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Examples of the Images: Regular potatoes (left) and irregular potatoes (right)
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Görüntü Örnekleri: Düzgün şekilli patatesler (sol), bozuk şekilli patatesler (sağ)
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Ankara Üniversitesi
ZİRAAT FAKÜLTESİ

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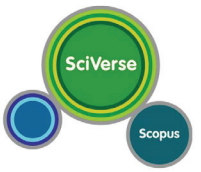

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Determination of the Adulteration of Butter with Margarine by Using Fat Constants

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ABSTRACT

This study focused on the adulteration of butter by margarine. The samples of pure butter, pure margarine and replacement of butter with margarine at a ratio of 10, 20, 30, 40, 50, 60, 70, 80 and 90% were prepared and fat constants including the Melting Point (MP), Refractive Index (RI), Reichert-Meissl Number (R-MN), Polenske Number (PN), Saponification Number (SN) and Iodine Number (IN) were measured for 66 samples (2 margarine types × 11 margarine replacements × 3 replicates). The types and addition levels of margarine significantly influenced the MP, R-MN, PN, SN, IN ($P<0.01$) and RI ($P<0.05$) of the samples. The fat constants in the samples became closer to margarine by increasing the levels of margarine. For each margarine added samples, the MP, R-MN, SN and IN were found to be statistically significant. According to these results, the MP, R-MN, SN and IN can be reliably used to differentiate margarine added butter from pure butter. The results were also supported by correlation analysis. As a result of this research, taking advantage of the R-MN, the following formula was developed to determine the addition of margarine to butter. Margarine adulteration rate (%)= 100.73 - (3.84 × Reichert-Meissl number), (F= 12830.43**)

Keywords: Butter; Margarine; Adulteration; Analysis; Fat constants

Yağ Sabitleri Kullanılarak Tereyağının Margarinle Tağşişinin Tespiti

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ÖZET

Bu araştırma, margarin ile tereyağının tağşişinin tespitine odaklanmıştır. Yağ örnekleri, saf tereyağından sade margarine kadarki aralıkta, tereyağına % 10, 20, 30, 40, 50, 60, 70, 80 ve 90 margarin katkılanarak hazırlanmıştır. Yağ örneklerinde erime noktası (EN), kırılma indisi (Kİ), Reichert-Meissl sayısı (R-MN), Polenske sayısı (PS), Sabunlaşma sayısı (SS) ve iyot sayısı (İS) tespit edilmiştir. Deneme 2 tip margarin × 11 margarin katkı oranı × 3 tekerrür olarak düzenlenmiş ve toplam 66 örnekte yürütülmüştür. Margarin tipi ve katkı seviyesi örneklerin EN, R-MS, PS, SS, İS ($P<0.01$) ve Kİ ($P<0.05$) değerlerini önemli derecede etkilemiştir. Örneklerin yağ sabitleri margarin katkı oranı arttıkça margarine yaklaşmıştır. Margarin ilave edilen her örneğin EN, R-MN, SS ve İS değerleri istatistiki olarak önemli derecede farklı

bulunmuştur. Bu sonuçlara göre, EN, R-MS, SS ve İS değerleri tereyağı ile margarini ayırt etmede başarıyla kullanılabilir. Bu durum korelasyon analizleri ile de doğrulanmıştır. Bu araştırmanın bir sonucu olarak, tereyağına yapılan margarin tağşişinin anlaşılmasında R-MN ile aşağıda geliştirilen formül avantaj sağlayabilir. Margarin tağşiş oranı (%)= 100.73 - (3.84 × Reichert-Meissl sayısı), (F= 12830.43**)

Anahtar Kelimeler: Tereyağı; Margarin; Tağşiş; Analiz; Yağ sabitleri

1. Introduction

Butter is a type of dairy product which is used directly or as an ingredient in various foods (Mallia et al 2008). Milk fat, which has important macromolecules, affects the nutritional value, texture, flavour and shelf life of a food product (Peggy 2005). Butter is a concentrated source of milk fat (~ 80%) and contains water and non-fat milk solids. Butter contains milk fat, fatty acids, essential fatty acids, vitamin A, β-carotene and certain minerals. It also contains conjugated linoleic acid, which promotes immunity. As butter is nutritious, it also offers various health benefits. Butter, as well as having a high nutritional value, also has a pleasant taste and aroma. Aside from all the advantages of butter, its price is higher than other oils and fats. For this reason in order to gain illegal profits sometimes it can be mixed with margarine. Although in most cases adulteration of fats and oils does not pose a threat to public health, fundamental rights of consumers (incorrect information and expensive fat) are violated (Ulberth & Buchgraber 2000). Margarine is similar to butter in appearance, but possesses several basic differences with respect to nutritional, rheological, flavour and fatty acid compositional properties. Margarines and their derivatives are added to butter to decrease the cost of butter.

Butter has a pleasant aroma that is unique. In addition, it melts in body temperature and can be digested easily. It plays an important role in human nutrition because it is an important energy source and consists of essential fatty acids. There is currently no knowledge about the level of adulteration, even if there are attempts to gain qualitative information about the level of adulteration on the basis of differences in the physical and chemical properties of butter. This situation leads to cheating the consumers and consumers spend more money, although the adulterated product contains insufficient nutritional elements. In addition, there are no clear scientific and practical results in case of doubt. Therefore, consumers are often helpless to act. Certain rapid, yet expensive techniques were (Attenuated Total Reflectance-Mid Infrared (ATR-MIR) spectroscopy) studied for rapid estimation of butter adulteration has been used successfully (Koca et al 2010). Differential scanning calorimetry (DSC) technique could be used in order to determine the adulteration of butter with margarine (Aktaş & Kaya 2001). Also, Dıraman (2006) has determined adulteration in the butter and olive oil, by using capillary column gas chromatography method. In order to understand the origins of butter, the most effective methods, qualitative and quantitative determination of sterols and tocopherols are also indicated (Derewiaka et al 2011). However, in this study, fast, cheap and easy methods for understanding the adulteration of butter by margarine were studied. Therefore, this study, in order to solve this problem, was planned to understand the margarine supplement, which is cheaper than butter, on the basis of distinctive characteristics between butter and margarine with the help of fat constants. Fat constants values of milk fat and margarine are very different. For example, Reichert-Meissl Number (R-MN) of butter varies between 17-35 (Metin 2008), but R-MN for vegetable oils maximum is 7 (Kurt et al 2007). Therefore, it is thought that the fat constants values will help determining the adulteration of butter.

2. Material and Methods

2.1. Materials

The butter was produced in the Dairy Plant of Atatürk University. Two different margarines, which are used for cooking (CM) and pastry (PM), were obtained from MARSAN (Food Industry and Trade Joint-stock Company, Adana/Turkey). Butter

and margarine analysis results are shown in Table 1. The samples of pure butter, pure margarine and replacements of butter by margarine at a ratio of 10, 20, 30, 40, 50, 60, 70, 80 and 90% were prepared and certain fat constants including Melting Point (MP), Refractive Index (RI), R-MN, Polenske Number (PN), Saponification Number (SN) and Iodine Numbers (IN) were measured for 66 samples (2 different types of margarine × 11 different fat varieties × 3 replicates).

2.2. Butter production

Butter making and the stages of preparation of fat samples are shown in Figure 1.

Table 1- Average values of the analysis results of control fats

Çizelge 1- Kontrol yağların analiz sonuçları ortalamaları

	Fat types		
Properties	*B*[1]	*PM*[2]	*CM*[3]
Dry matter (%)	82.43±2.81 b	85.93±1.92 b	99.83±0.09 a
Fat (%)	81.50±2.65 c	85.17±0.76 b	99.50±0.00 a
pH	4.46±0.37 a	4.77±0.21 a	4.29±0.04 a
Melting point (ºC)	32.87±0.31c	40.10±0.20 b	37.06±0.42 a
Refractive index	1.4567± 0.0007 b	1.4619± 0.0016 a	1.4620±0.0013 a
Reichert-Meissl number	26.17±0.62 a	0.67± 0.06 b	0.42±0.17 b
Polenske number	0.90± 0.10 a	0.22±0.06 b	0.20±0.0 b
Saponification number	228.1±2.1 a	196.0±4.7 b	193.7±1.0 b
Iodine number	32.35±0.58 b	56.88±1.26 a	56.41±1.10 a

1, butter; 2, pastry margarine; 3, margarine for cooking; different letters represent significant differences among fat types according to the Duncan's Multiple Range Test (P≤0.05); data were reported as mean ± standard deviation (n= 3)

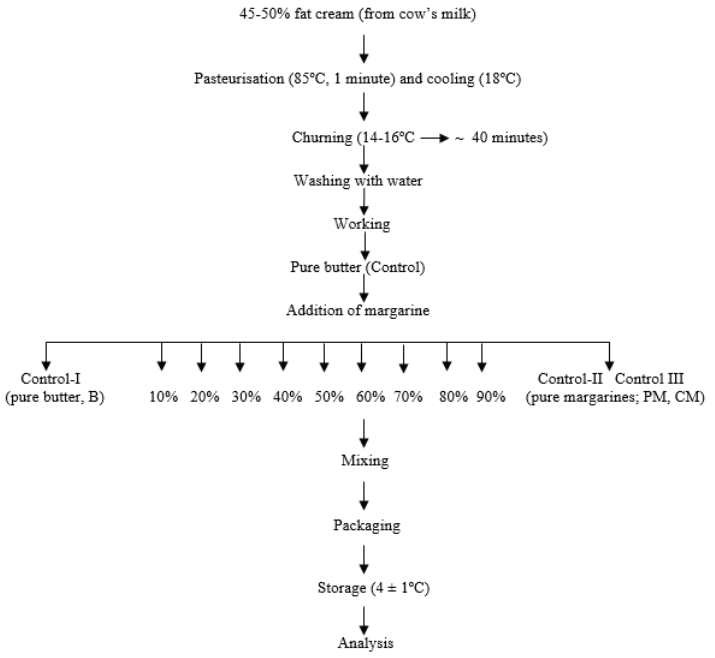

Figure 1- Fat samples preparation process

Şekil 1- Yağ örnekleri hazırlama işlemi

2.3. Pure fat analysis

The pH value of the control fat samples was determined electrometrically with a pH meter (HANNA instruments, Italy). Dry matter was found by heating in an oven at 102 °C until a constant weight was obtained. The fat contents (%) of margarine and butter samples were measured using the method of James (1995).

2.4. Preparation for the analysis of fat samples and other physicochemical analysis

Hundred grams or more of butter and margarines were melted and allowed to stand at 45 °C to 55 °C until the water and protein settle to the bottom. The melted fat was filtered through dry paper in a funnel heated by a water jacket and kept in a drying oven at approximately 60 °C. The filtered fat, which is free from turbidity, was poured into a wide-mouth bottle, the bottle was closed and kept in a cool place until it is analysed (Sherman 2009). RI, R-MN, PN, SN and IN analysis of fat samples were determined according to the standard methods (AOAC 1980) and as suggested by Sherman (2009). The melting point and refractive index analysis were made according to Anonymous (2009) and Atamer (1993), respectively.

R-MN is a measure of fatty acids which is volatile, water-soluble, or C4 and C6. PN is a measure of fatty acids which is volatile, water insoluble, or C8, C10, and C12 (Lawson 1995). SN is an indicator of molecular weight or size as a function of the chain lengths of the constituent fatty acids (Lawson 1995). MP is the level at which a solid fat becomes completely liquid and clear. Each individual pure fatty acid has a specific complete MP. Generally, MP increases with the increasing chain length. MP decreases with the increasing ratio of unsaturated fatty acid. IN is a measure of the number of double bonds or the degree of unsaturation (Lawson 1995). RI increases with the chain length and with the increasing unsaturation (Hamm & Hamilton 2000; Metin 2008).

2.5. Statistical analysis

The data was evaluated statistically using the analysis of variance (ANOVA) and the differences among the means were compared using the Duncan's multiple range tests (using SPSS statistical software program version 13 (SPSS Inc., Chicago, IL, USA; SPSS 1999). As a result the study, taking the advantage of R-MN, a formula was developed showing the addition of margarine to butter. We found this formula by using the simple linear regression model, which describes the statistical properties of estimators from the simple linear regression estimates, requires the use of a statistical model. Moreover, correlation coefficients were found between fat constants.

3. Results and Discussion

The analysis results of pure butter and pure margarine samples (control samples) are given in Table 1. The types and addition levels of margarine significantly influenced MP, R-MN, PN, SN, IN ($P<0.01$) RI ($P<0.05$) of the samples (Table 2). MP, RI, R-MN, PN, SN and IN were found to be 32.72 and 38.59, 1.4567 and 1.4620, 26.18 and 0.55, 0.88 and 0.21, 228.14 and 194.85, 32.36 and 56.65 in butter and margarine, respectively (Table 2). Therefore, the fat constant results mentioned above should be based on determining adulteration and its level. The effects of margarine addition rates on fat constants are shown in Figure 2-6. An increase of 10% altered samples of butter with margarine, PM and CM samples that are connected to both the contribution rate as well as the correlations between the fat constants, respectively, Tables 3 and 4 are given in the bulk.

The fat constants in the samples moved closer to margarine by increasing the levels of margarine. In each sample of added margarine, the MP, R-MN, SN and IN were found to be statistically significant ($P<0.01$). The fat samples of the rate of margarine contribution and main sources of variation of margarine have been effective on the MP, R-MN, PN, SN and statistically IN ($P<0.01$), and effective on the RI ($P<0.05$).

The constant values of fat samples moved closer to those of margarine as the contribution rate of margarine increased. In all contribution rates, MP, R-MN, SN and the IN were statistically different

Table 2- Average values of the analysis results related with adulterations of butter samples by margarine (n= 6)

Çizelge 2- Margarin ile tağşiş yapılan tereyağı örneklerinin analiz sonuçları ortalama değerleri (n= 6)

Margarine addition ratio (%)	*Melting point*	*Refractive index*	*R-M number*	*Polenske number*	*S.N.*	*Iodine number*
0	32.72±0.19^{a}	1.4567±0.00^{a}	26.18±0.09^{k}	0.88±0.11^{h}	228.14±2.07^{k}	32.36±0.42^{a}
10	33.48±0.40^{b}	1.4573±0.00^{ab}	23.57±0.20^{j}	0.77±0.08^{g}	222.44±1.47^{j}	34.80±1.24^{b}
20	34.06±0.57^{c}	1.4580±0.00^{bc}	20.97±0.48^{i}	0.67±0.09^{f}	219.20±2.41^{i}	37.40±2.12^{c}
30	34.57±0.79^{d}	1.4586±0.00^{cd}	18.42±0.61^{h}	0.58±0.13^{e}	216.92±2.64^{h}	40.51±1.97^{d}
40	35.08±1.14^{e}	1.4590±0.00^{de}	15.98±0.90^{g}	0.55±0.09^{e}	213.89±2.24^{g}	43.26±1.56^{e}
50	35.73±1.11^{f}	1.4596±0.00^{ef}	13.17±0.67^{f}	0.48±0.05^{d}	209.79±2.95^{f}	46.10±1.30^{f}
60	36.44±1.29^{g}	1.4600±0.00^{g}	10.43±0.68^{e}	0.42±0.07^{c}	206.53±2.25^{e}	48.66±0.93^{g}
70	37.00±1.35^{h}	1.4605±0.00^{gh}	8.01±0.58^{d}	0.39±0.03^{c}	203.83±3.02^{d}	50.66±0.57^{h}
80	37.50±1.37^{i}	1.4609±0.00^{hi}	5.16±0.60^{c}	0.30±0.05^{b}	199.87±4.49^{c}	53.15±0.58^{i}
90	38.02±1.49^{j}	1.4614±0.00^{ij}	2.83±0.39^{b}	0.28±0.04^{b}	197.33±4.41^{b}	54.78±0.80^{j}
100	38.59±1.64^{k}	1.4620±0.00^{j}	0.55±0.18^{a}	0.21±0.22^{a}	194.85±3.94^{a}	56.65±1.23^{k}
Margarine types (n= 33)						
CM	34.90±1.52^{a}	1.4600±0.00^{a}	12.80±8.46^{a}	0.52±0.22^{a}	209.36±11.32^{a}	45.84±7.78^{a}
PM	36.59±2.44^{b}	1.4590±0.00^{a}	13.61±8.32^{b}	0.48±0.21^{b}	211.15±10.49^{b}	44.77±8.38^{b}

Means followed with the same superscript letter within each column are not significantly different at P<0.01 probability levels; R-M number, Reichert-Meissl number; S.N., saponification number; PM, pastry margarine, CM, margarine for cooking

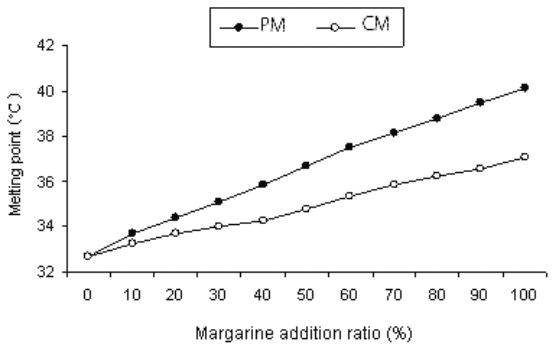

Figure 2- The effects of margarine contribution rates on the melting point

Şekil 2- Erime noktası üzerine margarin katkı oranının etkisi

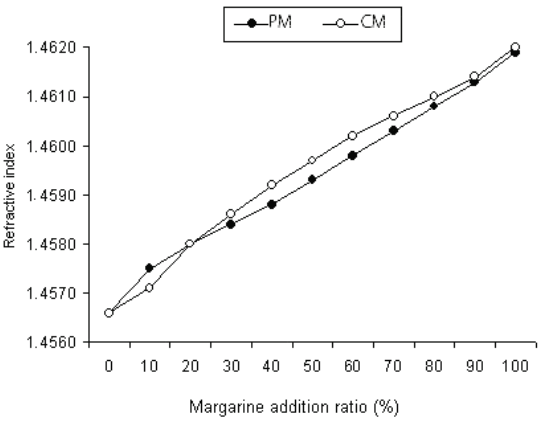

Figure 3- The effects of margarine contribution rates on the refractive index

Şekil 3- Kırılma indisi üzerine margarin katkı oranının etkisi

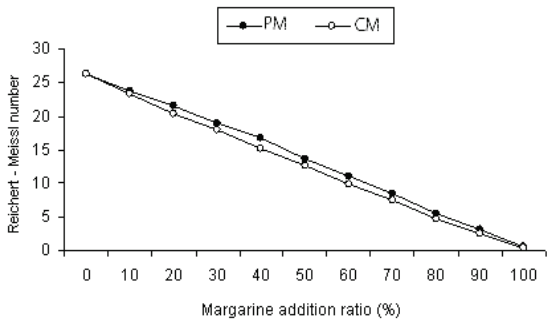

Figure 4- The effects of margarine contribution rates on the Reichert-Meissl number

Şekil 4- Reichert-Meissl sayısı üzerine margarin katkı oranının etkisi

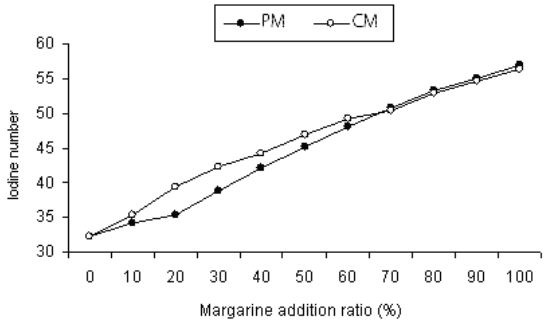

Figure 5- The effects of margarine contribution rates on the iodine number

Şekil 5- İyot sayısı üzerine margarin katkı oranının etkisi

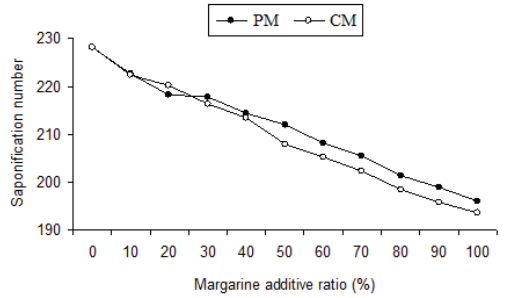

Figure 6- The effects of margarine contribution rates on the saponification number

Şekil 6- Sabunlaşma sayısı üzerine margarin katkı oranının etkisi

from each other. This situation has demonstrated that these four fat constants will give accurate results on detecting the degree to which margarine is added to butter, especially the R-MN (Table 2 and Figure 4).

R-MN of butter and margarine samples were found average as 26.18 and 0.55, respectively (Tables 1 and 2). These values are consistent with the values specified by other researchers (Kurt et al 2007; Metin 2008). R-MN and PN of animal fats decreases with the addition of foreign fat (Kurt et al 2007). RI changes from 1.4538 to 1.4578 in cow milk fat (Metin 2008). If butter is blended with vegetable fat, RI of butter increases. IN of milk fat is between 26-35 and the value is quite low compared to other oils (except coconut and palm kernel oil). IN of the milk fat increases with the addition of vegetable fats (Metin 2008). SN of the milk fat decreases with the addition of other fats, except coconut oil (Kurt et al 2007).

The values are very different from each other will be of great help on the adulteration of butter by margarine. Therefore, the fat constant results mentioned above should be based on determining the adulteration and its level. In this study, the highest correlation coefficient (r= -0.999) was found between the rate of contribution of margarine and R-MN (Table 3 and 4). As a result of this research study, taking advantage of the R-MN, the following Equation was developed showing the contribution of margarine to butter:

Margarine adulteration rate (M.A.R.) (%)= 100.73 - (3.84 × Reichert-Meissl number), (F= 12830.43**)

This Equation was also supported by other fat constants but their correlation coefficients were lower than R-MN's (Tables 3 and 4), therefore, R-MN should be used. As the following, regression equations are created for the other fat constants:

M. A. R. (%)= -416.02 + (13.04 × melting point), (F= 201.94**)

M. A. R. (%) = -21350.73 + (14663.50 × refractive index), (F= 193.43**)

M. A. R. (%)= 120 – (139.46 × polenske number), (F= 451.53**)

Table 3- Some correlations values between PM and calculated fat constants (n= 33)

Çizelge 3- Pastacılık margarini ve hesaplanmış yağ sabitleri arasındaki korelasyonlar (n= 33)

	M. P.	*R. I.*	*R-M. N.*	*P. N.*	*S. N.*	*I. N.*
M. A. R. (%)	0.988**	0.872**	-0.999**	-0.906**	-0.958**	0.992**
R. I.	0.838**					
R-M. N.	0.987**	-0.870**				
P. N.	-0.929**	-0.719**	0.905**			
S. N.	-0.950**	-0.916**	0.958**	0.863**		
I. N.	0.980**	0.873**	-0.992**	-0.895**	-0.954**	

**, correlation is significant at P<0.01 probability levels; M.A.R., margarine addition ratio; M. P., melting point; R. I., refractive index; R-M.N, Reichert-Meissl rumber; P. N., Polenske number; S. N., saponification number; I. N., Iodine number

Table 4- Some correlations values between CM and altered fat constants (n= 33)

Çizelge 4- Yemeklik margarin ve değişmiş yağ sabitleri arasındaki korelasyonlar (n=33)

	M. P.	*R. I.*	*R-M. N.*	*P. N.*	*S. N.*	*I. N.*
M. A .R. (%)	0.960**	0.863**	-0.998**	-0.956**	-0.977**	0.971**
R.I.	0.728**					
R-M. N.	-0.966**	-0.857**				
P. N.	-0.928**	-0.842**	0.960**			
S. N.	-0.951**	-0.822**	0.979**	0.953**		
I. N.	0.953**	0.799**	-0.976**	-0.930**	-0.964**	

**, correlation is significant at P<0.01 probability levels; M.A.R., margarine addition ratio; M. P., melting point; R. I., refractive index; R-M.N, Reichert-Meissl number; P. N., Polenske number; S. N., saponification number; I. N., Iodine number

M. A. R. (%)= 660.73 – (2.90 × saponification number), (F= 1130.20**)

M. A. R. (%)= -124.57 + (3.85 × iodine number), (F= 1571.10**)

However, it should not be used as a criterion for determining the adulteration of a small amount of margarine.

4. Conclusions

According to the results, the four fat constants including MP, R-MN, SN and IN can be reliably used to differentiate the margarine added butter from pure butter. This study has shown that these four fat constants will provide accurate results on detecting the degree to which margarine is added to butter, particularly the related R-MN. Margarine addition rate if it is over 20%, accurate results can be obtained with this formula. In conclusion, certain objective data and analytical methods were obtained to understand the adulteration with butter by margarine for food analysts.

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References

Aktaş N & Kaya M (2001). Detection of beef body fat and margarine in butterfat by differential scanning calorimetry. *Journal of Thermal Analysis and Calorimetry* **66**: 795-801

Anonymous (2009). Determination of melting point in fat. Marsan Gıda Sanayi ve Ticaret A.Ş. Laboratory Notes. Adana, Turkey

AOAC (1980). Official methods of analysis. 13th ed. Association of Official Analytical Chemists, Washington DC

Atamer M (1993). Tereyağı Teknolojisi Uygulama Kılavuzu. Ankara Üniversitesi Ziraat Fakültesi Yayınları No: 1314, Ankara, Türkiye

Derewiaka D, Sosińska E, Obiedziński M, Krogulec A & Czaplicki S (2011). Determination of the adulteration of butter. *European Journal of Lipid Science of Technology* **113**: 1005-1011

Dıraman H (2006). Tereyağı ve zeytinyağında muhtemel tağşişlerin kapiler kolon gaz kromatografisi yöntemi kullanılarak *Cis–Trans* yağ asitleri düzeyi ile belirlenmesi üzerine bir çalışma. *Academic Food Journal* **4**(23): 3-10

Hamm W & Hamilton R J (2000). Edible Oil Processing. In: Hamilton R J, editor. The Chemistry and Technology of Oils and Fats. Sheffield Academic Press, UK, pp. 18-19

James C S (1995). Analytical Chemistry of Foods. Chapman & Hall, Oxford

Koca N, Kocaoglu-Vurma N A, Harper W J & Rodriguez-Saona L E (2010). Application of temperature-controlled attenuated total reflectance-mid-infrared (ATR-MIR) spectroscopy for rapid estimation of butter adulteration. *Food Chemistry* **121**: 778-782

Kurt A, Çakmakçı S & Çağlar A (2007). Süt ve Mamulleri Muayene ve Analiz Metodları Rehberi. Atatürk Üniversitesi Ziraat Fakültesi Yayınları: 18, Ziraat Fakültesi Ofset Baskı, Erzurum, Türkiye

Lawson H (1995). Food Oils and Fats. Technology, Utilisation, and Nutrition. by New York: Chapman & Hall, pp. 30-31; 285-292

Mallia S, Piccinali P, Rehberger B, Badertscher R, Escher F & Schlichtherle-Cerny H (2008). Determination of storage stability of butter enriched with unsaturated fatty acids/conjugated linoleic acids (UFA/CLA) using instrumental and sensory methods. *International Dairy Journal* **18**: 983-993

Metin M (2008). Süt Teknolojisi-Sütün Bileşimi ve İşlenmesi. Ege Üniversitesi Mühendislik Fakültesi Yayın No: 33, Ege Üniversitesi Basımevi, Bornova, İzmir, Türkiye

Pegg R B (2005). Measurement of primary lipid oxidation products. In: Wrolstad RE, Acree TE, Decker EA, Penner MH, Reid D S, Schwartz SJ, Shoemaker CF, Smith DM, Sporns P, editors. Handbook of Food Analytical Chemistry: Water, proteins, enzymes, lipids, and carbohydrates. New York: John Wiley & Sons, Inc, pp. 515–529

Sherman H C (2009). Methods of Organic Analysis. Amazon.co.uk

Ulberth F & Buchgraber M (2000). Authenticity of fats and oils. *European Journal of Lipid Science of Technology* **102**: 687-694

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Chlorophylls Reductions in Fresh-Cut Chard (*Beta vulgaris* var. *cicla*) with Various Sanitizing Agents

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ABSTRACT

Safety of fresh-cut products is a widespread health concern and can be achieved by washing treatments with various agents. However, use of these agents can adversely affect the product quality depending on the processing and subsequent storage conditions. The effects of washing treatments with chlorine (50-200 mg L^{-1}), hydrogen peroxide (5.00-15.0%) and ozone (6.50 and 10.0 mg L^{-1}) followed by a cold storage (15 days/4 °C) period on chlorophylls contents of fresh-cut *Beta vulgaris* var. *cicla* (chard) were investigated by HPLC-DAD. In this study, treating samples with the sanitizing agents resulted in reductions in both chlorophyll a and chlorophyll b contents. These reductions generally increased with increasing the agent concentration. Chlorophyll a was found to be more sensitive than chlorophyll b to oxidation reactions with the agents used. Chlorophyll reductions of samples treated with ozone were at the higher level than samples treated by using other agents. Since the differences between chlorophylls contents of the samples treated with chlorine and hydrogen peroxide are very small, hydrogen peroxide can be suggested as an alternative to chlorine for sanitizing chard ($P<0.05$).

Keywords: *Beta vulgaris* var. *cicla;* Chard; Chlorophyll; Chlorine; Hydrogen peroxide; Ozone

Farklı Sanitasyon Ajanları Kullanımı ile Taze, Yıkanmış ve Doğranmış Pazılarda (*Beta vulgaris* var. *cicla*) Klorofil Düzeyinin Azalması

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ÖZET

Taze, yıkanmış ve doğranmış (fresh-cut) ürünlerde gıda güvenliği yaygın bir sorun olup, farklı yıkama ajanları kullanımı ile bu sorunun giderilmesi mümkün olabilir. Bununla birlikte, bu ajanların kullanımının proses ve daha sonra saklama

koşullarına bağlı olarak, ürün kalitesini olumsuz etkileyebilir. Bu çalışmada, farklı düzeylerde klor (50-200 mg L^{-1}), hidrojen peroksit (% 5.00-15.0) ve ozon (6.50 ve 10.0 mg L^{-1}) yıkama ajanı olarak kullanımının, soğuk depolama süresince (15 gün/4 °C) taze, yıkamış ve doğranmış *Beta vulgaris var. cicla* (pazı) klorofil içeriğine etkileri HPLC-DAD kullanılarak incelenmiştir. Çalışma sonucunda, sanitasyon (yıkama) ajanlarının kullanımı ile örneklerin klorofil a ve klorofil b içeriğinde düşüş belirlenmiştir. Bu düşüş genel olarak kullanılan ajan konsantrasyonu değişim düzeyi ile aynı yönde olmuştur. Klorofil a'nın, klorofil b'ye göre kullanılan ajanlardan kaynaklanan oksidasyona daha hassas olduğu tespit edilmiştir. Ozonla muamele edilen örneklerdeki klorofil kaybı, diğer ajanların kullanıldığı örneklere göre daha yüksek olarak belirlenmiştir. Klor ve hidrojen peroksit ile muamele edilmiş örneklerin klorofil içeriği arasındaki farklar çok küçük olduğu için, hidrojen peroksit pazı sanitasyonunda klora alternatif olarak önerilebilir ($P<0.05$).

Anahtar Kelimeler: *Beta vulgaris* var. *cicla;* Pazı; Klorofil; Klor; Hidrojen peroksit; Ozon

1. Introduction

Washing fruits and vegetables with sanitizing agents, like chlorine (Cl), ozone (O_3), hydrogen peroxide (H_2O_2), is a common practice to reduce the number of microorganisms and to extend the shelf life of the product. There are many scientific studies dealing with the effects of these washing processes most of which have focused on the microbial inactivation efficacy (Achen & Yousef 2001; Singh et al 2002; Garcia et al 2003; Selma et al 2007; Zorlugenc et al 2008; Olmez 2010; Zhou et al 2012; Elizaquivel et al 2012; Luo et al 2012). However, very little is known about the effects of these agents on the physical and chemical characteristics of the produce. Use of these agents at high concentrations in order to achieve higher microbial inactivation can cause deleterious effects on product quality such as losses in color, aroma, and nutritional value.

Chlorine (Cl), usually as hypochlorous acid HOCl, formed by dissociation of sodium hypochlorite (NaClO) in water, is the most commonly used disinfectant agent in the food industry. It is quite effective in inactivation food-borne microorganisms. However, it leads to the formation of toxic compounds on food contact surfaces and in wash water. For instance, trihalomethane compounds formed by the reaction of free-Cl with soluble organic compounds are proved to be carcinogenic (Kim et al 1999). For this reason, some restrictions in the use of Cl for washing agricultural products are implemented (Beltran et al 2005). Researchers and food processors investigate alternative applications to chlorination.

Ozone (O_3) and H_2O_2 appear to be promising alternatives with great potential applications in the food industry. After being used for years to disinfect water for drinking purposes, O_3 was approved for use as a disinfectant or sanitizer in food processing (FR 2001). Due to its quick decomposition to oxygen with no safety concerns about residues, it could be an acceptable technology to use with commodities marketed under "organic" classification (Gabler et al 2010). H_2O_2 is another chemical that can be used for disinfection of food (Kim et al 2007) and food contact surfaces (Khadre & Yousef 2001). Both O_3 and H_2O_2 are GRAS (generally recognized as safe) substances with high oxidation-reduction potentials, 2.1 and 1.8 mV, respectively (Kim et al 2003). Probably, because of these strong oxidizing activities, oxidations of color pigments such as carotenoids (Henry et al 2000) and anthocyanins (Simmons et al 1997) were reported.

Chlorophylls, principal color pigments in green vegetables, have two main types, namely chlorophyll a and chlorophyll b. Chlorophyll a is usually present at a concentration of 2-3 times higher than chlorophyll b in agricultural products (Kirca et al 2006). Minimizing chlorophyll degradation is an industrial challenge since chlorophylls are susceptible to chemical and physical changes during processing of vegetables. For instance, during thermal processing, the natural cellular structures disintegrate resulting in amenability of the pigment to various reactions

such as conversion of chlorophyll a and chlorophyll b to their corresponding pheophytins (Turkmen et al 2006). Also, reactions can occur through the removal of phytol group from chlorophylls and pheophytins by the action of enzyme chlorophyllase, resulting in the less stable chlorophyllides and pheophorbides, respectively (Kirca et al 2006). Bleaching of chlorophylls by oxidative reactions is another means of chlorophyll degradation. Procedures, in which strong oxidizing agents take place, may adversely affect nutritional and chemical product quality (e.g. may cause discoloration) depending on the concentration and time of exposure to the sanitizing solution.

The aim of this study was to investigate the effects of washing treatments with Cl, O_3, and H_2O_2 solutions on chlorophylls contents of fresh-cut chard throughout cold storage for 15 days and to compare these agents and their different doses in terms of chlorophyll degradation.

2. Material and Methods

2.1. Materials

Chard (*Beta vulgaris* var. *cicla*) used in the study was obtained from a local farmers market in Ankara and used immediately in the experiments. Chlorophyll a (Sigma C-5753) and chlorophyll b (Sigma C-5878) standards were purchased from Sigma Co (St. Louis, MO, USA). Methanol and chloroform were obtained from Riedel-de Haen (Seelze, Germany) whereas hexane was obtained from Sigma Aldrich (Steinheim, Germany). All solvents were either analytical or high performance liquid chromatography (HPLC) grade.

2.2. Preparation of chard samples for the treatments

Leaves that are uniform in size and color were selected and washed under running tap water to remove dirt, soil, etc. Midribs (white sections) were excised with a sharp stainless-steel knife and discarded. The rest of the leaves (leaflets) were cut into 1.00-1.50 cm wide strips with the knife. Cut leaf pieces were blended (mixed) for uniformity and separated into four lots for different treatments. One of the lots was directly used for chlorophyll analyses (controls) whereas the others were immediately treated with Cl, O_3 or H_2O_2 solutions.

2.3. Preparation of washing solutions and treatment procedures

High purity water (better than ASTM Type 2) obtained from a TKA Pacific UP/UPW water purification system (TKA Water Purification Systems GmbH, Niederelbert, Germany) was used for preparing all aqueous washing solutions. Washing solutions of Cl (50, 100 and 200 mg L^{-1}) and H_2O_2 (5.00, 10.0 and 15.0%) were prepared by appropriate dilutions of sodium hypochlorite solution (Sigma Aldrich, available Cl 10.0-13.0%) and H_2O_2 solution (Riedel de Haen, 30%, v v^{-1}), respectively. Cl levels in the treatment solutions were determined by Cl test strips (Quantofix, 1-100 mg L^{-1}, Macherey Nagel, Duren, Germany). The most effective ozonation method mentioned in the literature (Kim et al 1999; Achen & Yousef 2001; Olmez 2010), bubbling, was used for O_3 treatments of chard samples. O_3 was produced by a corona discharge generator (OG 20, Opal, Ankara, Turkey) with a production capacity of 20 g h^{-1}. The generator had an oxygen concentrator inside and used oxygen gas concentrated from the air for O_3 production. The generator could be run at two different levels, 10 and 20 g h^{-1}. Gaseous O_3, passing through silicone hose, was bubbled into the water by the help of a stainless-steel sparger with 10 µm pore size (Solvent inlet filter, Fisher Scientific, Fair Lawn, NJ, USA). The gas flow was controlled at 827 mL min^{-1} by a Riteflow flowmeter (150 mm, Size 2, Bel-Art Products, Pequannock, NJ, USA). O_3 concentrations in water were determined by using indigo blue dye, which is based upon Standard Methods (APHA, 1992). For this method, a stock indigo solution was prepared with potassium indigo trisulfonate (234087, Sigma Aldrich) and phosphoric acid (Riedel-de Haen). Indigo trisulfonate was decolorized by O_3 and the color changes were measured in spectrophotometer (UV-1601, Shimadzu, Kyoto, Japan) at 600 nm. At the end of ozonation processes, the O_3 concentrations in

water were 6.50±0.12 and 10.0±0.14 mg L^{-1} in case of running the O_3 generator at low (10 g h^{-1}) and high (20 g h^{-1}) levels, respectively.

Treatments were conducted in 1-L borosilicate glass jars containing the sanitizing solutions. For O_3 treatments, gaseous O_3 was bubbled into the water present in the jar. Jars containing 20 g of cut chard sample and 800 mL of sanitizing solution were shaken with an orbital shaker (Biosan OS-10, Riga, Latvia) at a speed of 200 rpm. All treatments were conducted at room temperature (22 °C) for 15 min. This treatment time is quite long and maybe impractical for the industry. However, it was necessary to use an extended treatment time to observe any detrimental effects of the agents -if they have- to chlorophylls contents of chard.

Treated chard samples were soaked in distilled water (1 L) for 1 min for rinsing. After removing the excessive water with a manual salad spinner, samples were placed in plastic zip-lock freezer bags and stored at 4±1 °C for 15 days.

2.4. Extraction of chlorophylls and HPLC analysis

Chlorophyll extractions were carried out according to the method of Teng & Chen (1999) with modifications. Extraction of chlorophylls was performed under dim light and at low temperatures to minimize photo degradation of the pigments. All the leaves from each treatment were homogenized using a lab blender (Waring blender) for 1 min. Some of the homogenized sample (~5 g) was put into a mortar and the tissue was mashed with a pestle. Mashed sample (0.20±0.001 g) was weighed in a test tube, to which 3.00 mL of methanol were added. After vortexing for 1 min at high speed, the methanol-phase containing the chlorophylls was transferred to a 25 mL volumetric flask. The residue in the test tube was re-extracted with 3.00 mL of methanol. This extraction procedure was repeated several (6-7) times until the residue became colorless. Then, all the extracts were pooled and brought to volume with methanol. This crude extract was centrifuged (Sigma, Model 2-16, Osterode, Germany) at 4000 rpm for 10 min and filtered through a hydrophilic PTFE Millex-LCR membrane filter (Millipore, Bedford, MA, USA) with 0.45 µm pores into an amber flask and immediately injected to HPLC.

2.5. HPLC analysis

HPLC analysis was carried out using a Shimadzu system (Kyoto, Japan) consisting of a LC-20AD pump, a DGU-20A5-E degasser and a UV-VIS photo diode array detector (SPD-M20). The chromatograms were recorded at 430 and 460 nm for chlorophyll a and chlorophyll b, respectively (Teng & Chen 1999). A Phenomenex (Torrance, CA, USA) analytical column (C18, 5 µm, 250 mm x 4.6 mm i.d.) was used in the experiments. A mixture of methanol:chloroform:n-hexan (85:7.5:7.5) at a flow rate of 1 mL min^{-1} under isocratic conditions was used as the mobile phase.

2.6. Standard solutions

Stock solutions of chlorophyll a (40 mg L^{-1}) and chlorophyll b (20 mg L^{-1}) were prepared by dissolving chlorophyll a and chlorophyll b standards, respectively, in methanol. Standard solutions of chlorophyll a (1.00, 5.00, 10.0, 15.0 and 20.0 mg L^{-1}) and chlorophyll b (1.00, 5.00, 7.00, 10.0 and 15.0 mg L^{-1}), prepared by appropriate dilutions of stock solutions with methanol, were injected in to HPLC and calibration curves were prepared.

2.7. Dry matter determination

To eliminate the variations in water content occurring due to respiration, transpiration, etc. during storage, all calculations were made on dry matter (DM) basis. DM contents of chard samples were determined according to Gornicki & Kaleta (2007), in triplicate.

2.8. Statistical analysis

The data were subjected to analysis of variance (ANOVA) to determine the significant differences between means by using Minitab statistical package (v.13, MINITAB Inc., USA). Values (n= 3) were reported as mean degradation rate ± standard error. Duncan's multiple range test, at a significance level of P= 0.05, was conducted for the separation

of means by using MSTAT-C statistical software (MSTAT 1991, Michigan State University, MI, USA).

3. Results and Discussion

Retention times for chlorophyll a and chlorophyll b were determined as 8.1 and 5.8 min, respectively. Chromatograms of chlorophyll a and chlorophyll b in untreated chard samples are shown as an example in Figure 1.

The chlorophyll a contents in chard samples treated with various doses of different sanitizing agents are shown in Table 1. Chlorophyll a levels significantly decreased just after treatments with the agents in all samples. Statistical analysis revealed that disinfectant doses and storage time significantly affected the levels of chlorophyll a in chard samples. Moreover, these factors showed a significant interaction for Cl and H_2O_2 treatments ($P<0.05$), but not for O_3 ($P>0.05$).

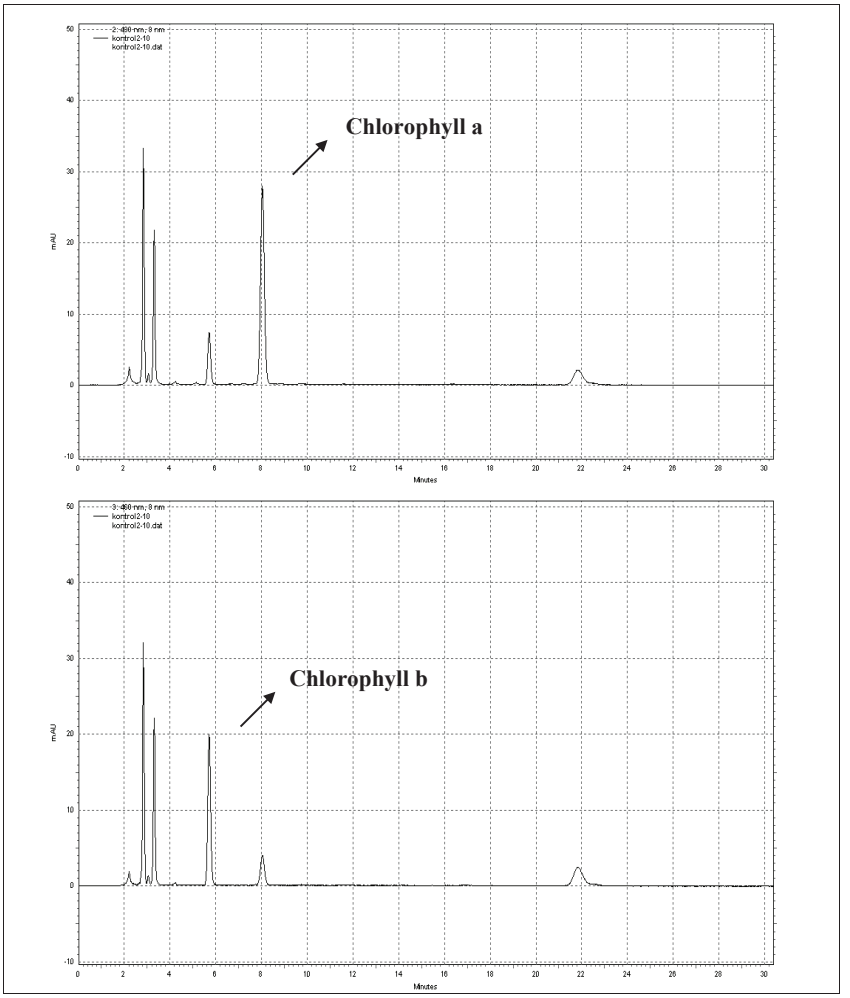

Figure 1- Chlorophyll a (at 430 nm) and chlorophyll b (at 460 nm) peaks in untreated chard

Şekil 1- İşlem görmemiş pazıda klorofil a (430 nm) ve klorofil b (460 nm) pikleri

Table 1- Chlorophyll a contents (mg kg^{-1}, DM) in chards throughout storage after treating with various doses of sanitizers (mean ± standard error, n= 3)

Çizelge 1- Farklı sanitasyon ajanları ile işlem sonrası depolama süresince pazıların klorofil a içeriklerindeki (mg kg^{-1}, KM) değişimler (ortalama ± standard hata, n= 3)

Disinfectant	*Disinfectant dose*	*Chlorophyll a*			
		Storage time (days)			
		0	*5*	*10*	*15*
Control	-	15650±250^{Aa*}	13330±87^{Ab}	12370±114^{Ac}	11730±149^{Ac}
Chlorine	50 mg L^{-1}	12810±287^{Ba}	12490±82^{Bab}	11820±139^{ABb}	10740±411^{Bc}
	100 mg L^{-1}	12690±326^{Ba}	11770±83^{Cb}	11250±90^{BCb}	10520±196^{Bc}
	200 mg L^{-1}	12140±283^{Ba}	11710±54^{Ca}	10770±370^{Cb}	9837±238^{Cc}
Ozone	6.50 mg L^{-1}	12980±177^{Ba}	12412±190^{Ba}	11567±139^{Bb}	10022±201^{Bc}
	10.0 mg L^{-1}	12138±205^{Ba}	10895±178^{Cb}	9369±88^{Cbc}	8705±99^{Cc}
Hydrogen peroxide	5.00%	13260±126^{Ba}	12820±93^{Aa}	11740±329^{ABb}	10700±411^{Bc}
	10.0%	11990±472^{Ca}	11680±332^{Bab}	11120±278^{BCb}	9981±196^{Bc}
	15.0%	11280±296^{Ca}	11060±227^{Ba}	10630±422^{Ca}	9105±238^{Cb}

*, means with different letters shown with lower case (a-c) show significant differences among sampling days (P<0.05) and means with different letters shown with upper case (A-C) show significant differences among doses and types of the agents (P<0.05)

Chlorophyll a content decreased in all samples including control throughout storage (Table 1). In control samples, 15.0, 21.0 and 25.0% reductions of chlorophyll a were observed at day 5, 10 and 15, respectively. Chlorophyll b contents of the samples treated by various doses of different sanitizers during 15-day storage are given in Table 2.

Similar to the results observed for chlorophyll a, chlorophyll b content decreased in all samples including control throughout storage. Reduction rates of chlorophyll b were 12.0, 17.0 and 23.0% at day 5, 10 and 15, respectively, in control samples. Disinfectant dose and storage time did not show any significant interaction on chlorophyll content of

Table 2- Chlorophyll b contents (mg kg^{-1} DM) in chards throughout storage after treating with various doses of sanitizers (mean ± standard error, n= 3)

Çizelge 2- Farklı sanitasyon ajanları ile işlem sonrası depolama süresince pazıların klorofil b içeriklerindeki (mg kg^{-1}, KM) değişimler (ortalama ± standard hata, n= 3)

Disinfectant	*Disinfectant dose*	*Chlorophyll b*			
		Storage time (days)			
		0	*5*	*10*	*15*
Control	-	5641±79^{Aa*}	4964±66^{Ab}	4682±65^{Abc}	4344±62^{Ac}
Chlorine	50 mg L^{-1}	5026±101^{Ba}	4200±68^{Bb}	4102±92^{Bb}	4062±117^{Bb}
	100 mg L^{-1}	4654±68^{BCa}	4117±105^{BCb}	4030±88^{BCbc}	3781±49^{Cc}
	200 mg L^{-1}	4297±77^{Ca}	4052±66^{Cb}	3802±89^{Cb}	3778±65^{Cb}
Ozone	6.50 mg L^{-1}	4970±103^{Ba}	4570±82^{Bb}	4290±101^{Bc}	3892±95^{BCd}
	10.0 mg L^{-1}	4443±111^{Ca}	3825±92^{Db}	3628±93^{Db}	3328±90^{Dc}
Hydrogen peroxide	5.00%	4617±75^{BCa}	4296±95^{Bb}	4233±88^{Bb}	4118±114^{ABb}
	10.0%	4519±77^{Ca}	4093±109^{Cb}	3811±86^{Cbc}	3610±82^{Cc}
	15.0%	4065±89Da	3830±79^{Db}	3779±64^{Cb}	3497±69^{Dc}

*, means with different letters shown with lower case (a-d) show significant differences among sampling days (P<0.05) and means with different letters shown with upper case (A-D) show significant differences among doses and types of the agents (P<0.05)

chard (P>0.05). Chlorophyll loss in leafy vegetables during storage is an ordinary consequence of senescence due to the disintegration of the plant tissues. Reductions in green colors of lettuce (Bolin & Huxsoll 1991), chard (Roura et al 2000), chicory and rocket (Ferrante et al 2004) during storage were also reported in previous studies.

3.1. Effect of Cl treatment on chlorophyll content in chard samples throughout storage

Treating chard samples with Cl solutions at various concentrations (50.0-200 mg L^{-1}) resulted in reductions in both chlorophyll a and chlorophyll b contents (Table 1 and Table 2). These reductions increased with increasing Cl concentration. At the end of storage period (15 days), reduction rates were 31.0, 33.0 and 37.0% for chlorophyll a and 28.0, 33.0 and 33.0% for chlorophyll b in samples treated with Cl solutions of 50.0, 100 and 200 mg L^{-1}, respectively. Reducing chlorophylls levels after Cl treatments in green bell peppers were also reported by Nunes & Emond (1999). They dipped green bell peppers into Cl solutions (0.00-200 mg L^{-1}) for varying time (0-45 min) and observed that total chlorophyll contents decreased with increasing time of dipping and Cl concentration.

In our study, the differences between Cl doses on chlorophyll a degradation were not significant at the beginning of storage, but became significant from day 5 on (P<0.01). Likewise, chlorophyll a and chlorophyll b levels in parsley samples treated with chlorinated (100 mg L^{-1}) and ozonated (12 mg L^{-1}) water significantly decreased compared to control samples beginning from the fifth day of storage (Karaca 2010). The author claimed that cellular fluids released due to cutting or vigorous washing were removed by water rinse. Kenny & O'Beirne (2009) reported that color loss was more pronounced in water-dipped and Cl-dipped lettuce than the samples subjected to a milder treatment (tap-rinsing). In intact cell tissues, chlorophyll is separated spatially from chlorophyllase, a key enzyme in chlorophyll metabolism. When cells of fresh produce are ruptured, as occurs during cutting or vigorous washing, chemical reactions are initiated that shorten storage life (Bolin & Huxsoll 1991). Chlorophyllase and its substrate, chlorophyll, come into contact and chlorophyll degradation reactions occur particularly in tissues adjacent to those that are damaged by cutting action, when acids and hydrolyzing enzymes of the vacuoles are released (Roura et al 2000).

In all samples including control, chlorophyll b levels determined at day 5 were significantly lower than those determined at the beginning of storage. It shows that chlorophyll b degradation takes place very rapidly in chard tissues. There were no significant differences between chlorophyll a contents of the samples treated with 50.0 and 100 mg L^{-1} Cl and between chlorophyll b contents of the samples treated with 100 and 200 mg L^{-1} Cl (P>0.05). Hence, it can be said that using higher concentrations of Cl would not result in any additional chlorophyll degradation, in other words, would not cause color loss in chard. Enhancing Cl concentration can be useful for achieving higher microbial inactivation levels. However, excessive use of Cl can also result in higher formation of toxic residues on produce surface and in wash water.

3.2. Effect of O_3 treatment on chlorophyll content in chard samples throughout storage

Similar to the results of Cl treatments, treating chard samples with O_3 solutions resulted in reductions in chlorophyll a and chlorophyll b contents (Table 1 and Table 2). At the end of storage period, reduction rates were 36.0 and 44.0% for chlorophyll a and 31.0 and 41.0% for chlorophyll b in samples treated with O_3 concentrations of 6.50 and 10.0 mg L^{-1}, respectively. These results show the susceptibility of chlorophyll a and chlorophyll b in chard to O_3. Philosoph-Hadas et al (1994) also claimed that chlorophylls are extremely sensitive to oxidative compounds such as O_3 and free radicals. In addition, recognizable discolorations were reported in many products such as broccoli (Skog & Chu 2001), lettuce (Singh et al 2002; Olmez & Akbas 2009), spinach (Klockow & Keener 2009; Vurma et al 2009) and *Arabidopsis thaliana* (Kubo et al 1995) after O_3 treatments.

The chlorophyll a contents of chard samples treated with high O_3 dose (10.0 mg L^{-1}) were significantly lower at day 5 than that at day zero. On the other hand; when treated with low O_3 dose (6.50

mg L^{-1}), the chlorophyll a level was maintained on the fifth day of storage and a decline was observed at day 10. In all samples, decreases were determined in chlorophyll b content on each day of sampling. Reduction in chlorophyll a content was slightly higher than that in chlorophyll b (3.00-5.00%) after treating with both O_3 doses.

3.3. Effect of H_2O_2 treatment on chlorophyll content in chard samples throughout storage

Treating chard samples with H_2O_2 solutions at various concentrations (5.00-15.0%) also resulted in reductions in both chlorophyll a and chlorophyll b contents (Table 1 and Table 2). In samples treated with 5.00, 10.0 and 15.0% of H_2O_2 solutions 32.0, 37.0 and 42.0% reductions of chlorophyll a and 27.0, 36.0 and 38.0% reductions of chlorophyll b were observed, respectively, at the end of storage.

Chlorophyll a levels determined just after washing treatments with H_2O_2 were maintained at day 5 after treatments with the solutions of 5.00 and 10.0% and at day 10 after treatments with the solutions of 15.0%. There were no significant differences between chlorophyll a contents of the samples treated with 10.0 and 15.0% of H_2O_2 at day zero, 5 and 10 ($P>0.05$). Moreover, no significant differences were observed between the chlorophyll b contents of samples treated with the solution of 5.00% H_2O_2 and that of control at the end of storage period ($P>0.05$). This means that the solution of H_2O_2 at 5.00% concentration did not cause any additional loss of chlorophyll b in chard samples stored for 15 days. The reductions in chlorophylls content in chard samples increased with increasing the concentration of H_2O_2 as well as other agents. Likewise, many other researchers (Simmons et al 1997) reported that when used as a surface disinfectant, H_2O_2 caused degradation of pigments (chlorophylls, anthocyanins, etc.) and this detrimental effect increased with increasing the agent concentration. In addition, H_2O_2 is claimed to be involved in a system (phenolic-peroxidase-H_2O_2 system) in *in vitro* bleaching of chlorophylls (Kato & Shimizu 1987). By the function of this system, chlorophyll is oxidized to colorless, low-molecular weight compounds (Yamauchi et al 2004).

3.4. Comparison of susceptibilities of chlorophylls against different sanitizing agents

For both chlorophyll a and chlorophyll b, the differences between treatments were less pronounced in the beginning of storage, but became more evident on the last sampling day, especially in the treatments with high O_3 dose. Chlorophyll a and chlorophyll b retentions in chards treated with various agents after 15-day storage are presented in Figure 2 and Figure 3, respectively. Chlorophyll a and chlorophyll b retentions in control samples were 75.0 and 77.0%, respectively, at the end of storage period. Chlorophyll a retention rates in Cl, O_3 and H_2O_2 treated samples were 63.0-69.0, 56.0-64.0, and 58.0-68.0%, respectively, at the end of storage period. Corresponding rates for chlorophyll b were 67.0-72.0, 59.0-69.0, and 62.0-73.0%. It shows that the decrease of chlorophyll a is slightly more marked than that of chlorophyll b.

At the end of storage period, chlorophyll a and chlorophyll b reductions were 32.0 and 27.0%, respectively, in samples treated with the solution of H_2O_2 at 5.00%. In samples treated with 10.0 and 15.0% H_2O_2 solutions 37.0 and 42.0% chlorophyll a reduction and 36.0 and 38.0% chlorophyll b reduction were observed, respectively. Similar results were obtained for Cl and O_3 treatments. For instance, in samples treated with 200 mg L^{-1} Cl, 6.50 mg L^{-1} and 10.0 mg L^{-1} O_3, chlorophyll a and chlorophyll b reductions were 37.0 and 33.0%, 35.0 and 31.0%, and 43.0 and 41.0%, respectively, at the end of storage. Overall, it can be said that chlorophyll a is more intensely degraded than chlorophyll b after all treatments, suggesting that chlorophyll a is more susceptible to oxidation with these agents. In previous studies, many authors suggested that chlorophyll a is more sensitive than chlorophyll b to heat (Weemaes et al 1999), sulphur dioxide and ethylene (Zhou et al 2010) treatments. Although nearly one-third of chlorophyll a and one-fourth of chlorophyll b reduced at the end of storage period, formation of pheophytins was not observed after any treatments. Pheophytins are the main degradation products of chlorophylls that form mainly during thermal processes (Turkmen et al 2006). Probably, in the degradation of chlorophylls with oxidizing agents such as Cl, O_3 and H_2O_2,

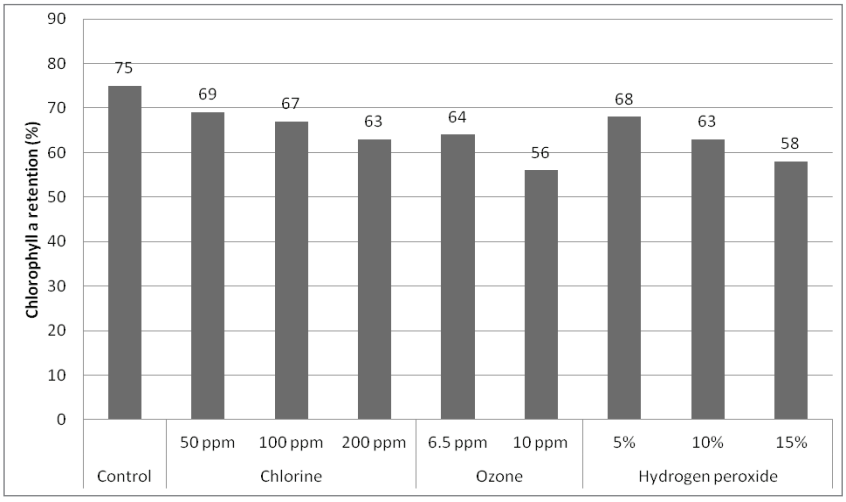

Figure 2- Chlorophyll a retention in chards treated with various agents after 15-day storage [The chlorophyll a content in untreated samples at the beginning of storage (15650 mg kg^{-1} DM was assumed as 100%, ppm= mg kg^{-1})

Şekil 2- 15 günlük depolama sonrası farklı ajanlarla muamele edilen pazılarda belirlenen klorofil a düzeyleri [İşlem uygulanmamış örneklerde depolama başlangıcındaki klorofil a içeriği (kuru maddede 15650 mg kg^{-1} % 100 olarak kabul edilmiştir, ppm= mg kg^{-1})

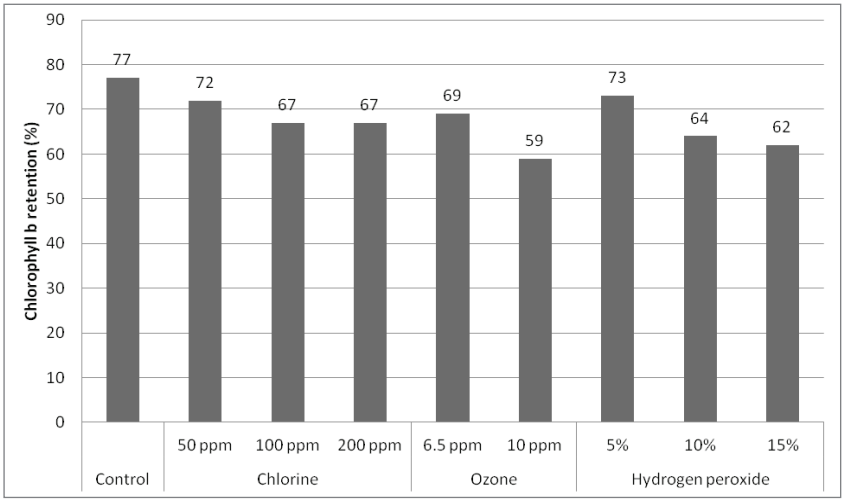

Figure 3- Chlorophyll b retention in chards treated with various agents after 15-day storage [The chlorophyll b content in untreated samples at the beginning of storage (5641 mg kg^{-1} DM was assumed as 100%, ppm= mg kg^{-1})

Şekil 3- 15 günlük depolama sonrası farklı ajanlarla muamele edilen pazılarda belirlenen klorofil b düzeyleri [İşlem uygulanmamış örneklerde depolama başlangıcındaki klorofil a içeriği (kuru maddede 5641 mg kg^{-1} % 100 olarak kabul edilmiştir, ppm= mg kg^{-1})

different pathways dominate the oxidative degradation mechanisms. Beltran et al (2005) and Lopez-Galvez et al (2010) did not determine any significant differences between chlorophyll contents of lettuce treated with different agents like O_3, Cl and Cl-dioxide. According to our results, since the chlorophyll contents of the samples treated with Cl and H_2O_2 are so close (1.00-5.00% difference), H_2O_2 can be suggested as an alternative of Cl.

4. Conclusions

In conclusion, both chlorophyll a and chlorophyll b contents decreased in all samples including controls during storage. At the end of 15-day storage, 25.0% of chlorophyll a and 23.0% of chlorophyll b reductions were observed in control (untreated) samples. Chlorophyll a reductions in Cl, O_3, and H_2O_2 treated samples were 31.0-37.0, 36.0-44.0, and 32.0-42.0%, respectively, at the end of storage. These rates were 28.0-33.0, 31.0-41.0, and 27.0-38.0% for chlorophyll b. Results revealed that chlorophyll a is more sensitive than chlorophyll b to oxidation reactions with the sanitizers used. H_2O_2 appears to be a good alternative of Cl in terms of color retention in chard. In addition, O_3 use can be appropriate at low dose and for short storage times.

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References

Achen M & Yousef A E (2001). Efficacy of ozone against Escherichia coli O157:H7 on apples. *Journal of Food Science* **66**: 1380-1384

APHA (1992). Standard methods for the examination of water and wastewater (18th edition): 4500-O3. Indigo Colorimetric Method, Washington, USA

Beltran D, Selma M V, Marin A & Gil M I (2005). Ozonated water extends the shelf life of fresh-cut lettuce. *Journal of Agricultural and Food Chemistry* **53**: 5654-5663

Bolin H R & Huxsoll C C (1991). Effect of preparation procedures and storage parameters on quality retention of salad-cut lettuce. *Journal of Food Science* **56**: 60-62

Elizaquivel P, Sanchez G, Selma M V & Aznar R (2012). Application pf propidium monoazide-qPCR to evaluate the ultrasonic inactivation of Escherichia coli 0157:H7 in fresh-cut vegetable wash water. *Food Microbiology* **30(1)**: 316-320

Ferrante A, Incrocci L, Maggini R, Serra G & Tognoni F (2004). Colour changes of fresh cut leafy vegetables during storage. *Journal of Food Agriculture Environment* **2**: 40-44

FR (Federal Register) (2001). Secondary direct food additives permitted in food for human consumption. Rules and Regulations June 26, 66, 123, Sec:173.368 Ozone, Final Rule

Gabler F M, Smilanick J L, Mansour M F & Karaca H (2010). Influence of fumigation with high concentrations of ozone gas on postharvest gray mold and fungicide residues on table grapes. *Postharvest Biology and Technology* **55**: 85-90

Garcia A, Mount J R & Davidson P M (2003). Ozone and chlorine treatment of minimally processed lettuce. *Journal of Food Science* **68**: 2747-2751.

Gornicki K & Kaleta A (2007). Modelling convection drying of blanched parsley root slices. *Biosystem Engineering* **97**: 51-59

Henry L K, Puspitasari-Nienaber N L, Jaren-Galan M, van Breemen R B, Catignani G L & Schwartz S J (2000). Effects of ozone and oxygen on the degradation of carotenoids in an aqueous model system. *Journal of Agricultural and Food Chemistry* **48**: 5008-5013

Karaca H (2010). Effect of ozonation on microbial inactivation and shelf life of lettuce, spinach, and parsley. Unpublished Ph. D. Thesis, pp. 91, Ankara University, Ankara, Turkey

Kato M & Shimizu S (1987). Chlorophyll metabolism in higher plants. VII-Chlorophyll degradation in senescing tobacco leaves–phenolic-dependent peroxidative degradation. *Canadian Journal of Botany* **65**: 729-735

Kenny O & O'Beirne D (2009). The effects of washing treatment on antioxidant retention in ready-to-use iceberg lettuce. *International Journal of Food Science Technology* **44**: 1146-1156

Khadre M A & Yousef A E (2001). Decontamination of a multilaminated aseptic food packaging material and stainless steel by ozone. *Journal of Food Safety* **21**: 1-13

Kim H J, Fonseca J M, Kubota C & Choi J H (2007). Effect of hydrogen peroxide on quality of fresh-cut tomato. *Journal of Food Science* **72**: 463-467

Kim J G, Yousef A E & Dave S (1999). Application of ozone for enhancing the microbiological safety and quality of foods: A review. *Journal of Food Protection* **62**: 1071-1087

Kim J G, Yousef A E & Khadre M A (2003). Ozone and its current and future application in the food industry. *Advances in Food Nutrition Research* **45**: 167-218

Kirca A, Yemis O & Ozkan M (2006). Chlorophyll and colour changes in grapevine leaves preserved by passive modification. *European Food Research and Technology* **223**: 387-393

Klockow P A & Keener K M (2009). Safety and quality assessment of packaged spinach treated with a novel ozone-generation system. *LWT-Food Science and Technology* **42**: 1047-1053

Kubo A, Saji H, Tanak K & Kondo N (1995). Expression of arabidopsis cytosolic ascorbate peroxidase gene in response to ozone or sulfur dioxide. *Plant Molecular Biology* **29**: 479-489

Lopez-Galvez F, Allende A, Martinez-Sanchez A, Tudela J A, Selma M V & Gil M V (2010). Suitability of aqueous chlorine dioxide versus sodium hypochlorite as an effective sanitizer for preserving quality of fresh-cut lettuce while avoiding by-product formation. *Postharvest Biology and Technology* **55**: 53-60

Luo Y, Nou X, Millner P, Zhou B, Shen C, Yang Y, Wu Y, Wang Q, Feng H & Shelton D (2012). A pilot plant scale evaluation of a new process aid for enhancing chlorine efficacy aganist pathogen survival and cross-contamination during wash. International *Journal of Food Microbiology* **158(2)**: 133-139

Nunes M C N & Emond J P (1999). Chlorinated water treatments affects postharvest quality of green bell peppers. *Journal of Food Quality* **22**: 353-361

Olmez H & Akbas M Y (2009). Optimization of ozone treatment of fresh-cut green leaf lettuce. *Journal of Food Engineering* **90**: 487-494

Olmez H (2010). Effect of different sanitizing methods and incubation time and temperature on inactivation of Escherichia coli on lettuce. *Journal of Food Safety* **30**: 288-299

Philosoph-Hadas S, Meir S, Akiri B & Kanner J (1994). Oxidative defense systems in leaves o three edible herb species in relation to their senescence rates. *Journal of Agricultural and Food Chemistry* **44**: 2376-2381

Roura S I, Davidovich L A & del Valle C E (2000). Quality loss in minimally processed Swiss chard related to amount of damaged area. *LWT-Food Science and Technology* **33**: 53-59

Selma M V, Beltran D, Allende A, Chacon-Vera E, Gil, M I (2007). Elimination by ozone of *Shigella sonnei* in shredded lettuce and water. *Food Microbiology* **24**: 492-499

Simmons G F, Smilanick J L, John S & Margosan D A (1997). Reduction of microbial populations on prunes by vapor-phase hydrogen peroxide. *Journal of Food Protection* **60**: 188-191

Singh N, Singh R K, Bhunia A K & Stroshine R L (2002). Efficacy of chlorine dioxide, ozone, and thyme essential oil or a sequential washing in killing *Escherichia coli* O157 : H7 on lettuce and baby carrots. *LWT- Food Science and Technology* **35**: 720-729

Skog L J & Chu C L (2001). Effect of ozone on qualities of fruits and vegetables in cold storage. *Canadian Journal of Plant Science* **81**: 773-778

Teng S S & Chen B H (1999). Formation of pyrochlorophylls and their derivatives in spinach leaves during heating. *Food Chemistry* **65**: 367-373

Turkmen N, Poyrazoglu E S, Sari F & Velioglu Y S (2006). Effects of cooking methods on chlorophylls, pheophytins and colour of selected green vegetables. *International Journal of Food Science and Technology* **41**: 281-288

Vurma M, Pandit R B, Sastry S K & Yousef A E (2009). Inactivation of Escherichia coli O157:H7 and natural microbiota on spinach leaves using gaseous ozone during vacuum cooling and simulated transportation. *Journal of Food Protection* **72**: 1538-1546

Weemaes C A, Ooms V, Loey A M V & Hendrickx M E (1999). Kinetics of chlorophyll degradation and color loss in heated broccoli juice. *Journal of Agricultural and Food Chemistry* **47**: 2404-2409

Yamauchi N, Funamoto Y & Shigyo M (2004). Peroxidase-mediated chlorophyll degradation in horticultural crops. *Phytochemistry Reviews* **3**: 221-228

Zhou J Y, Sun C D, Zhang L L, Dai X A, Xu C J & Chen K S (2010). Preferential accumulation of orange-colored carotenoids in Ponkan (Citrus reticulata) fruit peel following postharvest application of ethylene or ethephon. *Scientia Horticulturae* **126**: 229-235

Zhou B, Feng H & Pearlstein A J (2012). Continuous-flow ultrasonic washing system for fresh produce surface decontamination. *Innovative Food Science and Emerging Technologies* **16**: 427-435

Zorlugenc B, Zorlugenc F K, Oztekin S & Evliya I B (2008). The influence of gaseous ozone and ozonated water on microbial flora and degradation of aflatoxin B1 in dried figs. *Food and Chemical Toxicology* **46**: 3593-3597

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Yavaş Gelişen Sentetik Etlik Piliç Genotipleri ile Ticari Etlik Piliçlerin Büyüme, Karkas Özellikleri ve Bazı Ekonomik Parametreler Bakımından Karşılaştırılması

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ÖZET

Bu çalışmada yavaş gelişen 2 etlik piliç genotipinin (SG1, SG2) 49 günlük ve hızlı gelişen bir ticari etlik piliç genotipinin 42 (FG1) ve 49 günlük (FG2) besi süresi sonunda besi performansları, karkas özellikleri ve bazı ekonomik parametreleri karşılaştırılmıştır. İki dönem yürütülen denemede; her dönemde her genotipten 260, toplam 1560 (260 x 3 x 2) civciv kullanılmıştır. Civcivler her genotipte 10 tekerrür olacak şekilde yer bölmelerinde büyütülmüştür. Besi süresince gruplarda (SG1, SG2, FG1, FG2) canlı ağırlık, yem tüketimi, yemden yararlanma oranı ve ölüm oranı, besi süresi sonunda ise kesim ve karkas özellikleri belirlenmiştir. Üretim masrafları ve piliç eti satış değerlerine ait verilerden yararlanılarak gruplarda maliyet, karlılık ve teknik etkinlik skorları hesaplanmıştır. Grupların teknik etkinlik skorları stokastik sınır modeli (SSM) ile tahmin edilmiştir. SG1 ve SG2 genotipleri kesim canlı ağırlığına (2.0-2.5 kg) 49 günde ulaşmış; yem tüketimleri ise FG1'in yem tüketim düzeyine yakın olmuştur. Göğüs oranı FG1 ve FG2'de, but oranı ise SG1 ve SG2'de yüksek bulunmuştur. En yüksek nispi karlılık FG1 grubunda olmuş, bunu FG2, SG2 ve SG1 izlemiştir. SG1, SG2, FG1 ve FG2'nin teknik etkinlik skorları 0.9632, 0.9639, 0.9664 ve 0.9699 bulunmuştur ($P<0.05$). Net ve nispi karlılıkta ilk sıralarda yer alan FG1 ve FG2'de % 5.00 ve % 6.54 düzeyindeki ölümler önemli kayıplar olarak düşünülebilir. SG1 ve SG2 genotiplerinde 49 günlük yaşta ölüm oranı % 1.15 ve % 2.69 seviyesinde gerçekleşmiştir ($P<0.05$). Daha yavaş gelişerek 49 günlük besi süresinde kesim ağırlığına ulaşabilen SG1 ve SG2 genotipleri bu açıdan avantaj sağlamışlardır.

Anahtar Kelimeler: Ticari etlik piliç; Yemden yararlanma; Karkas özellikleri; Canlı ağırlık; Karlılık; Teknik etkinlik skoru

Comparison of Slow Growing Synthetic Broiler Genotypes with Commercial Broilers in Terms of Growth, Carcass Traits and Some Economic Parameters

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ABSTRACT

In this study, 2 slow growing broiler genotypes (SG1 and SG2) slaughtered at 49 days of age were compared with a fast growing commercial genotype slaughtered at 42 days of age (FG1) or 49 days of age (FG2) with respect to fattening performance, carcass characteristics and some economic parameters. The study was carried out in 2 periods and 260 chicks from each genotype in each period (a total of 1560 chicks; 260 x 3 x 2) were used. Ten replicates of chicks from each genotype were reared on litter system. Live weight, feed consumption, feed efficiency and mortality in groups (SG1, SG2, FG1, FG2) were determined throughout the production periods, while slaughter and carcass traits were determined at the end of production periods. Cost, efficiency and technical efficiency scores were calculated by using production expenses and meat sale prices. Stochastic frontier analysis (SFA) was used to estimate the technical efficiency scores of groups. SG1 and SG2 genotypes reached to slaughtering live weight (2.0-2.5 kg) at 49 days of age, and the feed consumptions of these 2 genotypes were similar to the consumption of F1. The breast ratio was higher in FG1 and FG2, whereas the thigh ratio was higher in SG1 and SG2. The highest relative profit was determined in FG1 genotype and FG2, SG2 and SG1 followed this genotype, respectively. The technical efficiency scores of SG1, SG2, FG1 and FG2 were 0.9632, 0.9639, 0.9664 and 0.9699 ($P<0.05$), respectively. The mortality in FG1 (5.00%) and FG2 (6.54%) can be interpreted as significant losses, although net and relative profits of these groups were superior to SG1 and SG2. At the end of 49 days, the mortality in SG1 and SG2 genotypes occurred as 1.15% and 2.69%, respectively ($P<0.05$). Despite the fact that SG1 and SG2 genotypes reached to slaughtering weight at 49 days of age, they were advantageous due to their lower mortality levels.

Keywords: Commercial broiler; Feed efficiency; Carcass traits; Live weight; Profitability; Technical efficiency score

1. Giriş

Etlik piliç üretimi tüm dünyada 1940'lı yıllardan itibaren gelişme göstererek, domuz etinden sonra en çok üretilen ürün haline gelmiştir (Yang & Jiang 2005). Gelecekte de bu trendin devam ederek, tavuk eti üretim ve tüketimindeki artışların diğer etlerden daha yüksek olacağı beklenmektedir (Anonymous 2013). Domuz eti tüketimi olmayan ülkelerde ise tavuk etinin payı % 65-70'e ulaşmıştır (Sarıca et al 2014a). Taleplerin tavuk eti lehine artmasındaki önemli etkenler; üretimin kolaylığı, arazi ihtiyacının düşüklüğü, tüm toplumlarda hiçbir yasak olmadan tüketilmesi, üretim süresinin kısalığı, sağlıklı olduğu imajı ve ucuzluğudur. Bugün üretimi yapılan etlik piliçlerde gelişme, yemden yararlanma ve karkas özelliklerinde geçmişe göre ciddi değişiklikler olmuştur. Bu değişikliklerde seleksiyon, bakım ve besleme ve sağlık korumadaki gelişmelerin önemli katkıları bulunmaktadır. Genetik seleksiyonun buradaki payının % 85-90 olduğu kabul edilmektedir (Havenstein et al 2003; Arthur & Albers 2003; Blagojevic et al 2009).

Gelişme düzeyi, yemden yararlanma ve et üretimi artmasına rağmen, piliçlerde yaşama gücü azalmış, iskelet gelişimi ve uyumu bozulmuş, kardiyovasküler hastalıklar artmış, bağışıklık sisteminde bozulmalar görülmüştür (Havenstein et al 2003; Cheema et al 2003; Decuypere et al 2003; Bessei 2006; Shim et al 2012).

Performans özelliklerine ilave olarak, tüketiciyi ve ürün işleme endüstrisini ilgilendiren karkas parçalarında, yağlanma ve organoleptik özelliklerin değişmesinde de hızlı gelişme yönünde seleksiyonun ciddi etkileri olmuştur (Remignon & Le Bihan-Duval 2003; Phongpa-ngan et al 2014). Diğer taraftan kesim yaşının 35-40 güne kadar düşürülmesi hayvan hakları savunucuları, tüketiciler, sağlıkçılar gibi değişik kesimlerce sorgulanmaktadır. Özellikle bazı Asya ve Avrupa ülkelerindeki tüketiciler yavaş gelişen, 56-81 gün arasında kesilen piliçlerin etlerine yüksek fiyat ödemeyi tercih etmektedirler (Chin 2003; Fanatico et al 2005a, 2005b; Fanatico et al 2006). Hızlı gelişen etlik piliç üretimi, alternatif üretimlerle karşılaştırılamayacak kadar etkin olmasına rağmen, bazı ülkelerde yavaş-orta düzeyde gelişen etlik piliç üretimi ve bunlar için uygulanan üretim sistemleri giderek yaygınlaşmaktadır (Mikulski et al 2011;

Chen et al 2013; Sarica et al 2014a). Büyüme, yem tüketimi, besi süresi ve karkas özellikleri bakımından önemli farklılıklara sahip olan yavaş-orta düzeyde gelişen genotiplerin üreticiye ekonomik getirisi de farklıdır (Shim et al 2012). Hızlı gelişen piliçlerde kesim yaşının düşüklüğü nedeniyle yıllık üretim kapasitesinin artması, entansifleşmeye bağlı olarak yüksek yerleşim sıklığı ve başarılı entegrasyon uygulamalarıyla gerçekleşen kesintisiz üretim sayesinde yüksek gelir sağlanmaktadır. Bu nedenle, et kalitesi, tüketici talebi, hayvan refahı, yaşama gücünün yüksekliği, dengeli büyüme gibi avantajlara sahip olan yavaş-orta düzeyde gelişen etlik piliçlerin hızlı gelişenlerle ekonomik açıdan da rekabet edebilmeleri önem taşımaktadır.

Bu çalışmada, yavaş gelişen etlik piliç üretiminde kullanmak için melezleme yoluyla üretilen 2 ebeveyn genotipinin (ROSS x Rhode Island Red ve ROSS x Barred Plymouth Rock) tavukları, ROSS-308 ebeveyninin horozlarıyla çiftleştirilerek orta düzeyde gelişen 2 genotip elde edilmiştir. Bu genotipler performans, karkas özellikleri, yaşama gücü ve net ekonomik gelir açısından ticari etlik piliç genotipiyle karşılaştırılmıştır.

2. Materyal ve Yöntem

Çalışma, Ondokuz Mayıs Üniversitesi Ziraat Fakültesi'nde 2013 yılının Şubat-Temmuz ayları arasında, 3 farklı genotiple yürütülmüştür. Bunlardan ikisi; yavaş gelişen etlik piliç üretimi için geliştirilmekte olan ikili melez ROSSxRhode Island Red (ROSSxRIR) ve ROSSxBarred Plymouth Rock (ROSSxBAR) genotiplerinin ROSS ebeveynleri ile çiftleştirilmesi sonucu üretilmiştir. Bu üçlü melezlerden biri SG1 (ROSSx(ROSSxRIR)), diğeri ise SG2 (ROSSx(ROSSxBAR)), olarak kodlanmıştır (Yamak et al 2014; Sarica et al 2014b). Elde edilen bu 2 genotip hızlı gelişen ROSS-308 hibritleri ile performans, karkas özellikleri ve ekonomik parametreler bakımından karşılaştırılmıştır.

Araştırma aynı kümeste Şubat-2012'de birinci dönem, Nisan-2013'te ise ikinci dönem üretimi olmak üzere 2 dönemde gerçekleştirilmiştir. SG1 ve SG2 genotipleri, ebeveynlerin 35 ve 42 haftalık yaşlarında elde edilen kuluçkalık yumurtalarından üretilmiştir. Araştırma-uygulama çiftliğindeki kuluçkahanede elde edilen civcivler ile aynı günde kuluçkadan çıkan ROSS-308 civcivleri alınarak deneme başlatılmıştır. Yetiştirilen sentetik genotiplerin ebeveynleri çalışmanın yürütüldüğü işletmede bulunduğundan ebeveyn yaşları bilinmektedir. Ancak, kıyaslamada kullanılan hızlı gelişen etlik piliçler ticari bir işletmeden alındığından ebeveyn yaşları bilinmemektedir. Buna rağmen, 2 üretim dönemi ardı sıra gerçekleştirilmiş, her 2 dönemde de etlik piliçler aynı firmadan temin edilmiştir. Dolayısıyla her 2 dönemde alınan hızlı gelişen piliçlerin aynı sürüden olma ihtimali oldukça yüksektir. Ancak, bunu ispatlayacak bir veri bulunmamaktadır. Her 2 üretim dönemi de 49 gün olarak uygulanmış, SG1 ve SG2 genotipleri 49. günde, ROSS-308 piliçlerinin yarısı 42. günde, diğer yarısı ise 49. günde kesilmişlerdir. Tüm genotiplerin performans kıyaslamasını daha anlaşılır kılmak için 42. günde kesilen ROSS-308 piliçleri FG1, 49. günde kesilen ROSS-308 piliçleri ise FG2 olarak kodlanmıştır.

Deneme pencereli, yapay havalandırılan, beyaz tasarruf ampulleri ile aydınlatılan, elektrikli infrared ısıtıcılarla ısıtılan, 20x12 m boyutlarında, 2.5 m yüksekliğinde bir kümeste yürütülmüştür. Her dönemde her genotipten erkek-dişi karışık 260'ar civciv (260 x 3= 780 civciv) üretime alınmıştır. Civcivler; 1.5 x 1.5 x 2.0 m (en x boy x yükseklik) boyutlarındaki tel ile kapalı 10'ar bölmeye, her bölmede 26 civciv olacak şekilde yerleştirilmiştir. İki üretim döneminde toplam 1560 civciv (780 x 2) kullanılmıştır. Böylece, 2 dönemde SG1 ve SG2 genotiplerinden 520'şer, ROSS-308 genotipinin FG1 ve FG2 grubunda 260'ar civciv denemeye alınmıştır.

Her bölmede birer askılı tüp yemlik ve 8 nipel suluk bulundurulmuştur. Yem ve su deneme boyunca serbest olarak verilmiştir. Her bölmeye eşit ağırlıkta, 8 cm kalınlığında kaba rende talaşı altlık olarak serilmiştir. Aydınlatma; ilk gün 24 saat, 21 güne kadar 23 saat ve kesim yaşına kadar 18 saat uygulanmıştır. Sağlık koruma uygulamaları ticari üretimdeki aşılama programına uyularak gerçekleştirilmiştir. Denemede kullanılan yemlerin

Çizelge 1- Değişik yaşlarda kullanılan yemlerin besin maddesi düzeyleri*

Table 1- Nutrients of diets used at different ages

Besin maddeleri	*Etlik civciv başlangıç (1-7. gün)*	*Etlik civciv (8-28. gün)*	*Etlik piliç (29-35. gün)*	*Etlik piliç bitiş (36-kesim)*
Ham protein (%)	23	22	21	18
ME (kcal kg^{-1})	3000	3100	3100	3100
Ham selüloz (%)	4.00	4.00	4.00	6.00
Ham kül (%)	5.00	5.00	5.00	8.00
Ca (%)	1.00	0.95	0.80	0.80
Yarar. fosfor (%)	0.50	0.50	0.45	0.60
Methionin (%)	1.00	0.45	0.40	0.40
Lysin (%)	1.35	1.20	1.10	1.00

*, hesaplanmış değerler

içerikleri Çizelge 1'de verilmiştir. Yemler bir ticari fabrikadan alınmıştır.

Denemede; canlı ağırlık, yem tüketimi, yemden yararlanma oranı, ölüm oranı, kesim ve karkas özellikleri belirlenmiş, üretim yapılan dönemde üretim maliyetini belirleyen unsurlara ait veriler ve bütün ve parçalanmış piliç eti piyasa fiyatları elde edilmiştir. Yem tüketimleri ve canlı ağırlıklar bölmeler bazında haftalık belirlenmiştir. Bu verilerden yemden yararlanma oranları hesaplanmıştır. Ölümler günlük olarak kaydedilmiş ve ölüm oranları belirlenmiştir. SG1 ve SG2 genotiplerinin ve FG2 ticari piliçlerinin kesimleri 49. günde, FG1 ticari piliçlerinin ise genel uygulamada olduğu üzere 42. günde yapılmıştır. SG1 ve SG2 genotiplerinde her bölmeden 2 erkek, 2 dişi piliç kesilmiştir. Her üretim döneminde SG1 ve SG2 genotiplerinden 40'ar piliç, toplamda 2 üretim döneminde 160 piliç kesilmiştir. Her üretim döneminde her bölmeden 4 erkek, 4 dişi olmak üzere FG1 ve FG2 genotiplerinden 5'er bölmeden toplam 80 piliç, 2 üretim döneminde toplam 160 piliç kesilmiştir. Piliçlerde kesim ağırlığı ve yenilebilir iç organlar (kalp, karaciğer ve temizlenmiş taşlık) ağırlığı kesim esnasında, soğuk karkas ağırlığı ise karkaslar +4 °C'de 24 saat bekletildikten sonra belirlenmiştir. Yenilebilir iç organların karkas ağırlığına oranları hesaplanmıştır. Abdominal yağ düzeyi; abdominal kaslara bağlı ve kloak çevresindeki yağlar ve iç organlar etrafındaki yağlar olarak belirlenmiş, abdominal yağın canlı ağırlığa ve karkas ağırlığına oranı hesaplanmıştır (Sarıca 1997; Sarıca et al 2009; Sarıca et al 2011). Karkas parçalamada standart uygulama yapılmış (Yamak et al 2014), but, göğüs, kanat, sırt ve boyun parçaları tartılmış ve karkas ağırlığına oranlanarak karkas parça oranları hesaplanmıştır. Tüm karkas parçaları, deri ve kemik ile birlikte tartılmıştır.

Performans ve karkas özelliklerine ait veriler tesadüf parselleri deneme deseninde dönemleri de ele alacak şekilde analiz edilmiştir. Oran ile ifade edilen verilere açı (arcsinüs) transformasyonu yapılmıştır. Ortalama değerler arasındaki farklılıkların çoklu karşılaştırılması Duncan testi ile yapılmıştır. Verilerin analizinde SPSS paket programı (Version 16) kullanılmıştır.

Ekonomik değerlendirmede maliyet; civciv, yem, aşı, işçilik, altlık, ısıtma, aydınlatma ve havalandırma giderlerinden oluşmuştur. Gelirler ise karkas ve karkas parça satış fiyatlarına göre hesaplanmıştır. Gelir ve gider hesabında ayrıca yaşama gücü (%) ve besi süresi (gün) kullanılmıştır. Maliyetler her genotip için ayrı, kg $piliç^{-1}$ ve günlük olarak hesaplanmıştır. Bunlardan mutlak ve nispi karlar belirlenmiştir. Genotiplerde teknik etkinliğin ölçümünde stokastik sınır modeli (SSM) kullanılmıştır (Coelli et al 1998). Teknik yetersizlik;

genotiplerdeki gerçek karkas üretim değeri ve farklı genotipler için stokastik sınır modeli ile tahmin edilen karkas üretim değeri arasındaki uzaklık olarak tanımlanmıştır. SSM'de bağımlı değişken olarak "karkas üretim değeri (TL adet^{-1})" alınmıştır. SSM'nin üretim fonksiyonu tahmininde diğer şartlar sabit kılınmış, bağımsız değişken olarak yem (kg piliç$^{-1}$) kullanılmıştır. Stokastik sınır modelinin yapısı Eşitlik 1'deki gibidir (Battese 1992; Battese & Coelli 1995; Coelli et al 1998).

$$\ln(T_i) = \ln(X_i)\beta + V_i - U_i,\ i = 1,\ldots\ldots,260 \qquad (1)$$

Burada; T_i, i'nci pilicin üretim değerini; X_i, i'nci pilicin girdilerini; β, girdi ile çıktı arasındaki ilişkiye ait parametreyi; U_i, negatif olmayan hata değişkenini ifade etmektedir. Bu değişken 0 ile 1 arasında olup teknik etkinliği göstermektedir. Formülde yer alan V_i ölçüm hatası gibi işletmenin kontrolünde olmayan, sıfır ortalamaya sahip hata terimini ifade etmekte ve U_i'den bağımsızdır. Bu yönteme göre; her bir civciv için teknik etkinlik, gözlenen üretim değerini olması gereken üretim değerine oranlayarak bulunmaktadır. Teknik etkinliğin hesaplanmasında Eşitlik 2 kullanılmıştır:

$$TE_i = \frac{Y_i}{Y_i^*} \qquad (2)$$

Burada; TE_i, i'nci pilicin teknik etkinliğini; Y_i, gözlenen üretim değerini; Y_i^*, ise tahmin edilen ve olması gereken üretim değerini ifade etmektedir. Stokastik sınır modelinde Cobb-Douglas tipi üretim fonksiyonu kullanılmış ve parametreler "En Yüksek Olabilirlik" yöntemi ile tahmin edilmiştir. Stokastik sınır modelinin tahmininde "Front 41, Version 4.0" paket programı kullanılmıştır (Coelli et al 1998).

3. Bulgular ve Tartışma

3.1. Performans ve karkas özellikleri

Genotip gruplarının haftalık canlı ağırlık ortalama değerleri arasındaki farklılıklar önemli (P<0.05) bulunmuştur. Ticari genotiplerin (FG1 ve FG2) 42 günlük yaşta ulaştıkları canlı ağırlıklara SG1 ve SG2 genotipleri 49 günlük yaşta yaklaşabilmişlerdir. (Çizelge 2). Birinci dönem canlı ağırlık ortalama değerleri ikinci döneme göre daha düşük (P<0.05) olmuştur. Birinci dönemde tüm genotiplerde denemeye alınan civcivlerin genç ebeveyn sürüsünün yumurtalarından çıkmaları nedeniyle civciv ağırlıkları daha düşük olmuştur. Ayrıca yetiştirme dönemleri arasındaki farklılıklar ile diğer yetiştirme faktörlerinin de canlı ağırlığı etkileyebildiği bilinmektedir (Türkoğlu & Sarıca 2014). Ancak, uygulamalar tüm genotipler için her dönemde sabit tutulmuştur. Ticari etlik piliçlerde 2.0-2.5 kg kesim

Çizelge 2- Gruplarda yaşa bağlı canlı ağırlık (kg) değişimi

Table 2- The variation in live weights (kg) of groups at different ages

		Yaş (hafta)							
		Çıkış	*1*	*2*	*3*	*4*	*5*	*6*	*7*
Grup	SG1	41.68 b	159.73 b	393.96 b	621.87 b	1016.74 b	1455.77 b	1951.76 b	2360.29 b
	SG2	40.74 b	154.82 b	388.13 b	621.94 b	1010.94 b	1483.28 b	1957.33 b	2363.07 b
	FG1	46.55 a	169.61 a	455.74 a	807.76 a	1379.98 a	2047.09 a	2669.99 a	-
	FG2	46.69 a	174.92 a	460.85 a	817.38 a	1380.04 a	2033.89 a	2716.49 a	3295.28 a
Dönem	1	40.11	127.55	361.72	691.04	1160.70	1737.94	2263.15	2585.53
	2	47.74	201.99	487.63	743.44	1233.15	1772.08	2384.64	2760.24
St. Hata		0.271	1.003	2.297	4.510	6.658	8.927	10.383	13.012
Etkiler									
Dönem		**	**	**	**	**	öd	**	**
Genotip		**	**	**	**	**	**	**	**
Dönem x Genotip		**	*	öd	*	öd	**	*	*

*, P<0.05; **, P<0.01; öd, P>0.05; farklı harflerle gösterilen ortalamalar arasındaki fark önemlidir

ağırlığına ulaşma 40.-50. günlerde, orta düzeyde gelişenlerde ise 50.-60. günlerde gerçekleşirken, yavaş gelişenlerde kesim yaşı genotipik yapıya göre değişebilmektedir (Grashorn & Clostermann 2002; Yang & Jiang 2005; Dou et al 2009). Çalışmada SG1 ve SG2 genotiplerinin, ortalama canlı ağırlık değerleri, Fanatico et al (2007)'nın orta düzeyde gelişenlerden elde ettiği değerlerden daha yüksek görülmektedir. Hatta Phangpa-nagan et al (2014)'nın hızlı gelişen broiler hatlarından elde ettikleri canlı ağırlık değerleriyle benzer veya daha yüksektir.

Genotiplerin ortalama canlı ağırlık değerlerinde olduğu gibi, yem tüketimi ve yemden yararlanma oranları tüm yaşlarda genotipler arasında farklı bulunmuştur (P<0.05). FG1 ve FG2'nin 6. hafta yem tüketimleri ile SG1 ve SG2'nin 7. hafta yem tüketimleri benzerlik göstermiştir (Çizelge 3). FG1 ve FG2'nin 7. haftada yemden yararlanma oranları SG1 ve SG2'nin 6. hafta yemden yararlanma oranları ile benzer bulunmuştur (Çizelge 4). Yem tüketimleri bazı yaşlarda dönemlere göre farklılık (P<0.05) göstermesine karşın, yemden yararlanma

Çizelge 3- Gruplarda yaşa bağlı yem tüketimlerindeki değişim

Table 3- The variation in feed consumption of groups at different ages

		Yaş (hafta)						
		1	*2*	*3*	*4*	*5*	*6*	*7*
Grup	SG1	163.16 b	487.07 b	933.67 b	1646.91 b	2519.05 b	3545.19 b	4583.88 b
	SG2	156.58 c	471.99 b	920.10 b	1621.15 b	2515.73 b	3516.08 b	4568.60 b
	FG1	176.58 a	537.67 a	1075.85 a	1984.11 a	3126.80 a	4476.07 a	-
	FG2	175.30 a	549.28 a	1102.19 a	2001.72 a	3156.45 a	4531.92 a	5890.38 a
Dönem	1	133.81	514.05	985.47	1761.30	2796.83	3930.29	4917.75
	2	201.99	508.97	1030.45	1865.66	2861.19	4104.35	5110.84
St. Hata		1.077	3.558	6.538	11.797	17.157	20.274	26.904
Etkiler								
Dönem		**	öd	**	**	öd	**	**
Genotip		**	**	**	**	**	**	**
Dönem x Genotip		öd	**	**	öd	öd	öd	öd

*, P<0.05; **, P<0.01; öd, P>0.05; farklı harflerle gösterilen ortalamalar arasındaki fark önemlidir

Çizelge 4- Gruplarda yaşa bağlı yemden yararlanma oranlarındaki değişim

Table 4- The variation in feed conversion ratios of groups at different ages

		Yaş (hafta)						
		1	*2*	*3*	*4*	*5*	*6*	*7*
Grup	SG1	0.98 ab	1.26 a	1.50 a	1.62 a	1.73 a	1.81 a	1.94
	SG2	0.95 bc	1.24 ab	1.48 a	1.60 a	1.69 a	1.79 a	1.93
	FG1	1.00 a	1.20 b	1.33 b	1.44 b	1.52 b	1.67 b	-
	FG2	0.94 c	1.21 b	1.35 b	1.45 b	1.55 b	1.66 b	1.79
Dönem	1	1.05	1.42	1.44	1.53	1.63	1.75	1.91
	2	0.891	1.05	1.40	1.53	1.63	1.73	1.87
St. Hata		0.006	0.008	0.011	0.011	0.009	0.008	0.008
Etkiler								
Dönem		**	**	*	öd	öd	öd	*
Genotip		*	*	**	**	**	**	**
Dönem x Genotip		öd	öd	öd	öd	öd	öd	öd

*, P<0.05; **, P<0.01; öd, P>0.05; farklı harflerle gösterilen ortalamalar arasındaki fark önemlidir

oranları 4., 5. ve 6. haftalarda dönemlere göre farklılık göstermemiştir. Etlik piliçlerde gelişme hızına bağlı olarak, besi süresinin yükselmesiyle yem tüketimi artarken, yemden yararlanma düşmektedir (Fanatico et al 2005b). Gelişme hızı ve besi süresine göre yemden yararlanmada büyük varyasyon görülmektedir. Abdullah et al (2010), 3'ü hızlı gelişen, 1'i yavaş gelişen 4 genotipin 43 günlük yaşta canlı ağırlıklarını sırasıyla; 2038, 1911, 1806 ve 1621 g, yemden yararlanma oranlarını ise 2.21, 2.33, 2.49 ve 2.62 olarak belirlemişlerdir. Mikulski et al (2011), hızlı ve yavaş gelişen genotiplerin 42 günlük yaşta canlı ağırlıklarını; 2.41 ve 1.94 kg, yemden yararlanma oranlarını ise 1.69 ve 1.70 olarak saptamışlardır. Shim et al (2012), 4 baba ve 3 ana broiler hattından üretilen ikili melez 7 broiler genotipinin 48 günlük yaşta canlı ağırlıklarını 3.09-3.40 kg; yemden yararlanma oranlarını ise 1.74 ile 1.84 arasında belirlemişlerdir. Çalışmamızda, tüm genotiplerin gerek 42, gerekse 49 günlük yaşlarda yemden yararlanma oranlarının kabul edilebilir düzeyde olduğu belirlenmiştir.

FG1 ve FG2 gruplarının karkas ağırlıkları ve karkas randımanları SG1 ve SG2 gruplarına göre daha yüksek (P<0.05) bulunmuş, yenilebilir iç organ ağırlıklarında da benzer bir durum görülmüştür. FG2 grubunun ortalama karkas ağırlığı ile karaciğer ve taşlık ağırlıkları FG1 grubu, SG1 ve SG2 genotiplerinden daha yüksek (P<0.05) bulunmuştur. En düşük abdominal yağ oranı FG1 grubunda saptanmış, FG1 grubunun abdominal yağ oranı tüm grupların abdominal yağ oranı ortalamalarından farklı (P<0.05) bulunmuştur (Çizelge 5).

Yenilebilir iç organların karkasın %'si olarak verilmesi için analizler yapılmış, ancak gerçek durumun ortaya konulması için ağırlık değerleri tercih edilmiştir. Oranlar ile ağırlık değerleri arasında benzer eğilimler görüldüğü için böyle bir değerlendirme yolu tercih edilmiştir. Karkas ağırlığı, karkas randımanı, yenilebilir iç organ ağırlıkları ve abdominal yağ oranları bakımından dönemler arasında farklılıklar önemli (P<0.05) bulunmuştur. Karkas ağırlığı ve karkas randımanı daha yüksek olan ticari genotipin abdominal yağ düzeyinin de düşük olması; SG1 ve SG2 genotiplerinin yavaş ve orta gelişme gösteren hatlarla benzerliğinin bir

Çizelge 5- Gruplarda karkas özelliklerine ait değişim

Table 5- The variation in carcass traits of groups at different ages

		Özellikler							
		Canlı ağırlık (g)	*Karkas (g)*	*Karkas (%)*	*Kalp (g)*	*Karaciğer (g)*	*Taşlık (g)*	*Abdominal yağ[1] (%)*	*Abdominal yağ[2] (%)*
Grup	SG1	2411.6 c	1773.4 c	73.51 b	14.6 b	48.5 c	29.0 b	1.80 ab	2.45 ab
	SG2	2518.1 c	1841.6 c	73.13 b	13.2 c	50.9 c	29.1 b	1.93 a	2.64 a
	FG1	2756.3 b	2066.6 b	74.95 a	18.1 a	59.3 b	28.8 b	1.43 c	1.91 c
	FG2	3384.1 a	2549.9 a	75.38 a	18.7 a	71.4 a	31.6 a	1.70 b	2.25 b
Dönem	1	2662.45	1988.30	74.55	15.16	54.82	28.39	1.58	2.12
	2	2872.67	2127.48	73.95	17.17	60.33	30.96	1.85	2.51
St. Hata		20.16	15.46	0.10	0.17	0.54	0.32	0.033	0.045
Etkiler									
Dönem		**	**	**	**	**	**	**	**
Genotip		**	**	**	**	**	**	**	**
Dönem x Genotip		öd	öd	öd	**	**	**	öd	öd

*, P<0.05; **, P<0.01; öd, P>0.05; farklı harflerle gösterilen ortalamalar arasındaki fark önemlidir; [1], canlı ağırlığa oran; [2], karkas ağırlığına oran

göstergesidir. Böyle hatlarda gelişme düzeyine bağlı olarak karkas randımanının değiştiği, hızlı gelişmeye paralel olarak karkas randımanının arttığı ve abdominal yağ düzeylerinin azaldığı daha önceki araştırmalarda (Fanatico et al 2005a; Kokoszynski & Bernacki 2008; Abdullah et al 2010; Mikulski et al 2011) ortaya konulmuştur.

SG1, SG2 ve FG1 gruplarının but oranları benzer olmasına karşın; FG1 ve FG2 gruplarının göğüs oranları SG1 ve SG2 genotiplerinden daha yüksek (P<0.05); yine kanat, sırt ve boyun oranları SG1 ve SG2 genotiplerinde FG1 ve FG2 gruplarından daha yüksek (P<0.05) bulunmuştur (Çizelge 6). But, göğüs, kanat gibi karkas parça

Çizelge 6- Gruplarda karkas parçalarına ait değişim

Table 6- The variation in carcass parts of groups at different ages

		Özellikler							
		But (%)	*Göğüs (%)*	*Kanat (%)*	*Sırt (%)*	*Boyun (%)*	*But (g)*	*Göğüs (g)*	*Kanat (g)*
Grup	SG1	27.7 a	36.1 b	10.8 a	18.9 a	6.7 a	491.42 c	638.90 c	191.92 b
	SG2	27.9 a	35.6 b	10.6 b	19.2 a	6.6 a	514.87 c	654.25 c	195.83 b
	FG1	27.6 a	40.5 a	9.3 d	17.2 c	5.2 c	570.71 b	836.33 b	192.62 b
	FG2	26.1 b	40.4 a	9.6 c	18.2 b	5.8 b	667.37 a	1029.77 a	243.23 a
Dönem	1	28.01	38.31	9.97	17.99	5.80	555.75	765.73	196.13
	2	26.68	37.97	10.22	18.73	6.36	566.44	813.90	215.68
St. Hata		0.088	0.110	0.036	0.093	0.038	4.912	6.076	1.594
Etkiler									
Dönem		**	öd	**	**	**	öd	**	**
Genotip		**	**	**	**	**	**	**	**
Dönem x Genotip		öd	öd	öd	**	**	öd	öd	öd

*, P<0.05; **, P<0.01; öd, P>0.05; farklı harflerle gösterilen ortalamalar arasındaki fark önemlidir

ağırlıklarında da benzer bir eğilim gözlenmiştir. Karkas parça oranlarında göğüs oranı dışındaki tüm özelliklerde dönemler arasında farklılıklar önemli (P<0.05) olmuştur. Birinci dönemde but oranları, ikinci dönemde ise kanat, sırt ve boyun oranları daha yüksek belirlenmiştir. Bu çalışmada ticari genotiplerin göğüs oranının yavaş gelişen SG1 ve SG2 genotiplerinin göğüs oranlarına göre yüksek bulunması, uzun süreli seleksiyonun karkasta göğüs oranının artmasını sağladığını bildiren araştırmacıları (Bessei 2006; Castellini et al 2008; Phongpa-ngan et al 2014) desteklemektedir. Çalışmamızda SG1 ve SG2 genotiplerinde but oranların yüksek bulunması, değişik araştırma bulgularında da ortaya koyulan yavaş ve orta düzeyde gelişen genotiplerin yüksek but oranlarına benzer bulunmuştur. (Castellini et al 2008; Abdullah et al 2010; Sarıca et al 2014b; Sarica et al 2014c; Yamak et al 2014).

3.2. Ekonomik değerlendirmeler

3.2.1. Genotiplerde maliyet ve karlılık

Tüm genotiplerde en önemli maliyet unsuru değişken masraflar olmuştur. Toplam masrafların % 92'sini değişken, % 8'ini sabit masraflar oluşturmuş; değişken masraflar içinde en önemli payı (% 75) yem maliyeti almıştır (Çizelge 7). Bunu, civciv maliyeti ve üretim dönemine hazırlık (altlık, dezenfeksiyon, vb.) için yapılan harcamalar takip etmiştir. Değişken masraflar toplamı dönem başına FG2, FG1, SG2 ve SG1 için sırasıyla 10.241, 8.337, 8.433 ve 8.453'dir. Sabit masraflar içinde en yüksek payı faizler oluşturmuş, bunu amortisman izlemiştir. Sabit masraflar toplamı FG2, SG2 ve SG1

Çizelge 7- Gruplara ait maliyet unsurları ve birim maliyet

Table 7- Cost elements of groups cost unit

Masraflar	*SG1*			*SG2*			*FG1*			*FG2*		
	Değer	*%*	*%*	*Değer*	*%*	*%*	*Değer*	*%*	*%*	*Değer*	*%*	*%*
Değişken masraflar												
Civciv (TL gün^{-1})	0.0204	11.83		0.0204	11.85		0.0274	13.80		0.0234	11.20	
Yem (TL gün^{-1})	0.1278	74.09		0.1274	74.03		0.1456	73.35		0.1613	77.18	
İşçilik (TL gün^{-1})	0.0034	1.98		0.0034	1.98		0.0040	2.02		0.0034	1.63	
Aşı ve ilaç (TL gün^{-1})	0.0024	1.39		0.0024	1.39		0.0024	1.21		0.0024	1.15	
Su (TL gün^{-1})	0.0001	0.05		0.0001	0.05		0.0001	0.05		0.0001	0.05	
Isıtma (TL gün^{-1})	0.0042	2.44		0.0042	2.44		0.0036	1.81		0.0042	2.01	
Aydınlatma (TL gün^{-1})	0.0001	0.05		0.0001	0.05		0.0001	0.05		0.0001	0.05	
Üretime hazırlık (TL gün^{-1})*	0.0103	5.97		0.0103	5.98		0.0120	6.05		0.0103	4.92	
Değişken masraf faizi (TL gün^{-1})	0.0038	2.20		0.0038	2.21		0.0033	1.66		0.0038	1.81	
Toplam değişken masraf (TL gün^{-1})	0.1725	100.00	91.08	0.1721	100.00	91.05	0.1985	100.00	93.19	0.2090	100.00	92.52
Sabit masraflar												
Genel idari giderler (TL gün^{-1})	0.0053	31.36		0.0053	31.36		0.0046	31.72		0.0053	31.36	
Amortisman** (TL gün^{-1})	0.0048	28.40		0.0048	28.40		0.0041	28.28		0.0048	28.40	
Faiz** (TL gün^{-1})	0.0068	40.24		0.0068	40.24		0.0058	40.00		0.0068	40.24	
Toplam sabit masraf (TL gün^{-1})	0.0169	100.00	8.92	0.0169	100.00	8.95	0.0145	100.00	6.81	0.0169	100.00	7.48
Toplam Masraflar (TL gün^{-1})	0.1894		100.00	0.1890		100.00	0.2130		100.00	0.2259		100.00
Besi süresi (gün)	49.00			49.00			42.00			49.00		
Toplam masraf (TL besi dönem^{-1})	9.281			9.261			8.946			11.0691		
Karkas ağırlığı (kg dönem^{-1})	1.773			1.842			2.067			2.550		
Canlı piliç maliyeti (TL kg^{-1})	5.235			5.028			4.328			4.341		

*, altlık, dezenfeksiyon, işçilik masraflarını kapsamaktadır; **, kümes, suluklar, yem makinesi, yemlikler için hesaplanan amortisman ve faiz masraflarıdır

için 0.828 iken, FG1 için 0.606 TL dönem^{-1} olarak hesaplanmıştır. Piliç/kg maliyeti ise FG2, FG1, SG2 ve SG1 için sırasıyla 4.341 TL, 4.328 TL, 5.028 TL ve 5.235 TL olmuştur. Buna göre maliyeti en düşük grup FG2 iken, en yüksek grup SG1 olmuştur (Çizelge 8).

Karlılık analizleri karkas ve parça satışına göre gerçekleştirilmiştir. Bütün karkasa göre pazarlamada FG2 ve FG1 genotipleri pozitif net kara sahip iken, SG1 ve SG2 genotiplerinde negatif net kar belirlenmiştir. Parçalanmış karkas değerlerinde ise bütün genotiplerde pozitif net kar bulunmuştur. Nispi kar açısından en yüksek kar elde edilen grup FG1 olmuş, bunu FG2, SG2 ve SG1 grupları izlemiştir (Çizelge 8).

3.2.2. Genotip gruplarında teknik etkinlik

Araştırmada tahmin edilen stokastik üretim fonksiyonu parametrelerinin işaretleri beklentiye uygun çıkmıştır. Üretim fonksiyonundaki yem değişkenine ait parametre, karkas üretim değeri ile yem arasında pozitif bir ilişki olduğunu göstermektedir. Yem için tahmin edilen elastikiyet katsayısı 0.28 bulunmuştur ($P<0.01$; Çizelge 9).

FG1, FG2, SG2 ve SG1'in teknik etkinlik skorları, 0.9664, 0.9699, 0.9639 ve 0.9633 olarak bulunmuştur ($P<0.01$). Buna göre FG2 grubu en iyi sonucu verirken, bunu FG1 izlemiştir. SG1 ve SG2 genotipleri arasındaki fark ise önemsiz bulunmuştur (Çizelge 10). Ekonomik değerlendirmelerde de

Çizelge 8- Farklı gruplarda maliyet ve karlılık

Table 8- Cost and profitability of different genotypes

Genotipler	Maliyet unsurları					Gelir unsurları				Net kar (TL dönem^{-1})		Nispi kar (TL dönem^{-1})	
	Değişken masraf (TL dönem^{-1})	Sabit masraf (TL dönem^{-1})	Toplam masraf (TL dönem^{-1})	Karkas ağırlığı (kg)	Maliyet (TL kg^{-1})	Parça fiyatı* (TL kg^{-1})	Karkas fiyatı (TL kg^{-1})	Toplam gelir parça (TL kg^{-1})	Toplam gelir karkas (TL kg^{-1})	Parça karkas	Bütün karkas	Parça karkas	Bütün karkas
SG1	8.453	0.828	9.281	1.773	5.235	5.798	4.50	10.280	7.979	0.563	-0.735	1.108	0.860
SG2	8.433	0.828	9.261	1.842	5.028	5.784	4.50	10.654	8.289	0.756	-0.528	1.150	0.900
FG1	8.337	0.609	8.946	2.067	4.328	5.925	4.50	12.247	9.302	1.584	0.159	1.369	1.040
FG2	10.241	0.828	11.069	2.550	4.341	5.858	4.50	14.938	11.475	1.530	0.172	1.350	1.036

*, her bir ebeveyn hattı için ağırlıklı ortalama ile hesaplanmıştır

Çizelge 9- Farklı gruplar için oluşturulan Cobb-Douglass tipi stokastik sınır modeline ait parametre tahminleri

Table 9- Parameter estimates of stochastic frontier model for different groups

Değişkenler	Parametreler	Standart hata	t- değeri
Üretim fonksiyonu			
Sabit	0.56	0.20	2.80**
Ln (Yem)	0.28	0.05	5.60**
Varyans parametreleri			
σ^2	0.22	0.06	3.67**
γ	0.71	0.03	3.55**
Log likelihood	2.83*		

*, P<0.05; **, P<0.01

Çizelge 10- Farklı gruplar için belirlenen teknik etkinlik skorları

Table 10- Technical efficiency scores for different genotype and groups

Grup	Ortalama	Standart sapma	Minimum	Maksimum
SG1	0.9633 c	0.0025	0.959	0.968
SG2	0.9639 c	0.0027	0.959	0.968
FG1	0.9664 b	0.0026	0.962	0.974
FG2	0.9699 a	0.0023	0.962	0.970

Farklı harfle gösterilen genotiplerin etkinlik skorları arasındaki farklılıklar önemlidir (P<0.05)

önemli olan ölüm oranlarında belirgin farklılıklar ortaya çıkmıştır. Hızlı gelişen genotipte 42 ve 49 günlük yaşlarda % 5.00 ve % 6.54 düzeyinde görülen ölüm oranları, SG1 ve SG2 genotiplerinde 49 günlük yaşta % 1.15 ve % 2.69 seviyesinde gerçekleşmiştir (P<0.05). Dönemler arasında farklılık olmamakla birlikte, hızlı gelişen genotipte kesim yaşının artması (FG2) her iki dönemde de ölüm oranlarının yükselmesine neden olmuştur. Ölümlerde en önemli etkenin hızlı gelişmeye bağlı olarak görülen metabolik bozukluklar olduğu belirtilmektedir (Mikulski et al 2011). Shim et al (2012), ölüm oranlarının gelişme hızı ile bağlantılı olduğunu belirtmişlerdir. Shim et al (2012), 48 günlük yaşta gelişme hızı farklı olan piliçlerde % 6.09 ile % 13.40 arasında ölüm ortaya çıktığını, gelişme hızı yüksek olan hatlarda daha yüksek ölüm oranı görüldüğünü ortaya koymuşlardır. Mikulski et al (2011), yavaş gelişen piliçlerde 65 günlük yaşta % 2.25 ile % 2.50 arasında ölüm oranlarına karşın, hızlı gelişen piliçlerde 42 günlük yaşta % 4.52 ile % 6.03 arasında ölümler belirlemişlerdir.

4. Sonuçlar

Etlik piliç yetiştiriciliğinde kullanılan ebeveyn hatları ve üretim materyalinin seçiminde en önemli husus maliyetler, özellikle de yem maliyetidir. Yüksek gelişme hızına sahip olan piliçler pazarlama ağırlığına çok erken ulaşmasına karşın, yaşa bağlı

olarak ortaya çıkan ölümler ciddi kayıplara neden olmaktadır (Shim et al 2012). Çalışmamızda net karlılık açısından ilk sırada yer alan FG1 grubunda % 5.00, FG2 grubunda % 6.54 düzeyindeki ölümler önemli kayıplar olarak değerlendirilebilir. Daha düşük gelişme düzeyine ve 7 haftalık besi süresinde kesim ağırlığına ulaşan SG1 ve SG2 genotipleri bu açıdan avantaj sağlamış, ticari genotipe göre daha az kayıpla üretim sağlamışlardır. Bu görüşler doğrultusunda, benzer genotipler üzerinde çalışılarak sonuçlar yaygınlaştırılabilir. Hızlı gelişen etlik piliç üretiminde kullanılan materyalin ebeveynleri % 100 ithalat yoluyla temin edildiğinden, ulusal ıslah çalışmalarının yaygınlaşması ile elde edilebilecek sonuçlar ülke ekonomisine katkı sağlayacaktır. Özellikle, ülkemizde üretimi henüz düşük seviyelerde bulunan yavaş gelişen etlik piliçlerin kullanımında bu genotiplere şans verilmesi dışa bağımlılığı azaltacaktır.

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Kaynaklar

Abdullah A Y, Al-Beitawi N A, Rjoup M M S, Qudsieh R I & Ishmais M A A (2010). Growth performance, carcass and meat quality characteristics of different commercial crosses of broiler strains of chicken. *Journal Poultry Science* **47**: 13-21

Anonymous (2013). The Statistical Reference for Poultry Executives. WATT Executive Guide to World Poultry Trends. www.WATTAgNet.com

Arthur J A & Albers G A A (2003). Industrial perspective on problems and issues associated with poultry breeding. Poultry Genetics, Breeding and Biotechnology (Edited by W.M. Muir and S.E. Aggrey), 1-12, CABI Publishing, USA

Battese G E (1992). Frontier production functions and technical efficiency: A survey of empirical applications in agricultural economics. *Agricultural Economics* **7**: 185-208

Battese G & Coelli T (1995). A model for technical inefficiency effects in a stochastic frontier production function for panel data. *Empirical Economics* **20**: 325-332

Bessei W (2006). Welfare of broilers: A review. *World's Poultry Science Journal* **62**: 455-466

Blagojevic M, Pavlovski Z, Skrbic Z, Lukic M, Milosevic N & Peric L (2009). The effect of genotype of broiler chickens on carcass quality in extensive rearing systems. *Acta Veterinaria* **59**(1): 91-97

Castellini C, Berri C, Le Bihan-Duval E & Martino G (2008). Qualitative attributes and consumer perception of organic and free-range poultry meat. *World's Poultry Science Journal* **64**: 500-512

Cheema M A, Qureshi M A & Havenstein G B (2003). A comparison of immune response of a 2001 commercial broiler with a 1957 randombred broiler strain when fed representative 1957 and 2001 broiler diets. *Poultry Science* **82**: 1519-1529

Chen X, Jiang W, Tan H Z, Xu G F, Zhang X B, Wei S & Wang X Q (2013). Effects of outdoor access on growth performance, carcass composition, and meat characteristics of broiler chickens. *Poultry Science* **92**: 435-443

Chin V (2003). Patterns of chicken consumption in South-East China. *British Poultry Sci*ence **44**: 784-785

Coelli T, Rao D S P & Battese G E (1998). An introduction to efficiency and productivity analysis. Kluwer Academic Publishers, Massachusetts, USA

Decuypere E, Bruggeman V, Barbato G F & Buyse J (2003). Growth and reproduction problems associated with selection for increased broiler meat production. Poultry Genetics, Breeding and Biotechnology (Edited by W.M. Muir and S.E. Aggrey), 13-28, CABI Publishing, USA

Dou T C, Shi S R, Sun H J & Wang K H (2009). Growth rate, carcass traits and meat quality of slow-growing chicken grown according of three raising systems. *Animal Science Papers and Reports* **27**(4): 361-369

Fanatico A C, Cavit L C, Pillai P B, Emmert J L & Owens C M (2005a). Evaluation of slower- growing broiler genotypes grown with and without outdoor access: Meat Quality. *Poultry Science* **84**: 1785-1790

Fanatico A C, Pillai P B, Cavitt L C, Owens C M & Emmert J L (2005b). Evaluation of slower-growing broiler genotypes grown with and without outdoor access: Growth performance and carcass yield. *Poultry Science* **84**: 1321-1327

Fanatico A C, Pillai P B, Cavitt L C, Emmert J L, Meullenet J F & Owens C M (2006). Evaluation of slower-growing broiler genotypes grown with and without outdoor access: Sensory attributes. *Poultry Science* **85**: 337-343

Fanatico A C, Pillai P B, Emmert J L, Gbur E E, Meullenet J F & Owens C M (2007). Sensory attributes of slow- and fast-growing chicken genotypes raised indoors or with outdoor access. *Poultry Science* **86**: 2441-2449

Grashorn M A & Clostermann G (2002). Performance and slaughter characteristics of broiler breeds for extensive production. *Archiv Geflügelkunde* **66**(4): 173-181

Havenstein G B, Ferket G B & Qureshi M A (2003). Growth, livability, and feed conversion of 1957 versus 2001 broilers when feed representative 1957 and 2001 broiler diets. *Poultry Science* **82**: 1500-1508

Kokoszynski D & Bernacki Z (2008). Comparison of slaughter yield and carcass tissue composition in broiler chickens of various origin. *Journal of Central European Agriculture* **9**(1): 11-16

Mikulski D, Celej J, Jankowski J, Majewska T & Mikulska M (2011). Growth performance, carcass traits and meat quality of slower-growing and fast-growing chickens raised with and without outdoor access. *Asian-Australasian Journal of Animal Sciences* **24**(10): 1407-1416

Phongpa-ngan P, Jung J, Antony N B, Karnuah A B, Mulligan J H, Aggrey S E & Wicker L (2014). Quality attributes in breast muscle from broilers of an Arkansas randombred line varying in growth rate. *Poultry Science* **93**: 187-193

Remignon H & Le Bihan-Duval E (2003). Meat quality problems associated with selection for increased production. Poultry Genetics, Breeding and Biotechnology (Edited by W.M. Muir and S.E. Aggrey), 53-66, CABI Publishing, USA

Sarica M (1997). The effect of slaughter age on carcass traits of broilers. *Turkish Journal of Veterinary and Animal Science* **21**(5): 413-420

Sarica M, Karacay N, Ocak N, Yamak U, Kop C & Altop A (2009). Growth, slaughter age and gastrointestinal tract traits of three turkey genotypes under barn and free-range housing systems. *British Poultry Science* **50**: 487-494

Sarica M, Ocak N, Turhan S, Kop C & Yamak U S (2011). Evaluation of meat quality from 3 turkey genotypes reared with or without outdoor access. *Poultry Science* **90**: 1313-1323

Sarica M, Türkoglu M & Yamak U S (2014a). Tavukçuluktaki gelişmeler ve Türkiye tavukçuluğu: Tavukçuluk Bilimi: Yetiştirme, besleme, hastalıklar (Editörler: M. Türkoglu & M. Sarıca) s: 1-39, Bey Ofset, Ankara

Sarica M, Yamak U S, Turhan S, Boz M A, Saricaoglu F T & Altop A (2014b). Comparing slow-growing chickens produced by two- and three-way crossings with commercial genotypes. 2. Carcass quality and blood parameters. *European Poultry Science* **78**. DOI: 10.1399/eps.2014.XX

Sarica M, Yamak U S, Boz M A & Uçar A (2014c). Geriye melezlemeyle üretilen etçi genotiplerin bir ticari etlik piliç genotipiyle büyüme, kesim ve karkas özellikleri bakımından karşılaştırılması. *Tavukçuluk Araştırma Dergisi* **11**(1): 5-9

Shim M Y, Tahir M, Karnuah A B, Miller M, Pringle T D, Aggrey S E & Pesti G M (2012). Strain and sex effects on growth performance and carcass traits of contemporary commercial broiler crosses. *Poultry Science* **91**: 2942-2948

Türkoğlu M & Sarıca M (2014). Damızlık tavuk yetiştiriciliği. Tavukçuluk bilimi, yetiştirme besleme hastaklıklar. Editörler: M. Türkoğlu, M. Sarıca. s. 344-350. Bey Ofset. Ankara

Yamak U S, Sarica M & Boz M A (2014). Comparing slow-growing chickens produced by two- and three-way crossings with commercial genotypes. 1. Growth and Carcass Traits. *European Poultry Science* **78**. DOI: 10.1399/eps.2014.XX

Yang N & Jiang R S (2005). Recent advances in breeding for quality chickens. *World's Poultry Science Journal* **61**: 373-381

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Identifying Irregular Potatoes by Developing an Intelligent Algorithm Based on Image Processing

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ABSTRACT

The objective of this study was to develop an algorithm based on image processing for detecting misshapen potatoes from the mass of potatoes and obtaining homogeneous products. The database used in this research included the digital images acquired from Agria variety of Ardabil (Iranian northern-west) potato with different sizes and shapes. A combination of morphological features including geometrical features like length, width and features related to shape such as roundness were taken into consideration in identifying irregular potatoes from others employing elongation and Fourier descriptors. Using statistical principal component analysis (PCA), seven features were selected as the most prominent for classification. The experimental results showed that the proposed method achieves a high level of accuracy with merely seven selected discriminative features, obtaining an average correct classification rate of 98% for training set. Additionally, regular potatoes were separated into small, medium and large categories with 100% accuracy. According to the results, the developed algorithm based on image processing can be used in classifying products with no proper shape.

Keywords: Classification; Image processing; Irregularity; Potato; Shape

Görüntü İşleme Tabanlı Akıllı Algoritma Geliştirerek Şekilsiz Patateslerin Belirlenmesi

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ÖZET

Bu çalışmanın amacı görüntü işleme tabanlı akıllı algoritma geliştirerek şekilsiz patateslerin belirlenmesi ve homojen şekilli patates elde edilmesidir. Materyal olarak İran'ın kuzeybatısında bulunan Ardabil bölgesinin Agria patates çeşidinin farklı boyut ve farklı görüntüleri kullanılmıştır. Şekilsiz patateslerin belirlenmesinde uzunluk, genişlik, yuvarlaklık gibi farklı özellikler göz önüne alınmış ve uzama ile Fourier tanımlayıcılarından yararlanılmıştır. İstatistik analize (PCA)

dayalı olarak sınıflandırmada çok önemli olan 7 özellik seçilmiştir. Araştırma sonucunda önerilen 7 yöntemin yüksek bir doğruluğa sahip olduğu, sınıflandırmada ortalama % 98 doğruluk oranına ulaştığı görülmüştür. Ayrıca patatesler % 100 oranında küçük, orta ve büyük gruplara ayrılabilmiştir. Elde edilen sonuçlara göre geliştirilen görüntü işleme tabanlı algoritma şekilsiz ürünlerin sınıflandırılmasında kullanılabilir.

Anahtar Kelimeler: Sınıflandırma; Görüntü işleme; Şekil bozukluğu; Patates; Biçim

1. Introduction

After wheat, maize, and rice, potato is the fourth most produced food in the world. Moreover, with over 4 million tons of potatoes Iran is ranked eighth in the world and third in Asia after China and India in terms of producing potato (FAO 2011). The high variety in size and shape along with damage-vulnerability has turned potato into a difficult crop as far as handling and grading. As a part of food industry the appearance and shape of the product play a significant role. This issue is particularly important concerning potatoes because the misshapen tubers have a lot of losses during peeling and subsequent processing such as cutting and drying. From of the view of a consumer, a good product is that which has a proper shape and appearance. Moreover, customers and food inspectors prefer regular potatoes. Hence, to achieve a homogeneous product, identifying and separating irregular potatoes is required in food production chain.

For reasons such as inconsistency, subjectivity and variability of potato sorting by workers, manual processes are, more often than not, considered extremely harsh and easily affected by environmental conditions such as changes in temperature and moisture (Razmjooy et al 2012). Often, separation and classification of misshapen potatoes from sound tubers is done by workers based on their indigenous knowledge, a fact which increases labor cost as well as human errors and production losses (Zhou et al 1998). In developed countries, such artificial intelligences as machine vision, with their many benefits, are widely used in the inspection of agri-production (Moreda et al 2012).

Advantages such as high accuracy, high speed, relatively low cost and repeatability have led most modern manufactures to utilize machine vision systems. In other words, in order to have a better quality control in food industry, machine vision technique has replaced other methods (Cubero et al 2011). Regarding the application of these systems in controlling and monitoring agricultural productions, numerous studies have been conducted (Aleixos et al 2002; Choudhary et al 2008; Al-Mallahi et al 2010; Barnes et al 2010; Liming & Yanchao 2010; Arribas et al 2011; Li et al 2012; Nashat et al 2014).

Determining shape is one of the most common processes of food quality assessment (Du & Sun 2004). It should be noted that shape is a major problem in potato inspection as potato tubers have various shapes affected by the environment resulting in irregularities. Incoming damage to the tuber during harvesting and transportation causes the formation of different shapes. Potato tubers are normally spherical or rectangular with smooth surface while those with more outgrowth and dip are considered to be misshapen. The shape can be analyzed through such different methods as invariant moments, boundary encoding, bending energy, fractals and Fourier description among which invariant moments and Fourier description are most commonly used owing to the fact that they are not dependent on scale and orientation.

As a result of the aforementioned points and also the need for sorting out units and food factories in Iran, particularly concerning potato production, the aim of this research was to separate irregular potatoes from other tubers and grade well-shaped (regular) potatoes in various categories based on their size and manner of offline machine vision.

2. Material and Methods

2.1. Preparing potato samples

A set of potato tubers of Agria variety (as a common variety) related to 2012 harvest was randomly collected from Ardabil (Iranian northern-west) Central Agricultural Research Center in different sizes and shapes. The tubers were removed from adherent clods and other waste materials during harvesting, carrying, and packaging so that all the samples had relative cleanliness. For the final evaluation, potatoes were primarily classified manually into well-shaped and misshapen with relative irregularity as the criterion. Figure 1 shows some of these regular and irregular potatoes. For the 'train and test' method, the available data is generally divided into two parts:training set and test set (Bramer 2007). First, the training set is used to construct a classifier (decision tree, neural net etc.). The classifier is then used to predict the classification for the instances in the test set. Accordingly, the database was divided into two parts, a training set and a test set, where the former was composed of 252 potatoes with 210 voted as regular and 42 as irregular (small, medium and large sizes). The testing set included 202 tubers with 178 regular and 24 irregular potato tubers so as to validate the prediction model in an offline mode. The aim of set was to evaluate and prepare the system for real-time applications.

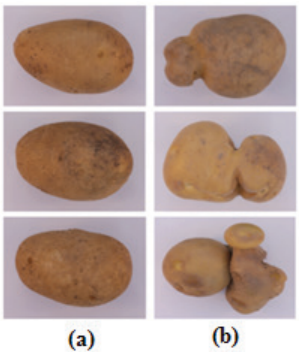

Figure 1- Examples of the database images: a, regular potatoes, and b, irregular potatoes

Şekil 1- Görüntü örnekleri: a, şekli düzgün patatesler; b, şekli bozuk patatesler

2.2. Image acquisition system

Image acquisition system shown in Figure 2 is a dome-shaped chamber, in which LED light bulbs and fluorescent are used to supply the lighting conditions. The lighting chamber is made of a stainless steel sheet. Lighting is an important and effective factor as far as image quality is concerned since lighting method and light source influence subsequent process and the obtained results. Hence, the LED bulbs were located at an angle of 45 degrees from the potatoes as there was no shadow around samples in this angle. Therefore, in order to eliminate shade and other undesired information, direct radiation was not used. The camera used in this research was a CCD camera (SONY ∝200) with the resolution of 10.1 mega pixel and 40 mm close-up lens with focal a length of 70 mm and aperture setting of F5.6. Such a well-chosen lighting system allowed the potatoes to be properly recognized and analyzed in the images. Therefore, there was no need for extra image processing. Due to the light reflection lake, Steinbach white paper was employed as background. The main advantage of this type of paper is light absorption rather than reflection. Digital images were taken with the resolution of 3872x2592 pixels under the same lighting condition and 12 VDC; next, they were transferred to the hard disc of a computer via USB

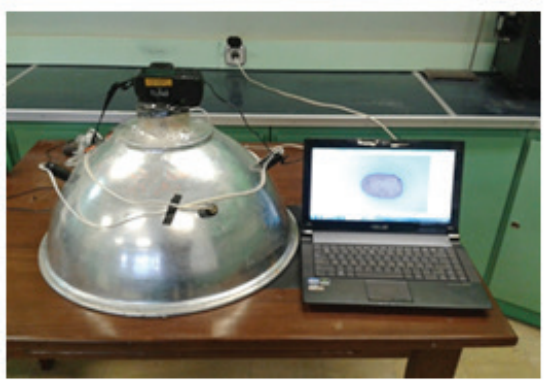

Figure 2- Image acquisition system

Şekil 2- Görüntü işleme sistemi

cable. Because of the CPU limitations, the images were converted into 968×648 resolution by resize operator. The images were processed and analyzed using the image processing toolbox of MATLAB R2012 software. The process of identifying irregular potatoes is shown in the flowchart of Figure 3.

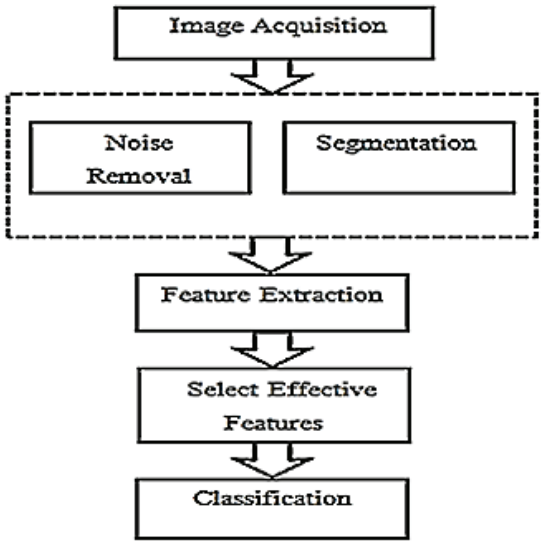

Figure 3- Flowchart of the image processing algorithm for discriminating irregular potatoes

Şekil 3- Şekli bozuk patateslerin belirlenmesinde kullanılan görüntü işleme algoritmasının akış diyagramı

2.3. Image pre-processing and segmentation

Although our image acquisition camera produced high contrast images, a pre-processing was needed in order to extract the usable features of each image for the analysis of the potato shape. This pre-processing included noise and background removal from the potato image in which they have redundant information. If noise and other undesired information were not removed, the image analysis would face different problems which would ultimately make it harder to obtain homogeneity. In other words, most of the subsequent processing such as image filtering, boundary detection, segmentation and feature extraction would fail to accurately determine the objects real boundaries due to the non-uniform illumination and noise in the image background. Hence, correcting non uniform illumination in the background can be useful in this regard. A linear Gaussian low pass filter (Castleman 1996) and morphological operations such as opening were used to reduce the noise and obtain images with a uniform background.

Image segmentation is the process of partitioning a digital image into multiple segments (set of pixels). In other words, in segmentation, pixels are clustered into salient image regions (i.e., regions corresponding to individual surfaces, objects, or natural parts of objects). The goal of segmentation is to simplify and/or change the representation of an image into something that is more meaningful and easier to analyze (Shapiro & Stockman 2001). The color of potatoe arils varies from yellow to red, corresponding to high R values. Hence, the segmentation used a pre-defined threshold in the R band. In fact, segmentation was approached by attempting to find the boundaries between regions and use global thresholding in the red ban. The pixels lower than this value belonged to the background (displayed in black pixels) and higher pixels were considered as foreground (the object displayed in white pixels). Since the intensity distributions of potatoes and background pixels were sufficiently distinct (after noise removal), it was possible to use a single (global) threshold applicable over the entire images. Thus, the algorithm was able to automatically estimate the required threshold value for each image. The results of pre-processing and segmentation and also the boundary or contour image of a potato are shown in Figure 4. It should be noted that both noise removal and segmentation processes did not take place simultaneously, rather in a tandem at a specific stage titled pre-processing.

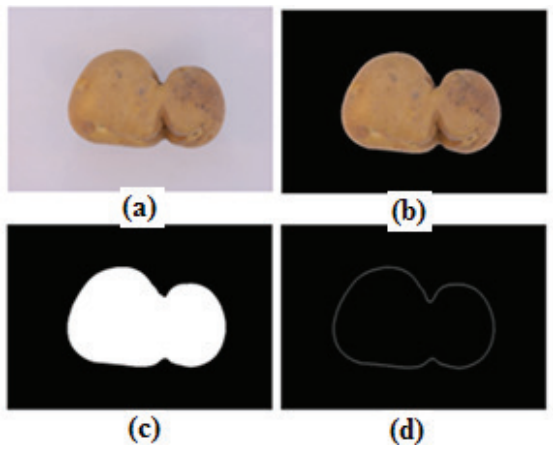

Figure 4- Results of pre-processing and segmentation operations: a, original image; b, uniformed background; c, segmented image; d, boundary of potato

Şekil 4- Ön işleme ve dilimleme işlem sonuçları: a, original görüntü; b, uniform olmayan arka görüntü; c, dilimlenmiş görüntü; d, patates görüntü çizgisi

2.4. Extracting morphological features

2.4.1. Calculating geometrical characteristics

Extracting different features is a major stage after pre-processing. Certain geometrical features such as perimeter, area, length and width were calculated from the binary images. For instance, the perimeter can be measured by tracing the boundary of the potato and summing all the steps of length 1 or $\sqrt{2}$ taken from Figure 4d. In fact, length 1 is the distance between two adjacent pixels and $\sqrt{2}$ is the distance at the corners of the boundary in rectangular shapes. The area equaled the total number of white pixels in the binary image. For length and width we computed the Euclidean distance transform of the binary image. In fact for each pixel in the binary image, the distance transform is assigned a number that is the distance between that pixel and the nearest nonzero pixel of binary image shown in Figure 5. In the two-dimensional area, the Euclidean distance between (x_1, y_1) and (x_2, y_2) was calculated by the Equation 1 (Gonzalez & Woods 2008). Moreover, through combining geometrical characteristics, several features were obtained such as elongation, roundness, extent and eccentricity which equation exist in the appendix.

$$\sqrt{(x_1 - x_2)^2 + (y_1 - y_2)^2} \quad (1)$$

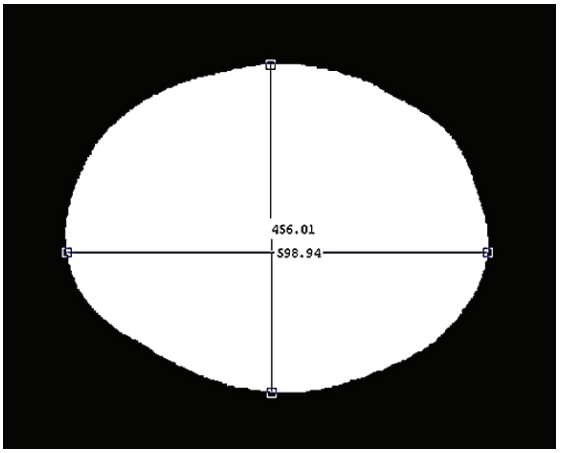

Figure 5- Length and width of a sample potato extracted from binary image

Şekil 5- İkili görüntüden elde edilen örnek patatesin uzunluğu ve genişliği

2.4.2. Extracting fourier descriptors

Describing the shapes of crops, while often simple and easy for human eyes and brains, is difficult for a computer. In other words, computer descriptors are deterministic and quantitative yet, they are subjective forman (Ying et al 2002). A 1-D functional representation of a boundary namely signature was employed. Regardless of how a signature is generated, however, the basic idea is to reduce the boundary representation to a 1-D function which is presumably easier to describe than the original 2-D boundary (Gonzalez & Woods 2008). Thus, as shown in figure 6 the radius signature r(k) was obtained from potato boundaries and the starting point in the contour was determined at the minimum distance of r(k) on clockwise. As mentioned in the previous section, Fourier descriptor is a common and reliable technique for the analysis of agri-productions shapes. Hence, after obtaining the radius signature, it was transferred into Fourier domain in order for coefficients to be calculated as Fourier descriptors of the boundary.

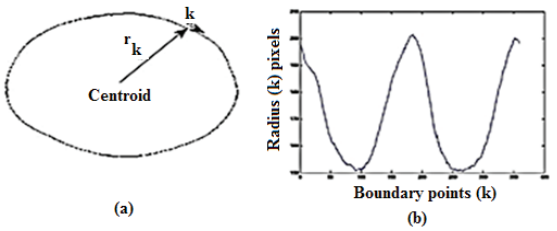

Figure 6- a, external boundary of a potato with its centroid point; b, corresponding boundary signature

Şekil 6- a, merkezinden itibaren bir patatesin dış sınırları; b, ilgili sınır işaretleri

In order to analyze the potato shapes, fast Fourier transform of r(k) was calculated using Equation (2) (Gonzalez & Woods 2008):

$$F(h) = \frac{1}{N}\sum_{k=1}^{N} r(k)e^{-j2\pi hk/N} \qquad h = 0,1,2,\ldots,N-1 \quad (2)$$

Where; N is the number of pixels in the boundary. The inverse Fourier transform of these coefficients restores r(k) that is, from Equation (3) (Gonzalez & Woods 2008):

$$r(k) = \frac{1}{N}\sum_{h=1}^{N} F(h).e^{j2\pi hk/N} \quad (3)$$

It is known from discussions of the Fourier transform that instead of all the Fourier coefficients, only the first P coefficients are employed. This is equivalent to setting $F(h)= 0$ for $h > P-1$ in Equation (3). The result is the Equation 4 with the approximation to r(k) (Gonzalez & Woods 2008):

$$\hat{r}(k) = \frac{1}{P}\sum_{h=1}^{P} F(h)e^{j2\pi hk/P} \quad (4)$$

Although only P terms were used to obtain each $\hat{r}(k)$, k still ranges from 1 to $N-1$. That is meaning the same number of points exists in the approximate boundary, but not as many terms were used in the reconstruction of each point (Gonzalez & Woods 2008). In this research, P was 10 meaning that the first 10 harmonics could describe the shape of potato tubers. Figure 7(b) shows the boundary of an irregular potato, consisting of 2384 points. The corresponding 2384 Fourier descriptors were obtained for this boundary using Equation (2). The objective of this illustration was to examine effects of reconstructing the boundary based on decreasing the number of Fourier descriptors (Gonzalez & Woods 2008).

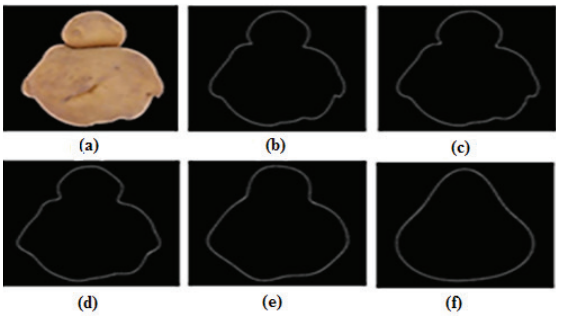

Figure 7- a, color segmented image of an irregular potato; b, boundary of potato tuber (2384 points); c-f, boundaries reconstructed using 72, 36, 18, and 8 Fourier descriptors, respectively. These points are approximately 3%, 1.5%, 0.75%, and 0.34% of 2384, respectively

Şekil 7- a, şekilsiz patatesin renk dilimli görüntüsü; b, 2384 noktadaki patates sınırı; c-f, 8, 18, 36 ve 72 Forier tanımlayıcıları kullanarak yeniden yapılan sınırlardır. Bu noktalar sırasıyla 2384 noktanın yaklaşık olarak % 3, % 1.5, % 0.75 ve % 0.34'dür

The changes in the frequency of Potato boundary can be represented by the harmonic components ($F(h)$) in the Fourier domain. For instance, is $F(0)$ the average radius, $F(1)$ represents the bending of an object, and $F(2)$ shows the elongation of the object and so on. Interpretation of the first few Fourier coefficients of radius boundary is summarized in Table 1.

Table 1- Shape extraction from radius boundary Fourier coefficients

Çizelge 1- Yarıçap sınır Fourier katsayılarından şekil çıkarma

Fourier coefficient	*Implied shape information*
F(0)	Average radius
F(1)	Bending
F(2)	Elongation
F(3)	Triangle
F(4)	Square

A regular round potato has a high $F(0)$ value and all $F(h)[h\geq1]$ near zero. An oblong potato has high $F(0)$ and $F(2)$ values and all others are close to zero. For all irregular potatoes, the entire value of $F(h)[h\geq1]$ is high, while other frequency components near zero. In order to obtain effective shape information, a method was employed where harmonics was multiplied by its magnitude $F(h)*h^m$ for an effective heuristic. It is worth mentioning that there is a parity relationship between boundary points in spatial and frequency domain (Tao et al 1995). This method offered two significant advantages and concepts. First, the operation of $F(h)*h$ in the frequency domain was equivalent to the Fourier transform of r(k)'s derivative in the spatial domain. Second, h in h∗F(h) provided a weight to enhance high frequency components, i.e. the relatively small curve changed along the potato boundary so that low and high frequencies could be compared on the same scale. This concept turned out to be quite useful in the definition of shape separator. Accordingly, a separator S was defined as in Equation 5 for the degree of shape irregularity (Tao et al 1995).

$$S = \sum_{h=0}^{10} F(h) * h^m, \;\; m = 1{,}2 \; or \; 3 \qquad (5)$$

Where; m is the order of r(k)'s derivative in the spatial domain. It should be noted that the higher the S, the more severe the regularity of the potato shape. S_1 can be explained as the derivative of the boundary signature, S_2 represents the curvature of the boundary and S_3 represents the very small changes in the boundary equivalent to the higher frequency components. In fact, based on the visual evaluation of the quantitative values, the separator underwent shape transformation from. Also, values of m= 3 or higher significantly rose to higher frequency components, i.e. noise components. Since high-frequency components account for the fine detail and low-frequency components determine the global shape, the values of m were limited to 3. Furthermore, the advantage of using $F(h)*h$ is that it can greatly improve the signal-to-noise ratio of boundary curvature which refers to the digital nature of the boundary.

2.5. Selecting effective features

In order to reduce the dimensionality of the data set and select the features with more effect on classification, we employed the statistical method of principal component analysis (PCA). PCA is a statistical procedure elucidating the covariance structure of a set of variables. In particular it allows for the identification of the principal directions in which the data varies. The advantage of PCA as an optimal linear transform is in maintaining the subspace with the highest variance (Smith 2002). In our study, entire the data set was written as an M×N data matrix. The first step in PCA was to move the origin to mean of the data titled mean vector, which was then subtracted from the data matrix to create the mean centered data vector. Next, the covariance matrix was computed using the mean centered data matrix with the subsequent obtaining of eigenvectors and eigenvalues from the covariance matrix. Finally, the principal components were obtained by computing the variances of these eigenvectors and sorting them in decreasing order. Out of the 34 included features, seven were chosen as the most effective in determining potato shapes: four size-dependent features (roundness, elongation, extent, and eccentricity) and three separators based on Fourier descriptor (S_1, S_2, S_3).

2.6. Confusion matrix

A confusion matrix is a simple methodology for displaying the classification results of a classifier. The confusion matrix is defined by labeling the desired classification on the rows and the predicted classifications on the columns (Stehman 1997). The diagonal elements represent the number of points for which the predicted label is equal to the true label, while off-diagonal elements are those that are mislabeled by the classifier. The higher the diagonal values of the confusion matrix the more the correct predictions. The strength of a confusion matrix is that it identifies the nature of the classification errors, as well as their quantities.

2.7. Grading regular potatoes by size

After all the potatoes were classified as regular and irregular, the well-shaped potatoes themselves were further graded. In our study, three thresholds were

given to the algorithm to separate three different size grades, namely small, medium, and large as listed below. In this case, USDA standard was used for grading well-shaped potatoes (d is the minimum diameter in a tuber).

$d \leq 3.81$ cm	Small
$3.81\text{cm} < d \leq 5.71\text{cm}$	Medium
$5.71 < d$	Large

The results of grading were compared to these three pre-classified groups. A correct classification would show that the threshold values were accurately chosen.

3. Results and Discussion

3.1. Morphological features

As it is demonstrated in Table 2, the main statistical components were average and standard deviation of all effective shape features extracted from the testing set (178 regular and 24 misshapen potatoes). Results indicated that because all shape features of the irregular potatoes had high standard deviation values, this group had a wide range of variation compared to the regular ones.

Table 2- Shape features (mean ± standard deviation) of regular and irregular potato tubers

Çizelge 2- Düzenli ve düzensiz patates yumrularının şekil özellikleri (ortalama ± standart sapma)

	Shape feature	*Regular*	*Irregular*
Size-shape features	Roundness	1.81±0.732	1.581±0.914
	Elongation	0.160±0.121	0.205±0.190
	Eccentricity	0.644±0.157	0.724±0.159
	Extent	0.307±0.112	0.314±0.203
Fourier-shape features	S_1	499.557±101.021	521.318±123.021
	S_2	2047.478±414.781	2107.427±1246.428
	S_3	19825.417±3933.572	21144.207±11738.426

3.2. Fourier descriptors

Fourier transform was used in order to estimate and determine the changes in the potato boundary signature. The main aim of using Fourier transform was to reduce the dimensionality so that through the translation from spatial domain to the frequency domain, the compression of many boundary data could be achieved. In fact, owing to the symmetric nature of Fourier transform, at least N/2 terms of F(h) were removed. In addition, each term of Fourier transform had new information by itself as Fourier coefficients are orthogonally positioned. Results showed that, irregular tubers had a higher F(h) magnitude compared with regular tubers.

Table 3 shows the results of classifying potato shapes into regular and irregular under confusion matrix for the training set where the total accuracy is 98%. As can be seen, this amount reaches 100% and 88.1% for regular and irregular potatoes respectively.

Table 3- Confusion matrix of discriminant analysis model to classify the shape of training set potatoes using stepwise discrimination (STEPDIS procedure of SPSS)

Çizelge 3- Diskriminant analiz karışıklık matrisi ayrımı kullanarak incelenen patates seti şeklini (SPSS'in STEPDIS yöntemi) sınıflandırma modeli

From/to	*Regular*	*Irregular*	*Total*	*% Correct*
Regular	210	0	210	100
Irregular	5	37	42	88.1
Total	215	37	252	98

Table 4 presents the accuracy values of the developed intelligent image processing for discriminating irregular potatoes. Identification efficiency of irregular tubers and well-shaped tubers were 90.3% and 98.6%, respectively. These results indicate the fact that, by adding several size-shape parameters to Fourier descriptors, the classification efficiency of the shape of potato tubers rose by 1.1% compared to the study of Tao et al (1995), who reached less than 90% efficiency in the shape determination of potato tubers.

Table 4- Classification accuracy of the image processing algorithm for the discrimination of potato shape in the testing set

Çizelge 4- İncelenen patates setinde görüntü işleme algoritması ayrımının doğruluk sınıflandırması

Real/estimated	*Regular*	*Irregular*
Regular	98.6	1.4
Irregular	9.7	90.3

3.3. Grading well-shaped potatoes

The developed algorithm was also able to grade regular potatoes after their discrimination from irregular tubers based on pre-defined thresholds. All the regular potatoes of the testing set (178 tubers) were graded as small, medium and large with 100% accuracy. It is quite obvious that through changing the threshold values, the system will able to grade in different categories which is arbitrary for operators and industry units.

4. Conclusions

In this study, we attempted to discriminate irregular potatoes by developing an image processing algorithm. Since only boundary information was needed for shapes, this approach provided accurate estimations of shapes because there was no need to consider such image textures as blemishes within an object. The combination of geometrical features and Fourier descriptors proved to be effective in shape determination. In addition, we found that only the first 10 harmonics of Fourier transform contained most of the tuber information. Also, through applying Fourier descriptors there was no need to check all the boundary points of the potatoes. What is more, due to the symmetry of Fourier transform, the advantage of the existing data compression on potato boundaries was used in reducing the volume of boundary information. The accuracy of the discrimination of misshapen potatoes was calculated as 98%. Also, it was concluded that the concurrent use of size-shape features and Fourier transforms would increase shape-classification efficiency of potatoes. Ultimately, the applied method in this research could probably be used in identifying the shapes of other agricultural and horticultural products in which case, the values of the thresholds should be modified alongside some other details.

Appendix

Feature	Formula/Explanation
Roundness	$\frac{Perimeter^2}{4.\pi.Area}$
Elongation	$\frac{Length - Width}{Length + Width}$
Eccentricity	$\sqrt{1 - \frac{(SemiMajor)^2}{SemiMinor^2}}$
Extent	Scalar that specifies the ratio of pixels in the region to pixels in the total bounding box. Computed as the Area divided by the area of the bounding box.

References

Aleixos N, Blasco J, Navarron F & Molto E (2002). Multispectral inspection of citrus in real-time using machine vision and digital signal processors. *Computers and Electronics in Agriculture* **33**(2): 121-137

Al-Mallahi A, Kataoka T, Okamoto H & Shibata Y (2010). An image processing algorithm for detecting in-line potato tubers without singulation. *Computers and Electronics in Agriculture* **70**: 239-244

Arribas J I, Sanchez-Ferroro G V, Ruiz-Ruiz G & Gomez-Gil J (2011). Leaf classification in sunflower crops by computer vision and neural networks. *Computers and Electronics in Agriculture* **78**: 9-18

Barnes M, Duckett T, Cielniak G, Stroud G & Harper G (2010). Visual detection of blemishes in potatoes using minimalist boosted classifiers. *Journal of Food Engineering* **98**: 339-346

Bramer M (2007). Principles of data mining. Ecole polytechnique, France and King's college London, UK

Castleman K (1996). Digital image processing. Englewood Cliffs, NJ: Prentice-Hall, 667 pp

Choudhary R, Paliwal J & Jayas D S (2008). Classification of cereal grains using wavelet morphological, colour and textural features. *Biosystems Engineering* **99**: 330-337

Cubero S, Aleixos N, Moltó E, Gómez-Sanchis J & Blasco J (2011). Advances in machine vision applications for automatic inspection and quality evaluation of fruits and vegetables. *Food and Bioprocess Technology* **4**(4): 487-504

Du C.J & Sun D W (2004). Recent developments in the applications of image processing techniques for food quality evaluation. *Trends in Food Science & Technology* **15**(5): 230-249

FAO (2011). Food and Agriculture Organization Statistics, FAOSTAT

Gonzalez R C & Woods R E (2008). Digital Image Processing. Pearson Education, Inc., Upper Saddle River, NJ, USA

Li Y, Dhakal S & Peng Y (2012). A machine vision system for identification of micro- crack in egg shell. *Journal of Food Engineering* **109**: 127-134

Liming X & Yanchao Z (2010). Automated strawberry grading system based on image processing. *Computers and Electronics in Agriculture* **715**: 532-539

Smith L I (2002). A Tutorial on principal components analysis. Available on the Internet at the following URL:http//www.cs.otago.ac.nz/cosc453/student_tutorials/principal_components

Moreda G P, Muñoz M A, Ruiz-Altisent M & Perdigones A (2012). Shape determination of horticultural produce using two-dimensional computer vision – A review. *Journal of Food Engineering* **108**(2): 245-261

Nashat S, Abdullah A & Abdullah M Z (2014). Machine vision for crack inspection of biscuits featuring pyramid detection scheme. *Journal of Food Engineering* **120**: 233-247

Razmjooy N, Mousavi B S & Soleymani F (2012). A real-time mathematical computer method for potato inspection using machine vision. *Computers and Mathematics with Applications* **63**(1): 268-279

Shapiro L G & Stockman G (2001). Computer vision. New Jersey, Prentice-Hall, USA

Stehman S V (1997). Selecting and interpreting measures of thematic classification accuracy. *Remote Sensing of Environment* **62**(1): 77-89

Tao Y, Morrow C T, Heinemann P H & Sommer H J (1995). Fourier-based separation technique for shape grading of potatoes using machine vision. *Transactions of the ASAE* **38**(3): 949-957

Ying Y, Jing H, Tao Y & Zhang N (2002). Detecting stem and shape of pears using Fourier transformation and an artificial neural network. *Information & Electrical Technologies Division of the ASAE* **46**(1): 157-162

Zhou L, Chalana V & Kim Y (1998). PC-Based machine vision system for real-time computer-aided potato inspection. *International Journal of Imaging Systems and Technology* **9**(6): 423–433

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Elicitor Applications to Cell Suspension Culture for Production of Phenolic Compounds in Grapevine

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ABSTRACT

In this study, the effects of cadmium sulphate ($CdSO_4$), fleuresans irradiation, methyl jasmonate (MeJA) and sucrose treatments on the production of phenolic compounds in grapevine cell suspension cultures initiated from callus from petiole tissues of *Vitis vinifera L. cvs*. Gamay, Kalecik karası and Öküzgözü were investigated. As the elicitors of $CdSO_4$ (0, 1 and 1.5 mM), MeJA (0 and 10 µM) and sucrose (0, 0.20 and 0.25 M) were applied. Cell suspensions were exposed to visible light (10,000 lux) for fleuresans irradiation or cultured in dark constantly. Total phenolics, total flavanols, total flavonols and anthocyanin were determined spectrophotometrically while *trans-r*esveratrol was quantified by HPLC. $CdSO_4$ at 1.5 mM concentration and MeJA at 10 µM concentration yielded the highest phenolic productions in all cultivars. Especially, Kalecik Karası treated with $CdSO_4$ at 1.5 mM had the highest total phenolic (3.144 mg g^{-1}), anthocyanin (1.672 CV g^{-1}) and *trans*-resveratrol (3.650 µg g^{-1}) contents. MeJA application at 10 µM provided the *trans*-resveratrol accumulation as high as 11.681 µg g^{-1} in Öküzgözü. 0.20 M sucrose concentration resulted in the highest total phenolics (4.215 mg g^{-1}) and *trans*-resveratrol (7.550 µg g^{-1}) in Kalecik Karası cultures while the most anthocyanin accumulation (2.024 CV g^{-1}) was achieved from Gamay. Darkness had strongly increased *trans*-resveratrol content in all cultivars, whereas total phenolics and anthocyanin synthesis were induced by light. Elicitor applications of $CdSO_4$, MeJA, sucrose and fleuresans irradiation can be an efficient approach for the production of phenolics in grapevines.

Keywords: Grapevine; Cell suspension; Cadmium; Methyl jasmonate; Anthocyanin, *trans*-resveratrol

Asmada Hücre Süspansiyon Kültürlerine Elisitör Uygulamaları ile Fenolik Bileşiklerin Üretilmesi

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ÖZET

Bu araştırmada asmada hücre süspansiyon kültürlerinde fenolik bileşiklerin üretiminde kadmiyum sülfat ($CdSO_4$) floresan radyasyonu, metil jasmonat (MeJA) ve sukroz uygulamalarının etkileri incelenmiştir. Gamay, Kalecik karası ve

Öküzgözü üzüm çeşitlerine ait yaprak saplarından elde edilen kallus hücre süspansiyon kültürlerine $CdSO_4$ (0, 1 ve 1.5 mM), MeJA (0 ve 10 µM) ve sukroz (0, 0.20 ve 0.25 M) uygulanmıştır. Floresan radyasyonu için hücre süspansiyonları tümüyle karanlıkta ya da 10000 lux ışık altında tutulmuştur. Toplam fenolik, toplam flavanol, toplam flavonol ve antosiyanin içerikleri spektrofotometrik olarak; *trans-*resveratrol içeriği ise HPLC ile belirlenmiştir. 1.5 mM konsantrasyonundaki $CdSO_4$ ve 10 µM konsantrasyonundaki MeJA bütün çeşitlerde en yüksek fenolik bileşik üretimini sağlamıştır. Özellikle 1.5 mM $CdSO_4$ uygulanmış Kalecik karası en yüksek toplam fenolik madde (3.144 mg g^{-1}), antosiyanin (1.672 CV g^{-1}) ve *trans*-resveratrol (3.650 µg g^{-1}) içeriğine sahip olmuştur. 10 µM konsantrasyonundaki MeJA uygulaması ise Öküzgözü çeşidinde *trans*-resveratrol miktarının 11.681 µg g^{-1} gibi yüksek bir değere çıkmasını sağlamıştır. Sukroz uygulamaları içinde 0.20 M dozu en yüksek toplam fenolik (4.215 mg g^{-1}) ve *trans*-resveratrol (7.550 µg g^{-1}) miktarını Kalecik karasında, en yüksek antosiyanin birikimini ise Gamay çeşidinde sağlamıştır. Karanlık uygulaması bütün çeşitlerde *trans*-resveratrol birikimini kuvvetli bir şekilde artırmıştır. Toplam fenolik ve antosiyanin sentezinin ışık tarafından uyarıldığı belirlenmiştir. Sonuçlar $CdSO_4$, MeJA, sukroz ve floresan radyasyonu gibi elisitör uygulamalarının üzümde fenolik bileşiklerin üretiminde önemli bir yaklaşım olabileceğini göstermiştir.

Anahtar Kelimeler: Asma; Hücre süspansiyonu; Kadmiyum; Metil jasmonat; Antosiyanin, *trans*-resveratrol

1. Introduction

Secondary metabolites are chemical compounds produced by plants. These compounds are not essential for cell structure, photosynthesis, respiratory metabolism or other primary functions. The main role of them is natural defense system against biotic and abiotic stresses (Rispail et al 2005). The interest in these metabolites has increased in recent years since many researchers reported that certain compounds will have a positive impact on preventing cancer and age-related disorders, such as certain neurological diseases and metabolic disorders (Dzhambazova et al 2011). In addition, some compounds were implicated in important biological functions in the body such as antioxidant defense system, immunological regulation and anti-inflammatory processes. Among the secondary metabolites, phenolic compounds characterized by having at least one aromatic ring and one or more hydroxyl groups attached to an aromatic ring, are one of the most important secondary metabolites (Cartea et al 2011). Plant phenolics comprise simple phenols, phenolic acids, coumarins, flavonoids, stilbenes, up to hydrolysable and condensed tannins, lignans and lignins.

Anthocyanins are an important group of natural pigments within the flavonoid family. They play a major role in plants by attracting insects for the purpose of pollination, and they serve as a UV screen for protecting the plant's DNA from damage by sunlight (Isley 1987). Many environmental factors (light, temperature, nutrition, drought and infection) have an effect on the synthesis of anthocyanins (Ismail & Mohamed 2010). Anthocyanins exhibit antioxidant properties, free radical scavenging properties and suppression of proliferation of human cancer cells (Dai et al 2007). Thus, they are widely used in food, beverages, cosmetics, pharmaceuticals etc. Resveratrol is a group of polyphenolic secondary metabolites within the stilbene family which is produced as a defensive reaction in response to biotic and abiotic stresses (Jeandet et al 2002). Resveratrols were detected approximately in 72 plants species (Jang et al 1997). They possess many functions including antioxidant and antimicrobial activities (Daroch et al 2001). Thus, *trans*-resveratrol has great potential in various industries such as medical, pharmaceutical, food and cosmetics.

Secondary metabolites can be obtained by direct extraction from plant organs (leaf, root, flower, fruit etc.) using traditional methods. On the other hand, cell cultures are potential sources in secondary metabolite production (Ramachandra & Ravishankar 2002). These are reliable and continuous techniques but the desired end metabolite content is often low. It is possible to increase the secondary metabolite accumulation in

the cell cultures by application of elicitors (Qu et al 2006; Ahmed & Baig 2014). Cadmium sulphate ($CdSO_4$), a heavy metal, is one of the elicitors. Heavy metals inhibit many physiological processes in plants (Zornoza et al 2002). Cadmium causes oxidative stress by disruption of the electron transport chain or induction of lipid peroxidation. Another elicitor is fleuresans irradiation which induces a photooxidative stress. There is no study on the effect of these two elicitors on production of phenolic compounds in grape cell cultures. Methyl jasmonate (MeJA) is another compound used as an elicitor source in order to increase the secondary metabolite synthesis. Jasmonic acid and MeJA are key compounds of the signal transduction system of plant defense reactions (Krisa et al 1999). Sucrose is a general source of carbohydrates and it is used for creation of osmotic stress.

The aim of this study was to investigate the effect of elicitors $CdSO_4$, fleuresans irradiation, MeJA, and sucrose on phenolic (total phenolic, total flavanols, total flavonols, anthocyanin and *trans*-resveratrol) accumulation in cultured cells of Gamay, Kalecik karası and Öküzgözü grape *(Vitis vinifera L.)* cultivars.

2. Material and Methods

Kalecik karası and Öküzgözü grapevine cultivars were chosen as plant material because they are among red wine cultivars grown widely in Turkey. Gamay, mostly used in studies for production of secondary metabolites in cell suspension cultures (Do & Cormier 1990; Larronde et al 1998; Krisa et al 1999; Zhang et al 2002), was also selected in order to compare our findings with previous studies and to allow ranking the cultivars in terms of secondary metabolite production capacities.

2.1. Callus and cell suspension cultures

Callus tissues were obtained from leaf petioles of Gamay, Kalecik karası and Öküzgözü cultivars by following procedures. The petioles were surface sterilized with commercial bleach (15%) for 15 min and rinsed with sterile distilled water. Petioles were cut into 1 cm pieces and placed onto a B5 culture medium (Gamborg et al 1968) with 30 g L^{-1} sucrose and 8 g L^{-1} bacto agar supplemented with 0.5 mg L^{-1} benzylaminopurine and 0.5 mg L^{-1} indole acetic acid (Shure & Acree 1994). The pH was adjusted to 5.75 before autoclaving. Explants were incubated at 25 °C under dark conditions. Induced calli were subcultured on the same media in order to maintain sufficient stock cultures. Cell suspensions were initiated by inoculating fresh friable fragments of calli (2.5 g each) into 50 mL of liquid media in 250 mL Erlenmeyer flasks. Media were supplemented with macro elements (B5 medium), micro elements (Murashige & Skoog 1962), vitamins (Morel 1970), 0.1 mg L^{-1} naphthalene acetic acid, 0.2 mg L^{-1} kinetin, 250 mg L^{-1} casein hydrolizate and 20 g L^{-1} sucrose. Then, they were placed in a rotary shaker (100 rpm). Incubation conditions were 16/8 h light/dark cycle and 6000 lux light intensity except from fleuresans irradiation. Then, these cultures were used for elicitor applications.

2.2. Elicitor applications

$CdSO_4$, dissolved in water, were applied at 1.0 and 1.5 mM concentrations to cell cultures in exponential growth phase at day 7. Studies show that the amount of metabolite production varied with the duration of incubation time with elicitors. Because of the differences in metabolite levels the cells were harvested at every 2 days until day 6. For fleuresans irradiation, cell cultures at day 7 were placed under continuous fluorescent light at 10,000 lux or cultured in dark (control) on shaker. Cells were harvested to determine metabolite levels at every 3 days until day 15. For MeJA treatments, MeJA was dissolved in 99% ethanol and added into autoclaved media after filter-sterilization. MeJA was added to cell culture media at day 7 of incubation at 10 µM concentration. Cells were harvested at every 3 days until day 15. For sucrose treatment, 0.20 M and 0.25 M sucrose concentrations were applied to cultures at day 5. Control treatment contained only autoclaved distilled water. The cells were harvested at every 3 days until day 15. After elicitor applications, harvested cells were weighed and kept at -20 °C

until the extraction and analysis. For each treatment three replicates and three 250 mL Erlenmeyer flasks for each replication were used in the experiments that 54 flasks for sucrose (3 concentrations and 6 sampling dates) and 36 flasks for each $CdSO_4$ (3 concentrations and 4 sampling dates), fleuresans irradiation (2 different irradiation regimes and 6 sampling dates) and MeJA (2 concentrations and 6 sampling dates) treatments were used and samples were taken from the separate flasks per treatment.

2.3. Extraction of phenolic compounds from harvested cells

Cell samples (2 g) were dried and powdered by liquid nitrogen and were extracted with 10 mL of 96% EtOH for 24 h at 40-45 °C. The incubated mixture is centrifuged at 4.000 rpm. Supernatant was concentrated with the rotary evaporator until dryness and resuspended in a methanol (Kiselev et al 2007). Amounts of total phenolics, total flavanols, total flavonols and anthocyanin were determined spectrophotometrically, and *trans*-resveratrol content was quantified by HPLC. Spectrophotometric readings were performed by a PG Instruments spectrophotometer (T70 Plus Dual Beam/Arlington, USA) and conducted with five repetitions. Folin-Ciocalteu reagent was used to estimate total phenolic content (Singleton & Rossi 1965) which was expressed as gallic acid equivalents (mg GAE g^{-1} fresh cell weight, FCW). Total flavanols were determined by the method of Arnous et al (2001) and expressed as catechin equivalents (mg CE g^{-1} FCW). Total flavonols were determined with Neu's reagent solution by the method of Dai et al (1995). The flavonol contents were expressed as rutin equivalent (mg RE g^{-1} FCW). Anthocyanin accumulation was determined by the method of Qu et al (2006), and it was represented as color value (CV) which was calculated with the Equation 1.

$$CV\ (CV\ g^{-1}\ FCW) = 0.1 \times Absorbance \times Dilution\ factor \quad (1)$$

Separation of *trans*-resveratrol was performed by the modified method of Caponio et al (1999). Reversed phase (RP)-HPLC analysis was done using a SCL-10Avp system controller, a SIL-10AD vp autosampler, a LC-10AD vp pump, a DGU-14 A degasser, a CTO-10 A vp column heater, and a Diode Array Detector set at 278 nm. The 250 x 4.6 mm i.d. 5 µm column was filled with Agilent Eclipse XDB-C18. The flow rate was 0.8 mL min^{-1}, the injection volume was 20 µL, and the column temperature was set at 30 °C. For gradient elution, mobile phase A contained 2% acetic acid; solvent B contained methanol. The gradient program reported by Göktürk-Baydar et al (2011) was used. The data were analyzed using the Shimadzu Class-VP Chromatography Laboratory Automated Software system. The amount of *trans*-resveratrol content in the cells were calculated as µg g^{-1} FCW using external calibration curves obtained for *trans*-resveratrol standard. HPLC determinations were done in triplicate. Data were analyzed by using analysis of variance (ANOVA) using SPSS 16.0 for Windows Software Package and the means were separated by Duncan's multiple range tests.

3. Results and Discussion

In this study, the effects of elicitor applications of $CdSO_4$, fleuresans irradiation, MeJA and sucrose on phenolic accumulation in cell suspension cultures of Gamay, Kalecik karası and Öküzgözü cultivars were determined. $CdSO_4$ treatments positively influenced ($P \leq 0.05$) the syntheses of all of the phenolics depending on its concentrations and sampling dates (Table 1). $CdSO_4$ treatment of 1.5 mM gave the highest phenolic contents in Gamay (2.044 mg g^{-1}) and Kalecik karası (3.144 mg g^{-1}) while both 1.0 and 1.5 mM treatments were suitable concentrations for total flavanols and total flavonols in Öküzgözü. $CdSO_4$ treatment of 1.5 mM resulted in the highest anthocyanin (1.672 CV g^{-1}) and *trans*-resveratrol (3.650 µg g^{-1}) in Kalecik karası cultures. There is no study about the effects of $CdSO_4$ applications on secondary metabolite production of grape cell cultures. On the other hand $CdCl_2$, another compound of cadmium, can be used for enhancing phenolic compounds and tocopherols in grape cell cultures depending on the $CdCl_2$ concentrations and exposure times (Çetin et al 2014) that $CdCl_2$ at 1.0 mM concentration was increased phenolics

Table 1- The effects of $CdSO_4$ application on production of phenolic compounds in grapevine cell cultures

Çizelge 1- $CdSO_4$ uygulamasının asma hücre kültürlerinde fenolik bileşiklerin üretimine etkileri

		Sampling Date (day)	*Total phenolic (mg g^{-1})*	*Total flavanols (mg g^{-1})*	*Total flavonols (mg g^{-1})*	*Anthocyanin (CV g^{-1})*	*Trans-resveratrol (µg g^{-1})*
					GAMAY		
Control		0	0.925 g*	0.064 d	0.045 d	0.624 i	0.721 e
		2	0.677 j	0.051 e	0.087 b	0.699 h	0.703 e
		4	0.884 h	0.048 e	0.044 d	0.787 g	0.862 d
		6	1.505 e	0.071 c	0.064 c	0.685 hi	0.700 e
1.0 mM		0	0.946 g	0.085 b	0.088 b	0.925 f	0.990 c
		2	1.727 b	0.060 d	0.090 b	0.971 ef	1.236 b
		4	1.598 d	0.037 f	0.050 d	1.125 b	0.872 d
		6	0.801 i	0.031 g	0.025 e	1.037 cd	1.613 a
1.5 mM		0	0.990 f	0.074 c	0.091 b	1.013 de	1.194 b
		2	2.044 a	0.093 a	0.107 a	1.099 bc	0.961 c
		4	1.672 c	0.060 d	0.083 b	1.291 a	1.642 a
		6	0.643 k	0.031 g	0.025 e	1.061 bcd	1.261 b
					KALECİK KARASI		
Control		0	1.330 h	0.101 c	0.022 g	0.763 g	0.873 f
		2	1.441 g	0.107 c	0.029 fg	0.776 g	0.850 f
		4	1.474 g	0.110 c	0.040 e	0.773 g	0.851 f
		6	1.302 h	0.099 c	0.033 ef	1.043 e	1.827 e
1.0 mM		0	1.616 f	0.076 de	0.062 d	1.152 d	1.672 e
		2	1.284 h	0.074 de	0.101 c	0.928 f	1.823 e
		4	2.226 c	0.082 d	0.098 c	1.224 d	1.856 e
		6	2.468 b	0.147 b	0.107 c	1.376 c	1.852 e
1.5mM		0	1.696 e	0.104 c	0.032 ef	1.336 c	2.331 c
		2	2.017 d	0.105 c	0.106 c	1.525 b	2.483 c
		4	2.234 c	0.110 c	0.138 b	1.672 a	3.650 a
		6	3.144 a	0.213 a	0.219 a	1.579 b	3.165 b
					ÖKÜZGÖZÜ		
Control		0	1.028 f	0.045 f	0.057 de	0.653 f	1.382 g
		2	1.059 f	0.054 ef	0.063 cd	0.869 d	1.393 g
		4	1.283 de	0.079 d	0.065 cd	0.824 d	2.10 cd
		6	1.221 e	0.094 c	0.066 cd	0.736 e	2.160 cd
1.0 mM		0	1.263 e	0.057 e	0.049 e	0.651 f	1.342 g
		2	1.503 c	0.103 bc	0.078 ab	1.269 b	1.664 e
		4	1.582 b	0.135 a	0.082 a	0.835 d	2.220 bc
		6	1.330 d	0.072 d	0.058 de	1.021 c	2.046 d
1.5 mM		0	1.247 e	0.071 d	0.071 bc	1.077 c	1.512 f
		2	1.524 bc	0.110 b	0.067 c	1.317 ab	2.843 a
		4	1.585 b	0.106 b	0.080 ab	1.360 a	2.962 a
		6	1.754 a	0.136 a	0.079 ab	1.059 c	2.284 b

*, differences between means indicated by the same letters are not statistically significant (P≤0.05)

and tocopherols when cells were harvested at day 2 and 4, respectively. Cadmium toxicity can promote altered metabolism which can include the formation of reactive oxygen species (ROS) in plants under stress conditions (Bergmann et al 2001). Metal ions act as abiotic elicitors and induce biosynthesis of phytoalexins in plant cell cultures (Radman et al 2003). Kidd et al (2001) reported that maize roots exposed to aluminium were exuded high levels of phenolics.

The effects of fleuresans irradiation on phenolic accumulation varied according to types of phenolics (Table 2). Anthocyanin biosynthesis was stimulated considerably by light irradiation and the maximum anthocyanin productions in Gamay (1.584 CV g^{-1}) Kalecik karası (1.893 CV g^{-1}) and Öküzgözü (2.667 CV g^{-1}) were obtained from the cells harvested at day 9. Light induced anthocyanin biosynthesis in cell cultures were reported in *Vitis vinifera* (Zhang et al 2002), *Daucus carota* (Takeda 1990) and *Perilla frutescens* (Zhong et al 1993). Fleuresans irradiation induces photooxidative stress and anthocyanin production is expressed in response to light treatment (Song & Lee 1998). Whereas, the contents of total phenolics, total flavanols, total flavonols and *trans*-resveratrol were the highest on the cells incubated at dark conditions ($P \leq 0.05$). Accordingly, the maximum total phenolic, total flavanol and total flavonol contents of cells cultured in darkness were found as 2.448 mg g^{-1}, 0.106 mg g^{-1} and 0.148 mg g^{-1} respectively in Öküzgözü. The results showed that the dark condition induced *trans*-resveratrol accumulation at all genotypes. The greatest *trans*-resveratrol contents were detected on Gamay (2.473 µg g^{-1}) and Kalecik karası (3.165 µg g^{-1}) cells at days 9 and Öküzgözü cells (9.483 µg g^{-1}) at day 6 (Table 2). Resveratrol can be found in the *cis* or *trans* configurations. Its *trans* form exists in plants, but in red wines a small amount of *cis* form has been detected. The *trans f*orm may change to *cis* form which it's isomer, when after exposure to the UV light (Lopez-Hernandez et al 2007) and high white light (Burns et al 2002).

MeJA positively influenced syntheses of all phenolic compounds on the grape cells (Table 3). Generally, higher concentrations of phenolic compounds were detected at towards the end of the culture. Total phenolic compounds were the highest on the cells applied MeJA and harvested at day 15 in all genotypes and their amounts changed between 1.972 mg g^{-1} (Kalecik karası) and 2.808 mg g^{-1} (Öküzgözü). Total flavanols, total flavonols and anthocyanin contents of MeJA applied cells were higher than those of the controls. MeJA also significantly enhanced *trans*-resveratrol content depending on the sampling date ($P \leq 0.05$) and it was the greatest on Kalecik karası (2.872 µg g^{-1}) and Öküzgözü (11.681 µg g^{-1}) cells harvested at day 9. Maximum anthocyanin accumulation reported after 20 µM jasmonic acid addition to Gamay cells (Zhang et al 2002). In Vinhao grapevine cell cultures, MeJA treatment increased stilbenic production 9-fold compared to the control (Lima et al 2012). MeJA induced anthocyanin accumulation in soybean seedlings as a result of the over-expression of chalcone synthase (Creelman et al 1992). Several elicitors could be used in cell suspension cultures as signaling molecules for *trans*-resveratrol production such as MeJA, cyclodextrins or chitosan (Donnez et al 2009). Jasmonic acid and MeJA are key compounds of the signal transduction system of plant defense reactions (Krisa et al 1999).

Sucrose treatments were applied to create osmotic stress (Do & Cormier 1990) hence to produce secondary metabolites. Sucrose is also essential to induce the expression of the chalcone-synthase gene, one of the genes in the anthocyanin's biosynthetic pathway (Takeuchi et al 1994). The effects of sucrose treatments on the phenolic compounds of grape cells were given in Table 4. Total phenolic, total flavanol, total flavonol and *trans*-resveratrol biosynthesis in Gamay cells increased when they treated with 0.25 M sucrose and harvested at day 9. Otherwise 0.20 M sucrose concentration gave the highest anthocyanin content (2.024 CV g^{-1}) in Gamay. In Kalecik karası, the most abundant total phenolic (4.215 mg g^{-1}), total flavanol (0.164 mg g^{-1}) and total flavonol (0.418 mg g^{-1}) contents were obtained from the cells cultured at 0.20 M sucrose treatment and harvested at day

Table 2- The effects of fleuresans irradiation on production of phenolic compounds in grapevine cell cultures

Çizelge 2- Işık radyasyonu uygulamasının asma hücre kültürlerinde fenolik bileşiklerin üretimine etkileri

	Sampling Date (day)	*Total phenolic (mg g^{-1})*	*Total flavanols (mg g^{-1})*	*Total flavonols (mg g^{-1})*	*Anthocyanin (CV g^{-1})*	*Trans-resveratrol (µg g^{-1})*
				GAMAY		
Dark	0	1.638 c*	0.045 h	0.097 bc	0.771 g	1.791 c
	3	1.637 c	0.055 fg	0.071 e	0.552 h	1.263 d
	6	1.081 i	0.056 f	0.092 c	0.435 i	2.104 b
	9	1.108 h	0.086 c	0.059 f	0.944 f	2.473 a
	12	1.018 j	0.042 i	0.074 e	1.067 e	2.390 a
	15	1.203 g	0.080 d	0.069 e	0.973 f	1.976 b
Light	0	1.262 f	0.053 g	0.088 c	1.075 e	0.974 f
	3	1.480 e	0.075 e	0.098 bc	1.213 d	1.023 ef
	6	1.556 d	0.091 b	0.082 d	1.384 c	0.622 g
	9	1.627 c	0.096 a	0.090 c	1.584 a	0.248 h
	12	1.718 b	0.090 b	0.103 ab	1.347 c	1.023 ef
	15	2.019 a	0.094 a	0.106 a	1.477 b	1.182 de
				KALECİK KARASI		
Dark	0	1.609 c	0.042 e	0.078 c	1.251 fg	1.971 c
	3	1.161 i	0.036 h	0.053 e	1.267 efg	1.633 de
	6	1.192 h	0.026 j	0.051 e	1.235 fg	1.474 e
	9	1.359 g	0.039 f	0.054 e	1.355 d	3.165 a
	12	1.470 e	0.038 g	0.067 d	1.320 de	2.442 b
	15	0.806 j	0.028 i	0.030 f	1.109 h	1.632 de
Light	0	1.741 b	0.060 d	0.080 c	1.208 g	2.411 b
	3	1.486 e	0.090 a	0.076 c	1.360 d	2.483 b
	6	1.416 f	0.083 c	0.066 d	1.736 b	1.662 d
	9	1.510 d	0.090 a	0.088 b	1.893 a	2.011 c
	12	1.793 a	0.090 a	0.111 a	1.501 c	1.590 de
	15	1.737 b	0.086 b	0.063 d	1.293 ef	1.463 e
				ÖKÜZGÖZÜ		
Dark	0	1.666 g	0.072 g	0.113 d	1.981 e	7.890 b
	3	1.785 ef	0.094 d	0.121 c	1.707 g	6.422 d
	6	1.179 i	0.063 h	0.103 e	2.493 b	9.483 a
	9	1.019 j	0.081 f	0.057 h	1.725 g	7.826 b
	12	1.752 f	0.072 g	0.081 g	1.637 h	7.152 c
	15	1.630 h	0.045 i	0.077 g	1.275 i	7.750 b
Light	0	1.822 de	0.090 e	0.107 e	2.179 d	3.192 f
	3	1.800 e	0.091 d	0.095 f	2.149 d	2.384 g
	6	2.232 c	0.100 c	0.138 b	2.248 c	1.665 h
	9	2.407 b	0.106 a	0.104 e	2.667 a	2.583 g
	12	2.448 a	0.104 b	0.148 a	1.952 e	2.742 g
	15	1.835 d	0.080 f	0.104 e	1.859 f	4.351 e

*, differences between means indicated by the same letters are not statistically significant (P≤0.05)

Table 3- The effects of MeJA application on production of phenolic compounds in grapevine cell cultures

Çizelge 3- MeJA uygulamasının asma hücre kültürlerinde fenolik bileşiklerin üretimine etkileri

	Sampling Date (day)	*Total phenolic (mg g^{-1})*	*Total flavanols (mg g^{-1})*	*Total flavonols (mg g^{-1})*	*Anthocyanin (CV g^{-1})*	*Trans-resveratrol (µg g^{-1})*
				GAMAY		
Control	0	1.738 g*	0.036 h	0.098 c	0.587 gh	1.198 f
	3	1.804 f	0.082 f	0.090 cd	0.531 hi	0.862 g
	6	1.527 j	0.066 g	0.049 h	0.491 i	1.561 d
	9	1.769 fg	0.083 ef	0.067 g	0.720 e	1.234 ef
	12	1.584 i	0.091 d	0.097 c	0.667 ef	1.485 d
	15	1.675 h	0.087 e	0.074 fg	0.851 d	1.232 ef
10 µM	0	1.782 f	0.098 c	0.115 b	0.648 fg	1.341 e
	3	2.138 c	0.080 f	0.082 ef	0.637 fg	1.530 d
	6	2.042 e	0.099 c	0.074 fg	0.968 c	1.962 bc
	9	2.086 d	0.110 b	0.085 de	1.163 a	1.900 c
	12	2.321 b	0.126 a	0.112 b	1.069 b	2.083 ab
	15	2.478 a	0.126 a	0.155 a	0.805 d	2.201 a
				KALECİK KARASI		
Control	0	1.506 f	0.088 c	0.059 c	0.541 f	1.914 cd
	3	1.108 h	0.089 c	0.058 c	0.427 g	1.291 f
	6	0.967 i	0.080 de	0.044 g	0.568 ef	0.382 i
	9	1.242 g	0.091 c	0.050 e	0.725 c	0.473 hi
	12	0.642 k	0.082 d	0.052 de	0.637 d	0.562 h
	15	0.697 j	0.067 f	0.040 g	0.595 de	1.060 g
10 µM	0	1.766 b	0.078 e	0.057 cd	0.627 d	2.563 b
	3	1.697 c	0.082 d	0.074 b	0.571 ef	1.752 e
	6	1.665 d	0.097 b	0.049 ef	0.728 c	1.880 cd
	9	1.556 e	0.101 a	0.060 c	1.056 b	2.872 a
	12	1.690 c	0.095 b	0.064 c	1.123 a	1.860 de
	15	1.972 a	0.100 a	0.098 a	0.709 c	2.000 c
				ÖKÜZGÖZÜ		
Control	0	1.330 i	0.050 g	0.057 ef	0.504 f	1.492 ef
	3	1.345 hi	0.030 j	0.058 def	0.520 f	1.681 ef
	6	1.371 gh	0.051 fg	0.053 f	0.552 f	2.860 d
	9	1.398 g	0.065 d	0.065 bc	0.616 e	4.095 c
	12	1.211 j	0.073 c	0.085 a	0.549 f	2.134 e
	15	1.647 f	0.053 ef	0.059 cdef	0.627 e	1.106 f
10 µM	0	1.821 d	0.047 h	0.060 cde	0.608 e	1.854 e
	3	1.951 c	0.055 e	0.066 bc	0.781 d	2.963 d
	6	1.744 e	0.056 e	0.068 b	0.856 bc	6.822 b
	9	1.622 f	0.056 e	0.081 a	0.888 b	11.681 a
	12	2.453 b	0.093 b	0.083 a	1.077 a	3.432 d
	15	2.808 a	0.101 a	0.080 a	0.821 cd	3.060 d

*, differences between means indicated by the same letters are not statistically significant (P≤0.05)

Table 4- The effects of sucrose application on production of phenolic compounds in grapevine cell cultures

Çizelge 4- Asmada hücre kültürlerinde fenolik bileşiklerin üretiminde sukroz uygulamasının etkileri

	Sampling Date (day)	*Total phenolic (mg g^{-1})*	*Total flavanols (mg g^{-1})*	*Total flavonols (mg g^{-1})*	*Anthocyanin (CV g^{-1})*	*Trans-resveratrol (µg g^{-1})*
				GAMAY		
Control	0	0.740 fg*	0.024 f	0.044 e	0.904 de	0.964 f
	3	1.189 e	0.051 d	0.066 d	0.851 de	1.235 e
	6	1.234 e	0.068 d	0.076 cd	1.029 d	1.413 d
	9	1.000 ef	0.031 e	0.022 fg	0.757 f	0.668 h
	12	1.010 ef	0.054 d	0.061 d	0.693 f	1.114 ef
	15	0.808 f	0.026 ef	0.045 e	0.467 g	0.553 i
0.20 M	0	0.650 g	0.028 ef	0.032 f	0.771 ef	0.802 g
	3	2.074 b	0.094 bc	0.103 b	2.024 a	1.981 c
	6	1.800 c	0.095 bc	0.103 b	1.493 b	1.934 c
	9	1.633 cd	0.066 d	0.084 c	1.101 cd	1.450 d
	12	1.605 cd	0.059 d	0.080 c	0.741 ef	1.022 f
	15	1.900 c	0.083 bc	0.115 b	0.765 ef	1.171 e
0.25 M	0	0.731 fg	0.023 f	0.023 fg	1.328 bc	0.973 f
	3	0.727 fg	0.032 e	0.021 fg	1.056 d	1.000 f
	6	1.234 e	0.054 d	0.050 e	0.752 ef	0.990 f
	9	3.510 a	0.164 a	0.361 a	1.480 b	3.740 a
	12	2.457 b	0.158 a	0.089 c	0.765 ef	1.262 e
	15	1.520 d	0.117 b	0.084 c	1.288 c	2.351 b
				KALECİK KARASI		
Control	0	1.683 c	0.058 d	0.023 f	0.925 f	1.793 fg
	3	1.879 c	0.067 c	0.063d	1.128 e	2.491 e
	6	1.474 cd	0.075 c	0.052 de	0.925 f	1.616 fg
	9	1.723 c	0.071 c	0.065 d	0.760 g	1.894 f
	12	1.417 cde	0.058 d	0.044 e	0.875 f	1.040 hi
	15	1.545 cd	0.061 cd	0.052 de	0.992 ef	1.990 f
0.20 M	0	1.588 cd	0.041 de	0.025 f	0.741 g	3.891 b
	3	1.088 e	0.064 cd	0.055 de	1.909 a	7.550 a
	6	2.064 b	0.095 bc	0.082 bc	1.728 ab	1.662 fg
	9	2.397 b	0.099 bc	0.097 b	1.096 e	3.973 b
	12	4.215 a	0.164 a	0.418 a	0.875 f	3.454 c
	15	1.524 cd	0.041 de	0.064 d	0.925 f	3.200 cd
0.25 M	0	1.338 de	0.043 de	0.023 f	0.832 f	0.881 i
	3	1.281 de	0.052 d	0.054 de	1.459 c	1.376 h
	6	1.497 cd	0.057 d	0.039 e	1.819 a	1.372 h
	9	1.682 c	0.112 b	0.083 bc	1.301 d	1.880 f
	12	1.192 e	0.057 cd	0.061 d	1.213 de	1.711 fg
	15	0.742 f	0.039 e	0.034 e	1.088 e	0.733 j
				ÖKÜZGÖZÜ		
Control	0	2.004 ef	0.117 cd	0.083 de	0.877 f	3.472 e
	3	2.366 cd	0.109 d	0.087 de	1.165 d	4.855 b
	6	1.992 ef	0.096 d	0.086 de	1.013 ef	3.476 e
	9	1.320 h	0.075 e	0.027 g	0.875 f	3.030 fg
	12	1.051 i	0.030 g	0.034 fg	0.869 f	2.283 j
	15	1.113 i	0.057 f	0.047 f	0.979 ef	3.190 f
0.20 M	0	2.073 e	0.117 cd	0.083 de	1.061 e	2.692 h
	3	2.463 c	0.098 d	0.091 de	1.496 b	2.270 j
	6	1.545 g	0.103 d	0.097 de	0. 912 ef	2.613 h
	9	2.067 e	0.116 cd	0.132 c	0.712 g	2.940 g
	12	2.151 de	0.140 b	0.112 d	1.104 e	4.042 cd
	15	2.462 c	0.147 b	0.163 b	1.192 c	3.843 d
0.25 M	0	1.869 f	0.094 d	0.086 de	0.979 ef	2.514 hi
	3	2.259 d	0.105 d	0.121 c	1.192 cd	2.435 i
	6	2.275 d	0.098 d	0.106 d	1.093 e	3.254 ef
	9	2.686 bc	0.125 c	0.169 b	1.245 c	4.172 c
	12	2.802 a	0.137 bc	0.217 a	1.629 a	4.91 b
	15	2.706 ab	0.187 a	0.204 a	1.088 e	5.531 a

12 while the greatest anthocyanin contents were obtained from both at 0.20 M and 0.25 M sucrose treatments as 1.909 CV g^{-1} and 1.819 CV g^{-1}, respectively. For Öküzgözü cell cultures, 0.25 M sucrose concentration was found as the most suitable sucrose concentration in terms of all secondary metabolites. The maximum *trans*-resveratrol content (7.550 µg g^{-1}) was obtained from Kalecik karası cell cultures treated with 0.20 M sucrose and harvested at day 3, which represent a 3 fold increase compared with the control cultures (2.491 µg g^{-1}) harvested on the same date. Similarly Larronde et al (1998) reported that total stilbene content was 1.5 times greater at 0.10 M sucrose than that of cells grown without added-sucrose while anthocyanin contents increased 12-fold from control to 0.15 M added sucrose. The production of secondary metabolites by increasing the concentration of carbohydrates has generally attributed to increased precursors available for secondary metabolite. On the other hand, it was also demonstrated that sucrose concentration of 4% decreased cell growth and hence may stimulate metabolite biosynthesis through an osmotic stress phenomenon biosynthesis (Knobloch & Berlin 1983). The synthesis of anthocyanins has been shown to be stimulated by sucrose in cells (Hirasuna et al 1991) of grapevine, and seems to result from an osmotic stress (Do & Cormier 1990). The mechanisms by which plant cells detect and respond to sucrose are very poorly understood. It is known that sucrose was found to modulate other metabolites such as polyphenol accumulation in *Vitis vinifera* cell cultures. Ferri et al (2011) reported the high levels of many flavonoids and stilbenes in Barbera cell suspensions treated with increased sucrose concentrations. The effect of sugars on plant cells seems to be due to the coupling of two mechanisms: osmotic stress and disturbed cellular metabolism (Do & Cormier 1990). *Trans*-resveratrol contents were affected by all sucrose concentrations and sampling dates. Donnez et al (2009) also reported that the amount of *trans*-resveratrol fluctuates widely according to plant species, elicitor and culture conditions.

4. Conclusions

The results showed that fleuresans irradiation, $CdSO_4$, MeJA and sucrose are potent elicitors in cell suspension cultures of Gamay, Kalecik karası and Öküzgözü grapevine cultivars. $CdSO_4$ at 1.5 mM concentration and MeJA at 10 µM concentration compared to controls yielded the highest total phenolics, anthocyanin and *trans*-resveratrol productions in all cultivars while 0.20 and 0.25 M sucrose concentrations were found as the most suitable concentrations depending on the cultivars. Light irradiation resulted in a significant synergistic enhancement of anthocyanin accumulation whereas dark conditions stimulated the total phenolic, total flavanol, total flavonol and *trans*-resveratrol synthesis in all cultivars tested. Further experiments should be studied to examine the relationship between elicitor and metabolite production in cell cultures. The results also demonstrate an efficient avenue for the development of similar strategies to advance the plant cell culture process for commercial production.

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References

Ahmed S A & Baig M M V (2014). Biotic elicitor enhanced production of psoralen in suspension cultures of *Psoralea corylifolia* L. *Saudi Journal of Biological Sciences* **21**(5): 499-504

Arnous A, Makris D P & Kefalas P (2001). Effect of principal polyphenolic components in relation to antioxidant characteristics of aged red wines. *Journal of Agriculture and Food Chemistry* **49**(12): 5736-5742

Bergmann H, Machelett B, Lippmann B & Friedrich Y (2001). Influnce of heavy metals on the accumulation of trimethylglycine, putrescine and spermine in food plants. *Amino Acids* **20**: 325-329

Burns J, Yokota T, Ashihara H, Lean M E J & Crozier A (2002). Plant foods and herbal sources of resveratrol.

Journal of Agriculture and Food Chemistry **50**: 3337-3340

Caponio F, Alloggio V & Gomes T (1999). Phenolic compounds of virgin olive oil: Influence of paste preparation techniques. *Food Chemistry* **64**: 203-209

Cartea M E, Francisco M, Soengas P & Velasco P (2011). Phenolic compounds in *Brassica* vegetables. *Molecules* **16**: 251-280

Creelman R A, Tierney M L & Mullet J E (1992). Jasmonic acid/methyl jasmonate accumulate in wounded soybean hypocotyls and modulate wound gene expression. *Proceeding of the Natural Academy of Sciences* USA **89**: 4438-4491

Çetin E S, Babalık Z, Hallaç-Türk F & Göktürk Baydar N (2014). The effects of cadmium chloride on secondary metabolite production in *Vitis vinifera* cv. cell suspension cultures. *Biological Research* **47**:47-53

Dai G H, Andary C, Mondolot L & Boubals D (1995). Involment of phenolic compounds in the resistance of grapevine callus to downy mildew (*Plasmopara viticola*). *European Journal of Plant Pathology* **101**: 541-547

Dai J, Patel J D & Mumper R J (2007). Characterization of blackberry extract and its antiproliferative and anti-inflammatory properties. *Journal of Medicinal Food* **10**(2): 258-265

Daroch F, Hoeneisen M & Gonzalez C L (2001). *In vitro* antibacterial activity of Chilean red wines against *Helicobacter pylori. Microbiology* **104**: 79-85

Do C B & Cormier F (1990). Accumulation of anthocyanins enhanced by a high osmotic potential in grape (*Vitis vinifera* L.) cell suspensions. *Plant Cell Report* **9**: 143-146

Donnez D, Jeandet P, Clement C & Courot E (2009). Bioproduction of resveratrol and stilbene derivatives by plant cells and microorganisms. *Trends in Biotechnology* **27**(12): 706-713

Dzhambazova T, Kondakova V, Tsvetkov I & Batchvarova R (2011). Grape secondary metabolites benefits for human health. In: Chuen R & Chang C (Eds). Advanced understanding of neurodegenerative diseases. Chapter 13, pp. 285-298

Ferri M, Righetti L & Tassoni A (2011). Increasing sucrose concentrations promote phenylpropanoid biosynthesis in grapevine cell cultures. *Journal of Plant Physiology* **168**: 189-195

Gamborg O L, Miller R A & Okajima K (1968). Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Research* **50**: 151-156

Göktürk Baydar N, Babalık Z, Hallaç Türk F & Çetin E S (2011). Phenolic composition and antioxidant activities of wines and extracts of some grape varieties grown in Turkey. *Tarım Bilimleri Dergisi-Journal of Agricultural Sciences* **17**: 67-76

Hirasuna T J, Shuler M L, Lackney V K & Spanswick R M (1991). Enhanced anthocyanin production in grape cell cultures. *Plant Science* **78**: 107-120

Isley P T (1987). Tillandsia: The world's most unusual air plants. Illustrated edition. Gardena, California, Botanical Press.

Ismail G S M & Mohamed H E (2010). Alteration in growth and thylakoid membrane lipid composition of *Azolla caroliniana* under phosphate deficiency. *Biologia Plantarum* **54**: 671-676

Jang M, Cai L, Udeani G O, Slowing K V, Thomas C F, Beecher C W, Fong H H, Farnsworth N R, Kinghorn A D, Mehta R G, Moon R C & Pezzuto J M (1997). Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. *Science* **275**: 218-220

Jeandet P, Douillet Breuil A C, Bessis R, Debord S, Sbaghi M & Adrian M (2002). Phytoalexins from the *Vitaceae*: Biosynthesis, phytoalexin gene expression in *trans*genic plants, antifungal activity and metabolism. *Journal of Agriculture and Food Chemistry* **50**: 2731-2741

Kidd P S, Llugany M, Poschenrieder C, Gunsé B & Barceló J (2001). The role of root exudates in aluminium resistance and silicon-induced amelioration of aluminium toxicity in three variety of maize (*Zea mays* L.). *Journal of Experimental Botany* **52**: 1339-1352

Kiselev K V, Dubrovina A S, Veselova M V, Bulgakov V P, Fedoreyev S A & Zhuravlev Y N (2007). The rolB gene-induced overproduction of resveratrol in *Vitis amurensis trans*formed cells. *Journal of Biotechnology* **128**: 681-692

Knobloch K H & Berlin J (1983). Influence of phosphate on the formation of the indole alkaloids and phenolic compounds in cell suspension cultures of *Catharanthus roseus*. *Plant Cell Tissue and Organ Culture* **2**: 333-340

Krisa S, Larronde F, Budzinsky H, Decendit A, Deffieux G & Mérillon J M (1999). Stilbene production by *Vitis*

vinifera cell suspension cultures: Methyl jasmonate induction and 13C biolabeling. *Journal of Natural Products* **62**: 1688-1690

Larronde F, Krisa S, Decendit A, Cheze C, Deffieux G & Merillon J M (1998). Regulation of polyphenol production in *Vitis vinifera* cell suspension cultures by sugars. *Plant Cell Rep*orts **17**: 946-950

Lima M R M, Ferreres F & Dias A C P (2012). Response of *Vitis vinifera* cell cultures to *Phaeomoniella chlamydospora*: Changes in phenolic production, oxidative state and expression of defence-related genes. *European Journal of Plant Pathology* **132**: 133-146

Lopez-Hernandez J, Losada P P, Sanches Silva A T & Lage Yusty M A (2007). Study of the changes of *trans*-resveratrol caused by ultraviolet light and determination of *trans*- and *cis*-resveratrol in Spanish white wines. *European Food Research Technology* **225**: 789-796

Morel G (1970). Le probleme de la transformation tumorale chez les végétaux. *Physiologium Vegetale* **8**: 189-191

Murashige T & Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15**(3): 472-497

Qu J G, Zhang W, Jin M F & Yu X J (2006). Effect of homogeneity on cell growth and anthocyanin biosynthesis in suspension cultures of *Vitis vinifera. Chinese Journal of Biotechnology* **22**(5): 805-810

Radman R, Saez T, Bucke C & Keshavarz T (2003). Elicitation of plants and microbial cell systems. *Biotechnology and Applied Biochemistry* **37**: 91-102

Ramachandra R S & Ravishankar G A (2002). Plant cell cultures: Chemical factories of secondary metabolites. *Biotechnology Advances* **20**: 101-153

Rispail N, Nash R & Webb K J (2005). Secondary Metabolite Profiling. *Lotus japonicus* Handbook. Chapter 7.4. pp. 341-348

Shure K & Acree T (1994). Production of ß-damascenone precursors in cell cultures of *Vitis labrusca* cv. Concord grapes. *Plant Cell Reports* **13**: 477-480

Singleton V L & Rossi J R (1965). Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid. *American Journal of Enology and Viticulture* **16**: 144-158

Song J S & Lee C S (1998). An, expression of CHS, CHI, and DFR genes in response to light in small radish seedlings. *Journal of Plant Biology* **41**: 277-282

Takeda J (1990). Light induced synthesis of anthocyanin in carrot cells in suspension-II. Effects of light and 2,4-D on induction and reduction of enzyme activities related to anthocyanin synthesis. *Journal of Experimental Botany* **41**: 749-755

Takeuchi A, Matsumoto S & Hayastu M (1994). Chalcone synthase from *Camellia sinensis*: Isolatyion of the cDNAs and the organ-specific and sugar-responsive expression of the genes. *Plant & Cell Physiology* **35**: 1011-1018

Zhang W, Curtin C, Kikuchi M & Franco C (2002). Integration of jasmonic acid and fleuresans irradiation for enhancement of anthocyanin biosynthesis in *Vitis vinifera* suspension cultures. *Plant Science* **162**: 459-468

Zhong J J, Yoshida M, Fujiyama K, Seki T & Yoshida T (1993). Enhancement of anthocyanin production by *Perilla frutescens* cells in a stirred bioreactor with internal light irradiation. *Journal of Fermentation and Bioengineering* **75**: 299-303

Zornoza P, Vázquez S, Esteban E, Fernández Pascual M & Carpena R (2002). Cadmium stress in nodulated white lupin: Strategies to avoid toxicity. *Plant Physiology and Biochemistry* **40**: 1003-1009

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Mineral Nutrient Content of Sweet Corn under Deficit Irrigation

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ABSTRACT

The present study was conducted to determine the effects of deficit irrigation (I) ($I_{100:}$ full irrigation; I_{85}: 15% deficit; I_{70}: 30% deficit; I_{55}: 45% deficit and I_{40}: 60% deficit) on mineral nutrient contents of fresh sweet corn (*Zea mays saccharata*) grain in two vegetation seasons, years of 2011 and 2012, in Isparta ecological conditions. The experiment was set up according to the Randomized Complete-Block Design with three replicates. The species of Lumina F_1 was used as the sweet corn cultivar. The results revealed statistically significant effects of water deficit on mineral nutrient contents of fresh sweet corn grain. The highest nitrogen content (2.29% in 2011 and 2.32% in 2012), the highest phosphorus content (0.332% in 2011 and 0.331% in 2012), the highest potassium content (0.855% in 2011 and 0.837% in 2012), the highest calcium content (0.031% in 2011 and 0.029% in 2012), the highest magnesium content (0.123% in 2011 and 0.132% in 2012), the highest iron amount (27.27 mg kg^{-1} in 2011 and 26.12 mg kg^{-1} in 2012), the highest copper amount (3.99 mg kg^{-1} in 2011 and 4.12 mg kg^{-1} in 2012) and the highest manganese amount (10.92 mg kg^{-1} in 2011 and 11.68 mg kg^{-1} in 2012) were obtained from the "I_{70}" irrigation level. The highest zinc amount (34.77 mg kg^{-1} in 2011 and 30.14 mg kg^{-1} in 2012) and the highest boron amount (5.389 mg kg^{-1} in 2011 and 5.306 mg kg^{-1} in 2012) were determined in I_{85} and I_{40} irrigation levels, respectively. Generally, the mineral nutrient contents were increased with water deficit up to a certain level "I_{70}" and he lower irrigation levels (I_{55} and I_{40}) than "I_{70}" resulted in decreased mineral nutrient contents, except for B, of sweet corn in both years.

Keywords: Sweet corn; Irrigation; Macro nutrient; Micro nutrient

Kısıntılı Sulamada Şeker Mısırın Mineral Besin İçeriği

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ÖZET

Çalışma; 2011 ve 2012 vejetasyon döneminde taze şeker mısır (*Zea mays saccharata*) tanesinin mineral besin içeriğine kısıntılı sulamanın (S) (S_{100}: tam sulama; S_{85}: % 15 kısıntı; S_{70}: % 30 kısıntı; S_{55}: % 45 kısıntı ve S_{40}: % 60 kısıntı) etkisini

belirlemek amacıyla Isparta ekolojik koşullarında yürütülmüştür. Deneme tesadüf blokları deneme desenine göre üç tekerrürlü olarak kurulmuştur. Şeker mısır çeşidi olarak Lumina F1 kullanılmıştır. Sonuçlar kısıntılı sulamanın taze şeker mısır tanesinin mineral besin içeriğine etkisinin istatistiksel olarak önemli olduğunu göstermiştir. En yüksek azot oranı (2011'de % 2.29 ve 2012'de % 2.32), fosfor oranı (2011'de % 0.332 ve 2012'de % 0.331), potasyum oranı (2011'de % 0.855 ve 2012'de % 0.837), kalsiyum oranı (2011'de % 0.031 ve 2012'de % 0.029), magnezyum oranı (2011'de % 0.123 ve 2012'de % 0.132), demir miktarı (2011'de 27.27 mg kg^{-1} ve 2012'de 26.12 mg kg^{-1}), bakır miktarı (2011'de 3.99 mg kg^{-1} ve 2012'de 4.12 mg kg^{-1}) ve mangan miktarı (2011'de 10.92 mg kg^{-1} ve 2012'de 11.68 mg kg^{-1}) "S_{70}" sulama seviyesinden elde edilmiştir. En yüksek çinko miktarı (2011'de 34.77 mg kg^{-1} ve 2012'de 30.14 mg kg^{-1}) ve bor miktarı (2011'de 5.389 mg kg^{-1} ve 2012'de 5.306 mg kg^{-1}) sırasıyla I_{85} ve I_{40} sulama seviyesinde belirlenmiştir. Genellikle her iki yılda da S_{70} sulama seviyesine kadar su kısıtlamasıyla şeker mısırın besin içeriği artmıştır ve S_{70} den daha düşük sulama seviyelerde (S_{55} ve S_{40}) bor hariç tanenin mineral besin içeriği azalmıştır.

Anahtar Kelimeler: Şeker mısır; Sulama; Makro besin; Mikro besin

1. Introduction

Sweet corn is used as a fresh or processed vegetable. Sweet corn contains higher kernel protein, oil, starch, sugar contents and many other nutrients than the other maize types. Sweet corn is favorable for fresh consumption because of its delicious taste, delicate crust and soft and sugary texture compared to other corn varieties. The human body needs nutritious food to stay healthy. The foods come from plants, fruit, vegetables, grains, legumes, nuts, seeds and animals, diary food, eggs and meat. To maintain good health, we need macronutrients, micronutrients, vitamins and minerals. Macronutrients are our main source of energy. Vitamins and minerals are essential for the body's many biochemical processes. Both vitamins and minerals are needed to maintain optimal health. Minerals assist the body in energy production and other biochemical processes. Minerals are critically important to the maintenance of human health. Because the human body cannot manufacture minerals, deficiencies are common. Minerals supports healthy immune system, is necessary to synthesize DNA, is essential for wound healing, supports healthy grow and development of body during adolescence, childhood and pregnancy (John & Jeanne 2012).

Sweet corn requires some specific environmental and cultural conditions such as an irrigation, which must be respected for the high productive and marketable yields. Corn grown mostly under irrigated conditions in Turkey. Mineral nutrient contents of grain is direct related to the available nutrient in the root zone in growing period. Available water in root zone is a significant factor in nutrient uptake of plants. Eryüce & Kılıc (2001) determined that inadequate irrigation result in low nutrient uptake in maize. According to the researches, to produce 1 kg of corn's dry matter, 368 liters of water is needed (House 1985). Water supply has a significant effect in the grain filling period (Adrienn & Janos 2012). Drought during this period usually leads to smaller grains, and the amount of dry matter accumulation decreases (Andrade et al 2005). During the seed filling period due to transfer of food to the seeds, an increase in growth intercepting hormones, an increase in ratio of abscisic acid to cytokinins in leaves, a decrease in leaf strength, an increase in the death of plant tissues, the falling of lower bush leaves, increasing respiration speed due to shadowing and reducing light, decreasing the accumulation of dry matter, and increasing the retransfer in plant are observed (Yin et al 2000; Murchie et al 2002). Szirtes et al (1977) stated that the grain nutrient content was significantly determined by the weather. The most influential factors on protein and the other mineral nutrient content are heat units and the quantity and distribution of precipitation in growing period (Asghari & Hanson 1984). Barber & Jessop (1987) found that wheat grain protein content decreased by irrigation. Andrade et al (2005) stated that

unfavorable water supply decreased dry matter accumulation in corn. Many factors influence the quality of crop products which serve as a basis for food, the most important of which are the variety, climatic factors and the production technology (Nagy 2009). Grain quality parameters have come to be more greatly preferred in recent years, making it necessary to know the effects of agro-technical factors on grain quality (Adrienn & Janos 2012). The aim of this research was to evaluate the effects of different irrigation levels on mineral nutrient content of fresh sweet corn.

2. Material and Methods

2.1. Experimental conditions

The study was conducted in the Isparta ecological conditions of Turkey during the 2011 and 2012 growing seasons. In the study, Lumina F_1 hybrid cultivar was used as the sweet corn variety.

Isparta has a territorial climate (cold winters and dry hot summers) with an annual mean rainfall of 524.4 mm. The long-term average temperature from April to end of August is 18.5 °C. The vegetative periods (from April to end of August) in 2011 and 2012 had average temperatures of 18.5 and 19.2 °C, total precipitation of 162.4 and 214.1 mm respectively (Table 1). Some physical and chemical characteristics of the experimental soil were presented in Table 2.

Seeds were sown at 5-6 cm depths using a dibbler in 70x20 cm row space on 9th and 5th May in 2011 and 2012 years, respectively. Each plot area was 25.2 m^2 and consisted of 6 rows. The experiments were arranged according to a randomized complete block design with three replicates.

Table 1- Climatic data of the experimental area*

*Çizelge 1- Deneme alanının iklim verileri**

Climatic factors	*Years*	*Months*					*Total or average*
		April	*May*	*June*	*July*	*August*	
Precipitation (mm)	2011	54.7	43.1	62.2	1.8	0.6	162.4
	2012	53.2	107.4	18.1	0.8	34.6	214.1
	Long term	56.6	50.8	28.4	18.4	0.8	155.0
Average Temperature (^{0}C)	2011	10.2	14.1	19.5	24.7	24.0	18.5
	2012	10.8	14.5	22.5	25.4	22.8	19.2
	Long term	10.8	15.6	20.1	22.3	23.9	18.5
Relative humudity (%)	2011	70.0	68.0	59.0	44.0	40.0	56.2
	2012	57.0	66.0	46.0	42.0	43.0	50.8
	Long term	64.2	50.3	53.0	45.8	44.5	51.5

*, records of the Isparta Meteorology Station

Table 2- Some physical and chemical characteristics of the experimental soil

Çizelge 2- Deneme toprağının bazı fiziksel ve kimyasal özellikleri

Depth cm	*ρ_b g cm^{-3}*	*FC θ_{fc}*	*WP θ_{pwp}*	*pH*	*EC dS m^{-1}*	*$CaCO_3$ g kg^{-1}*	*OM g kg^{-1}*	*K cmol kg^{-1}*	*Texture*
0-30	1.12	23.9	13.5	7.8	0.378	292	16.9	1.48	CL
30-60	1.18	24.7	14.6	7.8	0.381	221	12.8	1.13	CL
60-90	1.20	26.8	15.6	7.9	0.404	309	14.3	0.70	SiCL

ρ_b, soil bulk density; FC, field capacity; WP, wilting point; OM, organic matter; SiCL, silty-clay loam; CL, clay-loam

200 kg ha^{-1} N, 100 kg ha^{-1} P and 100 kg ha^{-1} K were applied to the rows in the form of ammonium sulphate, triple super phosphate and potassium chloride, respectively. The total quantity of phosphorus and potassium was applied at the time of sowing and nitrogen was applied in three equal amounts at the time of sowing, 10 cm seedling height and 35-40 cm height stages.

2.2. Irrigation

The irrigations were made on 29 June and 11 August in the 2011, and for 2012 year were made on 13 June and 9 August. The plants had been watered 7 and 8 times with 7 day intervals, respectively in both 2011 and 2012 (Table 3).

The 16 mm diameter lateral pipes with carrying 2 L h^{-1} of water had inline drippers located at 33 cm intervals. Soil water contents were measured by the gravimetric method in 30 cm increments to a depth of 90 cm in each plot at planting, before irrigations, and at the final harvesting date.

After sowing, the irrigation was watered using a sprinkler irrigation system at the beginning for uniform plant establishment. In this stage, irrigation was carried out two times. After the emergence of maize seedlings, subsequent irrigations were applied according to the prescribed irrigation rates. In study, irrigation treatments were consisted to according to five different deficit rates of available soil water before irrigation (1- $I_{100:}$ full irrigation and K_1: 1.00, 2- I_{85}: 85% of full irrigation and K_2: 0.85, 3- I_{70}: 70% of full irrigation and K_3: 0.70, 4- I_{55}: 55% of full irrigation and K_4: 0. 55 and 5- I_{40}: 40% of full irrigation and K_5: 0.40). Levels of irrigation water for treatments were computed using Equation 1.

$$I_r = W_{sd} \times K \quad (1)$$

Where; I_r, the irrigation water (mm); W_{sd}, soil water deficit in the irrigation before (mm); and K, the rate of water cuts.

The plant water consumption (Et) was estimated using Equation 2 (James 1988).

$$E_t = I_r + P + C_r - D_p - R_f \pm \Delta s \quad (2)$$

Where; E_t, plant water consumption (mm); I_r, irrigation water (mm); P, the precipitation (mm); C_r, the capillary rise (mm); D_p, the deep percolation losses (mm); R_f, the runoff losses (mm); and Δs, the moisture storage in soil profile (mm).

2.3. Analysis of mineral nutrient contents

When the kernel moisture was about 72% (Olsen et al 1990), five ears in the center of each plot were harvested manually. The cobs were immediately frozen by liquid nitrogen to prevent changing from sugar to starch. Later, grains were removed from ear by using cutter, and the samples were dried in an air-forced oven at 70 ^{0}C until became stable weight. Then dried samples were ground to pass through a 1-mm screen. Mineral nutrient contents including nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), manganese (Mn), zinc (Zn) and boron (B) were determined. N content was determined by using Kjeldahl method. Phosphorus content

Table 3- Seasonal irrigation water, rainfall, total water and evapotranspiration (ET) of sweet corn under different irrigation levels

Çizelge 3- Farklı sulama seviyelerinde şeker mısırın sulama suyu, yağış, toplam su ve buharlaşma miktarları

Irrigation levels	*Applied irrigation water (mm)*		*Rainfall (mm)*		*Total water (mm)*		*ET (mm)*	
(mm)	*2011*	*2012*	*2011*	*2012*	*2011*	*2012*	*2011*	*2012*
I_{40}	151.93	181.34	102.30	160.70	254.23	342.04	239.79	406.12
I_{55}	180.78	223.50	102.30	160.70	283.08	384.20	261.50	426.12
I_{70}	209.63	265.67	102.30	160.70	311.93	426.37	290.35	447.91
I_{85}	238.48	307.65	102.30	160.70	340.78	468.35	319.20	476.06
I_{100}	267.33	350.00	102.30	160.70	369.63	510.70	348.04	504.01

was determined according to the Molibdovanado-Phosphoric Acid Method (Kacar & İnal 2008). K content was measured by Flame Emission Spectrophotetry. Ca, Mg, Fe, Cu, Mn, Zn and B contents were measured with an Atomic Absorbtion Spectrofotometer.

2.4. Statistical analysis

All the data were analyzed according to the analysis of variance (ANOVA) using SAS Statistical Package Program; the significant differences between the group means were separated using the LSD (Least Significant Difference) test.

3. Results and Discussion

The effects of the different irrigation levels on the mineral nutrient contents, except for Ca and Mg in 2011, of sweet corn were found to be significant ($P<0.05$ and $P<0.01$) for both years. No significant differences between the two subsequent years in all mineral nutrient contents were found (Table 4).

Table 4- Effect on mineral nutrient contents of sweet corn of deficit irrigation

Çizelge 4- Kısıtlı sulamanın şeker mısırın mineral besin içeriğine etkisi

Irrigation	*N (%)*		*P (%)*		*K (%)*		*Ca (%)*	
levels (mm)	*2011*	*2012*	*2011*	*2012*	*2011*	*2012*	*2011*	*2012*
I_{40}	1.90 c	1.93 c	0.220 c	0.217 c	0.689 b	0.688 b	0.024	0.023 b
I_{55}	2.05 b	2.06 b	0.247 bc	0.265 b	0.744 ab	0.768 a	0.026	0.028 a
I_{70}	2.29 a	2.32 a	0.332 a	0.331 a	0.855 a	0.837 a	0.031	0.029 a
I_{85}	1.88 c	1.89 c	0.284 ab	0.287 b	0.823 ab	0.816 a	0.029	0.028 a
I_{100}	1.81 c	1.80 d	0.271 bc	0.273 b	0.814 ab	0.818 a	0.027	0.026 ab
Years	1.98	1.99	0.272	0.274	0.785	0.786	0.028	0.027
Lsd	0.118	0.057	0.055	0.027	0.150	0.075	ns	0.004
CV (%)	5.160	5.048	7.476	3.688	6.988	3.506	13.097	6.473
F value	57.42	84.01	13.00	49.75	4.50	14.34	1.85	6.65
P value	0.001	0.001	0.004	0.001	0.033	0.001	0.213	0.011

Irrigation	*Mg (%)*		*Fe (mg kg^{-1})*		*Cu (mg kg^{-1})*		*Mn (mg kg^{-1})*	
levels (mm)	*2011*	*2012*	*2011*	*2012*	*2011*	*2012*	*2011*	*2012*
I_{40}	0.104	0.103 b	20.95 b	20.88 b	3.10 b	3.09 c	6.57 b	6.65 c
I_{55}	0.117	0.117 ab	21.01 b	21.49 b	3.61 ab	3.68 b	7.38 b	7.36 c
I_{70}	0.123	0.132 a	27.27 a	26.12 a	3.99 a	4.12 a	10.92 a	11.68 a
I_{85}	0.118	0.119 ab	25.14 ab	25.78 a	3.89 ab	3.80 ab	10.86 a	10.59 a
I_{100}	0.120	0.121 ab	23.66 ab	23.98 ab	3.80 ab	3.73 ab	8.90 ab	9.06 b
Years	0.117	0.118	23.57	23.65	3.68	3.69	8.93	9.07
Lsd	ns	0.017	6.233	3.115	0.813	0.407	2.576	1.284
CV (%)	10.581	5.278	9.652	4.808	8.064	4.030	10.530	5.168
F value	1.05	8.04	4.08	13.37	4.17	19.12	13.28	61.05
P value	0.439	0.006	0.043	0.001	0.040	0.001	0.001	0.001

Irrigation	*Zn (mg kg^{-1})*		*B (mg kg^{-1})*	
levels (mm)	*2011*	*2012*	*2011*	*2012*
I_{40}	24.19 c	24.46 b	5.389 a	5.306 a
I_{55}	25.27 c	26.99 ab	3.761 b	3.528 b
I_{70}	29.70 b	28.55 ab	3.543 b	3.527 b
I_{85}	34.77 a	30.14 a	3.223 bc	3.126 c
I_{100}	28.39 bc	28.45 ab	2.483 c	2.267 d
Years	28.5	27.7	3.680	3.551
Lsd	4.344	4.122	0.741	0.370
CV (%)	5.570	5.427	7.351	3.808
F value	20.80	6.04	47.00	201.32
P value	0.003	0.015	0.001	0.001

Means in the same columns followed by the same letters are not significantly different at the 0.05 and 0.01 level as statistically. I_{100}, full irrigation; I_{85}, 85% of full irrigation; I_{70}, 70% of full irrigation; I_{55}, 55% of full irrigation and I_{40}, 40% of full irrigation

In both subsequent years (2011 and 2012) the "I_{70}" irrigation level resulted in the highest N content (2.29 and 2.32%, respectively), the highest P content (0.332 and 0.331%, respectively), the highest K content (0.855 and 0.837%, respectively), the highest Ca content (0.031 and 0.029%, respectively), the highest Mg content (0.123 and 0.132%, respectively), the highest Fe amount (27.27 and 26.12 mg kg^{-1}, respectively), the highest Cu amount (3.99 and 4.12 mg kg^{-1}, respectively) and the highest Mn amount (10.92 and 11.68 mg kg^{-1}, respectively) (Table 4). The highest Zn amount (34.77 and 30.14 mg kg^{-1}, respectively) and the highest B amount (5.389 and 5.306 mg kg^{-1}, respectively) were obtained from I_{85} and I_{40} irrigation levels, respectively, in both years (Table 4).

While the lowest N content (1.81 and 1.80%, respectively) and the lowest B content (2.483 and 2.267 mg kg^{-1}, respectively) were obtained from I_{100} irrigation level in both years, the others mineral nutrient contents of sweet corn decreased in the lowest irrigation level in both 2011 and 2012: the lowest P content (0.220 and 0.217%, respectively), the lowest K content (0.689 and 0.688%, respectively), the lowest Ca content (0.024 and 0.023%, respectively), the lowest Mg content (0.104 and 0.103%, respectively), the lowest Fe amount (20.95 and 20.88 mg kg^{-1}, respectively), the lowest Cu amount (3.10 and 3.09 mg kg^{-1}, respectively), the lowest Mn amount (6.57 and 6.65 mg kg^{-1}, respectively) and the lowest Zn amount (24.19 and 24.46 mg kg^{-1}, respectively) were obtained from the "I_{40}" irrigation level (Table 4).

N content was significantly increased with the water deficit up to "I_{70}". N content was decreased in the lower water levels (I_{55} and I_{40}) than "I_{70}", however the lowest N content was determined in "I_{100}" irrigation level in both 2011 and 2012. This can be explained by nitrogen decrease in the root zone due to nitrogen washing at the high irrigation levels (I_{85} and I_{100}). On the other hand, plants can not sufficiently benefit from nitrogen in the upper soil layers due to low water at the lower irrigation levels (I_{55} and I_{40}). Adrienn & Janos (2012) reported that irrigation up to a certain level significantly increased the nitrogen content of maize hybrids. Asghari & Hanson (1984) determined that the nitrogen content of corn was affected from the quantity and distribution of precipitation-irrigation-in growing period. Barber & Jessop (1987) found that increasing the number of irrigations up to three reduced grain nitrogen content. Oury et al (2003) stated that there is a negative effect of irrigation on grain nitrogen concentration. This can be explained by the strong association between the amount of minerals transported by the water flow in the plant, and transpiration (Misra et al 2006).

Water deficit up to "I_{70}" irrigation resulted in increased P, K, Ca, Mg, Fe, Cu and Mn content. These minerals in the "I_{100}" and "I_{85}" irrigation levels were higher than "I_{55}" and "I_{40}". Drought stress caused the concentration of P, K, Ca, Mg, Fe, Cu and Mn decreases in sweet corn grain. The lowest mineral contents (P, K, Ca, Mg, Fe, Cu and Mn) were obtained from the "I_{40}" irrigation level, the lowest irrigation level in both 2011 and 2012. Zn content was increased with the increasing irrigation water up to "I_{85}". The lowest Zn content was obtained from the "I_{40}" irrigation level, the lowest irrigation level in both 2011 and 2012. Yazar et al (2002) also reported the highest average corn grain yield from the full irrigation treatment with 6-day irrigation interval. Yıldırım & Kodal (1998) were stated that applications of excessive water were not increased grain yields at the important level. Limited water supply during the growing season results in soil and plant water deficits and reduces maize yields (Patel et al 2006). Water deficit delays physiological processes of corn, tasseling initiation and silking, and reduces plant height and vegetation growth of maize as a result decrease grain yields (Singh et al 2007; Payero et al 2009).

Especially, in the dripping irrigation system, salts in root zone and the irrigation water accumulate at edge of wetted front (Ertek & Kanber 2002). Therefore, in the higher irrigation levels (I_{100} and I_{85}) than "I_{70}" might be decreased to nutrient uptake of maize due to edge of wetted front by washed of plant nutrient elements. On the other hand, in the lower irrigation levels (I_{55}

and I_{40}) than "I_{70}" might be fall to nutrient uptake of maize due to insufficient dissolution of plant nutrients. Under water stress macro-micronutrients decrease due to increased remobilization of stored assimilates. Rezaei et al (2012) found that the Zn concentration of grains increased with decreasing water availability, and micronutrients such as Fe, Zn and Cu in shoots decreased as water supply limited but Mn concentration of grain and straw was not or only little affected by water stress. Andrade et al (2005) found that unfavorable water supply of corn, the speed and duration of dry matter accumulation decrease. Rouphael et al (2008) reported that under water stress conditions, higher concentration of K and Mg were observed. Some researchers stated that the high irrigation levels resulted in decreased mineral nutrient contents (Simonne et al 1998; Kirnak et al 2001; Kaya et al 2003; Rouphael et al 2008). B content was decreased with the increasing irrigation water (I_{55}, I_{70}, I_{85} and I_{100}). The highest B content was determined in "I_{40}" irrigation level, the lowest irrigation level; the lowest B content was obtained from "I_{100}" level, the highest irrigation level in both 2011 and 2012. There is close relationship between boron uptake of plants and its transport at different plant organs with water intake of plant (Marschner 1976). Therefore, it is possible to say that B content of sweet corn increased with lower irrigation levels. Zubaidi et al (1999) reported that plants were marginally deficient in N P and Zn, but boron concentration was high under drought stress condition.

4. Conclusions

The results obtained from present study indicated that irrigation levels (I_{40}, I_{55}, I_{70}, I_{85} and I_{100}) had significant effects on mineral nutrient contents (N, P, K, Ca, Mg, Fe, Zn, Mn, Zn and B) of sweet corn. The mineral nutrient contents were increased with water deficit up to a certain level "I_{70}". The lower irrigation levels (I_{55} and I_{40}) than "I_{70}" resulted in decreased mineral nutrient contents, except for B, of sweet corn. Based on the results of the research, it is possible to say that mineral nutrient contents of sweet corn increased with water deficit up to "I_{70}". "I_{70}" irrigation application for sweet corn can be accepted as the optimum water level due to higher nutrient content.

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This study is a part of research belonging to effects on performance of sweet corn of deficit irrigation.

References

Adrienn V S & Janos N (2012). Effects of nutrition and water supply on the yield and grain protein content of maize hybrids. *Australian Journal of Crop Science* **6**(3): 381-390

Andrade F H, Sadras V O, Vega C R C & Echarte L (2005). Physiological determinants of crop growth and yield in maize, sunflower and soybean: Their application to crop management, modeling and breeding. *Journal of Crop Improvement* **14**: 51–101

Asghari M & Hanson R G (1984). Climate, management and nitrogen effect on corn leaf nitrogen, yield and grain nitrogen. *Agronomy Journal* **76**: 911-916

Barber J S & Jessop R S (1987). Factors affecting yield and quality in irrigated wheat. *Journal of Agriculture Science Cambridge* **109**: 19-26

Ertek A & Kanber R (2002). Effects on salt distribution in soil to irrigate cotton by drip irrigation. *Journal Agriculture Science* **12**(2): 21-31

Eryüce N & Kılıç C C (2001). A review on potassium and stress relations in plants. Regional Workshop on: Potassium and water management in West Asia and North Africa. Amman, Jordan. Ed: A.E. Johnston, IPI Bern. Switzerland, pp. 103-110

House L R (1985). A Guide to Sorghum Breeding. International Crops Research Institute for Semiarid Tropics. Patancheru, India, pp. 344-502

James L G (1988). Principles of Farm Irrigation System Design. Wiley, New York

John B M & Jeanne R W (2012). Human Health, the Nutritional Quality of Harvested Food and Sustainable Farming Systems. http://www.nutritionsecurity.org/PDF/NSI_White%.pdf (accessed September 2012)

Kacar B & İnal A (2008). Bitki Analizleri. Nobel Yayın No: 1241, Fen Bilimleri Yayın No: 63, Ankara

Kaya C, Higgs D, Kirnak H & Tas I (2003). Mycorrhizal colonization improves fruit yield and water use efficiency in watermelon (*Citrullus lanatus* Thunb.)

grown under wellwatered and water-stressed conditions. *Plant Soil* **253**: 287–292

Kirnak H, Cengiz K, Davi H & Sinan G (2001). A long term experiment to study the role of mulches in physiology and macro-nutrition in strawberry grown under water stress. *Australian Journal Agriculture Research* **52**: 937–943

Marschner H (1976). Mineral metabolism, short and long distance transport. *Fortschr Botany* **38**: 71-80

Misra S C, Randive R, Rao V S, Sheshshayee M S, Serraj R & Monneveux P (2006). Relationship between carbon isotope discrimination, ash content and grain yield in wheat in the Peninsular Zone of India. *Journal of Agronomy and Crop Science* **192**: 352-362

Murchie E H, Yang J, Hubbart S, Horton P & Peng S (2002). Are there associations between grain-filling rate and photosynthesis in the flag leaves of field-grown rice? *Journal of Experimental Botany* **53**(378): 2217-2224

Nagy J (2009). Effect of sowing date on the yield and quality of maize hybrids with different growing seasons. *Acta Agronomy Hungarica* **57**: 389-399

Olsen J K, Giles J E & Jordan R A (1990). Post-harvest carbohydrate changes and sensory quality of three sweet corn cultivars. *Scientia Horticulture* **44**: 179-189

Oury F X, Berard P, Brancourt H M, Depatureaux C, Doussinault G, Galic N, Giraud A, Heumez E, Lecomte C, Pluchard P, Rolland B, Rousset M & Trottet M (2003). Yield and grain protein concentration in bread wheat: a review and a study of multi-annual data from a French breeding program. *Journal of Genetics and Breeding* **57**: 59-68

Patel J B, Patel V J & Patel J R (2006). Influence of different methods of irrigation and nitrogen levels on crop growth rate and yield of maize (*Zea mays* L.). *Indian Journal Crop Science* **1**(1-2): 175-177

Payero J O, Tarkalson D D, Irmak S, Davison D & Petersen J L (2009). Effect of timing of a deficit-irrigation allocation on corn evapotranspiration, yield, water use efficiency and dry mass. *Agricultural Water Management* **96**: 1387-1397

Rezaei M, Mohammadi S & Eivazi A (2012). Effect of different levels of irrigation on yield and micronutrients uptake by bread wheat genotypes**.** *Research on Crops* **13**(2): 432-438

Rouphael Y, Cardarelli M, Rea E & Colla G (2008). Grafting of cucumber as a means to minimize copper toxicity. *Environmental and Experimental Botany* **63**: 49–58

Simonne E H, Joseph D E & Harris C E (1998). Effects of irrigation and nitrogen rates on foliar mineral composition of bell pepper. *Journal of Plant Nutrition* **21**: 2545–2555

Singh A K, Roy A K & Kaur D P (2007). Effect of irrigation and NPK on nutrient uptake pattern and qualitative parameter in winter maize-potato intercropping system. *International Journal of Agriculture Science* **3**(1): 199-201

Szirtes V, Pongor S & Penczi E (1977). Effect of trace element fertilization on protein yield and lysine ration in maize. *Növenytermeles* **26**: 49-59

Yazar A, Sezen S M & Gencel B (2002). Drip irrigation of corn in the Southeast Anatolia Project (GAP) area in Turkey. *Irrigation and Drainage* **51**: 293-300

Yıldırım Y E & Kodal S (1998). Effect of irrigation water on corn grain yield in Ankara conditions. *Turkish Journal of Agriculture and Forestry* **22**: 65-70

Yin X, Schapendonk A D, Kropff M J, Van O M & Bindraban P S (2000). Generic equation for nitrogen-limited leaf area index and its application in crop growth models for predicting leaf senescence. *Annals of Botany* **85**: 579-585

Zubaidi A, McDonald G K & Hollamby G J (1999). Nutrient uptake and distribution by bread and durum wheat under drought conditions in South Australia. *Austrian Journal Plant Physiology* **39**: 721-732

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The Effect of Sward Structure and N Fertilization on the Grass-legume Silage Quality

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ABSTRACT

The effect of sward structure of the mixtures and nitrogen fertilization on grass-legume silage quality was investigated over two years. The study included Lucerne (*Medicago sativa*) in monoculture and in mixtures with cocksfoot (*Dactylis glomerata*), tall fescue (*Festuca arundinacea*) and sainfoin (*Onobrychis sativa*). Nitrogen fertilizer was applied to the field plots at four different rates: 0, 70, 140 and 210 kg ha^{-1}. The quality of lucerne silage was inferior to lucerne silage mixed with grasses due to the greater content of ammonia nitrogen (NH_3-N), acetic (AA) and butyric acids (BA), and reduced content of the lactic acid (LA). Gradual increase of N fertilization significantly has increased the content of NH_3-N, AA and BA and decreased the content of LA.

Keywords: Grass-legume mixture; Silage quality; Sward mixtures; Fertilization

Çim Yapısı ve N Gübrelemesinin Çim-Baklagil Silaj Kalitesine Etkisi

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ÖZET

Silaj karışımındaki çim yapısı ve N gübrelemesinin silaj kalitesine etkisi 2 yıllık bir çalışma ile araştırılmıştır. Çalışmada monokültür olarak adi yonca (*Medicago sativa*) ve karıım olarak da kamışsı yumak (*Festuca arundinacea*) domuz ayrığı (*Dactylis glomerata*) ve korunga (*Onobrychis sativa*) bulundurulmuştur. Azotlu gübre 0, 70, 140 ve 210 kg ha^{-1} dozlarında uygulanmıştır. Yüksek düzeyde amonyak azotu (NH_3-N), asetik asit (AA), butirik asit (BA) ve daha düşük laktik asit (LA) içeriği nedeniyle adi yonca silajının kalitesi, adi yonca ile çim karışımı silajlarından düşük olmuştur. Artan miktarda N uygulamaları NH_3-N, AA ve BA miktarında önemli düzeyde artışa LA miktarında ise önemli düzeyde azalışa neden olmuştur.

Anahtar Kelimeler: Çim-baklagil karışımı; Silaj kalitesi; Çim karışımı; Gübreleme

1. Introduction

Silage is animal feed rich in essential nutrients, resulting from fermentation of the chopped mass of different plant species by lactic acid bacteria fermentation in a given time period. The main substrates essential for increasing of lactic acid bacteria in number are sugars. These bacteria are one of the most important determinants for successful fermentation and production of high quality silage. Contrary to grasses that typically contain sufficient amounts of fermentable sugars, legumes are poor in sugars, which makes them difficult to ensile. This can be overcome by mixing legumes and grasses, which increases the sugar content in the silo mass, thus allowing ensiling of legumes. Therefore, the combined cultivation of legumes and grasses is very useful even it has some difficulties. However, there are many factors that directly or indirectly affect the fermentable characteristics of silage crops. For instance, different types of plants, and even different plant varieties, are characterized by different contents of easily fermentable sugars and fermentative properties, producing ensiled material of differing qualities. Weissbach (2003) argues that *Lolium* species have a high sugar content, so that these species may be added to the silo material of low fermentable value. Cocksfoot silage and mixtures of cocksfoot with lucerne have better quality characteristics than that of pure lucerne silage. Han et al (2006) have shown that silage made from cocksfoot and its mixtures with lucerne have a higher content of fermentable sugars compared to lucerne silage in two cuts. Testing a mixture of red clover with perennial (*Lolium perenne*) and annual ryegrass (*Lolium multiflorum*) and cocksfoot (*Dactylis glomerata*), Wyss (2004), concluded that ryegrasses had positive, and cocksfoot had negative impacts on the silage quality. Also, the same author Wyss (2006), comparing the fermentability of three legumes proved that white and red clover have higher fermentable coefficient than lucerne. Applied cultivation techniques and practices influence the fermentable properties of crops, and the content of water soluble sugars. N fertilization is one of the most common agricultural measures used to increase crop yields; however, it often has degrading effects on the fermentation process and silage quality. The negative effects of nitrogen are reflected through: increasing in the buffer capacity, the content of non-protein nitrogen, readily soluble nitrogen and NH_3-N (Tremblay et al 2005), reduction of fermentable sugar content in the silo material, reduction of the digestibility of silage (Keady et al 2000), as well as greater presence of harmful microorganisms and the production of mycotoxins (Bijelić 2009). In order to balance between high yield and good silage quality, it is necessary to find the levels of nitrogen that are required to meet these two objectives.

Therefore, the aim of this study was to evaluate the effect of botanical composition of the lucerne-grass mixtures and fertilization with different amounts of nitrogen on the herbage silage quality of lucerne and it mixtures with different grasses and legume.

2. Material and Methods

2.1. Field and laboratory experiment

This study was conducted at the Institute for Animal Husbandry, Belgrade, Serbia (44° 49′ 10″ N, 20° 18′ 45″ E) during 2010-2011. The study was organized as a randomized complete block design with four replications (4x4). In each replication, lucerne (*Medicago sativa*) was sown in monoculture (L) and in mixtures with grasses (*Dactylis glomerata* and *Festuca arundinacea*) and sainfoin (*Onobrychis sativa*) [LC-lucerne, cocksfoot (50:50); LCT-lucerne, cocksfoot, tall fescue (33.3:33.3:33.3); LCTS-lucerne, cocksfoot, tall fescue, sainfoin (25:25:25:25)] in field plots of 10 m^2. N fertilizer was applied to the field plots at four different rates: 0, 70, 140 and 210 kg ha^{-1}. Half of the N fertilizer was applied at the beginning of the growing season and the other half after the first cut. Prior to cutting, samples of plant material were collected from 1 m^2 areas from all plots for analysis of botanical composition. Plants were cut at the early flowering stage of lucerne.

Silage was prepared from the second cut. After 24 h wilting, the silage mass was chopped, and ensiled in 64 laboratory silos of 10 L volume (containers).

To encourage better fermentation, bacterial-enzyme inoculant was used in the amount of 10 g+2 L H_2O t^{-1} of fresh herbage. The silo containers were closed immediately after filling. After 90 days silages were opened and samples were taken for chemical analyses.

2.2. Determination of silage quality

After the fermentation period, laboratory silos were opened and two samples of silage material were taken from each silo for chemical analysis. Dry matter content was determined by drying the samples at 105°C overnight. Crude protein content was determined according to Kjeldahl (AOAC 1990). Lactic acid (LA) and volatile fatty acids [acetic (AA) and butyric acid (BA)] were quantified by a gas chromatographic system (GC-2014, Shimadzu, Kyoto, Japan) equipped with flame-ionization detector and auto sampler and injection system, using a Nukol™ (30 m×0.53 mm×0.5 µm) capillary column (Supelco, Sigma-Aldrich Co.) (Faithfull 2002). Ammonia nitrogen was determined using the distillation method using a Kjeltec 1026 analyser and the pH value was measured with a Hanna Instruments HI 83141 pH meter. Silage quality class was determined by the Flieg score (Kılıç 1986). Flieg score= [220+(2×silage dry matter (%)-15)]-40×silage pH value. Flieg Score values between 85 and 100 denote very good quality silage; between 60 and 80, good quality silage.

2.3. Statistical analysis

The data obtained were analysed using two-factorial analysis of variance (ANOVA) and the mean differences were tested with Fisher's Least Significant Difference (LSD) test (StatSoft 2007).

3. Results and Discussion

3.1. Impact of sward plant mixture

The structure of lucerne mixtures during the research changed (Table 1), depending on the sward maturity, competitive ability of species, resistance to various climatic conditions and their response to applied management practices.

Table 1- Botanical composition of grass-legume mixtures prior to cutting

Çizelge 1- Çim baklagil karışımlarının biçim öncesi botanik kompozisyonları

	2010			*2011*		
	L	*G*	*W*	*L*	*G*	*W*
Mixture (M)						
LC	61.0	37.9	1.1	57.7	31.2	2.2
LCT	64.7	34.7	0.6	64.0	34.6	1.4
LCTS	70.2	28.8	1.0	59.7	38.6	1.7

L, legumes; G, grasses; W, weeds

The grass-legume mixtures had no significant impact on the content of crude protein, pH or Flieg score of the resultant silages. Highly significant differences occurred in the content of NH_3-N, lactic, acetic and butyric acid (Table 2). One of the most important properties for sufficient fermentation in grasses and legumes is content of dry matter. By increasing the DM content, good silages can be made from legumes and grasses even with low acidification potential. However, DM content should be from 400-450 g kg^{-1}, everything beyond that should be avoided (Weissbach 2003). In our research, DM content was higher in grass-legume silages than in lucerne silages. Obtained values did not exceed the value of 450 g kg^{-1}, but in the second year of the study were slightly lower (Table 3). Lucerne silage had a significantly higher content of NH_3-N compared to the other silages. The content of NH_3-N in other silages ranged from 109.9 g kg^{-1} TN in the lucerne and cocksfoot mixture, to 120.5 g kg^{-1} TN in mixtures with sainfoin. The content of LA was the highest in the silage of lucerne and cocksfoot mixture (31.5 g kg^{-1} DM) and the lowest in pure lucerne silage (24.3 g kg^{-1} DM). Lucerne silage had a significantly higher content of AA and BA compared to the other silages.

The sward mixture had significant impact on the contents of NH_3-N and lactic acid in the silages, in the second year of study. Lucerne silage had the highest content of NH_3-N (115.4 g kg^{-1} TN) which was significantly higher than that of the mixture of

Table 2- Impact of sward structure on grass-legume silage quality in 2010

Çizelge 2- 2010 yılında çim-baklagil karışımında çim yapısının silaj kalitesi üzerine etkisi

Mixture (M)	*LC*	*LCT*	*LCTS*	*L*	*Level of significance*
					M
DM (g kg^{-1})	415.4	416.9	429.3	360.8	*
CP (g kg^{-1} DM)	167.1	168.0	173.0	165.1	ns
NH_3-N (g kg^{-1} TN)	112.8	109.9	120.5	123.5	**
LA (g kg^{-1} DM)	31.5	26.6	27.3	24.3	**
AA (g kg^{-1} DM)	15.0	18.6	14.9	19.7	**
BA (g kg^{-1} DM)	0.00	0.04	0.00	0.07	**
pH	4.8	4.8	4.8	4.8	ns
Flieg score	95.9	95.7	97.7	90.7	ns

DM, dry matter; CP, crude protein; TN, total nitrogen; LA, lactic acid; AA, acetic acid; BA, butyric acid; ns, non significant; *, P≤0.05; **, P≤0.01

lucerne with cocksfoot and lucerne with cocksfoot and tall fescue. The content of LA, as in the first year of the study, was the highest in lucerne–cocksfoot mixture silage and lowest in the pure lucerne, respectively. The content of BA was higher than in the first year of the study, while the pH was lower, what is the probably related to lower DM content. All silages, as in the previous year, were scored as very good quality (Table 3).

In the research of Heikkilä et al (1992), the addition of legumes to the plant mixture reduced the content of NH_3-N in silage. Therefore, silage obtained from the mixture of meadow fescue and timothy had a higher content of NH_3-N than grass-clover silage (48 g kg^{-1} TN and 36 g kg^{-1} TN, respectively). However, in research by Orozco-Hernández et al (1997), lucerne silage had a significantly higher content of NH_3-N, from 59 and 216 g kg^{-1} TN in relation to timothy silage of 46 and cocksfoot of 172 g kg^{-1} TN. According to Lättemäe & Tamm (2002) the content of NH_3-N in silage from mixed swards increases if the legumes ratio in the mixture increases. The physiological characteristics of legumes, the fact that they contain more N substances, and grasses contain more soluble sugars, can explain the significant differences in acid contents between lucerne silage and silage of its mixtures (Wilman & Wright 1983). In fact,

Table 3- Impact of sward mixture on grass-legume silage quality in 2011

Çizelge 3- 2011 yılında çim-baklagil karışımında çim yapısının silaj kalitesi üzerine etkisi

Mixture (M)	*LC*	*LCT*	*LCTS*	*L*	*Level of significance*
					M
DM (g kg^{-1})	317.6	320.1	340.7	311.3	ns
CP (g kg^{-1} DM)	156.9	154.9	172.5	174.6	*
NH_3-N (g kg^{-1} TN)	91.3	104.8	113.6	115.4	**
LA (g kg^{-1} DM)	31.4	27.4	27.6	25.8	**
AA (g kg^{-1} DM)	10.3	11.4	11.8	12.7	ns
BA (g kg^{-1} DM)	0.01	0.01	0.02	0.09	ns
pH	4.6	4.6	4.6	4.6	ns
Flieg score	85.2	85.2	89.8	84.2	ns

DM, dry matter; CP, crude protein; TN, total nitrogen; LA, lactic acid; AA, acetic acid; BA, butyric acid; ns, non significant; *, P≤0.05; **, P≤0.01

Heikkilä et al (1992), comparing the quality of silage of clover-grass mixtures with grass mixtures, concluded that clover-grass mixtures contained more CP and less crude fibre and lactic acid content. In this study, a mixture of grass and lucerne yielded better quality silage compared to pure lucerne. Also a mixture of lucerne and grasses with sainfoin had better silage performance than pure lucerne, which is consistent with the results of Wang et al (2007), who claim that incorporation of sainfoin into lucerne forage improve fermentation.

3.2. Impact of nitrogen fertilization and its interaction with sward mixtures on silage quality

N fertilization had a significant impact ($P<0.01$) on the content of NH_3-N, LA and BA. The control silage had a significantly lower content of NH_3-N in comparison to the other silages, whereas the silage fertilized with 210 kg N ha^{-1} had significantly higher NH_3-N content compared to the silages which received less N (Table 4). Keady & Kiely (1998) concluded that N fertilization has a detrimental effect on the quality of silage, because it increases the level of protein degradation, and the content of NH_3-N. In a study of the silage quality of timothy fertilized with 0, 60, 120 and 180 kg N ha^{-1}, Tremblay et al (2005) concluded that the NH_3-N content increased under the influence of the fertilization by 0.85 times.

In well-fermented clover - grass silages, the LA content ranges from 2.28-3.90%, the AA content up to 5.5% and BA from 0-0.12 g kg^{-1} DM (Djordjević & Dinić 2003). In relation to this statement, the LA content in the silages produced in the current study was satisfactory. The highest LA content occurred in the control and silage fertilized with 70 kg N ha^{-1}, and the lowest in silage fertilized with 210 kg N ha^{-1}. The BA content was relatively low (Table 4). According to Weissbach (2003), this could be explained by the fact that crop fertilized with N always contains some nitrate. That nitrate is partly reduced to nitrite in the silo which protects silage from butyric acid fermentation. Interaction of investigated factors by increasing share of legumes in the mixture and addition of N fertilizer significantly increased the concentration of ammonia nitrogen and acetic acid and decreased the concentration of lactic acid.

In the second year, fertilization led to significant changes ($P<0.01$) in NH_3-N content, and the contents of LA and AA (Table 5). The content of NH_3-N was significantly higher in silages fertilized with 210 kg N ha^{-1} (129.4 g kg^{-1} TN) compared to the other silages. Silages from the control and from sward fertilized with 70 kg N ha^{-1} contained below 100 g kg^{-1} TN of NH_3-N, which is characteristic of high-quality silage (Haigh & Parker 1985) LA and AA contents in silage, influenced by N-fertilization, were affected differently. Gradual increase of N fertilization has increased the content of acetic acid and decreased the content of lactic acid in silage. This is probably associated with the reduction of

Table 4- Impact of N fertilization on grass-legume silage quality in 2010

Çizelge 4- 2010 yılında N gübrelemesinin çim-baklagil karışımında silaj kalitesi üzerine etkisi

N fertilization (N)	*0*	*70*	*140*	*210*	*Level of significance*	
	kg N ha^{-1}				*N*	*Interaction MxN*
DM (g kg^{-1})	411.5	370.9	422.4	417.5	ns	ns
CP (g kg^{-1} DM)	174.2	169.6	172.3	171.2	ns	ns
NH_3-N (g kg^{-1} TN)	95.0	116.2	114.8	140.6	**	**
LA (g kg^{-1} DM)	30.0	30.8	25.1	23.8	**	**
AA (g kg^{-1} DM)	16.7	16.6	17.4	17.5	ns	**
BA (g kg^{-1} DM)	0.00	0.01	0.10	0.00	**	**
pH	4.8	4.8	4.7	4.9	ns	ns
Flieg score	96.1	92.2	100.5	91.1	ns	ns

DM, dry matter; CP, crude protein; TN, total nitrogen; LA, lactic acid; AA, acetic acid; BA, butyric acid; ns, non significant; *, $P\leq0.05$; **, $P\leq0.01$

Table 5- Impact of N fertilization on grass-legume silage quality in 2011

Çizelge 5- 2011 yılında N gübrelemesinin çim-baklagil karışımında silaj kalitesi üzerine etkisi

N fertilization (N)	*0*	*70*	*140*	*210*	*Level of significance*	
	kg N ha^{-1}				*N*	*Interaction MxN*
DM (g kg^{-1})	323.3	317.8	312.0	336.6	ns	ns
CP (g kg^{-1} DM)	153.1	160.4	170.4	174.9	ns	ns
NH_3-N (g kg^{-1} TN)	86.5	97.7	114.5	129.4	**	**
LA (g kg^{-1} DM)	32.0	28.6	25.1	25.2	**	**
AA (g kg^{-1} DM)	10.1	9.6	12.8	11.8	**	**
BA (g kg^{-1} DM)	0.00	0.01	0.03	0.09	ns	ns
pH	4.6	4.5	4.6	4.6	ns	ns
Flieg score	84.1	89.2	82.3	88.9	ns	ns

DM, dry matter; CP, crude protein; TN, total nitrogen; LA, lactic acid; AA, acetic acid; BA, butyric acid; ns, non significant; *, P≤0.05; **, P≤0.01

herbage water soluble carbohydrates in nitrogen treatments (WSC) pre-ensiling (King et al 2013) and according to Keady et al (2000) lactic acid concentration is positively correlated with herbage WSC. The pH of the silages was higher in the first year of the study, which is more advantageous from the viewpoint of quality.

The results of the present study indicate that nitrogen fertilization had no effect on the pH value. The authors, Keady & O´Kiely (1996), in their research report that N fertilization increased the pH value of the silage probably as a result of an increase of ammonia nitrogen content, which is of an alkali nature. In the present study, the lack of effect of N fertilization on the pH of silage, according to the Keady et al (2000) could be explained by an increase in the crude protein content fraction of the herbage at ensiling with increasing N fertilizer rate (Bijelić et al 2014). In assessing the quality of silage according to Flieg, taking into account the dry matter content and pH, silages were shown to be influenced by fertilization treatment better in the first than in the second year of the study.

The amount of NH_3-N in pure lucerne silage, mixtures thereof and from different fertilization treatments, is usually above the established values of 100 g kg^{-1} TN for high-quality silages. Considering this fact, in the evaluation of the silage quality, it would be beneficial to use a score that includes this parameter of quality, especially when it comes to ensiling plants with high protein content such as the mixtures included in the present study.

The interaction of N-fertilization rates and sward content in both study years had a highly significant impact (P≤0.01) on NH_3-N and volatile fatty acid contents. Lucerne and mixture silages with a higher share of legumes had higher contents of NH_3-N, AA and BA, and lower contents of LA if fertilized with high rates of N, which is in accordance with the results of King et al (2013).

4. Conclusions

Flieg quality silage score showed no significant difference in the quality of lucerne silage and its mixtures with grasses. However, the content of the quality parameters that are not included in the assessment indicate that the lucerne silage was of lower quality than silages from mixed sward. Lucerne silage had a significantly higher content of ammonia nitrogen, acetic and butyric acid. Although it is not clear in the quality assessment of the silage that adding high N rates diminished the quality of silage, they did act negatively on the content of some quality parameters, such as increasing the levels of NH_3-N and AA, and reducing the amounts of LA. Considering that the main objective of nitrogen fertilization is obtaining high yields, in order to meet the economic aspect of production and still get good quality silage, in the production of grass-

legume mixture one should opt for the lower levels of nitrogen fertilization. In agroecological conditions where the lucerne is superior to its mixtures in regard to the DM yield, owing to better quality of silages made of mixtures compared to pure lucerne silage, the use of lucerne mixtures is justified.

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References

AOAC (1990). Official methods of analysis. K. Helrich, ed. 15th ed. AOAC, Arlington,VA

Bijelić Z (2009). The influence of the mixture structure, nitrogen fertilization and utilization phase on quality parameters of grass-legume silages. PhD Thesis, University of Belgrade, R. Serbia

Bijelić Z, Tomić Z, Ružic-Muslić D, Krnjaja V, Mandić V, Vučković S & Nikšić D (2014). Forage quality and energy content of perennial legume-grass mixtures at three level of N fertilization. *Biotechnology in Animal Husbandry* **30**: 539-547

Djordjević N & Dinić B (2003). Siliranje leguminoza, Institut za istraživanja u poljoprivredi SRBIJA, Beograd

Faithfull N (2002). Methods in Agricultural Chemical Analysis: A Practical Handbook, CABI Publishing, Wallingford

Haigh P M & Parker J W G (1985). Effect of silage additives and wilting on silage fermentation, digestibility and intake, and on liveweight change of young cattle. *Grass and Forage Science* **40**: 429-436

Han K J, Collins M, Vanzant E S & Dougherty C T (2006). Characteristics of baled silage made from first and second harvests of wilted and severely wilted forages. *Grass and Forage Science* **61**: 22-31

Heikkilä T, Toivonen V & Mela T (1992). Comparison of red clover-grass silage with grass silage for milk production. In: *Proceedings of the 14th General meeting of the European Grassland Federation*, June 8-11, Lahti, pp. 388-391

Keady T W J & Kiely P O (1996). An evaluation of the effects of rate of nitrogen fertilization of grassland on silage fermentation, in-silo losses, effluent production and aerobic stability. *Grass and Forage Science* **51**: 350-362

Keady T W J & Kiely P O (1998). An evaluation of potassium and nitrogen fertilization of grassland and date of harvest on fermentation effluent production, dry matter recovery and predicted feeding value of silage. *Grass and Forage Science* **53**: 326-337

Keady T W J, Mayne C S & Fitzpatrick D A (2000). Prediction of silage feeding value from the analysis of the herbage at ensiling and effect of nitrogen fertilizer, date of harvest and additive treatment on grass silage composition. *Journal of Agricultural Science, Cambridge* **134**: 353-368

Kılıç A (1986). Silo Yemi (Öğretim, Öğrenim ve Uygulama Önerileri). 340 s. Bilgehan Basımevi, Izmir, Turkey.

King C, McEniry J, Richardson M & O′Kiely P (2013). Silage fermentation characteristics of grass species grown under two nitrogen fertilizer inputs and harvested at advancing maturity in the spring growth. *Grassland Science* **59**: 30-43

Lättemäe P & Tamm U (2002). The improvement of lucerne silage quality by using additives and lucerne-grass mixtures. *Agraarteadus* **13**(6): 337–342

Orozco-Hernandez J R, Brisson G J & Girard V (1997). Timothy grass or lucerne silage for cows in midlactation: effect of supplementary barley. *Journal of Dairy Science* **80**: 2876–2884

StatSoft (2007). STATISTICA, version 8.0, from http://www.statsoft.com

Tremblay G F, Belanger G & Drapeau R (2005). Nitrogen fertilizer application and developmental stage affect quality of timothy (*Phleum pratense* L.). *Grass and Forage Science* **60**: 337-355

Wang Y, Barbieri L R, Berg B P & McAllister T A (2007). Effect of mixing sainfoin with alfalfa on ensiling, ruminal fermentation and total tract digestion of silage. *Animal Feed Science and Technology* **135**: 296-314

Weissbach H F (2003). Theory and practice of ensuring good quality of silages from grass and legumes. In: *Proceedings of the XI. International scientific symposium "Forage Conservation",* September 9-11, Nitra, pp. 31-36

Wilman D & Wright P T (1983). Some effects of applied nitrogen on the growth and chemical composition of temperate grasses. *Herbage Abstracts* **53**: 387-393

Wyss U (2004). Ensilability of different grass-clover mixtures. *Grassland Science in Europe* **9**: 966-968

Wyss U (2006). Ensilability and silage quality of different grasses and legumes. *Agrarforschung* **13**: 442-447

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Çan Biberinde (*Capsicum baccatum* var. *pendulum*) Meyve Olgunluk Dönemleri ile Tohum Gelişimi ve Kalitesi Arasındaki İlişkilerin Belirlenmesi

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ÖZET

Bu araştırma Çan biberinde (*Capsicum baccatum* var. *pendulum*) farklı meyve olgunluk dönemlerinde hasat edilen tohumların, tohum gelişimi ile tohum kalitesi arasındaki ilişkilerin belirlenmesi amacıyla yürütülmüştür. Biber meyveleri 2011 ve 2012 yılı vejetasyon döneminde (1) yeşil olum, (2) renk dönüşümü, (3) turuncu olum, (4) kırmızı olum ve (5) aşırı olum dönemlerinde hasat edilmiştir. Renk gelişim dönemlerine göre tohum nemi % 37.2-69.0 arasında değişmiş, en düşük tohum nemi birinci ve ikinci yılda sırasıyla % 37.2 ve % 39.0 olarak aşırı olum döneminde belirlenmiştir. Meyve gelişim dönemlerine göre çimlenme oranı % 0-94 arasında değişmiş; maksimum çimlenme oranı 2011 yılında % 94 iken 2012 yılında % 79 bulunmuştur. Ortalama çimlenme süresi ise 4 ila 13 gün arasında olmuştur. Her iki yıl için çıkış oranı % 1-97 arasında değişmiş; maksimum çıkış oranı 2011 yılında % 97 ve 2012 yılında % 76 olarak belirlenmiştir. Buna bağlı olarak ortalama çıkış süresi 7 ila 18 gün arasında değişmiştir. Çıkış sonrası fide boyu 3.0-5.7 cm arasında, fide yaş ağırlığı 122.75-255.0 mg ve fide kuru ağırlığı 8.75-33.66 mg arasında belirlenmiştir. Sonuç olarak, meyvenin olgunlaşması ile tohum kalite özelliklerinin arttığı ve tohum kalitesi açısından en uygun meyve hasat zamanının kırmızı olum dönemi olduğu belirlenmiştir.

Anahtar Kelimeler: Fizyolojik olgunluk; Tohum nemi; Tohum gücü; Fide kalitesi

Determination of Relationships Between Fruit Maturity Stages, and Seed Development and Quality in Aji Pepper (*Capsicum baccatum* var. *pendulum*)

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ABSTRACT

This study was conducted to determine the relationships between the seeds harvested at different fruits maturity stages and the seed development and quality in Aji pepper (*Capsicum baccatum* var. *pendulum*). The fruits were harvested at

green ripe (1), breaker (2), orange ripe (3), red ripe (4) and over red ripe (5) maturity stages in 2011 and 2012 vegetation seasons. Seed moisture content was changed between 37.2% and 69.0% based on development stage, and over maturity stage had the lowest moisture content of 37.2% and 39.0% in both years, respectively. Germination rate was between 0% and 94% with the maximum of 94% in 2011 and 79% in 2012. The average germination time ranged from 4 to 13 days. The seedling emergence rate was between 1% and to 97% with the maximum of 97% in 2011 and 76% in 2012 that the average seedling emergence time changed between 7 and 18 days. Seedling length ranged from 3.0 to 5.7 cm, seedling fresh weight ranged from 122.75 to 255.00 mg and seedling dry weight ranged from 8.75 to 33.66 mg. The results showed that the seed quality increased as maturity level progressed, and the best fruit harvest date was red ripe stage for maximum seed quality in Aji pepper.

Keywords: Physiological maturity; Seed moisture; Seed vigor; Seedling quality

1. Giriş

Biber, gerek dünyada ve gerekse ülkemizde sevilerek tüketilen, içerdiği vitamin ve mineral maddeler yönünden zengin ve insan beslenmesine olumlu katkısı olan bir sebze türüdür. Ülkemiz üretimine konu olan biberlerin tamamına yakını *C. annuum* türüne aittir. *Capsicum* cinsi içerisinde yaklaşık 30 tür bulunmakla birlikte *Capsicum annuum* L., *C. baccatum* L., *C. chinense* Jacq., *C. frutescens* L., ve *C. pubescens* Ruiz & Pav. türleri kültüre alınmıştır (Bosland 1994).

C. baccatum kültürü yapılan bu beş türden biridir ve ülkemizde meyveleri çana benzediği için çan biberi veya gül biberi olarak adlandırılmaktadır. *C. baccatum* türü, *C. baccatum* var. *baccatum, C. baccatum* var. *pendulum* ve *C. baccatum* var. *praetermissum* olmak üzere üç alt türe ayrılmaktadır. *C. baccatum* var. *pendulum* alt türünün meyveleri taze tüketim yanında, salsa sosu, acı biber sosu ve toz biber üretiminde kullanılmaktadır (Jarret 2007). Tür adı olan 'baccatum' üzümsü meyve anlamına gelmektedir. Değişik meyve şekillerine, eşsiz bir lezzete ve farklı aromalara sahip olmasına rağmen Güney Amerika dışında pek tanınmaması ve yetiştirilmemesi büyük bir kayıptır. Bitki genellikle hızla büyümekte ve fazla boylanmaktadır. Ülkemizde yetiştiriciliği yapılan bir çeşidi bulunmamaktadır.

Biberde yapılan tüm çalışmalar *Capsicum annuum* türüne ait çeşitler üzerinde yürütülmüştür (Demir & Ellis 1992; Sanchez et al 1993; Demir & Ellis 1994; Cavero et al 1995; Yanmaz & Demir 1998; Demir 2002; Sarıyıldız 2003; Vidigal et al 2011). Diğer kültürü yapılan biber türlerinde ise tohum gelişimi ile ilgili olarak taranan kaynaklarda az sayıda çalışmaya rastlanmaktadır. Bir diğer önemli konu da meyve renklerinin daha objektif olarak belirlenmesinin sağlanmasıdır. Çünkü aynı bitki üzerinde farklı gelişim evrelerindeki meyvelerin bulunduğu biber gibi türlerde aynı gelişim dönemindeki meyvelerin daha objektif olarak belirlenmesinin daha kaliteli tohumlukların elde edilmesine yardımcı olabileceği düşünülmektedir.

Bu çalışma, *Capsicum baccatum* var. *pendulum* türüne ait bir hatta, farklı gelişim dönemlerinde hasat edilen meyvelerden elde edilen tohumların tohum kalite değişimini belirlemek, tohumluk gelişim sürecinde çıkış oranını tespit etmek ve bu veriler ışığında tür için en uygun tohumluk hasat dönemini saptamak amacıyla yürütülmüştür.

2. Materyal ve Yöntem

Çalışma Mustafa Kemal Üniversitesi, Ziraat Fakültesi, Bahçe Bitkileri Bölümünde 2011 ve 2012 yıllarında yürütülmüştür. Bitkisel materyal olarak süs biberi genetik koleksiyonunda bulunan MKÜ-19 (Çan biberi) nolu genotipi kullanılmıştır (Mavi & Mavi 2012). Tohumlar, fide dikim tarihinden bir ay önce içinde Klasman torf bulunan viyollere ekilmiş ve ısıtmasız cam serada çimlendirilmiştir. Dikim büyüklüğüne gelen fideler 2011 yılında 2 Mart tarihinde, 2012 yılında ise 10 Nisan tarihinde önceden hazırlanmış arazideki yerlerine dikilmiştir. Kültürel işlemlerin rahat yapılabilmesi ve denemelerde kullanılacak yeterli tohumun elde

edilebilmesi için 300 adet fide 35x45 cm sıra üzeri ve arası mesafelerde dikilmiştir. Dikimden hasat sonuna kadar bitkilerde kültürel işlemler düzenli bir şekilde yapılmıştır. Yetiştirme dönemi boyunca sıcaklık ve nem değerleri kaydedilmiştir (Çizelge 1).

Ana bitkinin ilk dört boğumu içerisindeki meyveler (1) yeşil olum, (2) renk dönüşümü, (3) turuncu olum, (4) kırmızı olum ve (5) aşırı olum olgunluk dönemlerinde hasat edilmiş ve tohumları ayrılmıştır. Meyve rengi her olgunluk dönemi için 30'ar meyvede Minolta renk ölçerle (*L**, *a**, *b**, hue (*h**) açı ve *C**) belirlenmiştir. Elde edilen tohumların kalitesi; tohum nemi, 1000 tohum ağırlığı, çimlenme oranı (%), ortalama çimlenme süresi (gün), kontrollü bozulma testi (% 24 nem, 45 ^{o}C, 24 h^{-1}), çıkış oranı (%), ortalama çıkış süresi (gün), fide boyu (cm), fide yaş ve kuru ağırlığı (mg) ve elektriksel iletkenlik (µS cm^{-1} g^{-1}) testleri ile belirlenmiştir (Eken 2014). Fide değerlendirmeleri tohum ekiminden 20 gün sonra yapılmıştır.

Denemeler tesadüf parselleri deneme desenine göre 4 tekerrürlü ve her tekerrürde 50 tohum olacak şekilde yürütülmüştür. Elde edilen veriler SPSS paket programı ile variyans analizine tabi tutulmuş ve yüzde şeklindeki veriler analiz öncesinde açı değerine çevrilmiştir. Ortalamalar arasındaki farklılıklar Duncan testi ile 0.05 önem düzeyinde karşılaştırılmıştır.

3. Bulgular ve Tartışma

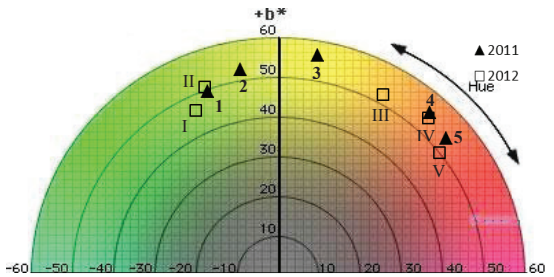
Bugüne kadar yapılan tohum gelişimi çalışmalarında meyve renkleri gözle (subjektif) belirlenmiştir. Çalışmamızda ise, daha sonra yapılacak çalışmalarda bir örneklik sağlamak amacıyla farklı hasat dönemlerinde meyvelerin renkleri dijital olarak Minolta renk ölçerle belirlenmiştir (Şekil 1 ve Çizelge 2). Kırmızı olum dönemindeki renk değerleri, Mavi & Mavi (2012)'nin kırmızı olum dönemindeki meyveleri ile benzer bulunmuştur. Kırmızı olum döneminin, Mavi & Mavi (2012) tarafından belirlenen meyve gelişim durumları ile karşılaştırıldığında, çiçeklenmeden sonra 65. güne denk geldiği tahmin edilmektedir. Hue açı değerleri ise Tadesse et al (2002) ile çok benzer bulunurken *C**

Çizelge 1- Denemenin yürütüldüğü Antakya ilçesine ait 2011 ve 2012 yılları iklim verileri

Table 1- The climatic data of Antakya where the experiments were conducted in 2011 and 2012

Yıllar ve aylar		*Aylık ortalama sıcaklık değerleri*			*Ortalama nem*	*Toplam yağış*
		Maksimum	*Minimum*	*Ortalama*		
		(°C)	*(°C)*	*(°C)*	*(%)*	*(mm)*
2011	Mart	26.3	3.1	13.7	64.2	143.5
	Nisan	28.9	5.4	17.0	66.1	130.4
	Mayıs	30.8	11.8	20.9	64.4	65.1
	Haziran	32.5	16.6	24.7	66.7	86.3
	Temmuz	35.0	22.8	27.7	65.9	0.0
	Ağustos	33.9	23.7	28.6	63.4	0.0
	Eylül	38.9	16.3	26.5	62.0	34.7
	Ekim	34.6	8.8	16.8	48.5	82.5
2012	Mart	23.8	-2.9	10.4	65.7	105.2
	Nisan	33.3	5.7	16.9	61.1	16.5
	Mayıs	34.0	15.5	21.3	64.3	97.6
	Haziran	43.2	17.5	26.0	60.7	1.6
	Temmuz	40.1	23.0	28.8	60.4	14.1
	Ağustos	37.3	23.2	29.4	59.2	0.0
	Eylül	38.4	19.4	27.4	58.9	0.0
	Ekim	38.5	12.0	22.1	60.2	85.1

değerleri daha yüksek olmuştur. Biberlerde, dijital renk ölçümü ile meyve renginin en uygun sınırları tespit edilebileceği ve bu dönemde yapılacak hasat ile kaliteli tohum üretimi mümkün olabilecektir.



Şekil 1- Farklı gelişme aşamalarında hasat edilen Çan biberi meyvelerinin 2011 (1, 2, 3, 4, 5) ve 2012 (I, II, III, IV, V) yıllarındaki a* ve b* renk değişimleri

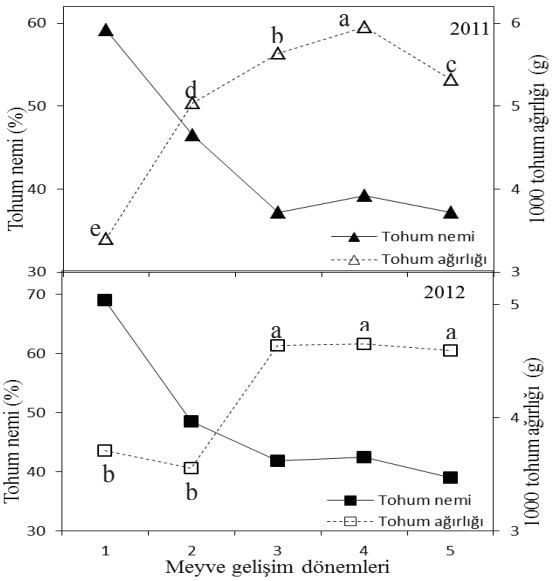
Figure 1- a and b* color variation in Aji peppers harvested at different development stages in 2011 (1, 2, 3, 4, 5) and 2012 (I, II, III, IV, V)*

Çizelge 2- Çan biberi meyvelerinde 2011 ve 2012 yıllarında L*, hue (h*) açı ve C* renk değerlerindeki değişimler

Table 2- L, h* and C* color variation of harvested Aji pepper fruits in 2011-2012*

Meyve gelişim dönemleri	*L**		*h**		*C**	
	2011	*2012*	*2011*	*2012*	*2011*	*2012*
Yeşil olum	65.6	63.5	109.1	114.5	52.8	49.0
Renk değişimi	68.5	67.1	99.4	109.4	56.8	54.2
Turuncu olum	65.6	58.3	81.1	63.0	61.2	55.4
Kırmızı olum	52.3	52.8	50.2	49.6	57.3	56.1
Aşırı olum	47.8	49.0	42.3	40.3	55.0	51.0

Tohum nemi 2011 yılında yeşil olum döneminde % 59.2 iken olgunlaşmanın ilerlemesi ile birlikte turuncu ve aşırı olum dönemlerinde % 37.2'ye düşmüştür. 2012 yılında ise yeşil olum döneminde % 69.0 olan nem içeriği aşırı olum döneminde % 39.0 olarak belirlenmiştir. Genellikle biber türlerinde tohum neminin % 40 seviyelerine düşmesi ile kalite artmaktadır. Demir & Ellis (1992); Blasiak et al (2006); Pagamas & Nawata (2007); Vidigal et al (2011), tür ve çeşitlere göre farklılık olmakla birlikte tohum neminin % 37-50 düzeyinde olduğu belirtilmiştir. Gelişim döneminin uzamasıyla birlikte tohum nemi azalırken, tohum ağırlığında ve kuru maddesinde artış meydana gelmektedir. Öte yandan tohum ağırlığının yüksek sıcaklıkta yetiştirilen bitkilerde düşük kaldığı Pagamas & Nawata (2007) tarafından belirlenmiştir. Yüksek sıcaklıklar, meyvelerin erken renklenmesin sağlayıp olgunlaşma süresini kısaltırken bünyesindeki tohumların kuru madde, yağ ve karbonhidrat birikimlerini azaltmaktadır. Yeterli gelişmesini tamamlamamış tohumlar kararmakta, tohum ağırlığı başta olmak üzere çimlenme oranı, tohum gücü ve sonuçta da tohum kalitesi düşmektedir. Çan biberinde de bu durum gözlenmiş olup (Şekil 2) çalışmanın ikinci yılındaki tohum ağırlıklarının nispeten düşük kalmasının bir nedeni olarak yüksek sıcaklıklar düşünülmektedir (Şekil 2).



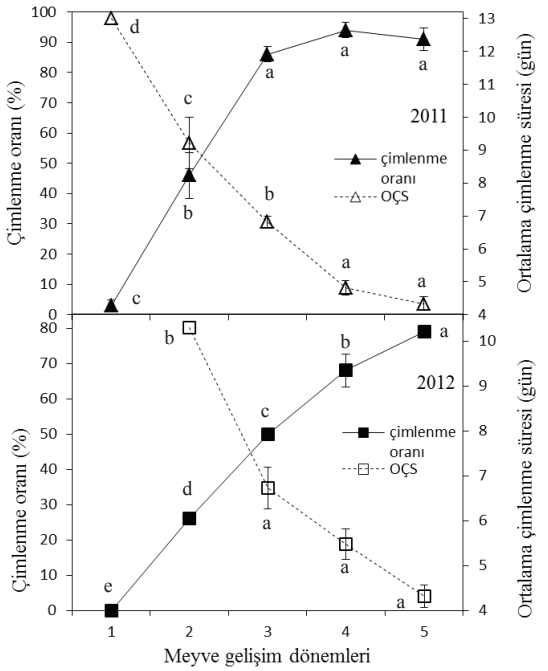
Şekil 2- Çan biberinde meyve gelişim dönemlerine göre tohumların tohum nemi (%) ve 1000 tohum ağırlığındaki (g) değişim

Figure 2- The change of moisture content (%) and 1000-seed weight (g) of Aji pepper seeds during fruit development stages

Tohum çimlenme oranı, gelişim dönemlerine bağlı olarak 2011 yılında % 3-% 94 2012 yılında ise % 0-% 79 arasında değişmiş ve aralarındaki fark istatistiksel olarak ($P<0.05$) önemli bulunmuştur. Özelikle çiçeklenmeden sonraki yaklaşık 30. güne denk gelen yeşil olum hasat döneminde tohumların henüz çimlenme yeteneğini kazanmadığı, renk dönüşümü döneminde ise çimlenme kabiliyeti kazanılmasına rağmen çimlenme oranı ve tohum gücünün düşük kaldığı gözlenmiştir. Çimlenme oranındaki değişim her iki yılda da meyve olgunluk döneminin ilerlemesi ile birlikte artmış ve meyve renginin tam kırmızı olduğu kırmızı ve aşırı olum dönemlerinde maksimum seviyeye ulaşmıştır.

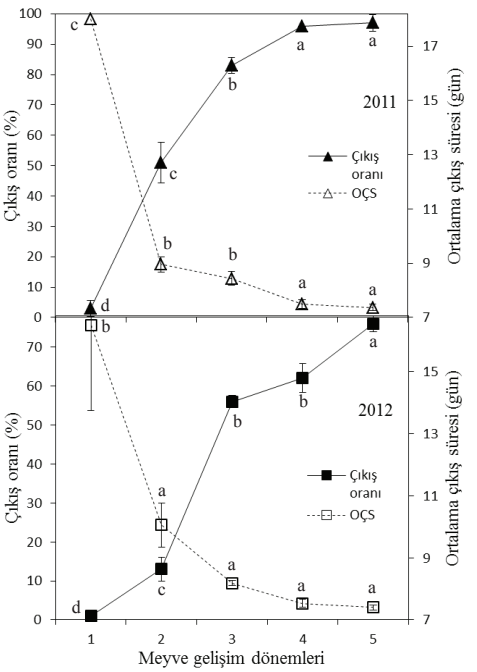
Ortalama çimlenme süresi ise meyve olgunluk dönemlerinin ilerlemesiyle kısalma göstermiştir (Şekil 3). Diğer araştırıcılar da genelde benzer sonuçlar bulmuştur. Sanchez et al (1993) aşırı olgun kırmızı dönemdeki tohumların çimlenme oranlarının çeşitlere bağlı olarak % 49-% 93 arasında değiştiğini ve 5 ila 7 günde çimlendiklerini belirtmiştir. Cavero et al (1995) ise iki farklı biber çeşidinde tohumların olgun ve aşırı olgun dönemlerde % 95, yarı olgun dönemde ise % 40-50 civarında çimlendiğini tespit etmiştir. Nascimento et al (2011) BRS Mari çeşidinde (*Capsicum baccatum*) çiçeklenmeden 70 gün sonra hasat edilen meyvelerin tohumlarının % 70 çimlenme oranına sahip olduğunu ve çiçeklenmeden sonra 80. günden itibaren çimlenme oranının düştüğünü saptamıştır.

Tohum çıkış oranında olgunlaşmanın ilerlemesi ile birlikte yükselme ve ortalama çıkış süresinde kısalma tespit edilmiştir (Şekil 4). 2011 yılında yeşil olum döneminde % 3, aşırı olum döneminde ise



Şekil 3- Çan biberinde meyve gelişim dönemlerine göre tohumların çimlenme oranı (%) ve ortalama çimlenme süresindeki (gün) değişim

Figure 3- The change of germination percentage (%) and germination time (day) of Aji pepper seeds during fruit development stages



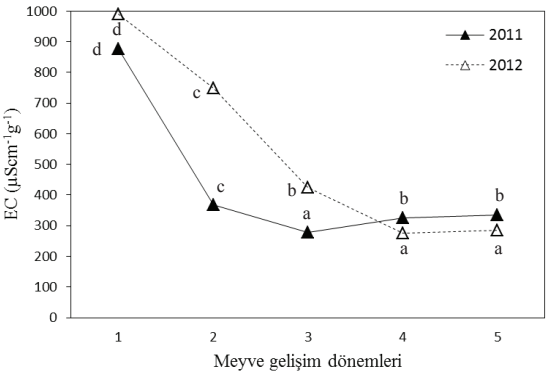
Şekil 4- Çan biberinde meyve gelişim dönemlerine göre tohumların çıkış oranı (%) ve ortalama çıkış süresindeki (gün) değişim

Figure 4- The change of seedling emergence rate (%) and the average emergence time (day) of Aji pepper seeds during fruit development stages

% 97 olarak belirlenmiştir. 2012 yılında ise bu değerler % 1 ve % 76 arasında değişmiş ve dönemler arasındaki fark istatistiksel olarak ($P<0.05$) önemli bulunmuştur. Demir & Ellis (1992) de California Wonder çeşidine ait biber tohumlarında benzer ilişki gözlemiştir.

Fide boyu 2011 yılında en yüksek (4.1 cm) aşırı kırmızı olum döneminde iken, en düşük (3.0 cm) renk dönüşümü döneminde ölçülmüştür. Fide yaş ağırlığı da kırmızı olum dönemi hariç olmak üzere meyve olgunlaşma dönemlerine bağlı olarak artmış ancak bu artış istatistiki olarak önemli bulunmamıştır. Fide kuru ağırlığı ise en yüksek aşırı olum (33.7 mg) ve kırmızı olum dönemlerinde (30.7 mg), en düşük ise renk dönüşümü döneminde (21.9 mg) tespit edilmiştir. 2012 yılında, renk dönüşümü dönemindeki fide boyu (3.2 cm), turuncu olum (5.5 cm), kırmızı olum (5.7 cm) ve aşırı olum (5.8 cm) dönemlerine göre daha düşük bulunmuştur. Fide yaş ağırlığı, en düşük renk dönüşümü döneminde (122.8 mg) tespit edilirken, en yüksek turuncu olum döneminde (238.5 mg) belirlenmiştir. Turuncu olum, kırmızı olum ve aşırı olum arasında farklılık önemsizdir. Fide kuru ağırlığı, en düşük renk dönüşümü döneminde (8.8 mg) ve en yüksek kırmızı olum döneminde (18.3 mg) bulunmuştur (Çizelge 3). Vidigal et al (2011) sadece fide boyu ile gelişme dönemlerini değerlendirmiş ve çiçeklenmeden sonra 50. günde elde edilen tohumların fidelerinin en kısa fide boyuna sahip olduğunu, diğer grupların ise birbirine yakın ve daha uzun fide oluşturduklarını bildirmiştir. Fide kuru ağırlığı ise Demir & Ellis (1992)'in çalışmasındaki sonuçlar ile benzerlik gösterirken, Demir (2002)'e göre daha yüksek bulunmuştur. Bu durumun fidelerin yetiştirilme koşulları ile ilişkili olabileceği düşünülmektedir.

Elektriksel iletkenlik test sonuçlarına göre, Çan biberinde meyve olgunluğundaki artışla birlikte bu meyvelerden elde edilen tohumlarda elektriksel iletkenlik değerleri azalmıştır. Elektriksel iletkenliği 800 µS cm^{-1} g^{-1} üzerinde olan tohumların ekim değerini kaybettiği, 400 µS cm^{-1} g^{-1} altında ise tohum gücünün yüksek olduğu söylenebilir. 270-300 µS cm^{-1} g^{-1} ise güçlü tohumlar için kabul edilebilir sınır olarak görülmektedir (Şekil 5). Ancak



Şekil 5- Çan biberinde meyve gelişim dönemlerine göre tohumların elektriksel iletkenlik (µS cm^{-1} g^{-1}) değerindeki değişim

Figure 5- The change of electrical conductivity of Aji pepper seeds during fruit development stages

Çizelge 3- Çan biberinde farklı olgunluk dönemlerinde hasat edilen biberlerin tohumlarının, fide boyu (cm) ile fide yaş ve kuru ağırlığı (mg) üzerine etkileri

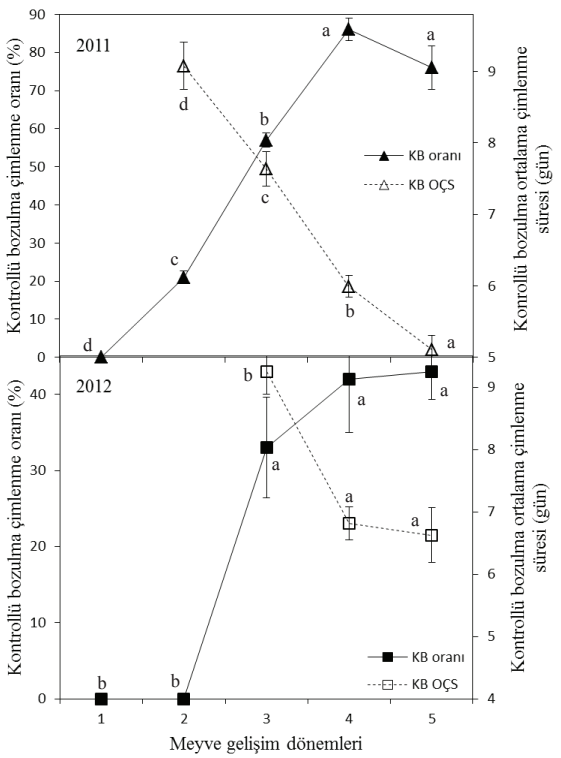
Table 3- The effect of Aji pepper seeds harvested at different fruit maturity stages on seedling length (cm), and seedling fresh and dry weight (mg)

Meyve gelişim dönemleri	*Boy (cm)*		*Yaş ağırlık (mg)*		*Kuru ağırlık (mg)*	
	2011	*2012*	*2011*	*2012*	*2011*	*2012*
Yeşil olum*	-	-	-	-	-	-
Renk değişimi	3.0 c	3.2 b	222.1	122.8 b	21.9 b	8.8 b
Turuncu olum	3.4 b	5.5 a	249.1	238.5 a	26.7 ab	18.0 a
Kırmızı olum	3.7 b	5.7 a	239.6	232.5 a	30.7 a	18.3 a
Aşırı olum	4.1 a	5.8 a	255.0	214.8 a	33.7 a	16.8 a

*, gelişme döneminde yeterli fide elde edilememesi nedeniyle veri alınamamıştır; aynı sütunda farklı harflerle gösterilen ortalamalar arası fark önemlidir ($P\leq0.05$)

değerlerin optimize edilmesi gerekli görülmektedir. Bu konuda çok az sayıda çalışma bulunmaktadır. Demir & Ellis (1992) ve Vidigal et al (2011) tohum olgunluğundaki ve gelişim dönemlerindeki artışla birlikte elektriksel iletkenlik değerlerinde azalma olduğunu göstermiştir ancak Vidigal et al (2011)'in değerleri oldukça yüksektir.

Kontrollü bozulma testi küçük tohumlu türler için uygun bir tohum gücü testidir. Kontrollü bozulma testine göre çimlenme oranı en yüksek kırmızı olum döneminde % 86 ve % 43 olarak sırasıyla 2011 ve 2012 yıllarında belirlenmiştir (Şekil 6). Her iki yılda da kontrollü bozulma çimlenme oranları gelişim dönemlerindeki artışa paralel olarak artmıştır.



Şekil 6- Çan biberinde, meyve gelişim dönemlerine göre tohumların kontrollü bozulma çimlenme oranı (%) ve ortalama çimlenme süresi (gün) değerlerindeki değişim

Figure 6- The change of germination rate (%) and germination time (day) at controlled deterioration tests of Aji pepper seeds during fruit development stages

Ancak 2012 yılındaki yüksek sıcaklıklar nedeniyle kontrollü bozulma çimlenme oranları daha düşük kalmıştır. Vidigal et al (2011) ise aynı mantıksal temele dayalı olan hızlandırılmış yaşlandırma testini kullanmış ve benzer şekilde erken meyve olgunluğunda elde edilen tohumların hızlandırılmış yaşlandırma testinde de düşük değerlere sahip olduğunu tespit etmiştir.

4. Sonuç

Çan biberinde (MKÜ 19 nolu genotip) yüksek çimlenme kabiliyeti için tohumluk meyve hasadının kırmızı olum ve kırmızı renklenme sonrasındaki 10 gün içerisinde, tohum neminin % 37-39'a düştüğü dönemde yapılması gerekmektedir. Yüksek sıcaklıkların tohum kalitesine olan olumsuz etkisi gözlendiğinden tohum üretimi için, bitkilerin gece-gündüz sıcaklık farkının daha yüksek olduğu ancak gece ve gündüz sıcaklıklarının nispeten düşük olduğu bölgelerde yapılması önerilmektedir. Elektriksel iletkenlik testinin biber tohumlarında optimize edilmesine ihtiyaç bulunmaktadır. Böylece ek bir tohum gücü testi olarak kullanılması mümkün olacaktır. Bu çalışma ile tohumluk meyve renginin objektif bir yöntemle tayini ilk kez yapılmıştır ve diğer biber tür ve çeşitlerinde kullanılması ve optimize edilmesi önerilmektedir.

Teşekkür

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Kaynaklar

Blasiak J, Kuang A, Farhangi C S & Musgrave M E (2006). Roles of intra-fruit oxygen and carbon dioxide in controlling pepper (*Capsicum annuum* L.) seed development and storage reverse deposition. *Journal of the American Society for Horticultural Science* **131**(1): 164-173

Bosland P W (1994). Chiles: History, Cultivation and Uses Spices. (Ed. G.Charalambous). *Herbs and Edible Fungi,* Elseiver Science, B.V. New Mexico

Cavero J, Ortega R G & Zaragosa C (1995). Influence of fruit ripeness at the time of seed extraction on pepper

(*Capsicum annuum*) seed germination. *Scientia Horticulturae* **60**: 345-352

Demir I (2002). The effect of controlled hydration treatment on germination and seedling emergence of unaged and aged pepper seeds during development. *Israel Journal of Plant Science* **50**: 251-257

Demir I & Ellis R H (1992). Development of pepper (*Capsicum annuum*) seed quality. *Annals of Applied Biology* **121**: 385-399

Demir I & Ellis R H (1994). The effects of priming on germination and longevity of sequentially harvested pepper seed lots. *Turkish Journal of Agriculture and Forestry* **18**: 213-217

Eken N İ (2014). Çan biberinde (*Capsicum baccatum* var. *pendulum*) meyve olgunluk dönemleri ile tohum gelişimi ve kalitesi arasındaki ilişkilerin belirlenmesi. Yüksek Lisans Tezi, Mustafa Kemal Üniversitesi Fen Bilimleri Enstitüsü (Basılmamış), Hatay

Jarret R L (2007). Morphologic variation for fruit characteristics in the USDA/ARS *Capsicum baccatum* L. germplasm collection. *HortScience* **42**(5): 1303-1305

Mavi K & Mavi F (2012). *Capsicum baccatum* var. *pendulum* türüne ait biber hattının tohumlarında çimlenme için uygun sıcaklığın belirlenmesi. *MKÜ Ziraat Fakültesi Dergisi* **17**(2): 79-86

Nascimento W M, Silva P P, Justino E V & Freitas R A (2011). Physiological seed quality and stand establishment of hot pepper (*Capsicum baccatum* L. var. *pendulum*) under tropical conditions. *Acta Horticulturae* **898**: 307-310

Pagamas P & Nawata E (2007). Effect of high temperature during the seed development on quality and chemical composition of chili pepper seeds. *Japanese Journal of Tropical Agriculture* **51**(1): 22-29

Sanchez V M, Sundstrom F J, McClure G N & Lang N S (1993). Fruit maturity, storage and postharvest maturation treatments affect bell pepper (*Capsicum annuum* L.) seed quality. *Scientia Horticulturae* **54**: 191-201

Sarıyıldız Z (2003). Farklı dönemlerde hasat edilmiş biber tohumlarında ABA değişimi ve kontrollü nemlendirme uygulamasının stres sıcaklıklarında çimlenme performansı ve tohum depo ömrü üzerine etkisinin belirlenmesi. Yüksek Lisans Tezi, Ankara Üniversitesi Fen Bilimleri Enstitüsü (Basılmamış), Ankara

Vidigal D S, Dias D C F S, Dias L A S & Finger F L (2011). Changes in seed quality during fruit maturation of sweet pepper. *Scientia Agricola* **68**(5): 535-539

Yanmaz R & Demir İ 1998. Bazı biber çeşitlerinde olgunluk dönemi ve kurutma sıcaklıklarının tohum kalitesine etkisi. *2. Sebze Tarımı Sempozyumu,* Tokat, s. 93-99

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Paprika Pepper Yield and Quality as Affected by Different Irrigation Levels

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ABSTRACT

This study was carried out to determine the effects of irrigation levels on yield and quality of paprika pepper under Harran plain conditions of Turkey. Different water stress levels under drip irrigation system were created by applying 125% of cumulative Class-A Pan evaporation (CAP_{125}), 100% (CAP_{100}), 75% (CAP_{75}) and 50% (CAP_{50}) of cumulative class-A-pan (CAP) evaporation in every 3 days. Besides, an irrigation treatment with IRSIS (Irrigation Scheduling Information System) computer program was created and applied as an alternative irrigation program. Three paprika pepper cultivars (Ace, King and Queens) were tested. Experiments were conducted in randomized blocks split plots design with 3 replications in 2005 at Koruklu Station of GAP Regional Development Administration located in Harran Plain of Şarlıurfa, Turkey. Applied irrigation water amount and ET (Evapotranspiration) values for IRSIS treatment were 874 mm and 908 mm, respectively. Applied irrigation amount and ET values of CAP_{125} and CAP_{50} were between 254-568 mm and 368-602 mm, respectively. The highest yield was obtained from IRSIS treatment with 25.63 t ha^{-1} and the lowest yield was obtained from CAP_{50} with 11.72 t ha^{-1}. The yield was significantly affected by cultivar, irrigation and cultivar × irrigation interactions. The average moisture, ASTA (American Spice Trade Association), capsaicin, vitamin C and beta-carotene contents were respectively varied between 83.96 – 84.76%, between 225.76–286.22 mg kg^{-1}, between 1404.11–2408.11 mg kg^{-1} and between 77.88–113.00 mg kg^{-1}. Beta-carotene contents were not affected significantly by the cultivars, irrigations and interactions. The effects of irrigation and interactions on vitamin C and capsaicin were not also significant while the effects of cultivar on vitamin C and capsaicin were significant at $P<0.05$ level. It was observed in this study that sufficient yield levels of paprika peppers might be achieved through implementation of proper irrigation and care practices.

Keywords: Evapotranspiration; Vitamin C; Capsicine; Class-A pan; Deficit irrigation

Farklı Sulama Seviyelerinin Paprika Biberinde Verim ve Kalite Üzerine Etkileri

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ÖZET

Bu çalışma, Harran Ovası koşullarında paprika biberinde sulama suyu seviyesinin verim ve kalite üzerine etkilerini belirlemek amacıyla yürütülmüştür. Farklı sulama suyu düzeyleri 3 günde bir Class-A-Pan (CAP)'dan olan kümülatif buharlaşmanın % 125 (CAP_{125}), % 100 (CAP_{100}), % 75 (CAP_{75}) ve % 50 (CAP_{50})'sinin damla sulama sistemi kullanılarak uygulanması şeklinde oluşturulmuştur. Ayrıca IRSIS paket programına göre oluşturulan bir sulama programı da (IRSIS) sulama konusu olarak seçilmiştir. Denemede üç paprika biber çeşidi (Ace, King ve Queens) kullanılmıştır. IRSIS konusundaki sulama suyu ve bitki su tüketimi (ET) değerleri sırasıyla 874 mm ve 908 mm' dir. CAP_{125} - CAP_{50} konularına uygulanan sulama suyu ve ET değerleri ise sırasıyla, 568-254 mm ve 602-368 mm aralığında değişim göstermiştir. En yüksek verim 25.63 t ha^{-1} ile IRSIS (Irrigation Scheduling Information System) sulamasından elde edilirken en düşük verim 11.72 t ha^{-1} ile CAP_{50} sulamasından elde edilmiştir. Çeşit, sulama ve çeşit x sulama interaksiyonun verim üzerine etkileri istatistiksel olarak önemli bulunmuştur. Ortalama nem, ASTA, kapsaisin, C-vitamini ve beta-karoten içeriği değerleri sırasıyla % 84.1, 261.1, 0.4 mg kg^{-1}, 1890.4 mg kg^{-1} and 98.9 mg kg^{-1} olarak elde edilmiştir. Beta-karoten içerikleri çeşit, sulama ve interaksiyonlardan önemli düzeyde etkilenmemiştir. Sulama ve interaksiyonların C vitamin ve kapsaisin üzerine etkileri de önemsizken çeşidin C vitamini ve kapsaisin üzerine etkileri $P<0.05$ düzeyinde önemli bulunmuştur. Sonuçlar uygun sulama ve bakım işlemlerinin zamanında yerine getirilmesiyle paprika biberinde arzu edilen verim düzeylerinin yakalanabileceğini göstermiştir.

Anahtar Kelimeler: Bitki su tüketimi; C-vitamini; Kapsaisin; A-sınıfı buharlaşma; Kısıntılı sulama

1. Introduction

Irrigation is the most significant input in agricultural activities to improve the yields. Throughout the world, about 70% of available water resources are allocated to agricultural activities, especially to irrigation. Today, it is almost impossible to increase the cultivated lands, therefore researches have to be done to improve unit area-yields to increase the total yields.

Şanlıurfa Province of Turkey has insufficient precipitations during the growth seasons of vegetables. Therefore, irrigation is an essential component of plant production activities of the region. Irrigation scheduling (amount and timing) is the key issue to get the highest yields per unit area. Plant water consumption is the essential parameter of irrigation scheduling works and it may vary based on climate factors and plant growth stages. Thus, plant water consumption values should separately be determined for each climate zone and such a case is especially significant in arid and semi-arid regions.

Unconscious irrigations have various negative impacts on soil and water resources (salinity, environmental pollution, waste of water) and sustainability of agricultural activities. Such irrigations also result in root crown rot *(Phytophthora capsici L.)* especially in peppers (Rista et al 1995).

Pepper is the largest culture crop in Harran Plain of Turkey and mostly surface irrigation methods are used in pepper irrigation. However, there is an urgent need to move on to drip irrigation to prevent excessive water use and resultant plant diseases. Statistical data reveal that Turkey has about 2% of world pepper production and of this amount 15% is produced in GAP (Southeastern Anatolia Project) region and of that amount 37% is produced in Şanlıurfa Province (FAOSTAT 2005).

Paprika peppers are sensitive to low temperatures and usually considered as a proper plant for the regions with temperature ranges between 24-30 °C. They constitute a significant raw material of world food industry and usually consumed as ground pepper, chili pepper and chili sauces. They are largely consumed in developed countries, especially in the USA. The greatest producers are Mexico, Zambia, Hungary and Spain. Problems experienced in production and processing of paprika peppers in these countries enforced the search for new production sites. Recently, Israel achieved to

get paprika pepper oil with new technologies and such a development has made this oil an essential raw material for drug and cosmetic industries. Southeastern Anatolia Region of Turkey has highly available climate conditions for paprika pepper production and paprika pepper has newly been getting popular throughout the region. Therefore, the present study was conducted to investigate the effects of different irrigation levels on yield, quality and plant physiological characteristics of paprika pepper cultivars of Ace, Queen and King.

2. Material and Methods

The present research was conducted over the experimental fields of Koruklu Station of GAP Regional Development Administration located in Harran Plain of Şanlıurfa Province. The station is located at 36° 42' North latitude and 38° 58' East longitude and has an altitude of 410 m. Although the province is within Southeastern Anatolia climate zone, Mediterranean climate is dominant in the region. Summers are hot and dry and winters are mild and precipitated.

With regard to climate data, long-term averages and the averages for growth period are provided in Table 1. The region has an annual total precipitation of 365.2 mm and of this amount 17.3% is received in fall, 52.8% in winter, 28.8% in spring and 1.1% in summer. Annual average temperature is 17.2 °C, the highest average temperature is 46.8 °C and the lowest average temperature is -16.8 °C. Annual average relative humidity is 51% and annual total evaporation is 1848.8 mm.

The research site has clayey soils with pH values between 7.3-7.8, low organic matter content, high cation exchange capacity and infiltration rates of between 12-116 mm h^{-1}. Physical characteristics of experimental soils are provided in Table 2. Available

Table 1- Climate data for pepper growth period and long-term averages

Çizelge 1- Büyüme sezonu ve uzun dönem iklim verileri

Climate parameter	*June*	*July*	*August*	*September*
	Long-term (1929-2004)			
Minimum temperature (°C)	9.4	11.0	9.2	3.4
Maximum temperature (°C)	45.4	46.8	46.7	44.0
Average temperature (°C)	28.0	31.4	30.4	25.6
Precipitation (mm)	2.5	0.1	-	0.1
Relative humidity (%)	35	33	36	34
Wind speed (m s^{-1})	2.5	2.6	2.1	1.5
	Growth period (2005)			
Minimum temperature (°C)	15.1	20.4	20.0	14.1
Maximum temperature (°C)	38.5	43.7	43.5	32.8
Average temperature (°C)	27.4	33.0	32.1	24.3
Precipitation (mm)	0	0	0	0
Relative humidity (%)	35.9	32.8	44.7	47.0
Wind speed (m s^{-1})	2.6	2.8	1.7	1.1

Table 2- Soil physical characteristics of the research site

Çizelge 2- Araştırma sahası toprak fiziksel özellikleri

Soil layers		*Particle size distribution*			*Texture class*	*FC*	*PWP*	*WHC*
(cm)	*Bulk density (g cm^{-3})*	*Sand (%)*	*Silt (%)*	*Clay (%)*		*(%)*	*(%)*	*(%)*
0-30	1.32	8.8	30.5	60.7	Clay	33.74	20.28	13.46
30-60	1.37	10.0	33.5	56.5	Clay	32.27	21.12	11.15
60-90	1.31	10.3	27.9	61.8	Clay	32.39	21.85	10.54

water holding capacity of the soil at 90 cm soil profile was calculated as 120 mm.

Three different world-wide common pepper cultivars (Ace, Queen and King) were used as the plant material of the study. Papri-Queen, Papri-King and Papri-Ace are considered high quality cultivars and can command relatively high prices under good management. Papri-Queen is more disease resistant, but lower yielding than Papri-King, so more appropriate for small-scale farmers. Papri-Queen seeds may have problems in uniformity, pungency and yield and a "genetic shift" in seed may lead to deterioration in quality (Langmead 2003). Seedlings were planted on 10th of June. Irrigation water was supplied from a well within the research station. The water quality class was found to be as C_2S_1 with medium salinity and low alkalinity (U.S. Salinity Lab. Staff 1954). Drip irrigation was used in irrigations.

Experimental site was prepared for planting with a cultivator and gobble-disc in May. Three manual hoeing were performed, the first one at the end of June, the second one on 15th of July and the last one on 10th of August. A plant disease was not encountered during the experiments. Troper was applied at a rate of 2500 g ha^{-1} to prevent root diseases and Mica super was applied at 1000 cc ha^{-1} dose for weed control just before seedling transplantation on the soil surface of experimental plots.

Plots were 70 m long and there were 10 rows in each plot with 70 cm row spacings and 25 cm on-row plant spacing. A 3-meter spacing was provided between the plots. Middle 6 rows were hand-picked since side rows were omitted as side effect. A drip line (with 50 cm dripper spacing and 4 L h^{-1} dripper discharge) was placed to each row. Daily evaporations were measured through a Class-A-Pan (CAP) within the research station. As base fertilizer, 400 kg ha^{-1} ammonium sulphate (33%) was applied just before sowing and 300 kg ha^{-1} 20-20-0 composed fertilizer was applied through fertigation at five equal doses as dressing fertilizer. Experiments were conducted in randomized blocks split plots design with 3 replications. Cultivars were placed in main plots and irrigation levels were placed in sub-plots.

Seasonal water deficit treatments were arranged as the 50% (CAP_{50}), 75% (CAP_{75}), 100% (CAP_{100}) and 125% (CAP_{125}) of 3-days cumulative evaporation from Class-A-Pan (CAP). Another irrigation treatment was created by using IRSIS irrigation scheduling program. Thus, 5 different irrigation treatments were applied to 3 different pepper cultivars. Experimental irrigation treatments were initiated when the plant cover ratio reached to 30% level (for better plant emergence) (15th of July). Until such a ratio, entire plants were irrigated at the optimum levels with equal amounts of water. A total of 162.5 mm irrigation water was applied until July 15, 2005 as not to create a water stress over the plants. IRSIS irrigation treatment was created by using long-term meteorological data of Akçakale meteorology station and FAO Penman-Monteith model. Amount of water to be applied in each irrigations was calculated by using the Equation 1.

$$I = E_{pan} * A * K_{cp} * P \quad (1)$$

Where; I, amount of irrigation water (L); E_{pan}, cumulative evaporation (mm); K_{cp}, plant-pan coefficient (1.25, 1, 0.75 and 0.50); P, plant cover ratio (obtained by dividing canopy width with row spacing); A, plot size (m^2).

Soil moisture measurements were taken up to 120 cm soil depth at planting, before each irrigation and at hand picking, gravimetrically. Actual water consumption of pepper was calculated by using water-budget equation (Equation 2).

$$ET = I + P - D \pm R \pm \Delta s \quad (2)$$

Where; ET, evapotranspiration (mm); I, irrigation water (mm); P, precipitation (mm); D, deep percolation (mm); R, runoff (mm); Δs, soil moisture variation between two sampling (mm).

Although the effective root depth of pepper was 60 cm, soil moisture measurements were made till 120 cm soil depth in order to consider deep percolation and runoff was taken as zero since drip

irrigation was used. Capillary rise was not also taken into consideration since there were not any groundwater problems in the field.

Plant height and stem diameter were measured over 5 plants selected from the middle sections of all treatment plots. Plant height was measured as the height from the root collar to top of the plant and average of replications was taken. A digital caliper was used to measure stem diameters at 5 cm above the root collar. Plant moisture analysis was carried out in accordance 1997-ASTA Method 2.0, color value with 1997-ASTA Method 20.1, Capsaicin analysis with 1997-ASTA Method 21.3 (ASTA 1997), vitamin C with Ruckemann (1980), Beta Carotene analysis with Bushway (1986) at Ankara Municipal Food Control Laboratories.

3. Results and Discussion

3.1. Irrigation water-ET-yield relationships

A total of 162.5 mm water was applied to each treatment until 30% plant cover level on 15th of July. Total seasonal irrigation water applications for the treatments IRSIS, CAP_{125}, CAP_{100}, CAP_{75} and CAP_{50} were respectively realized as 874, 568, 506, 383 and 254 mm. Seasonal ET values varied based on cultivars. The highest ET value was observed in IRSIS treatment of Queen cultivar with 927 mm and the lowest value in CAP_{50} of Queen with 376 mm (Table 3).

Optimal irrigation scheduling is very crucial for conserving water, nutrients, as well as improving the productivity and quality of the plants. Many studies investigated the effect of irrigation frequency on the growth and yield of paprika plants grown in the fields (Jaimez et al 1999; Sezen et al 2006). Yildirim et al (1994) carried out a drip irrigation study on pepper and reported the applied amounts of waters as between 395.4 and 718.6 mm. Kirnak et al (2003) implemented a drip irrigation study on local peppers of Şanlıurfa Province with three different irrigation intervals (2, 4 and 6 days) and three different Pan-coefficients (1.25, 1.0 and 0.75) and reported seasonal water applications of between 652-1010 mm, seasonal ET of between 726-1069 mm. Similar results were also observed by Degirmenci & Sozbilici (1995) with researches over the same plain. Wierenga & Hendrickc (1985) investigated the impacts of irrigation levels on yield and quality of chili peppers and observed the highest yields at irrigation levels of between 800-950 mm.

Average marketable yield also varied based on cultivar and irrigation water. The highest value was observed in IRSIS treatments of Ace with 38.13 t ha^{-1} and the lowest value was seen in CAP_{50} of King with 8.74 t ha^{-1} (Table 4). A second degree polynomial relationship was identified between marketable yield and ET (Figure 1). Increasing irrigation water resulted in increasing discard ratios.

Table 3- Irrigation water, ET and yield values for paprika pepper

Çizelge 3- Paprika biberi sulama suyu, ET ve verim değerleri

Treatment	*Seasonal irrigation water (mm)*	*Seasonal ET (mm)*			*Average marketable yield (t ha^{-1})*			*Discard (kg ha^{-1})*		
		Ace	*King*	*Queen*	*Ace*	*King*	*Queen*	*Ace*	*King*	*Queen*
IRSIS	874	920	907	927	38.13	19.57	22.33	750	1680	700
CAP_{125}	568	613	615	609	27.60	14.50	26.48	710	1280	550
CAP_{100}	506	567	548	561	18.03	12.07	19.54	350	640	360
CAP_{75}	383	469	570	477	14.84	10.29	16.69	340	350	190
CAP_{50}	254	377	381	376	12.95	8.74	14.24	210	310	240

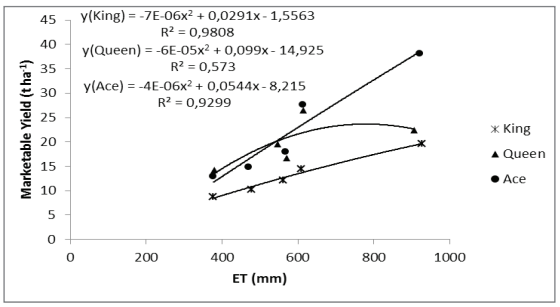

Figure 1- ET-yield relationships for paprika pepper cultivars

Şekil 1- Paprika biber çeşitleri için ET-verim ilişkileri

Considering the actual climate data of the growth season, irrigation treatments (IRSIS, CAP_{125}, CAP_{100}, CAP_{75} and CAP_{50}) were evaluated by IRSIS software and effects of water deficit (Eta ETm^{-1}) on yield (Ya Ym^{-1}) and stress periods were investigated. Water-yield relationships and water application efficiencies are provided in Table 4.

According to Table 4, (1) Since the water deficit in CAP_{50} treatment already exceeded 50%, the treatment should not be implemented, (2) IRSIS evaluations based on actual seasonal data revealed that excessive water was applied and therefore deep percolation was seen (77 mm), (3) Yield response factor (ky) was assumed to be 1.10 while running IRSIS simulation model, but actual data revealed a ky value of 1.29. Such a case indicated that lower value was selected in IRSIS simulations for the region.

Seasonal variations in water depletion within root region of different irrigation treatments were determined by IRSIS simulations and presented in Figure 2. As indicated in figure, water deficit was observed in all irrigation treatments, except for IRSIS treatment, from planting to end of flowering period with intensive vegetative growth. Moisture depletion within the root zones of deficit irrigation treatments increased with the level of deficit.

Shmueli & Goldberg (1972) carried out a drip irrigation research on peppers in Israel under dry conditions and applied four different irrigation levels (0.83, 0.95, 1.33 and 1.75 times of Class-A-Pan

Table 4- Evaluation of irrigation treatments with IRSIS software

Çizelge 4- IRSIS programı ile sulama uygulamalarının değerlendirilmesi

	Eta ETm^{-1}	*Irrigation water (mm)*	*Ya Ym^{-1}*	*Irrigation efficiency (%)*	*Drainage water (mm)*	*Runoff water (mm)*
IRSIS planning based on long-term climate data	1.00	794	100.0	100.0		
IRSIS planning based on actual climate data of the growth season	0.96	858	96.2	91.0	77	0
Evaluation of CAP_{125} treatment with IRSIS	0.74	583	65.9	98.1	11	0
Evaluation of CAP_{100} treatment with IRSIS	0.68	505	58.6	99.8	1	0
Evaluation of CAP_{75} treatment with IRSIS	0.55	363	44.0	100.0	0	0
Evaluation of CAP_{50} treatment with IRSIS	0.38	220	27.0	100.0	0	0

Eta, actual evapotranspiration; ETm, maximum evapotranspiration; Ya, actual yiled; Ym, maximum yield

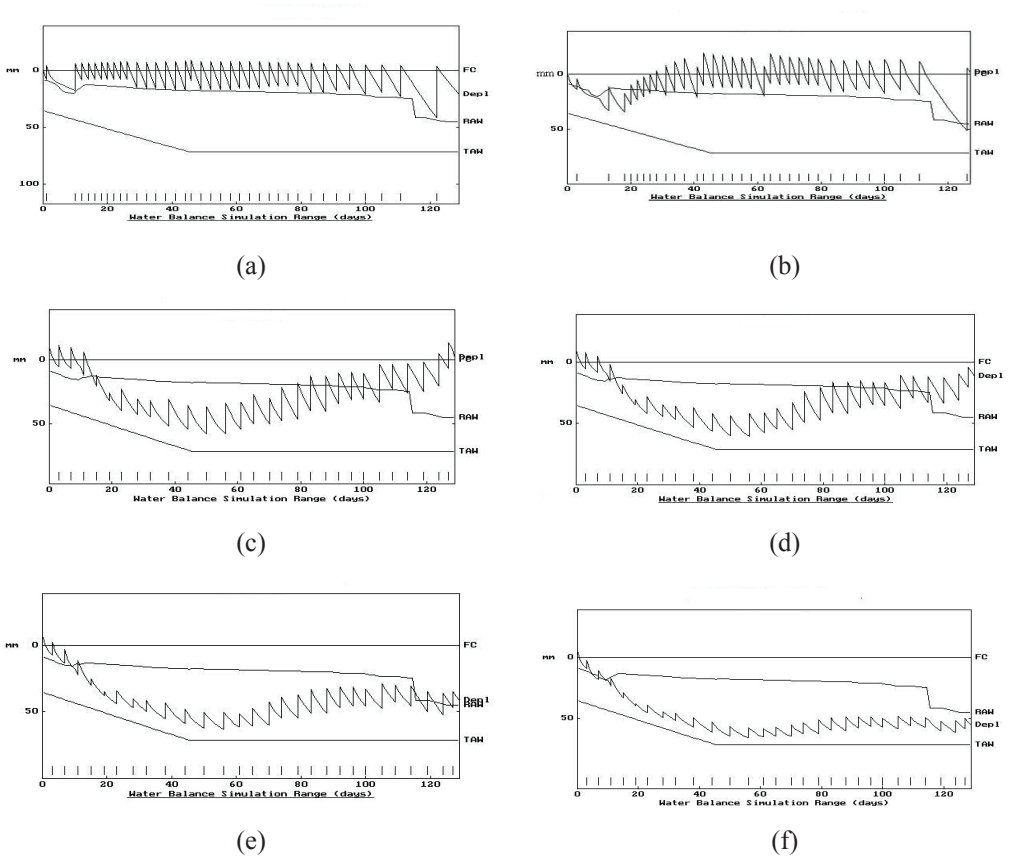

Figure 2- IRSIS simulations of irrigation treatments a, based on long-term climate data; b, based on actual growing season data; c, CAP_{125} based on actual data; d, CAP_{100} based on actual data; e, CAP_{75} based on actual data; f, CAP_{50} based on actual data (FC, field capacity; RAW, readily available water; TAW, total available water; Dep1, moisture depletion)

Şekil 2- Sulama uygulamalarının IRSIS sulama programında değerlendirilmesi a, uzun-yıllar iklim verilerine göre; b, 2005 yılı gerçek verilerine göre; c, 2005 yılı gerçek verilerine göre CAP_{125}; d, 2005 yılı gerçek verilerine göre CAP_{100}; e, 2005 yılı gerçek verilerine göre CAP_{75}; f, 2005 yılı gerçek verilerine göre CAP_{50}. (FC, tarla kapacitesi; RAW, mevcut yarayışlı Su; TAW, toplam yarayışlı su; Dept1, nem azalması)

evaporation) and indicated a yield response factor of 1.33. Palada & O'Keefe (2001) investigated the response of hot pepper cultivars to levels of drip irrigation in the Virgin Islands and observed increasing yield trends with increasing amounts of irrigation water. Similarly, increasing yield levels were also reported with drip irrigation in previous studies (Palada et al 2001; Palada & O'Keefe 2003). Park & Jung (2000) carried out a study in Korea and indicated the significance of moisture profile within root region for optimum irrigation programs especially for dry regions.

Effects of irrigation, cultivar and interactions on yield and plant physical characteristics are provided in Table 5. While the effects of cultivar on yield were significant at $P<0.05$ level, effects of irrigation and irrigation x cultivar interaction were found to

Table 5- Effects of irrigation, cultivar and interactions on yield and physiological characteristics

Çizelge 5- Sulama, çeşit ve interaksiyonların verim ve fizyolojik özellikler üzerine etkileri

Treatments	*Mean yield (t ha^{-1})*	*Plant height (cm)*	*Stem diameter (mm)*	*Canopy diameter (cm)*
Irrigations				
IRSIS	25.63 a*	65.22 b	13.75 b	54.65 b
CAP_{125}	22.01 b	67.88 a	15.21 a	64.85 a
CAP_{100}	16.10 c	65.00 b	13.22 bc	65.83 a
CAP_{75}	13.65 cd	66.88 ab	12.95 cd	63.88 a
CAP_{50}	11.72 d	59.44 c	12.60 d	55.65 b
Mean	17.82	64.889	13.549	60.978
LSD (0.05)	0.4830	2.379	0.6184	2.319
CV (%)	19.99	3.77	4.69	3.91
Cultivar				
Ace	21.84 a	68.26 a	14.46 a	63.93 a
King	12.18 b	63.06 b	13.65 a	61.13 b
Queen	19.45 a	63.33 b	12.52 b	57.87 c
Mean	17.82	64.88	13.549	60.978
LSD (0.05)	0.4830	2.762	1.093	2.581
CV (%)	19.99	3.77	4.69	3.91
Interactions				
Ace × IRSIS	37.38 a	65.00 de	14.00 bcd	55.90 c
Ace × CAP_{125}	26.89 b	67.33 bcde	15.00 ab	64.33 b
Ace × CAP_{100}	17.68 cde	71.00 ab	14.90 abc	67.97 ab
Ace × CAP_{75}	14.50 defg	72.66 a	13.53 d	67.07 ab
Ace × CAP_{50}	12.74 efgh	65.33 de	14.90 abc	64.50 b
King × IRSIS	17.89 cde	63.66 ef	13.90 cd	63.07 cd
King × CAP_{125}	13.22 defgh	66.33 cde	15.53 a	65.27 b
King × CAP_{100}	11.43 fgh	60.33 fg	13.267 d	65.53 b
King × CAP_{75}	9.94 gh	69.00 abcd	13.900 cd	69.87 a
King × CAP_{50}	8.43 h	56.00 h	11.67 e	51.83 de
Queen × IRSIS	21.63 bc	67.00 bcde	13.37 d	55.00 cd
Queen × CAP_{125}	25.93 b	70.00 abc	15.10 a	64.97 b
Queen × CAP_{100}	19.18 cd	63.66 ef	11.50 e	64.00 b
Queen × CAP_{75}	16.50 cdef	59.00 gh	11.43 e	54.73 cd
Queen × CAP_{50}	14.00 defgh	57.00 gh	11.23 e	50.633 e
Mean	17.94	64.88	13.549	60.978
LSD (0.05)	13.86	4.12	1.071	4.017
CV (%)	11.24	3.77	4.69	3.91

*, the means indicated with the same letter are not statistically significant

be significant at P<0.01 level. Average pepper yield was observed as 17.82 t ha^{-1}. The highest yield was observed in IRSIS irrigation with 25.63 t ha^{-1} and it was followed by CAP_{125} irrigation with 22.01 t ha^{-1}. Decreasing yields were observed in CAP irrigations with decreasing irrigation waters. While IRSIS and CAP_{125} were placed in different yield groups, CAP_{100} - CAP_{75} and CAP_{50} - CAP_{75} were placed into same groups. The lowest yield was seen in CAP_{50} with 11.72 t ha^{-1}.

While the cultivars Ace and Queen were placed in the same yield group, King was placed in a

different group. The highest yield was observed in Ace with 21.84 t ha^{-1} and the lowest yield was seen in King with 12.19 t ha^{-1}. With regard to interactions, while Ace × IRSIS interaction had the highest yield with 37.38 t ha^{-1}, King × CAP_{50} interaction had the lowest yield with 8.43 t ha^{-1}.

3.2. Effects of irrigations on physiological characteristics

While the cultivar had significant effects on plant height and diameter at $P<0.05$, irrigation and irrigation × cultivar interaction had significant impacts at $P<0.01$ level. Average plant height was observed as 64.8 cm. The highest value was seen in CAP_{125} irrigation with 67.8 cm and it was followed by IRSIS irrigation with 65.2 cm. The lowest value was observed in CAP_{50} treatment with 59.4 cm. IRSIS, CAP_{100} and CAP_{75} were placed into the same plant height group.

While the cultivars King and Queen were placed into the same height group, Ace was placed into another group. The highest plant height was observed in Ace with 68.2 cm and the lowest in King with 63.1cm. With regard to interactions, while Ace × CAP_{75} interaction yielded the highest plant height with 72.6 cm, the lowest value was observed in King × CAP_{50} interaction with 56 cm.

Average plant stem diameter was observed as 13.5 mm with the highest value in CAP_{125} irrigation (15.2 mm) and the lowest value in CAP_{50} irrigation (12.6 mm). While IRSIS and CAP_{125} were placed into different diameter groups, CAP_{75} and CAP_{100} were placed into the same group.

While the cultivars King and Ace were placed into the same diameter group, Queen was placed into a different group. The highest value was observed in Ace with 14.4 mm and the lowest in Queen with 12.5 mm. With regard to interactions, King × CAP_{125} interaction yielded the highest plant stem diameter with 15.5 mm and Queen × CAP_{50} interactions had the lowest diameter with 11.2 mm.

Effects of irrigation, cultivar and irrigation × cultivar interactions on plant canopy diameter were found to be significant at $P<0.01$ level. Average canopy diameter was observed as 60.9 cm. The highest value was obtained from CAP_{100} treatment with 65.8 cm and it was followed by CAP_{125} treatment with 64.8 cm. The lowest value was seen in CAP_{50} treatment with 55.6 cm. While IRSIS and CAP_{50} were placed into the same canopy diameter group, other irrigation treatments (CAP_{125}, CAP_{100} and CAP_{75}) were placed into another group.

With regard to canopy diameters of cultivars, all of them were placed into different groups. The highest value was observed in Ace with 63.9 cm and the lowest in Queen with 57.8 cm. Considering the interactions, Ace × CAP_{100} had the highest canopy diameter with 67.9 cm and Queen × CAP_{50} had the lowest canopy diameter with 50.6 cm.

Yohannes & Tadesse (1998) investigated yield and yield components of tomato under different irrigation systems and indicated drip irrigation as the best method for vegetable irrigation. Rista et al (1995) reported lower root collar (*Phytophthora capcisi*) incidences in drip irrigation than furrow irrigation. Current findings regarding the entire vegetative characteristics and their relationships with irrigation water comply with the findings of previous studies carried out on peppers (Bracy et al 1995; Degirmenci & Sozbilici 1995; Cevik et al 1996).

3.3. Effects of irrigations on quality parameters

As the quality parameters of paprika peppers, moisture content, ASTA value (color), capsicine, vitamin C and beta-carotene were investigated. Effects of irrigations, cultivars and interactions on quality parameters are presented in Table 6. Mean moisture content was observed as 84.1%, ASTA value as 261.1, capsicine content as 0.4 mg kg^{-1}, vitamin C content as 1890.4 mg kg^{-1} and beta carotene content as 98.9 mg kg^{-1}.

While the effects of irrigations on moisture contents were insignificant, effects of cultivar and interactions were found to be significant. The highest moisture content was observed in Ace with 85.3%. The cultivars Queen and King were statistically

placed into the same moisture content group. With regard to moisture contents of interactions, Ace × CAP_{100} interaction had the highest moisture content with 85.9% and King × IRSIS interaction had the lowest value with 81.8%.

The highest ASTA value was observed in IRSIS irrigation with 286.2 and it was followed by CAP_{50} irrigation with 282.5. The lowest value was seen in CAP_{100} irrigation with 225.7. While IRSIS and CAP_{50} were placed into the same ASTA group, CAP_{75} and CAP_{125} were placed into another same group and CAP_{100} was placed into a different group.

With regard to ASTA values of the cultivars, all of them were placed into different groups. The highest value was observed in Ace with 285.5 and the lowest value was seen in Queen with 236.0. With regard to interactions, Ace × CAP_{50} had the highest value with 327.1 and Queen × CAP_{100} interaction had the lowest value with 185.3. Other interactions had values in between them (Table 6).

Table 6- Effects of irrigation, cultivar and interactions on quality parameters

Çizelge 6- Sulama, çeşit ve interaksiyonların kalite özellikleri üzerine etkileri

Treatment	*Moisture content (%)*	*ASTA*	*Capsicine (mg kg^{-1})*	*Vitamine C (mg kg^{-1})*	*Beta carotene (mg kg^{-1})*
Irrigation					
IRSIS	83.96 ns	286.22 a*	0.44 ns	2117.88 ns	77.88 ns
CAP_{125}	83.70	264.34 b	0.53	2408.11	96.22
CAP_{100}	84.76	225.76 c	0.43	1840.77	113.00
CAP_{75}	84.25	247.04 b	0.41	1681.11	99.11
CAP_{50}	84.01	282.51 a	0.43	1404.11	108.44
Mean	84.140	261.178	0.452	1890.400	98.93
LSD (0.05)	-	17.85	-	-	-
CV (%)	1.250	7.03	20.81	40.27	34.08
Cultivar					
Ace	85.38 a	285.56 a	0.37 b	1837.00 ab	92.80 ns
King	83.70 b	261.93 b	0.53 a	2437.00 a	116.73
Queen	83.32 b	236.03 c	0.44 ab	1397.20 b	87.26
Mean	84.140	261.178	0.452	1890.400	98.93
LSD (0.05)	0.8561	18.51	0.1014	685.5	-
CV (%)	1.250	7.03	20.81	40.27	34.08
Interaction					
Ace × IRSIS	85.93 ab	307.33 ab	0.38 ns	2309.00 ns	72.00 ns
Ace × CAP_{125}	85.33 abc	265.36 cdef	0.37	2020.00	107.66
Ace × CAP_{100}	85.97 a	261.50 def	0.36	1715.66	105.00
Ace × CAP_{75}	85.16 abcd	266.53 cde	0.37	1682.66	71.67
Ace × CAP_{50}	84.53 abcde	327.10 a	0.39	1457.66	107.67
King × IRSIS	81.83 ef	258.20 defg	0.49	2701.66	94.67
King × CAP_{125}	83.80 cde	274.80 cde	0.71	2866.66	95.67
King × CAP_{100}	85.43 abc	230.50 gh	0.55	2358.00	146.00
King × CAP_{75}	83.43 def	260.66 defg	0.45	2201.66	136.67
King × CAP_{50}	84.03 cde	285.50 bcd	0.45	2057.00	110.67
Queen × IRSIS	84.13 cde	293.13 bc	0.46	1343.00	67.00
Queen × CAP_{125}	81.96 ef	252.86 efg	0.51	2337.66	85.33
Queen × CAP_{100}	82.90 ef	185.30 ı	0.37	1448.66	88.00
Queen × CAP_{75}	84.16 bcde	213.93 hı	0.41	1159.00	89.00
Queen × CAP_{50}	83.46 def	234.93 fgh	0.45	697.66	107.00
Mean	84.140	261.178	0.45	1890.400	98.93
LSD (0.05)	1.773	30.92	-	-	-
CV (%)	1.250	7.03	20.81	40.27	34.08

*, the means indicated with the same letter are not statistically significant; ns, not significant

Effects of irrigation, cultivar and interactions on beta carotene content were found to be insignificant. While the effects of irrigation and interactions on vitamin C and capsicine contents were found to be insignificant, effects of cultivar on those parameters were found to be significant at $P<0.05$ level. The highest vitamin C and capsicine contents were observed in cultivar King respectively with 2437.0 and 0.5 mg kg^{-1}.

The quality of paprika pepper is the key to its value and is mostly graded by the industry in terms of ASTA value. Moisture content is a factor effective in pricing. Burt (2006) reported a loss of quality in terms of not only appearance, pungency, fruit splitting and decay but more importantly of the concentration of capsanthin, capsorubin and some carotenoids resulting from increased water levels. Delfine et al (2000) reported that Capsicum *annum L* is one of the most susceptible crops to water stress because of wide transpiring leaf surface and elevated stomatal openings and yet relatively copious amounts of water may be undesirable in terms of resultant fruit yield and quality. The quality of paprika therefore depends on a moisture regime that may not be similar to the level that results in increased yields of the crop. This poses a dilemma for a farmer who is mindful of the fact that quality probably counts more than yield for the paprika crop.

4. Conclusions

Although pepper planting was performed in June, it was pointed out in this study that paprika peppers could reliably be produced in Harran Plain and it could be recommended as an alternative crop for local farmers. Irrigation program prepared by IRSIS simulation model based on long-term annual averages yielded reliable outcomes for the experimental year. It was recommended that yield-response factor (ky) should be selected as 1.29 and used in the model accordingly. Compared to IRSIS simulation model, water stress was observed in Class-A-Pan-based irrigations. Since water stress was at relatively effective levels for yield during root development and flowering periods, the 30% threshold canopy coverage ratio assumed for the initiation of irrigation programs should be increased. Since constant Kc coefficients were used in Pan-based irrigations throughout the entire season, increased water stress was observed. Therefore, different coefficients should be used for each growth period. It was observed in this study that sufficient yield levels of paprika peppers might be achieved through implementation of proper irrigation and care practices.

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Abbreviations and Symbols	
ASTA	American spice trade association
GAP	Southeastern anatolia project
CAP	Class-A-pan
IRSIS	Iirrigation scheduling information system
FAO	Food and agriculture organization
ET	Evepatranspiration
ETa	Actual evapotranspiration
ETm	Maximum evapotranspiration
FC	Field capacity
RAW	Readily available water
TAW	Total available water
Ya	Actual yield
Ym	Maximum yield

References

ASTA (1997). American Spice Trade Assiciation, Official Anaythical Methods of the American Spice Trade Association

Ruckeman (1980). Bestimmung von L-Ascorbinsäure mittels Hochleistungs-Flüssigchromatographie (HPLC), Lebensm Unters Forsch, **171**: 446-448

Bracy R P, Ediling R J & Moser E B (1995). Drip-irrigation management and fertizilition of bell pepper in a humid area. *Procedings of V. International Conference on Micro Irrigation*, Orlondo, Florida, pp. 181-185

Burt J (2006). Growing Capsicums and Chillies. Available in http://www.agwest.org [20 March 2007]

Bushway R J (1986). Determination of beta-carotene in some raw fruits and vegetables by high performance

liquid chromatography. *Journal of Agricultural Food Chemistry* **34**: 409-412

Cevik B, Abak K, Sari N, Kirda C & Topaloglu F (1996). Harran ovası koşullarında damla sulama yöntemiyle sulanan sebzelerde farklı su düzeylerinin verim ve kaliteye etkileri. Çukurova Üniversitesi Ziraat Fakültesi Genel Yayın No: 169, Adana

Degirmenci V & Sozbilici Y (1995). GAP Bölgesi Harran ovası koşullarında biberin sulama programı. Toprak ve Su kaynakları Araştırma yıllığı 1996. T.C. Başbakanlığı Köy Hizmetleri Genel Müdürlüğü APK Daire Başkanlığı Toprak ve Su kaynakları Araştırma Şube Müdürlüğü Yayınları No: 98, Ankara

Delfine S, Loreto F, Centritto M, Santarelli G & Alvino A (2000). Effects of water stress on the yield and photosynthesis of field-grown sweet pepper (*Capsicum annuum* L). *Acta Horticulture* **537**: 223–229

FAOSTAT (2005). Agriculture Database: htpp://apps.Fao.org

Jaimez R E, Rada F & Caria Nunez C (1999). The effect of irrigation frequency on water and carbon relations in three cultivars of sweet pepper (*Capsicum chinense* Jacp) in a tropical semiarid region. *Scientica Horticulture* **81**: 301-308

Kirnak H, Kaya C, Higgs D & Taş I (2003). Responses of drip irrigated bell pepper to water stress and different nitrogen levels with or without mulch cover. *Journal of Plant Nutrition* **26**: 263-277

Langmead P (2003). Appraisal of diversification opportunities, The Zambian Paprika Case Study. AGS Project 214A1, FAO

Palada M C & O'Keefe D A (2001). Response of hot pepper cultivars to levels of drip irrigation in the Virgin Islands. *Proceedings of Caribbean Food Crops Society* **37**: 190-196

Palada M C, Crossman S M A & Collingwood C D (2001). Raising vegetable production with micro-irrigation. *International Water & Irrigation* **21**(2): 41-44

Palada M C, O'Keefe D A & Mitchell J M (2003). Yield and water use of hot peppers under three irrigation regimes. *Horticultural Science* **38**: 707-710

Park S H & Jung H W (2000). Optimal irrigation scheduling of dripper system in the rainfed area. *Proceeding of VI International Conference on Micro Irrigation*, Capetown South Africa, Oct 22-27

Rista L M, Sillon M & Fornasero L (1995). Effect of different irrigation strategies on the mortaily of pepper by phytophtora capsici leonian in greenhouses. *Horticulture Argentina* **14**: 44-51

Sezen S M, Yazar A & Eker S (2006). Effect of drip irrigation regimes on yield and quality of filed grown bell pepper. *Agricultural Water Management* **81**: 115-131

Shmueli M & Goldberg D (1972). Response of trickle-irrigated pepper in an arid zone to various water regimes. *HortScience* **7**: 241-243

U.S Salinity Laboratory Staff (1954). Diagnosis and improvement of saline and alkali soils: U.S. Department of Agriculture Handbook 60, 160 p

Wierenga P J & Hendrickx J M H (1985). Yield and quality of trickle-irrigated chili peppers. *Agricultural Water Management* **9**(4): 339-356

Yildirim O, Yanmaz R, Aldemir D & Atak H (1994). Damla yöntemiyle sulanan biber bitkisinde uygun sulama aralığı ve sulama suyu ihtiyacının belirlenmesi. Ankara University Agricultural Faculty Publication No: 1372, Ankara

Yohannes F & Tadesse T (1998). Effects of drip and furrow irrigation and plant spacing on yield of tomato at dire dawa, Ethiopia. *Agricultural Water Management* **35**: 201-207

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Influence of Year, Parity and Birth Type on Milk Yield and Milk Components of Bandırma Sheep (German Black Head Mutton x Kıvırcık)

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ABSTRACT

This study was conducted to investigate the effects of year, parity and birth type (BT) on lactation milk yield (LMY), adjusted lactation milk yield based on 150 days lactation length (LMY150), lactation length (LL), average daily milk yield (ADMY) and milk components (MC) of Bandırma sheep. The study was carried out with 54 ewes in 2011 and 70 ewes in 2012 under semi-intensive conditions. LMY, LMY150, LL and ADMY of Bandırma sheep were determined as 70.31 kg, 73.29 kg, 142.02 days and 488.19 g, respectively. The effect of the year on LL was significant ($P<0.01$), but the effects of parity and BT were not significant. The effects of the year and parity were significant ($P<0.01$ and $P<0.05$, respectively) on LMY, LMY150 and ADMY, but the effect of BT was not significant. Fat, protein, lactose, total dry matter (DM) and non-fat dry matter (NFDM) were determined as 5.26%, 6.11%, 3.29%, 15.49% and 10.23%, respectively. Highly significant positive correlations were determined between fat content and DM ($r= 0.998$; $P<0.01$) and between fat content and NFDM ($r= 0.949$; $P<0.01$), whereas fat content was negatively correlated with ADMY ($r= -0.992$; $P<0.01$) and lactose content ($r= -0.957$; $P<0.01$). Significant negative correlations ($P<0.01$) were found between lactose content and other milk components except for protein content and ADMY. The results indicated that despite being a mutton type crossbred, lactation characteristics and MC of Bandırma sheep are also considerable.

Keywords: Mutton sheep; Synthetic breed; Lactation; Parity; Birth type

Bandırma Koyununun (Alman Siyah Başlı Et Koyunu x Kıvırcık) Süt Verimi ve Süt Bileşenlerine Yıl, Laktasyon Sırası ve Doğum Tipinin Etkisi

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ÖZET

Bu araştırma, Bandırma koyunlarının laktasyon süt verimi (LSV), 150 gün laktasyon uzunluğuna göre düzeltilmiş süt verimi (LSV150), laktasyon uzunluğu (LU), günlük ortalama süt verimi (GOSV) ve süt bileşenlerine (SB) yıl, laktasyon sırası ve doğum tipinin etkisini araştırmak için gerçekleştirilmiştir. Araştırma, 2011 ve 2012 yıllarında sırasıyla 54 ve 70 koyun ile yarı entansif koşullar altında yürütülmüştür. Bandırma koyunlarının LSV, LSV150, LU ve GOSV'i sırasıyla 70.31 kg, 73.29 kg, 142.02 gün ve 488.19 g olarak tespit edilmiştir. LS üzerine yılın etkisi önemli (P<0.01), laktasyon sırası ve doğum tipinin (DT) etkileri ise önemsiz bulunmuştur. LSV, LSV150 ve GOSV üzerine yıl ve laktasyon sırasının etkisi önemli (sırasıyla P<0.01 ve P<0.05), ancak DT'nin etkisi önemsiz bulunmuştur. Yağ, protein, laktoz, toplam kuru madde (KM) ve yağsız kuru madde (YKM) içerikleri sırasıyla % 5.26, % 6.11, % 3.29, % 15.49 ve % 10.23 olarak tespit edilmiştir. Sütteki yağ içeriğinin KM ve YKM ile korelasyonu önemli (P<0.01) ve pozitif (sırasıyla 0.998 ve 0.949), GOSV ve laktoz içeriği ile korelasyonu ise önemli (P<0.01) ve negatif (sırasıyla -0.992 ve -0.957) olarak tespit edilmiştir. Sütteki laktoz içeriği protein ve GOSV hariç diğer tüm süt bileşenleri ile önemli (P<0.01) ve negatif korelasyonlar göstermiştir. Etçi bir melez tip olmasına rağmen Bandırma koyununun laktasyon özellikleri ve süt bileşenleri bakımından da dikkate değer olduğu sonucuna varılmıştır.

Anahtar Kelimeler: Et koyunu; Sentetik ırk; Laktasyon; Laktasyon sırası; Doğum tipi

1. Introduction

Domestic sheep breeds in Turkey usually have low milk yield. Despite this fact, breeders have a tendency to milk the sheep even for a short time. Milk yield is important for both dairy products and rapid growth of the lambs during the suckling period in sheep breeding. Growth performance of the lambs is closely related to milk yield of the ewes. In mutton type sheep, fertility is as important as the milk yield. The ability of the lamb to reach slaughter age earlier is closely related to the amount of milk that the lamb receives from its mother for 2-3 months after birth. Sheep breeding in Turkey is practiced mainly in the form of pasture breeding. As a source of protein and calcium, milk is an important source of nutrition for societies with relatively lower income levels. Sheep milk and products are usually emphasized with their health benefits in industrialized countries.

In the Mediterranean, Balkan and Middle East countries, milk efficiency of sheep is utilized to a great extent. Especially in rural areas in Turkey, local or crossbred sheep breeds are mainly kept for milk and dairy product by local people (Ünal et al 2002). Sheep breeding is practiced mainly with local breeds and crossbred genotypes in Turkey. While meat, milk and wool efficiency vary depending on the breed, most of local breeds are not prolific. Only among them, Sakız (Chios) breed has high milk yield and reproductive ability, while Kıvırcık breed is raised for producing high quality lamb meat, and İvesi (Awassi) breed has high milk efficiency (Kaymakçı 2010).

The results of previous studies indicated that year (Özder et al 2004; Koncagül et al 2012a), birth type (Özder et al 2004), live weight and breed (Esen & Özbey 2002; Ceyhan et al 2007), lactation length (Kuchtik et al 2008) and parity (Koncagül et al 2012a) have significant effects on lactation milk yield (LMY). Moreover, year (Özder et al 2004), lactation period and length (Şahan et al 2005; Kuchtik et al 2008; Yılmaz et al 2011; Kralickova et al 2012) and nutrition (Morand-Fehr et al 2007; De Renobales et al 2012) are factors affecting the milk components as fat, protein and lactose.

Bandırma sheep is a synthetic breed originated from German Black Headed Mutton (GBM) rams and Kıvırcık (K) ewes. At first, K ewes were mated with GBM rams to obtain F_1 genotype. Then, F_1 ewes were divided in to two groups. The F_1 ewes in the first group were mated with GBM rams to obtain backcross offspring $GBMB_1$. Then, Bandırma-I genotype was obtained by pairing $GBMB_1$ ewes with $GBMB_1$ rams (Figure 1). The F_1 ewes in the second group were mated with $GBMB_1$ rams to

obtain the backcross offspring ($GBMB_1F_1$). The ewes and rams from $GBMB_1F_1$ were mated to obtain Bandırma-IIgeno type (Figure 2). Then, all the ewes and rams in Bandırma-I and Bandırma-II genotypes were united as a single flock in the mating season of 2008. The mating of ewes and rams of this single flock were repeated to get synthetic flock named as Bandırma sheep (Ceyhan et al 2011).

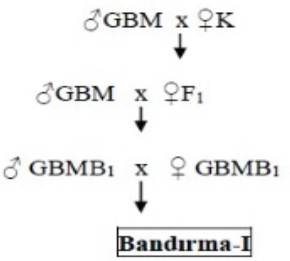


Figure 1- The breeding scheme to form Bandırma-I genotype

Şekil 1- Bandırma-I genotipini yetiştirme şeması

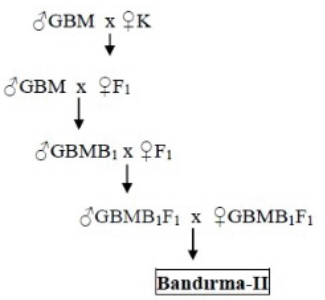


Figure 2- The breeding scheme to form Bandırma-II genotype

Şekil 2- Bandırma-II genotipini yetiştirme şeması

Fertility and growth performance (Ceyhan et al 2006a; Ceyhan et al 2011), fattening performance (Ceyhan et al 2008), primary reproductive characteristics (Sezenler et al 2009) and physiological reactions (Ceyhan et al 2006b) of Bandırma sheep in Marmara Region were investigated in details. But, not much literature is available on lactation characteristics and milk components of Bandırma sheep. Therefore, we aimed to investigate some lactation characteristics and milk components of Bandırma sheep that is a crossbred of German Black Head Mutton and Kıvırcık sheep.

2. Material and Methods

2.1. Animal material and management

The study was carried out with 54 and 70 ewes from Bandırma sheep flock kept in Bandırma Sheep Breeding Research Institute in 2011 and 2012. The Research Farm is located in Balıkesir province in Marmara region of Turkey at longitude of 40^0 21 E, the latitude of 27^0 52 N, and at altitude of 65 m. The mean relative humidity in the region ranges from 20% to 88% and the ambient temperature ranges from -14 to 42.4 ^{0}C. The annual rainfall in the region varies from 500 to 900 mm with an erratic distribution throughout the year (TSMS 2012).

Different feeding and management procedures were applied to the experimental sheep at two consecutive periods. The first period was from birth to the end of the second months of the lactation. During this period, care and feeding procedures of the sheep were carried out indoor. In addition to 1 kg concentrate feed per animal, 1 kg common vetch was given to each animal in two doses of 500 grams in the morning and 500 grams in the evening. The second period was from the beginning of the third month of the lactation to the end of the lactation. In this period, the sheep were kept mainly in the pasture. In addition to the pasture conditions, 1 kg common vetch was given to each animal.

The control day milk yield was taken by means of hand-milking (one day in the morning and the second day in the evening). The control day milk yields were measured every 14 days until the end of the lactation. The first control milk was measured within the first month of the lactation. The lambs were separated from their mothers in the evening and the ewes were milked in the next day morning for control milk. Then, the lambs were kept with their mothers until the morning of the following day and separated from each other, and then the ewes were milked in the evening. After weaning, morning and evening milking were done at 7 am and 7 pm,

respectively in the same control day. The milk was weighed with a weighing instrument sensitive to 5 g. Milking was terminated, when daily milk yield of the sheep fell below 100 g. In every control milking, 50 mL milk sample was taken from each ewe and kept in +4 °C for fat, protein, lactose, dry matter (DM) and non-fat dry matter (NFDM) analyses. The analysis of milk components (MC) was carried out using MIRIS Dairy Milk Analyzer device.

Fleishmann method (Barillet et al 1992) was used to obtain lactation milk yield for each ewe, and the model described as in Equation 1.

$$Yield = Y_1 x T_1 + \sum_{i=2}^{n} \frac{Y_i + Y_{i-1}}{2} x T_i + Y_n x 15 \quad (1)$$

Where; Yield is the LMY or MC; T_1 is the interval (day) from lambing to first milk control-day; T_i is the interval (day) from the $(i-1)^{th}$ milk control day to $(i)^{th}$ milk control day; Y_i is the control day milk yield or MC in the $(i)^{th}$ milk control day, and the 15 is the number of days assumed to proceed from last milk control day to the end of the lactation for a ewe. After obtaining yields for each ewe, all yields and milk components were adjusted to 150 days lactation length.

2.2. Statistical analyses

A general linear model mode l (SAS 2000) was used for the analyses of LMY, LMY150, LL, ADMY and MC. Tukey-Kramer test was applied for *post hoc* analyses. In the analyses, the Equation 2 was used.

$$Y_{ijkl} = \mu + y_i + bt_j + p_k + e_{ijkl} \quad (2)$$

Where; Y_{ijkl}= LL, LMY, ADMY, LMY150 or MC (fat, protein, lactose, DM or NFDM); μ, expected mean of the trait in the analysis; y_i, effect of year (i= 2011, 2012); bt_j, effect of birth type (j= 1, 2; single or twin); p_k, effect of parity (k= 1st, 2nd, 3rd, 4th and 5th lactation); e_{ijkl}, random residual.

Pearson correlation coefficients between ADMY and MC were also calculated (SAS 2000).

3. Results

Least square means and standard errors of LL, ADMY, LMY and LMY150 of Bandırma sheep are given in Table 1. The mean LL, ADMY, LMY and LMY150 were found to be 142.02 day, 488.19 g, 70.31 kg and 73.29 kg, respectively. ADMY, LMY and LMY150 were significantly affected by both

Table 1- Least squares means and standard errors of LL, ADMY, LMY and LMY150 by year, BT and parity

Çizelge 1- LU, GOSV, LSV ve LSV150 özelliklerinin yıl, DT ve laktasyon sırasına göre en küçük kareler ortalamaları ve standart hataları

		LL (days)	*ADMY (g)*	*LMY (kg)*	*LMY150 (kg)*
Factors	*n*	$\overline{X} \pm S_{\overline{x}}$	$\overline{X} \pm S_{\overline{x}}$	$\overline{X} \pm S_{\overline{x}}$	$\overline{X} \pm S_{\overline{x}}$
Years		**	**	**	**
2011	54	149.82±1.446[a]	557.17±17.414[a]	83.63±2.757[b]	83.57±2.612[a]
2012	70	135.00±1.211[b]	414.39±14.576[b]	56.28±2.308[a]	62.16±2.186[b]
BT		ns	ns	ns	ns
Single	80	141.79±1.123	575.06±17.483	70.40±2.140	73.33±2.028
Twin	44	143.03±1.561	591.17±24.260	69.51±2.976	72.41±2.819
Parity		ns	*	*	*
1	22	138.44±2.177	396.05±26.217[a]	55.45±4.151[a]	59.41±3.933[a]
2	27	141.68±1.992	476.43±23.985[ab]	68.12±3.797[ab]	71.47±3.598[ab]
3	23	146.07±2.169	486.11±26.115[ab]	71.28±4.134[ab]	72.92±3.917[ab]
4	26	144.14±1.982	514.94±23.864[b]	75.42±3.778[b]	77.24±3.580[b]
5	26	141.71±1.934	555.38±23.284[b]	79.51±3.686[b]	83.31±3.493[b]
Overall	124	142.02±2.043	488.19±24.516	70.31±3.790	73.29±3.747

[a, b], means within the levels of main factors with different letters differ significantly (*, P<0.05; **, P<0.01; ns, not significant); LL, lactation length; ADMY, average daily milk yield; LMY, lactation milk yield; LMY150, adjusted lactation milk yield according to 150 days lactation length; BT, birth type

year (P<0.01) and parity (P<0.05). The effect of year on LL was also significant (P<0.01), whereas LL was not significantly affected by parity. It was observed that ADMY, LMY and LMY150 increased in relation to increasing number of lactations (parity). However, this increase from 2nd parity to 5th parity was not significant. BT did not significantly affect LL, ADMY, LMY and LMY150 of ewes.

The means of fat, protein, lactose, DM and NFDM content were 5.26%, 6.11%, 3.29%, 15.49% and 10.23%, respectively (Table 2). The effect of year on all MC was significant (P<0.01) except for NFDM, whereas parity and BT did not affect MC of ewes.

Control day curves of average MC (%), milk yield (g) and MC (g) during the lactation period

Table 2- Least squares means and standard errors of milk components (MC) by year, BT and parity

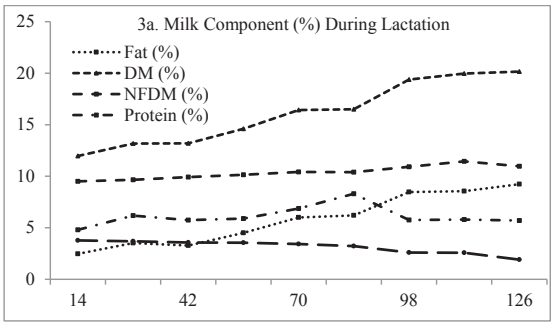
Çizelge 2- Süt bileşenlerinin (SB) yıl, DT ve laktasyon sırasına göre en küçük kareler ortalamaları ve standart hataları

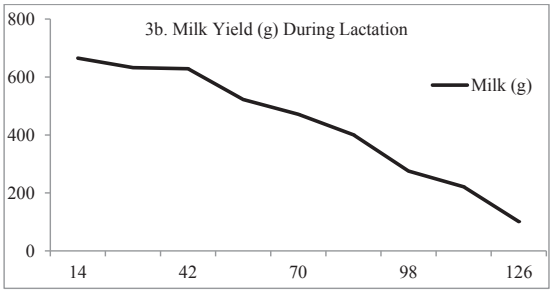
		Fat (%)	*Protein (%)*	*Lactose (%)*	*DM (%)*	*NFDM (%)*
Factors	*n*	$\overline{X} \pm S_{\overline{x}}$	$\overline{X} \pm S_{\overline{x}}$	$\overline{X} \pm S_{\overline{x}}$	$\overline{X} \pm S_{\overline{x}}$	$\overline{X} \pm S_{\overline{x}}$
Years		**	**	**	**	ns
2011	54	5.77±0.123[a]	6.40±0.097[a]	3.63±0.031[a]	16.03±0.157[a]	10.26±0.052
2012	70	4.80±0.103[b]	5.93±0.081[b]	2.95±0.026[b]	15.00±0.131[b]	10.22±0.043
Parity		ns	ns	ns	ns	ns
1	22	5.32±0.186	6.20±0.147	3.29±0.047	15.54±0.236	10.14±0.078
2	26	5.08±0.170	6.26±0.134	3.27±0.043	15.43±0.216	10.37±0.071
3	23	5.32±0.185	6.22±0.146	3.22±0.047	15.60±0.235	10.29±0.078
4	26	5.35±0.169	6.07±0.133	3.34±0.043	15.62±0.215	10.30±0.071
5	27	5.37±0.165	6.09±0.130	3.32±0.042	15.39±0.210	10.11±0.069
BT		ns	ns	ns	ns	ns
Single	80	5.41±0.096	6.07±0.076	3.30±0.024	15.66±0.122	10.22±0.040
Twin	44	5.16±0.133	6.26±0.105	3.27±0.034	15.37±0.169	10.27±0.056
Overall	124	5.26±0.088	6.11±0.063	3.29±0.036	15.49±0.107	10.23±0.033

[a,b], means within the same column and factor with different letters differ significantly (*, P<0.05; **, P<0.01; ns, not significant); NFDM, non-fat dry matter; DM, total dry matter

were presented in Figure 3a, 3b and 3c, respectively. In Figure 3a, DM, NFDM and fat content were found to be lower at the beginning of lactation and then increased towards the end of lactation period. However, the lactose content was initially higher but decreased later on. Slight increase was observed in the protein content during lactation period. However, as light decrease in control day average milk yield (CDMY) was observed during the first two months of the lactation, and then the decrease was higher and steady until the end of the lactation (Figure 3b). Similarly, the amount of MC decreased also during the lactation (Figure 3c). On the other hand, the rate of decrease in MC was smaller in comparison to CDMY due possibly to the reason that total amount of MC in CDMY increased gradually during the lactation.

CDMY and MC variation during lactation period with regard to year, parity and BT were presented in Figure 4. CDMY was higher for the first two months of the lactation and then a continuous decrease was observed until the end of the lactation regardless of year, parity and BT. Different lactation curves were observed for years and parities, but not for BT. On the other hand, increases or decreases in MC were very similar in years, parity or BT.





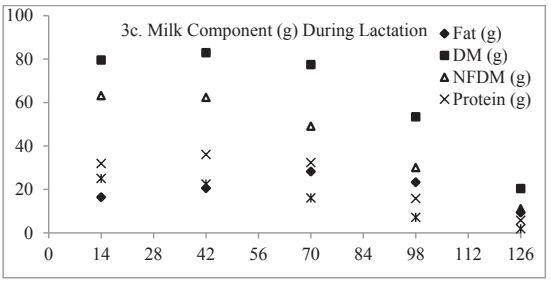


Figure 3- Control day average milk components (%, 3a), milk yield (g, 3b) and milk components (g, 3c) throughout the lactation

Şekil 3- Laktasyon süresince kontrol günü ortalama süt bileşenleri (%, 3a), süt verimi (g, 3b) ve süt bileşenleri (g, 3c)

Phenotypic correlations between ADMY and MC were given in Table 3. Negative and significant correlations were observed between ADMY with DM, NFDM and fat content (ranged from -0.927 to -0.992: P<0.01), while a high and positive correlation was found between ADMY and lactose content (0.965; P<0.01). Highly significant positive correlations were determined between fat content and DM (r= 0.998; P<0.01) and between fat content and NFDM (r= 0.949; P<0.01), whereas fat content was negatively correlated with ADMY (r= -0.992; P<0.01) and lactose content (r= -0.957; P<0.01). Significant negative correlations (P<0.01) were found between lactose content and other milk components except for protein content and ADMY.

4. Discussion

We determined higher LL, LMY and LMY150 incrossbred Bandırma sheep in comparison to LL, LMY and LMY150 values reported for Tuj (Karaoğlu et al 2001), Rambouillet (Ochoa-Cordero et al 2002), Kıvırcık, Gökçeada (Imroz) and Sakız (Ceyhan et al 2007), and for Awassi sheep (Iniguez & Hilali 2009). On the other hand, some authors reported longer LL, but lower LMY in Kıvırcık (Evrim et al 1992), Akkaraman and Hamdani-Akkaraman (F_1) crossbreds (Altın 2001), Akkaraman (Esen & Özbey 2002), Akkaraman, Sakız-Akkaraman (F_1), Kıvırcık-Akkaraman (F_1) and Sakız-Karayaka (B_1) crossbred ewes (Ünal et al 2002), Kıvırcık-Akkaraman (B_1), Akkaraman and Sakız-Akkaraman (B_1) sheep (Mundan & Özbeyaz 2004).The mean LMY and LL of Bandırma sheep in present study were lower than those of Sakız-

Table 3- Phenotypic correlation coefficients between ADMY (kg) and milk components (MC) throughout the lactation

Çizelge 3- Laktasyon süresince GOSV ve süt bileşenleri (SB%) arasındaki fenotipik korelasyon katsayıları

Parameters	*DM (%)*	*NFDM (%)*	*Fat (%)*	*Protein (%)*	*Lactose (%)*
ADMY	-0.986**	-0.927**	-0.992**	-0.254	0.965**
DM %		0.967**	0.998**	0.222	-0.945**
NFDM %			0.949**	0.203	-0.856**
Fat %				0.231	-0.957**
Protein %					-0.093

ADMY, average daily milk yield (kg); NFDM, non-fat dry matter; DM, total dry matter; **, P<0.01

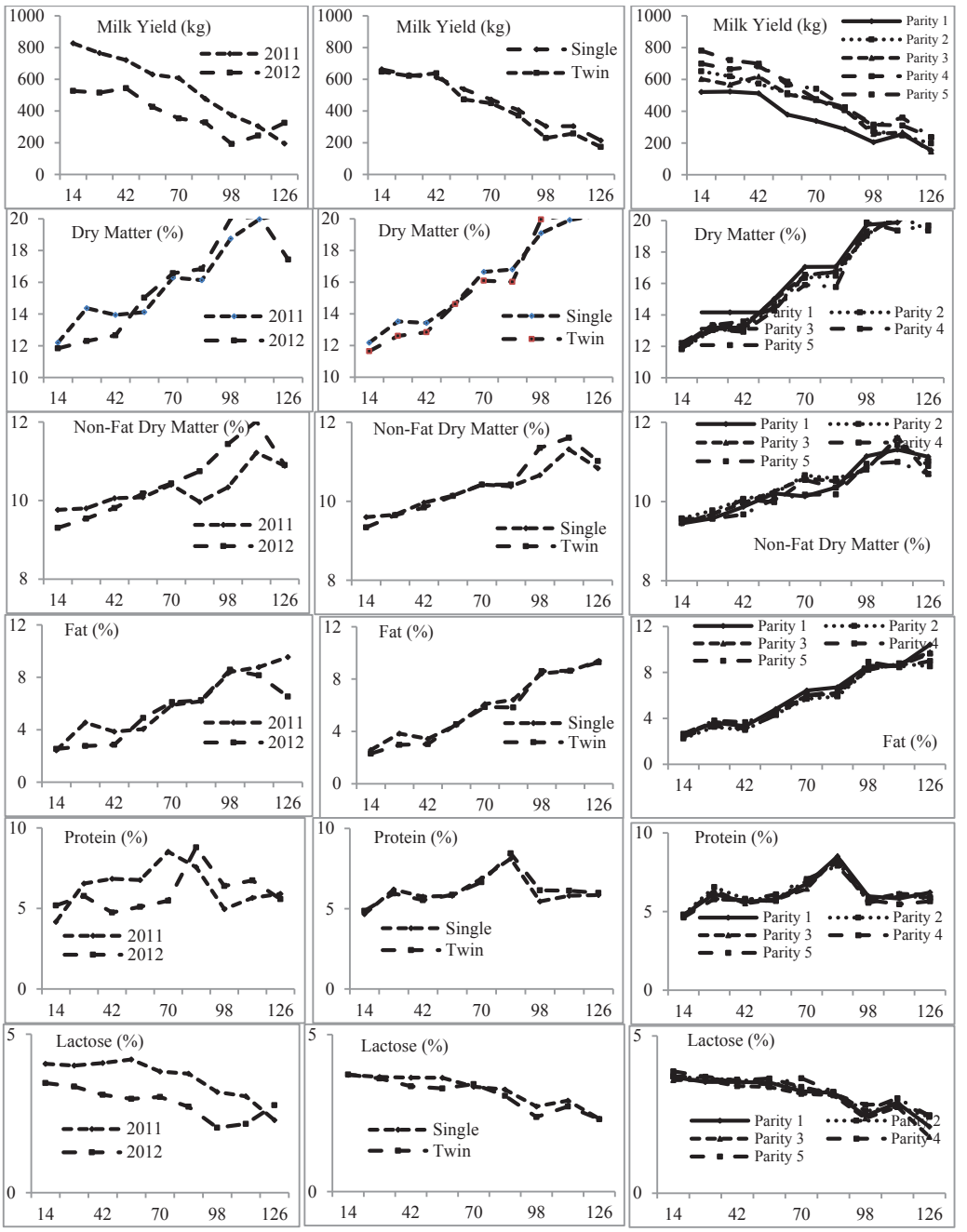


Figure 4- Control day average milk yields (g) and milk components (%) throughout the lactation by year, birth type and parity

Şekil 4- Kontrol günü ortalama süt verimi (g) ve süt bileşenlerinin (%) yıl, doğum tipi ve laktasyon sırası bakımından laktasyon süresince değişimi

Akkaraman (F_1) crossbreds (Esen & Özbey 2002), Sönmez (Kaymakçı et al 2002), Türkgeldi (Özder et al 2004), Awassi (Iniguez & Hilali 2009), Norduz (Ocak et al 2009; Koncagül et al 2012a), Zom sheep (Koncagül et al 2012b), Awassi, Gökçeada and Sakız (Kaymakçı et al 2005), Latxa sheep (Ruiz et al 2000), Awassi sheep (Pollott & Gootwine 2001) and Awassi sheep (Reidal et al 2010).

The previous studies demonstrated that LL depended on both the breed differences and the environmental conditions where sheep were raised. Thus, the lactation milk yield and length of Bandırma sheep raised in semi-intensive conditions were higher and longer than those of some mutton type and native breeds, while lower and shorter than those of milk type breeds and their crosses with native breeds.

The mean ADMY of Bandırma sheep in this study (488.19 g) was higher than those reported for Akkaraman and Hamdani-Akkaraman (F_1) crossbreds (Altın 2001), Tuj (Karaoğlu et al 2001), Akkaraman, Kıvırcık-Akkaraman and Sakız-Akkaraman (Mundan & Özbeyaz 2004), Kıvırcık and Gökçeada sheep (Ceyhan et al 2007). However, the mean ADMY was lower than the findings reported for Sönmez (Kaymakçı et al 2002), Sakız (Ceyhan et al 2007), Awassi (Al-Jundi 2010) and Zom sheep (Koncagül et al 2012b). Our results demonstrated that Bandırma sheep has higher milk yield and longer lactation period than Akkaraman breed and its crossbreds.

Main components of milk vary based on factors such as feeding and management, birth type, season, lactation period, breed and breast health (Park et al 2007). One of the most important components of milk is milk fat. In the current study, the mean fat content of Bandırma sheep milk (5.26%) was higher than those reported for Akkaraman (Esen & Özbey 2002), Rambouillet (Ochoa-Cordero et al 2002), Norduz (Ocak et al 2009) and Awassi sheep (Al-Jundi 2010). On the other hand, milk fat content found in the current study was lower than those reported for Tuj (Karaoğlu et al 2001), Karakaş (Karaca et al 2003) and Morkaraman sheep (Yılmaz et al 2011). Yılmaz et al (2011) reported that milk fat and protein contents were the lowest at the beginning of lactation (6.20% and 5.72%) and the highest at the end of the lactation (6.44% and 6.80%) in Morkaraman sheep (Yılmaz et al 2011). Our findings in this study are consistent with the findings of Yılmaz et al (2011). High or low fat content in the milk is closely related to the milk flavor and milk energy value. In particular, ewe producing milk with enough fat content is of great importance in fulfilling the energy needs of the lambs during the suckling period. From this perspective, it can be stated that Bandırma sheep is in desired level for milk fat content in comparison to mutton type Rambouillet, dual purpose (meat and milk) Akkaraman and Norduz, and milk type Awassi sheep breeds.

Protein content of milk determined in this study (6.11%) was higher than those reported for Rambouillet (Ochoa-Cordero et al 2002),but lower than those reported for Norduz (Ocak et al 2009), Morkaraman (Yılmaz et al 2011), Türkgeldi (Özder et al 2004) and Awassi (Şahan et al 2005; Murray et al 2009). The mean lactose content of Bandırma sheep milk (3.29%) determined in this study was lower than those of Ramboillet (5.5%; Ochoa-Cordero et al 2002), Awassi (4.34%; Şahan et al 2005, 5.01%; Al-Jundi 2010) and Morkaraman (5.12%; Yılmaz et al 2011). Protein is the most expensive part of a diet. Because the rumen manufactures protein from amino acids, the amount of protein is considerably more important than the quality of protein for lambs during suckling period due to the reason that the protein requirements are highest for growing lambs since they are building muscle. From the results of this study, it can conveniently be said that Bandırma sheep produce milk with sufficient protein content required for growth of their lambs.

High growth performance of lambs until weaning is desired in mutton type sheep. Growth and development of lamb during the suckling period depends greatly on the fattening method applied by the mother's milk yield. To reach the desired target body weight ina short period time as much as possible, pre-fattening developments of lambs must

also be well besides the survival of each lamb born alive in order to make economic lamb fattening. The findings in this study showed that Bandırma sheep have sufficient milk production to ensure the growth and development of lambs until weaning. Bandırma sheep might also be advantageous for lamb fattening after weaning under extensive or semi-intensive conditions in Marmara region in addition to its being an alternative crossbred for sheep farms benefiting both from sheep milk and lamb meat.

5. Conclusions

Bandırma sheep can be considered sufficient in terms of LMY, LL and ADMY in addition to its mutton properties. On the other hand, except for lactose content Bandırma sheep has comparable MC values with other domestic breeds and crossbreds. It can also be concluded that this sheep has sufficient milk yield to suckle their lambs during pre-weaning growth period due to the reason that CDMY was the highest and stable during the first two months of lactation.

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References

Altın T (2001). Variation of milk yield in sheep during lactation period and estimation of lactation milk yield by means of different methods. *YYU Journal of Agricultural Science* **11**(2): 1-7

Al-Jundi A (2010). Milk yield and lamb growth of Syrian-Awassi sheep graze on cereal and cereal-legume mixture forages. MSc. Thesis. Çukurova Unversity, Institute of Natural and Applied Science. Adana

Barillet Astruc F J M, de Brauwer P, Casu P S, Fabbri G, Feddersen E, Frangos K, Gabina D, Gama L T, Ruiz T J L & Sanna S R (1992). Int. Committee for Animal Recording Guidelines: International Regulations for Milk Recording in Sheep. pp. 15, Ins, de Elevage, Paris, France

Ceyhan A, Erdoğan İ, Kaptan C, Taluğ A M & Ada M (2006a). A study on production performance of Pure Kıvırcık, German Blackhead, F_1 (German Black Head x Kıvırcık) and B_1 (German Blackhead x F_1) ewes under Bandırma Condition. ÇÜ *Journal of Faculty of Agriculture* **21**(1): 37-44

Ceyhan A, Kaptan C, Ada M, Erdoğan İ & Taluğ A M (2006b). The physiological reactions in Bandırma environment conditions of Kıvırcık, German Black Headed Mutton, (GBH x Kıvırcık) F_1 and (GBH x F_1) B_1 ewe. *Tarım Bilimleri Dergisi-Journal of Agricultural Sciences* **12**(2): 113-120

Ceyhan A, Erdoğan İ & Sezenler T (2007). Some production characteristics of Kıvırcık, Gökçeada and Sakız breeds of sheep conserved as gene resources. *Journal of Tekirdag Agricultural Faculty* **4**(2): 211-218

Ceyhan A, Hanoglu H, Sezenler T & Erdogan İ (2008). The improvement studies on mutton sheep for Marmara region conditions. 2. Fattening and carcass characteristics of lambs. *Bulgarian Journal of Agricultural Science* **14**(6): 606-615

Ceyhan A, Sezenler T, Erdogan İ & Torun O (2011). The improvement studies on Mutton sheep for Marmara Region conditions: I. Fertility, lamb survival and growth performance of lambs. *Turkish Journal of Veterinary and Animal Sciences* **35**: 79-86

De Renobales M, Amores G, Arranz J, Virto M, Barrón L J R, Bustamante M A, Ruiz de Gordoa J C & Nájera A I (2012). Part-time grazing improves sheep milk production and its nutritional characteristics. *Food Chemistry* **130**(1): 90-96

Esen F & Özbey O (2002). Fertility and milk yield characteristics in White Karaman and Chios x White Karaman (F_1) Crossbred Sheep. *Turkish Journal of Veterinary and Animal Sciences* **26**: 503-509

Evrim M, Demir H & Başpınar H (1992). Production performance of Kıvırcık sheep under semi-intensive conditions II. Fertility, milk production, body weight and wool characteristics of ewes. *Journal of the Faculty of Veterinary University İstanbul* **17**(2): 13-24

Iniguez L & Hilali M (2009). Evaluation of Awassi genotypes for improved milk production in Syria. *Livestock Science* **120**: 232-239

Karaca O, Akyüz N, Andıç S & Altın T (2003). Milk yield characteristics of Karakaş sheep. *Turkish Journal of Veterinary and Animal Sciences* **27**: 589-594

Karaoğlu M, Macit M & Aksoy A (2001). Milk production traits of Tushin sheep in semi-intensive conditions. *Turkish Journal of Veterinary and Animal Sciences* **25**: 249-253

Kaymakçı M, Koşum N & Taşkın T (2002). Type fixation at Sönmez sheep (2. Some Body Measurements and Milk Yield Traits). *EU Faculty of Agriculture* **39**(2): 95-101

Kaymakçı M, Eliçin A, Işın F, Taşkın T, Karaca O, Tuncel E, Ertuğrul M, Özder M, Güney O, Gürsoy O, Torun O, Altın T, Emsen H, Seymen S, Geren H, Odabaşı A & Sönmez R (2005). Thecnical and economical approaches on Turkey's small ruminant breeding. *Thecnical Congress of TMMOB Agricultural Engineering Association*. 3-7 January, Ankara, pp.707-726

Kaymakçı M (2010). Advanced Sheep Breeding. Improved Second Edition. Sheep and Goat Breeders Association of Publications. Published by University of Ege. Bornova, İzmir

Koncagül S, Daşkıran İ & Bingöl M (2012a). Factors affecting lactation milk yield and lactation curve of Norduz sheep in farmer condition. *Kafkas Universitesi Veteriner Fak*ültesi *Dergisi* **18**(4): 677-684

Koncagül S, Karataş A, Akça N, Vural M E & Bingöl M (2012b). Factors affecting lactation milk yield and some lactation characteristics of Zom sheep in farmer conditions. *Iğdır University Journal of Institute of Science and Technology* **2**(4): 87-93

Kralickova S, Pokorna M, Kuchtik J & Filipcik R (2012). Effect of parity and stage of lactation on milk yield, composition and quality of organic sheep milk. *Acta Universitatis Agriculturae Et Silviculturae Mendelianae Brunensis* **9**(1): 71-78

Kuchtik J, Sustova K, Urban T & Zapletal D (2008). Effect of the stage of lactation on milk composition, its properties and the quality of rennet curdling in East Friesian ewes. *Czech Journal of Animal Science* **53**(2): 55-63

Morand-Fehr P, Fedele V, Decandia M & Le Frileux Y (2007). Influence of farming and feeding systems on composition and quality of goat and sheep milk. *Small Ruminant Research* **68**: 20-34

Mundan D & Özbeyaz C (2004). Milk yield of White Karaman (WK), Kıvırcık x WK B1 and Chios x WK B1 ewes and growth and livability of crossbred lambs. *Journal of Lalahan Livestock Research Institute* **44**(2): 23-35

Murray T L, Blache D B & Bencini R (2009). The selection of dairy sheep on calm temperament before milking and its effect on management and milk production. *Small Ruminant Research* **87**: 45-49

Ocak E, Bingöl M & Gökdal Ö (2009). The milk yield and milk components of Nordos sheep breeding in Van. *YYU Journal of Agricultural Science* **19**(2): 85-89

Ochoa-Cordero M A,Torres-Hernández G, Ochoa-Alfaro A E,Vega-Roque L & Mandeville P B (2002). Milk yield and compostion of Rambouillet ewes under intensive managment. *Small Ruminant Research* **43**: 269-274

Özder M, Kaymakçı M, Taşkın T, Köycü E, Karaağaç F & Sönmez R (2004). Growth and milk yield traits of Türkgeldi sheep. *Turkish Journal of Veterinary and Animal Sciences* **28**: 195-200

Park Y, Juarez W M, Ramos M & Haenlein G F W (2007). Physico-chemical characteristics of goat and sheep milk. *Small Ruminant Research* **68**: 88-113

Pollotta G E & Gootwine E (2001). A genetic analysis of complete lactation milk production in improved awassi sheep. *Livestock Production Science* **71**: 37-47

Reidal K, Al-Azzawi W, Al-Najjar K, Masri Y, Salhab S, Abdo Z, El-Herek I, Omed H & Saatci M (2010). Factors ınfluencing the milk production of awassi sheep in a flock with the selected lines at the Agricultural Scientific Research Centrein Salamieh/ Syria. *Kafkas Universitesi Veteriner Fakültesi Dergisi* **16**(3): 425-430

Ruiz R, Oregui L M & Herrero M (2000). Comparison of models for describing the lactation curve of latxa sheep and an analysis of factors affecting milk yield. *Journal of Dairy Science* **83**: 2709-2719

SAS (2000). SAS/STAT. SAS Inst. Inc, Cary, NC, USA

Sezenler T, Ceyhan A, Yaman Y, Küçükkebabçı M & Yüksel M A (2009). Reproductive and growth characteristics in the first age of Bandırma-I and Bandırma-II crossbred ewe lambs. *Journal of Tekirdag Agricultural Faculty* **6**(3): 265-272

Şahan N, Say D & Kaçar A (2005). Changes in chemical and mineral contents of Awassi ewes' milk during lactation. *Turkish Journal of Veterinary and Animal Sciences* **29**: 589-593

TSMS (2012). Turkish State Meteorological Service. http://www.mgm.gov.tr. Access: 21.12.2012

Ünal N, Atasoy F, Aytaç M & Akçapınar H (2002). Milk production in Akkaraman, Sakız (Chios) x Akkaraman F_1, Kıvırcık x Akkaraman F_1 and Sakız (Chios) x Karayaka B_1 Ewes during the first lactation. *Turkish Journal of Veterinary and Animal Sciences* **26**: 617-622

Yılmaz O, Çak B & Bolacalı M (2011). Effects of lactation stage, age, birth type and body weight on chemical composotion of Red Karaman sheep milk. *Kafkas Universitesi Veteriner Fakültesi Dergisi* **17**(3): 383-386

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Volatile Compounds, Chemical and Sensory Properties of Butters Sold in Çanakkale

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ABSTRACT

The aim of this study was to determine physical, chemical and sensory properties and volatile components of butter samples sold in Çanakkale. For this purpose, color, viscosity, refractive index, melting point, moisture (%), total acidity (%), acid degree value and fat content (%) of the samples were determined in eleven butter samples. Volatile compounds were identified by gas chromatography-mass spectrometry. Sensory properties of samples were determined by Spectrum™ method. There were significant differences among butter samples in terms of physical, chemical and sensory properties. Viscosity, refractive index, melting point, moisture, total acidity and fat content of the samples ranged between 45.40-62.0 cP, 1.3331-1.4672, 32.50-37.50 ˚C, 15.03-19.06%, 0.24-0.42%, 82-89%, respectively. Diacetyl, acetoin, acetic acid, hexanoic acid, butyric acid and δ-decalactone were major volatiles in butter samples. In addition, cooked, creamy, rancid and margarine-like were the characteristic terms developed by the panelists.

Keywords: Butter; Volatiles; Sensory; Chemical properties

Çanakkale'de Satılan Tereyağlarının Uçucu Bileşenleri, Kimyasal ve Duyusal Özellikleri

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ÖZET

Bu çalışmanın amacı Çanakkale'de satılan tereyağı örneklerinin fiziksel, kimyasal ve duyusal özelliklerinin belirlenmesidir. Bu amaçla onbir tereyağı örneğinin, renk, viskozite, refraktif indeks, erime noktası, nem (%), toplam asitlik (%), asit değeri ve yağ içeriği belirlenmiştir. Uçucu bileşenler gaz kromatografisi-kütle spektrometresi kullanılarak belirlenmiştir. Örneklerin duyusal özellikleri Spectrum™ metodu ile ortaya konmuştur. Fiziksel, kimyasal ve duyusal özellikler bakımından örnekler arasında önemli farklar olduğu bulunmuştur. Örneklerin viskozite, refraktif indeks, erime noktası, nem, toplam asitlik ve yağ içeriği sırasıyla 45.40-62.0 cP, 1.3331-1.4672, 32.50-37.50 ˚C, % 15.03-19.06,

% 0.24-0.42, % 82-89 arasında değişmektedir. Diasetil, asetoin, asetik asit, hekzanoik asit, butirik asit ve δ-dekalakton tereyağı örneklerinin önemli uçucu bileşenleridir. Ayrıca, pişmiş, kremamsı, ransit ve margarin benzeri panelistler tarafından geliştirilmiş karakteristik terimlerdir.

Anahtar Kelimeler: Tereyağı; Uçucular; Duyusal; Kimyasal özellikler

1. Introduction

Butter has 80-90% of milk fat, 10-20% of water, 0.5-0.8% of lactose and lactic acid, 0.6-0.7% of milk proteins and 0.14% of mineral substances (Üçüncü 2010). It is used in processed foods as well as pastries and meals because of its high nutritional value and flavor make it acceptable by consumers (Hocalar 2011). Packaging materials and storage conditions affect physical, chemical and sensory properties of the butter (Krasue et al 2007). Many studies aimed to investigate physical, chemical and textural properties of butter in processing conditions during storage (Senel 2006; Altun et al 2011; Arslan et al 2011; Ronholt et al 2012). Sensory characteristics of the products as well as physical and chemical properties are important properties for consumers. For example, lactones generally give fruity, creamy and buttery odors that are desired characteristics for butter. However, hexanal was described to have an oxidized odour and contributed to undesirable flavour to butter (Mallia et al 2008). There were several researches also carried out on physical, chemical and sensory properties of butter during processing and storage period (Abdel-Mageed & Fadel 1994; Widder & Grosch 1997; Krause et al 2007; Krause et al 2008; Mallia et al 2009). Nevertheless, specifically differences in sensory properties and volatile profiles of butters manufactured by different producers had been discussed in limited studies (Atamer et al 2007; Arslan et al 2011; Şenel et al 2011). The main objective of this study was to investigate and reveal differences in some physical, chemical and sensory properties and volatile profiles of some butter samples provided from the local producers in Canakkale.

2. Material and Methods

2.1. Butter samples

Total eleven commercial butters were evaluated in this study. All samples were provided from local market in September 2012 in Canakkale-Turkey. Samples were packaged in plastic or aluminum foil and samples were approximately 250-350 g. Until analysis, all samples were stored in a freezer and analyzed as duplicate.

2.2. Physical and chemical analysis

L, *a,* and *b* values of butter samples were determined by using Minolta Chroma Meter CR-400 model colorimeter (Minolta Inc., Japan). Viscosity was measured by Brookfield viscometer (Model DV II+ Pro, Brookfield Engineering Laboratories, Inc., MA, USA) integrated with circulating water bath (GFL, Grossburgwedel, Germany) according to Arslan et al (2011). Melting point was measured by capillary tube method according to Nas et al (2001) with minor modification. Refractive index was measured by Abbe 5 Refractometer (Bellingham-Stanly Co., Great Britain) at 40 ^{o}C. Moisture (%), fat (%) and total acidity (lactic acid %) of butters were determined according to Bradley et al (1992). The acid value (AV, mg KOH g^{-1} oil) of butters was determined according to Nas et al (2001).

2.3. Analysis of volatile compounds

Representative six samples were chosen by trained panelists for volatile analysis. Volatile compounds from butters were isolated by SPME technique (Pawliszyn 2012) according to Guneser & Karagul-Yuceer (2011). Volatiles were identified and quantified by gas chromatography-mass spectrometry (HP 6890 GC and 7895 C mass selective detector, Agilent Technologies,

Wilmington, DE, USA). Nonpolar HP5 MS column (J & W Scientific, Folsom, CA) was used for separation of volatile compounds. The GC oven temperature was programmed from 40 to 230 °C at a rate of 10 °C min^{-1} with initial hold of 5 min and final hold time of 20 min. Helium was used as a carrier gas at 1.2 mL min^{-1}. Identification of volatiles was based on the comparison of the mass spectra of unknown compounds with those in the National Institute of Standards and Technology (NIST) and Wiley Registry of Mass Spectral Data, 7th Edition (Wiley). Amount of the compounds was calculated from relative abundances of flavor compounds according to Avsar et al (2004).

2.4. Sensory analysis

Spectrum™ method was used to determine the sensory attributes of the butters (Meilgaard et al 1999). Sensory evaluation was conducted by seven panelists. Panel members were staff and graduate students in the Department of Food Engineering at Canakkale Onsekiz Mart University; four were females and three were males and ages ranged from 27 to 45 years. The panel had approximately 200 h-experience on generation and definition of descriptive terms for dairy foods. The terms used to define taste and flavor of butter were shown in Table 1 (Meilgaard et al 1999; Bradley & Smukowski 2009). Duplicate samples were served in the different sessions.

2.5. Statistical analysis

Analysis of variance (one way ANOVA) was conducted to determine the differences among the butter samples with respect to physical, chemical and sensory properties. Welch test that is a non-parametric test was used for some data which did not meet the prerequisites (homogeneity of variance and equality of variance) for ANOVA. Least Significant Difference (LSD) test was used for separating means in data. Multidimensional Scaling Analysis (MDS, ALSCAL approach) was also conducted to reveal differences or similarities in butters in terms of volatile properties (Sheskin 2004). SPSS for Windows (version 17.0, SPSS Institute Inc., Chicago, IL, USA) (SPSS 2008) and Minitab 16.1 (Minitab Inc., State College, PA, USA) (Minitab 2010) were used for all statistical analyses.

3. Results and Discussion

3.1. Physical and chemical properties

Physical and chemical properties of butter samples were shown in Table 2. *L*, *-a* and *b* values of the samples were significantly different ($P \leq 0.05$). *L*, *-a* and *b* values ranged between 88.34-96.11, 1.92-4.42 and 15.07-33.11, respectively. Sample 2 had the lowest *L* and *b* values, meanwhile the lowest *-a* value was observed in the sample 3. Hence, the highest *-a* value was observed in sample 8. Similar results were reported by other studies (Shukla et al 1994;

Table 1- Descriptive terms used for sensory evaluation of butters

Çizelge 1- Tereyağlarının duyusal değerlendirmesinde kullanılan tanımlayıcı terimler

Descriptors	*Definition*	*References*
Cooked	Aromatics associated with cooked milk	Milk heated to 85 °C for 30 min.
Whey	Aromatics associated with whey powder	Solubilize five g whey powder in 100 mL water
Creamy	Creamy aromatics associated with milk fat	Milk cream
Rancid	Aromatics associated with butyric acid	10 µL butyric acid in methanol
Margarine-like	Aromatics associated with margarine	Margarine at 25 °C
Sweet aromatics	Aromatics associated with sweet	5 mg vanillin in milk
Oxidized	Aromatics associated with stale/oxidized fats	Stored butter or vegetable oil
Storage/plastic	Aromatics associated with warehouse	Assignment by panelist
Stale	Aromatics associated with stale flavor	Assignment by panelist
Sweet	Taste sensation elicited by sugars	2% sucrose solution in water

Jinjarak et al 2006). *L*, *a* and *b* values of butters were determined as 88.67, -1.07 and 24.12, respectively by Shukla et al (1994). Moreover, Jinjarak et al (2006) revealed that no significant differences in *L, a* and *b* values of sweet cream, whey and cultured butter. It was found that *L* values of sweet cream, whey and cultured butter were 82.62, 85.89 and 85.14, while *b* values were 20.04, 20.96 and 22.46, respectively. The color of butter is the most important sensory quality parameter in terms of consumer preference. The color changes depending on many processing factors such as seasonal variations in milk content, microbial quality and storage conditions. It was emphasized that more yellowish color in butter associated with natural and "easier to spread" by consumer. Moreover, lack of color uniformity and mottled color in butter occurred by mixed churnings and an uneven distribution of moisture respectively (Krasue et al 2007; Bradley & Smukowski 2009). In the present study, samples 4 and 7 had the higher *b* values than other butters.

There were significant differences in terms of viscosity, refractive index and melting points of butter samples. Viscosities of butters ranged between 45.4-62.7 cP. Sample 11 had the highest viscosity while the lowest viscosity was observed in sample 6. Melting points of butters ranged between 31-37.5 °C. While sample 4 had the highest melting point (37.50 °C), the lowest melting points were observed in sample 10 and 5. The lowest refractive indexes were observed in samples 1 and 5 (Table 2). Sagdıc et al (2004) determined the melting points of traditional yayik butters produced from goat's, ewe's and cow's milk. The melting points were found to be 31.75 °C, 33.05 °C and 32.05 °C for goat's, ewe's and cow's milk butters, respectively. Fatouh et al (2003) investigated the melting point of the various buffalo butter oil fractions obtained by multi-step dry fractionation. They determined that the slip melting points were ranged between 24.2-45.0 °C for liquid and solid fraction of butter oil that obtained at different temperatures (15-40 °C). Glibowski et al (2008) investigated the rheological and textural properties of some table fats. They indicated that apparent viscosities of butter (82% milk fat) and sweet cream butter (74% milk fat) were 457 and 427 Pa at 20 °C, respectively. Nikolova et al (2007) investigated the refractive indexes of total 11 butter and margarine samples by using a specially designed laser refractometer. Refractive indexes of butter samples ranged between 1.4347-1.4491 at 40 °C. This range was in agreement with our findings in the present study. Body and textural characteristics of butter were also important for acceptability

Table 2- Physical and chemical properties of butter samples (n= 2, ±SE)

Çizelge 2- Tereyağı örneklerinin fiziksel ve kimyasal özellikleri (n= 2, ±SE)

Butters	*Colour values*			*Melting point (°C)*	*Viscosity (cP)*	*Refractive index*	*Moisture (%)*	*Lactic acid (%)*	*Acid value (mg KOH g^{-1} oil)*	*Fat (%)*
	L	*-a*	*b*							
1	94.27±0.47	3.15±0.17	30.05±0.15	37.50±0.50	50.05±2.35	1.3331±0.01	15.87±0.41	0.42±0.01	0.88±0.01	84.0±0.01
2	88.34±0.78	3.49±0.12	15.07±0.18	32.50±0.50	52.65±0.25	1.4541±0.01	14.86±0.85	0.24±0.01	0.89±0.01	85.0±0.01
3	95.31±0.15	1.92±0.05	19.38±0.14	32.00±0.00	53.05±0.15	1.4541±0.01	15.23±0.57	0.22±0.01	0.55±0.01	83.5±2.12
4	93.23±1.13	2.75±0.16	33.14±0.59	33.50±0.50	50.65±0.15	1.4552±0.01	17.30±0.48	0.32±0.01	0.83±0.05	82.0±0.01
5	91.05±0.14	3.04±0.05	30.31±1.33	31.50±0.50	53.30±1.50	1.3334±0.01	16.67±0.20	0.38±0.01	1.11±0.01	81.5±1.50
6	96.11±0.01	2.93±0.00	31.34±0.02	33.00±1.00	45.40±1.30	1.4554±0.01	15.30±0.08	0.26±0.02	0.61±0.05	85.0±0.01
7	92.34±1.93	2.43±0.08	33.11±2.11	33.00±0.01	59.25±6.25	1.4562±0.01	12.67±0.74	0.30±0.01	0.89±0.01	86.5±0.70
8	95.37±1.22	4.42±0.14	31.05±0.34	33.50±0.50	54.35±1.15	1.4672±0.01	15.66±0.43	0.24±0.01	1.19±0.08	85.0±0.01
9	93.06±1.62	3.29±0.10	27.70±1.09	33.50±0.50	52.75±2.65	1.4551±0.01	14.36±0.54	0.24±0.02	1.22±0.15	86.0±1.00
10	93.09±2.83	3.12±0.05	30.78±1.49	31.00±1.00	54.10±2.80	1.4555±0.01	15.18±0.36	0.26±0.01	0.99±0.00	84.0±1.00
11	91.78±0.32	2.94±0.14	29.70±0.20	32.50±0.50	62.70±3.70	1.4551±0.01	16.45±0.07	0.30±0.01	1.17±0.05	83.0±0.01
LSD	4.00	0.34	3.01	1.82	0.06	0.02	1.24	0.05	0.18	ns

ns, not significant (P≥0.05); SE, standard error

by consumer. Body and textural characteristics of butter was effected by water content, chemical composition, fatty acid composition, polymorphism and structure of milk fat crystal network and also processing steps especially churning and tempering. For example, present of globular water in small droplets in the butter may cause an increase in viscosity or an increase in unsaturated fatty acids level in milk fat fraction may lead to decrease in melting point (Wright et al 2001). It was indicated that high quality butter should melt evenly and disappeared slowly. Moreover, body of high quality butter should be firm and show a distinct waxy and close-knit texture (Bradley & Smukowski 2009). In the present study, samples 5 and 10 had lowest melting points. Viscosities of the samples 2, 3, 5, 7-11 were higher than other samples. Significant differences were observed in moisture contents, total acidities and acid values (mg KOH g^{-1} oil) of the butters ($P \leq 0.05$) while there were no differences among the samples in terms of fat content (%) (Table 2). Moisture (%), total acidity and fat (%) contents ranged between 12.67-17.30%, 0.22-0.42 and 81.5-86.5%, respectively. The highest moisture (17.30%) and the lowest fat content (81.5%) were observed in the samples 4 and 5, respectively. Sample 7 had the highest fat content (86.5%) (Table 2).

Samples 2 and 9 had the lowest moisture contents (14.86% and 14.36%). Our results are in agreement with the findings of other studies (Hayaloglu 1999; Sancak et al 2002; Arslan et al 2011). Turkish Food Codex (Anonymous 2005) for butter, milk fat based spreadable products and anhydrous milk fat require up to 16% moisture and at least 80% milk fat in butter. Moisture contents of samples 4, 5 and 11 did not comply with the Codex. Moreover, Arslan et al (2011) showed that total acidities of traditional butters were ranged between 0.51-3.44%. Differences among the butter samples in terms of acidity and acid value may also be due to the changes in the production steps, storage periods and microbial quality. The acid values of butter samples ranged between 0.55-1.22 mg KOH g^{-1} oil in the present study. The lowest acid value was found in the samples 3 and 6. Atamer et al (2007) determined that acid values of churn butter samples which were produced by the traditional methods ranged between 1.06-2.67 mg KOH g^{-1} oil. The amount of lactic acid obtained from butter was related to the composition of raw materials and washing process used in the butter production (Atamer et al 2007). Acid value is associated with excess of free fatty acid due to hydrolysis of triglycerides in fat and oil. Adequate hydrolysis of triglyceride can be favorable for flavor formation. However, higher acid value is an indicator of undesirable processing and storage conditions such as high temperature and relative humidity in storage, high lipase activity in the butter due to insufficient thermal processing (Wilbey 2009). Low acid value and high acidity in butter indicate low quality butter and bad preservation condition (Bendixen 1940; Koczon et al 2008). Both parameters effect the consumption rate of butters. While, high acid value shows oxidation and rancidity in butters, high total acidity (% lactic acid) can be associated undesirable microflora and/or using of low quality milk for the production of butter.

3.2. Analysis of volatile compounds

The major volatile compounds determined in butter samples were acids, aldehydes, ketones and lactones (Table 3). It was found that the amount of diacetyl in the butter samples ranged between 64.40-648.50 µg kg^{-1} butter. Diacetyl is one of the most important aroma compounds in butter. It is also responsible for sweet and creamy flavor of other dairy products such as cheese, milk and fermented milks. In general, diacetyl was formed by lactose and citrate metabolism of lactic acid bacteria, especially *Lactococcus lactis* ssp. *Lactis biovar*. *diacetylactis* and *Leuconostoc* spp. (Jay et al 2005). Other aroma compounds that determined in butter samples at high levels were acetic acid (except sample 9), butyric acid and acetoin. They ranged between 15.67-62642 µg kg^{-1}, 1113-89605 µg kg^{-1} and 785-3880 µg kg^{-1}, respectively. Butyric acid is formed by hydrolysis of free fatty acid and frequently identified in many cheese types (Parliament & McGorrin 2000; Curioni & Bosset 2002), while acetic acid is produced by lactose metabolism of hetero fermantative lactic

Table 3- Some volatile compounds determined in butter samples

Çizelge 3- Tereyağı örneklerinde belirlenen bazı uçucu bileşenler

			Concentration of volatiles (μg kg^{-1} butter)[b]					
No	*Volatile compounds*	*RI[a]*	*6*	*9*	*11*	*2*	*4*	*5*
1	Ethanol	<600	211.8	354	382.4	1053	282	543
2	Diacetyl	<600	64.4	648.5	113.4	180	157.9	415.4
3	Acetic acid	615	4601.4	15.67	157891	13907	62642	23128
4	Acetoin	708	1818	915	2376	785	3880	2881
5	Hexanal	803	16.4	10.3	4.2	3.9	23.2	44.7
6	Butyric acid	832	1966.1	1113	89605	9451	19004	11787
7	*p*-xylene	875	20.9	87.7	54.01	287	69.8	168.2
8	2-Heptanone	893	85.7	165.7	172.8	640	2366	204.5
9	Oxime methoxy-phenyl	902	707.5	184	23.7	nd	nd	nd
10	Pinene	937	54.4	436	335	95.4	1136	65.8
11	Hexanoic acid	1006	342.8	587	43747	2945	3479	2611
12	Limonene	1032	61.03	267	84.9	403	3851	1200
13	N-(2-mercaptoethyl)-1,3-thiazolidine	1035	13.7	182	61.03	98.4	192.1	112.7
14	2,7,10- trimethyldodecane	1039	nd	nd	nd	151.8	761	113.1
15	Isoamylbutanoate	1155	204.7	218	70.2	77.3	380	396.5
16	3,5-dimethyl octane	1057	nd	nd	nd	58.5	1423	31.0
17	Terpinene	1061	795	61.2	122.1	235.8	731	153.0
18	2-Nonanone	1092	6.8	35.1	29.81	302.1	411	123.1
19	δ-Caprolactone	1096	30.9	40.0	47.85	14.57	75.1	125.9
20	Nonanal	1104	10.7	23.3	53.7	42.2	139	53.7
21	Octanoic acid	1172	19.0	15.7	16816	647.1	10952	916
22	Ethyl octanoate	1196	nd	nd	nd	46.3	167	36.9
23	δ-Octalactone	1285	8.4	21.4	13.2	44.1	135	20.5
24	Decanoic acid	1362	64.7	168.6	136.8	107.5	1470	728
25	δ-Decalactone	1504	5.6	15.3	8.7	8.5	157.7	16.7

[a], retention index on HP 5 MS column; [b], relative abundances of flavor compounds; nd, not determined

acid bacteria and/or citric acid metabolism of homo and hetero fermentative lactic acid bacteria (Mayo et al 2010). Similar to diacetyl, acetoin is produced by lactic acid bacteria by using α-acetolactate synthase and α-acetolactate decarboxylase enzymes in lactose or citrate metabolism (Hickey et al 1983). The amount of butyric acid and diacetyl in butter were found 3600 μg kg^{-1}, 340 μg kg^{-1}, respectively (Schieberle et al 1993). Samples 2, 4 and 5 and sample 11 had very high amount of butyric acid which is incompatible with Schieberle et al (1993). This difference may be related to types of butters, their age or storage conditions and extraction methods of volatile compounds.

Hexanoic, octanoic and decanoic acids were also determined in all samples. They ranged between 342.8-43747 μg kg^{-1}, 15.7-16816 μg kg^{-1} and 64.7-168.6 μg kg^{-1}, respectively. Sample 11 had higher amount of hexanoic and octanoic acids than the other samples. Peterson & Reineccius (2003) determined twenty aroma active compounds in fresh sweet cream butter by using static headspace extraction methods with GC-MS and GC/O analysis. The researchers stated that the amount of diacetyl, butyric acid and hexanoic acid in fresh sweet cream were 6.6 μg kg^{-1}, 192 μg kg^{-1} and 732 μg kg^{-1}, respectively.

Aldehydes including hexanal and nonanal were also detected in the butters. These compounds are formed by decarboxylation of branched-chain keto acids by the Strecker metabolic pathways of lactic acid bacteria and can also be formed by secondary oxidation of fatty acids. Hexanal is well known secondary oxidation product of linoleic acid and nonanal can be formed by β-oxidation of oleic acid. Both compounds cause off-flavor in milk and dairy products (Kochhar 1996; Guneser & Karagul-Yuceer 2011).

Limonene, pinene and terpinene were determined at high concentrations in butters (Table 4). Terpenes and sesquiterpenes were found in plants as secondary metabolites. Therefore, these compounds might be transferred directly from forage to dairy products (Mariaca et al 1997; Viallon et al 2000). Similar to our results, Rae-Lee et al (1991) identified limonene and *p*-cymene at low levels in unsalted butter heated at different temperatures (100-150 °C).

The other important contributors of volatiles in butter were lactones formed by hydrolysis and cyclisation of hydroxy-fatty acid triglycerides (Nursten 1997; Sarrazin et al 2011). δ-caprolactone, δ-octalactone and δ-decalactone were identified in butter samples (Table 3). δ-caprolactone was found at the highest concentration in sample 5 (125.9 μg kg^{-1} butter) while sample 4 had the highest concentration of δ-octalactone (135 μg kg^{-1} butter) and δ-decalactone (157.7 μg kg^{-1} butter). In a study conducted by Schieberle et al (1993) on different kinds of butters, the amount of δ-decalactone and (Z)-6-dodeceno-γ-lactone were determined between 2150-5000 μg kg^{-1} butter and 57-260 μg kg^{-1} butter, respectively. Lozano et al (2007) investigated the effect of cold storage and packaging material on commercial butter. The researchers identified δ-hexalactone, δ-octalactone, δ-decalactone, δ-dodecalactone and γ-nonalactone in sweet cream butter wrapped with foil and wax parchment paper. Researcher also observed an increase in these lactones for both butter samples during 6 and 12 months of storage at 4 °C and -20 °C. Figure 1 shows geometric representation of butters in terms of all volatile compounds. Butters 2, 5, 6 and 9 had similar flavor characteristics whereas butters 4 and 11 had quite different flavor profile than others in terms of volatile compositions.

3.3. Sensory analysis

Creamy, cooked and margarine were characteristic attributes in butter samples (Table 4). There were no significant differences in whey, margarine-like, sweet aromatic and sweet attributes among

Table 4- Sensory properties of butter samples (n= 2, ±SE)

Çizelge 4- Tereyağı örneklerinin duyusal özellikleri (n= 2, ±SE)

Sample	*Cooked*	*Whey*	*Creamy*	*Rancid*	*Margarine - like*	*Sweet aromatic*	*Oxidized*	*Storage/ plastic*	*Stale*	*Sweet*
1	2.03±0.23	1.15±0.27	2.31±0.21	1.19±0.31	1.25±0.44	0.56±0.24	1.12±0.31	1.69±0.40	1.28±0.28	1.44±0.17
2	1.66±0.12	1.16±0.33	2.69±0.31	2.69±0.27	1.50±0.35	0.47±0.18	1.94±0.18	1.12±0.24	1.12±0.22	1.09±0.15
3	2.22±0.13	1.03±0.41	2.37±0.24	1.12±0.51	1.72±0.35	0.19±0.13	1.03±0.43	1.44±0.36	1.50±0.23	1.25±0.22
4	2.34±0.16	1.12±0.49	2.62±0.18	1.50±0.50	1.91±0.51	0.56±0.24	1.09±0.34	0.87±0.21	1.81±0.19	1.47±0.18
5	2.84±0.20	1.34±0.40	3.19±0.27	1.53±0.23	1.56±0.44	0.59±0.27	1.31±0.37	0.87±0.31	1.41±0.26	1.47±0.18
6	2.72±0.17	0.78±0.21	3.56±0.20	0.37±0.12	2.84±0.49	1.00±0.16	0.53±0.17	1.44±0.26	0.72± 0.16	1.19±0.18
7	2.28±0.20	0.81±0.26	2.72±0.28	0.91±0.21	1.91±0.34	0.37±0.18	1.47±0.19	1.31±0.21	1.22± 0.22	1.19±0.13
8	2.66±0.23	0.84±0.28	3.84±0.17	0.53±0.17	2.75±0.50	0.69±0.23	0.31±0.12	0.28±0.14	0.62±0.24	1.65±0.17
9	2.37±0.19	0.72±0.22	3.06±0.22	0.84±0.19	2.62±0.39	0.75±0.30	0.87±0.33	0.59±0.25	1.22±0.24	1.50±0.17
10	2.50±0.22	0.94±0.36	3.25±0.23	0.28±0.09	2.34±0.45	0.69±0.26	0.41±0.15	0.18±0.07	1.03±0.31	1.59±0.17
11	1.87± 0.19	0.72±0.31	3.56±0.15	0.22±0.12	2.81±0.50	0.78±0.26	0.19±0.12	0.06±0.04	1.47±0.28	1.34±0.11
LSD value	0.53	ns	0.64	0.79	ns	ns	0.75	0.71	0.68	ns

ns, not significant (P≥0.05); SE, standard error

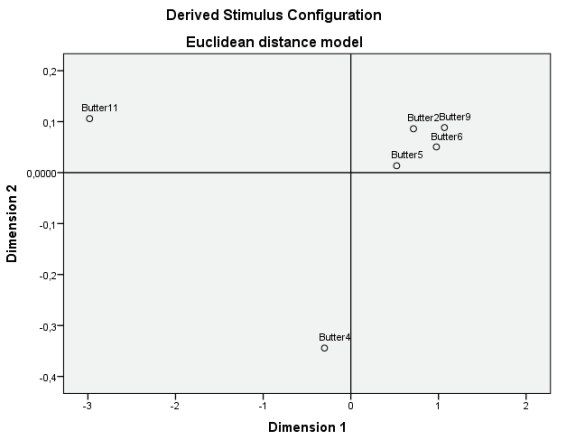

Figure 1- Multidimensional scaling map of butter samples in terms of volatile compounds

Şekil 1- Uçucu bileşenler açısından tereyağı örneklerine ait çok boyutlu ölçeklendirme haritası

the butters. Moreover, significant differences were observed among the butters in terms of cooked, creamy, rancid, oxidized, storage/plastic and stale ($P<0.05$). Whey and rancid were more intense attributes for the samples 1-5 than the other samples (Table 4). Sample 5 had the highest cooked aroma meanwhile sample 8 had the highest creamy attribute. Creamy and margarine-like attributes were higher in the samples 6-11 than others. However some undesirable flavors including rancid, storage/plastic, oxidized and stale were also determined in some samples. It was also performed a multidimensional scale analysis (MDS chart and MDS data were not shown) for volatile compounds and sensory attributes to reveal the relationship of between sensory attributes and characterization of volatile compounds. It was found that cooked, whey and creamy intensities of butter samples were related to diacetyl, 2-heptanone, octalactone, decalactone and caprolactone. Moreover, octalactone closely related with cooked and creamy flavors while the same relationship was observed between whey, rancid flavor and decalactone.

Unsuitable processing and storage conditions, packaging materials and using low quality milk in the production of butter may lead to increase in these undesirable attributes. Krause et al (2008) determined an increase in refrigerated/stale flavor and a decrease in milk fat and cooked/nutty flavor in stick and bulk butters stored at 5 °C and -20 °C for 24 months. Sensory language for 27 commercial butters was developed by Krause et al (2007). They found that diacetyl, cooked, grassy, milk fat flavors and salty taste were key characteristics for butters. Jinjarak et al (2006) characterized sensory attributes of sweet cream, whey and cultured butter. Significant differences were found in yellow, shiny, acidic odor, melt rate, porous, hard, spreadable, cheese odor, mouth coating, nutty, cardboard odors, acidic, nutty, diacetyl and grassy flavors of butters. They revealed that whey butter had more nutty and cardboard odor than sweet and cultured butters. Hence, cultured butter had more acidic odor and flavor, and grassy flavor than sweet and whey butter.

4. Conclusions

Some physical, chemical and sensory properties of butter samples collected from local market in Canakkale were investigated in this study. Significant differences among the samples in terms of physical, chemical and sensory characteristics were determined. Color measurements, melting points, acidities and viscosities of the samples varied. In general, samples had similar refractive indexes. On the basis of high concentrations of diacetyl, acetic acid, acetoin, δ-caprolactone, δ-octalactone and δ-decalactone played important roles in flavor of butter samples. All butter samples had similar sensory characteristics in terms of whey, margarine-like, sweet aromatic and sweet taste. Further studies are needed to determine the effects of textural properties, storage conditions, packaging types and processing conditions on butters.

References

Abdel-Mageed M & Fadel H H M (1994). Effect of freeze storage on the volatiles of butter. *Journal of Islamic Academy of Sciences* **7**: 185-188

Altun I, Andıc S, Tuncturk Y, Cecen A & Findik O (2011). Some chemical characteristics of butters obtained

from Van market. *Kafkas Universitesi Veteriner Fakültesi Dergisi* **17**: 645-648

Anonymous (2005). Turkish food codex for butter, milk fat based spreadable products and anhydrous milk fat. Codex number: 2005/19

Arslan N, Guneser O & Karagul-Yuceer Y (2011). Geleneksel ve endüstriyel tereyağlarının bazı kalite özellikleri (Some quality characteristics of traditional and industrial butters). *Hasad Gıda* **26**: 16-21

Atamer M, Senel E & Oztekin S (2007). Farklı tür sütlerden üretilen yayık tereyağlarının bazı nitelikleri (Some characteristics of churning butter produced from different types of milk). TUBITAK, Project no: 105O157, Ankara

Avsar Y K, Karagul-Yuceer Y, Drake M A, Singh T K, Yoon Y & Cadwallader K R (2004). Characterization of nutty flavor in Cheddar cheese. *Journal of Dairy Science* **87:** 1999-2010

Bendixen H A (1940). Acid values and acid ratios as related to the keeping quality of salted butter. *Journal of Dairy Science* **23**: 275-284

Bradley J R L, Arnold J E, Barbano D M, Semerad R G, Smith D E & Vines B K (1992). Chemical and physical methods. In: R T Marshal (Ed), *Standard methods for the examination of dairy products,* American Public Health Association, Washington, DC, pp. 363-532

Bradley J R L & Smukowski M (2009). Butter. In: S Clark, M Costello, M A Drake & F Bodyfelt (Eds), *Sensory Evaluation of Dairy Products,* Springer Science + Business Media, New York, pp. 135-167

Curioni P M G & Bosset J O (2002). Key odorants in various cheese types as determined by gas chromatography-olfactometry. *International Dairy Journal* **12:** 959-984

Fatouh A E, Singh R K, Koehler P E, Mahran G A, El-Ghandour M A & Metwally A E (2003). Chemical and thermal characteristics of buffalo butter oil fractions obtained by multi-step dry fractionation. *LWT-Food Science and Technology* **36**: 483-496

Glibowski P, Zarzycki P & Krzepkowska M (2008). The rheological and instrumental textural properties of selected table fats. *International Journal of Food Properties* **11:** 678-686

Guneser O & Karagül-Yüceer Y (2011). Characterization of aroma-active compounds, chemical and sensory properties of acid-coagulated cheese: Circassian cheese. *International Journal of Dairy Technology* **64:** 517-525

Hayaloglu A A (1999). A comparative study on physicochemical, microbiological and organoleptic qualities of butter produced from cream and yoghurt in Malatya region. M.Sc. Thesis, Çukurova University, Institute of Natural and Applied Sciences, Adana, Turkey

Hickey M, Hillier W A J & Jago G R (1983). Metabolism of pyruvate and citrate in lactobacilli. *Australian Journal of Biological Sciences* **36**: 487-496

Hocalar B T (2011). Tereyağı Teknolojisi (Technology of Butter). Izmir: Ege Universitesi Yayınları, Ege Meslek Yüksek Okulu Yayın No: 22

Jay J M, Loessner M J & Golden D (2005). Milk, fermentation, and fermented and nonfermented dairy products. In: J M Jay, M J Loessner & D Golden (Eds), *Modern Food Microbiology*, Springer Science, NY, USA, pp. 149-175

Jinjarak S, Walker J H, Olabi A & Jimenez-Flores R (2006). Sensory, functional, and analytical comparisons of whey butter with other butters. *Journal of Dairy Science* **89:** 2428-2440

Kochhar S P (1996). Oxidative pathways to the formation of off-flavours. In: M J Saxby (Ed), *Food Taints and Off-flavors,*Blackie Academic & professional, Chapman & Hall., Wester Cleddens Road, Bishopbriggs, Glasgow-UK, pp. 168-225

Koczon P, Gruczynska E, Kowalski (2008). Changes in the acid value of butter during storage at different temperatures as assessed by standard methods or by FT-IR Spectroscopy. *American Journal of Food Technology* **3**: 154-163

Krause A J, Lopetcharat K & Drake M A (2007). Identification of the characteristics that drive consumer liking of butter. *Journal of Dairy Science* **90:** 2091-2102

Krause A J, Miracle R E, Sanders T H, Dean L L & Drake M A (2008). The effect of refrigerated and frozen storage on butter flavor and texture. *Journal of Dairy Science* **91:** 455-465

Lozano L P, Miracle R E, Krause A J, Drake M & Cadwallader K R (2007). Effect of cold storage and packaging material on the major aroma components of sweet cream butter. *Journal of Agriculture and Food Chemistry* **55:** 7840-7846

Mallia S, Escher F & Schlichtherle-Cerny H (2008). Aroma-active compounds of butter: A review. *European Food Research and Technology* **226:** 315-325

Mallia S, Escher F, Schieberle P, Dubois S & Schlichtherle-Cerny H (2009). Characterization and quantification of odor-active compounds in unsaturated fatty acid/ conjugated linoleic acid (UFA/CLA)-enriched butter and in conventional butter during storage and induced

oxidation. *Journal of Agriculture and Food Chemistry* **57:** 7464-7472

Mariaca R G, Berger T F H, Gauch R, Imhof M I, Jeangros B & Bosset J O (1997). Occurrence of volatile mono- and sesquiterpenoids in highland and lowland plant species as possible precursors for flavor compounds in milk and dairy products. *Journal of Agriculture and Food Chemistry* **45:** 4423-4434

Mayo B, Aleksandrzak-Piekrczyk T, Fernandez M, Kowalezyk M, Alvarez-MartinP & Bardowski J (2010). Updates in the metabolism of lactic acid bacteria. In M. Fernanda, R R Raya & G M Vignolo (Eds), *Biotechnology of Lactic Acid Bacteria: Novel Applications*, Blackwell Publishing, State Avenue, Ames, Iowa, USA, pp. 3-35

Meilgaard M, Civille G V & Carr B T (1999). Descriptive analysis techniques. In M. Meilgaard, G V Civille & B T Carr (Eds) *Sensory Evaluation Techniques,* CRC Press, FL: Boca Raton

Minitab 16.1 (2010). PA, USA, Minitab Inc. State College

Nas S, Gokalp H S & Unsal M (2001). Bitkisel yağ teknolojisi (Technology of vegetable oils), Pamukkale Üniversitesi Ders Kitapları, Yayın No: 005, Denizli, Turkey

Nikolova K, Panchev I & Sainov S (2007). Refractometric investigation of butter and margarine. *European Food Research and Technology* **225**:139-143

Nursten H E (1997). The flavour of milk and dairy products: I. milk of different kinds, milk powder, butter and cream. *International Journal of Dairy Technology* **50:** 48-56

Parliament T H & McGorrin R J (2000). Critical flavor compounds in dairy products. In: S J, Risch & C T Ho (Eds), *Flavor Chemistry*. ACS Symposium Series 756, ACS Books, Washington, D.C

Pawliszyn J (2012). Theory of solid phase micro extraction. In: J Pawliszyn (Ed), *Handbook of solid phase microextraction*, Elsevier Inc., Waltham, MA, USA, pp. 13-57

Peterson D G & Reineccius G A (2003). Determination of the aroma impact compounds in heated sweet cream butter. *Flavour and Fragrance Journal* **18:** 320-324

Rae Lee S, Macku C & Shibamoto T (1991). Isolation and identification of headspace Volatiles Formed in Heated Butter. *Journal of Agriculture and Food Chemistry* **39:** 1972-1975

Ronholt S, Kirkensgaard J J K, Pedersen T B, Mortensen K & Knudsen J C (2012). Polymorphism, microstructure and rheology of butter: Effects of cream heat treatment. *Food Chemistry* **135:** 1730-1739

Sagdıc O, Donmez M & Demirci M. (2004). Comparison of characteristics and fatty acid profiles of traditional Turkish yayik butters produced from goats', ewes' or cows' milk. *Food Control* **6**: 485-490

Sancak C Y, Işleyici O, Alişarlı M, Akkaya L & Elibol C (2002). Van'da tüketime sunulan kahvaltılık tereyağlarının mikrobiyolojik ve kimyasal nitelikleri. *Yüzüncü Yıl* Üniversitesi *Veteriner Fakültesi Dergisi* **13:** 108-113

Sarrazin E, Frerot E, Bagnoud A, Aeberhardt K & Rubin M (2011). Discovery of new lactones in sweet cream butter oil. *Journal of Agriculture and Food Chemistry* **59:** 6657-6666

Schieberle P, Gassenmeier K, Guth H, Sen A & Grosch W (1993). Character impact odour compounds of different kinds of butter. *LWT-Food Science and Technology* **26:** 347-356

Sheskin D J (2004). Handbook of parametric and nonparametric statistical procedures, Chapman and Hall / CRC press, New York

Shukla A, Bhaskar A R, Rizvi S S H, & Mulvaney S J (1994). Physicochemical and rheological properties of butter made from supercritically fractionated milk fat. *Journal of Dairy Science* **1:** 45-54

SPSS (2008). SPSS Professional Statistics 17.0, Chicago, IL SPSS Inc

Senel E (2006). Bazı üretim parametrelerinin yoğurttan üretilen yayık tereyağının nitelikleri üzerine etkisi (The effect of some production parameters on yayık butter produced from yoghurt), Ph.D. Thesis, Ankara University Department of Dairy Technology, Institute of Natural and Applied Sciences, Turkey

Şenel E, Atamer M & Oztekin F S (2011). The oxidative and lipolytic stability of Yayık butter produced from different species of mammals milk (cow, sheep, goat) yoghurt. *Food Chemistry* **127:** 333-339

Üçüncü M (2010). Süt ve Mamülleri Teknolojisi. Sidas Yayıncılık, İzmir

Viallon C, Martin B, Verdier-Metz I, Pradel P, Garel J P & Coulon J B (2000). Transfer of monoterpenes and sesquiterpenes from forages into milk fat. *Le Lait* **80:** 635-641

Widder S & Grosch W (1997). Precursors of 2-nonenals causing the cardboard off-flavour in butter oil. *Nahrung* **41:** 42-45

Wilbey R A (2009). Butter. In: A Tamime (Ed), *Dairy fat and related products*, Wiley-Blackwell Publishing, Ames Iowa, USA, pp. 86-106.

Wright A J, Scanlon M G, Hartel R W & Marangoni A G (2001). Rheological properties of milkfat and butter. *Journal of Food Science* **66:** 1056-1071

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Effects of Zinc Application on Yield and Some Yield Components in Peanut *(Arachis hypogaea)* in the Easthern Mediterranean Region

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ABSTRACT

The effect of soil and foliar Zn fertilization on two varieties of peanut's (*Arachis hypogaea*) yield and some yield components were examined in this study. Soil applications of Zn doses were 0, 10, 20 and 40 kg ha^{-1} whereas 0, 0.5, 1 and 1.5 kg ha^{-1} Zn were sprayed to leaves. Applications of dose amounts of Zn lead to remarkable increase in yield and 100-seed weight. The effect of Zn treatment found to be statistically important at P<0.01 levels. The highest yield was obtained at COM variety as 6580.0 kg ha^{-1} with 0.5 kg ha^{-1} Zn foliar application. The lowest yield was measured at NC-7 variety's control plot with 3660.0 kg ha^{-1} in 2007. Foliar application Zn was statistically determined to be important to NC-7 variety peanut's grain Zn concentration at P<0.05 levels in each year. The economic analyses revealed that 0.5 kg ha^{-1} foliar application of Zn provided maximum profit with 10271.2 USD Dollars ha^{-1}.

Keywords: Micronutrient; Peanut; Yield; Zinc application

Doğu Akdeniz Bölgesinde Yer Fıstığında Çinko Uygulamasının Verim ve Bazı Verim Unsurlarına Etkileri

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ÖZET

Bu çalışmada topraktan ve yapraktan Zn gübrelemesinin COM ve NC-7 yerfıstığı çeşidinde verim ve bazı verim unsurlarına etkisi araştırılmıştır. Topraktan 0, 10, 20 ve 40 kg ha^{-1}; yapraktan 0, 0.5, 1 ve 1.5 kg ha^{-1} dozları uygulanmıştır. Zn uygulama dozları verimde ve 100 dane ağırlığında önemli miktarda artışlara neden olmuştur. Zn uygulamasının verim ve 100 dane ağırlığına etkisi istatistiksel olarak P<0.01 düzeyinde önemli bulunmuştur. En yüksek verim 6580 kg ha^{-1} ile 0.5 kg ha^{-1} yapraktan Zn uygulaması yapılan COM çeşitinde elde edilmiştir. En düşük verim 3660 kg ha^{-1} ile NC-7 çeşidinin control parselinde (0 doz) ölçülmüştür. Yapraktan uygulamanın NC-7 çeşidinde tanenin Zn içeriğine etkisi istatiksel olarak P≤0.05 düzeyinde önemli bulunmuştur. Ekonomik analizlerde 10271.2 US Dolar ha^{-1} maksimum gelir sağlayan yapraktan 0.5 kg ha^{-1} Zn dozu önerilmiştir.

Anahtar Kelimeler: Mikroelement; Yerfıstığı; Verim; Zn uygulaması

1. Inroduction

Zinc deficiency is responsible for many severe health problems. Among them are impairments of physical growth, immune system and learning ability, combined with increased risk of infections (Hotz & Brown 2004; Gibson et al 2008; Cakmak 2009; Cakmak et al 2010). 80% of peanut production of Turkey comes from the Cukurova region and Fe, Zn and other micronutrient deficiency is very common soil nutritional problem. However, Zn deficiency is one of the widespread nutritional disorders in crop production (Dağhan et al 2013). A number of factors affect the probability of a particular crop developing zinc deficiency such as very high lime content, low organic matter content, alkaline pH, very high Zn adsorption capacity of the calcareous soils etc. (Çakmak et al 1998; Srinivasara et al 2008). Also in soils with low Zn, the uptake of Zn is negatively affected by N, P, K, Ca, Mg, Na, Fe, Cu and Mn (Cakmak et al 1998; Farshid 2011; Dağhan et al 2013; Surucu et al 2013). Moreover, solubility of Zn is more influential than its amount for the occurrence of Zn deficiency in crops (Cakmak 2009; Rayo & Lucena 2009). Thus, zinc deficiency has common occurrence for the soils of the world and Turkey (Surucu et al 2013). More than half of the Turkish soils have less than 0.5 mg kg^{-1} zinc which is inadequate for plant growth (Cakmak et al 1998; Erdal et al 2000; Cakmak 2008).

Some researchers claimed that zinc fertilization of alfalfa increased herbage, hay, dry matter, crude protein yields and zinc concentration (Ceylan et al 2009). Peanut is widely produced in Cukurova Region of Southeastern Turkey, and micronutrients' deficiency symptoms are common due to high pH, lime and low organic matter content of the soils (Irmak et al 2008). Thus, this study aims to determine the effect of various amounts and types of Zn application on peanut's yield production along with fat and protein content in high pH and calcareous soils Cukurova Region of Turkey.

2. Material and Methods

The experiment was carried out using COM and NC-7 peanut varieties in Adana, Turkey. The Mediterranean climate with an average rainfall of 600 mm is dominating in the study area. Soils have "thermic" temperature with "xeric" moisture (Soil Survey Staff 2006). Wheat, corn, cotton, soybean, peanut, sunflower and rapeseed are commonly cultivated crops in the region. Soil sample was taken from 0-30 cm depth of each experimental plot for the analisis of routine properties. Soils were air dried and passed through 2 mm sieve. The DTPA (diethylene triamine pentaacetic acid)-extractable Zn of soil (Lindsay & Norvell 1978) and Zn concentrations of leaves and nut samples were analized by by using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, Perkin-Elmer) (Kacar & Inal 2008). Oil contents of nuts were analyzed according to Soxhelet method (James 1995). Protein contents of grain were analyzedsemi-micro Kjeldahl method (AOAC 2000).

In 2006 and 2007, dose experiments were established in split-split plot arrangement in completely randomized block design with three replications. In the experiment, the main plots were varieties, sub plots were applications and sub-sub plots were doses. The Zn fertilizer treatments were 0, 10, 20 and 40 kg Zn ha^{-1} as $ZnSO_4$ on soil before plowing. In the foliar Zn application rates were 0, 0.5, 1 and 1.5 kg Zn ha^{-1}. Respective amounts of $ZnSO_4$ were dissolved in 15 L de-ionised water and sprayed three times in twenty days interval in equal portions after flowering started. The soil and plant analysis data were subjected to two-way ANOVA by SPSS 13.0 statistical package programme.The mean separation was made by Duncan's test at $P \leq 0.05$.

Partial Budgeting and Marginal Profitability Ratio (PB and MPR) methods were used to determine the most economic application rate under current market conditions (Perin et al 1976; CIMMYT 1988). Partial budgeting process includes the average yield, gross production rate (GPR), fertilizer and fertilization costs, total variable input costs (TVIC) and net income (NI) parameters.

Marginal Profitability Analysis of applications was conducted following the partial budgeting process. Marginal Profitability Ratio indicates the extra income which will be taken in return for the invested 100 $ input towards increasing the yield (CIMMYT 1988).

The economical analyses were performed with the following equations:

GPR= Average Yield (kg ha^{-1}) x Product Price (kg $\$^{-1}$)

TVIC= Fertilizer Cost (kg ha^{-1}) + Fertilizing labor ($\$ ha^{-1}$)

NI= GSUD-TDGM

MPR= (Extra Net Income / Extra Total Variable Input Cost) x100

3. Results

3.1. The effect of zinc application on yield and 100-seed weight

The results of yields of experiment plots were presented in Table 1. The yield of NC–7 variety was 3781 kg ha^{-1} for control treatment (0 kg Zn ha^{-1}) and increased to 5406 kg ha^{-1} upon 10 kg Zn ha^{-1} application in 2006. Similar results were obtained for COM variety which the yield increased from 3876 kg ha^{-1} (control plot) to 4734 kg ha^{-1} with 10 kg ha^{-1} soil application of Zn. Moreover, all soil applied zinc significantly increased yield for both varieties (Table 1).

Foliar application of Zn was also effective on increasing yield. The yield was 3814 kg ha^{-1} in NC-7 variety without foliar application of Zn and increased to 4891 kg ha^{-1} with application of 0.5 kg ha^{-1} foliar-Zn and increased to 4953 kg ha^{-1} with 1 kg ha^{-1} foliar-Zn application in 2006. However, the yield of control plots for COM variety was higher than other three foliar Zn treatments and this is most probably due to the low sensitivity of COM variety to foliar applied Zn. This unexpected behaviour may be attributed to higher soil Zn concentration of the control plots (Table 4). However, differences in tolerance of species and varieties of some species to Zn were reported elsewhere (Cakmak et al 1998). Another suggestion to higher yield for control plot of COM variety may be due to the relatively high soil Zn content of the experimental plots. Similar results were obtained by soil and foliar Zn applications in 2007. Soil applied Zn, at the 10 kg ha^{-1} treatment, increased NC-7's yield from 3840 kg ha^{-1} (control) to 4840 kg ha^{-1}. Other Zn doses which 20 kg ha^{-1} and

Table 1- Effect of zinc application on crop yild

Çizelge 1- Çinko uygulaması verime etkisi

Mode of application	*Doses kg ha^{-1}*	*Crop yield* kg ha^{-1}*							
		NC-7 variety				*COM variety*			
		2006	*2007*	*Average*		*2006*	*2007*	*Average*	
Soil	0	3781	3840	1891	cd	3876	4340	4108	cd
	10	5406	4840	2708	ab	4734	5420	5077	ab
	20	4751	5440	2385	ab	4303	5890	5096	ab
	40	3912	5050	1976	bc	5685	5790	5737	ab
Foliar	0	3814	3660	1907	d	4738	5220	4979	ab
	0.5	4890	5360	2445	ab	4188	6580	5384	a
	1	4953	5340	2477	ab	4632	5890	5261	a
	1.5	4799	5330	4799	ab	4657	5580	5118	ab
CV		13%							
		Significant* ($P < 0.01$)							

40 kg ha^{-1} also resulted in further increases to 5440 and 5050 kg ha^{-1} for NC-7, respectively. 10 kg Zn ha^{-1} treatments increased the nut yield from 4340 kg ha^{-1} to 5420 kg ha^{-1} for COM variety. The obtained nut yield for 20 and 40 kg Zn ha^{-1} treatments were comparatively higher than the control treatment. 0.5 kg Zn ha^{-1} foliar treatment in 2007 for NC-7 resulted in 1700 kg ha^{-1} absolute yield increase comparing the control treatment. The gross yield obtained for 1 and 1.5 kg ha^{-1} foliar Zn applications were 5340 and 5330 kg ha^{-1}, respectively. The foliar application to COM variety somehow resulted in better yield performance for all of the treatments in the second year.

The varying Zn application methods either to soil or to leaves in 2006 increased 100-seed weight for both varieties (Table 2). This increase was detrimental for 10 kg Zn ha^{-1} treatment in NC-7 variety. However, the COM variety responded better to increasing Zn application rates for this trait such as 2.0, and 7.5 g absolute increase. The increasing foliar Zn treatments resulted in better performance in 100 seed weight for NC-7 variety. Ther were significant improvements in size of nuts upon foliar application of Zn. The recorded values were 105.0, 116.5, 119.9 and 118.6 g per 100 seeds for control, 0.5, 1.0, and 2.0 kg ha^{-1} treatments, respectively. Despite similar responses were obtained for COM variety it was not as detrimental as NC-7 variety. Fertilization of peanut by either way resulted in increases in the 100 seed weight in 2007. The most concrete performance was obtained for 10 kg Zn ha^{-1} treatment (128.0 g) whereas COM variety similarly responded to all Zn rates of soil treatments (134.0-135 g per 100 seeds). The obtained range of 100 seed weight for NC-7 upon spraying Zn was 117.0 and 125.0 g. Despite COM variety showed similar trend to spraying, the seed size of this variety was comperatively larger with a 100 seed weight range of 133.0-141 g.

3.2. Effect of zinc application on oil and protein contents of grain

Oil and protein contents of grain samples were presented in Table 3.The oil content ranges for Soil Zn treatments in 2006 were 41.3-45.7% and 44.4-45.4% for NC-7 and COM varities, respectively. The oil contents in the subsequent year were significantly increased to ranges 46.6-48.1% and 46.2-48.0% for the respective varieties. Spraying Zn increased the oil contents of seeds for both varieties (45.4-47.3% for NC-7 and 45.6-48.7% for COM) but these increases were not significant. It is shown that Zn applications have not affected oil contents of peanut grain in both years. Either the soil applications or spraying of Zn did not differ the protein content of seeds

Table 2- Effect of zinc applicatio on 100-seed weight

Çizelge 2- Çinko uygulamasının 100 tane ağırlığına etkisi

Mode of application	*Doses kg ha^{-1}*	*100-seed weight * g*							
		NC-7 variety				*COM variety*			
		2006	*2007*	*Average*		*2006*	*2007*	*Average*	
Soil	0	114.8	123.0	118.9	b	114.7	129.0	121.8	a
	10	119.5	128.0	123.8	a	116.7	135.0	125.8	a
	20	115.2	130.0	122.6	a	122.2	134.0	128.1	a
	40	117.7	124.0	120.9	a	122.2	136.0	129.1	a
Foliar	0	105.0	117.0	111.0	b	115.5	133.0	124.2	a
	0.5	116.5	125.0	120.8	a	121.3	141.0	131.2	a
	1	119.9	124.0	121.9	a	119.4	142.0	130.7	a
	1.5	118.6	125.0	121.8	a	121.0	135.0	128.0	a
CV		5%							
		Significant* ($P<0.01$)							

in 2006, but there was relatively higher deviation in protein content of NC-7 variety upon spraying (Table 3). In the second year of the experiment, the obtained protein contents were comperatively lower by 4.6-7.3% for NC-7 and 3.5-6.7% for COM. Despite positive relation between Zn treatments and protein contents of both varieties, Zn application did not affect protein content of seeds (Table 3). Some researchers reported that Zn application had a significant ($P<0.05$) effect on crude protein yield of alfalfa (Ceylan et al 2009).

3.3. Effect of zinc fertilization on zinc content of grain and leaf

The applicaition method, rates and year had significant effects on leaf and seed Zn concentrations (Table 4). Soil applied Zn was effective on both NC-7 variety's grain and leaf Zn concentration in 2006. The control plot's Zn concentration of grain was 37.0 mg kg^{-1} and raised to 55.0 mg kg^{-1} with increasing third doses which was 20 kg ha^{-1}. Similar to soil application, the foliar applied Zn enhanced grain and leaf Zn concentration of NC-7 variety which was 12.0 mg kg^{-1} at control plot and increased to 21.0 mg kg^{-1} at

Table 3- Effects of zinc application on oil and protein contents of peanut grain

Çizelge 3- Çinko uygulamasının yerfıstığı tohumlarının yağ ve protein içeriğine etkisi

Mode of application	*Dose kg ha^{-1}*	*Oil content of grain %*				*Protein content of grain %*			
		NC-7 variety		*COM variety*		*NC-7 variety*		*COM variety*	
		2006	*2007*	*2006*	*2007*	*2006*	*2007*	*2006*	*2007*
Soil	0	44.3	48.1	45.4	46.2	33.2	27.3	30.2	24.9
	10	44.7	47.8	46.0	47.9	33.8	27.5	29.7	24.2
	20	41.3	46.8	44.4	47.4	33.8	27.7	29.0	25.5
	40	45.7	46.6	45.9	48.0	33.2	26.7	29.4	24.0
Foliar	0	44.9	46.4	46.6	48.7	31.8	25.9	29.5	23.9
	0.5	45.4	45.4	45.5	48.4	31.7	27.1	29.7	23.9
	1	45.3	46.8	46.5	48.3	33.2	27.4	29.8	23.5
	1.5	44.4	47.3	47.2	45.6	34.0	26.7	29.2	22.5
CV		4%				7%			
		ns				ns			

ns, not-significant

Table 4- Effects of zinc application on Zn concentrations of soil, leaf and grain samples

Çizelge 4- Çinko uygulaması toprak, yaprak ve tane Zn içeriğine etkisi

Dose kg ha^{-1}	*Zn concentration of soil mg kg^{-1}*				*Zn concentration of leaf mg kg^{-1}*				*Zn concentration of grain mg kg^{-1}*			
	NC-7 variety		*COM variety*		*NC-7 variety*		*COM variety*		*NC-7 variety*		*COM variety*	
	2006	*2007*	*2006*	*2007*	*2006*	*2007*	*2006*	*2007*	*2006*	*2007*	*2006*	*2007*
0	0.24	1.01	0.20	0.24	14.0	7.0	24.0	6.0	37.0	74.1	34.0	80.4
10	0.20	0.90	0.18	0.35	10.0	12.0	24.0	5.0	39.0	77.9	39.0	64.7
20	0.20	0.96	0.18	0.75	15.0	6.0	17.0	6.0	55.0	72.2	42.0	126.2
40	0.24	0.29	0.20	0.90	17.0	6.0	22.0	4.0	51.0	51.9	40.0	55.5
0	0.36	0.20	0.36	0.25	12.0	7.0	25.0	11.0	33.0	61.9	38.0	69.3
0.5	0.80	0.53	0.18	0.26	21.0	9.0	28.0	8.0	42.0	97.4	40.0	65.7
1	0.20	0.34	0.18	0.29	25.0	12.0	27.0	12.0	43.0	73.0	40.0	76.1
1.5	0.24	0.51	0.20	0.38	10.0	16.0	24.0	13.0	30.0	75.0	44.0	72.6
					*		ns		*		ns	

*, $P < 0.05$; ns, not-significant

second dose which 0.5 kg ha^{-1} foliar Zn application in 2006. The Zn concentration of grain resembling to leaf Zn concentration, the control plot's 33.0 mg kg^{-1} Zn increased to 42.0 mg kg^{-1} with foliar 0.5 kg ha^{-1} Zn application. Foliar application Zn was statistically determined to be important to peanut's grain Zn concentration at 0.05 levels. In 2007, soil applied Zn increased control plot's 74.1 mg kg^{-1} value to 77.9 mg kg^{-1} at the second doses, 10 kg ha^{-1} application. The leaves Zn concentration is also increased from 7.0 mg kg^{-1} (control plot) to 12 mg kg^{-1} at 10 kg ha^{-1} Zn treatment in the same year. The foliar application also increased NC-7's leaves Zn concentration of control plot from 7.0 mg kg^{-1} to 16.0 mg kg^{-1} at fourth application which was 1.5 kg ha^{-1} in 2007. NC-7 variety's grain Zn concentration rose from 61.9 mg kg^{-1} of control plot amount to 97.4 mg kg^{-1} in 0.5 kg ha^{-1} foliar application (Table 4).

3.4. Economic analyses of zinc rates and fertilization methods

The effects of different Zn treatments on the marginal profit ratio (MPR) is provided in Tables 5 and 6. The maximum profit was determined at 0.5 kg ha^{-1} foliar Zn application with 10271.2 $ ha^{-1}. Based on MPR's in Table 5 and 6, Zn application doses of 20 and 40 kg ha^{-1} to soil and 1 and 1.5 kg ha^{-1} foliar spraying resulted in higher input expenses with lower income which gave negative MPR value. However, 10 kg ha^{-1} soil and 0.5 kg ha^{-1} foliar treatments revealed positive MPR values; the latter treatment has the maximum MPR with 1969.18% (Table 5 and 6).

Table 5- Economic analysis, partial budgeting and marginal profitability ratio of zinc dozes

Çizelge 5- Çinko dozlarının ekonomik analiz, kısmi bütçeleme ve marjinal karlılık oranına etkisi

Mode of application	*Dose kg ha^{-1}*	*Crop yield kg ha^{-1}*	*Gross production rate (GSUD ($ ha^{-1})*	*Variable input costs ($ ha^{-1})*	*Fertilizer and fertilization costs ($ ha^{-1})*	*Total variable input costs (TDM) ($ ha^{-1})*	*Net income (NG) ($ ha^{-1})*
Soil	0	3958.60	7798.40	00.00	00.00	00.00	7798.40
	10	5097.70	10042.50	216.00	50.00	266.00	9884.50
	20	5095.40	10037.90	432.00	50.00	482.00	9555.90
	40	4825.30	9505.80	648.00	50.00	698.00	8807.80
Foliar	0	4359.70	8588.60	00.00	00.00	00.00	8588.60
	0.5	5257.20	10356.70	05.40	80.00	85.40	10271.20*
	1	5203.90	10251.70	10.90	80.00	90.90	10160.80
	1.5	5094.20	10035.60	16.30	80.00	96.30	9939.20

*, the most profitable peanut yield

Table 6- Economic analysis and marginal profitability ratio of zinc applications

Çizelge 6- Çinko uygulamalarının ekonomik analizi ve marjinal karlılık oranı

Mode of application	*Dose kg ha^{-1}*	*Total variable input costs ($ ha^{-1})*	*Net income (NG) ($ ha^{-1})*	*Marginal profitability ratio*
Soil	0	00.00	7798.40	
	10	266.00	9884.50	1320.27
	20	482.00	9555.90	-104.19
	40	698.00	8807.80	-346.34
Foliar	0	00.00	8588.60	
	0.5	85.40	10271.20	1969.18*
	1	90.90	10160.80	-2018.87
	1.5	96.30	9939.20	-4084.31

*, the most profitable peanut yield

4. Discussion

Majority of the experimental soils have an inadequate Zn concentration for crop production. The Zn concentration of soils collected in 2006 varied from 0.18 mg kg^{-1} to 0.80 mg kg^{-1}. Except the experimental plot of NC-7 variety which Zn was applied 0.5 kg ha^{-1} via foliar fertilization, majority of soils' Zn concentration were below the critical level of 0.5 mg kg^{-1}. The soil Zn level was 0.80 mg kg^{-1} at 0.5 mg kg^{-1} foliar fertilizer treated NC-7 variety's plot whereas at COM variety experiment the Zn concentration was 0.18 mg kg^{-1} both in soil and foliar fertilizer applications. The Zn concentration ranged from 0.20 mg kg^{-1} to 1.01 mg kg^{-1} in 2007 soil samples and most of the plot's soil Zn level was below the critical deficiency threshold value of 0.5 mg kg^{-1} (Table 4). The researches undertaken in agricultural soils of Turkey revealed that more than half of the Turkish soils have Zn deficiency. Zn deficiency is more common in soils with high phosphorous concentration or excessively fertilized with phosphorus (Cakmak et al 2010). Irmak et al (2008) reported severe Zn deficiency in the soils of Cukurova region. The field experiment revealed that both soil and foliar applications of Zn significantly increased yield and 100-seed weight of peanut. The averages of two yearly data for both soil and foliar Zn treatments revealed statistically significant increases in nut size/yield at P= 0.01 (Table 1 and 2). The positive relations between soil-Zn and yield were reported by several researchers in different crops (Cakmak et al 1998; Kalayci et al 1999; Togay et al 2005; Dağhan et al 2013). Some researchers claimed that Zn fertilizers were also highly effective in increasing grain yield of wheat and alfalfa (Erdal et al 2003; Ceylan et al 2009; Cakmak 2010).

The increasing application of Zn both to soil and leaves found to be statistically significant at P<0.01 for NC-7 variety. Despite there was a positive relation between Zn application rates and 100-seed weight for COM variety, the relation was not statistically significant enough (Table 2). Several authors reported increase in yield and 100-seed weight of crops upon Zn fertilization. When Zn and phytin acid content were considered co-application of Zn to soil and leaves was suggested (Cakmak 2010). Some researchers studied the effect of various Zn fertilizers on some agronomical properties and corncob production of sweet corn (*Zea mays saccharatasturt.*). The highest corncob yield was achieved at 2.5 g L^{-1} (Borrechel) foliar treatment with 8927 kg ha^{-1} (Büyükerdem & Akman 2008). Togay et al (2005) investigated the response of yield of various wheat species and lines to Zn application in Van (highland with continental climate), and determined significant effects on plant height, seed number per spike, grain Zn concentration, 1000-seed weight, and grain yield.

Zn concentrations in Zn treated plots and their respective grain Zn concentrations gave statistically significant relations at P<0.05 (Table 4). Several researchers determined significant Zn-induced yield (seed/nut) increases in plants (Erdal et al 2000; Cakmak 2009; Farshid 2011). Some researchers reported that cereal crops are inherently very low in grain Zn and Fe concentrations (Cakmak et al 2010). However, soil and foliar application of Zn both in 2006 and 2007 were statistically insignificant for COM variety's grain and leaf Zn concentration which may be related to low response of COM variety to Zn fertilization or heterogeneity of the experimental field in two successive years. Studies undertaken in Southeastern Anatolia (semi-arid continental climate) and Central Anatolia (arid continental) on the effect of Zn on phytinacid (PA), Zn and P concentrations in wheat revealed the significance of Zn fertilization. Thus, the regulation of P concentration and PA/Zn ratioin P deficient calcareous soils results in inreases in the biological availablity of Zn (Cakmak et al 1998; Erdal et al 2000; Cakmak 2009). In this regard we suggest 0.5 kg ha^{-1} foliar Zn application to Easthern Mediterranean Region's farmers for maximizing the profit (Table 5 and 6) in peanut cultivation.

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References

AOAC **(**2000). Official Methods of Analysis. 17th ed. Gaithersburg, Maryland, USA, AOAC International

Büyükerdem N İ & Akman Z (2008). Effects of different zinc containing fertilizers applications on ear yield and some agronomic and quality characters of sweet corn. *Journal of Plant and Environmental Sciences* **1**: 21-27

Cakmak I, Torun B, Erenoglu B, Ozturk L, Marschner H, Kalayci M, Ekiz H & Yilmaz A (1998). Morphological and physiological differences in cereals in response to zinc deficiency. *Euphytica* **100**: 349-357

Cakmak I (2008). Enrichment of cereal grains with zinc: Agronomic or genetic biofortification. *Plant and Soil* **302**: 1-17

Cakmak I (2009). Enrichment of fertilizers with zinc: An excellent investment for humanity and crop production in India. *Journal of Trace Elements in Medicine and Biology* **23**: 281-289

Cakmak I, Pfeiffer W H & McClafferty B (2010). Biofortification of durum wheat with zinc and iron. *Cereal Chemistry* **87**: 10-20

Ceylan S, Soya H, Budak B, Akdemir H, & Esetlili B C (2009). Effect of zinc on yield and some related traits of alfalfa. *Turkish Journal of Field Crops* **14**(2): 136-143

CIMMYT (1988). From Agronomic Data to Farmer Recommendations: An Economics Training Manual. Completely revised edition, Mexico D.F

Dağhan H, Uygur V, Koleli N, Arslan M & Eren A (2013). The effect of heavy metal treatments on uptake of nitrogen, phosphorus and potassium in transgenic and non-transgenic tobacco plants. *Tarım Bilimleri Dergisi-Journal of Agricultural Sciences* **19**: 129-139

Erdal I, Bozkurt M A & Çimrin M K (2000). The effect of humic acid and P application on Fe, Zn, Mn and Cu contents of corn *(Zea mays*). *Tarım Bilimleri Dergisi-Journal of Agricultural Sciences* **6:** 91-96

Erdal U, Turan M A &Taban S (2003). Effect of zinc application on growth and nutrient concentrations of corn grown in soils with different characters. *Tarım Bilimleri Dergisi-Journal of Agricultural Sciences* **9**: 334-339

Farshid A (2011). Iron, copper and manganese concentration in maize leaf as influenced by soil and foliar application of zinc sulfate and boric acid. *International Journal of Academic Research* **3**: 1080-1087

Gibson R S, Hess S Y, Hotz C & Brown K H (2008). Indicators of zinc status at the population level: A review of the evidence. *British Journal of Nutrition* **99**: 14–23

Hotz C & Brown K H (2004). Assessment of the risk of zinc deficiency in populations and options for its control. *Food Nutrition Bulletin* **25**: 94-203

James C S (1995). Analytical chemistry of food. Publisher Blacie Academic and Professional, London

Irmak S, Surucu A K & Aydin S (2008). Zinc contents of soils and plants in the Cukurova Region of Turkey. *Asian Journal of Chemistry* **20**: 3525-3536

Kalayci M, Torun B, Eker S, Aydin M, Ozturk L & Cakmak I (1999). Grain yield, zinc efficiency and zinc concentration of wheat cultuvars grown in zinc-deficient calcareous soils in field and greenhouse. *Field Crops Research* **63**: 87-98

Lindsay W L & Norvell W A (1978). Development of a DTPA soil test for zinc, iron, manganese and copper. *Soil Science Society of American Journal* **42**: 421-428

Perin R, Winkelman D, Moscardi R & Anderson J (1976). From agronomic data to farmer recommendations An Economics Manual, Mexico

Rayo S L & Lucena J J (2009). Zn and Mn op-EDDHA chelates for soybean nutrition in hydroponics in high pH conditions. In: Proceedings of the International Plant Nutrition Colloquium XVI, Department of Plant Sciences, UC Davis

Soil Survey Staff (2006). Keysto Soil Taxonomy, United States Department of Agriculture Natural Resources Conservation Service, Tenth Edition, USDA, USA

Srinivasara C H, Wani S P, Sahrawat K L, Rego T J & Pardhasaradhi G (2008). Zinc, boron and sulphur deficiencies are holding back the potential of rain fed crops in semi-arid India: Experiments from participatory watershed management. *International Journal of Plant Production* **2**: 89-99

Surucu A, Ozyazıcı M A, Ozyazıcı G & Uygur V (2013). Determination of appropriate extraction methods for available iron, copper, zinc and manganese in acid soils. *Tarım Bilimleri Dergisi-Journal of Agricultural Sciences* **19**: 256-2678

Togay Y, Togay N, Kocakaya Z, Erdal I & Cig F (2005). Van koşullarında çinko uygulamasının farklı buğday çeşit ve hatlarında verim ve verim öğeleri üzerine etkisi. In: *Proceeding of VI Field Crop Congress of Turkey* **1:** 595-600

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Kiraz Anaçlarının *in vitro* Koşullarda Tuz Stresine Tolerans Mekanizmalarının Fizyolojik Parametreler ve Antioksidan Enzim İzoformları ile Belirlenmesi

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ÖZET

Tuzluluk, bitkilerde gelişim ve verimi sınırlayan önemli abiyotik stres koşullarının başında gelmektedir. Kiraz anaçlarının tuz stresi toleransları ile ilgili bilgiler oldukça sınırlıdır. Bu çalışmada, ülkemizde yetiştirilen üç kiraz anacının; Colt (*Prunus avium x Prunus pseudocerasus*), Gisela 5 (*Prunus cerasus x Prunus avium*) ve Maxma (*Prunus mahaleb x Prunus avium*), tuz stresine tolerans mekanizmaları *in vitro* koşullarda araştırılmıştır. Kiraz anaçlarının tuz stresine toleranslarını belirlemek amacıyla Murashige ve Skoog (MS) ortamına 0, 25 ve 50 mM NaCl uygulanmıştır. Anaçlarda; sürgün gelişimi, lipid peroksidasyonu (MDA), membran geçirgenliği (MG), toplam antioksidan aktivitesi (TAA), prolin içeriği, toplam klorofil içeriği, katalaz (CAT, EC 1.11.1.6) ve peroksidaz (POD, EC 1.11.1.7) antioksidan enzim izoformları ile Na ve Cl konsantrasyonları belirlenmiştir. Tuz stresi anaçların sürgün gelişimini ve toplam klorofil içeriğini kontrole göre azaltırken MDA içeriğini, MG, TAA ve prolin içeriğini artırmıştır. Anaçların stres koşullarında sürgün POD enzim aktivitesi artmış ve üç farklı POD izoformu elde edilmiştir. Maxma anacının POD aktivitesi diğer anaçların POD aktivitesinden daha düşük olmuştur. Anaçların CAT izoformlarında ise uygulamalar ve anaçlar arasında belirgin bir farklılık elde edilmemiştir. Bütün parametreler birlikte değerlendirildiğinde, NaCl stresine Maxma anacının hassas, Colt anacının orta derecede hassas ve Gisela 5 anacının da dayanıklı olduğu belirlenmiştir.

Anahtar Kelimeler: Enzim izoformu; Katalaz; Kiraz anaçları; Peroksidaz; Tuz stresi

Evaluation of Salt Tolerance Mechanisms with Physiological and Antioxidant Enzyme Isoform Parameters in *in vitro* Sweet Cherry Rootstocks

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ABSTRACT

Abiotic stress such as salinity is an important factor that limits plant growth and performance. Information regarding the genotypic variation for salinity stress tolerance in sweet cherry rootstocks is limited. In this study, salinity tolerance mechanisms of three sweet cherry rootstocks, namely; Colt (*Prunus avium x Prunus pseudocerasus*), Gisela 5 (*Prunus cerasus x Prunus avium*) and Maxma (*Prunus mahaleb x Prunus avium*), grown widely in Turkey were investigated under *in vitro* condition. The three rootstocks were cultured *in vitro* on MS medium supplemented with 0, 25 and 50 mM sodium chloride (NaCl). The Shoot growth, lipid peroxidation (MDA), membrane permeability (MP), total antioxidant activity (TAA), proline, total chlorophyll contents, catalase (CAT, EC 1.11.1.6) and peroxidase (POD, EC 1.11.1.7) antioxidant enzyme isoforms of rootstocks were studied. Compared to the control, salinity resulted in a reduction in the shoot growth and total chlorophyll contents. Contrary to this, MDA contents, MP, TAA and proline contents were increased by salinity. Activity of POD in the shoot of rootstocks was increased and three different POD isoforms were exhibited under saline conditions. The activity of POD was lower in the Maxma than the Colt and Gisela 5 rootstock. Salinity did not significantly change the CAT isoforms of the rootstocks. Regarding the all parameters studied, the rootstocks can be classified to their salt tolerance as sensitive (Maxma), moderately sensitive (Colt) and resistant (Gisela 5).

Keywords: Catalase; Enzyme isoform; Peroxidase; Salinity stress; Sweet cherry rootstocks

1. Giriş

Sulama suyundaki tuz düzeylerine bağlı olarak dünyanın pek çok yerinde özellikle kurak ve yarı kurak bölgelerde topraklar tuzlanmakta ve bunun sonucu olarak bitkisel üretimde önemli azalmalar meydana gelmektedir (Singh et al 2000). Dünyada 100-110 milyon hektar (ha) olan yarı kurak tarımsal alanın 20-30 milyon ha'ı tuz birikiminden önemli şekilde zarar görmekte ve her yıl 0.25-0.50 milyon ha tarımsal alanda tuz birikimi sonucu tarım yapılamamaktadır (FAO 2002).

Dünya kiraz üretimi 2012 yılı itibariyle 2,256,519 ton' dur. Kiraz üretimi bakımından 480,748 ton ile ilk sırada yer alan Türkiye'yi, Amerika Birleşik Devletleri, İran, İtalya ve İspanya takip etmektedir. Dünya kiraz üretiminde % 21.3'lük payıyla birinci sırada yer alan Türkiye'nin kiraz üretimindeki bu payı yıldan yıla artış göstermektedir (FAO 2012).

Tuzluluğa karşı hassas bitkiler gurubunda yer alan (Kotuby-Amachar et al 2000) ve ülkemiz için önemli bir ihracat ürünü olan kirazın, yetiştiriciliğinin yoğun olduğu Ege, Akdeniz ve Marmara bölgelerinin belirli yörelerinde karşılaşılan tarımsal sorunlar arasında; sulama suyu kalitesinin her geçen gün bozulması, fazla verim almaya yönelik aşırı gübreleme ve yanlış sulama yöntemlerinin kullanılması ilk sıralarda yer almaktadır. Bu sorunlardan kaynaklanabilecek olası tuzlulaşma riskine karşılık, tuz stresine toleranslı kiraz anaçlarının seçiminde, bitkilerin fizyolojik ve biyokimyasal mekanizmalarındaki değişimlerin bilinmesi oldukça önemlidir. Bu nedenle özellikle tarımsal açıdan sorunlu alanlarda stres koşullarına dayanıklı anaçların seçilmesi ve yetiştirilmesi büyük önem taşımaktadır. Giderek azalan tarım alanlarında, strese yol açan olumsuz çevre koşullarına karşı bitkisel üretimde verimliliği artırabilmek çok önemlidir. Verim artışı, stres koşullarına dayanıklı bireylerin seçilmesi veya ıslahıyla mümkündür.

Abiyotik stres koşullarında bütün bitkilerde oksidatif zararlanmalar meydana gelmekte ve anılan bu stres koşullarına dayanmak veya stresten kaçmak için bitki türlerinin ve çeşitlerinin geliştirmiş oldukları mekanizmalar birbirlerinden oldukça farklılık göstermektedir. Bu nedenle kimi bitki tür veya çeşitleri abiyotik stres koşullarından daha şiddetli etkilenirken kimi bitki tür veya çeşitleri dayanıklılık göstermektedir. Bu farklılıklar bitki türleri arasında olabileceği gibi aynı türün farklı çeşitleri arasında da önemli oranda görülebilmektedir.

Tuz stresine tolerans bakımından anaçlar ve çeşitler arasında önemli farklılıklar görüldüğü Troncoso et al (1999), Singh et al (2000) ve Fisarakis et al (2001) tarafından asma anaçları, Dragišić Maksimović et al (2013) tarafından arpa çeşitleri, Turner et al (2013) tarafından nohut çeşitleri ve Balal et al (2011) tarafından turunçgil anaçları ile yapılan çalışmalarda belirtilmiştir.

Abiyotik stres koşullarında, yaprak nispi nem içeriği ve yaprak su potansiyelinin düşmesi sonucu bitkilerde fotosentez oranı azalmaktadır (Lawlor 2001). Tuzlu koşullarda fotosentez oranının azalmasının temel sebebinin stomatal sınırlanmadan kaynaklandığı bilinmektedir (Cornic 1996). Tuz stresi altında stomaların kapanmasına bağlı olarak fotosentez oranı ve içsel CO_2 konsantrasyonunun azalması fotosentez metabolizmasını engellemektedir. Tuz stresinde stomaların kapanması bitkilerin beslenme durumlarını da olumsuz etkilemektedir (Oren et al 1999). Stres koşullarında stomaların kapanmasına bağlı olarak yaprakların mezofil dokularında CO_2 seviyesinin hızla düşmesi ve süperoksit radikallerinin (O_2•−) artmasıyla bitki dokularında moleküler oksijen ile rekabet eden nikotinamid adenin dinükleotit fosfat (NADP)'lar indirgenerek nikotinamid adenin dinükleotit fosfat hidrojen (NADPH) birikmektedir. Bu koşullarda bitki dokularında NADP miktarı azalmakta ve oksijen alternatif elektron alıcısı olarak görev yapmaktadır. Bu durumda bitki dokularında indirgenmiş oksijen türevleri olan süperoksit radikalleri (O_2•−), ve bunun indirgenmiş formu olan hidrojen peroksit (H_2O_2) ve hidroksil (OH•−) radikalleri oluşmaktadır (Cadenas 1989; Sairam & Saxena 2000). Aktif oksijen çeşitleri olarak adlandırılan bu radikaller, bitkilerde lipid peroksidasyonuna, membran zararlanmasına, proteinlerin bozunmasına, enzimlerin inaktivasyonuna, pigmentlerin azalmasına ve deoksiribonükleik asit (DNA) zincirlerinin bozunmasına yol açmaktadır (Fridovich 1986; Liebler et al 1986; Davies 1987; Imlay & Linn 1988). Bitkilerin süper oksit radikali ve H_2O_2 toksisitesini önleyebilmeleri, oksidatif strese karşı önemli savunma mekanizmaları olan antioksidatif enzimler aracılığıyla sağlanmaktadır. Bitkiler, hücrelerini toksik olan aktif oksijen çeşitlerinin zararlarından süperoksit dismutaz, askorbat peroksidaz, glutasyon redüktaz, katalaz enzimleri ve bunların metabolitleri olan glutasyon, askorbik asit, α-tokoferol ve karotenoidler aracılığıyla korumaktadırlar (Liebler et al 1986; Sairam et al 1998; Sairam & Saxena 2000). Antioksidan sistemleri güçlü olan çeşit veya türlerin stres koşullarına toleransları artmaktadır.

Bu çalışmada, üç farklı kiraz anacının (Colt, Gisela 5 ve Maxma) *in vitro* koşullarda tuz stresine toleransları; sürgünlerin toplam klorofil içeriği, membran geçirgenlikleri, lipid peroksidasyonu, toplam antioksidan aktivitesi, prolin içerikleri, katalaz ve peroksidaz antioksidan enzim izoformları gibi bazı fizyolojik ve biyokimyasal parametrelerle belirlenmesi amaçlanmıştır.

2. Materyal ve Yöntem

Bu araştırmada, bitki materyali olarak üç farklı kiraz anacına [Colt (*Prunus avium x Prunus pseudocerasus*), Gisela 5 (*Prunus cerasus x Prunus avium*), Maxma (*Prunus mahaleb x Prunus avium*)] ait sürgün uçları kullanılmıştır. İlkbahar döneminde bitkiler uyanmaya başladığında kiraz anaçlarından alınan sürgün uçları yüzeysel dezenfeksiyonunu sağlamak amacıyla, % 70'lik etanol içerisinde 2 dakika bekletilmiş, daha sonra 1-2 damla Tween-20 içeren % 5'lik sodyum hipoklorid çözeltisi içerisinde 10 dakika süre ile çalkalanmış ve steril saf su ile her biri 5'er dakika olmak üzere üç kez yıkanmıştır. Doku kültürü çalışmalarının bütün aşamalarında temel besin ortamı olarak Murashige ve Skoog ortamı (MS, Sigma-Aldrich) + 1 mg L^{-1} BAP (benzil amino purin) + 0.02 mg L^{-1} NAA (naftalen asetik asit) + % 3 sakkaroz + % 0.7 agar kullanılmıştır (Murashige & Skoog 1962). Besin ortamları, pH'ları 5.7'ye ayarlandıktan sonra. 121 ^{o}C sıcaklık ve 1.2 atm basınç altında 20 dakika süre ile otoklavda steril edilmişlerdir. Steril edilen sürgün uçları (20 mm) kurutma kâğıtları ile kurutulduktan sonra hazırlanan MS besin ortamı içerisinde kültüre alınmıştır. Gelişen bitkiler, tuz stresi yaratmak amacıyla 0, 25 ve 50 mM NaCl ilave edilmiş MS (800 mL iç hacme sahip cam kavanozlar içerisine

100'er mL olacak şekilde dağıtılmış) besin ortamı içerisinde dört hafta süreyle kültüre alınmıştır.

Deneme tesadüf parselleri deneme desenine göre dört tekerrürlü ve her tekerrürde 10 bitki olacak şekilde kurulmuştur. Çoğaltmanın tüm aşamalarında besin ortamlarına dikilen kültürler, sıcaklığı 25±1 ^{0}C ve gün uzunluğu 16/8 saat aydınlık/karanlık ve ışık yoğunluğu 3000 lux olarak ayarlanmış olan kültür odalarında gelişmeye bırakılmışlardır.

Dört haftalık gelişme döneminden sonra ortamlar içerisinden çıkarılan bitkilerin beş adedinde sürgün boyu, sürgün sayısı ve yaş ağırlıkları belirlendikten sonra sabit ağırlığa gelinceye kadar 65 ^{o}C sıcaklığa ayarlı havalı kurutma fırınında kurutularak kuru ağırlıkları belirlenmiştir. Geri kalan bitkiler içerisinden bir adet bitkinin tamamı alınarak membran geçirgenliği analizi Shen & Yan (2002)'a göre hasat sırasında yapıldıktan sonra kalan bitkiler bütün olarak enzim izoformlarının çıkarılması ve diğer analizler için -80 ^{o}C'de muhafaza edilmiştir.

Dondurulmuş bitki örneklerinde lipid peroksidasyonu (MDA) Heath & Packer (1968) ve Sairam & Saxena (2000), toplam antioksidan aktivite Prieto et al (1999), prolin Bates et al (1973) ve toplam klorofil Arnon (1946) ve Withan et al (1971) tarafından bildirildiği şekilde belirlenmiştir. Enzim (protein) ise Gulen & Eris (2004) tarafından belirtildiği şekilde ekstrakte edilmiştir.

Ekstraksiyon sonucunda elde edilen toplam protein miktarının belirlenmesinde Bradford (1976) yöntemi kullanılmıştır. Peroksidaz ve katalaz izoenzim kalıplarının belirlenmesi amacıyla Native-Page yöntemi kullanılmıştır. Ekstrakte edilen protein örnekleri % 7.5'lik poliakrilamid jelde Laemmli (1970) yöntemine göre sodyum dodesil sülfat (SDS) katılmaksızın elektroforezde yürütülmüştür. Elektroforez işlemi sonunda jeller POD izoenzimi için Wendel & Weeden (1989) ve CAT enzimi için Wolfe (1976)'e göre spesifik boyamalara tabi tutulmuştur.

Dört haftalık gelişme döneminden sonra ortamlar içerisinden çıkarılan bitkilerin beş adedinde sürgün boyu, sürgün sayısı ve yaş ağırlıkları belirlendikten sonra sabit ağırlığa gelinceye kadar 65 ^{o}C sıcaklığa ayarlı havalı kurutma fırınında kurutularak kuru ağırlıkları belirlendikten sonra kurutulmuş ve öğütülmüş bitki örnekleri mikro dalga fırında yaş yakma yöntemi (HNO_3+H_2O_2) ile yakıldıktan sonra Na fleymfotometre (Jenway PFP7, ELE Instrument Co. Ltd) ile, Cl ise öğütülmüş kuru bitki örneklerinde $AgNO_3$ ile titre edilerek belirlenmiştir (Johnson & Ulrich 1975).

Araştırma sonunda elde edilen veriler MINITAB paket programı kullanılarak varyans analizi ile değerlendirilmiş, ortalamalar arasındaki farkın önemlilik durumu ise MSTAT paket programı kullanılarak Duncan Çoklu Karşılaştırma Testi ile belirlenmiştir.

3. Bulgular ve Tartışma

3.1. Bitki gelişimi

In vitro koşullarda yetiştirilen kiraz anaçlarının yaş ve kuru ağırlığı ile sürgün sayısı üzerine anaç (A) ve uygulamanın (NaCl) (T) etkisi önemli olurken anaç x uygulama (AxT) interaksiyonunun etkisi önemsiz olmuştur (Çizelge 1). Tuz (NaCl) uygulaması ile birlikte anaçların yaş ve kuru ağırlıkları ile sürgün sayısı azalmıştır. Anaçların yaş ağırlık ortalamaları incelendiğinde en düşük yaş ağırlığın Maxma anacından elde edildiği görülürken Gisela 5 ve Colt anaçları arasındaki farkın istatistiki olarak önemsiz bulunmuştur. Tuz uygulaması kuru ağırlığı en fazla Maxma anacında azaltmış (% 30.7), bunu sırasıyla Colt (% 27.1) ve Gisela 5 (% 22.8) anacı izlemiştir. Anaçlar birlikte değerlendirildiğinde, ortalama sürgün sayısı 50 mM NaCl uygulaması ile kontrole göre önemli oranda azalmış, Gisela 5 ve Colt anaçlarının sürgün sayısı Maxma anacından daha düşük olmuştur. Anaçların sürgün boyu üzerine AxT interaksiyonunun etkisi önemli olmuştur. Tuz uygulaması ile anaçların sürgün boyu azalmış, en kısa sürgün boyu 50 mM NaCl uygulaması ile Maxma anacından elde edilmiştir (Çizelge 1).

Tuz stresi bitkilerde bir seri fizyolojik ve biyokimyasal sürecin olumsuz etkilenmesine yol açar. Termaat & Munns (1986) ve Ruiz et al

Çizelge 1- *In vitro* koşullarda NaCl uygulamasının kiraz anaçlarının yaş (YA) ve kuru ağırlığı (KA) ile sürgün sayısı (SS) ve sürgün boyu (SB) üzerine etkisi

Table 1- Effect of NaCl on fresh (FW) and dry weight (DW), number of shoots (NS) and shoot length (SL) of sweet cherry rootstocks grown in vitro

Anaçlar	*NaCl (mM)*	*YA (g 5 bitki^{-1})*	*KA (g 5 bitki^{-1})*	*SS (bitki^{-1})*	*SB (cm)*
Gisela 5	0	10.50	1.45	4.97	3.30 a
	25	9.31	1.18	4.92	2.97 b
	50	10.40	1.12	3.73	3.08 ab
Maxma	0	8.40	0.88	6.35	2.50 cd
	25	5.84	0.61	6.35	2.33 cd
	50	5.21	0.66	4.55	1.98 e
Colt	0	11.40	1.18	5.75	3.21 ab
	25	7.15	0.86	4.50	2.63 c
	50	8.62	0.97	3.55	2.31d
	Ortalama				
	0	10.10 a	1.67 a	5.69 a	3.00
	25	7.43 b	0.88 b	5.26 a	2.64
	50	8.07 b	0.92 b	3.94 b	2.46
Ortalama					
Gisela 5		10.07 a	1.25 a	4.54 b	3.12
Maxma		6.48 b	0.72 c	5.75 a	2.27
Colt		9.05 a	1.01 b	4.60 b	2.71
F-test					
Anaç (A)		9.20**	13.79**	7.66**	55.50**
Tuz (T)		5.22*	4.67*	13.71**	23.88**
AxT		0.96öd	0.13öd	0.84öd	3.96*

*, P<0.05; **, P<0.01; öd, önemli değil; A, anaç; T, tuz uygulaması; AxT, interaksiyon; aynı sütundaki farklı harfler Duncan Çoklu Karşılaştırma Testine göre farkların önemli olduğunu gösterir; değerler dört tekerrürün ortalamasıdır

(1999)'a göre tuz stresinin bitki gelişimi üzerine olan bu olumsuz etkileri ozmotik ve iyonik olmak üzere iki şekilde gerçekleşmektedir. Kiraz anaçlarının sürgün gelişimi, yaş ve kuru ağırlığı NaCl uygulaması ile önemli oranda azalmıştır. Benzer sonuçlar Gisela 5 anacında Erturk et al (2007) tarafından, kiraz anaçlarında [GF 677 (*Prunus persica* × *Prunus amygdalus*) ve Nemared (*Prunus persica*)] Sotiropoulos et al (2006a) ve M9 elma anacında Sotiropoulos (2007) tarafından da bildirilmiştir.

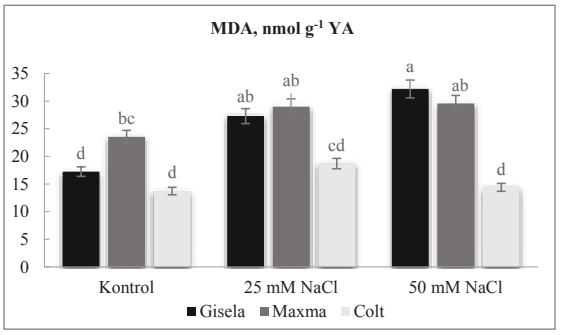
3.2. Lipid peroksidasyonu, toplam antioksidan aktivite, membran geçirgenliği, prolin ve toplam klorofil içeriği

In vitro koşullarda kiraz anaçlarının lipid peroksidasyonu ve toplam antioksidan aktivitesi üzerine AxT interaksiyonunun etkisi önemli olmuştur (Şekil 1 ve Şekil 2). Tuz uygulaması Gisela 5 anacının lipid peroksidasyonunu önemli derecede artırırken, Maxma ve Colt anaçlarının lipid peroksidasyonunda önemli bir değişikliğe neden olmamıştır. Ortalama lipid peroksidasyonunun

Maxma anacında diğer anaçlara göre daha yüksek olduğu belirlenmiştir. Abiyotik stres etmeni ile karşılaşan bitkilerde membran lipidlerinin peroksidasyonu hücresel boyutta stres içeren zararlanmanın bir göstergesidir. Bu sebeple MDA içeriği, membran lipidlerinin peroksidasyonunun ve oksidatif zararlanmaların bir göstergesi olarak değerlendirilmektedir (Gunes et al 2007). Tuz stresi altında MDA içeriğinin arttığı çeşitli çalışmalarda da bildirilmiştir (Gunes et al 2007; Sekmen et al 2007; Eraslan et al 2007; 2008). Tuza toleransı yüksek olan genotiplerin düşük MDA miktarına yani daha az lipid peroksidasyonuna sahip olduğu, lipid peroksidasyonu fazla olan genotiplerin ise tuza daha fazla duyarlılık gösterdikleri çeşitli araştırmacılar tarafından bildirilmiştir. Gossett et al (1994), tuz uygulaması ile hassas bir pamuk çeşidinde lipid peroksidasyonunun dayanıklı çeşitten % 51 oranında daha fazla olduğunu belirtmişlerdir. Benzer sonuçlar; Kuşvuran et al (2007) tarafından kavun genotiplerinde, Sekmen et al (2007) tarafından tuza hassas ve dayanıklı sinirotu çeşitlerinde ve Shalata & Tal (1998) tarafından ise domates genotiplerinde bildirilmiştir. Bu çalışmada da stres koşulunda en yüksek MDA içeriğinin Maxma anacında olduğu belirlenmiştir. Anaçların tuzdan etkilenme düzeyi ile sürgün MDA içeriği arasında ilişki olduğu, MDA içeriğindeki artışa göre yapılan sıralama ile tuza tolerans özelliğinin bağlantılı olduğu sonucuna varılmıştır.

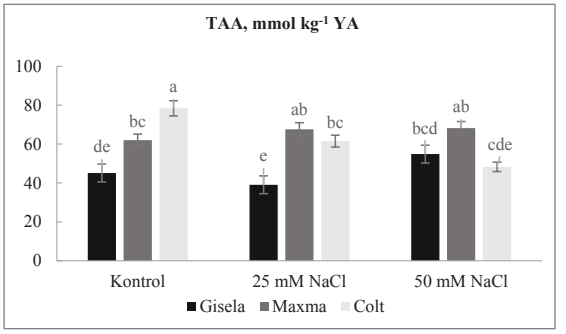
Tuz uygulaması Colt anacının toplam antioksidan aktivitesini azaltmış, Maxma anacının toplam antioksidan aktivitesinde önemli bir değişikliğe neden olmamıştır. Gisela 5 anacında ise yüksek tuz düzeyine göre düşük tuz düzeyinde toplam antioksidan aktivite daha düşük olmuştur (Şekil 2). Toplam antioksidan aktivite ortalama olarak Maxma anacında en yüksek seviyede olmuş bunu sırasıyla Colt ve Gisela 5 anaçları izlemiştir. Yapılan çeşitli araştırmalarda da yetişme ortamında NaCl konsantrasyonunun artması ile bitki yapraklarında toplam antioksidan aktivitenin arttığı bildirilmiştir (Molassiotis et al 2006; Eraslan et al 2007).

Kiraz anaçlarının membran geçirgenliği, prolin ve toplam klorofil içeriği üzerine tuz uygulamasının etkisi önemli olurken anaç ve AxT interaksiyonunun etkisi istatistiki olarak önemli bulunmamıştır. Anaçların membran geçirgenliğini 50 mM dozundaki tuz uygulaması, kontrol ve 25 mM tuz dozuna göre önemli oranda artırmıştır (Şekil 3). Abiyotik stres koşullarında bitkilerin membran



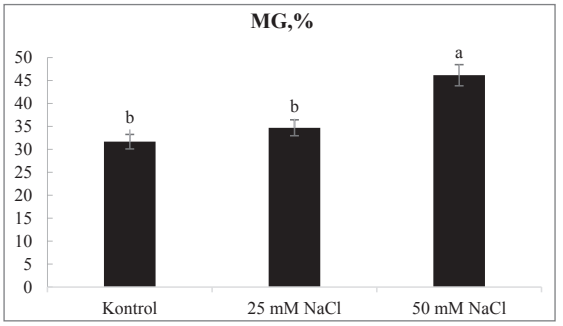
Şekil 1- *In vitro* koşullarda NaCl uygulamasının kiraz anaçlarının lipid peroksidasyonuna (MDA) etkisi [F-Test: Anaç: **; Tuz: **; AxT: * (, P<0.01; *, P<0.05)]**

*Figure 1- Effect of NaCl on lipid peroxidation (MDA) of sweet cherry rootstocks grown in vitro [F-Test: Rootstock: **; Treatments: **; RxT: * (**, P<0.01; *, P<0.05)]*



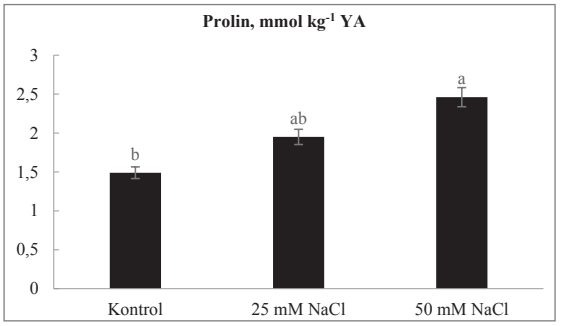
Şekil 2- *In vitro* koşullarda NaCl uygulamasının kiraz anaçlarının toplam antioksidan aktivitesine (TAA) etkisi [F-Test: Anaç: **; Tuz: öd; AxT: ** (, P<0.01; öd, önemli değil)]**

*Figure 2- Effect of NaCl on non-enzymetic total antioxidant activity (TAA) of sweet cherry rootstocks grown in vitro [F-Test: Rootstock: **; Treatments: ns ; RxT: ** (**, P<0.01; ns, non-significant)]*



Şekil 3- *In vitro* koşullarda NaCl uygulamasının kiraz anaçlarının ortalama membran geçirgenliğine (MG) etkisi [F-Test: Anaç: ^{öd}; Tuz: **; AxT: ^{öd} (, P<0.01; öd, önemli değil)]**

Figure 3- Effect of NaCl on average membrane permeability of sweet cherry rootstocks grown in vitro [F-Test: Rootstock: ^{ns}; Treatments: **; RxT: ^{ns} (**, P<0.01; ns, non-significant)]



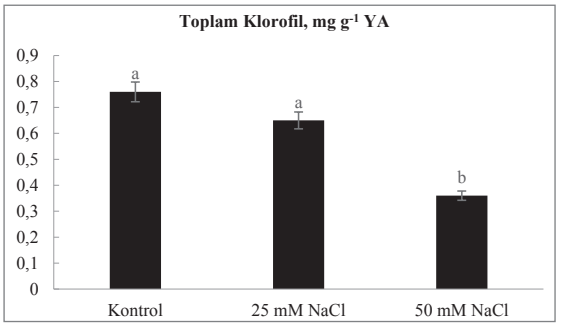
Şekil 4- *In vitro* koşullarda NaCl uygulamasının kiraz anaçlarının ortalama prolin içeriğine etkisi [F-Test: Anaç: ^{öd}; Tuz: **; AxT: ^{öd} (, P<0.01; öd, önemli değil)]**

Figure 4- Effect of NaCl on avarage proline concentrations of sweet cherry rootstocks grown in vitro [F-Test: Rootstock:^{ns}; Treatments:** ; RxT: ^{ns} (**, P<0.01; ns, non-significant)]

geçirgenliğinin arttığını bildiren pek çok araştırma bulunmaktadır (Alpaslan & Gunes 2001; Ismail 2004; Gunes et al 2007).

Artan dozda NaCl uygulaması anaçların prolin içeriklerini kontrole göre artırmış ancak prolin içeriğindeki artış yüksek tuz düzeyinde kontrole göre önemli olmuştur. (Şekil 4). Tarımsal ekosistemde herhangi bir stres etmeni (ağır metal toksisitesi, besin maddesi eksikliği, kuraklık, tuzluluk vb) ile karşılaşan bitkilerin olumsuz koşullara adapte olabilmek için bünyelerinde prolin miktarını artırdıkları belirtilmektedir (Hare & Cress 1997). Tuz stresi altında bitkilerde prolin içeriğinin arttığı Aziz et al (1999), Tarakcioglu & Inal (2002) ve Ismail (2004) tarafından da bildirilmiştir. Wang & Han (2009), yonca çeşitleri ile yaptıkları çalışmada prolin birikiminin tuza toleransın bir sonucu olabileceğini belirtmiştir.

Artan dozda NaCl uygulaması anaçların klorofil içeriklerini kontrole göre azaltmış ancak klorofil içeriğindeki azalış yüksek tuz düzeyinde kontrole göre önemli olmuştur (Şekil 5). Tuz (NaCl) stresinde klorofil içeriğinin azalması, tuzluluğun bitkiler tarafından Mg ve Fe gibi iyonların alımını olumsuz etkilemesi, bunun sonucu olarak kloroplast oluşumunun ve klorofil biyosentezinin azalması şeklinde açıklanmaktadır (Neocleous & Vasilakakis



Şekil 5- *In vitro* koşullarda NaCl uygulamasının kiraz anaçlarının ortalama toplam klorofil içeriğine etkisi [F-Test: Anaç: ^{öd}; Tuz: **; AxT: ^{öd} (**, P<0.01; öd, önemli değil)]

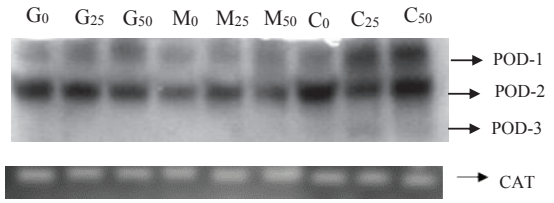
Figure 5- Effect of NaCl on average total chlorophyll concentrations of sweet cherry rootstocks grown in vitro [F-Test: Rootstock:^{ns} Treatments:**; RxT: ^{ns} (**:P<0.01; ns, non-significant)]

2007). Sotiropoulos et al (2006b), tuz uygulaması ile klorofil içeriğinin kiraz anaçlarında azaldığını ancak Gisela 5'in CAB 6P' ye göre daha fazla klorofil içerdiğini belirtmiştir. Tuz uygulaması ile bitkide klorofil içeriğinin azaldığına ait benzer sonuçlar Erturk et al (2007) tarafından Gisela 5 kiraz anacında ve Papadakis et al (2007) tarafından farklı kiraz çeşitlerinde de bildirilmiştir.

3.3. Peroksidaz (POD) ve katalaz (CAT) enzim izoformları

Farklı dozlarda NaCl uygulanan kiraz anaçlarının peroksidaz (POD) ve katalaz (CAT) enzim izoformları Şekil 6'da verilmiştir. Kiraz anaçlarının POD profillerindeki bantlarının farklı olarak yoğunlaştığı gözlenmiştir. Peroksidaz profillerindeki ilk bantlarda 25 ve 50 mM NaCl içeren MS ortamı üzerinde gelişen kiraz anaçlarının POD bandının kontrol grubuna göre daha yoğun olduğu ve Maxma anacının diğer anaçlara göre daha az yoğunlukta bant oluşturduğu gözlenmiştir. Özellikle 25 ve 50 mM NaCl içeren MS ortamı üzerinde gelişen Colt kiraz anacının yoğun bir peroksidaz bandı oluşturduğu tespit edilmiştir. Peroksidaz profillerindeki 3. bantlar sadece 25 ve 50 mM NaCl içeren MS ortamı üzerinde gelişen Colt anacında çok az bir yoğunlukta gözlenmiştir. Anaçlar arasında POD izoformu değerlendirildiğinde en düşük aktivasyonun Maxma anacında görüldüğü ve bunu sırasıyla Gisela 5 ve Colt anaçlarının izlediği belirlenmiştir.

Tuzlu ortamda POD enzim aktivasyonu ve izoform sayısındaki artış çeltik yapraklarında (Lee et al 2001), *Suaeda nudiflora*' nın kalluslarında (Cherian & Reddy 2003), ve MM106 elma anacının yaprak ve sürgünlerinde (Molassiotis et al 2006) tespit edilmiştir. Wang & Han (2009) tuzlu koşullarda yetişen yonca çeşitlerinden dayanıklı olanların POD aktivitelerini ve izoform sayısını artırarak tuza karşı tolerans geliştirebileceğini belirtmiştir.



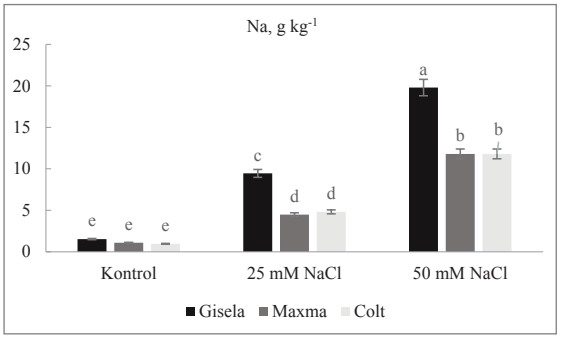
Şekil 6- *In vitro* koşullarda NaCl uygulanan kiraz anaçlarının peroksidaz (POD) ve katalaz (CAT) enzim izoformları. G, Gisela 5; M, Maxma; C, Colt; $_0$, kontrol; $_{25}$, 25 mM NaCl; $_{50}$, 50 mM NaCl

Figure 6- Effect of NaCl on peroxidase (POD) and catalase (CAT) antioxidant enzyme isoforms of sweet cherry rootstocks in vitro. G, Gisela 5; M, Maxma; C, Colt; $_0$, control; $_{25}$, 25 mM NaCl; $_{50}$, 50 mM NaCl

Stres koşullarında bitkilerde birikebilen hidrojen peroksidin (H_2O_2) parçalanmasında (detoksifikasyonu) etkili olan enzimler katalaz, askorbat-glutasyon döngüsüne katılan glutasyon reduktaz ve askorbat peroksidaz enzimleridir. Farklı dozlarda NaCl içeren ortamlar üzerinde gelişen Gisela 5, Maxma ve Colt kiraz anaçlarının CAT enzim izoformları, kiraz anaçlarının katalaz profillerinde bantlar tespit edildiğini fakat bantlar arasında belirgin bir fark olmadığını göstermiştir (Şekil 6). Tuza toleransı farklı olan karpuz çeşitlerinde tuz stresinin etkisini araştıran Yaşar et al (2008) tuz stresi koşullarında CAT aktivitesinin özellikle tuza toleranslı çeşitlerde çok ciddi artışlar gösterirken duyarlı çeşitlerde çok daha düşük seviyede artış göstermesinin tuz stresi altında CAT enzim aktivitesini artırma konusunda tuza toleranslı çeşitlerin daha yetenekli olmasından kaynaklandığını ve bu görüşün yaygın şekilde kabul edildiğini bildirmişlerdir. Chinta et al (2001), dut bitkisinde tuzlu koşullarda CAT enzim aktivitesinin arttığını, Lee et al (2001) ve Asish et al (2004) ise tuzlu ortamlarda katalaz enzim aktivasyonunun azaldığını belirtmişlerdir. Bu durum CAT enzim aktivitesinin bitki tür ve çeşidi ile araştırma koşullarına göre değişebildiğini göstermektedir.

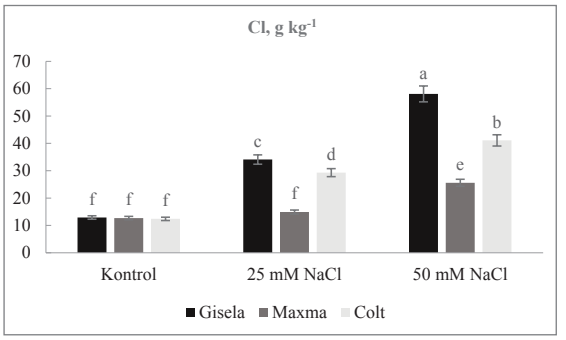
3.4. Bitki Na ve Cl konsantrasyonları

Kiraz anaçlarının Na ve Cl konsantrasyonları üzerine AxT interaksiyonunun etkisi önemli olmuştur. Uygulanan NaCl dozu arttıkça bitki Na ve Cl konsantrasyonları artmıştır (Şekil 7 ve 8). Tuzlu koşullarda Gisela 5 kiraz anacının Na ve Cl konsantrasyonlarının Maxma ve Colt kiraz anaçlarının Na ve Cl konsantrasyonlarından daha yüksek olduğu belirlenmiştir. Güneş et al (2003) asma anaçlarında, Papadakis et al (2007) farklı kiraz çeşitlerinde, Karakullukçu & Adak (2008) nohut çeşitlerinde, Karimi & Hasanpour (2014) nar çeşitlerinde, NaCl uygulaması ile sürgün Na ve Cl konsantrasyonlarının önemli oranda arttığını ve Na ve Cl birikimi yönünden anaç veya çeşitler arasında fark olduğunu belirtmişlerdir. Kuşvuran et al (2008), Na ve Cl konsantrasyonlarının tuza toleranslı ve hassas kavun genotiplerinin belirlenmesi açısından etkin bir parametre olabileceğini belirtmiştir.



Şekil 7- *In vitro* koşullarda NaCl uygulamasının kiraz anaçlarının Na konsantrasyonuna etkisi [F-Test: Anaç: **; Tuz: **; AxT: ** (, P<0.01)]**

*Figure 7- Effect of NaCl on Na concentrations of sweet cherry rootstocks grown in vitro [(F-Test: Rootstock:** Treatments:** ; RxT: ** (**, P<0.01)]*



Şekil 8- *In vitro* koşullarda NaCl uygulamasının kiraz anaçlarının Cl konsantrasyonuna etkisi [F-Test: Anaç: **; Tuz: **; A*T: ** (, P<0.01)]**

*Figure 8- Effect of NaCl on Cl concentrations of sweet cherry rootstocks grown in vitro [F-Test: Rootstock:** Treatments:** ; RxT: ** (**, P<0.01)]*

4. Sonuç

Bu çalışmadan elde edilen sonuçlara göre, tüm anaçların sürgün gelişimi tuz stresinden olumsuz etkilenmiştir. Stres koşullarında anaçların fizyolojik ve biyokimyasal yapılarında önemli değişikler meydana gelmiştir. Tuzluluğa toleransın belirlenmesinde sürgün gelişimi ile uyumlu bir şekilde sürgün lipid peroksidasyonu, toplam antioksidan ve POD enzim aktivitelerinin strese dayanım mekanizmasında seçim kriteri olarak kullanılabileceği belirlenmiş, stres koşulunda en yüksek lipid peroksidasyonu ve en düşük POD enzim aktivasyonu Maxma anacından elde edilmiştir. Tüm gelişim ve fizyolojik parametreler birlikte değerlendirildiğinde tuzluluk stresinden en fazla etkilenen anacın Maxma ve en az etkilenen anacın Gisela 5 olduğu, Colt anacının ise tuz stresinden orta derecede etkilendiği sonucuna varılmıştır.

Teşekkür

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Kaynaklar

Alpaslan M & Gunes A (2001). Interactive effects of boron and salinity stress on the growth, membrane permeability and mineral composition of tomato and cucumber plants. *Plant and Soil* **236**: 123-128

Arnon D M (1949). Copper enzymes in isolated chloroplasts polyphenoloxidase in *Beta vulgaris*. *Plant Physiology* **24**: 1-15

Asish K P, Anath B D & Prasanna M (2004). Defense potentials to NaCl in mangrove, *Bruguiera parviflora*: Differential changes of isoforms of some antioxidative enzymes. *Journal of Plant Physiology* **161**: 531-542

Aziz A, Martin-Tanguy J & Lather F (1999). Salt stress-induced proline accumulation and changes in tyramine and polyamine levels are linked to ionic adjustment in tomato leaf discs. *Plant Science* **145**: 83-91

Balal R M, Ashraf M Y, Khan M M, Jaskani M J & Ashfaq M (2011). Influence of salt stress on growth and biochemical parameters of citrus rootstocks. *Pakistan Journal of Botany* **43**(4): 2135-2141

Bates L S, Waldren R P & Teare I D (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil* **39**: 205-207

Bradford M M (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**: 248-254

Cadenas S E (1989). Biochemistry of oxygen toxicity. *Annual Review Biochemistry* **58**: 79-110

Cherian S & Reddy M P (2003). Evaluation of NaCl tolerance in the callus cultures of *Suaeda nudiflora* Moq. *Biologia Plantarum* **46**: 193-198

Chinta S, Lakshmi A & Giridarakumar S (2001). Change in the antioxidant enzyme efficacy in two high yielding genotypes of mulberry (*Morus alba* L.) under NaCl salinity. *Plant Science* **161**: 613-619

Cornic G (1996). Drought stress inhibits photosynthesis by decreasing stomatal aperture-not by affecting ATP synthesis. *Trends in Plant Science* **1**: 21-26

Davies K J A (1987). Protein damage and degradation by oxygen radicals. I. General aspects. *Journal of Biological Chemistry* **262**: 9895-9901

Dragišić Maksimović J, Zhang J, Zeng F, Živanović B D, Shabala L, Zhou M & Shabala S (2013). Linking oxidative and salinity stress tolerance in barley: Can root antioxidant enzyme activity be used as a measure of stress tolerance? *Plant and Soil* **365**: 141-155

Eraslan F, Inal A, Savasturk O & Gunes A (2007). Changes in antioxidative system and membrane damage of lettuce in response to salinity and boron toxicity. *Scientia Horticulturae* **114**: 5-10

Eraslan F, Inal A, Pilbeam D J & Gunes A (2008). Interactive effects of salicylic acid and silicon on oxidative damage and antioxidant activity in spinach (*Spinacia oleracea* L. cv. Matador) grown under boron toxicity and salinity. *Plant Growth Regulation* **55**: 207-219

Erturk U, Sivritepe N, Yerlikaya C, Bor M, Ozdemir F & Turkan I (2007). Responses of the cherry rootstock to salinity *in vitro*. *Biologia Plantarum* **51**(3): 597-600

FAO (2002). Crops and drops. Making the best use of water for agriculture. Food and Agriculture Organization of the United Nations, Rome, Italy

FAO (2012). Agricultural Statistics, Food and Agriculture Organization of the United Nations. http:\\www.faostat.fao.org., Accessed: 29 October 2014

Fisarakis I, Chartzoulakis K & Stavrakas D (2001). Response of Sultana vines (*V. vinifera* L.) on six rootstocks to NaCl salinity exposure and recovery. *Agricultural Water Management* **51**(1): 13-27

Fridovich I (1986). Biological effects of superoxide radical. *Archives of Biochemistry Biophysics* **247**: 1-11

Gossett D R, Millhollon E P & Lucas M C (1994). Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. *Crop Science* **34**: 706-714

Gulen H & Eris A (2004). Effect of heat stress on peroxidase activity and protein content in strawberry plants. *Plant Science* **166**(3): 739-744

Güneş A, Çelik H, Alpaslan M, Söylemezoğlu G, Eraslan F, Yaşa Z & Koç Ö (2003). Asmaların (*Vitis* spp.) bor toksisitesi ve tuzluluğa karşı toleransının belirlenmesine yönelik olarak bor, sodyum ve klor alımlarının karşılaştırılması. *Tarım Bilimleri Dergisi–Journal of Agricultural Sciences* **9**: 428-434

Gunes A, Inal A, Bagci E G & Pilbeam D (2007). Silicon-mediated changes of some physiological and enzymatic parameters symptomatic for oxidative stress in spinach and tomato grown in sodic-B toxic soil. *Plant and Soil* **290**: 103-114

Hare P D & Cress W A (1997). Metabolic implication of stress-induced proline accumulation in plants. *Plant Growth Regulation* **21**: 79-102

Heath R L & Packer L (1968). Photoperoxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics* **125**: 189-198

Imlay J A & Linn S (1988). DNA damage and oxygen radical toxicity. *Science* **240**: 1302-1309

Ismail A M (2004). Response of maize and sorghum to excess boron and salinity. *Biologia Plantarum* **47**(2): 313-316

Johnson J M & Ulrich A (1975). Analytical Methods for Use in Plant Analysis. II. California: *Agricultural Experimental Station Bulletin*

Karakullukçu E & Adak M S (2008). Bazı nohut (*Cicer arietinum* L.) çeşitlerinin tuza toleranslarının belirlenmesi. *Tarım Bilimleri Dergisi-Journal of Agricultural Sciences* **14**(4): 313-319

Karimi H R & Hasanpour Z (2014). Effects of salinity and water stress on growth and macro nutrients concentration of pomegranate (*Punica granatum* L.). *Journal of Plant Nutrition* **37**: 1937-1951

Kuşvuran Ş, Ellialtıoğlu Ş, Abak K & Yaşar F (2007). Bazı kavun (*cucumis* sp.) genotiplerinin tuz stresine tepkileri. *Tarım Bilimleri Dergisi-Journal of Agricultural Sciences* **13**(4): 395-404

Kuşvuran Ş, Yaşar F, Abak K & Ellialtıoğlu Ş (2008). Tuz stresi altında yetiştirilen tuza tolerant ve duyarlı *Cucumis* sp.'nin bazı genotiplerinde lipid peroksidasyonu, klorofil ve iyon miktarlarında

meydana gelen değişimler. *Yüzüncü Yıl Üniversitesi, Ziraat Fakültesi, Tarım Bilimleri Dergisi* **18**(1): 13-20

Kotuby-Amacher J, Koening R & Kitchen B (2000). Salinity and Plant Tolerance. Electronic Publication AG-SO-03, Utah State University Extension, Logan.

Laemmli U K (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**: 680-685

Lawlor D W (2001). Limitation of photosynthesis in water stressed leaves: Stomata vs. metabolism and the role of ATP. *Annals of Botany* **89**: 1-15

Lee D H, Kim T S & Lee C B (2001). The inductive responses of the antioxidant enzymes by salt stress in the rice (*Oryza sativa* L.). *Journal of Plant Physiology* **158**: 737-745

Liebler D C, Kling D S & Reed D J (1986). Antioxidant protection of phospholipid bilayers by α-tocopherol. Control of α-tocopherol status and lipid peroxidation by ascorbic acid and glutathione. *Journal of Biological Chemistry* **261**: 12114-12119

Molassiotis A N, Sotiropoulos T E, Tanou G, Kofidis G, Diamantidis G & Therios I (2006). Antioxidant and anatomical responses in shoot culture of the apple rootstock MM 106 treated with NaCl, KCl, mannitol or sorbitol. *Biologia Plantarum* **50**(1): 61-68

Murashige T & Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15**(3): 473-497

Neocleous D & Vasilakakis M (2007). Effects of NaCl stress on red raspberry (*Rubus idaeus* L. 'Autumn Bliss'). *Scientia Horticulturae* **112:** 282-289

Oren R, Sperry J S, Katul G G, Pataki D E, Ewers B E, Phillips N & Schaffer K V R (1999). Survey and synthesis of intra and inter specific variation of stomatal sensitivity to vapour pressure deficit. *Plant Cell & Environment* **22**: 1515-1526

Papadakis I E, Veneti G, Chatzissavvidis C, Sotiropoulos T E, Dimassi K N & Therios I N (2007). Growth, mineral composition, leaf chlorophyll and water relationships of two cherry varieties under NaCl-induced salinity stress. *Soil Science & Plant Nutrition* **53**(3): 252-258

Prieto P, Pineda M & Aguilar M (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry* **269**: 337-341

Ruiz D, Martinez V & Cerda A (1999). Demarcating specific ion (NaCl, Cl^-, Na^+) and osmotic effects in the response of two citrus rootstocks to salinity. *Scientia Horticulturae* **80**: 213-224

Sairam R K & Saxena D C (2000). Oxidative stress and antioxidants in wheat genotypes: Possible mechanism of water stress tolerance. *Journal of Agronomy & Crop Science* **184**: 55-61

Sairam R K, Deshmukhi P S & Saxena D C (1998). Role of antioxidant systems in wheat genotypes tolerance to water stress. *Biologia Plantarum* **41**(3): 387-394

Sekmen A H, Turkan I & Takio S (2007). Differential responses of antioxidative enzymes and lipid peroxidation to salt stress in salt-tolerant *Plantago maritima* and salt-sensitive *Palantago media. Physiologia Plantarum* **131**(3): 399-411

Shalata A & Tal M (1998). The effect of salt stress on lipid peroxidation and antioxidants in the leaf of the cultivated tomato and its wild salt-tolerant relative *Lycopersicon pennellii*. *Physiologia Plantarum* **104**: 169-174

Shen H & Yan X (2002). Membrane permeability in roots of crotalaria seedlings as affected by low temperature and low phosphorus stress. *Journal of Plant Nutrition* **25**(5): 1033-1047

Singh S K, Sharma H C, Goswami A M, Datta S P & Singh S P (2000). *In vitro* growth and leaf composition of grapevine cultivars as affected by sodium chloride. *Biologia Plantarum* **43**(2): 283-286

Sotiropoulos T E, Dimassi K N, Tsirakoglou V & Therios I N (2006a). Responses of two *Prunus* rootstocks to KCl induced salinity *in vitro*. *Biologia Plantarum* **50**(3): 477-480

Sotiropoulos T E, Therios I N, Almaliotis D, Papadakis I & Dimassi K N (2006b). Response of cherry rootstocks to boron and salinity. *Journal of Plant Nutrition* **29**: 1691-1698

Sotiropoulos T E (2007). Effect of NaCl and $CaCl_2$ on growth and contents of minerals, chlorophyll, proline and sugars in the apple rootstock M 4 cultured *in vitro*. *Biologia Plantarum* **51**(1): 177-180

Tarakcioglu C & Inal A (2002). Changes induced by salinity, demarcating specific ion ratio (Na/Cl) and osmolality in ion and proline accumulation, nitrate reductase activity and growth performance of lettuce. *Journal of Plant Nutrition* **25**(1): 27-41

Termaat A & Munns R (1986). Use of concentrated macronutrient solutions to separate osmotic from

NaCl-specific effects on plant growth. *Australian Journal of Plant Physiology* **13**: 509-522

Troncoso A, Matte C, Cantos M & Lavee S (1999). Evaluation of salt tolerance of in vitro-grown grapevine rootstock varieties. *Vitis* **38**: 55-60

Turner N C, Colmer T D, Quealy J, Pushpavalli R, Krishnamurthy L, Kaur J, Singh G, Siddique K H M & Vadez V (2013). Salinity tolerance and ion accumulation in chickpea (*Cicer arietinum* L.) subjected to salt stress. *Plant and Soil* **365**: 347-361

Wang X & Han J (2009). Changes of proline content, activity, and active isoforms of antioxidative enzymes in two alfalfa cultivars under salt stress. *Agricultural Sciences in China* **8**(4): 432-440

Wendel J F & Weeden N F (1989). Visualization and interpretation of plant isozymes. In: D E Soltis & P S Soltis (Eds), *Isozymes in Plant Biology*, Dioscorides Press, Portland, Oregon, pp. 5-44

Withan F H, Blaydes D F & Dewlin R M (1971). Experiments in Plant Physiology. New York, Von Nostrand Reinhold Company, pp. 55-56

Wolfe W H (1976). Identification of grape varieties by isozyme banding patterns. *American Journal of Enology Viticulture* **27**: 68-73

Yaşar F, Ellialtıoğlu Ş, Özpay T & Uzal Ö (2008). Tuz stresinin karpuzda (*Citrullus lanatus* (Thunb.) Mansf.) antioksidatif enzim (SOD, CAT, APX ve GR) aktivitesi üzerine etkisi. *Yüzüncü Yıl Üniversitesi, Ziraat Fakültesi, Tarım Bilimleri Dergisi* **18**(1): 51-55

TARIM BİLİMLERİ DERGİSİ-JOURNAL OF AGRICULTURAL SCIENCES

YAZIM KURALLARI

Genel

Tarım Bilimleri Dergisi, tarım bilimleri alanında ve yazım dili İngilizce olan özgün araştırma makaleleri yayınlar. Sonuçları önceden bilinen ve yenilik getirmeyen araştırma makaleleri, taksonomi ile sadece durum tespitine dayanan ve yöresel çalışmalar ile veri ve anket analizine dayanan çalışmalar derginin kapsamı dışındadır. Basılacak makalelerin daha önce hiçbir yerde yayınlanmamış olması ve yayın haklarının verilmemiş olması gerekir. Dergide yayımlanacak makalelerin her türlü sorumluluğu yazarına/yazarlarına aittir.

Yayımlanması için gönderilen eser, yayın ilkeleri doğrultusunda Dergi Editörler Kurulu tarafından ön incelemeye alınır. Dergi Editörler Kurulu, dergide yayınlanabilecek nitelikte bulmadığı makaleleri hakemlere göndermeden yazara/yazarlara iade kararı verme hakkına sahiptir. Ayrıca yazım kurallarına uymayan veya anlatım dili yetersiz olan makaleler, düzeltilmek üzere yazara/yazarlara iade edilir. Değerlendirmeye alınan makaleler, incelenmek üzere en az 2 hakeme gönderilir. Hakem değerlendirmesinden geçen makalelere ait düzeltmeler, düzeltmeler listesiyle birlikte en fazla 30 gün içerisinde sisteme yüklenerek gönderilmelidir. Bu süreden sonraki gönderimler kabul edilmez. Başeditör, hakem raporlarını ve/veya istenilen düzeltmelerin yeterli olup olmamasını dikkate alarak makalenin yayımlanıp yayımlanmamasına karar verir.

Makalede isimleri yer alan tüm yazarlar, yayım haklarını Tarım Bilimleri Dergisine verdiklerine dair **Makale Gönderme ve Telif Hakkı Devir Sözleşmesini** imzalamalıdır. Makalenin yayımlanması kabul edildikten sonra makale metninde, yazarlarında ve yazarların sıralamasında değişiklik yapılamaz. Makale yayıma kabul edildiğinde, sorumlu yazar Ankara Üniversitesi adına açılmış banka hesabına 300 TL yatırmalıdır. Makaleden sorumlu yazara banka hesap numarası, makalenin basıma kabul edilmesinden sonra bildirilir.

Makale Yükleme

Hazırlanan makaleler; sadece makaleden sorumlu yazar (makalenin yayım başlangıcından basım sonrasındaki her türlü yazışmalarda sorumluluğu bulunan) tarafından Tarım Bilimleri Dergisi web sayfasındaki çevrimiçi **Makale Gönderme ve Değerlendirme Sistemi** kullanılarak elektronik ortama yüklenmelidir. Makale yükleme bölümünün **"Başvuruyu Yükle"** bölümünde pdf formatındaki makale dosyasına ilave olarak "**Ek Dosyalar**" bölümüne aşağıdaki dosyaların da yüklenmesi gerekir.

- ✓ Makalenin Word (2003 veya daha üst versiyonları) formatındaki dosyası. Sisteme yüklenen makalenin hem pdf formatında ve hem de Word formatında iletişim, ad-soyad, kurum gibi yazarları tanıtıcı bilgiler bulunmamalıdır.
- ✓ Tüm yazarlar tarafından imzalanmış ve pdf formatında taranmış olan "Makale Gönderme ve Telif Hakkı Devir Sözleşmesi". Yayıma kabul edilmesi durumunda bu formların aslı da posta ile editöre gönderilmelidir.
- ✓ Yazar Makale Kontrol Listesi (pdf formatında),
- ✓ Yazarların ad-soyad, kurum ve iletişim bilgilerini içeren Word dosyası,
- ✓ Gerekiyorsa Etik Kurul Raporu eklenmelidir.

Derginin Kapsamı

Tarım Bilimleri Dergisi, tarım bilimleri alanında yapılan özgün araştırmaları ve yeni bulguları içeren makaleleri yayımlar. Sonuçları önceden bilinen ve yenilik getirmeyen araştırma makaleleri, taksonomi ile sadece durum tespitine dayanan ve yöresel çalışmalar ile veri ve anket analizine dayana çalışmalar derginin kapsamı dışındadır. Derleme makaleler, yayın komisyonunun çağrısı üzerine hazırlanmışsa normal inceleme ve değerlendirme sürecinden geçirilerek yayınlanır.

Makale Hazırlama

Makaleler, A4 boyutundaki kâğıdın tek yüzüne 12 punto Times New Roman yazı tipinde ve çift satır aralıklı yazılmalıdır. Sayfanın sağında, solunda, altında ve üstünde 3'er cm boşluk bırakılmalıdır. Makalenin her sayfası ve satırları numaralandırılmalıdır. Yazar ad(lar)ı açık olarak yazılmalı ve herhangi bir akademik unvan belirtilmemelidir. Editörler kurulu, anlatım dili yeterli olmayan makaleleri değerlendirme dışı tutabilir. Yazar(lar)ın makale göndermeden önce eseri dil yönünden bir dil bilimciye incelettirmesi tavsiye olunur. Sıralama olarak, İngilizce özet ve peşinden Türkçe özet verilir. Bu durum şekil ve çizelge başlıkları için de geçerlidir.

Makale; Türkçe Başlık, Türkçe Özet, Anahtar Kelimeler, İngilizce Başlık, İngilizce Özet, Keywords, 1.Giriş, 2.Materyal ve Yöntem, 3.Bulgular ve Tartışma, 4.Sonuçlar, Teşekkür (varsa), Kısaltmalar ve/veya Semboller (varsa), Kaynaklar bölümleri ile Şekil ve Çizelgelerden oluşmalıdır. Bölüm adları koyu yazılmalıdır.

Makale, "Kaynaklar" bölümü dâhil 16 sayfayı geçmemelidir. Yazar(lar), bu kısımların oluşturulmasında derginin web sayfasındaki **Makale Hazırlama Şablonunu** kullanmalıdır.

Başlık: Kısa ve açıklayıcı olmalı, 14 punto ve koyu, kelimelerin ilk harfi büyük olmalı, ortalanarak yazılmalı ve 15 kelimeyi geçmemelidir. İngilizce başlık Türkçe başlığı tam olarak karşılamalı, 13 punto ve koyu yazılmalıdır.

Özet ve Anahtar Kelimeler: Türkçe ve İngilizce özetlerin her biri 300 kelimeyi geçmemelidir. Türkçe ve İngilizce özetlerde sırasıyla "Özet" ve "Abstract" kelimeleri kullanılmalıdır. Özet, çalışmanın amacını, nasıl yapıldığını, sonuçları ve sonuçlar üzerine yazar(lar)ın yaptığı değerlendirmeleri içermelidir. Özetlerin 1 satır altına, her anahtar kelimenin ilk harfi büyük diğerleri küçük harflerle, mümkünse başlıkta kullanılmayan, çalışmayı en iyi biçimde tanımlayacak ve aralarında noktalı virgül (;) olacak şekilde en fazla 6 anahtar kelime yazılmalıdır.

1. Giriş: Bu bölümde; çalışma konusu, gerekçesi, konu ile doğrudan ilgili önceki çalışmalar ve çalışmanın amacı verilir.

2. Materyal ve Yöntem: Kullanılan materyal ve yöntem aynı başlıkta verilmelidir. Alt başlık verilecekse bölüm numarası ile birlikte numaralandırılmalı (2.1. gibi) ve italik yazılmalıdır. Yeni veya değiştirilmiş yöntemler, aynı konuda çalışanlara araştırmayı tekrarlama olanağı verecek nitelikte açıklanmalıdır.

3. Bulgular ve Tartışma: Elde edilen bulgular verilmeli, gerekirse çizelge, şekil ve grafiklerle desteklenerek bulgular açıklanmalıdır. Elde edilen bulgular tekrardan kaçınılması amacıyla ya çizelge ya da grafik olarak verilmelidir. İstatistikî olarak önemli bulunan faktörler, uygulanan istatistik analiz tekniğine uygun karşılaştırma yöntemi ile yorumlanarak ilgili istatistikler üzerinde harflendirme yapılmalıdır. İstatistiki analiz yönteminin doğru seçilmediği ve/ya analizin gereği gibi yapılmadığı durumlarda editörler kurulu makaleyi değerlendirme dışında tutabilir. Bulgular tartışılmalı ancak gereksiz tekrarlardan kaçınılmalıdır. Bulguların başka araştırmalarla benzerlik ve farklılıkları verilmeli, nedenleri açıklanmalıdır.

4. Sonuçlar: Elde edilen sonuçlar, bilime ve uygulamaya katkısıyla birlikte kısa ve öz olarak verilmelidir. Giriş ile Bulgular ve Tartışma bölümünde verilen ifadeler bu kısımda aynı şekilde tekrar edilmemelidir.

Teşekkür: Gerekli ise mümkün olduğunca kısa olmalı ve yapılan katkı ifade edilerek verilmelidir.

Kısaltmalar ve/veya Semboller: Makalede kısaltmalardan mümkün olduğunca kaçınılmalıdır. Semboller Makale Hazırlama Şablonunda belirtildiği gibi verilmelidir. Kısaltma ve semboller metin içinde ilk kez kullanıldığı yerde açıklanmalıdır. Uluslararası geçerliliği olan ve yerleşik kısaltmalar tercih edilmelidir. Kısaltmalar makalenin başlığında kullanılmamalıdır. Semboller SI sistemine göre verilmelidir.

Kaynaklar: Eserde yararlanılan kaynaklara ilişkin atıf metin içinde "(Yazarın soyadı yıl)" yöntemine göre yapılmalıdır. Örnek: (Doymaz 2003), (Basunia & Abe 2001). Yazara atıf yapılırsa sadece yayının yılı parantez içine alınmalıdır. Örnek: Doymaz (2003)'e göre ya da Basunia & Abe (2001). Üç ya da daha fazla yazar için makale içindeki atıfta "et al" kullanılmalıdır. Örnek: (Lawrence et al 2001) veya Lawrence et al (2001)'e göre. Aynı yazarın aynı yıl içinde 1'den fazla yayını varsa, yıldan sonra küçük harfler verilmelidir. Örnek: (Akpınar et al 2003a). Aynı yazarın birden fazla yayınına atıf yapılacaksa yıldan sonra noktalı virgül (;) işareti ile ayırt edilmelidir. Örnek: (Akpınar 2007; 2009; 2013). Birden fazla atıf yapılırsa atıflar arasında noktalı virgül (;) kullanılmalıdır. Örnek: (Perl et al 1987; Bailly et al 1996; Copeland & McDonald 2001; Goel & Sheoran 2003). Eğer bilginin, kaynağın belirli bir sayfasından ya da sayfalarından alındığı belirtilmek istenirse (Hardeman & Jochemsen 2012, s 657-674; Naess 1991, s 34) biçiminde gösterilmelidir. Kaynaklarda Anonim ya da Anonymous şeklinde gösterim yapılmamalıdır.

Kaynaklar bölümünde metin içinde atıfı yapılan tüm kaynaklar alfabetik olarak (yazarların soyadlarına göre) ve orijinal dilinde verilir. İki veya daha fazla yazarlı eserlerin bildiriminde son yazardan önce "&" kullanılmalıdır. Örnek: Lawrence K C, Funk D B & Windham W R (2001). Kaynağın sonuna nokta (.) işareti konulmamalıdır. Dergi isimleri kısaltma yapılmadan tam adı ile yazılma ve italik yazılmalıdır. Kongre kitaplarında Türkçe ya da yabancı dilde özeti yayınlanmış çalışmalara atıf yapılamaz. Makaledeki yanlış atıf ve kaynak gösterimlerine ait sorumluluk yazar(lar)a aittir. Kaynaklar bölümündeki her bir kaynağın sonuna nokta (.) konmamalıdır.

Dergi:

Doymaz I (2003). Drying kinetics of white mulberry. *Journal of Food Engineering* **61**(3): 341-346

Basunia M A & Abe T (2001). Thin-layer solar drying characteristics of rough rice under natural convection. *Journal of Food Engineering* **47**(4): 295-301

Lawrence K C, Funk D B & Windham W R (2001). Dielectric moisture sensor for cereal grains and soybeans. *Transactions of the ASAE* **44**(6): 1691-1696

Akpinar E, Midilli A & Bicer Y (2003a). Single layer drying behaviour of potato slices in a convective cyclone dryer and mathematical modelling. *Energy Conversion and Management* **44**(10): 1689-1705

Kitap:

Yıldırım O (1996). Bahçe Bitkileri Sulama Tekniği. Ankara Üniversitesi Ziraat Fakültesi Yayınları: 1438, Ders Kitabı: 420, Ankara

Mohsenin N N (1970). Physical Properties of Plant and Animal Materials. Gordon and Breach Science Publishers, New York

Kitapta Bölüm:

Fıratlı Ç (1993). Arı yetiştirme. (Ed: M Ertuğrul), *Hayvan Yetiştirme*, Baran Ofset, Ankara, s. 30-34

Rizvi S S H (1986). Thermodynamic properties of foods in dehydration. In: M A Rao & S S H Rizvi (Eds), *Engineering Properties of Foods*, Marcel Dekker, New York, pp. 190-193

Yazarı Belirtilmeyen Kurum Yayınları:

TUİK (2012). Tarım İstatistikleri Özeti. Türkiye İstatistik Kurumu, Yayın No: 3877, Ankara

ASAE (2002). Standards S352.2, 2002, Moisture measurement-unground grain and seeds. ASAE, St. Joseph, MI

İnternetten Alınan Bilgi:

FAO (2013). Classifications and standards. http://www.fao.org/economic/ess/ess-standards/en/ (Erişim tarihi:10.02.2013)

Tez:

Koyuncu T (1992). Tarım arabalarında kullanılan çarpma etkili frenlerin araştırılması. Yüksek lisans tezi, Ankara Üniversitesi Fen Bilimleri Enstitüsü (Basılmamış), Ankara

Berbert P A (1995). On-line density-independent moisture content measurement of hard winter wheat using the capacitance method. PhD Thesis, Crandfield University (Unpublished), UK

Tam Metin Kongre/Sempozyum Kitabı:

Yağcıoğlu A, Değirmencioğlu A & Cağatay F (1999). Drying characteristics of laurel leaves under different drying conditions. In: *Proceedings of the 7th International Congress on Agricultural Mechanization and Energy*, 26–27 May, Adana, Turkey, pp. 565–569

Kara Z & Beyoğlu N (1995). Konya ili Beyşehir yöresinde yetiştirilen üzüm çeşitlerinin göz verimliliklerinin belirlenmesi üzerine bir araştırma. *Türkiye II. Ulusal Bahçe Bitkileri Kongresi. Bildiriler (II)*: 3-6 Ekim, Adana, s. 524-528

Şekiller ve Çizelgeler: Şekil, grafik, fotoğraf ve benzerleri "Şekil", sayısal değerler ise "Çizelge" olarak belirtilmelidir. Tüm şekil ve çizelgeler makalenin sonuna yerleştirilmelidir. Şekil ve çizelgelerin boyu tek sayfa düzeninde en fazla 16x20 cm ve çift sütun düzeninde ise genişliği en fazla 8 cm olmalıdır. Şekil ve çizelgelerin boyutu baskıda çıkabilecek çözünürlükte olmalıdır. Araştırma sonuçlarını destekleyici nitelikteki resimler 600 dpi çözünürlüğünde "jpg" formatında olmalıdır. Renkli resimler yerine gri ya da siyah tonlu resimler tercih edilmelidir. Çizelgelerde düşey çizgi kullanılmamalı ve makale hazırlama şablonunda belirtildiği gibi hazırlanmalıdır. Her çizelge ve şekle metin içerisinde atıf yapılmalıdır. Tüm çizelge ve şekiller makale boyunca sırayla numaralandırılmalıdır (Çizelge 1 ve Şekil 1). Çizelge ve şekil başlıkları ve açıklamaları kısa ve öz olmalıdır. Çizelge ve şekillerin İngilizce başlıkları, Türkçe başlığın hemen altına italik olarak yazılmalı, ilk yazılan Türkçe başlık yazısı koyu olmalıdır. Şekil ve çizelge başlık yazıları 9.5 punto, şekil ve çizelgelerin içindeki yazılar 9 punto, çizelge altı yazılar 8 punto Times New Roman yazı karakterinde olmalıdır. Şekillerde yatay ve düşey kılavuz çizgiler ve rakamlar bulunmamalıdır. Ancak istatistiksel karşılaştırmalar yapılıyorsa küçük harfler bulunabilir. Çizelge ve şekillerde kısaltmalar kullanılmış ise hemen altına bu kısaltmalar açıklanmalıdır. Şekil ve çizelge başlıkları ile çizelge altı yazılarının sonuna nokta (.) konmamalıdır.

Birimler: Tüm makalelerde SI (Systeme International d'Units) ölçüm birimleri kullanılmalıdır. Ondalık kesir olarak nokta kullanılmalıdır (1,25 yerine 1.25 gibi). Birimlerde "/" kullanılmamalı ve birimler arasında bir boşluk verilmelidir (m/s yerine m s^{-1}, J/s yerine J s^{-1}, kg m/s^2 yerine kg m s^{-2} gibi). Sayı ile sembol arasında bir boşluk bırakılmalıdır (4 kg N ha^{-1}, 3 kg m^{-1} s^{-2}, 20 N m, , 1000 s^{-1}, 100 kPa, 22 °C ve % 29 gibi). Bu kuralın istisnaları düzlemsel açılar için kullanılan derece, dakika ve saniye sembolleridir (°, ′ ve ″). Bunlar sayıdan hemen sonra konmalıdır (10°, 45', 60" gibi). Litrenin kısaltması "l" değil "L" olarak belirtilmelidir. Cümle sonunda değillerse sembollerin sonuna nokta konulmamalıdır (kg. değil kg).

Formüller ve Eşitlikler: Formüller numaralandırılmalı ve formül numarası formülün yanına sağa dayalı olarak parantez içinde gösterilmelidir. Formüllerin yazılmasında Word programı matematik işlemcisi kullanılmalı, ana karakterler 12 punto, değişkenler italik, rakamlar ve matematiksel ifadeler düz olarak verilmelidir. Metin içerisinde atıf yapılacaksa "Eşitlik 1" biçiminde verilmelidir (…ilişkin model, Eşitlik 1' de verilmiştir).

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References

Cite references in the text as author's family name should be followed by the year of the publication in parentheses (Peter 2010; Basunia & Abe 2001). Use *et al* after the first author's family name for citations with three or more authors (Lawrence et al 2001). For citations of the same authors published on the same year, use letters after the year (Dawson 2009a).

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Journal Articles

Doymaz I (2003). Drying kinetics of white mulberry. *Journal of Food Engineering* **61**(3): 341-346

Basunia M A & Abe T (2001). Thin-layer solar drying characteristics of rough rice under natural convection. *Journal of Food Engineering* **47**(4): 295-301

Lawrence K C, Funk D B & Windham W R (2001). Dielectric moisture sensor for cereal grains and soybeans. *Transactions of the ASAE* **44**(6): 1691-1696

Akpinar E, Midilli A & Biçer Y (2003a). Single layer drying behavior of potato slices in a convective cyclone dryer and mathematical modeling. *Energy Conversion and Management* **44**(10): 1689-1705

Books

Mohsenin N N (1970). Physical Properties of Plant and Animal Materials. Gordon and Breach Science Publishers, New York

Book Chapter

Rizvi S S H (1986). Thermodynamic properties of foods in dehydration. In: M A Rao & S S H Rizvi (Eds.), *Engineering Properties of Foods*, Marcel Dekker, New York, pp. 190-193

Publications of Institutions / Standard Books

ASAE (2002). Standards S352.2, 2002, Moisture measurement - unground grain and seeds. ASAE, St. Joseph, MI

Internet Sources

FAO (2013). Classifications and standards. Retrieved in April, 12, 2011 from http://www.fao.org/economic/ess/ess-standards/en/

Thesis and Dissertations

Berbert P A (1995). On-line density-independent moisture content measurement of hard winter wheat using the capacitance method. PhD Thesis, Crandfield University (Unpublished), UK

Conference Proceedings (Full papers)

Yağcıoğlu A, Değirmencioğlu A & Cağatay F (1999). Drying characteristics of laurel leaves under different drying conditions. In: *Proceedings of the 7th International Congress on Agricultural Mechanization and Energy*, 26–27 May, Adana, pp. 565–569

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