The increase in the concentration of the PLP was noticed by the panellists in terms of colour and the concentration increase and the score given to the color decreased (Table 5). The patties were examined in terms of appearance; the highest score was given to the control group. The patties with MwP3 were less preferred by the panellists than the other groups. This situation is probably due to the increase in fibre ratio in leaves may have hardened the structure of patties. The highest general acceptability score was given to the MwP1 group; the lowest score was given to MwP3 (Table 5).

Table 5

The sensory evaluation of cooked beef	patties containing different levels of t	peach leaf powders

Treatments	Colour	Appearance	Odour	Flavour and taste	Texture	General acceptability
Control	7.25±0.49 <sup>AB</sup>	7.62±0.24 <sup>A</sup>	5.87±0.72	5.75±0.49 <sup>AB</sup>	$6.87 \pm 0.60$	6.87±0.33 AB
BHT	$7.50{\pm}0.47^{A}$	$6.75 \pm 0.46^{AB}$	$4.62 \pm 0.61$	$5.00{\pm}0.36$ AB	$5.62 \pm 0.46$	$5.87 \pm 0.37$ AB
AP	$5.87 \pm 0.74^{ABC}$	$6.62 \pm 0.39^{AB}$	$5.50 \pm 0.53$	$5.25 \pm 0.86$ AB	$5.50 \pm 0.81$	5.37±0.81 AB
MwP1	$6.62 \pm 0.61^{AB}$	7.25±0.42 <sup>A</sup>	$6.37 \pm 0.50$	6.75±0.46 <sup>A</sup>	6.75±0.29	$7.12\pm0.37^{\text{A}}$
MwP2	$4.75 \pm 0.68^{BC}$	$6.12 \pm 0.41^{AB}$	$5.50 \pm 0.40$	5.00±0.61 AB	4.87±0.51	5.62±0.43 AB
MwP3	$3.87 \pm 0.48^{\circ}$	$5.00{\pm}0.50^{B}$	$5.25 \pm 0.46$	3.75±0.81 <sup>B</sup>	$5.12 \pm 0.67$	4.87±0.41 <sup>B</sup>
Significant	**	**	NS	NS	NS	*

Values are means of triplicate samples ( $\pm$ SE). NS – not significant (P>0.05). <sup>A-C</sup> Means within columns with different superscript letters are significantly different (\*P<0.05; \*\*P<0.01).

Control: Raw patties without peach leaf powders (PLP) and BHT. BHT: Raw patties with 0.01% butylhydroxytoluene (BHT). AP: Raw patties 1% peach leaf powder dried in air. MwP1: Raw patties with 0.5% peach leaf powder dried in microwave oven. MwP2: Raw patties with 1% peach leaf powder dried in microwave. MwP3: Raw patties with 2% peach leaf powder dried in microwave.

### 4. Conclusions

The usage of ground peach leaf has an important effect on TBARS values througout the storage time. The PLP dried in microwave oven retarded the lipid oxidation of beef patties when compared to the AP group. Especially, MwP3 significantly prevented lipid oxidation. Sensory properties had been affected by the peach leaves, while the addition of PLP showed a hard texture. Further studies are required for revealing the microbiological properties of the beef patties.

#### 5. Acknowledgements

The authors would like to thank the Selçuk University Coordinating Office for Scientific Research Projects (SU-BAP. Konya TURKEY) for financial support (Project Number: 17201100). This research was produced from a part of the Master Thesis of Alime Yaldız Cabi.

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Selcuk Journal of Agriculture and Food Sciences

http://sjafs.selcuk.edu.tr/sjafs/index

**Review Article** 

SJAFS

(2022) 36 (1), 120-126 e-ISSN: 2458-8377 DOI:10.15316/SJAFS.2022.017

# Usage of Probiotics in the Poultry Industry and Effects on Meat Quality

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# **ARTICLE INFO**

Article history: Received date: 23.11.2021 Accepted date: 04.03.2022

Keywords: Probiotics Feed additives Poultry Meat Quality

# ABSTRACT

Currently, a significant survey field is the use of probiotics as feed additives. There are many essays about the effect of the use of probiotics on meat quality. There is common agreement that probiotics supplementation could improve meat quality. Probiotic treatment increases meat tenderness however probiotics on lipid composition and oxidation of meat and sensory properties may change. The products obtained can be presented to the consumer as a healthy, taste and aroma enhanced and safe food. Thus, while providing delicious and nutritious food to the consumer, it also has positive effects on consumer health. Especially today, consumption of functional foods containing probiotics is increasing rapidly. Consumer interest has accelerated research on probiotics. On the other hand, there is a continuous increase in the number of microorganisms used in the market as probiotics. The current situation will be taken one step further with the discovery of new and active microorganism varieties that can be used as probiotics in the future probiotics will be the subject of many studies in the future. Therefore, it is thought that this issue should be emphasized. Apart from all these; as a group of growth promoters, the supplement of probiotics to the diet of poultry has been found to develop growth performance, increase feed conversion yield and develop immune responses.

# 1. Introduction

The poultry industry has become a significant economic activity in a lot of countries (Kabir 2009). The manufacture and consumption of poultry meat has been dramatically increasing. This speedy growth is to an enormous degree associated to the requests of the consumers for a healthy diet and meat as it is basic component (Popova 2017). For the purpose of achieve income, efficient and economical production, safety and quality, beside essential nutrient, for several years, antibiotics have been added to poultry diets (Okanović et al 2014). This common use of antibiotics in poultry in order to promoting growth rate, increasing feed conversion efficiency and for the prevention of intestinal infections have led to an instability of the beneficial intestinal flora and the appearance of resistant bacteria (Gupta & Das 2013; Popova 2017).

After the prohibition of antibiotics, the search for alternative additives to antibiotics has gained momentum with the increasing concerns that the continuity and profitability of production may be adversely affected as a result of the losses that may occur in the performance of the animals (Üstündağ & Özdoğan 2017).

With rising attention about antibiotic resistance, there is rising attention in discovering alternatives to antibiotics for poultry production. Natural feed additives, such as live "probiotics" have potential to decrease enteric disease in poultry and latter contamination of poultry products (Gupta & Das 2013; Popova 2017). Thus, probiotics are being considered to fill this emptiness and several farmers are using them in prefer to antibiotics (Kabir 2009).

As a result of marketing studies based on the relationship between food and health, there is an increase in the interest of consumers in this direction. In this context, probiotics, one of the product groups that have the largest share in the development of new and functional foods, are also welcomed by the consumer (Doğu & Sarıçoban 2015).

Probiotics are healthy bacteria, yeast and other microorganisms that maintain the natural balance of the digestive system (Palamutoğlu & Sarıçoban 2013).

Probiotics are culture of living microorganisms that are used as functional ingredients to manipulate and maintain good health by controlling gut microflora and

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increasing digestive enzyme activity (Alloui et al 2013). The term 'probiotic' comes from the Greek words 'pro' and 'biotic,' meaning 'for life' (Gibson & Fuller 2000; Dhama et al 2011) and was first used in 1965 as contrary to the word antibiotic, to indicate unknown growth promoting substances produced by a ciliate protozoan that stimulated the growth of another ciliate (Krâl et al 2013; Popova 2017). Many definitions have been proposed for the term "probiotic". The more widely accepted one is "live microorganisms which, when consumed in adequate amounts, confer a health effect on the host" (FAO/WHO, 2002; Gaggia 2010; Popova 2017). This description implies that a health influence must be demonstrated for the probiotic (Francesca et al 2010; Krâl et al 2013; Park et al 2016).

Probiotics are live, in general non-pathogenic microorganisms supplemented to both human and animals diet (Getachew 2016). They are one by one microorganisms or groups of microorganisms which have positive influence on host by developing the

Table 1

Probiotic microorganisms (Holzapfel et al 2001)

properties of intestinal microflora. Their influence on production consequences reflects in reduction of risk of illnesses, probiotics develop the function of the immune system and display important effect on morphofunctional properties of intestines (Okanović et al 2014). Probiotics also prevent contamination of carcasses by intestinal pathogens during processing (Kabir 2009).

Aims of the use of probiotics as feed supplements can be listed as (Alloui et al 2013):

- Pathogenic bacteria control
- Improve health and production performance
- Reduce antibiotic use in poultry

Probiotic microorganisms that are generally used for animals (Table 1) include *Bifidobacterium*, *Lactococcus*, *Lactobacillus*, *Bacillus*, *Streptococcus* and yeasts such as *Candida*, which are usually found in the poultry intestine (Park et al. 2016). Apart from these, one of the most successful probiotic bacteria used in poultry are *Bacillus subtilis* (Alloui et al 2013).

Lactobacillus species	Bifidobacterium species	Other lactic acid bacteria	Nonlactic acid bacteria
L. acidophilus	B. adolescentis	Enterococcus faecalis	Bacillus cereus var. toyoi
L. amylovorus	B. animalis	Enterococcus faecium	Escherichia coli strain nissle
L. casei	B. bifidum	Lactococcus lactis	Propionibacterium freudenreichi
L. crispatus	B. breve	Leuconstoc mesenteroides	Saccharomyces cerevisiae
L. delbrueckii subsp. bulgari-	B. infantis	Pediococcus acidilactici	Saccharomyces boulardii
cus			
L. gallinarum	B. lactis	Sporolactobacillus inulinus	
L. gasseri	B. longum	Streptococcus thermophilus	
L. johnsonii			
L. paracasei			
L. plantarum			
L. reuteri			
L. rhamnosus			

Probiotics could be infectious, particularly in debilitated and immuno-compromised populations (Getachew 2016). Some species of *Lactobacillus, Bifidobacterium, Leuconostoc, Enterococcus* and *Pediococcus* have been isolated from infection areas. Lately, emphasis has been given to the selection, preparation and practice of probiotic strains, particularly lactic acid bacteria (Otutumi et al 2012).

# 2. Mechanisms of Action of Probiotics.

Probiotics show some significant ways of action. The mechanism of action of probiotics needs to be fully elucidated (Ülger et al 2015). However, how probiotics realize their mechanism of action is still a matter of debate (Kıran & Osmanağaoğlu 2012). A number of the recommended modes of action of probiotics are given below:

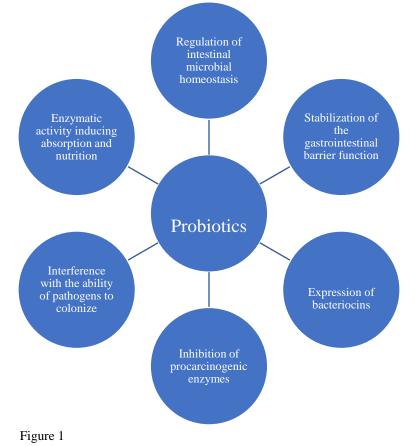
1) Maintaining a beneficial microbial population by "antagonism" and "competitive exclusion" (Ghadban 2002); an antagonistic effect towards pathogen bacteria by modification of gut pH, direct antimicrobial influence by secretion of products which prevent their improvement, such as organic acids, bacteriocins, and hydrogen peroxide, production of short chain fatty acids in the intestine, regulation of the immune system of the host, normalization of gut microbiota, and another metabolic effects (Alloui et al 2013).

2) Improving feed intake and digestion (Ghadban 2002; Apata 2008; Budak Bağdatlı & Kundakçı 2013);

3) Changing bacterial metabolism (Ghadban 2002).

4) Stimulating the immune system (Haghighi et al 2005; Kabir 2009).

Probiotic microorganisms have signified much healthy beneficial effects via in-vivo trials, accompanied by much promising recent facilities as advanced by invitro experiments. Generally, probiotics have been demonstrated to develop intestinal microbial stability, supply prevention against gut pathogens and modulate immune system (Park et al 2016). There are lots of effects of probiotics on health, including regulation of intestinal microbial homeostasis, enzymatic activity inducing absorption and nutrition, stabilization of the gastrointestinal barrier function, expressions of bacteriocins, interference with the ability of pathogens to colonize and inhibition of procarcinogenic enzymes (Figure 1) (Gaggia et al 2010). However probiotic use has not reached the expected prevalence in Turkey. The biggest reason for this is that the probiotics used are not only imported, but also maintain their viability for a long time in the process from production to use (added to feed and stored) and the problems associated with their use with other feed additives (Kocabağlı & Alp 2015).



Effects of probiotics on health (Gaggia et al 2010)

## 3. Effects on Growth Performance

Probiotics, which have been used to increase productivity since the 1970s, have commercially prepared preparations containing live bacterial, yeast and fungal cultures and various enzymes (Karademir & Karademir 2003).

There are studies investigating the effects of probiotics on the performance of poultry. As a result of these studies, it was reported that significant increases were observed in the growth performance of broiler chickens, layer chickens, ducks, turkeys, quails and ostriches with the addition of probiotics (Üstündağ & Özdoğan 2017).

Especially after the prohibition of the use of antibiotics as growth promoters, probiotics became one of the important feed additives used for this purpose. Indeed, Baidya et al (1993) reported that probiotics are the most effective growth accelerators. There are many reports that chickens fed diets containing probiotics gain more weight (Ülger et al 2015). As a group of growth promoters, the supplement of probiotics to the diet of poultry has been found to develop growth performance, increase feed conversion yield and develop immune responses (Dhama et al 2011).

In a study practice of probiotic to poultry resulted in 5–6% less mortality rates through the first week, completely suppressing the growth of *E. coli*, developing daily gain and feed conversion ratio. Used a probiotic bacterial culture from *Bifidobacterium* pseudulongum, *Bifidobacterium* thermophilium, and *L. acidophilus* in dose of  $6.8 \times 10^6$  for acquiring safe and healthy poultry products (Ghadban 2002).

For example addition of probiotic *Streptococcus faecium* M-74 to broiler diet (0.5 mill. CFU/g and 1.0 mill. CFU/g) from 14th to 21st day of age increased body weight, developed feed conversion ratio, and reduced mortality of the treated chickens. Containing the probiotic Lactosacc and *S. faecium* JMB 52 cultures (400 mill. CFU/g) to the feed of broilers leads to the

development of their productivity, consistent development (Ghadban 2002).

Stanley et al (1993) reported that the addition of 0.1% live yeast (S. cerevisiae) to broiler feeds caused an increase in carcass weight.  $\left[ \underbrace{sep} \right]$ 

In a study was evaluate effects on performance characteristics in quail by dietary addition of Saccharomyces cerevisiae and lactic acid bacteria (Pediococcus acidilactici). In this study performance parameters (body weight gain, feed intake and feed conversion ratio) were determined weekly. Performance characteristics were affected significantly by dietary addition of Saccharomyces cerevisiae and Pediococcus acidilactici throughout the experiment. Birds fed diet containing Saccharomyces cerevisiae and Pediococcus acidilactici significantly improved body weight gain, feed intake and feed conversion ratio. These results suggested that the usage together with yeast and bacteria in quail diets could be more effective than alone yeast or bacteria (Parlat & Göçmen 2010).

In contrast, in a study in the probiotic addition had no effect on feed conversion ratio and body weight gain during grower (16 to 29 d) and finisher (30 to 45 d) periods. Further, no significant differences in body weight at 29 and 44 d were found between chicken groups. Similarly, marginal effects of *Bacillus* spp. and a commercial probiotic (containing *Lactobacillus* spp. and *Bifidobacterium* spp.) (Kim et al 2016).

Karaoğlu and Durdağ (2005) investigated the effects of adding different levels (0.1% and 0.2%) yeast culture (*S. cerevisiae*) to the rations on carcass characteristics and performance in 19-day-old broiler chicks. rate, feed consumption and carcass yield did not create an effect in terms of reported.

Miles et al. (1981a, b) in their study by adding two different levels of probiotics (*L. acidophilus*) to breeder quail rations, egg production, feed consumption, reproduction, brood yield and mortality between quails fed with feeds containing probiotics and quails in the control group. found that there was no significant difference between the rates.

#### 4. Effects of Probiotics on Meat Quality

Physical and chemical properties of meat such as colour, flavor, odour, texture and pH are the basic parameters that determine meat quality.

Probiotic meat products have become one of the health-related products that have increased their importance today. In addition to the positive effects of these products on health, they also have features such as improving the taste, aroma and physical structure of the product as added value and being effective on the microbiological flora (Doğu & Sarıçoban 2015).

It is thought that probiotics and organic acids, which increase the number of beneficial microorganisms in the digestive tract and act by reducing the pH of the environment, can be used as an alternative to antibiotics in the poultry industry to increase performance and meat quality, and studies have been carried out on this subject (Dama 2019).

There is common agreement that probiotics supplementation could improve meat quality (Park et al 2016). Probiotics including *Bacillus licheniformis* in the poultry diet improved the meat colour, flavour and juiceness in fresh meat (Liu et al 2012), in spite of the fact that *Bacillus subtillis* indicated unimportant effect on the texture in cooked meat (Alfaig et al 2013; Popova 2017). Mahajan et al (2000) emphasize that the scores for the sensory properties of the meatballs; appearance, texture, juiciness and overall acceptability were significantly higher and those for flavour were lower in the probiotic fed group (Kabir 2009; Jadhav et al 2015; Park et al 2016).

## 4.1. Tenderness of Meat

Tenderness is known as one of the most significant properties of meat that extremely effect its consumer acceptability. As tenderness accounts as a main meat eating satisfaction, food scientists have always looked for effective tenderization processes that are capable of improving meat quality (Barekat & Soltanizadeh 2017).

Studies have shown that probiotic treatment increases meat tenderness. Improved tenderness which was shown by reduced shear force was determinated by Yang et al (2010) when probiotic *Clostridium butyricum* was added in diet of broiler (Park et al 2016).

Zhang et al (2005) conducted an experiment with 240, day-old, male broilers to search the effects of *Saccharomyces cerevisiae* cell components on the meat quality and they reported that meat tenderness could be improved by the whole yeast or *Saccharomyces cerevisiae* extract (Kabir 2009).

#### 4.2. Lipid Composition and Oxidation of The Meat

Lipid oxidation is an important issue related to offflavour, off-odour and warmed-over flavour seems to be relative to lipid oxidation in meat. Lipid autooxidative degradation gives products that alter the food quality, e.g. the colour, texture, flavour, aroma and the nutritive value.

Probiotics on lipid composition and oxidation of meat are changing. Latest research showed either positive or lack of adverse effect of the probiotics on the lipid stability of chicken meat. In spite of the reduced content of polyunsaturated fatty acids (PUFA) and the higher total fat content, *Aspergillus awamori* and *Aspergillus niger* reduced crucially the content of thiobarbituric acid reactive substances (TBARS) in broiler breast (Popova 2017).

Investigation on the influence of diverse probiotics on the fatty acid profile of meat is relatively limited, but the overall results point out positive influence of the probiotics, mostly related to decrease in saturated and increase of polyunsaturated fatty acids. Feeding broilers with *Aspergillus awamori* and *Saccharomyces cerevisiae* or combination of them led to important reduction in the saturated C16:0 and C18:0, and increase in C18:1 as well as in the polyunsaturated C18:2, C18:3, C20:4 (Saleh et al 2013). The same was observed when the diet of the birds included *Aspergillus awamori* and *Aspergillus niger* in diverse amounts (0.01%, 0.05%, 0.1%) as well as *Aspergillus awamori* in combination with selenium nanoparticles (Saleh 2014). Increase in the C18:3 in breast and C18:2 and C18:3 in the thighs after probiotic administration (Hossain et al 2012); however, in the other test, decrease in the n-6 polyunsaturated fatty acids (PUFA) in both breast and thigh (Popova 2017).

Endo & Nakano (1999) reported a greater tendency of higher ratio of unsaturated fatty acids to saturated fatty acids in breast and thigh meat of broilers fed with probiotics (including *Bacillus, Lactobacillus, Streptococcus, Clostridium, Saccharomyces* and *Candida*) (Park et al 2016).

#### 4.3. Microbiological Properties of Meat

In poultry meat production, some microorganisms present in meat deteriorate meat quality, shorten its shelf life, and pose a risk to human health. Therefore, one of the factors that affect the meat quality and is closely related to the shelf life of the meat is the microbial load of the meat (Dama 2019).

Since meat is a food of animal origin, the quality of raw materials is very important in terms of the quality of the product to be formed. The microbial load of meat products is closely related to the raw material (Doğu & Sarıçoban 2015).

Mahajan et al (2000) reported that broiler breast fed diets including probiotics had lower total aerobic bacterial counts than drumsticks (Aksu et al 2005). In a study in total bacterial counts of vacuum-packaged legs and drumsticks stored at 0°C for 16 days were lower compared with aerobically packaged samples along the first 8 days of storage. They rapidly increased. Using total bacterial counts, determined that vacuum packaged broiler carcasses could be preserved at 2°C for 10 days (Aksu et al 2005).

Concerning the microbiological quality of meat, competitive put out of cultures for broilers can be used to decrease contamination by *Salmonella enteritidis* in processed carcasses, decreasing thus the exposure of consumers to food-borne infections (Otutumi et al 2012).

#### 4.4. Sensory Properties of Meat

Probiotics on sensory properties of meat are changing. Some studies show a positive influence of probiotics on sensory properties whereas other studies show no influence of probiotics. Probiotics may have an influence on flavour of meat. In a study in a favorable influence of probiotics including *Bacillus licheniformis* and *Bacillus subtilis* spores on the flavor of broiler meat after cooling for 5 days however in a different study in probiotics fed with water and feed did not had any influences on sensory characteristics of meat. In a study

in probiotic addition significantly increased the meat tenderness and meat quality. Majority of the carcass characteristics are forthrightly commensurate to the increased body weight at the time of slaughter. In contrast, in the other experiment, no significant difference in carcass % between probiotic treated and untreated treatments on the sensory parameter basis (Jadhav et al 2015).

## 5. Conclusion

There is a popular opinion that consumers would prefer to buying poultry meat from animals processed with natural agents rather than antibiotics, hormones, or other chemicals. Probiotics can present enormous potential as alternatives for antibiotics to completely eliminate antibiotic use, because probiotics do not lead to microbial resistance. In addition to probiotics constitute a cost-effective alternative to antibiotic growth promoters. Probiotics seem to be the feed additives of the coming years, particularly under the politics of banning of antibiotics. Probiotics are gaining importance because they have a number of beneficial effects in poultry. These are: to supply nutrient to the feed, to improve immunity, to prevent intestinal tract disease, to promote growth and meat quality and stability, environmental friendly.

Find out more information and gaining experiences on comprehend probiotics and find out their overall practicability for poultry meat quality in the coming years would help in making further improvements.

In recent years, the use of probiotics has become clearer than in previous years. Probiotics can be seen as an important alternative to antibiotic agents for growth promotion in poultry. It is thought that this resource will be utilized more effectively in the future.

As it is a relatively new field of study, research on the subject continues. Considering the benefits, it is thought that more studies should be done on this subject.

Although the use of probiotics is quite old, there are still unexplained points in terms of the mechanism of action and measurement of effectiveness. However, it is predicted that the use of probiotics and prebiotics will increase in the future as a result of the restriction of antibiotics as growth promoters and also because consumers avoid products produced using antibiotics. Giving probiotics to animals, in particular, will stimulate the immune system, thereby reducing susceptibility to disease. In addition, both the positive results obtained and the economic nature revealed that the use of probiotics, at least at a certain level, will continue in the short and long term.

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