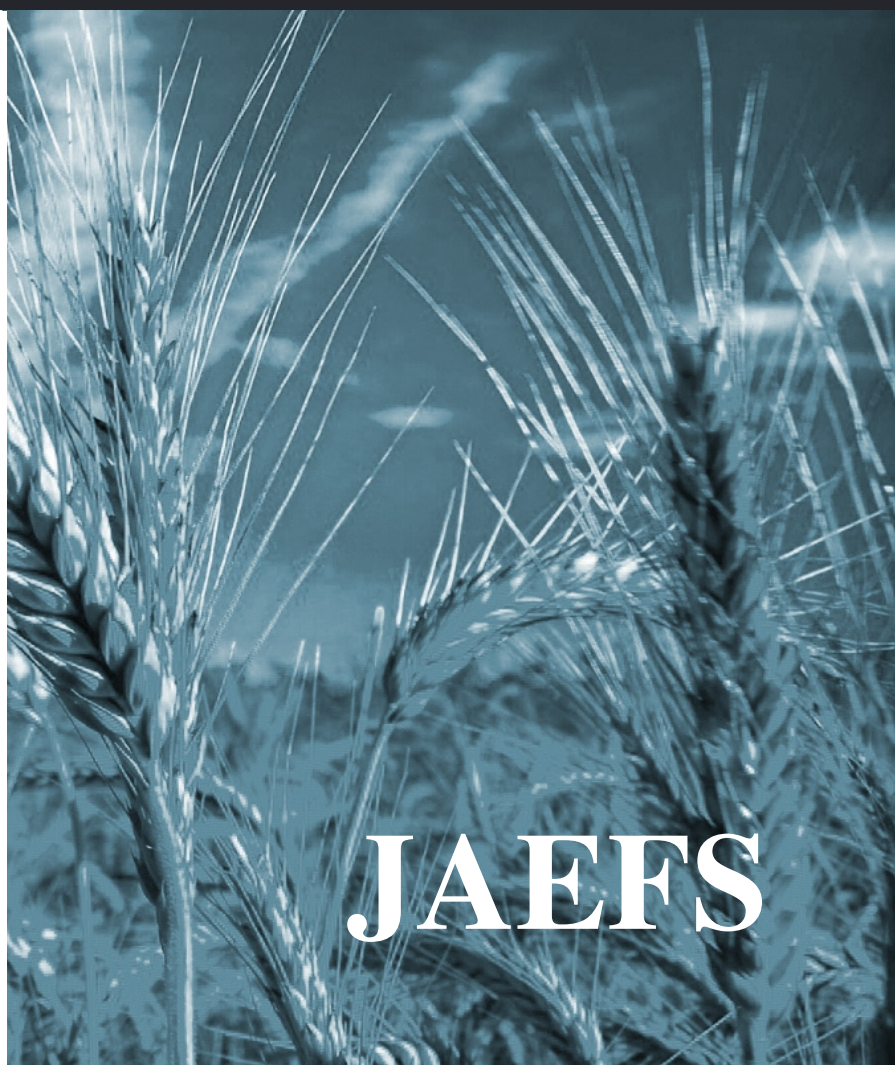
DECEMBER
2019

INTERNATIONAL JOURNAL OF AGRICULTURE, ENVIRONMENT AND FOOD SCIENCES

www.jaefs.com
dergipark.gov.tr/jaefs
editor@jaefs.com

YEAR : 2019
VOLUME : 3
ISSUE : 4



JAEFS

e-ISSN : 2618-5946
doi : 10.31015/jaefs

JAEFS

International Journal of Agriculture, Environment and Food Sciences

Int J Agric Environ Food Sci

e-ISSN : 2618-5946
DOI: 10.31015/jaefs

www.jaefs.com

December

Volume : 3 Issue : 4 Year : 2019

JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946

www.jaefs.com

Int J Agric Environ Food Sci 3 (4) December 2019

DOI: 10.31015/jaefs

JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946

www.jaefs.com

Int J Agric Environ Food Sci 3 (4) December 2019

DOI: 10.31015/jaefs

JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946

www.jaefs.com

Int J Agric Environ Food Sci 3 (4) December 2019

DOI: 10.31015/jaefs

JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946

www.jaefs.com

Int J Agric Environ Food Sci 3 (4) December 2019

DOI: 10.31015/jaefs

JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946

www.jaefs.com

Int J Agric Environ Food Sci 3 (4) December 2019

DOI: 10.31015/jaefs

JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946

www.jaefs.com

Int J Agric Environ Food Sci 3 (4) December 2019

DOI: 10.31015/jaefs

JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946

www.jaefs.com

Int J Agric Environ Food Sci 3 (4) December 2019

DOI: 10.31015/jaefs

Production Information

Journal Name	International Journal of Agriculture, Environment and Food Sciences
Abbreviation	Int J Agric Environ Food Sci
Subjects	Agriculture, Environment and Food Sciences
e-ISSN	2618-5946
Publisher	Gultekin Ozdemir
Owner	Gultekin Ozdemir
Language	English
Frequency	Quarterly (March, June, September, December)
Type of Publication	International, Scientific, Open Access Double-blinded peer review Widely distributed periodical
Manuscript Submission and Tracking System	JAEFS uses the submission system of TUBITAK-ULAKBIM JournalPark Open Journal Systems - http://dergipark.gov.tr/jaefs
License	Journal is licensed under a Creative Commons Attribution 4.0 International License
Legal Responsibility	Authors are responsible for content of articles that were published in Journal.
Indexed and Abstracted in	TÜBİTAK ULAKBİM TR Dizin, Crossref, Directory of Open Access Journal (DOAJ), AGORA (Access to Global Online Research in Agriculture), AGRIS (Agricultural Science and Technology Information), WorldCat, Google Scholar, SOBIAD, Scilit, ROAD (Directory of Open Access Scholarly Resources), Neliti, International Citation Index, ROOT Indexing, ResearchBib, Index Copernicus International, ESJI, JournalTOCs, TEELS, ResearchGate, Microsoft Academic
Address	International Journal of Agriculture, Environment and Food Sciences Prof.Dr. Gultekin Ozdemir Dicle University Faculty of Agriculture Department of Horticulture, 21280 Diyarbakir / TURKEY
Contact	Prof.Dr. Gultekin Ozdemir Phone: +90 532 545 07 20 E-mail: editor@jaefs.com jaefseditor@gmail.com Web : www.jaefs.com dergipark.gov.tr/jaefs

JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946

www.jaefs.com

Int J Agric Environ Food Sci 3 (4) December 2019

DOI: 10.31015/jaefs

Aim and Scope

"International Journal of Agriculture, Environment and Food Sciences" (JAEFS) is an international journal, which publishes original research and review articles dealing with Agriculture, Environment and Food Sciences.
JAEFS Journal is an international, scientific, open access periodical published in accordance with independent, unbiased, and double-blinded peer-review principles.
Journal publishes Quarterly (March, June, September, December).
The publication language of the journal is English and continues publication since December 2017.
A Digital Object Identifier (DOI) number has been assigned for each article accepted to be published in JAEFS, starting from December 2017.
Journal of JAEFS welcomes article submissions and does not charge any article submission or processing charges.

International Journal of Agriculture, Environment and Food Sciences areas of interest include, but are not limited to: Agricultural Engineering and Technology, Environmental Engineering and Technology, Food Engineering and Technology, Agriculture, Environment, Food, Agriculture History, Agricultural Economics, Agronomy, Animal Sciences, Aquaculture, Biochemistry, Biotechnology, Bioinformatics and Data Science, Crop Science, Dairy Science, Extension Science and Education, Entomology, Environmental Science, Fish and Fisheries, Food Processing, Food Chemistry, Food Culture, Food Health and Nutrition, Food History, Food Industry Development, Food Marketing, Food Policy and Practices, Food Safety, Forestry, Horticulture, Information Technologies and Systems, Irrigation, Molecular Biology, Organic Agriculture, Plant Physiology, Plant Breeding, Plant Protection, Plant Sciences, Precision Agriculture, Rural Development and Policy, Sustainable Agriculture, Soil Science, Plant Nutrition, Energy Crops, Veterinary, Water Resources.

Open Access Statement

International Journal of Agriculture, Environment and Food Sciences (JAEFS) publishes fully open access journal, which means that all articles are available on the internet to all users immediately upon publication.
All the original articles and review papers published in JAEFS journal are free to access immediately from the date of publication.
International Journal of Agriculture, Environment and Food Sciences (JAEFS) don't charge any fees for any reader to download articles and reviews for their own scholarly use.
Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author. This is in accordance with the BOAI definition of open access.
Copyright of the layout and design of International Journal of Agriculture, Environment and Food Sciences articles remains with the journal and cannot be used in other publications.
International Journal of Agriculture, Environment and Food Sciences also operates under the Creative Commons Licence CC-BY-NC-ND.
All authors publishing with the JAEFS accept these as the terms of publication.
Benefits of open access for Authors include:
Free access for all users worldwide
Authors retain copyright to their work
Increased visibility and readership
Rapid publication
No spatial constraints

Copyright Policy

Authors who publish with International Journal of Agriculture, Environment and Food Sciences (JAEFS) agree to the following terms:

Authors transfer the copyright to International Journal of Agriculture, Environment and Food Sciences (JAEFS) . Authors, however, upon the publication of their work, can republish it in an another journal or book provided that they obtain the permission of the Journal. Moreover, they are to include the note indicating that their work has first been published in International Journal of Agriculture, Environment and Food Sciences (JAEFS) and the link to their work in the journal.
Authors, upon the publication of their work, can publish it in online environments and/or social networks (personal webpages, course content websites, Facebook, etc.). However, they also provide the link to the work as published in the journal.
"International Journal of Agriculture, Environment and Food Sciences" journal is licensed under a

Creative Commons Attribution 4.0 International License 

JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946

www.jaefs.com

Int J Agric Environ Food Sci 3 (4) December 2019

DOI: 10.31015/jaefs

Contents

Research Articles

International Journal of Agriculture, Environment and Food Sciences

JAEFS

e-ISSN : 2618-5946

DOI: 10.31015/jaefs.2019.4.1

www.jaefs.com

Research Article

Int J Agric Environ Food Sci 3(4):204-210 (2019)

Efficacy study on the influence of Organic Manures and Tillage on Red Cowpea (*Vigna unguiculata* L.) performance in a Sahel Savannah region of Nigeria

Mahmud Sani[1,*] Ibraheem Alhassan[1] Dantata Ishaku James[1]

Auwalu Garba Gashua[1] Musa Adamu A[1].

[1]Department of Agronomy, Federal University Gashua, Yobe State, Nigeria

[*]Corresponding Author: mahmudsani17@gmail.com

Abstract

The objectives of this work were to determine the effects of tillage depths, manure types, and the nature of their relationships with some performance indices and Grain Yield of red Cowpea (*Vigna unguiculata* L.). The experiment was laid out on a Split plot design; with Tillage depth (Zero, Shallow, and Deep Tillage) as main the plot, while Manure Type (No Manure, Cow Dung and Poultry Manure) was assigned to subplots. Upon analyses using R-Software, Plots treated with Poultry Manure showed the highest positive response compared to no manure and cow dung in terms of growth parameters, above ground biomass (523.3 $Kgha^{-1}$), and Grain Yield (696.0 $Kgha^{-1}$). Zero Tillage performed better than shallow and deep tillage. It was concluded that interaction between zero tillage and poultry manure proved the best to improve cowpea grain yield with value of (1063.8 $Kgha^{-1}$), above ground biomass (766.7 $Kgha^{-1}$), and can increase farmer's income and feed for their livestock. Pearson's Multiple Linear Correlation indicated high positive relationship between organic manure and all Cowpea parameters measured ($r \geq +0.65 \leq +0.92$). Tillage depth was found to significantly correlate with Pod length ($r = -0.36^*$) and Single Pod Weight ($r = -0.40^*$) at $P \leq 0.05$ in a negative passion.

Keywords: Correlation, Cowpea, Organic Manure, Tillage, Yield

Introduction

Vigna Unguiculata L. Walp, commonly known as Cowpea, Wake, or Beans in Nigeria, is a major leguminous crop of the Savanna regions of West Africa commonly found in either white or red coloration. The seeds and fodder as major sources of plant protein, vitamins, and energy for farmers and livestock due to its healthy nutritional status (Samndi et al., 2014). It is also a source of income to small and large-scale farmers. Cowpea fodder is usually stored for sale at the peak of the dry season, and has been reported to increase or stabilize farmers' annual income by about 25% in West Africa (Dugje et al., 2009). Young cowpea leaves and immature pods of cowpea are consumed as protein source. Cultivation of cowpea, if at all, require only a small amount of nitrogen at the initial stage of growth before the start of nodules formation, which is the onset of nitrogen fixation through which nitrogen availability is improved. Intercropping with cereal crops is also a practice by farmers as it inhabits microbes that actively participate in processes, extraction and supply of nitrogen to mixture and succeeding crops (such as millet and sorghum) when grown in rotation especially in areas where poor soil fertility is a problem (Aikins and Afuakwa, 2012). Cowpea yield of 1500 - 2000 $Kgha^{-1}$ is attainable in the Savannah (Chude et al., 2012).

Cowpea can be grown under rainfall, irrigation or residual moisture conditions along river banks or flood plains and fadamas during the dry season. Its optimum temperature range is

Cite this article as:
Sani, M., Alhassan, I., James, D. I., Gashua, A. G., Adamu A., M. (2019). Efficacy study on the influence of Organic Manures and Tillage on Red Cowpea (Vigna unguiculata L.) performance in a Sahel Savannah region of Nigeria. Int. J. Agric. Environ. Food Sci., 3(4), 204-210
DOI: https://dx.doi.org/10.31015/jaefs.2019.4.1
Received: 07 March 2019 **Accepted:** 26 October 2019 **Published:** 23 December 2019
Year: 2019 **Volume:** 3 **Issue:** 4 (December) **Pages:** 204-210
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

between 28^{0}C and 30°C (night and day) during the growing season. The crop performs well in agro-ecological zones where the rainfall range is between 500 and 1200 mmyr^{-1}. However, with the development of extra-early and early maturing cowpea varieties, the crop can thrive in the Sahel where the cumulative rainfall is less than 800 mmyr^{-1}. Some of these varieties are tolerant to drought and well adapted to sandy loams and poorly fertile soils. However, best cowpea yields are obtained in well-drained sandy loam to clay loam soils with pH between 6 and 7 (Dugje et al., 2009).

Land preparation, especially the disturbance of agriculturally important soil layers by Tillage was reported to be among management practices that affect soil properties and general crop performances (Adekiya et al., 2014; Shahzad et al., 2014; Aikins and Afuakwa, 2012; Adeyemo and Agele, 2010). Positive influences of Organic (OM) quantity and source on the performance and productivity of vegetables were evidently established by Usman (2015), cereals by Adeyemo and Agele (2010), tubers, and legumes by Tanimu and Lyocks (2013). The interaction effects of tillage and OM were also reported to be beneficial to crop production (Adekiya et al., 2014; Adeyemo and Agele, 2010). A study by Alhassan et al., (2018) recommended the combined use of conservational tillage and organic matter incorporation for uplifting soil productivity in Sudano-Sahelian Savannah region.

Sahel savannah is a biogeographic zone of transition in Africa between the Sahara to the north and Sudanian Savannah to the south. A belt of up to 1000 km (620 miles) wide that spans the 5400 km (3360 miles) from Atlantic ocean to Red Sea. In West Africa, the Sahel covers (from west to east); parts of northern Senegal, central Mali, northern Burkina Faso, the larger part of Niger Republic, the extreme northeastern part of Nigeria and central Tchad republic (Wikipedia, 2018).

In the Sahel Savannah with Average Farmers Yield (AFY) between 300 – 1000 Kgha^{-1} of Cowpea (Chude et al., 2012) however, farmers tend to be unmindful of the of positive or negative effects of tillage practices and manure uses despite its abundance, the interaction, and relationship of both practices with performance parameters and yield of legumes, and especially Cowpea, a major crop in the region. Therefore, an understanding of how tillage practices and OM application affect cowpea production in the Sahel Savannah will help farmers to make decision which will provide for more precise practices to improve cowpea production towards higher attainable yields. The objectives of this study were to evaluate the response of some cowpea performance indices and grain yield to tillage and organic manure additions. Also, it aimed to determine the effect of tillage and OM interaction on the parameters, as well as determining the linear correlation between tillage and OM with the cowpea performance indices and Grain yield in the Sahel Savannah.

Materials and Methods

Location

The research was carried out at Madamuwan Gabas located on latitude 12.012^{0}N and longitude 10.564^{0}E, in the Nigerian Sahel Savannah agro ecological zone. The Nigerian Sahel spans over a significant land area in northern Yobe, Borno and Adamawa states (Wikipedia, 2018). The soils of the study location are classified as Alfisols according to USDA system and predominantly Aeric Tropaqualfs characterized by deep and imperfect drainage, and derived from Alluvial weathered-Basement Complex parent materials of sedimentary origin (FDALR, 1990). The climate of Sahel was Aw (Koppen system) signifying Tropical wet and dry seasons (Dugje et al., 2009). The cumulative annual rainfall in the past three seasons in the study area ranged from 550mm – 750 mm as a unimodal occurrence between May and October, with an average annual temperature range of 27^{0}C – 41.6^{0}C across the study area from 2015 to date as in Table 1 (NASA, 2018).

Table 1. Average annual values of some climatic factors in the study area

	Average annual climatic factors			
Year	Max. Temp. (^{0}C)	Min. Temp (^{0}C)	Cum. Rainfall (mmyr^{-1})	Sol. Rad. (KJm^{-2}d^{-1})
2015	43	27	673	16.7
2016	41	26	550	18.1
2017	43	28	750	17.4

Max. = maximum. Min. = minimum. Cum. = cumulative. Sol. = Solar. Rad. = Radiation.

Experimental Design

The experiment was laid out based on split plot design consisting of plots measuring 6m^2 each with Tillage as main plots while OM as sub-plots, replicated three times. Treatments consisted of three three tillage levels: Zero Tillage (ZT) achieved through hand picking, Shallow (0-15cm) Tillage (ST) soil disturbance and Deep (0-30cm) Tillage through disturbing top-soil using calibrated diggers and three types of manures: No manure (No), Cow Dung (CD) and Poultry Manure (PM) applied at the rate of 5.0 tonnesha^{-1} 3 weeks before planting,

Interaction studies were based on the following: Zero Tillage x No Manure (Control – ZTNo), Zero Tillage x Cow Dung (ZTCD), Zero Tillage x Poultry Manure (ZTPM), Shallow Tillage x No Manure (STNo), Shallow Tillage x Cow Dung (STCD), Shallow Tillage x Poultry Manure (STPM), Deep Tillage x No Manure (DTNo), Deep Tillage x Cow Dung (DTCD) and Deep Tillage x Poultry Manure (DTPM).

Sampling of soil and manures

Composite soil sample of the study location was collected using soil auger at 0-30cm depth prior to application of organic manures. Likewise, the manures used in the study were sampled at the application time.

Laboratory Analysis

Composite samples of the pre-planted Soil and Manures used in the experiment were prepared for physical and chemical properties analyses using standard laboratory samples preparation. Soil reaction (pH) and electrical conductivity (EC) were determined in 1:2.5 (Soil: Water) paste using glass electrode pH and EC meters respectively. Soil particle sizes were determined using the hydrometer method as described in Tanimu and Lyocks (2013). Bulk density (BD) was determined using core sampler (Aikins and Afuakwa, 2012). Organic carbon (OC) was determined using the Walkley-Black wet oxidation method (Walkley and Black, 1934). Total Nitrogen (N_T) was determined using the regular macro-kjeldhal distillation method as in Fedhasa and Tesfaya (2015). Available phosphorus (P) was determined using the method of Bray and Kurtz-Bray 1 extraction (Tanimu and Lyocks, 2013). Exchangeable Cations were extracted using 1M NH_4OAC. Calcium (Ca) and Magnesium (Mg) then read with Atomic Absorption Spectrophotometer (AAS), while Potassium (K) and Sodium (Na) were read using Flame Photometer (Ciesielski et al., 1997).

Agronomic Practices

Variety IT84E-108 was planted on flat land at the spacing of 45 x 45 cm as recommended by Chude et al., (2012). Plots were kept free of weeds to prevent competition with crop. Pod length (cm), Leaf area (cm^2), Plant Height (m), Single pod weight (g) were recorded at harvest while above ground biomass ($Kgha^{-1}$), and Grain yield ($Kgha^{-1}$) were weighed after harvest.

Statistical Analysis

Data collected were statistically analyzed using R software (3.4.3). Upon significant F-Test, Least Significant Difference (LSD) test was used for means separation (Amanullah et al., 2014). Pearson's multiple linear correlation analysis was performed using SPSS to determine the relationship between OM and Tillage, with cowpea performance parameters.

Result and Discussion

Physical and chemical properties of the experimental soil were shown in Table 2. The soil was found to be of Sandy Loam (SL) texture, the soil particle size distribution fall in the Sandy range classified by Hill Laboratories (2010). Sandy ranged soils indicate a high degree of weathering and clay eluviations from the surface layer (Nakao et al., 2009) it is difficult to detect such minor and progressive changes using conventional methods. We measured the amount of the frayed edge site (i.e. the weathering front of illitic minerals, with low OC content and probably nutrient loss through volatilization in the location as reported by Eghball and Power (1999). The findings on the experimental site soil conformed with those reported by Alhassan et al., (2018) on sahelian savannah soils of Bade, Nigeria.

Results in Table 3 showed that the Cow Dung used for the experiment had the least BD of 1.29 gcm^{-3} followed by the experimental PM with BD of 1.32gcm^{-3}. Both manures have lower densities than the soil (1.43gcm^{-3}). Organic carbon content and C: N ratio were higher in CD than PM. Bulk densities of the soil and experimental manures could be attributed to the OC contents of the three media analyzed, as seen in Tables 2 and 3. This attests to a report mentioned in Bouajila and Sanaa (2011) on improvement in BD as a result of high OC content.

A contrasting quality in Tables 2 and 3; is the highest N_T, P and K contents (fertility indices) found in PM as compared to CD and the Study Soil (SS). The soil of the site was very low in N_T, low in available P and high in exchangeable K. This implied that the SS may benefit from the N and P in the manures despite its low Effective Cation Exchange Capacity (ECEC) upon the summation of the basic cations in Table 2. The CD was lower than the soil in all basic cations while PM is higher than the soil in K but lower in all of Ca, Mg and Na contents.

Pod Length (cm)

Results presented in Table 4 indicated that OM had a significant effect on cowpea pod length. Mean pod length is higher in PM (18.2cm) than CD treated plots (16.5cm), while the least and significantly different pod length of 13.7cm was recorded in no manure plots. A study by Meena *et al.*, (2015) crop performance, energy relations and economics in greengram (Vigna radiata L. reported positive benefits of OM from crop residue on a leguminous crop. Tillage depth was also found to be significant. Although mean Pod length was statistically at par for all the depths, values obtained showed that the maximum (17.1cm) was recorded in ZT, while the minimum (14.7cm) was obtained with DT plots (Figure 1). In ST plot however, mean pod length was 16.5 cm. Tillage also established benefits to soil and Green gram (*Vigna radiata* L.) performance in the study of Meena et al., (2015).

Leaf Area (cm^2)

The results presented in Table 4 also showed that cowpea leaf area benefitted significantly from OM application. Mean leaf area values indicated that PM treated plot produced the maximum (138.5cm^2), CD treated plot was 86.8cm^2, and the control plot (No manure) had the minimum leaf area (70.2cm^2). Tillage has no significant effect on leaf area. The interaction of OM with Tillage was highly significant with highest leaf area (163.2cm^2) in ZTPM treated plots and least (54.9cm^2) in ZTNo treated plot as shown by Figure 2. The least performance of Non tilled plots attests to the report of Fuhrer and Chervet (2015).

Plant Height (m)

Statistical analysis of plant height in Table 4 showed the significance of OM application. Mean values of 0.54m and 0.65m that are statistically different for CD and PM were been observed over no manure plots with 0.43m as the mean height of the plants. This finding is at par with that of Idris et al., (2018) on cowpea in the Sahel Savannah of Niger with the addition of Phosphorus, and the study of Soretire and Olayinka (2013) for soya bean height in Abeokuta, Nigeria. Both Tillage type (Table 4) and the interaction between tillage and manure (Figure 3) were found to have no statistical significance on the plant height.

Single Pod Weight (g)

Mean values obtained for single pod weight in Table 4 indicated the significance of OM and that of Tillage for cowpea production in the Sahel savannah. The interaction of OM X Tillage was not significant from the analyzed data in the Table 4, but heavier pods were obtained upon OM interaction with the least soil disturbance (ZT) as seen in Figure 4. This could be attributed to the long term higher soil moisture retention with conservational (no tillage) reported in the study of Fuhrer and Chervet (2015).

Above Ground Biomass (Kgha^{-1})

Above Ground Biomass in CD plot (293.2 Kgha^{-1}) was not statistically different from the plot with no manure (233.0 Kgha^{-1}), while PM plot was statistically significant having a value of 523.3 Kgha^{-1}. ZT (400 Kgha^{-1}), ST (316.6 Kgha^{-1}) and DT (333.3 Kgha^{-1}) were all statistically insignificant in terms of biomass. Tillage X OM was highly significant as depicted by Figure 5. (Idriss et al., 2018) reported a significant increase in cowpea performance in both Niger and Burkina Faso due to OM. Mando and Vanlauwe (2005) also reported a significant long term interaction between tillage and manure on general crop performance and soil properties improvement in the dry Sahelian regions of West Africa.

Grain Yield (Kgha^{-1})

Results in Table 4 also showed that Grain Yield in CD-treated plots (394.7 Kgha^{-1}) was not statistically different from the plot with no manure (216.3 Kgha^{-1}), while yield of (696.0 Kgha^{-1}) from PM treated plots was statistically significant. Findings reported by Olatunji et al., (2012) were at par with this study on PM application. The ZT (503.6 Kgha^{-1}), ST (338.8 Kgha^{-1}) and DT (464.3 Kgha^{-1}) were all statistically insignificant. This conforms with the findings of a number of studies (Idriss et al., 2018; Mando & Vanlauwe, 2005; Meena et al., 2015). The interaction was significant in such a way that the grain yield produced is higher at PM plots in a succession of ZT, ST then DT plots from Figure 6. The yield obtained in ZTPM plot (1063.8 Kgha^{-1}) surpassed the AFY of between 300-1000 Kgha^{-1} highlighted in Chude et al., (2012).

Linear relationships

Correlation analysis between treatments and performance parameters showed that OM had a positive and highly significant correlation with all the cowpea performance parameters and grain yield (Table 5) in the order Plant Height (r = 0.92), Leaf Area (r = 0.86), Single Pod Weight (r = 0.76), Biomass (r = 0.72), Pod Length (r = 0.67) and Grain Yield (r = 0.65). Fertility indices in the OM treated soils are therefore important in such a way that PM gave the highest NPK content which produced the highest results in all the parameters when compared to CD and no manure accordingly.

Tillage, on the other hand, was found to have a negative correlation with the Cowpea performance parameters. Statistically insignificant correlation was observed with Biomass (r = -0.18), Leaf Area (r = -0.09), Grain Yield (r = -0.05) and Plant Height (r = -0.01) as in Table 3, and significant negative relationship was seen with both Single Pod Weight (r = -0.40) and Pod Length (r = -0.36) on Table 5.

Figure 1. Response of cowpea pod length to tillage and OM

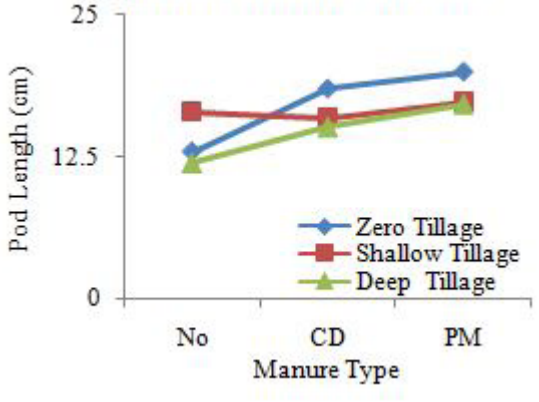


Figure 2. Response of cowpea Leaf Area to t'llage and OM

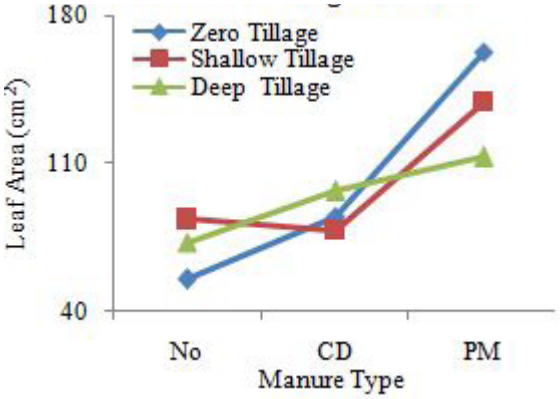


Figure 3. Response of cowpea plant height to tilage and OM

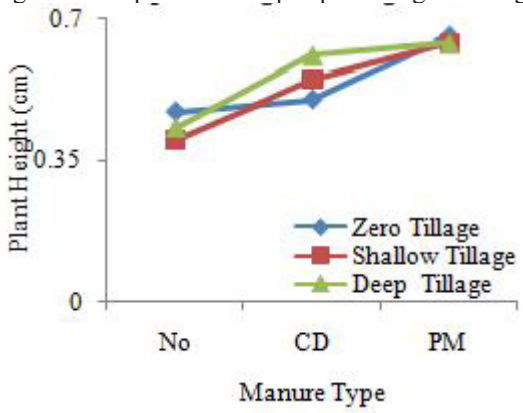


Figure 4. Response of cowpea single pod weight to tillage and OM

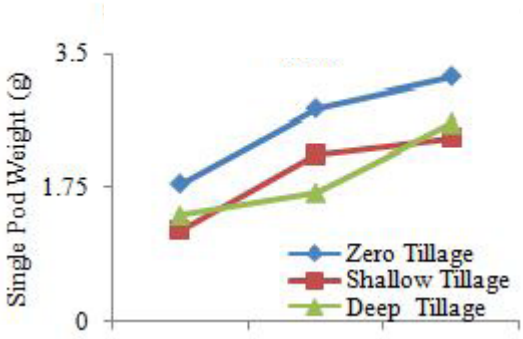


Figure 5. Response of cowpea above ground biomass to tillage and OM

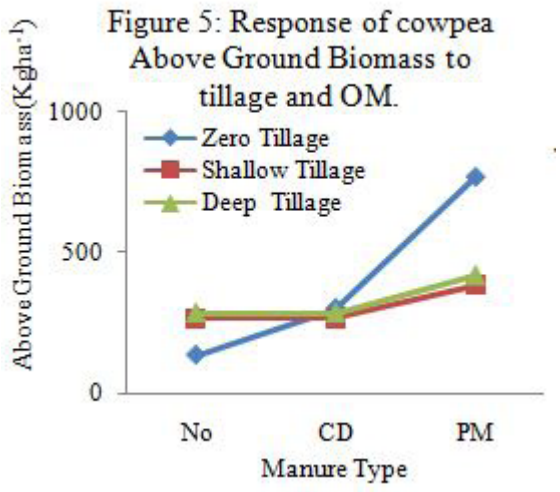
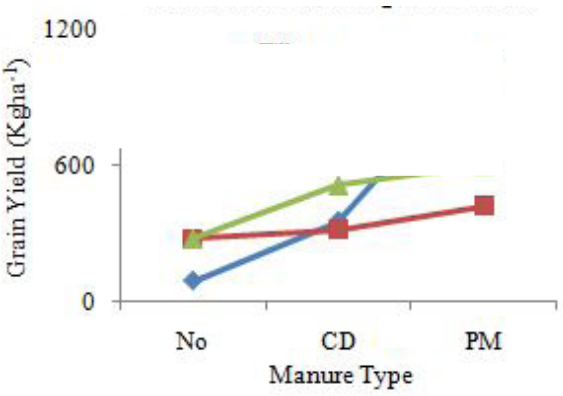


Figure 6.Response of cowpea grain yield to tillage and OM



Conclusion

In conclusion, Interaction between Zero Tillage (ZT) and Poultry Manure (PM) proved to be the best combination to improve cowpea Grain Yield ($Kgha^{-1}$) and above ground biomass ($Kgha^{-1}$) in the Sahel Savannah. Manure was found to show high correlation with all the growth parameters and grain yield of cowpea. Tillage depth was also found to significantly correlate negatively with Pod length (r = -0.36*) and Single Pod Weight (r = -0.40*) at $P \geq 0.05$.

Acknowledgement

The authors acknowledge with deep gratitude, the DISTRICT HEAD of Madamuwan Gabas for the provision of land and man-power to help in carrying out the field trial from pre planting operations to harvest.

References

Adeyemo, A. J., and Agele, S. O. (2010). *Effects of tillage and manure application on soil physicochemical properties and yield of maize grown on a degraded intensively tilled alfisol in southwestern Nigeria*.

Aikins, S., and Afuakwa, J. (2012). Effect of four different tillage practices on soil physical properties under cowpea. *Agriculture and Biology Journal of North America*. https://doi.org/10.5251/abjna.2012.3.1.17.24

Alhassan, I., Garba Gashua, A., Dogo, S., and Sani, M. (2018). Physical properties and organic matter content of the soils of Bade in Yobe State, Nigeria. *International Journal of Agriculture, Environment and Food Sciences*, *804*, 160–163. https://doi.org/10.31015/jaefs.18027

Amanullah, J., Shahzad, A., Mohammed, A., and Aziz, K. (2014). Growth and Yield Components of sesame (*Sesamum Indicum* L.) as influenced by Phosphorus Levels under different Row Spacing. *Journal of Environment and Earth Science*, *4*(22), 150–154.

Bouajila, K., and Sanaa, M. (2011). Effects of organic amendments on soil physico-chemical and biological properties. *J. Mater. Environ.Sci.*, *2*(S1), 485–490.

Chude, V. O., Olayiwola, S. O., Daudu, C., and Ekeoma, A. (2012). *Fertilizer Use and Management Practices for Crops in Nigeria* (4th Edition; V. O. Chude, S. O. Olayiwola, C. Daudu, & A. Ekeoma, eds.). Abuja: Federal Fertilizer Department.

Ciesielski, H., Sterckeman, T., Santerne, M., and Willery, J. P. (1997). A comparison between three methods for the determination of cation exchange capacity and exchangeable cations in soils. *Agronomie*, *17*(1), 9–16. https://doi.org/10.1051/agro:19970102

Dugje, I. Y., Kamara, A. Y., Ekeleme, F., and Ajeigbe, H. A. (2009). *Farmers' Guide to Cowpea Production in West Africa*. IITA, Ibadan, Nigeria.

Eghball, B., and Power, J. F. (1999). Composted and Non composted Manure Application to Conventional and No-Tillage Systems. *Agronomy Journal*, *91*(5), 819. https://doi.org/10.2134/agronj1999.915819x

FDALR. (1990). *The Reconnaisance Soil Survey of Nigeria (1:650,000) Soil Report*. Volume 2.

Fedhasa Benti and Tesfaya Balemi. (2015). Effects of land use types on soil quality and productivity . *STAR Journal*, *5*(1), 83. https://doi.org/http://dx.doi.org/10.4314/star.v4i3.13

Fuhrer, J., and Chervet, A. (2015). No-tillage: Long-term Benefits for Yield Stability in a More Variable Climate? *Procedia Environmental Sciences*, *29*(Agri), 194–195. https://doi.org/10.1016/j.proenv.2015.07.257

Hill Laboratories. (2010). Soil tests and interpretation- Technical note. *Hamilton, New Zealand*.

Idriss, S., Nouri, M., Maman, G., and Gonda, A. bdou. (2018). Cowpea response to nutrient application in Burkina Faso and Niger. *African Journal of Agricultural Research*, *13*(30), 1508–1515. https://doi.org/10.5897/AJAR2018.13207

Mando, A., and Vanlauwe, B. (2005). Long term effect of tillage and manure on soil organic fraction and crop performance under Sudano-Sahelian conditions. *Soil and Tillage Research*, *80*(1–2), 95–101. https://doi.org/10.1016/j.still.20004.03.002

Meena, J. R., Behera, U. K., Chakraborty, D., and Sharma, A. R. (2015). Tillage and residue management effect on soil properties, crop performance and energy relations in greengram (Vigna radiata L.) under maize-based crop

ping systems. *International Soil and Water Conservation Research*, *3*(4), 261–272. https://doi.org/10.1016/j.iswcr.2015.11.001

Nakao, A., Funakawa, S., and Kosaki, T. (2009). Soil Science. *European Journal of Soil Science*, *60*(December), 613–621. https://doi.org/10.1097/00010694-199207000-00011

NASA. (2018). Prediction of World Energy Resources. www.nasapower.com. retrieved on 10th november 2018.

Olatunji, O., Ayuba, S. A., Anjembe, B. C., and Ojeniyi, S. O. (2012). Effect of NPK and Poultry Manure on Cowpea and Soil composition. *Nigerian Journal of Soil Science NJSS*, *22*(1), 110–116.

Samndi, A. M., Ichi, J. O., Miko, S., & Adnan, A. A. (2014). Cowpa yield response to sowing date and irrigation intervals. *Nigerian Journal of Soil Science.* 24, 207–216.

Soretire, A. A., and Olayinka, A. (2013). Response of Soybean (Glycine max L.) to Cow dung and Wood ash application in Tropical acid soils of South-Western Nigeria. *Nigerian Journal of Soil Science NJSS*, *23*(2), 103–113.

Tanimu, J., and Lyocks, S. W. J. (2013). Assessment of Manure Management Practices and Nitrogen Levels on Soil Phosphorus in an Alfisol. *Journal of Agriculture and Sustainability*, *4*(2), 177–190.

Usman, M. (2015). Cow Dung, Goat and Poultry Manure and Their Effects on the Average Yields and Growth Parameters of Tomato Crop. *Journal of Biology, Agriculture and Healthcare*, *5*(5), 7–10.

Walkley, A., and Black, I. A. (1934). An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science*, *37*(1), 29–38.

Wikipedia. (2018). *The Sahel*. retrieved 11th december 2018.

Table 2. Physical and Chemical Properties of the Experimental Site

	Analyzed Properties													
Medium	Physical Properties						Chemical Properties							
Texture	Sand	Silt	Clay	BD	OC	EC	pH	N_T	P	K	Ca	Mg	Na	
SS	SL	72	17	11	1.43	1.38	0.88	6.75	0.13	0.36	1.82	4.61	15.4	1.38

SS = Study Soil. Sand, Silt, Clay, OC, and N_T are all in percentages (%). BD is in gcm^{-3}. EC is in $dScm^{-1}$. P, K, Ca, Mg and Na are in cmol (+) kg^{-1}. N_T = total Nitrogen.

Table 3. Analyzed Properties of the Manures used in the study

Manure	Analyzed Properties									
	BD	OC	EC	pH	N_T	P	K	Ca	Mg	Na
CD	1.29	15.1	Nd	6.70	0.36	0.83	1.31	2.41	0.86	0.98
PM	1.32	12.6	Nd	6.51	0.60	1.30	2.40	3.21	2.00	0.90

CD = Cow Dungs. PM = Poultry Manure. Sand, Silt, Clay, OC, and N_T are all in percentages (%). BD is in gcm^{-3}. EC is in $dScm^{-1}$. P, K, Ca, Mg and Na are in cmol (+) kg^{-1}. Nd = Not determined. N_T = total Nitrogen.

Table 4. Performance Parameters and Grain Yield of Cowpea as affected by OM and Tillage Methods

Treatments	Pod Length (cm)	Leaf Area (cm^2)	Plant Height (m)	Single Pod Weight (g)	Above Ground Biomass ($kgha^{-1}$)	Grain Yield ($Kgha^{-1}$)
OM (5.0 $tonha^{-1}$)						
Zero	13.7^b	70.2^b	0.43^c	1.4^c	233.0^b	216.3^b
Cow dung	16.5^a	86.8^b	0.54^b	2.2^b	293.2^b	394.7^b
Poultry manure	18.2^a	138.5^a	0.65^a	2.7^a	523.3^a	696.0^a
LSD (0.05)	2.0	17.1	0.04	0.5	115.0	232.2
Tillage						
Zero	17.1^a	101^{ns}	0.54^{ns}	2.6^a	400.0^{ns}	503.6^{ns}
Shallow	16.5^a	100^{ns}	0.53^{ns}	1.9^b	316.6^{ns}	338.8^{ns}
Deep	14.7^a	94^{ns}	0.54^{ns}	1.9^b	333.3^{ns}	464.3^{ns}
LSD (0.05)	2.5	-	-	0.7	-	-
Interaction						
OM X Tillage	Ns	**	Ns	Ns	**	*

Means in the same category followed by different letters are significantly different at $P \leq 0.05$ levels. Ns = non-significant, * significant at 0.05 probability level, ** significant at 0.01 probability level (highly significant).

Table 5. Linear Correlation between OM, Tillage and Cowpea Performance Parameters

Parameters	Organic Manure	Tillage
Grain Yield	0.65**	-0.05
Above Ground Biomass	0.72**	-0.18
Single Pod Weight	0.76**	-0.40*
Plant Height	0.92**	-0.01
Leaf Area	0.86**	-0.09
Pod Length	0.67**	-0.36*

*Correlation is significant at the 0.05 probability level.

** Correlation is significant at the 0.01 probability level (highly significant).

Appendix 1. Effects of OM and Tillage on Cowpea Performance indices as affected by OM and Tillage obtained at Harvest

Treatments	Pod Length (cm)	Leaf Area (cm^2)	Plant Height (m)	Single Pod Weight (g)	Above Ground Biomass ($Kgha^{-1}$)	Grain Yield ($Kgha^{-1}$)
Control	12.9	54.9	0.47	1.8	133.3	92.6
ZTCD	18.5	85.2	0.50	2.8	300.0	354.9
ZTPM	20.0	163.2	0.66	3.2	766.7	1063.8
STNo	16.4	83.4	0.40	1.2	266.7	277.5
STCD	15.9	78.5	0.55	2.2	266.7	317.2
STPM	17.3	139.3	0.64	2.4	383.3	421.7
DTNo	12.0	72.4	0.43	1.4	283.3	278.8
DTCD	15.1	96.7	0.61	1.7	283.3	511.8
DTPM	17.1	113.0	0.61	2.6	416.7	602.3

Control = Zero Tillage x No Manure, ZTCD = Zero Tillage x Cow Dung, ZTPM = Zero Tillage x Poultry Manure, STNo = Shallow Tillage x No Manure, STCD = Shallow Tillage x Cow Dung, STPM = Shallow Tillage x Poultry Manure, DTNo = Deep Tillage x No Manure, DTCD = Deep Tillage x Cow Dung and DTPM = Deep Tillage x Poultry Manure.

Appendix 2. Linear Correlation Matrix between OM, Tillage and measured Cowpea Performance Parameters

Parameters	OM	Tillage	Grain Yield	Above Ground Biomass	Single Pod Weight	Plant Height	Leaf Area	Pod Length
OM	1							
Tillage	-	1						
GY	0.65**	-0.05	1					
AGB	0.72**	-0.18	0.84**	1				
SPW	0.76**	-0.40*	0.59**	0.64**	1			
PH	0.92**	-0.01	0.59**	0.67**	0.71**	1		
LA	0.86**	-0.09	0.77**	0.90**	0.59**	0.78**	1	
PL	0.67**	-0.36*	0.54**	0.66**	0.64**	0.55**	0.67**	1

*Correlation is significant at the 0.05 probability level.

** Correlation is significant at the 0.01 probability level (highly significant).

GY = Grain Yield, AGB = Above Ground Biomass, SPW = Single Pod Weight, PH = Pant Height, LA = Leaf Area, and PL = Pod Length.

JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2019.4.2 www.jaefs.com

Research Article Int J Agric Environ Food Sci 3(4):211-216 (2019)

Contribution of roots to growth and physiology of watermelon grafted onto rooted and unrooted seedlings of various bottle gourd rootstocks

Abdullah Ulas[1,*] Alim Aydın[2] Firdes Ulas[2] Halit Yetisir[2]

[1]Erciyes University, Faculty of Agriculture, Department of Soil Science and Plant Nutrition, Kayseri, Turkey
[2]Erciyes University, Faculty of Agriculture, Department of Horticulture, Kayseri, Turkey

[*]Corresponding Author: agrulas@erciyes.edu.tr

Abstract

A hydroponic experiment was conducted between April and May in 2018 by using an aerated Deep Water Culture (DWC) technique in a controlled growth chamber of Erciyes University, Agricultural Faculty in Kayseri, Turkey. To evaluate contribution of roots for growth and physiology a commercial watermelon [*Citrullus lanatus* (Thunb.) Matsum. and Nakai] cultivar (Crimson Tide F_1) was grafted onto two different bottle gourd (*Lageneria siceraria*) genotypes (39-01 and 47-02) and one commercial rootstock genotype (Argenterio) by using two propagation techniques (unrooted or rooted seedlings). Plants were grown in 8 L pots filled continuously aerated nutrient solution, in Randomized Block Design with 4 replications for 6 weeks. Results indicated that shoot and root fresh (FW) and dry (DW) weights, main stem length, total leaf area, leaf chlorophyll index (SPAD), photosynthetic activity of leaves of watermelon were significantly ($P<0.001$) affected by rooting type, genotype and genotype x rooting type interaction. Irrespective of rooting type, the grafted genotypes usually showed significantly higher performance in growth and physiological development than ungrafted control plants. Among graft combinations, the highest growth performance was shown by C.Tide/Argenterio while the lowest was shown by C.Tide/39-01. In terms of rooting type, watermelon plants usually showed a better performance in growth and physiological development when they were used as rooted seedlings compared to unrooted ones. Grafting watermelon onto unrooted seedlings caused a significant reduction in shoot FW by 21.6%, in shoot DW by 12.8%, in root FW by 29.5%, in root DW by 33.7%, in stem length by 11.5%, in total leaf area by 26.3%, in SPAD by 11.2% and in photosynthesis by 18.2%. All these clearly indicate that roots are playing very essential role in contribution to growth and development of plants, particularly at the beginning of growth stage. Therefore, our study suggested that grafting with unrooted seedlings is not a useful application strategy for watermelon plants grown under hydroponic conditions, even when they are grafted onto vigorous rootstocks.

Keywords: Grafting, Genotype, Rootless cuttings, DWC, Hydroponic

Introduction

Grafting is an important and widely applied practice for the production of cucurbit and solanaceous vegetable crops which are usually propagated by using grafted seedlings (Alan et al., 2017). The first grafted vegetable was achieved in Korea and Japan in the late 1920s by grafting watermelon onto gourd rootstocks to manage the soilborne Fusarium wilt (*Fusarium* spp.) diseases (Sibomana et al., 2013). Later on, several studies were carried out on grafting which represents a feasible alternative propagation technique in fruit bearing vegetables such as in tomato, watermelon, cucumber and eggplant to solve issues related to biotic and/or abiotic stress factors that affecting the fruit yield and quality (Lee, 1994; Davis et al., 2008; Schwarz et al., 2010; Savvas et al., 2010). Depending on the scion cultivars, the effects of the rootstocks on plant growth, fruit yield and quality can be resulted diversely either

Cite this article as:
Ulas, A., Aydın, A., Ulas, F., Yetisir, H. (2019). Contribution of roots to growth and physiology of watermelon grafted onto rooted and unrooted seedlings of various bottle gourd rootstocks. Int. J. Agric. Environ. Food Sci., 3(4), 211-216
DOI: https://dx.doi.org/10.31015/jaefs.2019.4.2
Received: 08 September 2019 **Accepted:** 09 November 2019 **Published:** 23 December 2019
Year: 2019 **Volume:** 3 **Issue:** 4 (December) **Pages:** 211-216
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

positively in enhancement (Ozdemir et al., 2016) or negatively in decline (Edelstein, 1999; Lee and Oda, 2003). Since, not all the rootstock species are appropriate and useful for the all scion cultivars.

Generally, watermelon cultivars are grafted onto *Cucurbita moschata, C. maxima, Benincasa hispida* and *Lagenaria siceraria* rootstocks which are the widely used rootstock species for watermelon (Lee, 1994). In Turkey, grafting studies on watermelon plants were started in 2000s by testing 10 rootstocks consisting of Lagenaria, landrace and cucurbit hybrids (Yetisir, 2001). Ozdemir et al. (2016) reported that, watermelon varieties Crimson Tide and Crisby were grafted onto hybrid rootstocks of RS 841, Ferro, Argentario and Macis rootstocks and observed that more yield, fruit size, plant development and fruit quality were produced by the grafted plants.

Cucurbits grafting can be done by applying different propagation techniques, such as using by unrooted cuttings or rooted seedlings as rootstocks. However, there are several advantages and disadvantages of using some of these propagation techniques (unrooted cuttings or rooted seedlings) in rootstock grafting (Lee and Oda, 2003). The advantages of unrooted grafting are; quick and easy method, for some vegetables, very well seedling homogeneity, regulates able to stem length regulation, more hygienic. On the other hand, disadvantages of this method are; delay in root formation during healing of graft part, slow growth, and infection risk. The aim of this work was to evaluate the significance of roots for growth and physiology of watermelon grafted onto rooted and unrooted cuttings of various bottle gourd (*Lagenaria sicerari*) rootstock genotypes under hydroponic condition.

Materials and Methods

Plant Material

In this study a commercial watermelon cultivar (Crimson Tide F1) was used as scion and two different bottle gourd (*Lageneria siceraria*) landrace genotypes (39-01 and 47-02) and one commercial bottle gourd rootstock (Argenterio) genotype were used as rootstock materials (Table 1).

Experimental Site and Plant Growth Conditions

An experiment was conducted between April and May in 2018 by using an aerated Deep Water Culture (DWC) technique in a controlled growth chamber situated in the Plant Physiology Laboratory of Erciyes University, Faculty of Agriculture, central Anatolia in Turkey. For the vegetation period, the average day/night temperatures were 25/22 °C, the relative humidity was 65-70% and about 350 µmol m^{-2} S^{-1} photon flux was supplied in a photoperiod of 16/8 h of light/dark regimes in the controlled growth chamber. To produce homogenous seedling for hydroponic growth medium, seeds of watermelon were sown one week earlier than quickly germinating bottle gourd's seeds in a multi-pots contained a mixture of peat (pH: 6.0-6.5) and perlite in a 2:1 (v:v) ratio for 2 weeks. When the seedlings developed two or three true leaves, scions were grafted onto rootstocks. Some of the ungrafted watermelon (Crimson Tide) plants were used as scion control plants while some of them were grafted onto different rootstocks.

After grafting process, plants were healed and acclimatized in the tunnel covered with double-layered plastic film and shade cloth in the climate chamber for one week (Leoni et al., 1990). In order to prevent grafted plants from wilting by the excessive transpiration and to enhance healing, the tunnel was closed for the first three or four days of healing and acclimatization period. For the next three or four days, the opening and closing of the tunnel were done depending on the conditions of grafted plants and growth room. This was done for the acclimatization of grafted plants to environmental conditions outside tunnel. After the end of healing and acclimatization period, the grafted and ungrafted control plants were carefully freed from the growth medium with no root damage and then transferred into 8 L plastic pots filled with nutrient solution in growth chamber. Each pot was filled with 8 L nutrient solution that was aerated by an air pump to supply sufficient oxygen. The experiment was arranged in a completely randomized block design with four replications and three plants in each pot (replication). In the hydroponic experiment the total vegetation period from transplanting into 8 L plastic pots up to final harvest was almost six weeks.

The nutrient solution was prepared by using distilled water contained analytical grade (99% pure) chemicals according to modified Hoagland and Arnon formulation. In hydroponic experiment, 2000 µM nitrogen was supplied by using two different proportional N sources (75% $Ca(NO_3)_2$ and 25% $(NH_4)_2SO_4$). Furthermore, basic nutrient solution had the following composition (µM): K_2SO_4 (500); KH_2PO_4 (250); $CaSO_4$ (1000); $MgSO_4$ (325); NaCl (50); H_3BO_3 (8.0); $MnSO_4$ (0.4); $ZnSO_4$ (0.4); $CuSO_4$ (0.4); $MoNa_2O_4$ (0.4); Fe-EDDHA (80). All nutrients were replaced when the N concentration of the nutrient solution in the 2.0 mM N rate pots fell below 0.3 mM, as measured daily with nitrate test strips (Merck, Darmstadt, Germany) by using a NitracheckTM reflectometer. Distilled water was added every two days to replenish the water lost to evaporation, and the solution was changed weekly.

Harvest, Shoot- Root Fresh and Dry Weight, Root: Shoot Ratio Measurements

At the end of the experiment plants were harvested by separating them into shoot and roots. For the fresh weight determination plant organs were fractioned into the leaf, stem and roots and then weighted. After measuring the fresh weights of each shoot and root fraction, samples were stored separately in paper bags and dried in a ventilated oven at 70 °C for 72 hours. Root: shoot ratio was calculated from the dry weight.

Main Stem Length and Leaf Physiological Measurements

At the end of the experiment the main stem length and leaf physiological measurements of plants were determined destructively. Main stem length (cm) was measured by using a ruler. Total leaf area (cm^2) of harvested plants was measured destructively with a leaf area measuring device (LI-COR LI-3100C, Inc., Lincoln, NE, USA).

On the other hand, the leaf chlorophyll index (SPAD) was determined non-destructively by using a portable chlorophyll (SPAD) meter (Minolta SPAD-502). During the growth period, SPAD readings were performed on 3th and 4th week of the vegetation period at the center of the leaves on the fully expanded

youngest leaf of whole plants for each treatment.

The leaf-level CO_2 gas exchange (μmol CO_2 m^{-2} s^{-1}) measurements were done in controlled growth chamber by using a portable photosynthesis system (LI-6400XT; LI-COR Inc., Lincoln, NE, USA). The leaf photosynthesis measurement was performed on the most recent fully expanded leaves, using four replicate leaves per treatment on 3th and 4th week of the vegetation period.

Statistical Analysis

Statistical analysis of the nutrient solution experiment data was performed using SAS Statistical Software (SAS 9.0, SAS Institute Inc., Cary, NC, USA). A two-factorial analysis of variance was performed to study the effects of graft combination (genotype) and rooting type and genotype x rooting type interactions on the plants. Levels of significance are represented by $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, and ns means not significant. Differences between the treatments were analyzed using Duncan's Multiple Test.

Table 1. The scion, rootstock and their graft combinations under two propagation techniques

Genotypes	Scion/Rootstock (S/R)	Propagation (Root Type)
C.Tide	Ungrafted Control (Crimson Tide)	Rooted - Unrooted
C.Tide/39-01	Crimson Tide/Landrace bottle gourd (*L. siceraria*)	Rooted - Unrooted
C.Tide/47-02	Crimson Tide/ Landrace bottle gourd (*L. siceraria*)	Rooted - Unrooted
C.Tide/Argenterio	Crimson Tide/Commercial bottle gourd (*L. siceraria)*	Rooted - Unrooted

Results and Discussion

Shoot and Root Fresh Biomass Production and Main Stem Length

Results obtained from hydroponic experiment indicated that shoot and root fresh (FW) and main stem length of watermelon were significantly (P<0.001) affected by rooting type, genotype and genotype x rooting type interaction (Table 2). Irrespective of rooting type, the grafted genotypes usually showed significantly higher shoot (85% increase in shoot FW and 41% increase in stem length) and root growth (170% increase in FW) performance than ungrafted control plants. Among graft combinations, the highest shoot growth was shown by unrooted C.Tide/Argenterio while the lowest was shown by unrooted C.Tide/39-01. On the other hand, the highest root growth was shown by rooted C.Tide/39-01 graft combinations while the lowest was shown by rooted C.Tide/47-02. The lowest fresh biomass and shortest stem length of both rooted and unrooted C.Tide/39-01 graft combinations, might be due to differences in partitioning of dry matter between scion (watermelon) and rootstock (39-01). The vigor of the rootstock is important in conferring scion vigor (Gisbert et al., 2011), but its effect on scion may depend also on watermelon variety (Ozdemir et al., 2016). Our results clearly indicated that grafting with vigor rootstocks either with rooted seedlings or unrooted cuttings have pronounced positive effect on shoot and root growth.

A higher performance in shoot and root growth of grafted watermelon plants might be results of vigorous and active root system of bottle gourd rootstocks that contributed to water and mineral nutrient uptake (Rivero et al., 2003) which led to increase in leaf area formation and photosynthetic activity of leaves. Since, the leaf area formation plays an important role for the light interception and carbon assimilation by crops (Grosse, 1989). Consequently, biomass production and yield of a crop is strongly dependent on its leaf area as well as the rate of leaf photosynthesis (Hirasawa and Hsiao, 1999).

In collaboration with our study, Wei et al., (2009) reported similar results and stated that plants grafted onto *Lageneria siceraria* rootstock genotypes have significantly higher fresh matter in shoots and roots than those of ungrafted plants. Also, other authors stated similar reports about the grafting effects on plant growth and yield (Chouka and Jebari, 1999; Yetisir and Sarı, 2004; Yetisir et al., 2006). Irrespective of grafting process, watermelon plants usually showed a better performance in growth and physiological development when they were used as rooted seedlings compared to unrooted cuttings. Because, unrooting treatment caused a significant reduction in shoot FW by 21.6%, in root FW by 29.5%, and in stem length by 11.5% of watermelon plants. This might be due to lower water transport and mineral uptake (Rivero et al., 2003) from roots to shoots that caused a decline in leaf area formation and thus a low photosynthetic activity of scion leaves.

Shoot and Root Dry Matter Accumulation and Partitioning

The accumulation of shoot and root dry matter and its partitioning of watermelon were significantly (P<0.001) affected by rooting type, genotype and genotype x rooting type interaction (Table 3). Irrespective of rooting type, the grafted genotypes usually produced significantly higher shoot (96% increase), and root (119% increase) dry matter than ungrafted control plants. Graft combinations differed significantly and thus the highest shoot dry matter accumulation was shown by C.Tide/Argenterio while the lowest was shown by C.Tide/39-01. On the other hand, the highest root growth was shown by C.Tide/Argenterio and C.Tide/39-01 graft combinations while the lowest was shown by C.Tide/47-02. This is the similar variation existed also in shoot and root fresh matter production among the same graft combinations (Table 2).

Our results clearly indicated that grafting with vigor rootstocks either with rooted seedlings or unrooted cuttings have pronounced positive effect on shoot and root growth and hence on dry matter accumulations (Table 3). This might be results of stronger root growth of the rootstock (Yetisir and Sari, 2004; Khah, 2011) that contributed to water and mineral nutrient uptake (Rivero et al., 2003) and to augmented endogenous hormone production (Zijlstra et al., 1994) which led to increase in leaf area formation and photosynthetic activity of scion leaves. However there was no significant difference between ungrafted watermelon control plants and grafted C.Tide/Argenterio

and C.Tide/47-02 graft combinations in dry matter partitioning (root:shoot ratio), while significantly highest root:shoot ratio was demonstrated only by C.Tide/39-01 (Table. 3).

It has also been reported that grafting promotes vegetative growth at different levels depending on rootstock characteristics. Many studies reported that an interaction between rootstocks and scions exists resulting in high vigor of the root system and greater water and mineral uptake leading to an increased yield and to fruit growth enhancement (Besri, 2002; Kacjan Marsic and Osvald, 2004). Irrespective of grafting process, watermelon plants usually showed a better performance in growth and physiological development when they were used as rooted seedlings compared to unrooted cuttings. Because, unrooting treatment caused a significant reduction in shoot DW by 12.8%, in root DW by 33.7%, and in root:shoot ratio by 40.6% of watermelon plants. This might be due to lower water transport and mineral uptake (Rivero et al., 2003) from roots to shoots that caused a decline in leaf area formation and thus a low photosynthetic activity of scion leaves.

Physiological Leaf Development and Photosytetic Activity of Leaves

The total leaf area, leaf chlorophyll index (SPAD) and photosynthesis of watermelon were significantly (P<0.001) affected by rooting type, genotype and genotype x rooting type interaction in hydroponic experiment (Table 4). Irrespective of rooting type, the grafted genotypes significantly increased the total leaf area almost by 124%, the leaf SPAD value by 19% and the photosynthetic activity by 44% as compared to ungrafted control plants.

Among graft combinations, highly significant differences were found in physiological leaf development and photosynthetic activity of leaves. Significantly highest total leaf area, SPAD and photosynthesis were demonstrated consistently by the graft combination of C.Tide/Argenterio while the lowest was shown by C.Tide/39-01. The leaf area formation is evidently affected by the scion, but the rootstock may also have significant effects on plant growth (Davis et al., 2008).

Many researchers found that grafting on hybrid rootstocks promoted plant yield increase (Yetisir et al., 2006; Alan et al., 2007; Alexopoulos et al., 2007). In this study, an increase in leaf area formation was determined to be consistent with previous studies.

Since the interspecific hybrid rootstocks with vigorous root system are able to absorb water and nutrient elements more efficiently in addition to disease resistance, they are superior to ungrafted plants in terms of plant yield (Huitron et al., 2009).

Furthermore, concerning leaf chlorophyll index (SPAD) and photosynthesis, our results agreed with the finding of other researchers (Lee, 1994; Besri, 2008). The increased yield of grafted plants is also believed to be due to enhanced water and mineral uptake (Rivero et al., 2003). Pulgar et al. (2000) found that grafting influences absorption and translocation of phosphorus, nitrogen, magnesium, and calcium. Therefore, improving nutrient uptake increases photosynthesis, these conditions allow grafted plants to produce higher yields (Hu et al., 2006). Regardless of grafting process, watermelon plants usually showed a better performance in physiological leaf development and photosytetic activity when they were used as rooted seedlings compared to unrooted cuttings. Since, unrooting treatment caused a significant reduction in total leaf area by 26.3%, in SPAD by 11.2% and in photosynthesis by 18.2% of watermelon plants. This might be due to lower water transport and mineral uptake (Rivero et al., 2003) from roots to shoots that caused a decline in leaf area formation and thus a low photosynthetic activity of scion leaves.

Table 2. Shoot and root fresh matter and main stem length of rooted and unrooted control and grafted watermelon genotypes

Genotypes	Shoot Fresh Weight (g plant^{-1})		Root Fresh Weight (g plant^{-1})		Main Stem Length (cm plant^{-1})	
(Scion/ Rootstock)	Rooted	Unrooted	Rooted	Unrooted	Rooted	Unrooted
C.Tide	19.95 B	17.95 d	4.35 C	2.69 b	9.28 D	7.31 c
C.Tide/39-01	36.23 A	22.87 c	11.75 A	7.23 a	9.79 C	11.34 a
C.Tide/47-02	37.48 A	27.51 b	9.92 B	7.40 a	11.79 A	10.19 b
C.Tide/Argenterio	36.46 A	33.70 a	10.20 B	8.18 a	10.61 B	10.68 b
Genotype	***		***		***	
Rooting type	***		***		***	
Genotype X Ro.type	***		***		***	

Values denoted by different letters (lower and upper case letters for rooted and unrooted, respectively) are significantly different between genotypes within columns at P < 0.05: ns, non-significant. *P < 0.05, **P < 0.01 and ***P < 0.001.

Table 3. Shoot and root dry matter, root: shoot ratio of rooted and unrooted control and grafted watermelon genotypes

Genotypes	Shoot Dry Matter (g plant^{-1})		Root Dry Matter (g plant^{-1})		Root:Shoot (g g^{-1})	
(Scion/ Rootstock)	Rooted	Unrooted	Rooted	Unrooted	Rooted	Unrooted
C.Tide	1.62 C	1.16 d	0.62 C	0.24 b	0.38 B	0.21 c
C.T/39-01	2.38 B	1.45 c	1.17 A	0.72 a	0.49 A	0.50 a
C.T/47-02	2.57 A	2.34 b	0.99 B	0.74 a	0.39 B	0.32 b
C.T/Argenterio	2.50 A	2.97 a	1.02 B	0.82 a	0.41 B	0.28 b
Genotype	***		***		***	
Rooting type	***		***		***	
Genotype X Ro.type	***		***		***	

Values denoted by different letters (lower and upper case letters for rooted and unrooted, respectively) are significantly different between genotypes within columns at $P < 0.05$: ns, non-significant. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

Table 4. Total leaf area, leaf chlorophyll index (SPAD) and photosynthetic activity of rooted and unrooted control and grafted watermelon genotypes

Genotypes	Leaf area (cm^2 plant^{-1})		Leaf chlorophyll Index (SPAD)		Photosynthesis (µmol CO_2 m^{-2} s^{-1})	
(Scion/ Rootstock)	Rooted	Unrooted	Rooted	Unrooted	Rooted	Unrooted
C.Tide	300.79 C	240.52 d	36.77 D	32.88 d	6.47 D	5.83 d
C.T/39-01	596.50 B	340.10 c	40.22 C	35.78 c	8.59 C	6.40 c
C.T/47-02	605.37 B	440.19 b	42.46 B	37.26 b	9.48 B	7.59 b
C.T/Argenterio	647.71 A	565.02 a	43.91 A	39.24 a	9.63 A	8.12 a
Genotype	***		***		***	
Rooting type	***		***		***	
Genotype X Ro.type	***		***		***	

Values denoted by different letters (lower and upper case letters for rooted and unrooted, respectively) are significantly different between genotypes within columns at $P < 0.05$: ns, non-significant. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$.

Conclusion

Grafted vegetable production has become a common practice in many parts of the world. It is an effective agricultural approach to improve plant growth, due to that the yield and quality of the shoot system, partially, depend on the root system. The results of the present experiments demonstrate that how the rootstocks improve plant vigor and productivity whether grafted on to rooted seedlings or unrooted cutting plants. The grafted plants were more robust in terms of main stem length, leaf area, leaf chlorophyll index (SPAD), photosynthesis, fresh and dry weights than those of the ungrafted control plants. This effect, which is present only in some grafting combinations, therefore the scion x rootstock combination is of major importance in terms of growth and development, whereas the choice of the right combination could be a useful means in grafting vegetable production. Watermelon scion variety interacts significantly different when they are grafted onto rooted seedlings or unrooted cutting as a scion-rootstock combination under hydroponic conditions. Unrooting process on the cuttings caused a significant reduction in shoot and root growth, total leaf area, leaf SPAD value and in photosynthesis. All these clearly indicate that roots are playing very essential role in contribution to growth and development of watermelon plants, particularly at the beginning of growth stage. Therefore, our study suggested that grafting with unrooted cuttings is not a useful application strategy for growth and physiology of watermelon plants grown under hydroponic conditions, even when they are grafted onto vigorous rootstocks.

Acknowledgements: We thank to all staff members of the Plant Nutritional Physiology Laboratory of Erciyes University, Turkey for the technical supports and supplying all facilities during the experiments.

References

Alan, O., Ozdemir, N., Gunen, Y. (2007). Effect of grafting on watermelon plant growth, yield and quality. Journal of Agronomy, 6(2):362-365 [Google Scholar].

Alan, O., Duzyaman, E., Sen, F. (2017). How growing cycles affect plant growth and yield of grafted watermelon combinations. Fresenius Environmental Bulletin, 26 (6): 4214-4221 [Google Scholar].

Alexopoulos, A.A., Kondylis, A., Passam, H.C. (2007). Fruit yield and quality of watermelon in relation to grafting. Journal of Food Agriculture and Environment, 5(1):178-179 [Google Scholar].

Besri, M. (2002). Alternatives to methyl bromide for tomato production in the Mediterranean area. Proceedings of the International Conference on Alternatives to Methyl Bromide, Spain, 5-8 March, 2002, 177-181 [Google Scholar].

Besri, M. (2008). Cucurbits grafting as alternative to Methyl Bromide for cucurbits production in Morocco. In November 2008 Annual International Research Conference on Methyl Bromide Alternatives and Emissions Reductions, Orlando, Florida. pp. 60 (1-6) [Google Scholar].

Chouka, A.S. and Jebari, H. (1999). Effect of grafting on vegetative and root development, production and fruit quality. Acta Hortic. 492, 85–93 [Google Scholar].

Davis AR, Perkins-Veazie P, Hassell R, Levi A, King SR, Zhang X (2008). Grafting effects on vegetable quality. HortScience 43: 1670-1672 [Google Scholar].

Edelstein, M., Cohen, R., Burger, Y., Shriber, S., Pivonia, S., Shtienberg, D. (1999). Integrated management of sudden wilt in melons, caused by Monosporascus cannonballus, using grafting and reduced rates of methyl bromide. Plant disease, 83(12), 1142-1145 [Google Scholar].

Gisbert C, Prohens J, Raigón MD, Stommel JR, Nuez F (2011). Eggplant relatives as sources of variation for developing new rootstocks: effects of grafting on eggplant yield and fruit apparent quality and composition. Sci. Hortic. 128,14–22 [Google Scholar].

Grosse, F. (1989). Untersuchungen zur Ertragsbildung und Ertragsstruktur in einem Winterrapssortiment. Ph.D. Thesis. Christian-Albrechts-University, Agricult., Facult., Kiel, Germany, 1989 [Google Scholar].

Hirasawa, T., Hsiao, T.C. (1999). Some characteristics of reduced leaf photosynthesis at midday in maize growing in the field. Field Crops Research, *62*, 53-62 [Google Scholar].

Huitron RM, Ricardez-Salinas M and Camacho F (2009). Influence of grafted watermelon plant density on yield and quality in soil infested with melon necrotic spot virus. HortScience, 44(7):1838–1841 [Google Scholar].

Hu CM, Zhu YL, Yang LF, Chen SF, Hyang YM (2006). Comparison of photosyn-thetic characteristics of grafted and own-root seedling of cucumber under lowtemperature circumstances. Acta Bot. Boreali-Occident. Sin. 26, 247–253 [Google Scholar].

Kacjan Marsic N and Osvald J (2004). The influence of grafting on yield of two tomato cultivars (Lycopersicon esculentum Mill.) grown in a plastic house. Acta agriculurae Slovenia, 83-2. [Google Scholar]

Khah EM (2011). Effect of grafting on growth, performance and yield of aubergine (Solanum melongena L.) in greenhouse and open-field. Int. J. Plant Prod. 5 (4), 359-366. [Google Scholar]

Lee JM (1994). Cultivation of grafted vegetables I. Current status, grafting methods, and benefits. Hort.Sci. 29:235–239. [Google Scholar]

Lee JM and Oda M (2003). Grafting of herbaceous vegetables and ornamental crops. Hort. Rev. (Amer. Soc. Hort. Sci.) 28:61–87. [Google Scholar]

Leoni S, Grudina R, Cadinu M, Madedu B and Carletti MG (1990). The influence of four rootstocks on some melon hybrids and a cultivar in greenhouse. Acta Hort. 28:127–134. [Google Scholar]

Ozdemir AE, Çandır E, Yetisir H, Aras V, Arslan O, Baltaer O, Ustün D, Unlu, M. (2016). Effects of rootstocks on storage and shelf life of grafted watermelons. Journal of Applied Botany and Food Quality, 89:191-201 [Google Scholar].

Pulgar G, Villorar G, DA M and Romero, L (2000). Improving the mineral nutrition in grafted watermelon plants: Nitrogen metabolism. Biologia Plantarum 43:607-609 [Google Scholar].

Rivero RM, Ruiz JM and Romero L (2003). Can grafting in tomato plants strengthen resistance to thermal stress? J. Sci. Food Agric., 83, 1315–1319 [Google Scholar].

Savvas D, Colla G, Rouphael Y, Schwarz D (2010). Amelioration of heavy metal and nutrient stress in fruit vegetables by grafting. Sci. Hortic. 127, 156–161 [Google Scholar].

Schwarz D, Rouphael Y, Colla G, Venema JH (2010). Grafting as a tool to improve tolerance of vegetables to abiotic stresses: thermal stress, water stress and organic pollutants. Sci. Hortic. 127, 162–171 [Google Scholar].

Sibomana IC, Aguyoh JN and Opiyo AM (2013). Water Stress Affects Growth and Yield of Container Grown Tomato (Lycopersicon esculentum Mill) Plants. Global Journal of Bio-Science and Biotechnology. G.J.B.B., Vol.2 (4) 2013: 461-466. ISSN 2278 – 9103 [Google Scholar].

Wei GP, Yang LF, Zhu YL, Chen G (2009). Changes in oxidative damage, antioxidant enzyme activities and polyamine contents in leaves of grafted and non-grafted eggplant seedlings under stress by excess of calcium nitrate. Sci.Hortic. 120, 443–451 [Google Scholar].

Yetişir, H. (2001). Karpuzda Aşılı Fide Kullanımının Bitki Büyümesi, Verim ve Meyve Kalitesi Üzerine Etkileri ile Aşı Yerinin Histolojik Açıdan İncelenmesi. ÇÜ Fen Bilimleri Enstitüsü, Adana. 179 s. [Google Scholar].

Yetisir H and Sarı N (2004). Effect of hypocotyl morphology on survival rate and growth of watermelon seedlings grafted on rootstocks with different emergency performance at various temperatures. Turk. J. Agric. For (TUBITAK), 28:231-237 [Google Scholar].

Yetisir H, Caliskan ME, Soylu S, Sakar M (2006). Some physiological and growthresponses of watermelon [Citrullus lanatus (Thunb.) Matsum. and Nakai] graftedonto Lagenaria siceraria to flooding. Environ. Exp. Bot. 58, 1–8 [Google Scholar].

Zijlstra S, Groot SPC and Jansen J (1994). Genotypic variation of rootstocks for growth and production in cucumber. Possibilities for improving the root system by plant breeding. Sci. Hort. 56:195–196 [Google Scholar].

International Journal of
Agriculture, Environment and Food Sciences

JAEFS

e-ISSN : 2618-5946

DOI: 10.31015/jaefs.2019.4.3

www.jaefs.com

Research Article

Int J Agric Environ Food Sci 3(4):217-219 (2019)

Effects of some pesticides on *Bombus terrestris* under laboratory conditions

Derya Taplamacioglu[1] **Ismail Karaca**[1,*]

[1]Isparta University of Applied Sciences, Faculty of Agriculture, Department of Plant Protection, Isparta, Turkey

[*]Corresponding Author: ismailkaraca@isparta.edu.tr

Abstract

Pesticides have not only lethal effect on bees but they can also change their navigational behaviors. The bumblebees exposed to pesticides may not find their food and hive, and even their motor nervous system can acutely be affected. In this study, effects of some pesticides (abamectin, acetamiprid, deltamethrin, imidacloprid) on bumblebees were investigated. The bumblebee colonies were obtained from Koppert Biological Systems, Inc., Turkey. Six doses of each pesticide were tested on motor behaviors of some extremities of the bumblebees and bumblebees were fed on only 50% sucrose as a control group to compare with pesticide applications. Pesticides were applied to bees by feeding and spraying methods. Then, the situations of motor mobility of legs, antennae and proboscis extension of the bumblebees exposed to pesticides were scored. According to the results of the study, the pesticides used in the experiment had an impact on motor nervous system of the bumblebees, and the most effective pesticide was imidacloprid, followed by deltamethrin, acetamiprid, and abamectin, respectively. These results show that imidacloprid, all doses, demage basic motor coordination fundamental to locomotion and foraging and kill at label dose.

Keywords: Bumblebees, Pesticide, Imidacloprid, Proboscis extension reflex

Introduction

Globally, more than 25000 species of the Apoidea (bees) are the key agents of the ecosystem and agricultural pollination in diverse habitat (Donovan, 1980; Michener, 1979; 2007).

Pesticides used against pests in agricultural areas and especially in greenhouses have negative effects on pollinator bees. These negative effects occur as changes in the behaviors of bees, as well as colony losses and the death of bees (Karahan et al., 2015). Besides honey bees, bumble bees are the second most important pollinators for many plants in the natural flora. 239 species were identified and more than 30 countries in the world use these bees as pollinators on 25 different cultivated plants (Goodwin & Steiner, 1997; Williams, 1998; Benton, 2000; Aslan et al., 2017). Because of easy breeding and the large colony populations, *Bombus terrestris* L. (Hymenoptera: Apidae) is one of the main species used in greenhouses for pollination of plants, such as tomatoes, peppers, and melons (Gürel et al., 2001; Gösterit & Gürel, 2005). Populations of bees and other insect pollinators have fallen dramatically in recent years in many nations and there is growing scientific evidence that pesticides had a significant role (Matheson et al., 1996; Allen-Wardell et al., 1998). Currently, many studies were conducted to further understand the toxic effects of pesticides on bees (Kandemir, 2007; Özbek, 2010).

In Turkey, demand for bumblebees has been increased especially in the Mediterranean coastal region for the pollination of plants grown in greenhouses.

Tomato production is widespread in the region and approximately 150000 commercially produced B. terrestris colonies were used in 2012-2013 production season (Gösterit and Gürel, 2010).

Over the past years several laboratory and field tests have been developed to investigate the effect of neonicotinoid insecticides on motor and sensory functions linked to the foraging capacity of bees (Blacquiere et al., 2012).

The aim of this study was to investigate the sublethal effects of the most commonly used pesticides (abamectin, acetamiprid, deltamethrin, imidacloprid) on motor coordination of *B. terrestris*.

Cite this article as:
Taplamacioglu, D., Karaca, I. (2019). Effects of some pesticides on *Bombus terrestris* under laboratory conditions. Int. J. Agric. Environ. Food Sci., 3(4), 217-219
DOI: https://dx.doi.org/10.31015/jaefs.2019.4.3
Received: 04 October 2019 **Accepted:** 09 November 2019 **Published:** 24 December 2019
Year: 2019 **Volume:** 3 **Issue:** 4 (December) **Pages:** 217-219
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

Materials and Methods

Stock colonies of *Bombus terrestris* L. (Hymenoptera: Apidae) hives were provided from Koppert/ Turkey (http://www.koppert.com.tr/). Four pesticides used in this study were given in Table 1.

Experiments were carried out under laboratory conditions. Based on the recommended doses by the companies, 6 different concentrations were prepared by half diluting for each step (Table 2). Pesticides were applied to bees by feeding method. Glycose syrup used as control was provided by Koppert company.

Hives including approximately 60-70 bees were opened under red light in the dark. Five bees were taken into the falcon tubes and briefly cooled in the refrigerator at -20 °C until they show first signs of immobility. Immobile bees were taped at their thorax to fixing slots made with syringe. This taping process was performed without preventing the functions of antenna, head, legs and abdominal movements. After bee separation, healthy individuals were controlled for their response by applying water and syrup to their antenna. Then, 5 microliter syrup was mixed with 5 microliter insecticide solution, dropped on the Petri dishes and fed to the bees. Bees are kept under laboratory conditions for 2 hours. At the end of this period, motor movements (antenna, legs, abdomen and the proboscis extension reflex) of bumblebees were controlled and scored. In this scoring system, each body part was scored separately and these scores were totalized for each bee. Evaluation was carried out by giving minimum 0 and maximum 6 points. These points and explanations are given in Table 3.

Table 1. List of the four different pesticides tested, their active ingredients (AI) and commercial names, formulation types, and dosages

Active ingredient	Common name	Formulation	Dosage
Abamectin	Agrimec	EC	0.25 ml / l
Acetamiprid	Mostar	SP	0.30 ml / l
Deltamethrin	Decis	EC	0.10 ml / l
Imidacloprid	Confidor	SC	0.10 ml / l

Table 2. Doses of four pesticides used in the study for 100 ml suspension

AGRIMEG Abamectin	MOSTAR Acetamiprid	DECIS Deltamethrin	CONFIDOR Imidacloprid
Control	Control	Control	Control
0.781 µl	0.937 µl	0.312 µl	0.312 µl
1.652 µl	1.875 µl	0.625 µl	0.625 µl
3.125 µl	3.75 µl	1.25 µl	1.25 µl
6.25 µl	7.5 µl	2.5 µl	2.5 µl
12.5 µl	15 µl	5 µl	5 µl
25 µl	30 µl	10 µl	10 µl

Table 3. The motor movement scores of *Bombus terrestris* body parts

Scores	Explanation
0 point	Bees couldn't move any of their body parts
1 point	Bees move their proboscis, antenna, legs or abdomen slowly
2 point	Bees move their body parts normally

Experiments were replicated 5 times and 30 bees were used for each pesticide. Totally 120 bees were used for this experiment. Experiments were conducted in a climate chamber with 25°C temperature, 60% relative humidity and 16:8 h (light: dark) photoperiod.

Statistical analyses was conducted using the statistical program IBM SPSS (Ver. 20) SPSS Inc., Chicago, Illinois, ABD. Tukey's HSD test was used to detect significant differences among the doses and pesticides.

Results and Discussion

Effects of Abamectin, Acetamiprid, Deltamethrin and Imidacloprid on motor movements of *Bombus terrestris* are given in Table 4.

Abamectin didn't affect the motor movements of *B. terrestris* and all doses were statistically similar. Effects of Acetamiprid and Deltamethrin on the motor movements of *B. terrestris* individuals were intermediate level. The most efficient pesticide was Imidacloprid.

The results of the study in Table 4, showed that Imidacloprid has the maximum negative effect on motor movements

of bumble bees and lowest score was recorded with Abamectin treatment followed by Acetamiprid and Deltamethrin. The control, where there was no insecticide spray, had the highest score according to motor movements.

Recent research suggested that widespread agricultural use of imidacloprid and other pesticides may cause honeybee colony collapse disorder. For this reason, several countries have restricted the use of imidacloprid and other neonicotinoids (Woodcock et al., 2016). Likewise, worries are raising concern about the impact of neonicotinoids on wild bumble bee populations (Laycock et al., 2012). Cresswell et al. (2013) reported that imidacloprid reduced feeding and locomotory activity in bumblebees. In our study, imidacloprid showed most adverse effects against *B. terrestris*. Recommended dose (40 µl/100 ml) of imidacloprid used for the greenhouse pests was significantly different from lower doses and control group. Bees exposed to this recommended dose showed no mobility. As a result, it can be mentioned that this pesticide should not be applied during pollination in order to reduce severe effects on *B. terrestris*.

Conclusion

Tests, like the behavioral observations we report here, would be a rapid means of assessing the impact of longer-term exposure to pesticides on bee motor functions and could be used as a reliable bioassay for sublethal effects on pollinators. However, further research is required to establish imidacloprid's impact in greenhouse populations.

Table 4. Comparison of the motor movement scores of *Bombus terrestris* subjected to four insecticides

Doses	Abamectin	Acetamiprid	Deltamethrin	Imidacloprid
Control	6.00±0.00 a*	6.00±0.00 a	6.00±0.00 a	6.00±0.00 a
1	6.00±0.00 a	6.00±0.00 a	5.00±0.57 ab	2.66±0.33 b
2	6.00±0.00 a	6.00±0.00 a	4.66±0.33 ab	2.33±0.88 b
3	5.66±0.33 a	5.33±0.33 ab	4.00±0.00 b	1.33±0.33 bc
4	5.66±0.33 a	5.33±0.33 ab	4.00±0.00 b	0.66±0.33 bc
5	5.00±0.57 a	5.00±0.10 ab	2.00±0.57 c	0.66±0.33 bc
6	5.33±0.66 a	2.66±1.66 b	1.66±0.33 c	0.00±0.33 c

*Means in each row shown by the same letter (s) are not significantly different at (P< 0.05%) according to Tukey Multiple Range Test.

References

Allen-Wardell, G., Bernhardt, P., Bitner, R., Burquez, A., Buchmann, S., Cane, J., Cox, P.A., Dalton, V., Feinsinger, P., Ingram, M., Inouye, D., Jones, C.E., Kennedy, K., Kevan, P., Koopowitz, H., Medellin, R., Medellin-Morales, S., Nabhan, G.P., Pavlik, B., Tepedino, V., Torchio P., Walker, S. (1998) The Potential Consequences of Pollinator Declines on the Conservation of Biodiversity and Stability of Food Crop Yields. Conservation Biology, 12, 1, 8-17. [URL]

Aslan, M.M., Ücük, C., Candan, G. (2017) Kahramanmaraş İli Bombus Arı Türleri ve Bulundukları Bitki Örtüsünün Belirlenmesi (Determination of Bumble Bee Species and Their Foraging Plants in Kahramanmaraş Province). KSU J. Nat. Sci., 20(4): 334-338. [CrossRef]

Benton, T. (2000) The bumblebees of Essex. The Nature of Essex Series, No: 4, Loginga Books, Essex, p 179

Blacquiere T, Smagghe G, Van Gestel CAM, Mommaerts V (2012) Neonicotinoids in bees: A review on concentrations, side-effects and risk assessment. Ecotoxicology 21, 973–992. [CrossRef]

Cresswell, J.E., Robert, F.L., Florance, H., Smirnoff, N. (2013) Clearance of ingested neonicotinoid pesticide (imidacloprid) in honey bees (Apis mellifera) and bumblebees (*Bombus terrestris*). Pest Manag Sci, 70, 332–337. [CrossRef]

Donovan, B.J. (1980) Interactions between native and introduced bees in New Zealand. New Zealand Journal of Ecology, 3,104-116. [URL]

Goodwin, S., Steiner, M. (1997) Introduction of *Bombus terrestris* for biological pollination of horticultural crops in Australia. A submission to AQIS and Environment Australia, Gosford IPM Services, Gosford. [URL]

Gösterit, A., Gürel, F. (2005) *Bombus terrestris* (HYMENOPTERA: APIDAE) arilarinin Yayılmasının ekosistem üzerine etkileri (Effects Of Invasion Of *Bombus terrestris* (Hymenoptera: Apidae) On The Ecosystem) Uludag Bee Journal, 5, 115-121.

Gösterit. A., Gürel, F. (2010) Bombus Arıları ve Bitkisel Üretim Açısından Önemleri. Arıcılık Arastırma Dergisi, 4, 9-12.

Gürel, F., Gösterit, A., Talay, R., Efendi, Y. (2001) Bombus arısı (*Bombus terrestris*)'nın örtüaltı yetiştiricilikte ve ekolojik tarımda kullanımı. Türkiye 2. Ekolojik Tarım Sempozyumu, 14-16 Kasım 2001, Antalya, 245-255.

Kandemir, İ. (2007) Amerika Birleşik Devletleri'nde toplu arı .lümleri ve koloni çökme bozukluğu (KÇB) üzerine bir derleme. Uludağ Arıcılık Dergisi, 7, 63-69.

Karahan, A., Çakmak, İ., Hranitz, J.M., Karaca, İ., Wells, H. (2015) Sub-lethal imidacloprid effects on honey bee flower choices when foraging. Ecotoxicology, 24, 6, 1-9. [CrossRef]

Laycock, I., Lenthall, K.M., Barratt, A.T., Cresswell, J.E. (2012) Effects of imidacloprid, a neonicotinoid pesticide, on reproduction in worker bumblebees (*Bombus terrestris*). Ecotoxicology, 21, 1937–1945. [CrossRef]

Michener, C.D. (1979) Biogeography of the bees. Annals of the Missouri Botanical Garden. 66, 3, 277-347. [URL]

Michener, C.D. (2007) The Bees of the World. The Johns Hopkins University Press, Baltimore and London. p 953.

Özbek, H., (2010) Arılar ve insektisitler. İnsektisitlerin Arılara Olumsuz Etkileri. Uludağ Arıcılık Dergisi, 10, 3, 85-95.

Williams, P.H. (1998) An annotated checklist of bumblebees with an analysis of patterns of description. Bulletin of the Natural History Museum Entomology Series, 67, 79-152. [URL]

Woodcock, B.A., Isaac, N.J.B., Bullock, J.M. Roy, D.B., Garthwaite, D.G.Crowe, A., Pywell, R.F. (2016). Impacts of neonicotinoid use on long-term population changes in wild bees in England. Nature Communications, 7, 12459, 1-8. [CrossRef]

JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2019.4.4 www.jaefs.com

Research Article Int J Agric Environ Food Sci 3(4):220-225 (2019)

Assessment of quality characteristics of fried zucchini slices, pre-dried with osmotic dehydration

Sehriban Gul Ozcelik[1,2] **Erkan Karacabey**[2,*]

[1]Beykent University, School of Health Sciences, Nutrition and Dietetics, 34100, İstanbul, Turkey
[2]Suleyman Demirel University, Engineering Faculty, Food Engineering Department, 32200, Isparta, Turkey

[*]Corresponding Author: erkankaracabey@sdu.edu.tr

Abstract

In this study, it was aimed to investigate the quality characteristics of fried zucchini slices pre-dried using osmotic dehydration (OD) method at varying conditions (salt concentration, vacuum or ultrasound application, time). Textural properties (hardness, elasticity, cohesiveness, chewiness, firmness), surface color values (L*, a*, b*), moisture and oil content of the final product were determined. Textural properties did not change with OD applications ($p>0.05$). Vacuum and solution concentration applied during OD method had significant effects on surface color values ($p\leq0.05$). OD did not cause any significant change in final moisture and oil content of the fried product. In case of ultrasound assisted osmotic dehydration (US-OD), it was seen that the effect of time on moisture content was important the solution concentration of 5% ($p\leq0.05$). On the other hand, changes at salt concentration have created significant differences in both the application process ($p\leq0.001$). When solution concentration was 0%, effect of time was negligible to oil content ($p>0.05$). Notwithstanding, the solution concentration for both processing time was found to be significant ($p\leq0.05$).

Keywords: Osmotic dehydration, Ultrasound, Frying, Textural properties, Surface color values, Moisture content

Introduction

Frying is a treatment applied to enhance the taste and reliability of food (Blumenthal, 1991). In this process, the products are generally immersed into a hot oil, heated by a heat source to around 150 to 190 ° C and frying material is kept in oil until its color, flavor, and texture meet to the consumer's demand (Choe & Min, 2007; Dobarganes, Márquez-Ruiz, & Velasco, 2000). But, at this method, too much oil penetrates into the fried product and the foods having high oil content are associated with many diseases (Bingol, Zhang, Pan, & McHugh, 2012). This is the challenge according to new consumer trends.

Different frying processes have been developed and tested to reduce oil absorption (Da Silva & Moreira, 2008; Naz, Siddiqi, Sheikh, & Sayeed, 2005). One of the processes used is the pre-drying process. As a pre-treatment different methods could be used. One of them is the osmotic dehydration (OD). Mass transfer rate of OD process is generally low, when it is applied alone. Therefore, in order to accelerate the mass transfer taking place, OD may be conducted with processes like vacuum, microwave, ultrasound, and/or centrifugal force application (Corzo vd. , 2007; Rastogi vd. , 2002). It has been proven that ultrasound-assisted OD technology allows to work at low temperatures, with high water loss (Fernandes vd. , 2008; Garcia-Noguera vd. , 2010).

In this context, pre-drying of zucchini slices by OD applied alone or with ultrasound or under vacuum was the aim of the study. Its effects on moisture and oil content of fried zucchinis as well as on their quality characteristics were evaluated.

Materials and Methods

Materials

Zucchini (*Cucurbita pepo* L. cv) was purchased from a local market in Isparta, Turkey, and stored in a polyethylene bags in a refrigerator at 4°C before use.

Preparation of Zucchini Slices

About 100g zucchini, removed from the refrigerator, was

Cite this article as:
Ozcelik, S.G., Karacabey, E. (2019). Assessment of quality characteristics of fried zucchini slices, pre-dried with osmotic dehydration. Int. J. Agric. Environ. Food Sci., 3(4), 220-225
DOI: https://dx.doi.org/10.31015/jaefs.2019.4.4
Received: 08 July 2019 **Accepted:** 09 November 2019 **Published:** 24 December 2019
Year: 2019 **Volume:** 3 **Issue:** 4 (December) **Pages:** 220-225
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

washed, peeled and sliced (3×10^{-3} m thick) using an industrial type slicer (Arisco, HBS-200; Çiğili, ˙ Izmir, Turkey). Afterwards, zucchini slices were subjected to blanching for 90s in a hot water at 85°C for enzyme inactivation and excess surface water was removed using absorbent paper.

Osmotic Dehydration Method

Zucchini slices being at 3 mm slice thickness (1:4 (kg sample / L solution) ratio) was depth for 80 min in different NaCl solutions (10% and 15%) at constant temperature (40°C) water bath (J.P SELECTA S.A, Precisdig 6001238, SPAIN).

Vacuum-Assisted Osmotic Dehydration Method

Zucchini slices being at 3 mm slice thickness (1:4 (kg sample / L solution) ratio) were depth in different NaCl solutions (10% and 15%) at 40°C and then the atmospheric pressure in an application chamber was reduced to 31 kPa by vacuum pump (ROCKER, Rocker 300, CHINA) and held in these conditions for 10 min. Afterwards, the vacuum pressure was released up to atmospheric pressure and samples were continued to be kept for additional 70 min in the same solution.

Ultrasound Assisted Osmotic Dehydration Method

Zucchini slices in a 3 mm slice thickness (1:4 (kg sample / L solution) ratio) was depth for 10 or 20 min in different NaCl solutions (5%, 10% and 15%) which were kept at the constant temperature level of 40°C. During osmotic application, ultrasonic treatment was also conducted at constant power level of 50% by ultrasonic probe (Ultrasonic-Homogenizer, CY-500, Spain). Instead of osmotic solution, distilled water was used a control.

Deep-Fat-Frying

End of OD applications, partially dried zucchini slices was fried in 3L of sunflower oil (Marsa Oil Industry Co. Ltd., Istanbul, Turkey) at 180° C for 1 min. Temperature (180°C) and time (1 min) were the corresponding values of deep-fat-frying process which was determined according to our previous study (Özçelik, 2015). In that study, sensory analysis was conducted to determine the frying conditions to achieve the fried zucchini slices (3mm thickness) taking the highest score from general acceptance. An industrial type fryer (Remta Co. Ltd., Istanbul, Turkey) was used for deep-fat-frying. After frying, the fried zucchini slices were removed from the oil and kept for 300s over a wire screen to drain the most of the surface oil and then excess surface oil, still remaining, was removed by tissue paper. Oil content, moisture content, breaking force, and L*, a*, b* values were measured in these fried slices.

Analysis of Samples

Moisture Content

The method of AOAC (1995) was used. Zucchini slices were ground after frying. Five grams of ground zucchini was used for moisture content. Ground sample was dried in an oven (FN300; Nüve, Akyurt, Ankara, Turkey) at 105±0.5°C, until no weight change was attained. The test was performed in duplicate.

Oil Content

The oil determination method reported by James (1995) was used with modification. Briefly, oil extraction was performed in a Soxhlet extractor (Büchi Universal Extraction System B-811; Postfach, Flawil, Switzerland) using hexane as a solvent to determine the oil content of fried zucchini slices. Before extraction, fried zucchini (5g) was dried in a vacuum oven at 60°C and then ground. Oil content ($g.kg^{-1}$) was calculated as wet bases. The test was performed in duplicate.

Surface Color

Color measurements of examples were determined using Minolta Color Meter (CR-10, Konica Minolta, Osaka, Japan) and expressed by CIE (L *, a *, b *) color system (Robertson, 1977). Five zucchini slices were used for each measurement and at five different locations for each slice. Results were given as a mean of five slices.

Breaking Force

Breaking force for fried zucchini slices was determined according to Bourne (1978). A texture analyzer (TA.XTPlus; Stable Micro Systems Co. Ltd, Godalming, UK) was used for measurement of the breaking force of the samples. In order to measure the force requirement to break fried sample, a Perspex blade (A/LKB) was used. Probe movement speed was 1×10^{-3} ms^{-1} and initial distance from the platform was set as 25×10^{-3} m. The breaking force was expressed in gram force. All texture tests were immediately performed after frying process. Each result was given as a mean of five measurements.

Statistical Analysis

Results are the mean of two replicates. The influences of process conditions were evaluated by Tukey–ANOVA test comparing treatments. Differences between treatments were judged at the 5% significance level ($p\leq0.05$) using Minitab (Minitab 14.12.0) (Minitab Inc., State College, PA).

Results and Discussion

The influences of pretreatment for partial drying of zucchini slices were investigated in terms of textural properties, surface color, moisture and oil content. Both methods were conducted at different conditions, so the effects of process parameters were also evaluated.

Osmotic Dehydration

Effect of osmotic dehydration (OD) and simultaneous vacuum application on textural properties, moisture, oil content, surface color values of the final product were examined. All measured values and corresponding statistical analysis were given in Table 1a and Table 1b. Each application was coded with capital letter and these codes were also defined in Table 1a and Table 1b.

Textural Properties

Textural properties of any processed food are important due to their direct effects on consumer's perceptions. Thus, they should be determined and carefully evaluated to clearly figure out the influence of any intended process on final productquality in terms of textural characteristics. Fried food materials are also considered in this regard and their textural properties are required to be represented. In current study, OD was used for partial drying of zucchini slices before frying and its parameters NaCl concentration of osmotic solution and application of vacuum were investigated. In order to evaluate the effects of parameters on hardness, elasticity, cohesiveness, chewiness and firmness of zucchini slices, they were statistically analyzed and the results were given in Table 2. The results indicated that

neither the concentration of NaCl in the hypertonic solution nor the vacuum application creates any significant effect on elasticity, cohesiveness, chewiness and firmness of the fried zucchini slices ($p>0.05$) (Table 2). Additionally, OD, itself, also did not change the elasticity, cohesiveness, chewiness and firmness of fried slices compared to the control samples (K-21) ($p>0.05$) (Table 1a).

According to Table 1, hardness value of N-coded application (at 40°C, at 80 min, OD 15% NaCl) was different from hardness value of control group (K-21). However, other textural values of N-coded application and all textural values of all other OD applications (N, O, L, M) were not different from control group (K-21). The effects of time and concentration changes on the hardness values were shown at Figure 1.

As can be seen from Figure 1 that, an increase in NaCl concentration significantly increased the sample's hardness value ($p\leq0.05$), whereas vacuum did not create any change ($p>0.05$).

Surface Color Values

Another quality attribute is the surface color of food products, since it gives idea about the quality of product. Thus, it is used as a strong marketing tool. In this regard, surface color of fried products also gains high importance. In this study, surface color of fried zucchini slices was measured after frying process. The results were given in Table 1b. Surface color was described with three color parameters, L*, a*, and b*. From Table 1b it can be concluded that, surface color values of all OD applications (N, O, L, M) were different from control group (K-21) ($p\leq0.05$).

Table 1a. Textural properties, surface color values, moisture (%) and oil content (%) of osmotic pre dried combined fried zucchini slices

GROUP	Hardness (g.force)	Elasticity	Cohesiveness	Chewiness	Firmness (g.force)
B (K-21) (oil temprature of 180°C , frying tim of 3mm , thickness of 1min)	0.70±0.27^{b}	4.9±6.94^{a,b,d}	0.1±0.38^{a,b,c}	0.04±1.73^{a}	53.87±51.90^{b}
N (40°C 80min osmotic 15% NaCl)	176.87±146.14^{a}	0.00±0.0^{d}	0.00±0.00^{c}	0.00±0.00^{a}	104.22±35.35^{b}
O (40°C 80min osmotic 10% NaCl)	13.09±26.58^{b}	2.80±6.2^{b,c,d}	0.2±0.42^{a,b,c}	1.03±2.24^{a}	134.83±119.42^{b}
L (40°C 10min vacuum osmotic+70min osmotic 15% NaCl)	75.25±79.34^{a,b}	0.64±2.02^{d}	0.07±0.23^{b,c}	0.24±0.74^{a}	139.43±56.41^{b}
M (40°C 10min vacuum osmotic+70min osmotic 10% NaCl)	13.45±27.87^{b}	0.95±2.68^{c,d}	0.14±.03^{a,b,c}	0.24±1.42^{a}	89.35±62.40^{b}
P (50% power 40°C 20min ultrasound+osmotic 15% NaCl)	17.02±37.10^{b}	0.15±0.35^{c,d}	0.10±0.38^{c,d}	0.09±0.20^{a}	104.42±1.95^{b}
S (50% power 40°C 10min ultrasound+osmotic 15% NaCl)	0.68±0.37^{b}	0.21±0.46^{d}	0.04±.23^{c,d}	0.11±0.24^{a}	104.50±1.42^{b}
T (50% power 40°C 20min ultrasound+osmotic 10% NaCl)	47.06±103.68^{a,b}	0.10±0.228^{d}	0.04±0.10^{d}	0.01±0.02^{a}	125.41±3.00^{b}
U (50% power 40°C 10min ultrasound+osmotic 10% NaCl)	111.65±84.38^{a,b}	14.18±12.33^{a}	0.54±0.5^{a,b,c}	1789.7±43.7^{a}	137.66±9.01^{b}
V (50% power 40°C 20min ultrasound+osmotic 5% NaCl)	101.12±67.80^{a,b}	14.57±7.59^{a}	0.80±0.33^{a}	452.03±2.24^{a}	263.05±19.42^{a}
X (50% power 40°C 10min ultrasound+osmotic 5% NaCl)	173.52±2.02^{a}	12.4±0.35^{a,b}	0.6±0.26^{a,b,c}	1448.5±12.9^{a}	137.02±1.95^{b}
Y (50% power 40°C 20min ultrasound+osmotic 0% NaCl)	73.70±0.37^{a,b}	11.6±0.5^{a,b,c}	0.39±0.42^{a,b,c,d}	1774.1±0.06^{a}	97.63±1.42^{b}
Z (50% power 40°C 10min ultrasound+osmotic 0% NaCl)	68.02±03.68^{a,b}	15.87±0.22^{a}	0.72±0.10^{a}	1587.2±0.69^{a}	125.26±3.00^{b}
Oil percentages were calculated by way of wet weight					
Different lower case letters top of the numbers shown difference between groups					

Table 1b. Textural properties, surface color values, moisture (%) and oil content (%) of osmotic pre dried combined fried zucchini slices

GROUP	L*	a*	b*	Moisture (%)	Oil (%)
B (K-21) (oil temprature of 180°C , frying tim of 3mm , thickness of 1min)	62.24±7.10^{d}	2.00±3.91^{g}	25.03±2.86^{h}	19.65±1.92^{e}	73.09±1.33^{a}
N (40°C 80min osmotic 15% NaCl)	74.57±2.50^{b,c}	7.21±1.99^{a,b,c}	39.72±1.43^{b,c,d}	34.63±0.65^{d}	41.27±0.15^{c,d,e}
O (40°C 80min osmotic 10% NaCl)	74.94±1.69^{b}	5.15±1.66^{d,e}	41.42±2.68^{a,b}	36.22±4.65^{d}	47.93±1.66^{c,d}
L (40°C 10min vacuum osmotic+70min osmotic 15% NaCl)	78.38±2.30^{b}	3.33±2.05^{f}	34.32±3.35^{g}	32.66±1.00^{d}	43.53±3.32^{c,d}
M (40°C 10min vacuum osmotic+70min osmotic 10% NaCl)	73.35±1.94^{b,c}	6.13±1.60^{b,c,d}	42.59±1.74^{a}	34.20±4.65^{d}	43.68±4.30^{c,d}
P (50% power 40°C 20min ultrasound+osmotic 15% NaCl)	74.40±3.29^{a,b}	7.37±2.72^{a,b}	35.63±2.51^{f,g}	19.57±0.92^{e}	46.64±0.51^{c,d}
S (50% power 40°C 10min ultrasound+osmotic 15% NaCl)	72.56±2.85^{b}	6.44±1.99^{b,c}	39.29±2.37^{c,d}	24.31±0.65^{e}	48.76±0.37^{c,d}
T (50% power 40°C 20min ultrasound+osmotic 10% NaCl)	70.52±6.60^{d}	8.21±2.07^{a}	38.61±3.02^{c,d,e}	34.54±0.28^{d}	45.59±0.25^{c,d}
U (50% power 40°C 10min ultrasound+osmotic 10% NaCl)	74.77±1.69^{b}	4.00±1.32^{e,f}	37.97±3.38^{d,e}	44.94±3.70^{c}	37.41±1.65^{d,e}
V (50% power 40°C 20min ultrasound+osmotic 5% NaCl)	70.50±2.84^{d}	7.99±2.21^{a}	40.11±7.54^{b,c}	66.83±0.41^{b}	33.58±2.83^{e}
X (50% power 40°C 10min ultrasound+osmotic 5% NaCl)	74.46±1.73^{b,c}	5.04±1.66^{d,e}	37.29±2.72^{e}	49.52±1.08^{c}	46.17±2.30^{b}
Y (50% power 40°C 20min ultrasound+osmotic 0% NaCl)	74.73±1.55^{b}	4.99±1.22^{d,e}	35.80±2.33^{f,g}	70.77±0.13^{b}	56.65±10.02^{b}
Z (50% power 40°C 10min ultrasound+osmotic 0% NaCl)	74.12±1.31^{b,c}	6.04±1.13^{c,d}	38.37±2.00^{c,d,e}	79.22±0.16^{a}	42.94±0.16^{c,d}
Oil percentages were calculated by way of wet weight					
Different lower case letters top of the numbers shown difference between groups					

Table 2. Significance of effect of corresponding parameter on textural properties

Parameter	Elasticity	Cohesiveness	Chewiness	Firmness
Solution Concentration (SC)	0.286^{ns}	0.250^{ns}	0.361^{ns}	0.747^{ns}
Vacuum (Vc)	0.676^{ns}	0.941^{ns}	0.627^{ns}	0.865^{ns}
SC*Vc	0.392^{ns}	0.588^{ns}	0.369^{ns}	0.188^{ns}

ns: shown that negligible of the difference between groups

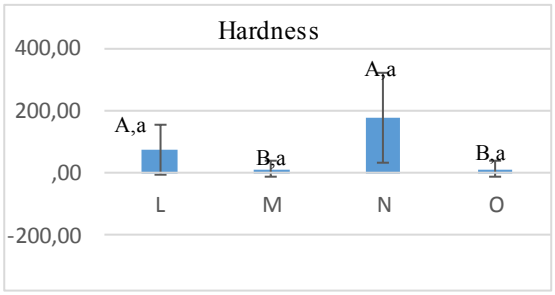


Figure 1. Effect of the vacuum and the solution concentration on the hardness.

L : 40°C 10min vacuum osmotic+70min osmotic 15% NaCl; M : 40°C 10min vacuum osmotic+70min osmotic 10% NaCl; N : 40°C 80min osmotic 15% NaCl; O : 40°C 80min osmotic 10% NaCl

Different lower case letters top of the bars indicate the significant effect of vacuum.

Different upper case letters top of the bars indicate the significant effect of solution concentration.

Figure 2 shows the change in each color parameter with the effects of NaCl concentration and vacuum application. The brightness, L* was found to be changed with NaCl concentration increased from 10% to 15%, when OD was conducted under vacuum ($p \leq 0.05$), whereas no significant difference was seen with changing concentration ($p > 0.05$) in case of no vacuum. The effect of vacuum was also analyzed and compared with OD under atmospheric pressure. Vacuum application caused a significant change in L* value for both cases, under vacuum or at atmospheric pressure ($p \leq 0.05$) (Figure 2).

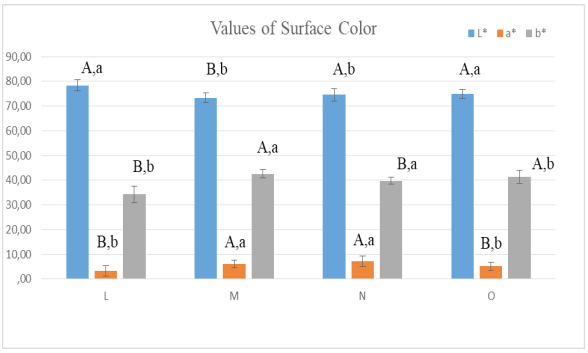


Figure 2. Effect of the vacuum and the solution concentration on the surface color values

L : 40°C 10min vacuum osmotic+70min osmotic 15% NaCl; M : 40°C 10min vacuum osmotic+70min osmotic 10% NaCl; N : 40°C 80min osmotic 15% NaCl; O : 40°C 80min osmotic 10% NaCl

Different lower case letters top of the bars indicate the significant effect of vacuum.

Different upper case letters top of the bars indicate the significant effect of solution concentration.

Vacuum and the solution concentration effects were important for a* and b* values ($p \leq 0.05$) (Figure 2). Vacuum effect was significant for both solution concentration (10% and 15%) on a* values ($p \leq 0.05$). Vacuum application caused an increase in a* value in case of 10% NaCl solution use, whereas it reduced a* values in case of 15% NaCl solution use ($p \leq 0.05$). The effect of solution concentration was also significant on a* values ($p \leq 0.05$). a* value increased with rise of solution concentration under atmospheric pressure, whereas it was decreased with NaCl concentration in vacuum assisted OD. b* was changed with NaCl concentration increased from 10% to 15% when OD was conducted under vacuum or atmospheric pressure ($p \leq 0.05$) (Figure 2). b* values were decrease with the increase in concentration of the solution irrespective of vacuum applied or not. A significant difference in b* values was seen depending on vacuum application, as well ($p \leq 0.05$) (Figure 2).

Moisture and Oil Content

The statistical values of moisture% and oil% were given at Table 1b. As can be seen from Table 1b that, moisture% and oil% values of all OD applications were different from moisture% and oil% values of control group (K-21). Table 3 displays the influences of NaCl concentration and vacuum application on moisture and oil content of fried zucchini slices. As can be seen from Table 3 that, none of the variables caused any significant change in moisture and oil content of zucchini slices ($p > 0.05$). In other words, OD caused a change in moisture and oil content of final fired zucchini slices, this effect was not related to its process parameters investigated in this study. Oil content of final fried zucchini slices was reduced to almost to half of that measured for control group (K-21). However, it should be considered that moisture content remained high compared to control group (Table 1b).

Table 3. Significance of effect of corresponding parameter on moisture (%) and oil (%)

Parameter	Moisture%	Oil%
Solution Concentration (SC)	0.665 ^{ns}	0.165 ^{ns}
Vacuum (Vc)	0.572 ^{ns}	0.648 ^{ns}
SC*Vc	0.994 ^{ns}	0.180 ^{ns}

ns: shown that negligible of the difference between groups.

Ultrasound-Assisted Osmotic Dehydration

Effect of ultrasound assisted osmotic dehydration (US-OD) applications on textural properties, moisture and oil content, surface color values of the final product were examined. Corresponding code for each application and all statistical data for these trials were given in Table 1a and Table 1b.

Textural Properties

From Table 1a, it is seen that all textural properties of US-OD applications (P, S, T, U, V, X, Y, Z) generally were not different from control group (K-21).

The effects of solution concentration and time on textural properties were shown in Table 4. Change of time significantly affected cohesiveness values of fried zucchini slices ($p \leq 0.001$). Changes in solution concentration and time did not create any significant effect on other textural properties ($p > 0.05$) (Table 4).

Surface Color Values

Statistically obtained surface color values of US-OD applications were in Table 1b. It has been seen that surface color values of US-OD applications (P, S, T, U, V, X, Y, Z) were

Table 4. Significance of effect of corresponding parameter on textural properties

Parameter	Hardness	Elasticity	Cohesiveness	Chewiness	Firmness
Solution Concentration (SC)	0.936ns	0.056ns	0.051ns	0.592ns	0.451ns
Vacuum (Vc)	0.092ns	0.061ns	0.001***	0.195ns	0.178ns
SC*Vc	0.173ns	0.281ns	0.417ns	0.749ns	0.119ns

ns: shown that negligible of the difference between groups.

***: shown that significance of the difference between groups.

different from control group (K-21) as a general trend.

The effects of changes in time and concentration on surface color values were shown in Figure 3. L* values of zucchini slices processed at all solution concentrations except for 0% concentration were significantly affected by time ($p \leq 0.05$). Also, different salt concentrations for each application time (10min and 20min) were found to be important ($p \leq 0.001$). Another color parameter, a* value was also found to be varied with time significantly ($p \leq 0.001$), except for the samples treated with 15% osmotic solution ($p > 0.05$). Time change caused a significant variation in b* values of the fried zucchini slices when they were subjected to osmotic treatment at solution concentration of 0% or 15% ($p \leq 0.05$). However, time effect on b* value disappeared when concentration of osmotic solution was 5% or 10% ($p > 0.05$).

Another process parameter, the concentration of osmotic solution was found to significantly affect the surface brightness of fried zucchini slices ($p \leq 0.01$). Similar trend was seen for color parameters of a* and b* values of samples and they significantly changed with concentration ($p \leq 0.01$).

Moisture and Oil

Moisture (%) and oil (%) values of zucchini slices processed by US-OD applications (P, S, T, U, V, X, Y, Z) were given in Table 1b, and compared with control group. When moisture (%) and oil (%) values of zucchini slices produced with US-OD application were compared with those for control group (K-21), it was seen that they were different form control group.

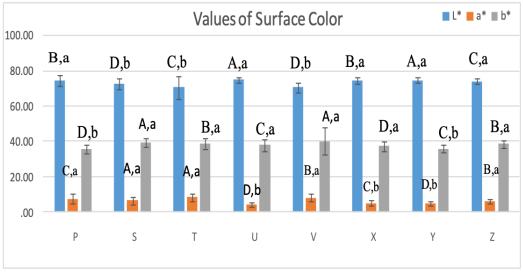


Figure 3. Effect of the time and the solution concentration on the surface color values

P 50% power 40°C 20 min ultrasound+osmotic 15% NaCl; S 50% power 40°C 10min ultrasound+osmotic 15% NaCl; T 50% power 40°C 20min ultrasound + osmotic 10% NaCl; U 50% power 40°C 10min ultrasound + osmotic 10% NaCl; V 50% power 40°C 20min ultrasound + osmotic 5% NaCl; X 50% power 40°C 10min ultrasound + osmotic 5% NaCl; Y 50% power 40°C 20min ultrasound + osmotic 0% NaCl; Z 50% power 40°C 10min ultrasound + osmotic 0% NaCl

Different lower case letters top of the bars indicate the significant effect of time.

Different upper case letters top of the bars indicate the significant effect of solution concentration.

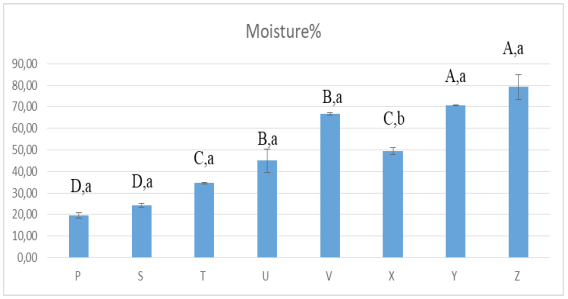


Figure 4. Effect of the vacuum and the solution concentration on the moisture (%) values

P 50% power 40°C 20 min ultrasound+osmotic 15% NaCl; S 50% power 40°C 10min ultrasound+osmotic 15% NaCl; T 50% power 40°C 20min ultrasound + osmotic 10% NaCl; U 50% power 40°C 10min ultrasound + osmotic 10% NaCl; V 50% power 40°C 20min ultrasound + osmotic 5% NaCl; X 50% power 40°C 10min ultrasound + osmotic 5% NaCl; Y 50% power 40°C 20min ultrasound + osmotic 0% NaCl; Z 50% power 40°C 10min ultrasound + osmotic 0% NaCl

Different lower case letters top of the bars indicate the significant effect of time.

Different upper case letters top of the bars indicate the significant effect of solution concentration.

Figure 4 and Figure 5 showed that the effect of time on moisture and oil content of zucchini slices. Time was only effective on moisture content of fried samples, when solution concentration was 5% ($p \leq 0.05$). On the other hand, oil content was found to be changed with time almost for all trials ($p \leq 0.05$), except for those performed when just water was used as an osmotic medium ($p > 0.05$). Changes in salt concentration caused significant differences in the final moisture and oil contents of processed zucchini slices ($p \leq 0.001$).

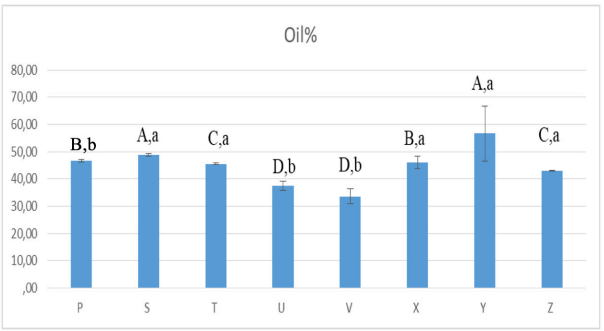


Figure 5. Effect of the vacuum and the solution concentration on the oil (%) values

P 50% power 40°C 20 min ultrasound+osmotic 15% NaCl; S 50% power 40°C 10min ultrasound+osmotic 15% NaCl; T 50% power 40°C 20min ultrasound + osmotic 10% NaCl; U 50% power 40°C 10min ultrasound + osmotic 10% NaCl;

V 50% power 40°C 20min ultrasound + osmotic 5% NaCl; X 50% power 40°C 10min ultrasound + osmotic 5% NaCl; Y 50% power 40°C 20min ultrasound + osmotic 0% NaCl; Z 50% power 40°C 10min ultrasound + osmotic 0% NaCl

Different lower case letters top of the bars indicate the significant effect of time.

Different upper case letters top of the bars indicate the significant effect of solution concentration.

Conclusion

Textural properties of OD applications and US-OD did not differ from that processed as a control group, whereas moisture (%), oil (%) and surface color values of OD applications and US-OD were different from those values determined for control group as general trend.

OD process parameters did not create any significant variation in textural properties for almost all OD applications in general extent. Effects of vacuum and osmotic solution concentration were found to be significant for surface color values, but in different levels. Nevertheless, different parameters of OD applications were negligible on moisture (%) and oil (%) values.

When the parameters' effects of US-OD processes were evaluated, it was found that textural properties, moisture (%) and oil (%) values were not affected by almost all OD applications in general extent. Surface color values were affected by the effects of vacuum and concentration of osmotic solution.

Unchanged textural properties and reduced oil (%) content for zucchini slices were obtained with OD and US-OD applications in this study.

Acknowledgements

This research was financially supported by "Suleyman Demirel University Scientific Research Projects Office", Turkey (Project number: 4201-YL1-14).

References

AOAC. (1995). *Official methods of analysis (16th ed.)*.

Bingol, G., Zhang, A., Pan, Z., & McHugh, T. H. (2012). Producing lower-calorie deep fat fried French fries using infrared dry-blanching as pretreatment. Food Chemistry, 132(2), 686-692 [CrossRef]

Bourne, M. C. (1978). Texture profile analysis [Food acceptability]. Food technology.

Choe, E., & Min, D. (2007). Chemistry of deep-fat frying oils. Journal of food science, 72(5), R77-R86 [CrossRef]

Corzo, O., Bracho, N., Rodríguez, J., & González, M. (2007). Predicting the moisture and salt contents of sardine sheets during vacuum pulse osmotic dehydration. Journal of food engineering, 80(3), 781-790 [CrossRef]

Da Silva, P. F., & Moreira, R. G. (2008). Vacuum frying of high-quality fruit and vegetable-based snacks. LWT-Food Science and Technology, 41(10), 1758-1767 [CrossRef]

Dobarganes, C., Márquez-Ruiz, G., & Velasco, J. (2000). Interactions between fat and food during deep-frying. European Journal of Lipid Science and Technology, 102(8-9), 521-528 [CrossRef]

Fernandes, F. A., Gallão, M. I., & Rodrigues, S. (2008). Effect of osmotic dehydration and ultrasound pre-treatment on cell structure: Melon dehydration. LWT-Food Science and Technology, 41(4), 604-610 [CrossRef]

Garcia-Noguera, J., Oliveira, F. I., Gallão, M. I., Weller, C. L., Rodrigues, S., & Fernandes, F. A. (2010). Ultrasound-assisted osmotic dehydration of strawberries: Effect of pretreatment time and ultrasonic frequency. Drying Technology, 28(2), 294-303 [CrossRef]

Hawkes, J., & Flink, J. M. (1978). Osmotic Concentration of Fruit Slices Priorto Freeze Dehydration. Journal of Food Processing and Preservation, 2, 265-284 [CrossRef]

James, C. (1995). Determination of fat by the Soxhlet methods. Analytical chemistry of foods, 91-92.

Naz, S., Siddiqi, R., Sheikh, H., & Sayeed, S. A. (2005). Deterioration of olive, corn and soybean oils due to air, light, heat and deep-frying. Food Research International, 38(2), 127-134 [CrossRef]

Rastogi, N., Raghavarao, K., Niranjan, K., & Knorr, D. (2002). Recent developments in osmotic dehydration: methods to enhance mass transfer. Trends in Food Science & Technology, 13(2), 48-59 [CrossRef]

Robertson, A. R. (1977). The CIE 1976 Color Difference Formulae. Color Research & Application, 2(1), 7-11 [CrossRef]

Özçelik, S.G. (2015). Optimization of frying process of zucchini slices by frying combined with predehydration in terms of oil absorption and product texture, Suleyman Demirel University Engineering Faculty, Isparta. 125.

Torreggiani, D. (1993). Osmotic dehydration in fruit and vegetable processing. Food Research International, 26(1), 59-68 [CrossRef]

JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2019.4.5 www.jaefs.com

Research Article Int J Agric Environ Food Sci 3(4):226-232 (2019)

Rheological behaviour and yield characterization of gum from local isolates: *Xanthomonas hortorum* pv. *pelargonii* and *Xanthomonas axonopodis* pv. *begonia*

Ahmet Sukru Demirci[1] Ibrahim Palabiyik[1] Demet Apaydin[2,*] Tuncay Gumus[1] Mustafa Mirik[3] Muhammet Arici[4]

[1]Namik Kemal University, Department of Food Engineering, Tekirdag, Turkey
[2]Hitit University, Department of Restaurant and Catering Services, Corum, Turkey
[3]Namik Kemal University, Department of Plant Protection, Tekirdag, Turkey
[4]Yıldız Technical University, Department of Food Engineering, Istanbul, Turkey

[*]Corresponding Author: demetapaydin22@gmail.com

Abstract

Xanthan production capacity of local isolates *X. hortorum* pv. *pelargonii* and *X. axonopodis* pv. *begonia* were investigated by systematically changing fermentation conditions. Optimum yields were found as 11.19 g/L, 9.72 g/L and 9.65 g/L and for standard isolate *X. campestris* DSM 19000, *X. hortorum* pv. *pelargonii* and *X. axonopodis* pv. *begonia*, respectively. Optimum agitation rate and inoculum volume were found as 180 rpm and 5%. Moreover, better gel forming and thickening properties were obtained for xanthan gum from local isolates. Higher K value was observed for gum solutions of the local isolates at all concentration when Ostwald de Waele model was used. Activation energies changed between 4.85 and 25.43 kJ/mol and it is the highest for gum from standard isolate. Moreover, K' and K'' values obtained from dynamic rheological analysis were higher for the local isolates than that of standard isolate. The results confirmed that the local isolates appeared to be suitable microorganisms for xanthan gum production.

Keywords: Xanthan gum, *X. hortorum* pv. *pelargonii*, *X. axonopodis* pv. *begonia*, Rheological behavior, Gum yield

Introduction

Xanthan gum is a natural and commercially important polysaccharide produced by isolates of *Xanthomonas* (Faria et al., 2010). Xanthan is widely used in food, cosmetic, oil recovery and pharmaceutical industries due to its ability to alter the rheological properties of aqueous solutions (Li et al., 2016).

Because of its wide applications, it becomes important to develop local isolate of *Xanthomonas* as a xanthan producer. It is known that different isolates of *Xanthomonas* can produce xanthan gums of different composition, viscosity and yield. The isolation and screening of *Xanthomonas* isolates from natural habitats is still the most efficient method of identifying isolates with high capacity of xanthan production and/or high rheological quality (Torrestiana et al., 1990; Antunes et al., 2003). It is necessary to investigate the rheological properties and chemical structure before deciding on their commercial applicability. For this reason, there is a need to search for new isolates especially local isolates that can produce high yields of good quality xanthan gum.

In addition to bacterial isolates used, operational conditions during fermentation influence the yield, rheological properties and structure of xanthan gum produced (Garcia-Ochoa et al. 2004). Culture conditions like temperature, pH, inoculum size, agitation rate, air flow rate and medium composition are parameters that should be evaluated to optimize xanthan production, and improve rheological properties of the gum, mainly when wild isolates of *Xanthomonas* are studied.

Hence, in the present study, novel local isolates of *Xanthomonas* (*Xanthomonas hortorum* pv. *pelargonii* and *Xanthomonas axonopodis* pv. *begonia*) were evaluated in terms of gum rheology and xanthan gum production at different conditions of agitation rate and inoculum volume and compared

Cite this article as:
Demirci, A.S., Palabiyik, I., Apaydin, D., Gumus, T., Mirik, M., Arici, M. (2019). Rheological behaviour and yield characterization of gum from local isolates: Xanthomonas hortorum pv. pelargonii and Xanthomonas axonopodis pv. begonia. Int. J. Agric. Environ. Food Sci., 3(4), 226-232
DOI: https://dx.doi.org/10.31015/jaefs.2019.4.5
Received: 21 June 2019 **Accepted:** 26 November 2019 **Published:** 24 December 2019
Year: 2019 **Volume:** 3 **Issue:** 4 (December) **Pages:** 226-232
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

with the *X. campestris* DSM 19000 standard isolate in order to determine the yield and quality of gums produced by the new isolate.

Materials and Methods

Isolation and identification of microorganisms

X. axonopodis pv. *begonia* (Xcb-9), and *X. axonopodis* pv. *dieffenbachia* (Xad-2) were isolated from infected plant parts of begonia (Begonia X tuber hybrid), and anthurium (Anthurium andraeanum), respectively.

Identification of the isolates was initially confirmed by morphological, biochemical, and physiological tests including potassium hydroxide solubility for Gram reaction, catalase reaction, oxidative/fermentative metabolism, and hypersensitive reaction on tobacco leaves. Identification of the isolates was confirmed by fatty acid methyl ester (FAME) analysis. The two isolates of *Xanthomonas* spp. were isolated in Turkey (Aysan & Sahin, 2003; Mirik et al, 2007).

X. campestris DSM 19000 (NRRL B-1459) was obtained from the the The Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures (Germany).

Maintenance of microorganisms and cell growth

The microorganisms were maintained in YM (Yeast Malt) agar containing (gL^{-1}): 3.0 yeast extract; 3.0 malt extract; 5.0 peptone; 10.0 glucose; 20.0 agar and distilled water (pH 7.2) (Mesomo et al. 2009). Cultures were transferred at 2-week intervals. Cell production was performed in liquid media YM.

Xanthan gum production

The production of xanthan gum was carried out in 1000 ml Erlenmeyer flasks with 400 ml of medium consist of 40.0 (g/L) glucose, 2.1 (g/L) citric acid, 2.866 (g/L) KH_2PO_4, 0.507 (g/L) $MgCl_2$, 0.089 (g/L) Na_2SO_4, 0.006 (g/L) H_3BO_3, 0.006 (g/L) ZnO, 0.020 (g/L) $FeCl_3 \cdot 6H_2O$, 0.020 (g/L) $CaCO_3$. Glucose was used as a carbon source for the fermentation studies (Liakopoulou-Kyriakides et al., 1999). The fermentations were commenced with inoculums size of 5% (v/v) and 10% (v/v), experiments were conducted at four different agitation rates (180, 200, 220 and 300 rpm) on a orbital shaker (STUART SI500). As showed in the review of Rosalam and England (Rosalam & England, 2006), several works indicated the optimal conditions as temperature at 28°C, fermentation time of 72 h and initial pH at 7.2. Based on the results presented in the literature, during the process, the temperature of the system was maintained at 28°C. This control was necessary since heat was released during the substrate consumption reactions, and thus the temperature of the medium tended to rise. The initial pH of the fermentation medium was 7.2. However, constant pH control was not possible in the shaker. Runs were terminated after 72 h of incubation. All experiments were performed duplicate.

Recovery of xanthan gum

The fermented broth was centrifuged 30 min for cell removal (SIGMA 2-16KL) at 4 °C and 10.000 rpm. Isopropanol (Merck) was added to the supernatant in the proportion of 1:3 (v/v) for precipitate of the biopolymer. The mixture was stored at 4 °C for 24 h and then centrifuged again at 10.000 rpm for 30 min at 4 °C to recover the precipitated gum, which was dried in an oven at 50 °C until constant weight to determine the xanthan gum content. The production of the biopolymers of each isolate at different conditions was evaluated by the weight of the dry product per liter of fermented broth and the averages expressed in g/L.

Rheological analysis of the xanthan gum

Frequently used concentrations in food systems (0.5%, 1%, and 2%) were used in all the rheological measurements. Samples were prepared by dissolving the desired amount of dry sample in deionized water with a magnetic stirrer at 40 °C. Prepared samples were tempered for 24 hr at room temperature before conducting any experiment. Reproducibility of the data was checked by repeating experiments between 3 and 5 times with new samples. Rheological analyses were conducted by suitable models to quantify the properties of xanthan gums.

Steady shear measurements

All rheological measurements were conducted using a controlled stress Discovery Hybrid Rheometer-2 (TA Instruments New Castle, DE, USA) fitted with a parallel-plate geometry (stainless steel, 40 mm diameter, 1000 µm gap). Shear rate range of 1-100 s^{-1} was used for xanthan solutions at 0.5, 1 and 2 wt % and shear rate, shear stress, normal force, torque and apparent viscosity data were collected during experiments. Ostwald de Waele model was fitted to flow behaviors of the samples;

$$\sigma=K(\gamma)^n \quad (1)$$

where σ is the shear stress (Pa), K is the consistency coefficient ($Pa.s^n$), γ is the shear rate (s^{-1}), and n is the flow behavior index (dimensionless).

Dynamic rheological measurements

Dynamic oscillatory shear rheometer Discovery Hybrid Rheometer-2 (TA Instruments New Castle, DE, USA) was used to conduct stress sweep and frequency sweep tests for all gum solution. Stress sweep test was conducted to determine linear viscoelastic region. Frequency sweep test was performed at 0.6 Pa over a frequency range of 0.05–100 rad/s. The elastic or storage modulus (G') and the viscous or loss modulus (G") were modeled by a power law;

$$G'=K'(\omega')^{n'} \quad (2)$$

$$G''=K''(\omega'')^{n''} \quad (3)$$

where K', ω' and n' were intercepts, angular frequency and elastic behavior index, respectively and K", ω" and n" were viscous counterparts.

Effect of temperature on the rheological parameters

The effect of temperature on viscosity of the gum solutions was also investigated and modeled by Arrhenius equation.

$$A=A_0\exp(E_a/RT) \quad (4)$$

where A is the parameter (Pa.s), A_0 is the constant of Arrhenius equation(Pa.s), E_a is the activation energy (kJ/mol), R is gas constant ($8{,}314*10^{-3}$ kJ/molK), and T is temperature (K).

Statistical analysis

Statistical analyses were conducted using Minitab for Windows Release 14®. Standard errors were calculated using the Duncan's multiple range test.

Results and Discussion

Effect of Agitation Rate and Inoculum Volume on Xanthan Gum Production

The results of experiments conducted in shake flasks in order to evaluate the effects of agitation rate and inoculum volume on the production of xanthan gum by *X. hortorum* pv. *pelargonii*, *X. axonopodis* pv. *begonia* and *X. campestris* DSM 19000 were shown in Figure 1-3. Based on the results of the experiments, both inoculum volume and agitation rate have been shown to be important factors, for xanthan concentration. Optimal xanthan production was found with agitation at 200 rpm and inoculum volume at 5 % for isolate *X. hortorum* pv. *pelargonii* as a yield of 9.72 g/L (Fig. 1). The corresponding optimum agitation rate and inoculum volume for other isolate *X. axonoopodis* pv. *begonia* were 180 rpm and 5% respectively, which resulted in a xanthan production of 9.65 g/L (Fig. 2). Similarly, the greatest production of 11.19 g/L for the isolate *X. campestris* DSM 19000 was found with agitation at 180 rpm and inoculum volume at 5%, corresponding to the central point, as can be observed in Fig 3. Generally, *X. campestris* DSM 19000 exhibited higher ability for xanthan gum production as compared to isolates at different conditions.

The results of previous studies (Torrestiana et al., 1990; Nitschke & Thomas, 1995; Sánchez et al., 1997; Souza & Vendruscolo, 1999) showed that the production depended on the isolate and that was also confirmed in this work. It can be deduced, therefore, that the selection of isolate should be the first stage in the xanthan search for the highest yield.

In inoculum experiments, the aim is to optimize the cell concentration to give maximum xanthan production. When xanthan gum production values were compared at 5% and 10% inoculum size of *Xanthomonas* species, higher yields were obtained for all microorganisms at the rate of 5% inoculum except for studies with 300 rpm. The inoculum size 10% might facilitate better the production of biomass than xanthan. High amount of inoculum was not good enough for the production as the nutrients and the space for them was not sufficient to grow actively. Depending up on the isolates, the inoculum size could vary. Papagianni et al. (2001) study xanthan production using 10% inoculum volume. However, Leela and Sharma (2000) and Fernandez-Silva et al. (2009) used 2-15% and 20% inoculum volumes, respectively for xanthan production. Our findings were in agreement with those obtained by Ben Salah et al. (2011), who observed maximum xanthan yield at 5% inoculum.

Furthermore, the graphs of Fig. 1-3 showed that, in general, agitation had a significant effect on xanthan production. Xanthan production was partly associated with metabolic growth; up to 180 rpm, an increase in xanthan production occurred due to oxygen transfer limitation. However, the xanthan yield dropped when higher rate from 220 rpm was applied, probably due to cellular damage by hydrodynamic stress. Agitation speed could be beneficial to the growth and performance of the microbial cells by improving the mass transfer characteristics with respect to substrates, products and oxygen (Garcia-Ochoa et al., 2000). Thus, agitation resulted in a better mixing of the fermentation broth, allowing maintaining a concentration gradient between the interior and the exterior of cells.

Some researchers reported that higher stirrer speed was necessary for xanthan production by *X. campestris* pv. *mangiferaeindicae* IBSBF 1230 (Mesomo et al., 2009), *X. arboricola* pv *pruni* 106 (Borges et al., 2009), *X. campestris* ATCC 33913 (Psomas et al., 2007), *X. campestris* PTCC 1473 (Gilani et al., 2011), *X. campestris* (Casas et al., 2000), *X. campestris* ATCC 1395 (Papagianni et al., 2001). Nevertheless, our results were in agreement with that of Ben Salah et al. (2011) who evaluated xanthan production at distinct stirrer speeds (50, 180 and 250 rpm) and obtained highest levels of xanthan gum at an agitation speed of 180 rpm.

Our results showed that; there is an optimum agitation rate which can not cause bacterial damage and at the same time not limit mass transfer for each bacteria used in xanthan gum production. These results confirmed that yield depended on fermentation parameters and a type of bacterial isolate.

Rheological properties of xanthan gums

Steady shear properties

The gums produced by *X. axonopodis* pv. *begonia* (Xcb-9), *X. axonopodis* pv. *pelargonii* (Xad-2) and standard isolate (*X. campestris* DSM 19000) were also evaluated rheologically and Ostwald de Waele model was used to fit experimental viscosity versus shear rate data to make comparison of non-Newtonian behavior of the solutions (Table 1). Determination coefficient values were higher than 0.98 indicating good fitting of the model and pseudoplastic behavior of gum solutions. Generally solutions of exopolysaccharides obtained from microorganisms showed shear thinning behavior (Kim & Yoo, 2011). The flow behavior index (n), obtained from the model equation, indicated the extent of shear thinning (pseudoplastic) behavior as it deviated from 1. At all gum concentrations of the solutions, gum from standard isolate (*X. campestris* DSM 19000) showed higher n value than those from the local isolates, indicating more pseudoplastic behavior of local isolates. This showed clearly different morphology of gums obtained from different isolates. Lee and Chang (2015) reported that structure of polysaccharides which inhibits the formation of intramolecular hydrogen bonding kept molecule in the extended form during the application of shear. Therefore, solution showed shear thinning behavior. However, when intramolecular hydrogen bonding was formed due to different branch groups in a molecule, it caused lower shear thinning behavior. This rheological feature is also desired property of xanthan solutions as it increases organoleptic qualities in food and causes high degree of mixability, pumpability, and pourability during their processing and/or actual use (Ki-Won et al., 2006). This showed that biopolymers obtained biotechnologically from *Xanthamonas* species isolated from begonia and anthurium plant would be a more suitable thickener or an efficient stabilizer for suspensions and emulsions in many industries (Garcia-Ochoa et al., 2000). As could be seen from K (consistency index) values, gum from local isolates formed higher viscosity solutions at all concentrations than standard isolate indicating that new gums had higher branching ratio as well as their greater hydration capacity and thickening properties.

Concerning the effect of temperature on the viscosity values of gum solutions, Arrhenius model was used. R^2 values were found between 0.96 and 0.99 as shown in Table 2. It was clearly seen that activation energies changed between 4.85 and

25.43 kJ/mol. At all concentrations activation energy of gum from standard isolate was higher than gums from local isolates indicating better temperature stability by lower viscosity decrease as temperature increased. Generally high temperature of solution increased intermolecular distances and decreased chain overlap and entanglement which resulted in viscosity decrease. A similar decrease in temperature was also observed in other polysaccharides such as pectin, starch and gums (Hosseini-Parvar et al., 2010).

Dynamic rheological properties

Gel properties of gums can be determined by investigating viscoelastic properties of gum solutions. Dynamic rheological behavior of the solutions was modeled according to power law and the corresponding viscoelastic parameters were shown in Table 3. *X. axonopodis* pv. *vesicatoria* showed weak gel-like behavior at all studied concentration as the slopes ($n' = 0.32–2.95$; $n'' = 0.18–3.62$) were positive and values of K' ($4.1*10^{-5}$–18) were much higher than those of K'' ($2*10^{-6}$–11) [32]. However, commercial xanthan obtained from *X. campestris* DSM 19000 only demonstrated weak gel-like behavior at high concentration (2%). At 0.5% concentration both gums showed fluid-like behavior as the value of the exponents describing the dependence of moduli with frequency, being higher than unity (Martinez-Padilla et al., 2004). K' values from local isolates were significantly higher than that of standard isolate for all concentrations except 0.5%. This showed that better gel quality of gum solutions obtained from local isolates, *X. axonopodis* pv. *begonia* and *X. hortorum* pv. *pelargonii.*

Table 1. Effect of xanthan gum concentration on Ostwald de Waele parameters and apparent viscosity of xanthan gum solutions obtained from different isolates at 20°C.

Xanthan gum conc. (%)	Isolate	K (Pa s^n)	n (-)	R^2
0.5	*X. axonopodis* pv. *begonia*	0.452a	0.55±0.1b	0.99
	X. hortorum pv. *pelargonii*	0.335a	0.532b	0.99
	X. campestris DSM 19000	0.154b	0.688a	0.99
1	*X. axonopodis* pv. *begonia*	6.321a	0.185c	0.99
	X. hortorum pv. *pelargonii*	2.155b	0.363b	0.99
	X. campestris DSM 19000	1.445b	0.457a	0.98
2	*X. axonopodis* pv. *begonia*	22.872a	0.171b	0.99
	X. hortorum pv. *pelargonii*	19.27b	0.183b	0.99
	X. campestris DSM 19000	16.295c	0.236a	0.99

K: consistency index; n: flow behavior index; R^2: determination coefficient
Different lowercase letters show differences between the columns (P<0.05).

Table 2. Activation energies of xanthan gum obtained from different isolates with different gum concentrations

Xanthan gum conc. (%)	Isolate	A_0 (Pa s^n)	Activation energy (kJ/mol)	R^2
0.5	*X. axonopodis* pv. *begonia*	$2.03*10^{-5}$b	21.21b	0.98
	X. hortorum pv. *pelargonii*	0.0001a	17.7c	0.98
	X. campestris DSM 19000	$1.98*10^{-6}$b	25.43a	0.99
1	*X. axonopodis* pv. *begonia*	0.001b	15.85b	0.96
	X. hortorum pv. *pelargonii*	0.17a	4.85c	0.96
	X. campestris DSM 19000	$7.4*10^{-5}$c	21.25a	0.98
2	*X. axonopodis* pv. *begonia*	0.15a	7.17b	0.97
	X. hortorum pv. *pelargonii*	0.24a	6.36b	0.97
	X. campestris DSM 19000	0.046b	10.12a	0.97

A: constant determined from the Arrhenius relationship; R^2: determination coefficient
Different lowercase letters show differences between the columns (P<0.05)

Table 3. Effect of xanthan gum concentration on *G'* (storage modulus), *G"* (loss modulus), R^2 (determination coefficient) values of different gum solutions obtained from different isolates at 20°C

Xanthan gum conc. (%)	Isolate	*K'*	*n'*	R^2	*K"*	*n"*	R^2
0.5	*X. axonopodis* pv. *begonia*	0.055b	1.192b	0.98	0.069b	0.98a	0.94
	X. hortorum pv. *pelargonii*	0.0013c	2.043a	0.99	0.362a	0.355b	0.84
	X. campestris DSM 19000	0.076a	1.01b	0.95	$5.24*10^{-3}$c	1.51c	0.97
1	*X. axonopodis* pv. *begonia*	6.943a	0.266c	0.99	3.077a	0.183c	0.98
	X. hortorum pv. *pelargonii*	0.560b	0.798b	0.99	1.415b	0.418a	0.99
	X. campestris DSM 19000	0.25b	0.99a	0.99	1.4b	0.34b	0.84
2	*X. axonopodis* pv. *begonia*	29.323a	0.254b	0.99	13.835a	0.172b	0.99
	X. hortorum pv. *pelargonii*	23.99b	0.290b	0.99	12.770a	0.180b	0.99
	X. campestris DSM 19000	13.32c	0.42a	0.99	10.98b	0.259a	0.99

K' and K": consistency index for storage and loss modulus, respectively; *n' and n"*: flow behavior index for storage and loss modulus, respectively; R^2: determination coefficient

Different lowercase letters show differences between the columns (P<0.05).

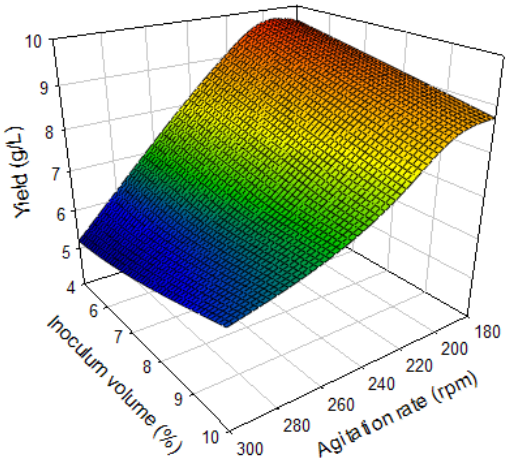


Figure 1. Effects of inoculum volume and agitation rate on the production of xanthan for *X. hortorum* pv. *pelargonii* (g/L)

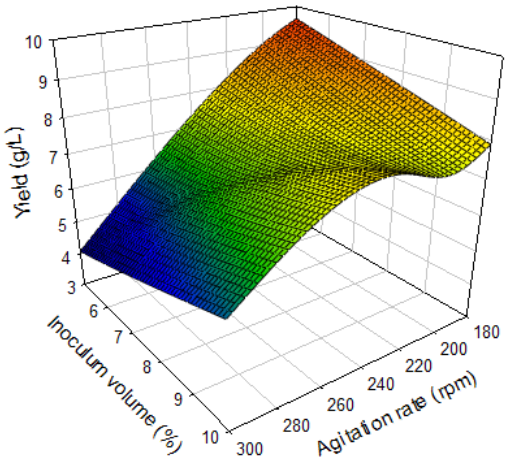


Figure 2. Effects of inoculum volume and agitation rate on the production of xanthan for *X. axonopodis* pv. *begonia* (g/L)

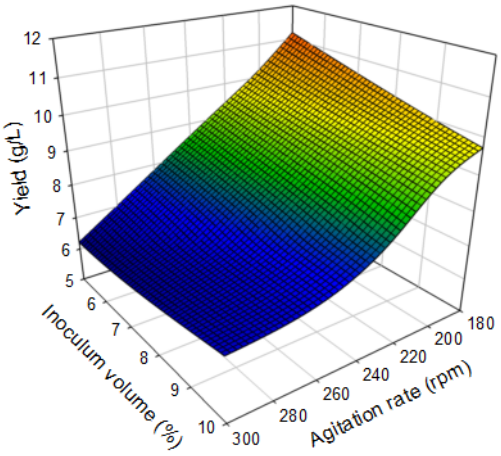


Figure 3. Effects of inoculum volume and agitation rate on the production of xanthan for *X. campestris* DSM 19000 (g/L)

Conclusion

Rheological behavior of xanthan produced from *X. hortorum* pv. *pelargonii* and *X. axonopodis* pv. *begonia* isolates and standart isolate *X. campestris* DSM 19000 was characterized and the effect of agitation levels and inoculum volume on xanthan gum yield was investigated. It was determined that the xanthan gum production potentials of the isolates used in this study were high and the xanthan yields obtained with the standard isolate *X. campestris* DSM 19000 were close to the results obtained with the isolates. The optimised conditions for the production of xanthan in terms of agitation and inoculum were 200 rpm and 5 % for *X. hortorum* pv. *pelargonii* and 180 rpm and 5 % for *X. axonopodis* pv. *begonia*, resulting in a mean production of 9.72 and 9.65 gL^{-1} gum respectively, in 72 h. Generally, it was found that studied isolates were fragile to high agitation rates.

Concerning the rheological behavior, shear thinning properties were observed for all gums. Gums form local isolates caused higher consistency of solution when used at same concentrations compared to gums from standard isolate. Temperature stability of solutions prepared by standard isolate was better. However, gel forming capacity of gums from local isolates, *X. hortorum* pv. *pelargonii* and *X. axonopodis* pv. *begonia* was observed to be better than standard isolates.

Rheological properties and yield values confirmed that isolate *X. hortorum* pv. *pelargonii* and *X. axonopodis* pv. *begonia* appeared to be suitable microorganisms for xanthan gum production when compared to standard isolate, *X. campestris* DSM 19000.

Acknowledgements

We thank The Scientific and Technological Research Council of Turkey (TUBITAK) for financial support (Project Number TOVAG-114O429)

References

Antunes, A.E.C., Moreıra, A.S., Vendruscolo, J.L., Vendruscolo, C.T. (2003). Screening of *Xanthomonas campestris* pv *pruni* strains according to their production of xanthan and its viscosity and chemical composition. Brazilian Journal Of Food Technology, 6, 17-322 [URL]

Aysan, Y., Sahin, F. (2003). First report of bacterial blight of anthurium caused by *Xanthomonas axonopodis* pv. *dieffenbachiae* in Turkey. Plant Pathology,52,783 [CrossRef]

Ben Salah, R., Chaari, K., Besbes, S., Blecker, C., Attia, H. (2011). Production of xanthan gum from *Xanthomonas campestris* NRRL B-1459 by fermentation of date juice palm by-products (Phoenix dactylifera L.). Journal of Food Process Engineering,34,457–474 [CrossRef]

Borges, C.D., de Paula, R.C.M., Feitosa, J.P.A., Vendruscolo, C.T. (2009). The influence of thermal treatment and operational conditions on xanthan produced by *X. arboricola* pv pruni strain 106. Carbohydrate Polymers. 75, 262-268 [CrossRef]

Borges, C.D., Vendruscolo, C.T. (2007). Xanthan synthesized by strains of *Xanthomonas campestris* pv *pruni*: Production, viscosity and chemical composition. *Bioscience Journal,* 23, 67–73 [URL]

Casas, J.A., Santos, V.E., Garcia-Ochoa, F. (2000). Xanthan gum production under several operational conditions: molecular structure and rheological properties. *Enzyme and Microbial Technology* 26, 282–291 [CrossRef]

Faria, S., Vieira, P.A., Resende, M.M., Ribeiro, E.J., & Cardoso, V.L. (2010). Application of a model using the phenomenological approach for prediction of growth and xanthan gum production with sugar cane broth in a batch process. LWT - Food Science and Technology,43,498–506 [CrossRef]

Fernandez-Silva, M., Fornari, R.C.G., Mazutti, M.A., De Olivera, D., Ferreira-Padhila, F., Jose Cichoski, A., et al. (2009). Production and characterization of xantham gum by *Xanthomonas campestris* using cheese whey as sole carbon source. Journal of Food Engineering, 90, 119–123 [CrossRef]

Garcia-Ochoa, F., Santos, V.E., Casas, J.A., Gomez, E. (2000). Xanthan gum: production, recovery, and properties. Biotechnology Advances, 18, 549-579 [CrossRef]

Gilani, S.L., Najafpour, G.D., Heydarzadeh, H.D., Zare, H. (2011). Kinetic models for xanthan gum production using *Xanthomonas campestris* from molasses. Chemical Industry and Chemical Engineering Quarterly, 17, 179−187 [CrossRef]

Gupte, M.D., Kamat, M.Y. (1997). Isolation of wild *Xanthomonas* strains from agricultural produce, their characterization and

potencial related to polysaccharide production. Folia Microbiologica, 42, 621-628 [CrossRef]

Hosseini-Parvar, S.H., Matia-Merino, L., Goh, K.K.T., Razavi, S.M.A., Mortazavi, S.A.(2010). Steady shear flow behavior of gum extracted from Ocimum basilicum L. seed: Effect of concentration and temperature. Journal of Food Engineering, 101, 236–243 [CrossRef]

Ki-Won, S., Yong-Seok, K., Gap-Shik, C. (2006). Rheology of concentrated xanthan gum solutions : steady shear flow behavior. Fibers and Polymers, 7, 129-138 [CrossRef]

Kim, W.W. and Yoo, B. (2011). Rheological and thermal effects of galactomannan addition to acorn starch paste. *LWT - Food Science and Technology*, 44, 759–764 [CrossRef]

Lee, Y., Chang, Y.H. (2015). Effects of galactomannan addition on rheological,pasting and physical properties of water chestnut starch. Journal of Texture Studies, 46, 58–66 [CrossRef]

Leela, J.K., Sharma, G. (2000). Studies on xanthan production from *Xanthomonas compestris*. Bioprocess Engineering, 23, 687–689 [URL]

Li, P., Li, T., Zeng, Y., Li, X., Jiang, X., Wang, Y., Xie, T., Zhang, Y. (2016). Biosynthesis of xanthan gum by Xanthomonas campestris LRELP-1 using kitchen waste as the sole substrate. Carbohydrate Polymers, 151, 684-691 [CrossRef]

Liakopoulou-Kyriakides, M., Psomas, S.K., Kyrakidis, D.A. (1999). Xanthan gum production by *Xanthomonas campestris* w.t fermentation from chestnut extract. *Applied Biochemistry and Biotechnology*, 82, 175-183. [CrossRef]

Lopez, M.J., Vargas-Garcia, M.C., Suarez-Estrella, F., Moreno, J. (2004). Properties of xanthan obtained from agricultural wastes acid hydrolysates. Journal of Food Engineering, 63, 111-115 [CrossRef]

Martinez-Padilla, L.P., Lopez-Araiza, F., & Tacente, A. (2004). Steady and oscillatory shear behavior of fluid gels formed by binary mixtures of xanthan and gellan. Food Hydrocolloid, 18(3), 471-481 [CrossRef]

Mesomo, M., Silva, M.F., Boni, G., Padilha, F.F., Mazutti, M.A., Mossi, A.J., Oliveira, D., Cansian, R.L., DiLuccio, M., Treichel, H. (2009). Xanthan gum produced by *Xanthomonas campestris* from cheese whey: production optimization and rheological characterization. Journal of the Science of Food and Agriculture, 89, 2440–2445 [CrossRef]

Mirik, M., Aysan, Y., Cinar, O. (2007). Copper-resistant strains of *Xanthomonas axonopodis* pv. *vesicatoria* (Doidge) Dye in the eastern Mediterranean region of Turkey. Journal of Plant Pathology, 89, 153-154 [URL]

Moreira, A.S., Vendruscolo, J.L.S., Gil-Tures, C., Vendruscolo, C.T. (2001). Screening among 18 novel strains of *Xanthmonas campestris* pv. *pruni*. Food Hydrocolloid, 15, 469-474 [URL]

Nitschke, M., Thomas, R. W.S.P. (1995). Xanthan gum production by wild-type isolates of *Xanthomonas campestris*. World Journal of Microbiology and Biotechnology, 11, 502-504 [CrossRef]

Papagianni, M., Psomas, S.K., Batsilas, L., Paras, S.V., & Kyriakidis, D.A. (2001). Liakopoulou- Kyriakides M. Xanthan production by *Xanthomonas campestris* in batch cultures. Process Biochemistry, 37, 73–80.

Psomas, S. K., Liakopoulou-Kyriakides, M., Kyriakidis, D.A. (2007). Optimization study of xanthan gum production using response surface methodology. Biochemical Engineering Journal, 35, 273–280 [CrossRef]

Rosalam, S., England, R. (2006). Review of xanthan gum production fromunmodified starches by *Xanthomonas comprestris* sp. Enzyme and Microbial Technology, 39, 197–207 [CrossRef]

Sánchez. A., Ramírez, M.E., Torres, L.G., Galindo, E. (1997). Characterization of xanthans from selected *Xanthomonas* strains cultivated under constant dissolved oxygen. World Journal of Microbiology and Biotechnology, 4, 443-451 [URL]

Souza, A.S., Vendruscolo, C.T. (1999). Produção e caracterização dos biopolímeros sintetizados por *Xanthomonas campestris* pv *pruni* cepas 24 e 58. Ciência e Engenharia, 8, 115-123.

Torrestiana, B., Fucikovsky, L., Galindo, E. (1990). Xanthan production by some *Xanthomonas* isolates. Letters in Applied Microbiology, 10, 81-83 [CrossRef]

Yoon, S. J., Lee, Y., Yoo, B. (2016). Rheological and pasting properties of naked barley flour as modified by guar, xanthan, and locust bean gums. Preventive Nutrition and Food Science, 21(4), 367-372 [CrossRef]

International Journal of
Agriculture, Environment and Food Sciences

JAEFS

e-ISSN : 2618-5946

DOI: 10.31015/jaefs.2019.4.6

www.jaefs.com

Research Article

Int J Agric Environ Food Sci 3(4):233-239 (2019)

Next Generation Sequencing (NGS) based variation analysis: A new practical biomarker for beef tenderness assessment

Esin Calik[1] **Volkan Baltaci**[2] **Kezban Candogan**[1,*]

[1]Ankara University, Faculty of Engineering, Department of Food Engineering, Ankara, Turkey
[2]Yüksek İhtisas University School of Medicine Department of Medical Genetics, Ankara, Turkey

[*]Corresponding Author: candogan@eng.ankara.edu.tr

Abstract

Evaluation of some meat quality attributes using genetic analysis is steadily increasing. PCR based targeted variation analysis is one of the most commonly preferred techniques for this purpose. Recently, Next Generation Sequencing (NGS) method has drawn considerable attention because of its' high analysis capacity. The purpose of the current study was to determine variations in CAST gene from Brangus and Simmental cattle by performing whole gene sequencing using NGS, and to investigate the potential of NGS method in evaluating meat tenderness based on the high genomic data it provides. Whole gene sequence analysis was performed on Calpastatin (CAST) gene of samples acquired from 52 Brangus and 52 Simmental beef cattle breeds using NGS method, and the variations detected were evaluated in terms of their potential in measuring meat tenderness and quality. NGS outputs were analyzed in Ensemble "cow" database platform and 13 variations were detected. One of these variations (EXON 8 c.439C>G/ p.L147LV) was evaluated as undeclared before. In 20 Brangus cattle and in 9 Simmental cattle, no variations were detected whereas 6 variations (V1, V2, V5, V8, V10 and V13) were found significantly different ($p<0.05$) based on their distribution in breeds. Bearing in mind the developments in bioinformatics and NGS method which provides high volume of genomic data, use of these methods in evaluating tenderness of meat was thought to be more practical than assessment based on sensory analyses and instrumental texture evaluations.

Keywords: Beef tenderness, Polymorphisms, Calpastatin (CAST) gene, Next Generation Sequencing (NGS)

Introduction

Flavor, juiciness, and tenderness are very essential attributes by meat consumers (Aaslyng and Meinert, 2017). The taste and quality of meat is effected by the animals' species, age, kind of nutrition, sex, muscle type and environmental conditions (Klont, Brocks, and Eikelenboom, 1998; Wheeler, Shackelford, and Koohmaraie, 2000). Quality assessment of these characteristics can only be obtained after slaughter using classic conventional tests (Lu et al., 2013). Today's meat industry is generally using traditional phenotypic characteristics for this purpose. At the same time, the information obtained from extended genomic variations and genetic markers have opened up a new path for animal breeding and selection to supply feed and growth efficiency and improved carcass quality (Mateescu, Garrick, and Reecy, 2017). Several studies have shown that the genetic constitution (genotype) has significant effect on meat texture (Gao, Zhang, Hu, and Li, 2007; Van Eenennaam et al., 2007). Though several genes impact the

Cite this article as:
Calik, E., Baltaci, V., Candogan, K. (2019). Next Generation Sequencing (NGS) based variation analysis: A new practical biomarker for beef tenderness assessment. Int. J. Agric. Environ. Food Sci., 3(4), 233-239
DOI: https://dx.doi.org/10.31015/jaefs.2019.4.6
Received: 13 September 2019 **Accepted:** 04 December 2019 **Published:** 24 December 2019
Year: 2019 **Volume:** 3 **Issue:** 4 (December) **Pages:** 233-239
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

meat quality, two genes in particular, Calpain (CALP) and Calpastanin (CAST), are directly associated with tenderness (Page et al., 2004; Barendse, 2002). The proteolytic enzyme system responsible for meat tenderness during postmortem aging is the calcium-dependent proteolytic system which includes three different constituents, m-calpain (CAPN1), μ-calpain (CAPN2) and a specific calpain inhibitor, calpastatin (CAST). Calpain activity is influenced by calpastatin which is the endogenous specific inhibitor for protease μ-calpain. Increased post-mortem calpastatin activity is correlated with reduced meat tenderness. Variations in calpastatin gene change the activity of the gene and affect the tenderness of postmortem meat (Zhou et al., 2017; Bhat, Morton, Mason, and Bekhit, 2018; Leal-Gutiérrez and Mateescu, 2019; Herrera-Mendez, Becila, Boudjellal, and Ouali, 2006; Enriquez-Valencia et al., 2017; Curi et al., 2009).

The genomic sequence of bovine CAST contains 35 exons spanning nearly 130 kb (Raynaud et al., 2005). Based on amino acid sequences, six different domains can be recognized some of which have been reported to be involved in binding calpastatin to biological membranes having a central role in the regulation of Ca^{2+} channels (Djadid, Nikmard, Zakeri, and Gholizadeh, 2011).

Targeted variation analysis has been used as a marker and detection tool to estimate meat quality traits including tenderness. One of the classical DNA methods, polymerase chain reaction (PCR) with targeted variation analysis techniques is applied for this purpose. This method serves to detect targeted variations by analyzing a restricted gene or the region of a gene. Associations between the genetic variations in relation to these components and meat tenderness have been determined in different meat types. Parra-Bracamonte, Martinez-Gonzales, Sifuentes-Rincon, and Ortega-Rivas (2015) detected the frequency of alleles of genetic markers related to tenderness in meat obtained from five Zebu breeds of beef cattle in Mexico and noted that there is a strong association between tenderness, and prevalence and distribution of polymorphisms in calpain and calpastatin. Zhou et al. (2017) investigated the associations of single nucleotide polymorphisms (SNPs) in CAPN1 and CAST genes from six generations broilers with some carcass characteristics and tenderness indicating that while in CAPN1 gene, SNP1, SNP2, SNP3 and SNP4 showed an association with carcass and tenderness characteristics, for CAST gene, SNP5 and SNP6 might be potential candidates as molecular markers for molecular assisted selection.

In recent years, next generation sequencing (NGS) technology has become a powerful tool as an accurate and rapid method in concurrent analysis of large amounts of genes or large DNA regions, and also in detection of new mutations or variations as well as noted variations. NGS technology has emerged as a promising approach for rapid detection of economically-motivated adulteration in meat mixtures (Cavin, Cotteneta, Cooperb, and Zbinden, 2018). However, the use of NGS technology in meat science and technology is very limited. NGS technology can provide abundant data thanks to its' high analysis capacity. However, analysis of this data and relevant bioinformatic studies are yet to keep up with the data provided by the method (Goodwin, Mcpherson, and Mccombie, 2016). NGS technology can provide reliable results as it has high reading capacity. However, compared to previous methods, due to high amount of DNA sequence data, high number of variations and errors are detected. On the other hand, variations detected by NGS must be confirmed using Sanger sequencing (Sanger, Nicklen, and Coulson, 1977). Analytic capacity and reliability of the two tests are certainly different; in targeted variation analysis, Sanger and NGS methods are equally reliable. However, if they are compared in a study concerning the sequence analysis of a larger region, Sanger sequencing is more reliable than NGS. As a matter of fact, in whole exon sequencing or whole genome sequence screening, analytic validation of NGS decreases and its' concordance and sensitivity rates drop to 95-97%. This is caused by the decline in reading depth, presence of artifact variants, repeat sequences and wide constitutional variants. Targeted Sanger sequencing panels provide higher coverage with a shorter test run time, and data set handling takes less time than NGS applications (Linderman et al., 2014; De Koning, Jongbloed, Sikkema-Raddatzand, and Sinke, 2014). As the NGS application is increasing, the acquired data will increase the bioinformatic analysis capabilities and due to its sensitivity, specificity and accuracy rates of the test will also increase. NGS technology allows us to include more genomic data and more variations in the test simply by using a nasal swab or blood sample. Because of this, when compared to other classic methods (based on sensory perception and instrumental texture analysis), it is much more practical and can provide faster results.

The purpose of the current study was to determine variations in CAST gene from Brangus and Simmental cattle slaughtered in Turkey by performing whole gene sequencing using NGS technology, instead of PCR based targeted variation analysis. Another purpose of the study was to research the potential of using high genomic data provided by NGS method

for evaluation of meat quality, particularly tenderness.

Materials and Methods

Sample Collection

Whole gene sequence analysis was performed on Calpastatin (CAST) gene on samples acquired from 52 Brangus and 52 Simmental beef cattle breeds using NGS method and the variations detected were evaluated in terms of their potential in measuring meat tenderness and quality. 52 Brangus breed cattle were provided from "Sakarya Meat Processing Plant of General Directory of Meat and Milk Board, Sakarya" and 52 Simmental breed cattle were provided from "Sincan Meat Industry Plant of General Directory of Meat and Milk Board, Ankara". The animals were 16 months old on average, weighing at least 450 kg male cattles. Two meat samples of 1x1x1 cm thickness were taken from the carcass of each animal, labeled, and stored at -20 ºC in Eppendorf tubes for further genetic analyses.

DNA isolation

The samples collected from both breeds and stored at -20 ^{0}C were collectively put through DNA isolation procedure. The meat samples were homogenized by being cut into pieces using a scalpel. Later on, DNA isolation procedures took place using QIAamp DNA FFPE Tissue Kit (50) (Qiagen). After isolation, concentration of DNA samples was measured and archived.

Spectrophotometric Measurement

The amount and concentration measurement of isolated DNA samples were performed by "NanoDrop Spectrophotometry".

PCR amplification and Gel Electrophoresis

Before NGS analysis, multiplex PCR method was used to amplify the target regions of the sample DNA. A Primer coupled with an adapter designed to encompass the exon-intron binding areas and the 31 exons of the CAST gene was used for this purpose. Owing to the primers with adapters, samples and regions could be differentiated (Table 1).

Table 1. 31 exons and exon-intron binding areas of the CAST gene with appropriate adapter primers

Region	Primer (forward)	Region	Primer (reverse)
CASTex1-F	GCCCTCGCTCCCTCCCAG	CASTex1-R	CCGGTCACCTGCCCAGAG
CASTex2-F	CCTATGTCAATGGAGAATTATTAACAGTTC	CASTex2-R	GGAGATATTTGCTACCTCATTATTTATTTCAT
CASTex3-F	TGCGGTTGACCACACTGTTAAG	CASTex3-R	TGCCCAGAAATGATACTTTGTTCCA
CASTex4-F	TCAGCCACGATTGAGTGACTAAC	CASTex4-R	AATCCTGTATAAGTATATAGATGGTGTTTGAG
CASTex5-F	TGTTAATTCGTGTTGCTTACTTGACT	CASTex5-R	AGCGTTACAGAAGATGGTGAACT
CASTex6-F	AAAGCATAATAATCTTAACTCACAACACT	CASTex6-R	AGCTATTCATTATTATTTCAAAGAATCCCA
CASTex7-F	AACCAGACACCAACAGCCATT	CASTex7-R	AATAACTGCCATTCTAGGTAGGACTT
CASTex8-F	AAGTGTTATGAATTGCTTTCTACTCCTC	CASTex8-R	TCATCTGTCTGCTTTATTTACCTTTGG
CASTex9-F	GCTAGTGACCATTTCCCTACAAGAT	CASTex9-R	GACGCACGCTCCTCTTCATC
CASTex10-F	TATCATTGTTATTATTACTTCTGCTGTTCTG	CASTex10-R	TTAAGCTAACCTCACCTCATATTGTT
CASTex11-F	AAGTGAAGGATGTGCAGCAAGTA	CASTex11-R	GCTACCACGGACGCTAACAG
CASTex12-F	ACTGCTGGCTTCTTAATGATTTGTAT	CASTex12-R	CCATCCAATCTGTAACACTCTGAC
CASTex13-F	ACACGACTGAGCGACTGAACT	CASTex13-R	CCCACCCTCTTCCTTTGAATAGATG
CASTex14-F	AATCTGTTCTGTCACTTAAATGGTTCC	CASTex14-R	AGCCTACACATCGCAACTAGAGA
CASTex15-F	TATGTTTCCTTCATCTGCCAGTCAA	CASTex15-R	GAGGTCTACGGGTATAATGCACTATT
CASTex16-F	GCCACAGCTCATTCCTAGAGATT	CASTex16-R	TATGTTGGGCATTAGTTTCGTAACC
CASTex17-F	TTCCTCCACCTCCAGTCTCC	CASTex17-R	TTCAGGGTTTCCAGAGTTGTTATCT
CASTex18-F	AATACAAACTCACTCAAACATATCAGAAA	CASTex18-R	TTCTCCTTAATACTAGGCTGGCATAT
CASTex19-F	ATTCATTACTTGTTGTGTGACATTTATCT	CASTex19-R	AATACGTTTGGTCCTGGCATTT
CASTex20-F	CCGTATTGTTGGTTCATTGTTGTC	CASTex20-R	GTAATACATTGGTAATACAGGAGGAAGG
CASTex21-F	TCTGAGTTGTTCGTTGTAGTCTCTT	CASTex21-R	CGCTCGCTCTGCTTCACTT
CASTex22-F	TTATCAGAGAACGAGGTACTAACACT	CASTex22-R	TGCTAACAGGATGTGAGTTAAGTAATAC
CASTex23-F	ATCATCAGCTATAACCTATCAACCTCT	CASTex23-R	TCTGCCCTTCCTAAATTAACCATCA
CASTex24-F	CGAGGTAGCGTTTGCTGACA	CASTex24-R	CACTGGCTCTATTAGTTACACTGTTG
CASTex25-F	TGCTGTGTTGCTGTGCTTCC	CASTex25-R	CCTATCTTGCCAGTCTTACCTCTTC
CASTex26-F	CTAAGGTGGCTAACCAGTGACTAAT	CASTex26-R	TCTCTGTTCGTTTCCAAGGCAAA
CASTex27-F	TCTGTTGACATTGTTGCTCTAAGTTAC	CASTex27-R	GAGTATAGATCCAACCTGGACACC
CASTex28-F	ACTGGATAAAGATCATGTAAATACTGACTTA	CASTex28-R	GCACAAGGTAGGCATTCACTGA
CASTex28-F	CTCAGCACCTTGTATAACAGAGTG	CASTex28-R	AAGTTTCCTAGGGCATTAATCAGTTTA
CASTex30-F	AGTTAATTGCTAGATGGAGTGTTGAC	CASTex30-R	TGGAGAAGGAAATGGCAACCC
CASTex31-F	GGGAAGAATTCAGTGTTTGGACTAAA	CASTex31-R	AGATTTCAGTGTCCCTTTCATTGC

Afterwards, a purification process was performed using magnetic beads. Then, DNA fragments containing 31 exons, identified as specific bands in 2% agarose gel electrophoresis were observed and inspected. Following this, multiplex PCR products were registered and stored at -20°C to be used in NGS screening.

Next Generation Sequencing (NGS) Analysis

Following the proliferation of DNA samples using adapter primers and the purification procedure, library preparation steps were applied. During the library preparation, Nextera V2 kit (Illumina, California) was used; afterwards, samples were uploaded to the NGS device (Miseq-Illumina, USA). NGS outputs were analyzed in the Ensembl data base. The detected variations' pathogenity was evaluated in the databases and outputs were recorded for each animal.

Interpretation of (NGS) Results

In the genome of every organism along with some common sequences there are several different sequences (variations). If there is a change especially in the coding sequences in functional regions of the gene (variations) that causes a malignant manifestation (that is, causing a change in the amino acid sequence and protein conformation), this change is referred to as "pathogen variation" (mutation) (Linderman et al., 2014). On the other hand, benign variations or polymorphisms can lead to favorable or unfavorable results (Richards et al., 2015). Whether a variation is pathogenic or not depends on factors such as its' localization (intronic, exonic or splice site), changes expression or post-translational defects and ultimately its frequency. The probability of a variation being pathogenic increases if it is Minor Allele Frequency (MAF) value is under 1%, whereas a frequency rate above 1% it is likely to be a polymorphism (Richards et al., 2015). Other determining factors for pathogenic variation are; along with the frequency of the change, its' localization (intronic or exonic); being a nonsense, frameshift change or being localized in start codon or it is a change concerning the deletions of a few exons. On the other hand, the definition of variation in different literatures and how it has been described by different researchers (pathogen/ polymorphism) is also an important criterion and it is possible to understand if the variation in question is a pathogen by referring to different databases (Linderman et al., 2014).

In today's world, several and very rich databases and analysis programs have been developed. There are also databases and analysis programs concerning plants and different animal species. These programs compare the reference sequence with the target region. One of these databases, Ensembl, is a platform that includes the DNA sequence information of cattle genome under the name "cow" (Ensemble Cow Database, 2019).

In this study, we performed the comparison and analysis of the DNA outputs we obtained using NGS analysis with the Ensemble "cow" cattle genome with the "mutation survayor" program (1-5 Ensembl web). Before the analysis, the quality scores of NGS raw data (such as amount of DNA and reading values) were checked using the Mutation Surveyor.

Results and Discussion

CAST gene has been proven to have an impact on the tenderness and textural features of the meat following the slaughter of the cattle (Yousefi and Azari, 2012). In this study, by using NGS method, the correlation between the CAST gene and meat tenderness was not only explained through polymorphic variations but also provided a chance to detect all possible polymorphisms. Thus, a dynamic and practical method based on genetic markers for meat cattle farming for breed or animal preferences was targeted. As a result of the analysis, 13 variations were determined. Unlike the Sanger sequencing and target polymorphism studies that were used in many previous surveys, a dynamic analysis was performed in this study. With the NGS technology, more variations were determined and included in the study and also new variations were identified (Table 2). The NGS outputs were analyzed in the Ensembl database.

When the analysis outputs are taken under consideration, one (EXZON 8 c.439C>G/ p.L147LV) of the 13 polymorphisms we detected is a variation that has not been reported before. One of the polymorphisms we detected (EXZON 22 c.1632A>G/p.E544E) is a splice region variation and therefore, in silico indicates "likely pathogenic" quality. This polymorphism has been detected in both Brangus and Simmental cattle breeds and observed that it has no pathogenic phenotypical effect. The c.439C>G/ p.L147LV variation was found only in 2 samples (numbered 9 and 26) of Brangus breed and was not observed in any cattle of Simmental breed. If the polymorphisms we detected and indicated in Table 8 are taken under consideration in terms of their frequency, all of them are above 1% (average frequency 25.55%) except three (EXON 20 c.1510C>T / p.P504S, INTRON 6 c.373-3C>CT and EXON 8 c.439C>G/ p.L147LV) qualify as polymorphisms. In 20 animals in Brangus group, and 9 animals in Simmental group, no variations were detected. The presence of variations and the group correlations were examined using cross tables and fisher exact test. (Table 3). Based on this; there is a statistically significant correlation ($p<0.05$) between the presence of V1, V2,

V5, V8, V10 and V13 variations and the study groups (Table 3).

V1 variation (EXON 20 c.1526T>C/p.V509A) was observed in 48.1% of Brangus cattle while 82.7% in Simmental cattle group (p=0.001). V2 variation (EXON 22 c.1632A>G/p.E544E) was detected in 30,8% of Brangus cattle breed whereas 80.82% in Simmental cattle group (p=0.000). V5 variation (EXON 9 c.616G>A/p.E206K) was observed in 13.5% of Brangus breed, and 36.52% of Simmental group (p=0.006). V8 variation (EXZON 26 c.1985G>C/p.S662T) was not observed in Brangus group while it was found as 21.2% in Simmental group (p=0.000). V10 variation (EXON13 c.895 G>A/p.A299T) was observed in %5 of Brangus group and not at all in Simmental group (p=0.028). While V13 variation (INTRON 18 c.1335+6G>A) was not detected in Brangus group, in Simmental group it was observed as 25% (p=0.000).

Table 2. Variations and their Minor Allele Frequency (MAF) values

#	VARIATIONS	RS NUMBER	MAF (Ensembl)	MAF (Current Study)
V1	EXON 20 c.1526T>C/p.V509A	rs109384915	38%	42.78%
V2	EXON 22 c.1632A>G/p.E544E	rs110712559	25%	36.53%
V3	EXON 14 c.934A>G/p.N312D	rs723916435	23%	1.92%
V4	EXON 9 c.583A>G/ p.T195A	rs210072660	44%	25.00%
V5	EXON 9 c.616G>A/p.E206K	rs384020496	19%	2.50%
V6	EXON 8 c.439C>G/ p.L147LV	undeclared	-	0.96 %
V7	INTRON 22 c.1714-3C>T	rs110711318	21%	2.88 %
V8	EXON 26 c.1985G>C/p.S662T	rs110914810	45%	8.65%
V9	EXON 9 c.630G>AG/ p.K210KK	rs378682309	15%	1.92%
V10	EXON13 c.895 G>A/ p.A299T	rs715323791	-	2.40%
V11	EXON 20 c.1510C>T / p.P504S	rs1116977475	-	0.48%
V12	INTRON 6 c.373-3C>CT	rs433558933	-	0.48 %
V13	INTRON 18 c.1335+6G>A	undeclared	-	7.69%

Table 3. Frequencies and level of significance for variations detected in Brangus and Simmental beef cattle

		Brangus		Simmental		P Value
Variation	Presence	n	%	n	%	
V1	No	25	48.1	9	17.3	0.001*
	Yes	27	51.9	43	82.7	
V2	No	35	67.3	10	19.2	0.000*
	Yes	17	32.7	42	80.8	
V3	No	48	92.3	52	100.0	0.059
	Yes	4	7.7	0	0.0	
V4	No	36	69.2	29	55.8	0.112
	Yes	16	30.8	23	44.2	
V5	No	45	86.5	33	63.5	0.006*
	Yes	7	13.5	19	36.5	
V6	No	50	96.2	52	100.0	0.248
	Yes	2	3.8	0	0.0	
V7	No	51	98.1	47	90.4	0.102
	Yes	1	1.9	5	9.6	
V8	No	52	100.0	41	78.8	0.000*
	Yes	0	0.0	11	21.2	
V9	No	52	100.0	50	96.2	0.248
	Yes	0	0.0	2	3.8	
V10	No	47	90.4	52	100.0	0.028*
	Yes	5	9.6	0	0.0	
V11	No	51	98.1	52	100.0	0.500
	Yes	1	1.9	0	0.0	
V12	No	51	98.1	52	100.0	0.500
	Yes	1	1.9	0	0.0	
V13	No	52	100.0	38	73.1	0.000*
	Yes	0	0.0	14	26.9	

* $p<0.05$

Conclusion

Among the factors that affect the flavor and tenderness of beef that are as significant as genetic factors are the geography of where the cattle are farmed, feeding and environmental factors. However, when the undeniable effect of genetic factors is taken under consideration, genetic based evaluation tools are an efficient method for beef or animal selection. The main purpose of these kinds of studies is that more genetic biomarkers (variations) are scanned with more practical and reliable methods, and the acquired data are analyzed to generate genotype and phenotype profiles. NGS technology provides an important potential in achieving this purpose thanks to its high analysis capacity.

This study focused on NGS based genetic profiling as a more tangible and practical approach compared to sensory perception and textural based methods. The CAST gene variation profiles of highly preferred Simmental and Brangus cattle breeds in our country were compared using NGS method and it was found that the difference in distribution of variations among the two breeds is statistically significant. In upcoming studies, the genetic variation profiles and the sensory and instrumental texture evaluations can be compared to find out the phenotypical outcome of the detected variations. Therefore, NGS technology would become a very practical and comprehensive technique in evaluating meat quality and tenderness. Over time the increase in these kinds of applications will enrich the current genomic data, increase the sharpness of bioinformatic analysis and the technique will eventually be completely validated. This method is DNA based and can be applied using any biological material (blood, nasal mucosa etc.) unlike sense perception and textural analysis methods. Thus, in practice it can be applied on live animals and allow adequate animal selection for slaughter.

NGS is a strong candidate considering the anticipated developments in bioinformatics in the near future. One of the advantages of preferring NGS method for evaluating the tenderness of meat and animal selection is that it can scan several gene loci and variations, and these data can be used as selection criteria. Over time, with the increase in genomic data, the precision of the test will increase even more. By analyzing NGS outputs with advanced analysis programs, not only can the already identified variations be defined but also new variations could be identified as pathogenic based on in silico parameters.

The method based on DNA analysis and the bioinformatic analysis of several variations is a validated, more reliable method when compared to other methods such as sensory evaluation which are subjective and not standardized and can lead to difficulties in their application (the education of the participants who will evaluate, subjective evaluation criteria, etc.). When all these advantages are taken under consideration, use of NGS technology will become more commonly preferred in selecting quality meat for consumption.

References

Aaslyng, M. D., and Meinert, L. (2017). Meat flavour in pork and beef – From animal to meal. Meat Science,132,112–117. [CrossRef]

Barendse, W. G. (2002). DNA markers for meat tenderness. International patent application. No. Vol. 1. PCT/AU02/00122. World Intellectual Property Organization Inernational Publication, No. WO 02/064820. [URL]

Bhat, Z. F., Morton, J. D., Mason, S. L., and Bekhit, A. E. A. (2018). Role of calpain system in meat tenderness: A review. Food Science and Human Wellness, 7, 196-204 [CrossRef]

Cavin, C., Cotteneta, G., Cooperb, K. M., and Zbinden, P. (2018). Meat Vulnerabilities to Economic Food Adulteration Require New Analytical Solutions. CHIMIA International Journal for Chemistry, 697–703 [CrossRef]

Curi, R. A., Chardulo, L. A., Mason, M. C., Arrigoni, M. D., Silveira, A. C., and de Oliveira, H. N. (2009). Effect of single nucleotide polymorphisms of CAPN1 and CAST genes on meat traits in Nellore beef cattle (Bos indicus) and in their crosses with Bos taurus. Animal Genetics, 40, 456–462 [CrossRef]

De Koning, T. J., Jongbloed, J. D., Sikkema-Raddatzand, B., and Sinke, R. J. (2014). Targeted next-generation sequencing panels for monogenetic disorders in clinical diagnostics: The opportunities and challenges. Expert Review of Molecular Diagnostic, 15(1), 61-70 [CrossRef]

Djadid, N. D., Nikmard, M., Zakeri, S., and Gholizadeh, S. (2011). Characterization of calpastatin gene in Iranian Afshari sheep. Iranian Journal of Biotechnology, 9(2), 145-149 [URL]

Enriquez-Valencia, C. E., Pereira, G. L., Malheiros, J. M., de Vasconcelos Silva, J. A. I., Albuquerque, L. G., de Oliveira, H. N., Chardulo, L. A. L., and Curi, R. A. (2017). Effect of the g. 98535683A> G SNP in the CAST gene on meat traits of Nellore beef cattle (Bos indicus) and their crosses with Bos Taurus. Meat Science, 123, 64–66 [CrossRef]

Ensembl Cow Database, 2019. [URL]

Gao, Y., Zhang, R., Hu, X., and Li, N. (2007). Application of genomic technologies to the improvement of meat quality of farm animals. Meat Science, 77, 36–45 [CrossRef]

Goodwin, S., McPherson, J.D., and Mc Combie, W.R. (2016). Coming of age: ten years of next-generation sequencing technologies. *Nature* Reviews *Genetics*, 17, 333–351 [CrossRef]

Herrera-Mendez, C. H., Becila, S., Boudjellal, A., and Ouali, A. (2006). Meat ageing: Reconsideration of the current concept. Trends Food Sci Technol, 17(8),

394-405 [CrossRef]
Klont, R. E., Brocks, L., and Eikelenboom, G. (1998). Muscle fibre type and meat quality. Meat Science, 49, 219–229. uscle fibre type and meat quality. Meat Science, 49, 219–229 [CrossRef]
Leal-Gutiérrez, J. D., and Mateescu, R. G. (2019). Genetic basis of improving the palatability of beef cattle: current insights. Food Biotechnology, 33(3), 193-216 [CrossRef]
Linderman, M. D., Brandt, T., Edelmann Jabado, O., Kasai, Y., Kornreich, R., Mahajan, M., Shah, H., Kasarskis, A. and Schadt, E. E. (2014). Analytical validation of whole exome and whole genome sequencing for clinical applications. BMC Medical Genomics, 7, 20 [CrossRef]
Lu, D., Sargolzaei, M., Kelly, M., Voort, G. V., Wang, Z., Mandell, I., Moore, S., Plastow, G., and Miller, S. P. (2013). Genome-wide association analyses for carcass quality in cross bred beef cattle. BMC Genetics, 14(80), 1471-2156 [URL]
Mateescu, R. G., Garrick, D. J., and Reecy, J. M. (2017). Network Analysis Reveals Putative Genes Affecting Meat Quality in Angus Cattle. Frontier in Genetics, 8, 171 [CrossRef]
Page, B. T., Casas, E., Quaas, R. L., Thallman, R. M., Wheeler, T. L., Shackelford, S. D., Koohmaraie, M., White, S. N., Bennett, G. L., and Keele, J. W. (2004). Association of markers in the bovine CAPN1 gene with meat tenderness in large cross bred populations that sample influential industry sires. Journal of Animal Science, 82, 3474–3481 [CrossRef]
Parra-Bracamonte, M., Martinez-Gonzales, J.C., Sifuentes-Rincon, A., and Ortega-Rivas, E. (2015). Meat tenderness genetic polymorphisms occurrence and distribution in five Zebu breeds in Mexico. Electronic Journal of Biotechnology,18(5) [CrossRef]
Raynaud, P., Gillard, M., Parr, T., Bardsley, R., Amarger, V., and Levéziel, H. (2005). Correlation between bovine calpastatin mRNA transcripts and protein isoforms. Archives of *Biochemistry* and *Biophysics*, 440, 46-53 [CrossRef]
Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W. W., Hegde, M., Elaine Lyon Spector, E., Voelkerding, K., and Rehm, H. L. (2015). Standards and Guidelines for the Interpretation of SequenceVariants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genetics in Medicine, 17(5), 405–424 [CrossRef]
Sanger, F., Nicklen, S., and Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. Proceedings of the *National Academy* of *Sciences,* USA, 74, 5463–5467 [CrossRef]
Van Eenennaam, A. L., Li, J., Thallman, R. M., Quaas, R. L., Dikeman, M. E., Gill, C. A., Franke, D. E., and Thomas, M. G. (2007). Validation of commercial DNA tests for quantitative beef quality traits. Journal of Animal Science, 85, 891–900 [CrossRef]
Wheeler, T., Shackelford, S., and Koohmaraie, M. (2000). Variation in proteolysis, sarcomere length, collagen content, and tenderness among major pork muscles. Journal of Animal Science, 78, 958–965 [CrossRef]
Yousefi, S., and Azari, M. A. (2012). Study of Calpastatin gene polymorphism in Holstein cattle and buffalo. Animal Sciences and Biotechnologies, 45 (1), 285-288 [URL]
Zhou, Y. G., Xiong, Y., WuYang, C., Jiang, X. S., Ran, J. S., Jin, J., Wang, Y., Lan, D., Ren, P., Hu, Y. D., and Liu, Y. P. (2017). Experimental verification of CAPN1 and CAST gene polymorphisms in different generations of Da-Heng broilers. Hindawi BioMed Research International. [CrossRef]

International Journal of Agriculture, Environment and Food Sciences

JAEFS

e-ISSN : 2618-5946

DOI: 10.31015/jaefs.2019.4.7

www.jaefs.com

Research Article

Int J Agric Environ Food Sci 3(4):240-244 (2019)

Sequence data characterization and development of DNA markers for sesame (*Sesamum indicum* L.)

Ayse Gul Ince[1] **Mehmet Karaca**[2,*]

[1]Vocational School of Technical Sciences, Akdeniz University, 07070 Antalya, Turkey
[2]Department of Field Crops, Akdeniz University, 07070 Antalya, Turkey

[*]Corresponding Author: mkaraca@akdeniz.edu.tr

Abstract

New DNA sequencing techniques enable researchers obtain large quantity of sequence information, which are deposited in digital storage or could be further mined for other purposes. Sesame (*Sesamum indicum* L.) is one of very important oilseed crops, its seed oil contains many antioxidant properties making sesame the queen of oil crops. Today, molecular plant breeding technology is indispensable for plant breeders and seed producers. Compared to other seed oil crops the available number of microsatellite markers in sesame is still not sufficient enough for the development of polymorphic markers for breeding and genetic studies. Thus, new approaches or resources are needed for development of microsatellite markers for sesame. In the present study, we utilized a total of 45099 transcribed genomic DNA sequences/expressed sequence tags and mined these sequences for studying frequency of microsatellite motifs, ranging from di- to hexa-nucleotides with four to ten tandem repeats, and repeat numbers greater than 10. Using mined transcribed data, 42 putative microsatellite markers were developed and characterized at the sequence level. However, we did not confirm these markers and have no information about the level of their polymorphisms in sesame *in vitro*. We discussed the biological meaning of the motif lengths and repeat numbers in the sesame genome.

Keywords: Microsatellite, Motif length, Repeating motif, Primer pairs, Tandem repeats

Introduction

Sesame (*Sesamum indicum* L.), belonging to the family *Pedaliaceae*, is one of the most important oil seed crops in tropical and subtropical regions of Asia, Africa and South America. Today, approximately 2.74 million tons of sesame seeds are produced in approximately 6.1 million hectares worldwide. Major sesame seed producing countries are India, China, Ethiopia, Sudan, Myanmar and Uganda. Sesame is sometime referred to as the 'queen of oilseeds' due to its superior quality of oil among the major oilseed crops including peanut, soybean and rapeseed (Wei et al., 2014). Seeds of sesame contain about 50–60% edible oil and 25% protein, with antioxidants such as lignans, sesamolin and sesamin. Sesame has small diploid genome size of approximately 350 Mb and contains 2n = 26 chromosomes. Although sesame is among the first oilseed crop utilized by human and has many varieties and ecotypes adapted to various ecological conditions throughout the world, it is one of the neglected crops and not widely cultivated. One of the main reasons behind its limited cultivation is its mechanical harvesting difficulties such seed shedding, uninform maturation of the seeds and very low yield (Yen, 1990; Cheung et al., 2007; Ali-Al-Somain et al. 2017).

DNA markers have been proven useful in plant breeding and maintenance of germplasm collections. Although it is very behind from other important agricultural crops, the use of DNA marker technologies has been carried out to estimate the genetic variation in sesame germplasm using random amplified polymorphic DNA (RAPD, Pham et al., 2011), amplified fragment length polymorphism (AFLP, Ali et al., 2007), inter-simple sequence repeat (ISSR, Kumar and Sharma, 2011), sequence-related amplified polymorphisms (SRAP, Ali-Al-Somain et al., 2017) and simple sequence repeat or also known

Cite this article as:
Ince, A.G., Karaca, M. (2019). Sequence data characterization and development of DNA markers for sesame (*Sesamum indicum* L.). Int. J. Agric. Environ. Food Sci., 3(4), 240-244
DOI: https://dx.doi.org/10.31015/jaefs.2019.4.7
Received: 28 September 2019 **Accepted:** 04 December 2019 **Published:** 24 December 2019
Year: 2019 **Volume:** 3 **Issue:** 4 (December) **Pages:** 240-244
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

as microsatellite (SSR, Yue et al. 2013). However, the use of DNA markers in sesame is limited at the genetic variation studies. Furthermore, most of DNA marker techniques used in sesame were carried out using random DNA markers along with a few co-dominant polymorphic DNA markers (Ali et al., 2007; Ince et al., 2010a; Kumar and Sharma, 2011; Wei et al., 2014).

Among DNA marker systems, microsatellites (SSRs) are considered the best DNA markers for understanding the genetic relationships, genetic mapping, hybrid detection and genetic purity testing of any given crop species, due to their abundance, random distribution within the genome, high polymorphism information content, high reproducibility and co-dominant nature (Ince et al., 2008; Ince et al., 2010a; Karaca and Ince, 2011). Microsatellites can be classified into genomic and expressed sequence tag (EST) based microsatellites, depending on their original sequences. Genomic microsatellites can be determined using costly, labor-intensive, and time-consuming traditional methods, but the inter-specific transferability of genomic microsatellites is limited. Whereas, EST-microsatellites obtained from expressed sequences tags have higher level of inter-specific transferability and polymorphism level in many plant species (Ince et al. 2010b). However, microsatellite markers have not been screened on whole-genome level in the *Sesamum* genus. Up to date, only 10 genomic microsatellite and 44 EST-microsatellite markers were developed in previous studies. Additionally, only one global transcriptomic analysis was performed (Wei et al., 2011). Development and utilization of microsatellite markers from ESTs deposited in public databases have several advantages such as identifiability of functional gene for suitable diagnostic markers, high transferability between species, suitable for medium-sized laboratory conditions, and their low development cost (Cloutier et. al., 2009; Karaca and Ince, 2011).

The main purpose of this study was to explore microsatellite sequences varying lengths and motifs in transcriptomic sequences of sesame. With this aim, a total of 45099 sequences in the transcribed region of sesame genome were analyzed. Additionally, new sets of microsatellite primer pairs were designed and these primer pairs could be very useful in sesame breeding and genetic studies.

Materials and Methods

Target sequences

A total of 45099 EST sequences of sesame (*Sesamum indicum* L.) stored in NCBI GenBank databases (ftp://ftp.ncbi.nih.gov/) were downloaded and stored in a personal computer. Data were analyzed to find ESTs with microsatellites or simple sequence repeats. In the present study, repeats in ESTs were identified using TRA 1.5 software (Bilgen et al., 2004). Tandem repeats with motif length di-, tri-, tetra-, penta and hexa-nucleotides were mined. The number of repeats in the motifs ranged from four to ten (Karaca et al., 2005).

Designing of microsatellite primer pairs

Microsatellite primer pairs were designed using Batch Primer 3 1.0 software (You et al., 2008) based on the following main parameters: GC content value was set between 40% and 80%, annealing temperature (Tm) was set between 59°C and 62°C, max self-complementary was set 4.00, max 3' self-complementary was set 3.00, and expected amplified product size was defined as 200–350 bp (Ince et al., 2010a). For primer pair selection, motif lengths were set di- to hexa-nucleotides. Other criteria including minimum number of microsatellite repeats for motifs di- to hexa-nucleotides were applied as previously used in Ince et al. (2010b).

Results and Discussion

In this study, we analyzed the pooled ESTs of sesame from NCBI EST databases and develop sets of EST-SSRs. DNA molecular markers play an important role in sesame breeding studies including identification of the genes responsible for desirable traits, determination of genetic variation, genetic relationships, genetic mapping, hybrid detection in germplasm (Karaca et al., 2017a).

In the present study, tandem repeats varied in their repeats numbers. Analysis results indicated that the number of repeat containing sequences decreased as the repeat number in a motif increased as shown in Table 1. In total 6082 sequences containing 7376 tandem repeats were identified from 45099 sequences when lowest repeat number was set to 4. On the other hands, only 80 tandem repeats were determined when lowest repeat number was set to 10. As they can be seen in Table 1, about 13.5% of sequences contained four repeats while 0.17% sequences contained 10 or more repeat numbers. Based on sesame transcribed sequences we noted that majority of repeating numbers were between 4 and 6. This indicates that tandem repeats of di- to hexa-nucleotides containing four to seven repeats may have biological functions more than other repeats.

In the present study, tandem repeat types found in the sesame genome were represented in Table 2. Results indicated that repeat numbers within each motif ranged from di- to hexa-nucleotides. When repeat finding criteria of repeat numbers was set to a minimum number of 4 repeats, the most abundant type of repeat motifs was di-nucleotide (5278, 71.55%), followed by tri-nucleotide (1935, 26.23%), tetra-nucleotide (115, 1.56%), hexa-nucleotide (27, 0.37%), and penta-nucleotide (21, 0.28%) repeat units (Table 2). The number of microsatellites with greater than 10 tandem repeats was very low but di-nucleotides (67, 83.8%) were the most common, followed by tri-nucleotides (12, 15.0%), hexa-nucleotide (1, 1.25%) and tetra- and penta-nucleotides were not existed (Table 2).

Among the tandem repeats, di- and tri-nucleotides contained greater than 10 repeats while penta-nucleotides contained few number of sequences containing 5 and 6 repeats (Table 2). This indicated that di- and tri-nucleotides have higher frequency in part of transcribed portion of sesame genome. In general, the number of tandem repeat containing repeat types were di-nucleotides. This was the most interesting findings of the present study because other research conducted on *in silico* data mining reported that tri-nucleotide repeats were the most abundant type. However, there were inconsistent results between the higher frequencies between di- and tri-nucleotides among research. Inconsistency was probably due to the different number of repeat searching criteria used among the studies (Karaca et al., 2017a; Karaca et al., 2017b). Zhang

et al. (2012) reported that di-nucleotide motifs (48.01%) were the most abundant, followed by tri- (20.96%), hexa- (25.37%), penta- (2.97%), tetra- (2.12%), and mono-nucleotides (0.57%) in the 42566 unique-transcript sequences. Total length covered was 47987 kbp in the sesame genome. These authors identified a total of 7324 SSRs, with motifs greater than 15 bp. When the motif length was changed to greater than 18, the number of SSRs detected were 4440.

Table 1. Summary of tandem repeats in part of transcribed portion of sesame genome (4509 sequences)

# RN	# Sequences	# Repeat Strings	Repeat Index	Repeat Strings/ Sequences	Total TR	Repeat (%)
4	6082	7426	0.165	1.221	7376	13.486
5	1643	1866	0.041	1.136	1854	3.643
6	760	845	0.019	1.112	867	1.685
7	447	471	0.010	1.054	466	0.991
8	296	307	0.007	1.037	305	0.656
9	208	213	0.005	1.024	213	0.461
10	143	145	0.003	1.014	126	0.317
>10	79	80	0.002	1.013	80	0.175

RN: repeats number, TR: Tandem Repeats

Table 2**.** Frequency of tandem repeat motifs (RMs) in part of transcribed portion of sesame genome revealed by *in silico* research

	Number of Repeats in Motifs							
RMs	4	5	6	7	8	9	10	>10
Di	5278 (71.55%)	1330 (71.7%)	630 (75.2%)	377 (80.9%)	257 (84.3%)	184 (86.4%)	126 (86.9%)	67 (83.8%)
Tri	1935 (26.23%)	469 (25.3%)	174 (20.8%)	80 (17.2%)	45 (14.8%)	26 (12.2%)	16 (11.03%)	12 (15%)
Tetra	115 (1.56%)	36 (1.94%)	19 (2.27%)	4 (0.85%)	na	na	na	na
Penta	21 (0.28%)	8 (0.43%)	6 (0.72%)	na	na	na	na	na
Hexa	27 (0.37%)	8 (0.43%)	6 (0.72%)	3 (0.64%)	3 (0.98%)	3 (1.4%)	3 (2.07%)	1 (1.25%)
Total	7376	1854	867	466	305	213	126	80

In the present study, tandem repeat contexts in the transcribed portion of sesame genome were also studied and findings were depicted in Table 3. We clearly noted that as the motif lengths increased from di- to hexa-nucleotides and the repeats numbers increased from four to ten, and greater than ten, motif contexts varied. Among the di-nucleotides AG/TC repeats contained 4, 8, 9, 10 and greater than 10 repeats were abundant while CT/AG repeats consisted of more repeats contained from 5 to 7 repeats in comparison to the other repeats. Tri-nucleotide motif context, CTT was the most abundant type in especially the repeats numbers from nine to ten. This finding revealed that tri-nucleotide motif context of CTT would be very important context and further transcriptomic studies should be focused on CTT context. CTT translates into leucine (L, Leu) amino acid that is an essential amino acid for human (Weber, 1990; Zhang et al., 2012; Karaca et al., 2017a; Karaca et al., 2017b). Tetra-nucleotide repeats ATAC/TATG consisted of more repeats comparison to other contexts (Table 3). Penta-nucleotides consisted of CTCCT/GAGGA while GCTCCC/CGAGGG is only one prominent sequence contexts in hexa-nucleotide repeats. However, there were no tetra- and penta-nucleotides with repeat number greater than seven in this study (Table 3).

In another study, Zhang et al. (2012) reported that the top four motif repeats for SSR marker were (AG/CT)n [1268 (34.51%)], (CA/TG)n [281 (7.65%)], (AT/AT)n [215 (5.85%)], and (GAA/TTC)n [131 (3.57%)] using RNA-Seq methods in sesame.

Finally, in the present study we also aimed to design microsatellite primer pairs for sesame using the transcribed sequences characterized in this study. A total of 42 microsatellite primer pairs from EST sequences were designed using criteria described in this study. These microsatellite primer pairs were called SUS primer pairs stand for Sesame Unique Simple Sequence Repeats (Table 4).

SUS primer pairs consisted of microsatellites ranging from di- to hexa-nucleotide repeats. Repeat numbers of microsatellite motifs varied from 6 to 17 for di-nucleotide motifs, 5 to 6 for tri-nucleotide motifs, 4 for tetra-nucleotide motifs, 3 for hexa-nucleotide motifs while only one penta-nucleotide motif consisted of 3 repeats. Melting temperature values of microsatellite primer pairs ranged from 59.32°C to 61.70°C. We strongly suggested that annealing temperature values of primer pairs used in polymerase chain reaction should be 3-4 degrees Celsius below the melting temperature given in Table 4 for efficient marker development.

Up to date, although several hundred EST-SSRs have previously been developed from EST sequences and utilized in sesame genetic diversity studies (Wei et al., 2011; Zhang et al., 2012; Wei et al., 2014). However, it is important to notice that polymorphic markers are not enough to use in marker-assisted plant breeding methods for important qualitative and quantitative traits in sesame. In this study, randomly selected a few SUS primer pairs were tested and results indicated that SUS markers developed in this study would be very useful in sesame breeding studies.

Table 4. List of SUS primer pairs developed from transcribed part of sesame genome

ID	Accession #	Sequences (5'Þ3')	RT	T	GC	S
SUS01F	JZ971789.1	TCGTCGTCTCTCAGCTCTCTC	$[AG]_{17}$	60.03	57.14	244
SUS01R	JZ971789.1	CGTGTATTGCTTTCCCTACCTC		60.02	50.00	
SUS02F	JZ971779.1	AGACGGTTGGGTCCTCTCAT	$[GT]_{9}$	60.91	55.00	243
SUS02R	JZ971779.1	TTTATCCAGACAAGCCAGCAG		60.39	47.62	
SUS03F	JZ971778.1	CAAAGGTGTCAATCTTAGCAAGG	$[GCA]_{5}$	60.17	43.48	228
SUS03R	JZ971778.1	CCACCCTCCCAAAACTCTTT		60.33	50.00	
SUS04F	JZ971778.1	CAAAGGTGTCAATCTTAGCAAGG	$[ATA]_{5}$	60.17	43.48	228
SUS04R	JZ971778.1	CCACCCTCCCAAAACTCTTT		60.33	50.00	
SUS05F	JZ971764.1	TATCAGCTTGCCACTTCCTTC	$[AT]_{6}$	59.47	47.62	260
SUS05R	JZ971764.1	CAACAATAGCAGCAGCATCAA		60.03	42.86	
SUS06F	JZ971762.1	GAGATCAAGAACGGCGGACT	$[GAAT]_{4}$	61.70	55.00	249
SUS06R	JZ971762.1	CATTTACATCAGAGACACCCACA		59.91	43.48	
SUS07F	JZ971752.1	AGCTTCCACTAGCAACAGCAA	$[CAA]_{5}$	60.20	47.62	219
SUS07R	JZ971752.1	TCAGTAGCTTGACCCCTTCTG		59.48	52.38	
SUS08F	JZ971751.1	AAGCGGTCATGTTTCTGCTAA	$[TCT]_{5}$	59.90	42.86	275
SUS08R	JZ971751.1	GAAGGGGTATTGGAAAGCAAC		59.83	47.62	
SUS09F	JZ971716.1	CAACAATCCAAACACAGTAGAAGC	$[GCT]_{5}$	60.10	41.67	254
SUS09R	JZ971716.1	CCAAGGACGAGAAGAAGAAGAA		59.99	45.45	
SUS10F	JZ971716.1	GCAAGCAACCAACGGTAGAGT	$[CTT]_{5}$	61.59	52.38	204
SUS10R	JZ971716.1	GATCGAGAAGATCAAGGACAAGA		59.84	43.48	
SUS11F	JZ971716.1	CAACAATCCAAACACAGTAGAAGC	$[TCGCTG]_{3}$	60.10	41.67	254
SUS11R	JZ971716.1	CCAAGGACGAGAAGAAGAAGAA		59.99	45.45	
SUS12F	JZ971714.1	TTCCGGCACTGACTTTAACA	$[TCT]_{5}$	59.32	45.00	229
SUS12R	JZ971714.1	AGGCGAGAAGACTGATTGGAT		60.23	47.62	
SUS13F	JK755189.1	GACCGCACAAAGCATTACAAG	$[GACGAG]_{3}$	60.68	47.62	247
SUS13R	JK755189.1	CCATGTTAAGCCAATCTTCCA		59.95	42.86	
SUS14F	JK755186.1	CTCTTGACATGCCCGAACTAC	$[GACGAG]_{3}$	59.75	52.38	245
SUS14R	JK755186.1	GCGTGTGATGCACTCTTTCTT		60.46	47.62	
SUS15F	JK755185.1	CTTGAAAGCAAACTCGACCAG	$[CT]_{7}$	60.04	47.62	227
SUS15R	JK755185.1	GCAGCTCATCTTGCACTTGA		60.30	50.00	
SUS16F	JK755185.1	CTTGAAAGCAAACTCGACCAG	$[TC]_{9}$	60.04	47.62	227
SUS16R	JK755185.1	GCAGCTCATCTTGCACTTGA		60.30	50.00	
SUS17F	JK755181.1	GCATCTATCTCTCCCGGTTCT	$[TC]_{8}$	59.69	52.38	272
SUS17R	JK755181.1	TCCTTCGATTGGCTTACAAGA		59.83	42.86	
SUS18F	JK755181.1	TAGATGGCTCGATTACCCTCA	$[CTTG]_{4}$	59.68	47.62	269
SUS18R	JK755181.1	CTCTCAAGTGGACGCAAAGAC		60.04	52.38	
SUS19F	JK755164.1	CTGCTGTTGCTGCTGTAAATG	$[AGA]_{5}$	59.69	47.62	236
SUS19R	JK755164.1	CCGCGACTTCTTTTTCTTCTT		60.01	42.86	
SUS20F	JK755222.1	GGGAGTGATTAGGGTTTGCTC	$[AG]_{6}$	59.95	52.38	233
SUS20R	JK755222.1	TTGAAGCGGAGGAGTTAAGGT		60.25	47.62	
SUS21F	JZ191039.1	AGATTTCTCGGCTGAAACAGG	$[TGGA]_{4}$	60.75	47.62	242
SUS21R	JZ191039.1	ATACATCGCTCGCATCAAAAC		60.12	42.86	
SUS22F	HO710201.1	GGGGCTGAGAATTTGAGAGAG	$[AG]_{6}$	60.33	52.38	279
SUS22R	HO710201.1	GGCCTCTTAGTTGACCAGACA		59.34	52.38	
SUS23F	HO710187.1	GCAAAAGGTGAGGGATGAACT	$[GAT]_{5}$	60.49	47.62	253
SUS23R	HO710187.1	CTGCTGGATGTCAGTTTCCTG		60.84	52.38	
SUS24F	HO710180.1	AATCCACAACCCTCTTCTTCG	$[CT]_{11}$	60.48	47.62	247
SUS24R	HO710180.1	CTATCCTGCGGACCCATTATC		60.67	52.38	
SUS25F	HO710174.1	CGGTCCTGCAAGTGAAGATAA	$[GA]_{6}$	60.26	47.62	223
SUS25R	HO710174.1	TCATAAAGACACCACACCACACT		59.43	43.48	
SUS26F	HO710172.1	TCTGTTGTAGGCGGAAAGTGT	$[GA]_{7}$	59.79	47.62	274
SUS26R	HO710172.1	ACGAGCAGTTTGTTTGGTACG		60.22	47.62	
SUS27F	HO710172.1	TCTGTTGTAGGCGGAAAGTGT	$[GA]_{7}$	59.79	47.62	274
SUS27R	HO710172.1	ACGAGCAGTTTGTTTGGTACG		60.22	47.62	
SUS28F	HO710168.1	GGAGACATTCTTGGCATGGT	$[TGA]_{5}$	59.93	50.00	248
SUS28R	HO710168.1	CCCTTCAGACCTCAACTCCTC		60.24	57.14	
SUS29F	HO710168.1	GGAGACATTCTTGGCATGGT	$[GAT]_{5}$	59.93	50.00	248
SUS29R	HO710168.1	CCCTTCAGACCTCAACTCCTC		60.24	57.14	
SUS30F	HO710168.1	GGAGACATTCTTGGCATGGT	$[CTG]_{6}$	59.93	50.00	248
SUS30R	HO710168.1	CCCTTCAGACCTCAACTCCTC		60.24	57.14	
SUS31F	HO710163.1	GAGGTACGGAGAGCACAAGC	$[AGA]_{5}$	60.02	60.00	243
SUS31R	HO710163.1	GCAAGCAACCAACGGTAGAGT		61.59	52.38	
SUS32F	HO710163.1	GAGGTACGGAGAGCACAAGC	$[CAG]_{5}$	60.02	60.00	243
SUS32R	HO710163.1	GCAAGCAACCAACGGTAGAGT		61.59	52.38	
SUS33F	HO710163.1	GAGGTACGGAGAGCACAAGC	$[CAGCGA]_{3}$	60.02	60.00	243
SUS33R	HO710163.1	GCAAGCAACCAACGGTAGAGT		61.59	52.38	
SUS34F	HO710139.1	GCTTGTGCTATGCTGCCTTAT	$[CAC]_{5}$	59.54	47.62	245
SUS34R	HO710139.1	ACTTCTTTTAACCACCGACAGC		59.70	45.45	
SUS35F	HO710139.1	AAATGGGCATGACAGGAGAC	$[CCACGG]_{3}$	59.93	50.00	249
SUS35R	HO710139.1	GTGTAATTGTGAACGGGCCTA		59.87	47.62	
SUS36F	JK085550.1	AATAGTAAAACGGATCGCTTGG	$[AGAC]_{4}$	59.54	40.91	257
SUS36R	JK085550.1	ATCATGTATCCCCGCTGCTT		61.76	50.00	
SUS37F	JK085549.1	TCTTCTCCGTGTCCGAAAGT	$[CACT]_{4}$	59.84	50.00	253
SUS37R	JK085549.1	CATCTTCACATGCTTTAGCCTCA		61.64	43.48	
SUS38F	JK085527.1	CTGGGCGCTCTATCGTTTAG	$[AAG]_{5}$	60.00	55.00	240
SUS38R	JK085527.1	TGAACCTTGACGCTTTGATCT		59.87	42.86	
SUS39F	JK085514.1	GCCAGAAGTGTGAGTGATGC	$[GAAGC]_{3}$	59.43	55.00	230
SUS39R	JK085514.1	TGATTTACCGAGTTTCCGATG		59.95	42.86	
SUS40F	JK085504.1	GGGGACTCACTCACTCACTCA	$[CT]_{8}$	60.30	57.14	256
SUS40R	JK085504.1	AGCCATCTTTATTGGGAGGTG		60.33	47.62	
SUS41F	JK085497.1	TTCATCACCCTCACCTGTTTC	$[TTC]_{5}$	59.96	47.62	242
SUS41R	JK085497.1	GTATTTGATCCCACACGCATT		59.70	42.86	
SUS42F	JK085451.1	GGGCTGGATTTTTATTTGTTCC	$[CATC]_{4}$	60.85	40.91	265
SUS42R	JK085451.1	CTTAGAGGGAAAGGGGGAGTT		59.95	52.38	

ID: Primer Identification, SUS stands for Sesamum indicum L., RT: Repeat Type, T: Temperature of Melting (°C), GC: Guanine-Cytosine content (%) and S: Expected amplified product size (bp).

Conclusions

In the present study, results revealed that transcribed portion of sesame genome contained significant amount of tandem repeats. Frequency of tandem repeat motifs and the number of repeats in motif varied. Data mining analysis revealed that di-nucleotides were dominant repeat types used in all searching criteria in sesame transcribed genome. Among the di-nucleotides AG/TC repeat motifs were dominant in comparison to the other repeat motifs. However, especially in the tri-nucleotide repeats CTT motif contexts were frequently existed in the repeats numbers from nine to greater than ten. Microsatellite primer pairs developed in the present study could be used in sesame breeding programs. Main breeding goals of sesame are higher yield and better quality oil seed. Genetic relationships and diversity among sesame germplasm have been searched mostly using RAPD, ISSR, AFLP, etc. Marker-assisted selection and molecular breeding studies in sesame have lagged behind in comparison with other plants. Thus, a rapid and cost-effective approach to develop molecular markers for sesame is required. Polymorphic primer pairs (SUS markers) could be used as tools in marker-assisted selection and sesame germplasm identification.

References

Ali, G.M., Yasumoto, S. and Katsuta, M.S. (2007). Assessment of genetic diversity in sesame (*Sesamum indicum* L.) detected by amplified fragment length polymorphism. Electronic Journal of Biotechnology, 10, 12-23 [CrossRef]

Ali-Al-Somain, B.H., Migdadi, H.M., Al-Faifi, S.A., Muharram, A.A., Mohammed, N.A. and Refay, Y.A. (2017). Assessment of genetic diversity of sesame accessions collected from different ecological regions using sequence-related amplified polymorphism markers. 3 Biotech, 7, 82 [CrossRef]

Bilgen, M., Karaca, M., Onus, A.N. and Ince, A.G. (2004). A software program combining sequence motif searches with keywords for finding repeats containing DNA Sequences. Bioinformatics, 20, 3379-3386 [CrossRef]

Cheung, S.C., Szeto, Y.T. and Benzie, I.F. (2007). Antioxidant protection of edible oils. Plant Foods Human Nutrition, 62, 39-42 [CrossRef]

Cloutier, S., Niu, Z., Datla, R. and Duguid, S. (2009). Development and analysis of EST-SSRs for flax (*Linum usitatissimum* L.). Theoretical Applied Genetic, 119, 53-63 [CrossRef]

Ince, A.G., Karaca, M., Bilgen, M. and Onus, A.N. (2008). Digital differential display tools for mining microsatellite containing organism, organ and tissue. Plant Cell Tissue and Organ Culture, 94, 281-290 [CrossRef]

Ince, A.G., Onus, A.N. and Karaca, M. (2010a). Polymorphic microsatellite markers transferable across *Capsicum* species. Plant Molecular Biology Reporter, 28, 285-291 [CrossRef]

Ince, A.G., Karaca, M. and Onus, A.N. (2010b). CAPS-microsatellites: use of CAPS method to convert non-polymorphic microsatellites into useful markers. Molecular Breeding, 25, 491-499 [CrossRef]

Karaca, M, Bilgen, M, Onus, A.N., Ince, A.G. and Elmasulu, S.Y. (2005). Exact tandem repeats analyzer (ETRA): a new program for DNA sequence mining. Journal of Genetics, 84, 49-54 [CrossRef]

Karaca, M. and Ince, A.G. (2011). New non-redundant microsatellite and CAPS-Microsatellite markers for cotton (*Gossypium* L.). Turkish Journal of Field Crops, 16, 172-178 [URL]

Karaca, M., Ince, A.G., Uygur-Göçer, E. and Aydin, A. (2017a). Microsatellite distribution and densities in promoter and gene body entities of some plant genes. The Journal of Scientific and Engineering Research, 4, 154-163 [URL]

Karaca, M., Ince, A.G., Uygur-Göçer, E. and Aydin, A. (2017b). Descriptive statistics and PIC values of genomic and transcriptomic-microsatellites in several plant species. The Journal of Scientific and Engineering Research, 4, 236-246 [URL]

Kumar, V. and Sharma, S.N. (2011). Comparative potential of phenotypic, ISSR and SSR markers for characterization of sesame (*Sesamum indicum* L.) varieties from India. Journal of Crop Science and Biotechnology, 14, 163-171 [CrossRef]

Pham, T.D., Geleta, M., Bui, T. M., Bui, T.C., Merker, A. and Carlsson, A.S. (2011). Comparative analysis of genetic diversity of sesame (*Sesamum indicum* L.) from Vietnam and Cambodia using agro-morphological and molecular markers. Hereditas, 148, 28-35 [CrossRef]

Weber, J.L. (1990). Informativeness of human (dC-dA)n·(dG-dT)n polymorphisms. Genomics, 7, 524-530 [CrossRef]

Wei, W., Qi, X, Wang, L., Zhang, Y., Hua, W., Li, D., Lv H. and Zhang W. (2011). Characterization of the sesame (*Sesamum indicum* L.) global transcriptome using Illumina paired-end sequencing and development of EST-SSR markers. BMC Genomics, 12, 451 [CrossRef]

Wei, X., Wang, L., Zhang, Y., Qi, X., Wang, X., Ding, X. and Zhang, J. (2014). Development of simple sequence repeat (SSR) markers of sesame (*Sesamum indicum*) from a genome survey. Molecules, 19, 5150-5162. doi: 10.3390/molecules19045150

Yen, G.C. (1990). Influence of seed roasting process on the changes in composition and quality of sesame (*Sesamum indicum* L.) oil. Journal of the Science of Food and Agriculture, 50, 563-570 [CrossRef]

You, F.M., Huo, N., Gu, Y.Q., Luo, M., Ma, Y., Hane, D., Lazo, G.R., Dvorak, J. and Anderson, O.D. (2008). BatchPrimer3: a high throughput web application for PCR and sequencing primer design. BMC Bioinformatics, 9, 253 [CrossRef]

Yue, W., Wei L., Zhang T., Li C., Miao H. and Zhang H. (2013). Genetic diversity and population structure of germplasm resources in sesame (*Sesamum indicum* L.) by SSR markers. Acta Agronomica Sinica, 38, 2286-2296 [CrossRef]

Zhang, H., Wei, L., Miao, H., Zhang, T. and Wang C. (2012). Development and validation of genic-SSR markers in sesame by RNA-seq. BMC Genomics, 13, 316 [CrossRef]

International Journal of Agriculture, Environment and Food Sciences

JAEFS

e-ISSN : 2618-5946

DOI: 10.31015/jaefs.2019.4.8

www.jaefs.com

Research Article

Int J Agric Environ Food Sci 3(4):245-249 (2019)

Determination of the friction force and energy for Karacadağ local rice cultivars for different surfaces

Resat Esgici[1,*]

[1]Bismil Vocational High School, Dicle University, Diyarbakır, Turkey

[*]Corresponding Author: resgici@dicle.edu.tr

Abstract

The friction properties of grain products are one of the important parameters that are considered in the design of transport, transmission and storage structures and equipment. In addition, the friction force and coefficients on different surfaces are important in the selection of the power supply required for such design of structures and in the calculation of their actual dimensions. These are the common materials used for handling and processing of grains and construction of storage and drying bins. In this study, the static friction force and static friction energy were measured on 4 different material surfaces (rubber, chrome, galvanized sheet and PVC) and 3 different moisture contents (9.30%, 18.50% and 29.00%) for Karacadağ Beyaz and Karacadağ Karakılçık local rice varieties. The pulling of the grain box on the surface and the friction forces and energy were measured at a constant speed of 20 mms^{-1} with Llyod tester having a capacity of 2500 N. There was no significant difference between the varieties in terms of frictional force and friction energy ($p > 0.05$). However, the effect of surface material and grain moisture content was found to be very important ($p < 0.01$). As the moisture content of the grain increased, the static friction force and friction energy values increased. The highest values were obtained in PVC and rubber material with a moisture content of 29.00%, the lowest value was obtained in chromium material and 9.30% moisture content.

Keywords: Rice, Friction force, Friction energy, Friction surface

Introduction

The friction values of grain products are among the important physico-mechanical properties that should be known for the design of the machines used in the sowing, planting, harvesting and post-harvesting processing of agricultural products as well as for the selection of the working parameters. Moreover, it also plays an important role in determining the conveyance, transportation and storage properties of agricultural products. Friction preserves its importance for determining the vertical loads on the lateral surfaces of bins and similar storage structures and between the material and lateral surfaces in pneumatic conveying as well as for the pressing and cutting operations of agricultural products (Beyhan et al., 1999; Sabahoglu and Ozturk., 1996; Guzel et al.,1996; Alayunt, 2000; Colak and Sacilik., 2002; Sessiz., 2005). Knowledge of the friction characteristics of various grain products at different surface materials and moisture contents is important for selecting the machine that will be designed or produced or the selection of the power source for the facility and calculating its actual dimensions.

Rice cultivation can be carried out in many provinces and especially those in the Marmara and Black Sea regions in Turkey which has a significant importance with regard to rice production and consumption in the world. Hence, the level of rice harvest and post-harvest drying, storage and processing facilities continues to increase every year. Southeastern Anatolia is another region where rice is produced in Turkey. Şanlıurfa, Diyarbakır and Mardin are among the provinces in the region with high rice cultivation. The geography located in between these three provinces is known as the Karacadağ region. Rice cultivation in this region is carried out mostly in natural and rocky areas where machines cannot be used and the use of machinery is limited in contrast with other regions of Turkey.

Cite this article as:
Esgici, R. (2019). Determination of the friction force and energy for Karacadağ local rice cultivars for different surfaces. Int. J. Agric. Environ. Food Sci., 3(4), 245-249
DOI: https://dx.doi.org/10.31015/jaefs.2019.4.8
Received: 22 September 2019 **Accepted:** 05 December 2019 **Published:** 24 December 2019
Year: 2019 **Volume:** 3 **Issue:** 4 (December) **Pages:** 245-249
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

The use of chemical pesticides and fertilizers is limited. Thus, rice with a higher quality with regard to human health can be produced in this region. That is why; local varieties are mostly preferred and consumed in the region (Esgici et al., 2016).

Even though it is such an important product, significant quality and quantity losses take place during the harvesting, transportation, drying, storage and factory processing stages. Hence, the design of transportation, conveyance and storage structures for rice is important for reducing product losses and operational costs. One of the physical-mechanical properties of grainy products is the friction property that is displayed when the product is moved on the surface of materials used in transportation-conveyance and storage structures. That is why, the friction characteristics of the agricultural products should be taken into consideration when selecting or designing equipment for harvest, transportation, storage and drying. The material slides on the material surface during the loading and unloading processes when transporting and conveying agricultural products. Sliding depends on the product content and surface material.

Knowledge of friction characteristics for various grain products on different surface materials and moisture contents is important for the selection of the power source and the calculation of the actual dimensions (Sabahoglu and Ozturk., 1996; Guzel et al.,1996; Caliskan and Vursavus., 2009). In addition, friction force between the material and the lateral surface plays an important role in pneumatic conveying.

In this study, 4 different surface materials were used for the Karacadağ Beyaz and Karacadağ Karakılçık local rice varieties cultivated in the Diyarbakır province and its environs for determining the friction force and friction energy for three different moisture contents and the relationships between them were examined.

Materials and Methods

Plant characteristics and measurement devices

Karacadağ Beyaz and Karacadağ Karakılçık rice varieties were used in the present study as plant material (Figure 1). Both varieties were obtained in 2018 from the production areas of an active producer in the city of Diyarbakır. The rice samples acquired from harvester storage during harvesting were transferred over to the Agricultural Machines and Technologies laboratory where experiments were conducted. Grain moisture contents were determined in accordance with the ASABAE (2008) standards with oven drying method at 103◦C for 24 hours (Figure 1). The trials were carried out for three different moisture values. The moisture contents of the grains obtained from the harvester storage during harvesting were taken into consideration during the first trials (29.00 %). The grains with high moisture content acquired during harvesting were left to wait for 5 days in the laboratory to decrease the grain moisture for the second moisture experiments. During this time frame, the moisture content decreased down to about 19.00 % and experiments were carried out at this moisture content. Rice grains were left to natural drying in the laboratory for 10 days for the third moisture experiments after which the experiments were carried out when the moisture content decreased by about 9.30 %.

Figure 1. Rice varieties used (Karacadağ Beyaz on the left, Karacadağ Karakılçık on the right).

Friction Characteristics

Static friction force has a greater value in comparison with the dynamic friction force. Since the higher value of static friction force or coefficient is taken into consideration for power selection and for the design of other agricultural equipment and structures, static friction force and static friction energy were measured during the present study.

The measurements were carried out on 4 different surface materials (galvanized sheet, PVC, Chrome and Rubber) and at three different moisture content values 9.30 %, 18.50 % and 29.00 %). A Lloyd plus brand test device with a measurement capacity of 2500 N and a special test setup shown in Figure 2 were used for pulling of the rice grains on the material and the measurement of the friction force and friction energy. This setup is comprised of three units. These are; product cup, friction surface and data measurement setup. The product box has dimensions of 250 x 250 x 90 mm3 with the lower part of the box containing the rice seeds left open and a setup with rails and wheels was placed underneath the box in order to avoid contact between the sides of the box and the surface. The weight of the box used in the experiment was 1.9 kg (19 N) and 1.00 kg (10 N) of rice grains were added inside for the trials. Therefore, the normal force was taken as 2.9 kg (29 N) for all trials.

The friction data of the tests were recorded automatically by the device as force-time graphs. The pulling operation was carried out with a constant speed of 20 mms^{-1} at a distance of 50 cm. The highest force value measured by the device when the box first started its movement was taken into consideration as static friction force. The average of 50 measurements recorded by the device was calculated as one repetition (Sessiz et al., 2018; Esgici et al., 2018). Figure 3 presents a typical force-time change graph.

Statistical analysis

SPSS 14 statistical package software was used for statistical analyses and comparisons. GLM model was applied for multiple variance analysis. Differences between the averages were determined according to TUKEY test results.

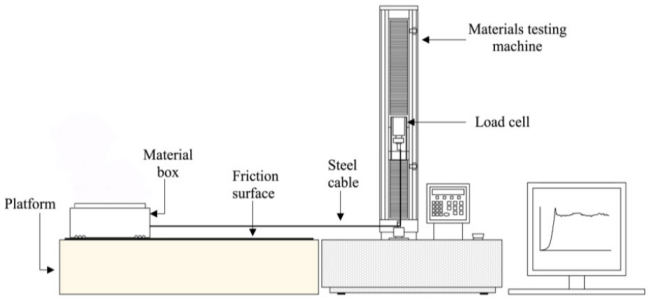


Figure 2. The setup and material test device used for determining the friction characteristics.

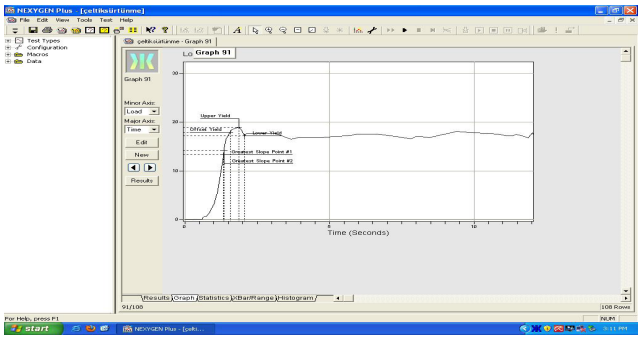


Figure 3. Typical force-time change graph.

Results and Discussion

The average values obtained for the friction force for both varieties are presented in Table 1. The difference between the varieties with regard to static friction force was observed to be statistically insignificant as a result of the variance analyses carried out. However, these values were relatively higher numerically for the Karacadağ Karakılçık variety (Table 1). The average values of both varieties are presented in Figure 3. As can be seen in Table 1 and Figure 4, the average static friction force values for both varieties increased on all surface materials with the increase of the grain moisture content. The difference between the moisture content was observed to be statistically significant. According to Guzel et al. (1996), friction force depends on the type and roughness type of the surfaces in friction. Therefore, friction force and energy increased with increasing moisture since the rice grain surfaces are rough and awned resulting in sliding on the material rather than rolling. According to Ozturk and Sabahoglu (1994) friction coefficients increase with increasing moisture content. Because the increase of friction force also corresponds to the increase in friction coefficients since the normal force remains constant. The highest values for both varieties were observed respectively in materials of PVC, rubber with smooth surface, galvanized sheet and chromium). While the lowest static force was obtained in chrome material with 11.77 N, the highest value was obtained as 30.30 N for 29.00 % grain moisture content in the Karacadağ Karakılçık variety and PVC material. PVC has similar surface characteristics with rubber material but is rougher. Hence, it has displayed a greater resistance in the opposite direction against normal movement. This was due to the adherence characteristics between the two surfaces as the grains slide on the surface as well as the awned structure of the grains. This was put forth by Ozturk and Sabahoglu (1994) as an increase in the friction resistance due to the fluid of the material transferring onto the surface material as agricultural products pass from the same surface repeatedly. Moreover, temperature increases at the interface as the product passes from the surface. Friction force increases since the increase in the contact time between the surfaces has an impact on adhesion and surface wear.

Data for the friction energy are presented in Table 1 and Figure 5. The difference between the varieties with regard to friction energy was observed to be statistically insignificant. However, as was the case for static friction force, friction energy on all surfaces increased with increasing moisture content in both varieties. The lowest friction energy was obtained as 2.061 Nm at a moisture content ratio of 9.30 % for the Beyaz variety on chrome surface material, whereas the highest value was obtained as 7.053 Nm at a moisture content ratio of 29.00 % for the Karakılçık variety on PVC material. While the PVC material and rubber with smooth surface displayed similar characteristics, chrome and galvanized sheet materials also displayed similar characteristics (Table 1). Since the difference between the two varieties was observed to be statistically insignificant, the average values for the two varieties are presented in Figure 5. As can be seen from the graph, increase in grain moisture content increased the friction energy (Nm) for all surfaces. This increase was observed to be statistically significant for all surfaces. The highest values were obtained for rubber and PVC materials. The lowest values were determined for chrome and galvanized sheet material with smoother friction surfaces. As can be observed, the structure of the friction surface, moisture content and the awn characteristics of the grain had an impact on friction energy. A similar result was also expressed by Ozturk and Sabahoglu (1994), Colak and Sacilik (2002), Guzel et al. (1996).

Table 1. Change in static friction force and friction energy subject to moisture content and variety.

	Karacadağ Beyaz			Karakılçık		
	Moisture content, %			Moisture content, %		
	9.30	19.00	28.60	9.30	19.00	28.60
Static friction force, N						
Rubber	15.35	18.86	24.48	13.95	15.32	24.72
Chrome	11.77	15.77	20.49	11.86	14.69	21.68
Galvanized sheet	12.65	16.77	23.30	12.83	17.52	24.68
PVC	18.40	22.54	29.20	17.60	21.48	30.30
Static friction force, N						
Rubber	3.094	4.552	5.799	3.303	4.696	5.926
Chrome	2.061	3.429	3.647	2.735	3.337	3.767
Galvanized sheet	2.888	3.297	3.866	2.541	4.413	5.722
PVC	4.454	5.564	6.448	4.478	5.275	7.053

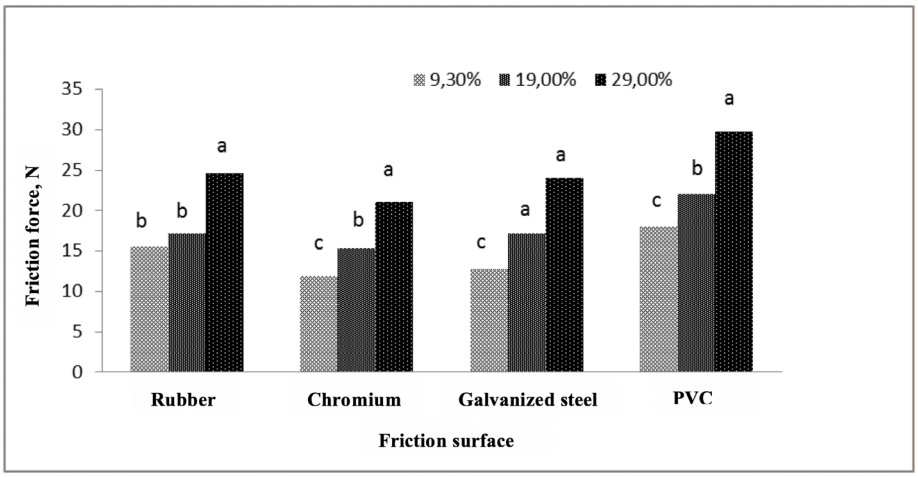


Figure 4. Change in friction force subject to material and moisture content

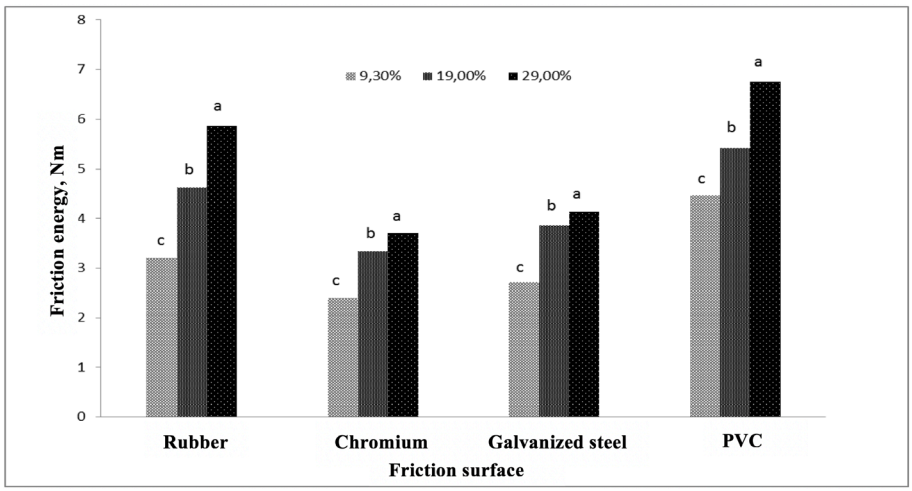


Figure 5. Change in friction energy subject to material and moisture content

Conclusion

The difference with regard to static friction force and friction energy between the varieties was determined to be statistically insignificant as a result of the variance analyses carried out. However, these values were relatively higher for the Karacadağ Karakılçık variety since the awns were larger. The average static friction force and static friction energy values increased with increasing moisture content for both varieties. The highest values for both varieties were observed on PVC, rubber with rough surface, galvanized sheet and chrome materials respectively. The lowest static force was obtained as 11.77 N on the chrome material, whereas the highest value of 30.30 N was obtained at 29.00 % grain moisture content, Karacadağ Karakılçık variety and on PVC material. As was the case for the static friction force, friction energy increased with increasing moisture content for both varieties. The lowest friction en-

ergy was obtained as 2.061 Nm at a moisture content of 9.30 % for the Beyaz variety on chrome surface material, whereas the highest value was obtained as 7.053 Nm at a moisture content of 29.00 % for the Karakılçık variety on PVC material.

References

Alayunt, F. (2000). Biological Materials. Ege University, Agriculture Faculty, Textbook, No: 541. İzmir-Turkey.

Beyhan, M. A., Nalbant, M., Tekguler, A. (1994). Determination of coefficient of friction in the grain and husk hazelnuts for different surfaces. Proc. XV. National Congree on Agricultural Mechanization' September 20-22, Antalya, Turkey, 343-352.

Caliskan, N., Vursavus, K. (2009). Determining the Physical and Frictional Properties of Washington Navel Orange Fruit Related to Post-Harvest Technology. Journal of Agricultural Machinery Science 5(1),83-92.

Colak, A. and Sacilik, K. (2002). Determination of the Coefficients of Friction of Olive (Olea europaea L.cv. Memecik). Journal of Agricultural Sciences, 8(2), 11-118.

Esgici, R., A. Sessiz, Y. Bayhan. (2016). The Relationship Between the Age of Combine Harvester and Grain Losses for Paddy. International Scientific Journal. Mechanization in Agriculture. S.18-21, ISSN 0861-9638, Sofia-Bulgaria.

Esgici, R., Pekitkan, F.G., Guzel, E., Sessiz, A. (2018). Friction Coefficients For Gundelia Tournefortii Seed on Various Surfaces. XIX. World Congress of CIGR(Commission Internationale du Génie Rural) "Sustainable Life for Children" 22-25 April 2018, Antalya, Turkey.

Guzel, E., Ulger, P., Kayisoglu, B. (1996). Product Processing and Evaluation Technique. Cukurova University, Agriculture Faculty, Textbook, No: 47 Adana-Turkey.

Mohsenin N. N. (1986). Physical Properties of Plant and Animal Materials. Gordon and Breach Science Publishers. New York [CrossRef]

Ozturk, R., Sabahoglu, Y. (1994). Evaluation of methods for determination of friction coefficients of biological materials. 15. National Congree on Agricultural Mechanization, S.353-362. Antalya-Turkey.

Sabahoglu, Y., Ozturk, R. (1996). Determination of Friction Coefficients of Some Wheat Cultivars. 6th. International Congress on Agricultural Mechanization and Energy, Ankara-Turkey.

Sessiz, A. (2005). Physical properties of some green olive cultivars. Proceedings of the International Congress on Mechanization and Energy in Agriculture &27th International Conference of CIGR Section IV: The Efficient Use of Electricity and Renewable Energy Sources in Agriculture, Sep.27-29,2005, İzmir-TURKEY.

Sessiz, A., E. Guzel., Bayhan, Y. (2018). Determination of Cutting Force and Energy in Grape Cane in Some Domestic and Foreign Grape Varieties. Turkish Journal of Agricultural and Natural Sciences. 5(4): 414–423 [CrossRef]

Sessiz, A., Pekitkan, F.G., Esgici, R. (2019). Comparison of Physical and Friction Properties of Local Paddy Varieties in Karacadağ Region. 32. National Congree on Agricultural Mechanization, S.353-362 Canakkale-Turkey

Sessiz, A., Pekitkan, F.G., Esgici, R., Guzel, E. (2018). Determination of Friction Characteristics of Kenger (Gundelia tournefortii) Seeds by Using Regression Technique for Various Surfaces. Journal of Agricultural Machinery Science, 14 (3), 143-148.

International Journal of
Agriculture, Environment and Food Sciences

JAEFS

e-ISSN : 2618-5946

DOI: 10.31015/jaefs.2019.4.9

www.jaefs.com

Research Article

Int J Agric Environ Food Sci 3(4):250-256 (2019)

Removal of Pb (II) from aqueous solution with Reactive Red 198 and carbonization of sugar beet pulp with citric acid

Bahdisen Gezer[1,*]

[1]Usak University, Faculty of Engineering, Department of Electrical and Electronics, Usak, Turkey

[*]Corresponding Author: bahdisen.gezer@usak.edu.tr

Abstract

In this study, a high carbon activated carbon was obtained from the sugar beet pulp by carbonizing with concentrated citric acid. The main purpose of this study is to evaluate the obtained catalyst for heavy metal removal in waste water. The adsorption studies of a basic dye Red 198 on the sugar beet pulp (SBP) were investigated. Balance and kinetic studies, carbon adsorbents obtained from sugar beet pulp were used for balance and kinetic studies. With carbonization of sugar beet pulp with citric acid was studied to remove Pb (II) from Reactive Red 198 dye aqueous solutions. Adsorption in researchswere performed to investigate the effects of solution concentration (20-30-40 mg/L), temperature (25,35,45°C) and pH (3-6-9) on the separation of Reactive Red 198 aqueous solution from beet pulp. Carbon balance adsorbent obtained from sugar beet pulp was done in equilibrium and kinetic studies. Langmuir and Freundlich isotherm models work were done to be appropriate for Reactive Red 198 adsorption. The maximal lead adsorption capacity obtained from Langmuir isotherm at pH 8 and 25°C 837.53 mg/g. Adsorption of Pb (II) is an endothermic process. The results showed that Reactive Red 198 from the beet pulp is an inexpensive and efficient adsorbent for the removal of aqueous solutions.

Keywords: Sugar beet pulp (SBP), Carbonization, Pb (II) adsorption, Citric acid, Reactive Red 198

Introduction

In the plastic, textile, dyeing and finishing processes, dyeing, coloring matter, leather, cosmetics, pharmaceuticals, food and paper manufacturing industry consume very big amounts of multicolored waste water at distinct stages (Yao et al., 2010).The use of paints in these industries constitutes an important class of pollutants (Hussien et al., 2016). The basic use of dyes is to change the color properties of different substrates such as paper, fabric, leather, and others. It has been determined that dyes affect photosynthetic activity to a great extent (Inastopulos eet al., 2014; Ferreira et al., 2014). In industry like this, it is seen that multicolored wastewater is discharged to water bodies such as streams and rivers and causes serious environmental effects. It also inhibits color light penetration, delays photosynthetic activity, and inhibits biota growth.Heavy metals in living life are considered to be one of the most toxic groups. In the food chain, it is possible to access wastes to water receptors or landfills. Heavy metals have been found to cause toxic effects, cancer and muscle-nerve diseases as they are very difficult to remove from the body (Petrović et al.,2014;Milojković et al., 2014).

SBP, a byproduct of sugar beet behind sugar extraction, produces approximate 14 million tons on dried matter basis every year in the European Economic Community, whichever consisting firstly of hemicellulose, pectin and cellulose (Gerente et al., 2000).This product is generally supplied to farmers for use as animal feed, but it is also used in various methods to increase the value of paper production (Vaccari et al., 1994; Vaccari et al., 1997), such as detergents (Petiti et al., 1993), fibers (Bertin et al., 1988; Michel et al., 1988) and pectins (Arslan 1995; Levigne et al., 2002).Removal of diverse organic and inorganic pollutants in industrial wastewater is ansignificant practice in adsorption processes using appropriate adsorbents. Nowadays, attention has been paid to the manufacture of

Cite this article as:
Gezer, B. (2019). Removal of Pb (II) from aqueous solution with Reactive Red 198 and carbonization of sugar beet pulp with citric acid. Int. J. Agric. Environ. Food Sci., 3(4), 250-256
DOI: https://dx.doi.org/10.31015/jaefs.2019.4.9
Received: 07 October 2019 **Accepted:** 17 December 2019 **Published:** 24 December 2019
Year: 2019 **Volume:** 3 **Issue:** 4 (December) **Pages:** 250-256
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

lowcost sorbents from agricultural waste or products. Cellulosic products grown in agriculture are used in carbon production in the separation and purification stages of industrial processes. Sugar beet produces wastewater, emissions, odors, and solid waste and causes environmental pollution to a large extent. Plant material and sludge washed in large mills are broken down in freshwater bodies, absorbing available oxygen (Gopal et al., 2009; Clay., 2013). The transformation of SBP to activated carbon is an economically inexpensive alternative. Since the beet pulp contains carboxyl groups and high metal cations, pH, presence, and concentration of organic and inorganic ligands which absorb nature are important factors Violante et al., 2010; Moubarik et al., 2015). In addition, the cationic species of polysaccharides and their modified products are effective in adsorbing aqueous solutions. Therefore, SBP is used in heavy metal adsorption to prevent environmental pollution (Sharma et al., 1994; Altundogan, 2005).

Processed agricultural materials have been shown to increase absorption capacities for metal ions of poly acids (such as citric, tartaric, phosphoric) at slightly high temperatures. With these processes, some extra acidic groups may form an ester linkage in the material. (Wong et al., 2003; Lehrfeldi 1997).

Adsorption of Pb (II) from aqueous solution with SBP was investigated by Pehlivan et al (2008).It is stated that the adsorption process is relatively fast and a 70-75% removal of Pb metal is achieved in 70 minutes.It is stated that adsorption density is pH dependent, 43.5 mg / g at pH 5 for Pb (II) and removal occurs in the form of ion exchange, physical adsorptionand chemical adsorption.In differentwork conducted by the similar researcher, sugar beet pulp was used for adsorption of Zn (II) from aqueous solutions (Pehlivan et al., 2006).In the study, it is stated that the process reaches equilibrium in 60 minutes and maximum adsorption concentrations are determined as 35.6 mg / g at pH 6.0 for Zn (II).

In this work, sugar beet meal was treated with citric acid to obtain a substance with a high carbon content. The main purpose of this work is to evaporate solid products or auxiliary products obtained during carbonization of divalent lead in gas, liquid and aqueous solutions. This purpose, the impact of pH, contact time, solution concentration and solution temperature wereresearched. Experimental data were associated with properties of binding capacity, kinetic and thermodynamic were discussed. The hexavalent lead adsorption properties of the obtained carbonaceous material were determined.

Materials and Methods

Materials

SBP wassupplied from Uşak sugar endustry. The SBP was dehumidified by blowing air for 20 hours. It was dried in an at 55 °C for 22 hours. The dough was milled in a mixer. Thesemilled material was siftedin sieve shaker and The samples to be used in the experiment are divided into micro grain sizes. 120 mesh (125μm) samples were used in each step of the experiment.

The chemical structure of Reactive Red 198 (MW=984.2 g/mol) dyestuff is given in Figure 1 and was chosen as the adsorbate. Firstly, a wavelength scan was performed for this dyestuff and the highest absorbance value was found to be 519 nm. In the following studies, measurements were made by keeping this value constant.

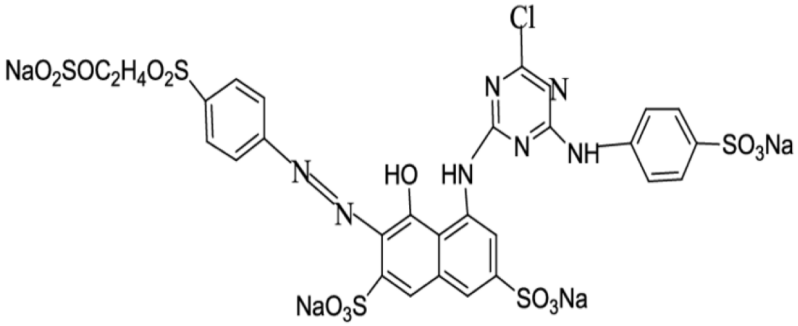


Figure 1. Chemical structure of Reagent Red 198 (Tunali et al., 2009)

Preparation of sugar beet pulp

The SBP was esterified with citric acid solution. For this purpose, 30 g beet pulp samples were mixed with 0.7 M citric acid ($C_6H_8O_7.H_2O$; Merck 100242; 99%) solution in a glass bowl at a rate of 8 times their weight. This mixture was mixed with the aid of a glass baguette to allow the citric acid solution to penetrate well into the beet pulp. After the solid had absorbed the solution, the mixture was left in the oven at 55 °C for 28 hours. The weight of the obtained material was determined. It was then heated in an oven at 110 °C. For 100 minutes to remove the water formed from the esterification with the internal water. The weight of the material was determined again. The resulting material was rinsed with distilled water at a rate of 45 times its weight each time for 1.5 hours until no citric acid was left in the wash water, and at the end of this time the filtrates were removed. To determine the presence of citric acid in the wash water, 15 mL of 0.25 M Pb $(NO_3)_2$ solution was added to 15 mL of wash water and precipitation (lead citrate precipitate) was observed. In the washing step where no precipitation was observed, it was concluded that citric acid did not pass into the washing water anymore and the washing process was terminated. This washed product was spread by thin film to remove coarse moisture by means of a fan and then dried to constant weighing (~ 24 hours) in an oven at 110°C. Once the final weight was determined, it was stored in a closed container for use in the experiments.

Adsorption study

Adsorption studies were performed at 25 °C by adding a constantquantity of adsorbent (2.0 g) into an amount of 250 ml of glass containing 150 ml of solution at distinct starting concentrations (20, 30 and 40 mg / L). The pH (3, 6 and 9) of this dye solution was determined using 0.12 M NaOH and 0.12 M HCl solutions and measurements were made using a pH meter. The test tubes were placed on a rotary shaker (150 rpm). An equilibrium was maintained for 200 minutes. These samples were puted in a centrifuge tube and centrifuged at 4000 rpm for 8 minutes. The concentration of Reactive Red 198 in the supernatant solution was investigated by measurementingthe optical density at 519 nmutilization a spectrophotometer.

Thequantity of lead adsorbed was detected according to the measured lead concentration before and after equilibration.The amount of adsorption at equilibrium q_e (mg/g) was providedby the following equation:

$$q_e = \frac{V}{m}(C_i - C_e) \qquad (1)$$

where C_i and C_e are the initial and equilibrium liquid phase concentrations (mg/L), V the volume of the solution (L) and m is the weight of the sugar beet pulp used (g).

Isotherm analysis

Adsorption isotherm explain the coactionamong the solution and isusage to determine the adsorbent and highest adsorption. The dried sugar beet meal reveals the binding capacity to Reactive Red 198. Results obtained were investigatedby applying the model Freundlich (Freundlich, 1906) ve Langmuir (Langmuir, 1916).Freundlich isotherm is the most widely used model for defining atadsorption method of heavy metal ions in aqueous solution. Freundlich isotherm occurs by the accumulation of metal ions with heterogeneous surface through multi-layer adsorption. Thequantity of adsorbed increased as a result of increasing concentration. Equilibrium data were investigatedwith the Freundlich equation:

$$\ln q_e = \ln K_f + \frac{1}{n}\ln C_e \qquad (2)$$

C_e (mg/L) and q_e (mg/g) equilibrium concentrations and the quantity of dye adsorbed at equilibrium, $K_f$adsorption capacity and n adsorption intensity are indicative of Freundlich constants (Kong et al., 2014). K_f and 1/n can be calculated from lnq_e and lnC_e graph.

The best of all sorption isotherms applicable to sorption processes is the Langmuir model. In this model, the amount of absorption on the surface of each sorbate molecule has equal absorption activation energy and can be calculation to define sorption of an ionic species of Pb (II) from an solution (Ed. 2).

$$\frac{C_e}{q_e} = \frac{1}{bq_{max}} + \frac{C_e}{q_{max}} \qquad (3)$$

where C_e is equilibrium concentration of Pb (II) (mg/ L), q_e the amount of Pb (II) adsorbed per unit mass of sorbent (mg/g) and q_{max} is the highest sorption capacity (mg/g). b (L/mg) is a constant connected to the concern of binding sites or bonding energy. If the value gives a straight slope with respect to CeIt intersects $1/q_{max}$ and $1/bq_{max}$ and shows sorption in the Langmuir model.

Results and Discussion

Some properties of SBP treated with citric acid are summarized in Table 1. In the experimental stage with citric acid, it is seen that the capacity to bind some extra carboxyl groups of cellulose maked strong in independent first alcohol groups is increased. Depending on the solubility of some organic materials for the extracted example, a measure of organic pollution may show a stabilization of a lower chemical oxygen demand value. The results show that abasis processing followed by a citric acid alteration stabilizes the sugar beet pulp because of crosslinking and increases the cation uptake resulting from carboxylic groups introduced into the molecules.

Table 1.Contents of SBP and citric acid-treated SBP

Parameter	Sugar beet pulp	Citric acid-treated sugar beet pulp
Water retention capacity (g/g)	8.21	5.07
Swelling capacity (ml/g)	7.24	4.13
Moisture content (%)	6.52	6.11
Ash content (%)	4.58	5.79
Bulk density (g/ mL)	0.302	0.373
Conductivity[b] (µs/cm)	156	242
pH[1]	4	8
Chemical oxygen demand[1] (mg-O_2/L)	169.7	72
Cation exchange capacity[2] (mequiv./g)	0.91	4.27

[1]Measurements in the liquid obtained by equilibrating 1g of substance with 100 mL of water for 24 hours
[2]On dry basis (at 110 °C)

Effect of contact time at Pb(II) adsorption

Different experiments were kept for equilibration with initial Red 198 dye concentration. Figure 2 shows the adsorption kinetics of the dye holding capacity of Reactive red 198 at 25, 35 and 45°C over time. The amount of dye getting adsorbed on the surface of activated carbon increases with increasing contact time till a tableland is achieved. It represents the state of dynamic equilibrium in which the amount of dye adsorbed onto the adsorbent is in equilibrium with that of the present in solution. Adsorption studies were performed for 8 hours

and It is seen from Figure 2 that the adsorption capacity increases with increasing temperature and time. More dye was removed during the initial 100 minutes contact time. Finally afast adsorption for all studied temperatures, the equilibrium was established in 130 minutes. After an equilibrium time of 150 minutes, the graph now shows that Reactive Red 198 is very well adsorbed.

The attainment of equilibrium takes a bit longer time due to a complex mechanism involved in the adsoption of dyes on macro and micro pores of activated carbon. The mechanism entails the confrontation of dye molecules to the boundary layer before diffusing onto the surface of adsorbent and then finally entering into the porous structure of carbon.

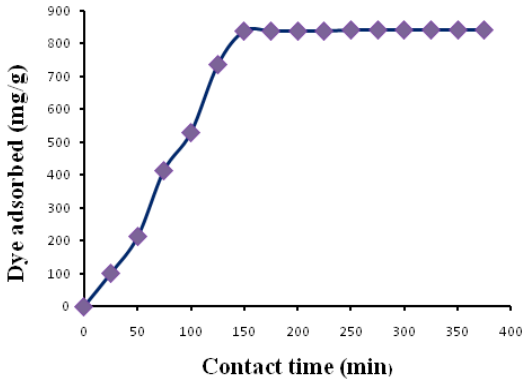


Figure 2. Influence of contact time on the removal of Pb(II) by using of citric acid-treated SBP (pH 8.0; adsorbent dosage: 5 g/l; initial Pb(II) concentration: 25–450 mg/L; temperature: 25 °C)

In most of the relevant studies published in the literature, it has been found that pH is an significant be effective component for dye adsorption. Experiments were implementedin the pH range from 1.0 to 10.0 to find a appropriate pH value for influence adsorption of the Reactive Red 198 dye by citric acid-treated sugar beet pulp. The increase in Pb(II) adsorption from 1 to 6 was due to increasing number of coordinate bonds between Pb(II) and nitrogen atoms of amine groups on SBP hydrogel. The decrease in adsorption from pH 8 to 10 was attributed to the gradual precipitation of Pb (II) ions to from $Pb(OH)_2$. Red 198 dye adsorption decreased with increasing pH which was due to the gradual deprotonation of NH^+_3 on SBP hydrogel to hydrophobic NH_2 thereby increasing electrostatic repulşion between the hydrogel and Red 198 dye molecules (Akiode et al., 2015).The change in equilibrium dye uptake with initial pH is shown in Figure 3. The highest equilibrium uptake value was obtained to be 837.53 mg/g at pH 8.0.

It was at the lowest level in pH 2 in dye adsorption, severely rised at pH 4 and reached its highest level at pH 8. Since weakest acidic groups are low in PH 2, the total surface charge on the SBPhappens less negative by being surrounded by hydronium ions. This reduces the interaction of the dye cations with the larger driving forces with the binding sites of the citric acid-treated SBP and decreases the attractiveness of positively charged cations (Pehlivan et al., 2008). This pH values greater than 4, the carboxyl groups of citric acid-treated sugar beet pulp are deprotonated and charged negatively. Therefore, it can be assumed that the maximum adsorption level is achieved that all carboxyl functions of citric acid-treated sugar beet pulp are deprotonated and possibly neutralized by electrostatic interaction between the negatively charged adsorbent and Reactive Red 198 molecules.

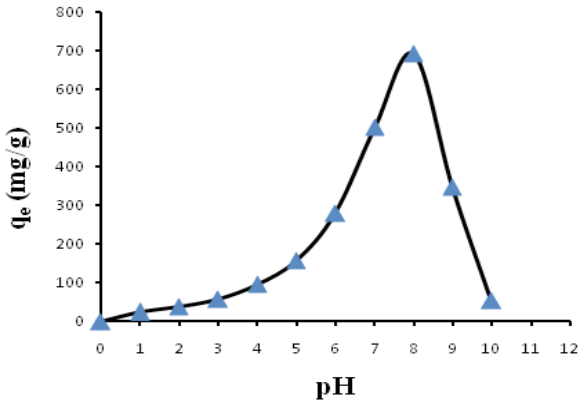


Figure 3. Influence of pH on the Reactive Red 198 adsorption (C_0 = 50 mg/L, T = 25°C)

Effect of temperature on adsorption

Temperature effects rate and degree of adsorption. In addition, For a contact period on the effect of temperature Pb (II) adsorption on citric acid-treated SBP, the range of 25-35-45°C was studied (Table 2).

As shown in Table 2, the equilibrium adsorption (q_e) rised with the rise of the initial Reactive Red 198 concentration. Temperature effectts the ratio and degree of adsorption. In addition, the temperature dependency of adsorption ensures thermodynamic and mechanicalknowledge regarding the sorption process. The influence of temperature on the effect of Pb (II) adsorption on citric acid-treated SBP was studied at 25-45 °C for a 6 hour contact period in solutions with initial concentrations ranging from 22 to 40 mg/L.

The highest yield is seen in Table 2, where the removal of the dye increases to 837.53 mg/g at 25 ° C. The increase in temperature, the increase in the number of active surface areas for adsorption on the surface of the adsorbent may be related to the increase in the pore volume of the adsorbent. It can be said that the increase in adsorption may be caused by the temperature drop in the thickness of the layer surrounding the sorbent. Therefore, the mass transfer in the adsorbate layer causes a decrease in resistance. At the same time, the increase in kinetic energy increases the activity of the dye molecule. The increase in dye concentration caused a decrease in the adsorption movement. At low concentrations, the dye had a low absorption rate and higher biosorption yields. At higher concentrations, the existing adsorption sites were reduced and saturation of the sorption sites appeared. Therefore, a decrease was observed in adsorption efficiency values (Table 2).

Adsorption equilibrium studies

Adsorption experiments were performed under agitation using activated carbon and aqueous Red 198 dye at 25, 35 and 45 °C. The adsorption experiments were implemented to work effects of diversity initial Red 198 concentration, SBP amount and temperature (25, 35 and 45 °C). The concentration of dye was monitored after fşxed interval of time (50-100 min) using UV-Visible spectrophotometer at absorption maxima of 519 nm. The unit adsorption capacity are shown in Table 2.

Table 2. Equilibrium uptake capacities and adsorption yields obtained at different concentrations and temperatures

C_0 (mg/L)	25°C		35°C		45°C	
	q_{eq} (mg/g)	Adsorption (%)	q_{eq} (mg/g)	Adsorption (%)	q_{eq} (mg/g)	Adsorption (%)
25	837.53	94.12	794.71	83.73	516.18	61.80
35	802.47	90.18	739.5	77.91	493.62	59.09
45	673.57	72.48	557.38	58.73	286.49	34.30

Isotherm study

Adsorption isoterm is the work of equilibrium relationship between adsorbate adsorbed on the surface of adsorbent and adsorbate remained in solution, if they are kept in contact with each other for longer periods of time. The data obtained in the adsorption experiment at distinct temperatures were investigated by placing Freundlich and Langmuir isotherm models (Langmuir, 1918; Freundlich, 1907). The Langmuir model is the most accepted on efor the application of activated carbon in waste water treatment. This model take on the monolayer formation on the surface of adsorbent which appling the more specific binding of adsrbate. All sites on the surface of adsorbent are equivalent and there occurs no interaction between adsorbate and adsorbent molecules. Freundlich isotherm is used in the adsorption of ions in heavy metal solutions since it assumes that the collection of metal ions occurs on a heterogeneous surface through multilayer adsorption and that the amount of adsorbate adsorbed rise infinitely. The best of all adsorption isotherms in adsorption processes is the Langmuir model. In this model, each sorbate molecule had the same absorption activation energy as the absorption capacity of the surface of the molecule. Langmuir adsorption isotherm can be used to define the absorption of an ionic species such as Pb (II) from aqueous solutions. Experimental datas investigated at 25 °C for pH 3-9 and Reactive Red 198 solutions, the concentration of 20-40 mg /L, equilibrium values of the liquid phase concentration and the values corresponding to the amount of adsorbed material were used to generate the adsorption isotherms of the concentration of the dye. Freundlich and Langmuir equations give the coefficients in Table 3. The isotherm models to the adsorption work is evaluated by the coefficient of determination (R^2) of each surface. It is seen from the data that Freundlich and Langmuir isotherm models provide a wellsuitable in all investigated pH values. The values of correlation coefficent were almost ideal (R^2>0.999) for pseudo second order kinetic model, which shows a good agreement in theoretical and experimental results as shown in Table 3. In addition to, a good correlation was observed between q_e values, calculated using pseudo second order model and the experimental observation.

Table 3. Isotherms constants for Reactive Red 198 adsorbed on citric acid-treated Sugar beet pulp (Eq. 1 and 2)

T (°C)	Langmuir model			Freundlich model		
	q_{max}	K_b	R^2	K_F	n	R^2
25	838.01	2.96	0.999	207.83	57.61	0.987
35	793.14	2.79	0.946	196.71	2.94	0.857
45	531.80	1.87	0.634	131.89	1.97	0.564

Conclusion

This article presents the improvements in the use of waste materials from the sugar industry as adsorbans and their performance in eliminating various water pollutants. The study found that surface modification with chemicals greatly improves the efficiency of favorable kinetic sequencing and adsorption yields and the extraction of adsorbans from sugar beet waste. Adsorbans' performance depends largely on pollutant types and experimental conditions. Most of the studies are aimed at determining maximum adsorption capacities using a single synthetic contaminant solution.

In this study, modified sugar beet pulp was investigated by treatment of lead absorption with citric acid. Characterization tests of the changed sugar beet pulp (SBP) showed improved cation exchange capacity, equilibrate hydration and dissolution properties. Adsorption studies were performed to investigate the effectiveness of modified sugar beet pulp in lead binding. The surface chemistry of carbons also change on varying the type of treatment. Langmuir and Freundlich isotherm models were analyzed according to temperature. The Langmuir model best correlates experimental data. Kinetic evaluations show that lead absorption follows the second order kinetic model. According to the results of the experiment, dye adsorption is exothermic depending on the temperature. The maximum adsorption capacity of the sugar beet pulp for Reactive Red 198 was 837.53 mg/g at 25 °C, pH 8. In general, this study showed that there may be a simple sorbent from SBP with high lead removal capacity.

The present work demonstrates a viable pproach for the preparation of tailor made carbon from the sugar beet pulp (SBP) and their potential in removing Red 198 from aqueous streams.

References

Altundogan, H.S. (2005). Cr(VI) removal from aqueous solution by iron (III) hydroxide-loaded sugar beet pulp. Process Biochem., 40, 1443–1452.

Akiode, O., Idowu, M., Omeike, S. and Akinwunmi, F. (2015). Adsorption and kinetics studies of Cu (II) ions removal from aqueous solution by untreated and treated sugarcane bagasse Global NEST J., 17, 583–593.

Anastopoulos, I. and Kyzas, G.Z. (2014). Agricultural peels for dye adsorption: a review of recent literature. J. Mol. Liq., 200, 381–389.

Arslan, N. (1995). Extraction of pectin from sugar-beet pulp and intrinsic viscosity molecular weight relationship of pectin solutions. Int. J. Food Sci. Technol., 32, 381–385.

Bertin, C., Rouau, X. and Thibault, J.F. (1988). Structure and properties of sugar beet fibres. J. Sci. Food Agric., 44, 15–29.

Clay, J. (2013). World Agriculture and the Environment: A Commodity-By-Commodity Guide to Impacts and Practices, Island Press.

Dronnet, V.M., Renard, C.M.G.C., Axelos, M.A.V. and Thibault, J.F. (1996). Characterisation and selectivity of divalent metal ions binding by citrus and sugar-beet pectins. Carbohyd. Polym., 30, 253–263.

Ferreira, A.M., Coutinho, J.A., Fernandes, A.M. and Freire, M.G. (2014). Complete removal of textile dyes from aqueous media using ionic- liquid-based aqueous two-phase systems. Sep. Purif. Technol., 128, 58–66.

Freundlich, H.M.F. (1906). Uber Die Adsorption in Losungen. Z Phys Chem (Leipzig), 7A:385.

Freundlich, H.N.F. (1907) Ueber die adsorption in Loesungen. Z. Phys. Chem., 57, 385–470.

Gerente, C., Couespel du Mesnil, P., Andres, Y., Thibault, J.F. and Le Cloirec, P. (2000). Removal of metal ions from aqueous solution on low cost natural polysaccharides sorption mechanism approach. React. Funct. Polym., 46, 135–144.

Gopal, A.R., Kammen, D.M. (2009). Molasses for ethanol: the economic and environmental impacts of a new pathway for the lifecycle greenhouse gas analysis of sugarcane ethanol. Environ. Res. Lett., 4, 044005.

Hussien, M., El-Bindary, A., El-Sonbati, A., Shoair, A. and El-Boz, R. (2016). Green removal of phenolic azo dye from aqueous solutions using rice straw fly ash. J. Mater. Environ. Sci., 7, 4214–4225.

Kong, W., Ren, J., Wang, S. and Chen, Q. (2014). Removal of heavy metals from aqueous solutions using acrylic-modified sugarcane bagasse-based adsorbents: equilibrium and kinetic studies. Bioresources, 9, 3184–3196.

Langmuir, I. (1916). The Constitution and Fundamental Properties of Solids and Liquids. J Am Chem. Soc.,38, 2221.

Langmuir, I. (1918) The adsorption of gases on plane surfaces of glass, mica and platinum. J. Am. Chem. Soc., 40, 1361–1403.

Lehrfeld, J. (1997). Cation exchange resins prepared from phytic acid. J. Appl. Polym. Sci., 66, 491–497.

Levigne, S., Ralet, M.C. and Thibault, J.F. (2002). Characterisation of pectins extracted from fresh sugar beet under different conditions using an experimental design. Carbohyd. Polym., 49, 145–153.

Michel, F., Thibault, J.F., Barry, J.L. and DeBaynast, R. (1988). Preparation and characterisation of dietary fibre from sugar beet pulp. J. Sci. Food Agric., 42, 77–85.

Milojković, J.V., Mihajlović, M.L., Stojanović, M.D., Lopičić, Z.R., Petrović, M.S., Šoštarić, T.D. and Ristić, M.D. (2014). Pb (II) removal from aqueous solution by Myriophyllum spicatum and its compost: equilibrium, kinetic and thermodynamic study. J. Chem. Technol. Biotechnol., 89, 662–670.

Moubarik, A. and Grimi, N. (2015). Valorization of olive stone and sugar cane bagasse by-products as biosorbents for the removal of cadmium from aqueous solution. Food Res. Int., 73, 169–175.

Pehlivan, E., Çetin, S. and Yanık, B.H. (2006). Equilibrium studies for the sorption of zinc and copper from aqueous solutions using sugar beet pulp and fly ash. Journal of Hazardous Materials B, 135, 193-199.

Pehlivan, E., Yanık, B.H., Ahmetli, G. and Pehlivan, M. (2008). Equilibrium Isotherm Studies for the Uptake of

Cadmium and Lead Ions onto Sugar Beet Pulp. Bioresour Technol,99, 3520.

Petit, S., Ralainirina, R., Favre, S. And De Baynast, R. (1993). World Patent, 02092.

Petrović, J.T., Stojanović, M.D., Milojković, J.V., Petrović, M.S., Šoštarić, T.D., Laušević, M.D. and Mihajlović, M.L. (2016). Alkali modified hydrochar of grape pomace as a perspective adsorbent of Pb 2+ from aqueous solution. J. Environ. Manag., 182, 292–300.

Sharma, D.C. and Forster, C.F. (1994). A preliminary examination into the adsorption of hexavalent chromium using low-cost adsorbents. Bioresource Technol., 47, 257–264.

Tunali, S., Akar, T. and Cabuk, A. (2009). Decolorization of a textile dye, Reactive Red 198 (RR198), by Aspergillus parasiticus fungal biosorbent. Brazilian Journal of Chemical Engineering, 26, 2, 399-405.

Vaccari, G., Nicolucci, C., Mantovani, G. and Monegato, A. (1994). Process for manufacturing paper from sugar-beet pulp and paper thus obtained. European Patent, 94114666.4.

Vaccari, G., Mantovani, G., Dosi, E., Nicolucci, C. and Monegato, A. (1997). Paper manufacture using beet pulp and factory waste lime. Int. Sugar J., 99, 532–536.

Violante, A., Cozzolino, V., Perelomov, L., Caporale, A. and Pigna, M. (2010). Mobility and bioavailability of heavy metals and metalloids in soil environments. J. Soil Sci. Plant Nutr., 10, 268–292.

Wong, K.K., Lee, C.K., Low, K.S. and Haron, M.J. (2003). Removal of Cu and Pb by tartaric acid modified rice husk from aqueous solutions. Chemosphere, 50, 23–28.

Yao, Y., Xu, F., Chen, M., Xu, Z. and Zhu, Z. (2010). Adsorption Behavior of Methylene Blue on Carbon Nanotubes. Bioresour Technol,101, 3040.

JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2019.4.10 www.jaefs.com

Research Article Int J Agric Environ Food Sci 3(4):257-264 (2019)

Effects of different propagation methods on the strawberry cv. 'Florida Fortuna' yield gown under low tunnel

Serhat Usanmaz[1,*]

[1]European University of Lefke, Faculty of Agricultural Sciences and Technologies, Gemikonağı, Northern Cyprus, via Mersin 10 Turkey

[*]Corresponding Author: susanmaz@eul.edu.tr

Abstract

This study was conducted to test the effects of three different propagation methods on the yield and fruit quality performance of strawberry cv. 'Florida Fortuna'. Experimental studies were conducted during the 2018-2019 growing period in Yedidalga village, located in Lefke region in Northern Cyprus. Tested methods of present study are: 1) bare root cold stored (frigo), 2) fresh/green bare root, and 3) strawberry plug propagation methods. Studies were conducted in a low-tunnel (4 m wide and 2 m height) with three replications according to the completely randomized block design. Each replication consisted of 20 plants. Regular measurements were carried to measure yield (g plant^{-1}), number of fruits (# plant^{-1}), total soluble solids (TSS) concentration, fruit firmness (kg cm^{-2}) and titratable acidity (g/100 g citric acid). Results showed that the propagation methods have high influence on the weekly and total yield of the strawberry plants. According to the obtained data, plug propagation method was found to provide earliness on fruit yielding and continuous yielding (except winter period). Moreover, frigo propagation method was found to be the latest for fruit bearing, but to have highest yield in the end of the growing period.

Keywords: Bare root cold stored (frigo), Fresh/green bare root, Strawberry plug, Total yield, Earliness

Introduction

Strawberry (*Fragaria x ananassa* Duch.) has a high adaptation ability to different ecological conditions (Hennion et al., 1997) and is highly favoured by consumers due to its unique flavour and health benefits, such as: antioxidant, anti-cancer and anti-inflammatory characteristics (Seeram et al., 2006). Due to the unique flavour and scientifically accepted health benefits, the production and consumption of the strawberry fruits has been increasing during the past decades. The non-climacteric fruits of strawberry do not ripen after harvest (Cordenunsi et al., 2005) and are very sensitive to storage conditions (Kahramanoğlu, 2019). Strawberry plants generates stolons (runners) as they grow and those stolons produce adventitious roots generally from the second node and leads the development of new plants. This is called as asexual propagation where "mother" plants clone itself and is called "daughter" plant. Therefore, propagation of the strawberry plants are generally and commercially carried out by asexually. However, during the commercial production, the stolons are being removed and do not let to produce daughter plants (Narváez-Ortiz et al., 2018).

The most important commercial way of strawberry propagation is the use of bare root cold stored (frigo) plants which are generally planted in summer and bear fruit in early spring (Lieten, 2002). Propagation from frigo plants requires long time, sensitive to diseases, and grows slowly (Pritts, 2001). On the other hand, strawberry production from plugs is known to be quick way of production where planting takes place in mid-autumn and harvesting starts in spring. The disadvantage of plugs is that, it is more expensive than frigo plants (Dolgun, 2006). The strawberry plugs had been used as an alternative to frigo propagation for a long time and reported to develop quickly and overcome some problems of bare root plants (Hennion et al., 1997; Dolgun, 2006). The use of strawberry plugs was also noted to extend the production period (Kadir et al.,

Cite this article as:
Usanmaz, S. (2019). Effects of different propagation methods on the strawberry cv. 'Florida Fortuna' yield gown under low tunnel. Int. J. Agric. Environ. Food Sci., 3(4), 257-264
DOI: https://dx.doi.org/10.31015/jaefs.2019.4.10
Received: 25 September 2019 **Accepted:** 17 December 2019 **Published:** 24 December 2019
Year: 2019 **Volume:** 3 **Issue:** 4 (December) **Pages:** 257-264
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

2006; Durner et al., 2002). Manipulating plugs with photoperiod also reported to enhance fruit bearing (Fernandez and Ballington, 2003; Takeda and Newell 2006). However, the field performance of these propagation methods would vary according to the varieties and climatic conditions (Pritts, 2001; Dolgun, 2006). Moreover, this is time consuming which requires intensive capital and labour and the results might be positive (Fernandez and Ballington, 2003) or even negative (Takeda and Hokanson, 2002) for improving fruiting. Previous studies also reported that the propagation method of the strawberry plants plays an important role on the nursery performance and yield of the plants (Paszko et al., 2014; Capocasa et al., 2019). In line with this information, present study aimed to study the yield performance of bare root cold stored (frigo), fresh/green bare root, and strawberry plug propagation methods on the strawberry cv. 'Florida Fortuna'.

Materials and Methods

Materials

Present study was conducted in the years of 2018-2019 growing period in Yedidalga village, located in Lefke region in Northern Cyprus. The area is specialized with the Mediterranean climate with hot and dry summers and mild and light rainy winters. Experimental studies were carried with 'Florida Fortuna' cultivar. The 'Florida Fortuna' cultivar is an early season cultivar with medium to large berry size, fair flavour and firm fruits. The smooth appearance fruits of 'Florida Fortuna' are bright to dark red in colour (Anonymous, 2019). The yield of cv. 'Florida Fortuna' was tested when plants are propagated with 1) bare root cold stored (frigo), 2) fresh/green bare root (bare root), and 3) strawberry plug propagation methods. The frigo and fresh/green bare root plant materials of present study were purchased from Lassen Canyon Nursery (USA) and transferred to Northern Cyprus with Air Cargo in one day. The frigo materials were kept at -2 C and the bare root plants at 22 C.

The strawberry plug materials of present study are propagated from actively growing terminal runner tips of stolons locally. First of all, high evolution mother plants of strawberries were imported from Lassen Canyon Nursery in mid of April 2018. These plants were planted onto the Trodos mountains (elevation 1.100 m). Climatic data for this site is given in Table 1. One month later, the un-rooted stolons (runners) of those mother plants with 2 to 4 leaves were planed into plug tray (45 units of round-conic cells). The cells were in 5 cm wide and 6 cm deep. Peat moss was used as a growing media for the plugs. The mother plants continued to receive normal irrigation and fertilization while the daughter plants in plugs were regularly misted 3 times in a day until September.

Table 1. Climatic data for the strawberry plug production area (Trodos mountain)

Parameter	Months					
	Apr	May	Jun	Jul	Aug	Sep
Mean Monthly Max. Temp.	24.8 °C	27.9 °C	30.8 °C	32.4 °C	32.4 °C	30.5 °C
Mean Monthly Min. Temp.	0.4 °C	4.4 °C	9.0 °C	13.2 °C	13.8 °C	10.0 °C
Mean Daily Sunshine Duration	7.5 h	8.8 h	10.4 h	10.5 h	10.0 h	8.4 h
Mean RH at 08:00 hrs LST	52 %	46 %	40 %	33 %	35 %	42 %
Mean RH at 13:00 hrs LST	53 %	48 %	42 %	36 %	41 %	43 %

Experimental Studies

Experimental studies were conducted with three different propagation methods of two different cultivars, as described above. Studies were conducted in a low-tunnel (4 m wide and 2 m height) with three replications according to the completely randomized block design. Each replication consisted of 20 plants. The frigo materials of cv. 'Florida Fortuna' of present study were transplanted on 15th of August, 2018 and the fresh/green bare root and strawberry plugs were transplanted on 15th of September, 2018. The frigo and bare root plants (after purchasing); and the strawberry plug plants (after producing at Trodos mountain) were directly planted into low-tunnels without any adaptation periods. Plants were set at 33 cm spacing in rows and 33 cm between rows with two rows per bed by using cross planting. Production beds were in 50 cm wide and 100 cm distance were given between the two production beds. Black polyethylene mulch was used for covering the beds and mulching was performed 3 days before planting. Weed management was done regularly by hand. Irrigation and fertilization were carried according to the below given program (Table 2.).

Data Collection and Analysis

Besides to the plant yield, the percentage of rooting was also measured in the present study. In the beginning, 20 plants were planted for each replication. Thus, regular observations were done to determine the number of alive plants. The number of alive (rooted) plants were used to determine the rooting percentages of each cultivar with different propagation method. Hereafter, 10 plants were selected from each replication to continue yield and quality measurements. Total yield and number of harvested fruits were regularly measured and noted throughout the growing period. Harvesting was performed regularly by hand at commercial maturity. Number of fruits harvested from each plant and their total weight was measured and noted. Apart from the yield, one fruit was selected from each replication at every harvest; and total soluble solids (TSS) concentration, fruit firmness (kg cm^{-2}) and titratable acidity (g/100 g citric acid) were measured.

Fruit weight was measured with a digital scale sensate to ±0.01 g. The fruit firmness (kg cm^{-2}) was determined by a hand penetrometer (cylindrical probe: 2 mm in diameter) by measuring the firmness from three distinct locations (around

the equatorial region) of a selected fruit from each replication. Hand refractometer was used to determine the total soluble solids (TSS) concentration of the fruits as % Brix. Titratable acidity (TA) of fruits was determined according to the procedure and formula reported by AOAC (1990) by titrating the juice samples with 0.1 N NaOH to an endpoint of pH 8.1.

Table 2. Irrigation and fertilization program of the present study

Months	Irrigation		Fertilization (Amount plant^{-1})	Pesticide Application
	Interval	Amount (L day^{-1} plant^{-1})		
August	2	0.50	15th of Aug: 0.9 g MAP (12-61-0)	N/A
			29th of Aug: 0.9 g MAP (12-61-0) + 0.9 g 20-20-20+ME	N/A
September	3	0.75	15th of Sep: 0.9 g MAP (12-61-0)	100 ml 100 L^{-1} Kresoxim-Methyl
			29th of Sep: 0.9 g MAP (12-61-0) + 0.9 L Humic acid	200 ml 100 L^{-1} Boscalid
October	3	0.75	15th of Oct: 0.9 g 19-19-19	100 ml 100 L^{-1} Deltamethrin
			29th of Oct: 0.9 g 19-19-19 + 0.9 L Humic acid	250 ml 100 L^{-1} Bupirimate
November	5	0.75	15th of Nov: 0.9 g 19-19-19	N/A
			29th of Nov: 0.9 g 19-19-19 + 0.9 L Humic acid	125 ml 100 L^{-1} Folpet 80%
December	5	0.75	11th of Dec: 0.9 g 19-19-19 + 0.9 L Humic acid	N/A
			18th of Dec: 0.9 g 19-19-19	400 ml 100 L^{-1} Copper oxychloride 50% WP
			25th of Dec: 0.9 g 19-19-19 + 0.20 g Fe	N/A
January	5	0.75	5th of Jan: 1.8 g 14-7-21 + 0.4 L MgO	250 ml 100 L^{-1} Spinosad 480
			12th of Jan: 1.8 g 14-7-21 + 0.4 L MgO + 0.2 Fe	N/A
			19th of Jan: 0.9 g 14-7-21 + 0.4 L MgO + 0.9 g KNO_3	250 ml 100 L^{-1} Azoxystrobin
			26th of Jan: 0.9 g 14-7-21 + 0.4 L MgO + 0.2 Fe	500 ml 100 L^{-1} Fenhexamid
February	3	0.75	2nd of Feb: 0.9 g K_2O	250 ml 100 L-1 Spinosad 480
			9th of Feb: 0.9 g 14-7-21 + 0.4 L MgO	N/A
			16th of Feb: 0.9 g $Ca(NO_3)_2$	500 h ha^{-1} Tebufenpyrad
			25th of Feb: 0.9 g 14-7-21	N/A
March	2	1.00	10th of Mar: 0.9 g 14-7-21 + 0.9 g KNO_3	250 ml 100 L^{-1} Spinosad 480
			16th of Mar: 0.9 g $Ca(NO_3)_2$	N/A
			23th of Mar: 0.9 g K_2O + 0.9 g 14-7-21	N/A
			30th of Mar: 0.9 g KNO_3	35 ml 100 L^{-1} Etoxazole 110
April	1	1.00	6th of Apr: 0.9 g 14-7-21	250 ml 100 L^{-1} Spinosad 480
			13th of Apr: 0.9 g $Ca(NO_3)_2$	N/A
			25th of Apr: 0.9 g K_2O + 0.9 g 14-7-21	100 ml 100 L^{-1} Milbemectin 9.3 g L^{-1}
			30th of Apr: 0.9 g 14-7-21	N/A
May	1	1.00	7th of May: 0.9 g 20-20-20	250 ml 100 L^{-1} Spinosad 480
			11th of May: 0.9 g 14-7-21 + 0.9 g Fe	N/A
			18th of May: 0.9 g $Ca(NO_3)_2$	35 ml 100 L^{-1} Etoxazole 110
			23th of May: 0.9 g 14-7-21 + 0.9 g Fe	N/A
			30th of May: 0.9 g $Ca(NO_3)_2$	N/A
June	1	1.20	8th of Jun: 0.9 g $Ca(NO_3)_2$	60 ml 100 L^{-1} Bifenazate 240 g L^{-1}

Results and Discussions

The first important result of study is the survival rates of the plants after transplanting (Table 3.).

According to the results obtained, the highest survival rate is obtained from strawberry plug propagation method with 86.67% and the lowest survival rage was measured from the frigo plants with 63.33%.

According to the results of present study, propagation of the strawberry plants with plug method provides earliness on the yielding. First yield was obtained in mid-November for

this propagation method (Figure 1.). The second fruit bearing treatment of present study is the fresh/green bare root propagated strawberries. Plant of this method gave yield 7 days after the plug propagated strawberries. However, the yield was too low and the interval between the other yields was high. The continuous yield for fresh/green bare root propagated plants was obtained at the end of December (about 1.5 months after plug method). Hereafter, plants went into a dormancy period during the winter time and provided less yield. The final treatment, frigo propagated plants gave first yields in the beginning of March. However, the yielding interval was close and total yield of this propagation method was very high, when compared with the other methods. Thus, the cumulative yield of frigo propagated plants reached the cumulative yield of bare roots in 30 days; and reached the cumulative yield of plug propagated plants in 60 days. At the end of the growing period, the highest total yield was obtained from the frigo plants with a mean of 960.0 g plant^{-1} yield, and is followed by the plug propagated plants with 864.2 g plant^{-1}. The plants which were propagated with fresh/green bare root method found to have a total of 550.0 g plant^{-1} yield.

Table 3. Survival rates of the strawberry plants propagated with different methods

Propagation methods	Survival rate
Strawberry plug	86.67 %
Fresh/Green bare root	76.67 %
Bare root cold stored (Frigo)	63.33 %

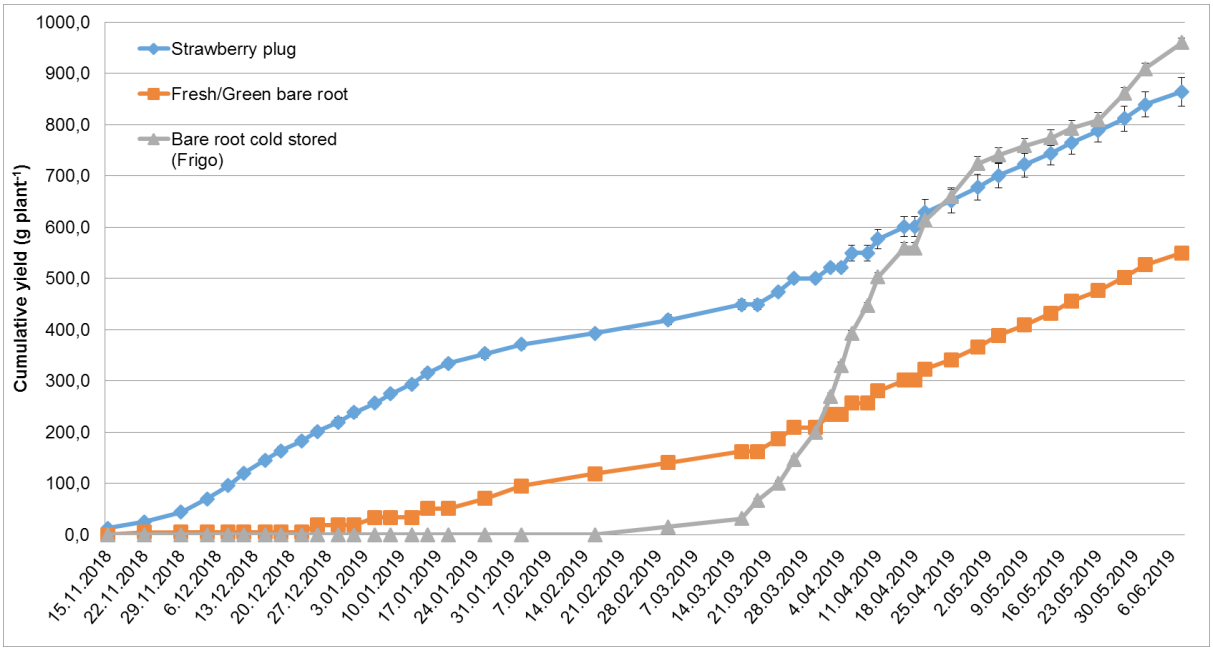


Figure 1. Cumulative fruit yield (g plant^{-1}) of strawberry plants cv. 'Florida Fortuna' propagated with different methods

Present results are in accordance with the findings of some other previous studies conducted in different environments and suggested that plug plants have similar yield with frigo plants (Bish et al., 1997; Hamann et al., 1997; Durner, 1999). Similar to present study, Takeda and Hokanson (2003) previously conducted a study about the strawberry production from plugs in greenhouse and reported that the plug production provides earliness on the yielding. In another study Durner (2016) reported that the plug propagation enhanced spring and total fruit production (weight and number) but have no significant effect on winter production. These results are also in accordance with the findings of present study. The main reason behind the earliness and continuous productivity of strawberries were reported to the chilling period received during transplant preparation and the photoperiod. It is known that if the plants receive the chilling injury in a short period of time, the flushing period (fruit bearing) happens in a short period and *vice versa* (Takeda, 1999). On the contrary, Takeda and Hokanson (2002) noted that the exposure of July propagated plugs of cv. 'Chandler' to chilling (10 °C) did not affect fall or winter greenhouse productivity. This would be due to that the chilling was applied to the plugs long after the development. In support to this idea, some previous studies (Takeda and Newell, 2007; Deyton et al., 2009;) suggest that earlier plug propagation provides higher yield at the strawberry fruits.

The results for the cumulative number of fruits plant^{-1} are in accordance with the results of cumulative yield of the plants (Figure 2.). The raw data behind the cumulative results showed that the highest number of fruits plant^{-1} at a harvest was obtained from the frigo propagated plants and is 3. The average number of harvested fruits plant^{-1} during the whole growing period was found to be 1.18 for fresh/green bare root method,

1.19 for plug method and 2.07 for frigo propagation method. This result together with the yield data suggests that plug method is better when farmers aim to have earliness and continuous yield during the growing season; and the frigo propagation method could be recommended when earliness in not targeted while high yield is important.

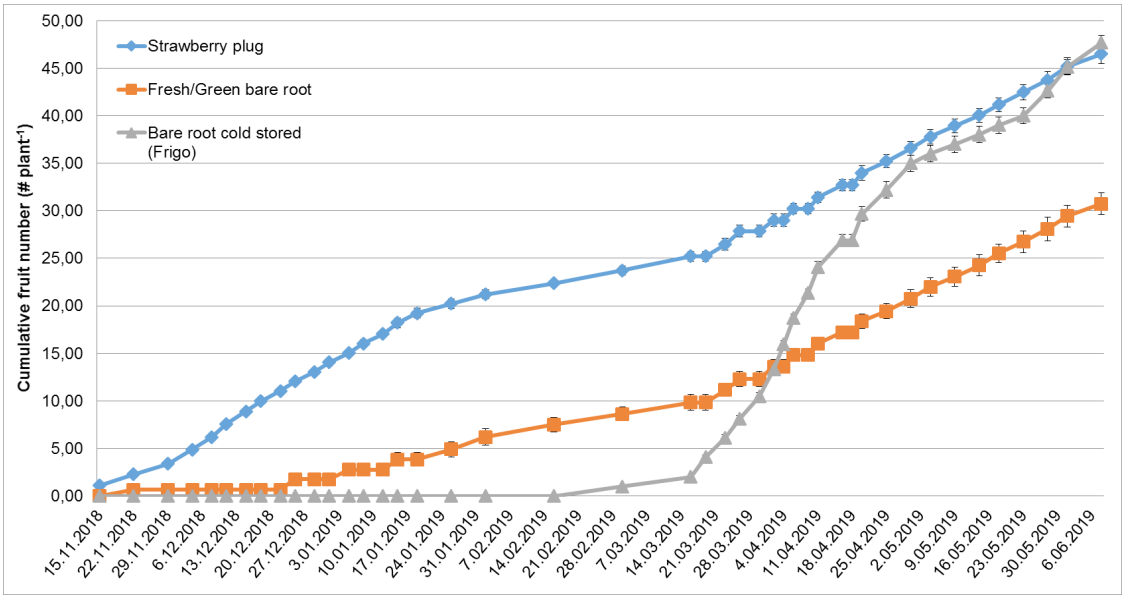


Figure 2. Cumulative fruit number (# plant^{-1}) of strawberry plants cv. 'Florida Fortuna' propagated with different methods

Another important result of present study is about the average fruit weight of the strawberries propagated with different methods. According to the results obtained, the frigo propagated plants have higher fluctuations than the other two methods in terms of the change in the average fruit weight (Figure 3.).

While there is an important difference among the average fruit weight of the different propagation methods, no significant difference was obtained for the average fruit weight of all growing period. At the end of the growing period, the average fruit weight of plants propagated with plug, bare root and frigo methods was found to be 18.53 g, 17.66 g and 19.45 g, respectively. Total soluble solids (%) of the strawberry fruits found to vary during the growing period. At the beginning of the harvesting period, the fruit TSS of the plants propagated with plugs and fresh/green bare root method were 9.83% and 9.65%, respectively (Figure 4.). Until the end of the February, the fruit TSS was similar for all plants. Hereafter, with the increase and decrease in yield, the fruit TSS started to vary.

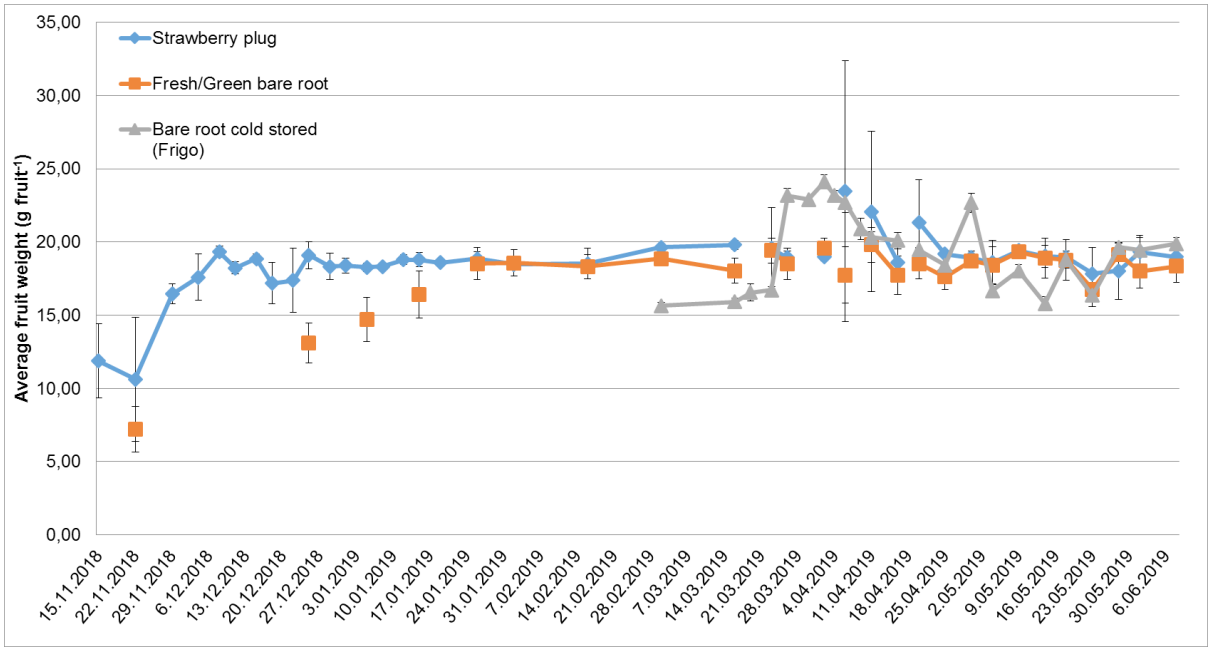


Figure 3. Average fruit weight (g plant^{-1}) of strawberry plants cv. 'Florida Fortuna' propagated with different methods

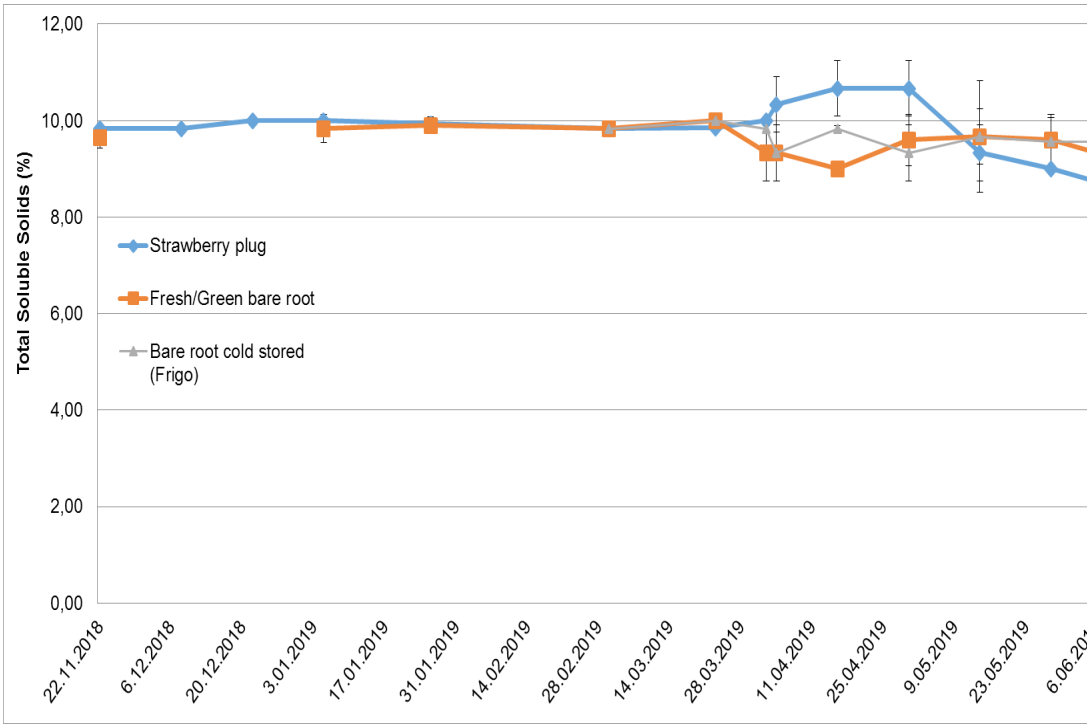


Figure 4. Total soluble solids (%) of strawberry plants cv. 'Florida Fortuna' propagated with different methods

According to the results of present study, the titratable acidity (TA) of the fruits did not vary during the growing period and also did not vary among the different propagation methods (Figure 5.). The results for TA varied from 0.60 to 0.61 during the studies.

Similar with the TA results, the fruit firmness of fruits in present study did not show any significant differences either among the propagation methods or during the growing period (Figure 6.). The results for fruit firmness varied from 0.74 to 0.77 during the studies.

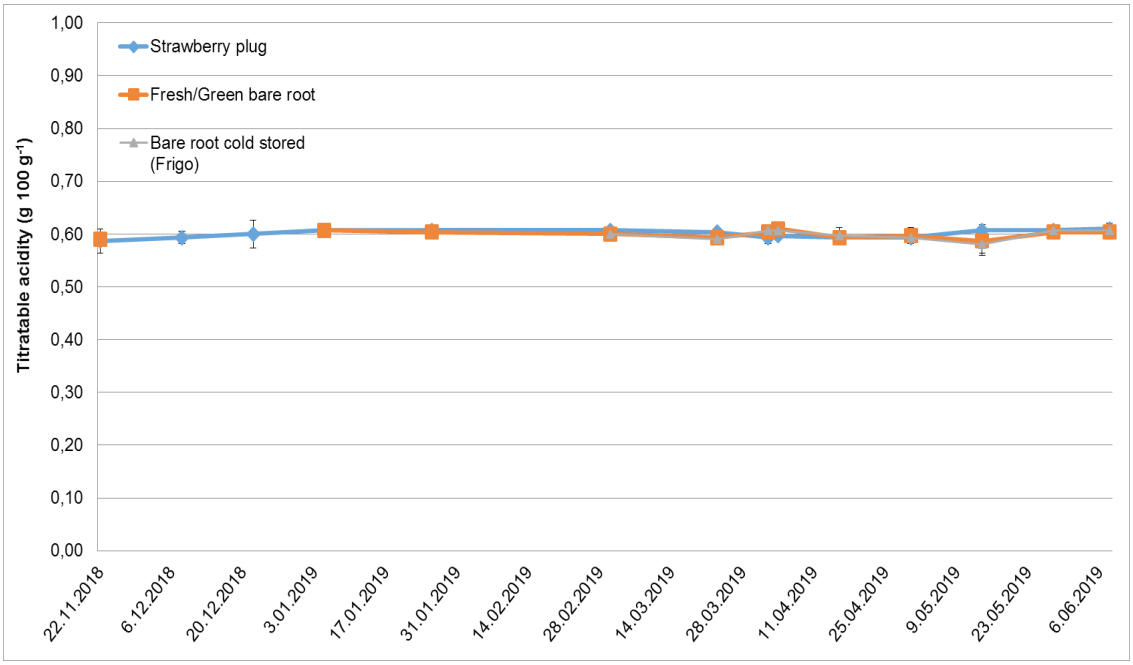


Figure 5. Titratable acidity (g 100 g^{-1}) of strawberry plants cv. 'Florida Fortuna' propagated with different methods

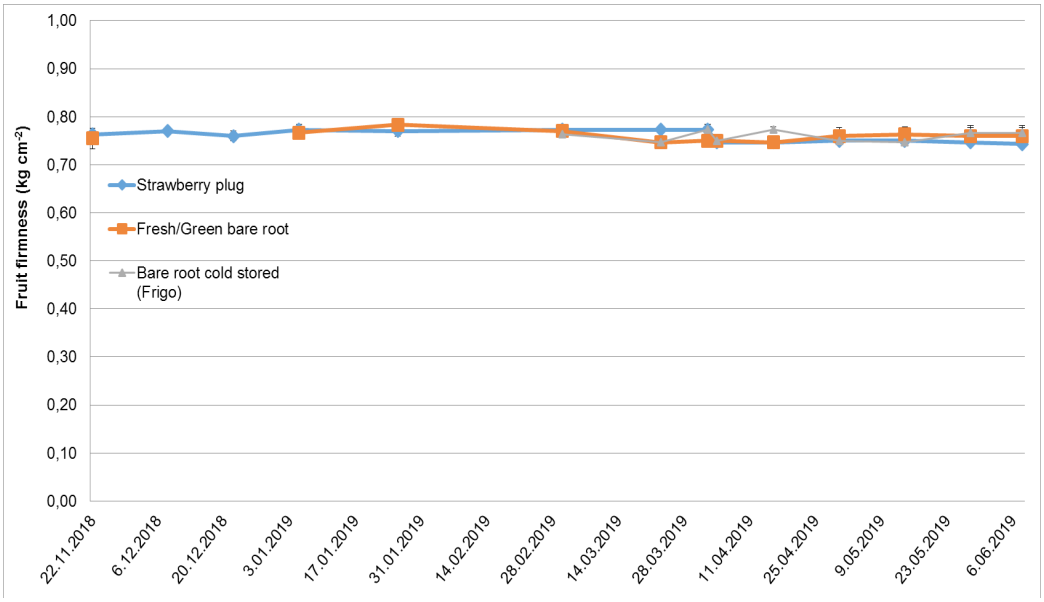


Figure 6. Fruit firmness (kg cm^{-2}) of strawberry plants cv. 'Florida Fortuna' propagated with different methods

Conclusion

According to the results obtained, strawberry plug propagation method provides better survival rate in the field conditions as compared with bare root and frigo methods. Another important result is that, plug propagation provides earliness on fruit yielding and continuous yielding (except winter period). The frigo propagation method was found to be the latest for fruit bearing, but to have highest yield. To sum up the results, plug method provides earliness and continuous yielding throughout the growing season, while the frigo propagation method delays fruit bearing but provides higher yield with less interval. Results also showed that there is slight difference in the average fruit weight of the plants throughout the growing period and the frigo plants have higher fluctuations in the weekly average fruit weights. Another important result of present study is that the increase in the weekly yield cause a decrease in TSS and *vice versa*.

References

Anonymous. (2019). Strawberry Varieties. In: website of Lassen Canyon Nursery. [URL] (Accessed on 21th of October, 2019).

Bish, E.B., Cantliffe, D.J., Hochmuth, G.J., Chandler, C.K. (1997). Development of containerized strawberry transplants for Floridas winter production system. Proceedings of the 3rd International Strawberry Syposium, Apr. 29-May 4, Veldhoven, Netherlands: 461-465 [CrossRef]

Capocasa, F., Balducci, F., Marcellini, M., Bernardini, D., Navacchi, O., Mezzetti, B. (2019). Comparing nursery behavior, feld plant yield and fruit quality of in vitro and in vivo propagated strawberry mother plants. Plant Cell, Tissue and Organ Culture (PCTOC) 136:65-74 [CrossRef]

Cordenunsi, B.R., Genovese, M.I., Nascimento, J.R.O., Hassimotto, N.M.A., Santos, R.J., Lajolo, F.M. (2005). Effects of temperature on the chemical composition and antioxidant activity of three strawberry cultivars. Food Chemistry 91:113-121 [CrossRef]

Deyton, D., Sams, C., Takeda, F., Cummins, J. (2009). Off-season greenhouse strawberry production. HortScience 44(4):1002 [URL]

Dolgun, O. (2006). Yield Performance of Strawberry (fragaria x ananassa) Plug Plants in Eastern Mediterranean Climatic Conditions. International journal of Agricultural Research 1(3):280-285 [CrossRef]

Durner, E.F., Poling, E.B., Maas, J.L. (2002). Recent Advances in Strawberry Plug Transplant Technology. HortTechnology, 12(4):545-550 [CrossRef]

Fernandez, G., Ballington, J. (2003). Double cropping of strawberries in an annual system using conditioned plug. Acta Horticulturae 614:547-552 [CrossRef]

Hamann, K.K., Poling, E.B., Scheer, H., Lieten, F., Dijkstra, J. (1997). The influence of runner order, night temperature and chilling cycles on the earliness of Selva plug plant fruit production. Acta Horticulturae, 439:597-603 [CrossRef]

Hennion, B., Schupp, J., Longuesserre, J., Scheer, H., Lieten F., Dijkstra, J. (1997). Fraisimotte strawberry plug plant developed by CIREF in France. Acta Horticulturae, 439: 469-474 [CrossRef]

Kadir, S., Carey, E., Ennahli, S. (2006). Influence of high tunnel and field conditions on strawberry growth and development. HortScience 41(2):329-335 [CrossRef]

Kahramanoğlu, İ. (2019). Effects of lemongrass oil application and modified atmosphere packaging on the postharvest life and quality of strawberry fruits. Scientia Horticulturae 256:108527 [CrossRef]

Lieten, P. (2002). The use of cold stored plant material in central europe. Acta Horticulturae 567: 553-559 [CrossRef]

Narváez-Ortiz, W.A., Lieth, J.H., Grattan, S.R., Benavides-Mendoza, A., Evans, R.Y., Preciado-Rangel, P.,

Valenzuela-García, J.R., Gonzalez-Fuentes, J.A. (2018). Implications of physiological integration of stolon interconnected plants for salinity management in soilless strawberry production. Scientia Horticulturae, 241:124-130 [CrossRef]

Paszko, D., Pawlak, J., Wróblewska, W. (2014). Yield of two strawberry cultivars depending on the cropping method on the example of a commercial plantation in a specialized horticultural farm. Acta Sci. Pol., Hortorum Cultus 13(6):149-159 [URL]

Pritts, M. (2001). Growing strawberries healty communities strong economies and clean environments What is the Role of the Researcher. Acta Horticulturae, 567: IV International Strawberry Symposium [CrossRef]

Seeram, N.P., Adams, L.S., Zhang, Y., Lee, R., Sand, D. Scheuller, H.S., Heber, D. (2006). Blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry extracts inhibit growth and stimulate apoptosis of human cancer cells in vitro. Journal of Agricultural Food Chemistry 54:9329-9339 [CrossRef]

Takeda, F. (1999). Out of season greenhouse strawberry production in soilless substrate. Adv Strawb Res 18:4-15 [URL]

Takeda, F., Hokanson, S.C. (2002). Effects of transplant conditioning on 'Chandler' strawberry performance in a winter greenhouse production system. In: Hokanson, S.C., Jamieson, A.R. (Eds.), Strawberry Research to 2001. ASHS Press, Alexandria, VA, pp. 132-135 [URL]

Takeda, F., Hokanson, S.C. (2003). Strawberry Fruit and Plug Plant Production in the Greenhouse. Acta Horticulturae, 626:283-285 [CrossRef]

Takeda, F., Newell, M. (2006). A method for increasing fall flowering in short-day 'Carmine' strawberry. HortScience 41:480-481 [URL]

Takeda, F., Newell, M. (2007). Effect of plugging date on fall flowering and fruiting in some short-day strawberry. Int J Fruit Sci 6:103-117 [URL]

International Journal of
Agriculture, Environment and Food Sciences

JAEFS

e-ISSN : 2618-5946

DOI: 10.31015/jaefs.2019.4.11

www.jaefs.com

Research Article

Int J Agric Environ Food Sci 3(4):265-271 (2019)

Aqueous and ethanolic extracts of propolis for the control of tyramine production by food-borne pathogens

Aykut Burgut[1,*]

[1]Department of Animal Science Technology, Faculty of Agriculture, University of Cukurova, Adana, Turkey

[*]Corresponding Author: burguta@cu.edu.tr

Abstract

The influences of aqueous and ethanolic extracts of propolis (1%) on growth of common Gram-negative (*Salmonella* Parathyphi A, *Campylobacter jejuni*, *Yersinia enterocolitica* and *Klebsiella pneumoniae)* and -positive *(Listeria monocytogenes, Staphylococcus aureus* and *Enterococcus faecalis*) food-borne pathogens and their biogenic amines (BAs) production were examined in tyrosine decarboxylase broth (TDB). The highest growth inhibitory activity was observed against Gram-negative *S.* Paratyphi A in the existence of ethanolic and aqueous extracts of propolis, with 2.49 and 1.9 log reduction, respectively. Ethanolic extracts of propolis were more effective than that of aqueous extract on growth inhibition of *L. monocytogenes* ($p<0.05$). Both extracts of propolis had significant effect on reducing ammonia production by bacteria ($p<0.05$). Tyramine, dopamine, agmatine and spermine were major amines formed in TDB. Tyramine production was the lowest with *S.* Paratyphi A (1.94 mg/L) and highest with *E. faecalis* (254.93 mg/L). The existence of ethanolic propolis extracts in TDB led to significantly fewer tyramine production by Gram-positive *S. aureus, L. monocytogenes* and *E. faecalis,* and Gram-negative *C. jejuni* ($p<0.05$). Histamine produced lower than 1.3 mg/L by all food-borne pathogens. Ethanolic extracts of propolis generally led to lower histamine production by bacteria. The influence of propolis on BAs production varied according to type of extracts, specific BAs and bacterial strains. However, the aqueous of propolis generally showed a synergistic effect on most of BAs mainly tyramine production by bacteria. Thus, the use of propolis ethanolic extracts appeared to be more suitable than aqueous extract to control tyramine production in foods.

Keywords: Propolis, Tyramine, Food-borne pathogens, Food safety

Introduction

Food safety is a major public health concern worldwide (Liu et al., 2019). Food-borne illnesses are often related with pathogens, toxins and chemicals, and are a global public health problem for a variety of causes. New risks constantly occur while others are inhibited (Camino-Feltes et al., 2017). Pathogenic bacteria have a capacity to form BAs via amino acid decarboxylation action. Some bacteria species including *Bacillus, Citrobacter, Klebsiella, Escherichia, Proteus, Pseudomonas* and *Photobacterium* may decarboxylate one or more amino acids (Silla-Santos, 1996, Karovičová and Kohajdová, 2005).

Digestion of food having excessive levels of BAs are occupied in several toxicological symptoms which resulted in various kinds of foodborne illness (headaches, low blood pressure, heart palpitations, edema, vomiting, and diarrhea) (Maintz and Novak, 2007). Therefore, the existence of BAs can influence both the feature and the safety of foods (Gram and Dalgaard, 2002). BAs have been found in many foods including fish, meat, cheese, vegetables and wines (Lorenzo et al., 2007). Tyramine is known as the most commonly accumulated BAs in cheese (Fernandez et al., 2007). Tyramine can cause physiological reactions such as peripheral vasoconstriction, improved cardiac output, elevated respiration, increased blood glucose, and release of norepinephrine (Shalaby, 1996). In view of the fact that the detection of the “cheese reaction” hyperten-

Cite this article as:
Burgut, A. (2019). Aqueous and ethanolic extracts of propolis for the control of tyramine production by food-borne pathogens. Int. J. Agric. Environ. Food Sci., 3(4), 265-271
DOI: https://dx.doi.org/10.31015/jaefs.2019.4.11
Received: 01 September 2019 **Accepted:** 17 December 2019 **Published:** 24 December 2019
Year: 2019 **Volume:** 3 **Issue:** 4 (December) **Pages:** 265-271
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

sive crisis caused by tyramine intake among individuals on monoamine oxidase inhibitor (MAOI) drugs, several studies have focused on the tyramine content in foods (Marcobal et al. 2012). Control of BAs is crucial to ensure the safety of fermented foods (Li et al., 2018). BAs production can be restricted by preventing bacterial proliferation or the decarboxylase action of bacteria (Wendakoon and Sakaguchi 1995).

Propolis is a natural constituent accumulated by bees from native flora (Majiene et al., 2007). Propolis have natural combinations of several secondary metabolites that exert a variety of bioactivity e.g. antibacterial, antiulcer, antioxidant, and anti-viral activities (De Figueiredo et al., 2015). Since propolis is safe for humans if extensive dose is not taken (Satoshi et al., 2005), it possesses a wide range of uses such as preservatives in food products (fruits, juice, soft drinks, fish and meat products) and also in veterinary pharmaceutical applications (Casquite et al. 2016). Antimicrobial activity of propolis is due to their primary ingredients of flavonoids, phenolic compounds diterpenic acids and aromatic acids (Afrouzan et al., 2018). Propolis extracts exhibited the highest antimicrobial activity towards the Gram-positive food-borne pathogen bacteria e.g. *Bacillus cereus* and *S. aureus* (Nedji and Loucif-Ayad, 2014). The propolis exerted a noticeable antibacterial activity against the Gram-positive strain (*L. monocytogenes*) and restricted action against Gram-negative *Salmonella* Enteritidis depending on different propolis dose (Temiz et al., 2011). Propolis extracts had an inhibitory effect towards *S. aureus* isolated from instant soups, although their antimicrobial effects varied depending on their geographical regions (Apaydın and Gümüş, 2018).

Although many studies have been done about the antibacterial properties of propolis, there are limited studies regarding its impact on tyramine and other BAs accumulation by bacteria. Thus, the aim of the study was to examine the impact of aqueous and ethanolic extracts of propolis on tyramine and other BAs produced by common Gram-negative and positive food-borne pathogens.

Material and Method

Food-borne pathogens

Enterococcus faecalis ATCC29212, *Staphylococcus aureus* ATCC29213, *Klebsiella pneumoniae* ATCC700603, *Campylobacter jejuni* ATCC 33560 and *Listeria monocytogenes* ATCC19112 were purchased from the American Type Culture Collection (Rockville, MD, USA). *Salmonella* Parathyphi A NCTC13 and *Yersinia enterocolitica* NCTC 11175 were provided from the National Collection of Type Cultures (London, UK, Özogul et al., 2011).

Preparation of propolis extracts

Propolis from *Apis mellifera* was obtained using a commercial plastic trap in August 2018 (Adana, Turkey). Crude propolis was ground into powder and extracted with ethanol (70%) or water (100%). They placed in daily shakable containers for 48 h. Solutions of propolis were prepared aseptically and protected from light. They were stored in a dark place at 4 ºC until analysis.

Culture Conditions and Bas Analysis

The production of BAs from all food-borne pathogens in this work was monitored using tyrosine decarboxylase broth (TDB) suggested by Klausen and Huss (1987). The extraction process and derivatisation of BAs were performed in accordance with the method of Kuley and Ozogul (2011). The mobile phase contained acetonitrile (Sigma 439134, Steinheim, Germany) and grade water for the amine analyses. A high-performance liquid chromatography (HPLC, Shimadzu, Kyoto, Japan) was used to detect BAs. ODS Hypersil (5µ, 250x6 mm, Phenomenex, UK) was used as column.

Monitoring bacterial growth in TDB

Estimation of total viable counts in TDB was made in triplicate. Plate count agar (Fluka 70152; Steinheim, Switzerland) was used to growth of bacteria. Spread plates with appropriate dilution of 0.1 ml were incubated for 2 days at 30˚C.

Statistical Analysis

Mean and standard deviation of three replicates were measured. The significance of differences ($p<0.05$) was determined using Oneway ANOVA with SPSS 15.0 version (SPSS Inc., USA).

Result and Discussion

Bacterial growth in TDB

Bacterial load in TDB in the existence or absence of propolis extracts was shown in Table 1. Bacterial loads in control groups were in range from 8.41 log cfu/ml for *S.* Paratyphi A to 8.95 log cfu/ml for *Y. enterocolitica*. Significant differences in bacterial load apart from *S. aureus* were observed between control and propolis treated groups ($p<0.05$). By contrast, propolis extracts exhibited different extents of inhibitory effects against *S. aureus,* depending on concentration, collecting area and time (Lu et al., 2005; Apaydın and Gümüş, 2018). Presence of water or ethanolic propolis extracts in TDB resulted in lower bacterial growth ($p<0.05$), although extracts statistically did not affect growth of *S. aureus*. The highest inhibitory effects were observed against Gram-negative *S.* Paratyphi A (>1.9 log reduction), *Y. enterocolitica* (>1.2 log reduction) and *C. jejuni* growth (>1 log reduction). Propolis acted against both Gram-positive and Gram-negative bacteria.

The antimicrobial action of propolis is related to its natural ingredients and is different in individual countries (Przybyłek and Karpiński, 2019). However, the propolis was also reported to have better action against Gram-positive bacteria than Gram-negative (Kim and Chung, 2011; Pobiega et al., 2019). Among Gram-positive bacteria, the highest growth inhibition of propolis extracts was found against *E. faecalis*, with about 0.7 log reduction. The effects of ethanolic and aqueous propolis extracts on bacterial growth were statistically similar except for *L. monocytogenes*. However, Al-Ani et al. (2018) found that aqueous extract of propolis exerted poor bactericidal action against Gram-negative bacteria. Similarly, ethanolic propolis extracts were more effective than that of water extract on growth inhibition of *L. monocytogenes* in TDB ($p<0.05$). Ethanol extract of propolis exhibited strong antilisterial activity (Pobiega et al., 2019; Temiz et al., 2011). The antibacterial action of propolis is as a consequence of the direct action on the microorganism and encouragement of the immune sys-

tem causing in initiation of natural defences of the organism (Sforcin and Bankova, 2011; Przybyłek and Karpiński, 2019).

Biogenic amine production by bacteria

Figure 1 shows tyramine accumulation by food-borne pathogens. Tyramine production was the lowest with *S.* Paratyphi A (1.94 mg/L) and highest with *E. faecalis* (254.93 mg/L). *Enterococcus* spp. are important tyramine producer in fermented foods and able to yield TYR more than 520 mg/L (Connil et al., 2002; Özogul and Özogul, 2007; de Palencia et al., 2011). Tyramine accumulation was the weakest (6.42 mg/L) with *K. pneumoniae* among food-borne pathogens tested (Özogul et al., 2015). In the present study, *K. pneumoniae* accumulated tyramine at a moderate level (29.53 mg/L).

Presence of propolis ethanolic extracts in TDB led to significantly fewer tyramine production by Gram-positive *L. monocytogenes, E. faecalis, S. aureus* and Gram-negative *C. jejuni* ($p<0.05$). However, aqueous extracts of propolis caused considerably higher tyramine formation by all of food-borne pathogens, mainly Gram negative-bacteria. The highest stimulating effect of aqueous propolis extracts was found for *S.* Paratyphi A, with 146-fold higher tyramine production, which was not consistent with result of bacterial load in TDB. Tyramine production by *K. pneumonia* and *Y. enterocolitica* were also 11 and 13 fold higher with propolis aqueous extracts. The presence of 6 mg tyramine in one or two usual servings of food is thought to be sufficient to cause a mild adverse event while 10–25 mg will produce a severe adverse event in those using MAOI drugs (Da Prada et al., 1988). A limit of 200–800 mg in one or two usual servings has been proposed for tyramine in foods (Da Prada et al., 1988; Marcobal et al., 2012). Food borne-pathogens produced tyramine between 4.93 (*Y. enterocolitica*) and 37.64 mg/L (*L. monocytogenes*) in the presence of ethanolic propolis extract.

Table 1. Bacterial growth in tyrosine decarboxylase broth with or without propolis extracts (log cfu/mL)

	Control	Ethanolic extracts of propolis	Water extracts of propolis
Gram-positive bacteria			
L. monocytogenes	8.69±0.01a	8.51±0.02c	8.62±0.01b
E. faecalis	8.56±0.01a	7.88±0.10b	7.77±0.01b
S. aureus	8.55±0.05a	8.51±0.00a	8.50±0.00a
Gram-negative bacteria			
S. Parathyphi A	8.41±0.22a	6.51±0.16b	5.92±0.21b
K. pneumoniae	8.58±0.00a	7.83±0.08b	7.70±0.09b
Y. enterocolitica	8.95±0.08a	7.68±0.03b	7.72±0.06b
C. jejuni	8.90±0.05a	7.68±0.07b	7.85±0.11b

*Data are stated as mean value of three samples, Mean value±Standard deviation.
[a–c] Show statistically significant differences ($P < 0.05$) between control and treated group in a column.

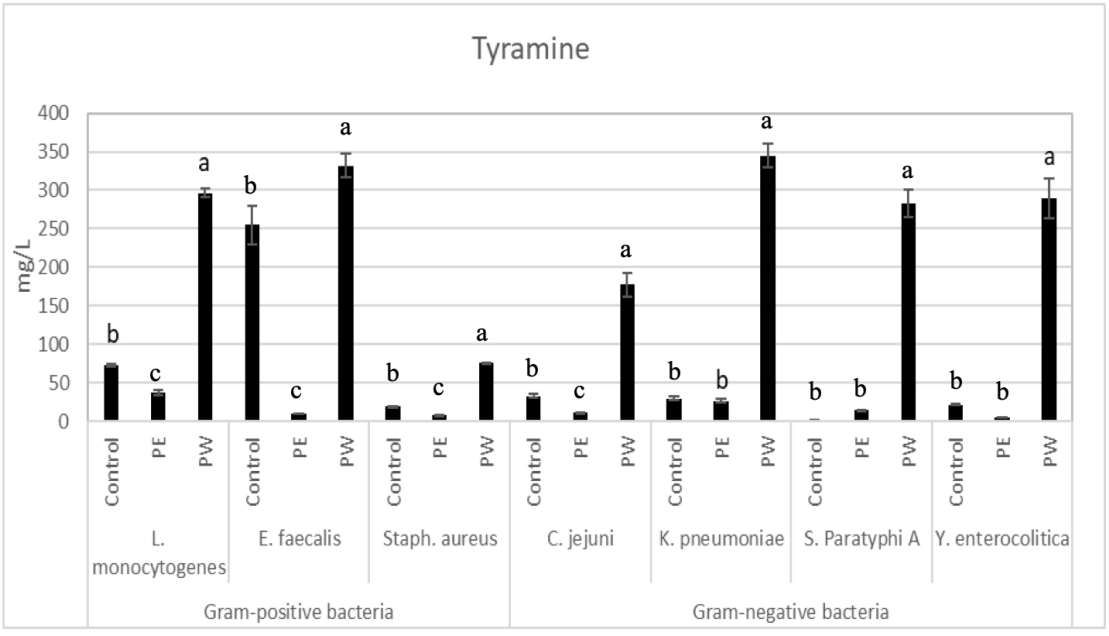


Figure 1. Tyramine accumulation by food borne pathogens in the absence or existence of propolis extracts. PE: group treated with ethanolic extract of propolis, PW: group treated with aqueous extract of propolis. [a–c] Show statistically significant differences ($P < 0.05$) between control and treated group

Table 2 and 3 illustrate ammonia and BAs production by Gram-positive and -negative food-borne pathogen in the absence or presence of propolis extracts, respectively. Ammonia production was in range from 167.63 mg/L by *C. jejuni* to 469.57 mg/L by *K. pneumoniae*. The highest ammonia accumulation was reported for *K. pneumoniae* and *S. aureus* (470-525 mg/L) in TDB (Kuley and Özogul, 2011), which was consistent with current results. Both extracts of propolis were significantly effective on reducing ammonia production by bacteria ($p<0.05$). Ethanolic extracts of propolis was more effective on reducing ammonia production by *E. faecalis* (2 fold-lower) and *S. aureus* (3-fold lower) than that of aqueous extracts ($p<0.05$), although both extracts of propolis showed statistically similar inhibitory effect on ammonia production by most of the bacteria.

Food-borne bacteria produced all amine tested apart from tryptamine. Tyramine, dopamine, agmatine and spermine were main amines produced in TDB, which was in agreement with result of Özogul et al. (2015). Putrescine production was the highest with *Y. enterocolitica* (35.75 mg/L) and *C. jejuni* (32.50 mg/L), whilst *C. jejuni* (40.32 mg/L) and *L. monocytogenes* were main cadaverine producer. *S. aureus* and *S.* Paratyphi A produced considerably higher concentrations of putrescine than other food-borne pathogens (De las Rivas et al., 2006). In the current study, *S. aureus* and *S.* Paratyphi A produced putrescine at the level of 15.29 and 2.70 mg/L, respectively. Putrescine and cadaverine production by *L. monocytogenes* and *C. jejuni* was significantly inhibited by both propolis extracts. Moreover, presence of ethanolic extracts generally led to considerably lower putrescine and cadaverine production, although aqueous extracts of propolis mostly induced higher putrescine and cadaverine accumulation in TDB broth. Spermidine production by bacteria was above 4 mg/L and generally suppressed by both extracts. Spermine was produced at the highest level by *L. monocytogenes* and *E. faecalis* (about 60 mg/L). Although spermine production by *L. monocytogenes* and *Y. enterocolitica* considerably suppressed by presence of aqueous or ethanolic extracts of propolis, aqueous extracts of propolis resulted in higher spermine production by Gram-positive *S. aureus* and Gram-negative *C. jejuni* and *K. pneumoniae.*

Histamine is known as the causative agent of histamine intoxication and causes diarrhea, headache, rhinoconjunctival symptoms and other reactions in immunocompromised patients (Maintz and Novak, 2007; Comas-Basté et al., 2019). The US Food and Drug Administration (1995) recommended an upper limit of histamine to 5 mg/100 g (50 ppm) in fish (Al-Bulushi et al., 2009), whilst the European Commission (Commission Regulation EC No. 1441/2007, 2007) has suggested that the mean content of histamine in fish should not be above 10 mg/100g. Histamine produced lower than 1.3 mg/L by all food-borne pathogens. Ethanolic extracts of propolis was generally led to lower histamine production by bacteria. However, inhibitory effect of aqueous extract of propolis on histamine formation was just detected for *Y. enterocolitica* and *C. jejuni*, whilst it stimulated histamine production by *E. faecalis*, *S. aureus* and *K. pneumoniae*. The highest histamine production (3.24 mg/L) was found for *E. faecalis* in the existence of aqueous extracts of propolis. Serotonin production by bacteria was <6 mg/L in TDB. Serotonin accumulation by the most of bacteria was also inhibited by ethanolic propolis extract. Suppression effects of aqueous extract on serotonin production were observed for *L. monocytogenes*, *S. aureus* and *C. jejuni*.

TMA production by bacteria was between 1.25 and 50.34 mg/L by *S.* Paratyphi A and *K. pneumoniae,* respectively. Apart from *K. pneumoniae* and *Y. enterocolitica*, stimulatory effect of propolis extracts on TMA production was found. *K. pneumoniae* and *S.* Paratyphi A had good ability to produce dopamine in TDB, with corresponding value of 554.84 and 523.52 mg/L, whilst Gram-positive bacteria produced dopamine below 145 mg/L.

Propolis ethanolic extract had a significant effect on suppression of dopamine production by Gram-negative bacteria mainly *C. jejuni*, but ineffective against Gram-positive bacteria apart from *L. monocytogenes*. However, aqueous extract of propolis increased dopamine production of all Gram-positive bacteria tested, whilst it did not affect dopamine formation by *C. jejuni* and *K. pneumoniae*. Agmatine accumulation was the uppermost by *C. jejuni* and *K. pneumoniae*, with corresponding value of 95.50 and 90.81 mg/L. Both propolis extracts resulted in lower agmatine production by these bacteria ($p<0.05$), whereas there were no substantial differences in agmatine production by these bacteria between control and aqueous extracts groups. However, agmatine production by Gram-positive bacteria increased with aqueous extracts of propolis ($p<0.05$). The antimicrobial mechanisms of propolis are multiple and complex, being determined by the synergistic effects of phenolic compounds and other biologically active components (Hazem et al., 2017). Increase in most of BAs in the presence of aqueous extracts of propolis may be due to the fact that the basic biologically active constituents of propolis are hardly soluble in water (Kubiliene et al., 2015). Other reasons for the increase in BAs may be due to the presence of other synergistic conditions, such as changes in water activity and pH. Therefore, more detailed studies are needed to determine this effect.

Conclusions

Propolis extracts showed good antimicrobial activity against food-borne pathogens apart from *S. aureus* in TDB. The highest growth inhibitory activity of propolis extracts was observed for Gram-negative *S.* Paratyphi A, *C. jejuni* and *Y. enterocolitica*. Bacterial growth did not generally associate well with BAs production. The influence of propolis on BAs formation varied according to the type of extracts, specific BAs and bacterial strains, although ammonia production by bacteria was suppressed in the existence of propolis extract. The study results revealed that aqueous extract of propolis showed synergistic effects on the most of BAs production by bacteria mainly tyramine. In conclusion, it has been suggested that the use of the ethanolic propolis extract in food products may be more suitable than the aqueous extract of propolis.

Table 2a. Ammonia and BAs accumulation by Gram-positive food borne pathogen in the absence or presence of propolis extracts (mg/L).

	AMN	PUT	CAD	SPD	PHEN	SPN
LM	206.42±8.17a 123.08±7.59b 129.44±5.23b	3.99±0.10a 0.50±0.00c 0.87±0.02b	33.27±1.07a 5.17±0.08c 19.60±0.80b	7.53±0.04a 0.00±0.00b 0.00±0.00b	2.73±0.12b 0.55±0.07c 24.28±0.95a	62.21±4.15a 24.68±1.16c 45.99±3.19b
EF	213.70±3.80a 89.13±2.90c 115.92±4.99b	3.45±0.28b 0.00±0.00c 12.42±0.12a	7.38±0.86b 6.25±0.08b 37.42±0.44a	21.25±0.43a 13.95±0.15b 0.00±0.00c	8.62±0.63b 0.92±0.03c 32.47±0.58a	61.23±5.07a 53.91±4.85ab 44.50±0.91c
SA	274.58±11.76a 88.92±5.35c 135.25±13.35b	15.29±1.31b 7.45±0.12c 26.47±0.36a	10.15±0.03b 2.42±0.08c 17.52±1.26a	4.16±0.13a 2.01±0.00b 3.86±0.21a	0.93±0.03c 2.23±0.05b 5.38±0.07a	4.82±0.14b 5.19±0.44b 27.07±0.06a

*Data are expressed as mean value of three samples, Mean value±Standard deviation.

[a–c] Show statistically significant differences ($P < 0.05$) between control and treated group in a row.

LM: *Listeria monocytogenes*, EF: *Enterococcus faecalis*, SA: *Staphylococcus aureus*, C: control group without propolis extract addition, PE: group treated with ethanolic extract of propolis, PW: group treated with aqueous extract of propolis. AMN, ammonia; PUT, putrescine; CAD, cadaverine; SPD, spermidine; PHEN, 2-phenylethyl amine; SPN, spermine

Table 2b. Ammonia and BAs accumulation by Gram-positive food borne pathogen in the absence or presence of propolis extracts (mg/L).

	HIS	SER	TMA	DOP	AGM	Groups
LM	1.29±0.04a 0.28±0.00b 1.28±0.03a	4.06±0.16a 1.85±0.04c 3.38±0.05b	1.88±0.02c 3.44±0.06b 5.64±0.84a	70.50±3.69c 128.82±6.92b 317.28±14.66a	39.79±2.75c 60.44±0.64b 76.34±7.14a	C PE PW
EF	0.89±0.05b 0.42±0.01c 3.24±0.06a	1.56±0.04b 1.42±0.08b 3.72±0.20a	8.28±0.77c 21.99±0.16b 27.76±1.60a	62.35±1.77b 64.17±3.77b 155.24±1.94a	25.15±1.13c 54.33±1.94b 64.54±1.31a	C PE PW
SA	0.38±0.00b 0.13±0.01b 2.43±0.23a	3.25±0.13a 1.08±0.01c 2.42±0.14b	3.06±0.16c 3.75±0.02b 13.20±0.27a	144.87±7.37b 129.87±7.88b 459.91±29.80a	15.79±0.86b 16.74±0.77b 64.39±2.91a	C PE PW

*Data are expressed as mean value of three samples, Mean value±Standard deviation.

[a–c] Show statistically significant differences ($P < 0.05$) between control and treated group in a row.

LM: *Listeria monocytogenes*, EF: *Enterococcus faecalis*, SA: *Staphylococcus aureus*, C: control group without propolis extract addition, PE: group treated with ethanolic extract of propolis, PW: group treated with aqueous extract of propolis. HIS: histamine, SER, serotonin; TMA, trimethylamine; DOP, dopamine, AGM, agmatine

Table 3a. Ammonia and BAs accumulation by Gram-negative food borne pathogen in the absence or presence of propolis extracts (mg/L)

	AMN	PUT	CAD	SPD	PHEN	SPN
YE	272.12±21.91a 118.28±8.15b 82.75±3.28b	35.75±0.07b 1.91±0.13c 60.20±2.78a	17.05±0.10a 15.21±1.50a 14.20±1.35a	19.63±0.80a 8.86±0.46b 0.00±0.00c	10.21±0.23b 0.00±0.00c 11.45±0.48a	55.85±1.43a 27.70±0.02c 36.53±0.15b
CJ	167.63±11.25a 97.37±4.66b 98.08±6.87b	32.50±2.76a 0.00±0.00c 25.97±1.37b	40.32±1.89a 7.39±0.17c 33.73±2.36b	10.27±0.29a 0.00±0.00c 1.26±0.01b	0.72±0.05b 0.54±0.06b 1.11±0.10a	35.35±2.31b 35.04±0.27b 67.37±2.65a
KP	469.57±24.54a 94.62±6.89b 107.91±1.24b	1.36±0.14b 0.35±0.03b 61.94±5.38a	4.85±0.49b 3.29±0.02b 35.43±2.26a	31.23±2.13a 0.00±0.00b 2.16±0.13b	0.39±0.01c 0.90±0.13b 2.48±0.16a	32.36±2.46b 30.52±2.45b 48.01±3.92a
SP	268.48±23.57a 102.63±7.72b 143.94±12.71b	2.70±0.18b 0.69±0.02c 26.71±0.33a	24.57±1.70a 13.69±0.15c 19.18±1.80b	18.39±1.07a 17.55±0.07a 4.57±0.00b	0.78±0.00b 0.00±0.00b 16.82±1.29a	53.30±2.78a 31.89±1.27b 50.91±3.90a

*Data are expressed as mean value of three samples, Mean value±Standard deviation.

[a–c] Show statistically significant differences ($P < 0.05$) between control and treated group in a row.

YE: *Yersinia enterocolitica*, CJ: *Campylobacter jejuni*, KP: *Klebsiella pneumoniae*, SP: *Salmonella* Paratyphi A, C: control group without propolis extract addition, PE: group treated with ethanolic extract of propolis, PW: group treated with aqueous extract of propolis. AMN, ammonia; PUT, putrescine; CAD, cadaverine; SPD, spermidine; PHEN, 2-phenylethyl amine; SPN, spermine

Table 3b. Ammonia and BAs accumulation by Gram-negative food borne pathogen in the absence or presence of propolis extracts (mg/L)

	HIS	SER	TMA	DOP	AGM	Groups
YE	0.99±0.01a 0.63±0.05c 0.78±0.04b	2.66±0.27b 1.10±0.08c 3.93±0.32a	12.27±0.55b 3.84±0.22c 16.77±1.05a	263.42±15.39b 125.20±7.35c 308.47±16.99a	74.88±1.52b 85.09±5.02ab 86.22±2.43a	C PE PW
CJ	1.18±0.03a 0.47±0.01c 0.86±±0.07b	5.79±0.28a 0.73±0.03b 1.02±0.04b	5.39±0.19b 24.97±0.01a 6.12±0.50b	393.80±14.95a 0.00±0.00c 270.91±3.14b	95.50±8.19a 48.31±3.24b 85.92±0.73a	C PE PW
KP	0.38±0.00b 0.42±0.02b 0.84±0.06a	2.48±0.02b 1.10±0.00c 3.71±0.46a	50.34±0.81a 13.40±1.27c 35.32±0.36b	554.84±37.36a 205.63±7.97b 630.83±29.43a	90.81±8.81a 64.23±3.84b 93.57±2.62a	C PE PW
SP	0.70±0.03a 0.31±0.01b 0.77±0.02a	2.24±0.16b 0.77±0.08c 4.95±0.29a	1.25±0.00c 3.15±0.14b 15.25±0.26a	523.52±13.94a 296.97±2.15b 521.92±3.47a	25.57±1.00b 36.07±0.37b 131.67±6.35a	C PE PW

*Data are expressed as mean value of three samples, Mean value±Standard deviation.

[a–c] Show statistically significant differences ($P < 0.05$) between control and treated group in a row.

YE: *Yersinia enterocolitica*, CJ: *Campylobacter jejuni*, KP: *Klebsiella pneumoniae*, SP: *Salmonella* Paratyphi A, C: control group without propolis extract addition, PE: group treated with ethanolic extract of propolis, PW: group treated with aqueous extract of propolis. HIS: histamine, SER, serotonin; TMA, trimethylamine; DOP, dopamine, AGM, agmatine

References

Afrouzan, H., Tahghighi, A., Zakeri, S. and Es-haghi, A. (2018). Chemical Composition and Antimicrobial Activities of Iranian Propolis. Iranian Biomedical Journal, 22, 1, 50-65 [CrossRef]

Al-Ani, I., Zimmermann, S., Reichling, J. and Wink, M. (2018). Antimicrobial activities of European propolis collected from various geographic origins alone and in combination with antibiotics. Medicines, 5, 1, 2. [CrossRef]

Al Bulushi, I., Poole, S., Deeth, H.C., Dykes, G.A. (2009). Biogenic amines in fish: Roles in intoxication, spoilage, and nitrosamine formation. Critical Reviews in Food Science and Nutrition, 49, 369–377.

Apaydin, H. and Gümüş, T. (2018). Inhibitory effect of propolis (bee gum) against *Staphylococcus aureus* bacteria isolated from instant soups. Journal of Tekirdag Agricultural Faculty, 15, 1, 67-75.

Camino Feltes, M. M., Arisseto-Bragotto, A. P. and Block, J. M. (2017). Food quality, food-borne diseases, and food safety in the Brazilian food industry. Food Quality and Safety, 1, 1, 13-27. [CrossRef]

Casquete, R., Castro, S. M., Jácome, S. and Teixeira, P. (2016). Antimicrobial activity of ethanolic extract of propolis in "Alheira", a fermented meat sausage. Cogent Food and Agriculture, 2, 1, 1125773. [CrossRef]

Comas-Basté, O., Latorre-Moratalla, M. L., Sánchez-Pérez, S., Veciana-Nogués, M. T. and del Carmen Vidal-Carou, M. (2019). Histamine and other biogenic amines in food. From scombroid poisoning to histamine intolerance. In Biogenic Amines. IntechOpen. [CrossRef]

Commission Regulation (EC) (2007). No: 1441/2007 Amending regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. Official Journal of European Union L, 322, 12–29.

Connil, N., Breton, Y. L., Dousset, X., Auffray, Y., Rincé, A. and Préevost, H. (2002). Identification of the *Enterococcus faecalis* tyrosine decarboxylase operon involved in tyramine production. Applied and Environtal Microbiology, 68, 3537–3544. [CrossRef]

Da Prada, M., Zurcher, G. and Wuthrich, I.(1988). On tyramine, food, beverages: the reversible MAO inhibitor monoclobemide. Journal of Neural Transmission, 26, 31–36.

De Figueiredo, S.M., Binda, N.S., Almeida, B.D.M., Lemos Abreu, S.R., de Abreu, J.A.S., Pastore G.M., Sato H.H., Toreti V.C., Tapia, E.V. and Park, Y.K. (2015). Green propolis: Thirteen constituents of polar extract and total flavonoids evaluated during six years through RP-HPLC. Current Drug Discovery Technologies, 12, 229–239.

De las Rivas, B., Marcobal, A., Carrascosa, A. and Munoz, R. (2006). PCR detection offood bacteria producing the biogenic amines histamine, tyramine, putrescineand cadaverine. Journal of Food Protection, 69, 2509–2514. [CrossRef]

De Palencia, P. F., Fernández, M., Mohedano, M. L., Ladero, V., Quevedo, C., Alvarez, M. A. and López, P. (2011). Role of tyramine synthesis by food-borne Enterococcus durans in adaptation to the gastrointestinal tract environment. Applied and Environmental Microbiology, 77, 2, 699-702. [CrossRef]

Fernández, M., Linares, D. M., del Río, B., Ladero, V. and Alvarez, M. A. (2007). HPLC quantification of biogenic amines in cheeses: correlation with PCR-detection of tyramine-producing microorganisms. Journal of Dairy Research, 74,3, 276-282. [CrossRef]

Gram, L. and Dalgaard, P. (2002). Fish spoilage bacteria-problems and solutions. Current Opinion in Biotechnology, 13, 262–266. [CrossRef]

Hazem, A., Popescu, C.V., Crisan, J., Popa, M., Chifiriuc, M., Pircalabioru, G.G. and Lupuliasa, D. (2017). Antibacterial efficiency of five propolis extracts on planktonic and adherent microbial strains. Farmacia, 65, 5, 813-818.

Karovičová, J. and Kohajdová, Z. (2005). Biogenic amines in

food. Chemical Papers, 59, 1, 70-79.
Kim, Y. H. and Chung, H. J. (2011). The effects of Korean propolis against foodborne pathogens and transmission electron microscopic examination. New Biotechnology, 28, 6, 713-718. [CrossRef]
Klausen, N.K. and Huss, H.H. (1987). A rapid method for detection of histamine-producing bacteria. International Journal of Food Microbiology, 5, 137-146.
Kubiliene, L., Laugaliene, V., Pavilonis, A., Maruska, A., Majiene, D., Barcauskaite, K. and Savickas, A. (2015). Alternative preparation of propolis extracts: comparison of their composition and biological activities. BMC Complementary and Alternative Medicine, 15, 156, 1-7. [CrossRef]
Kuley, E. and Özogul, F. (2011). Synergistic and antagonistic effect of lactic acid bacteria on tyramine production by food-borne pathogenic bacteria in tyrosine decarboxylase broth. Food Chemistry, 127, 3, 1163-1168. [CrossRef]
Li, L., Wen, X., Wen, Z., Chen, S., Wang, L. and Wei, X. (2018). Evaluation of the biogenic amines formation and degradation abilities of Lactobacillus curvatus from Chinese bacon. Frontiers in Microbiology, 9, 1015, 1-9. [CrossRef]
Liu, Y., Cao, Y., Wang, T., Dong, Q., Li, J. and Niu, C. (2019). Detection of 12 common food-borne bacterial pathogens by TaqMan real-time PCR using a single set of reaction conditions. Frontiers in Microbiology, 10, 222, 1-9. [CrossRef]
Lorenzo, J. M., Martínez, S., Franco, I. and Carballo, J. (2007). Biogenic amine content during the manufacture of dry-cured lacón, a Spanish traditional meat product: Effect of some additives. Meat Science, 77, 2, 287-293. [CrossRef]
Lu, L. C., Chen, Y. W. and Chou, C. C. (2005). Antibacterial activity of propolis against *Staphylococcus aureus*. International Journal of Food Microbiology, 102, 2, 213-220. [CrossRef]
Maintz, L. and Novak, N. (2007). Histamine and histamine intolerance. The American Journal of Clinical Nutrition 85, 1185–1196. [CrossRef]
Majiene, D., Trumbeckaite, S., Pavilonis, A., Savickas, A. and Martirosyan, D. M. (2007). Antifungal and antibacterial activity of propolis. Current Nutrition and Food Science, 3, 4, 304-308.
Marcobal, A., De Las Rivas, B., Landete, J. M., Tabera, L. and Muñoz, R. (2012). Tyramine and phenylethylamine biosynthesis by food bacteria. Critical Reviews in Food Science and Nutrition,52, 5, 448-467. [CrossRef]
Nedji, N. and Loucif-Ayad, W. (2014). Antimicrobial activity of Algerian propolis in foodborne pathogens and its quantitative chemical composition. Asian Pacific Journal of Tropical Disease, 4, 6, 433-437. [CrossRef]
Özogul, F. and Özogul, Y. (2007). The ability of biogenic amines and ammonia production by single bacterial cultures. European Food Research and Technology, 225, 385–394. [CrossRef]
Özogul, F., Kaçar, Ç. and Kuley, E. (2015). The impact of carvacrol on ammonia and biogenic amine production by common foodborne pathogens. Journal of Food Science, 80, 12, M2899-M2903. [CrossRef]
Pobiega, K., Kraśniewska, K., Przybył, J. L., Bączek, K., Żubernik, J., Witrowa-Rajchert, D. and Gniewosz, M. (2019). Growth biocontrol of foodborne pathogens and spoilage microorganisms of food by polish propolis extracts. Molecules, 24, 16, 2965. [CrossRef]
Przybyłek, I. and Karpiński, T. M. (2019). Antibacterial properties of propolis. Molecules, 24, 11, 1-17. [CrossRef]
Satoshi, M., Yoshikazu, I., Yukio, N., Shozo, O., Takashi, S., Yoko, A. and Yoshinori, N. (2005). Identification of caffeoylquinic acid derivatives from Brazilian propolis as constituents involved in induction of granulocytic differentiation of HL-60 cells. Bioorganic and Medicinal Chemistry, 13, 5814–5818. [CrossRef]
Silla-Santos, M. H. (1996). Biogenic amines: their importance in foods. International Journal of Food Microbiology, 29, 2-3, 213-31. [CrossRef]
Sforcin J.M. and Bankova V. (2011). Propolis: Is there a potential for the development of new drugs? Journal of Ethnopharmacology, 133, 253–260. [CrossRef]
Shalaby, A.R. (1996). Significance of biogenic amines to food safety and human health. Food Research International, 29, 7, 675-690. [CrossRef]
Temiz, A., Şener, A., Tüylü, A. Ö., Sorkun, K. and Salih, B. (2011). Antibacterial activity of bee propolis samples from different geographical regions of Turkey against two foodborne pathogens, Salmonella enteritidis and *Listeria monocytogenes*. Turkish Journal of Biology, 35, 4, 503-511. [CrossRef]
Wendakoon, C.N. and Sakaguchi, M. (1995). Inhibition of amino acid decarboxylase activity of Enterobacter aerogenes by active components in spices. Journal of Food Protection, 58, 3, 280-283. [CrossRef]

JAEFS

International Journal of Agriculture, Environment and Food Sciences

e-ISSN : 2618-5946 DOI: 10.31015/jaefs.2019.4.12 www.jaefs.com

Research Article Int J Agric Environ Food Sci 3(4):272-278 (2019)

Production Economy of Pomegranate in Manisa Province

Mehmet Ugurlu[1,*]

[1]Republic of Turkey Ministry of Trade, Turkey

[*]Corresponding Author: mehmet.ugurlu@cbu.edu.tr

Abstract

In this study, pomegranate production economy, input use in pomegranate production, cost, profitability and productivity analyzes were discussed. The survey data for 2018 were obtained from face to face interviews with 82 pomegranate producers selected by a stratified sampling method. In this study, demographic structure, input usage; fertilizer and chemigation usage amounts, machinery and labor use, production activity costs and profitability were examined. As a result of the study, pomegranate production cost was calculated as 1950.4 TL per decare and 2440 kg per decare pomegranate was obtained in response to these production costs. As a result of marketing activities, gross profit was 1496.8 TL per da and net profit was 834.5 TL per decare.

Keywords: Pomegranate, Production economics, Cost, Profitability, Manisa

Introduction

Pomegranate production in Manisa, between 2004 and 2018, both in terms of production area and production amount and number of pomegranate trees with or without fruit showed positive and negative developments. As can be seen in Table 1 as the number of pomegranate trees in fruit-bearing age, it has started to increase gradually since 2004 (35.380 units). In 2013, it increased by 134% compared to the previous year 2012 and by 653% increased (231.198) number of fruit-bearing pomegranate trees were reached. As a result of this rapid change, as of 2014, pomegranate has become one of the most important fruits grown in Manisa province, which has high potential in terms of climate and other geographic and agricultural conditions for many varieties of fruits, especially viticulture. In 2014, 6.214 ton production quantities reaching the highest point of pomegranate production, according to the data 2018 to about 1% of the total amount of pomegranate production in Turkey with 4,867 tons were produced in Manisa (Turkstat 2018).

As can be seen from the statistical information above, increases have been observed compared to the previous year until 2014 and after 2014 there has been a decrease. However, according to the 2004 index, the number of pomegranate trees yielding fruit increased continuously.

Table 2 provides information regarding to number of pomegranate trees not producing fruits (2004-2018). As can be seen from Table 2, although the number of non-fruit trees in Manisa in 2015 decreased by -19% compared to the previous year and increased by 1164% compared to 2004.

Table 3 shows the pomegranate production areas in Manisa between the years 2004-2018. Accordingly, the highest production area was reached in 2014 with 5,568 da. This area means an increase of 1,591% compared to 2004. The year in which the highest increase was compared to the previous year was 2008. In parallel with the increase in the number of fruitless pomegranate trees, the production area increased by 659% in 2008 compared to 2004.

In Table 4, yields per fruit-bearing tree for 2004 to 2018 are given. Accordingly, the yield varies between 18 and 25 kg. However, as shown in Table-7 in our study in the production areas, the yield was determined to be 45.9 kg per fruit-bearing tree. Even if the average yield per tree is 25 kg, which is the highest given statistics, producers cannot afford to pay half of their costs.

Table 5 shows the annual production of pomegranate between 2004 and 2018. Accordingly, the highest production

Cite this article as:
Ugurlu, M. (2019). Production Economy of Pomegranate in Manisa Province. Int. J. Agric. Environ. Food Sci., 3(4), 272-278
DOI: https://dx.doi.org/10.31015/jaefs.2019.4.12
Received: 08 September 2019 **Accepted:** 17 December 2019 **Published:** 24 December 2019
Year: 2019 **Volume:** 3 **Issue:** 4 (December) **Pages:** 272-278
Available online at : http://www.jaefs.com - http://dergipark.gov.tr/jaefs

amount was reached in 2014 with 6,214 tons. This amount means an increase of 802% compared to 2004. Although the increase in pomegranate production in 2014 was parallel to the increase in the number of fruit trees in 2014, it remained low compared to the increase in pomegranate production in 2014. Determination of input usage amounts, costs and revenues in agricultural products is of great importance for micro level producers and economic policy makers. The results of research on agricultural product costs are an important tool that governments can use to determine price policies. In addition, agricultural product costs are widely used in planning activities such as determining the usage levels of physical production inputs, workforce planning, making financing programs, preparing product budgets and investment projects, etc. (Ozkan and Yilmaz 1999; Ozalp and Yilmaz 2013).

In this study, it was aimed to determine the input usage, cost, annual activity results and comparative analysis of pomegranate production in Manisa province and to make profitability and productivity analyzes. In addition, it is aimed to reveal the reasons why pomegranate production is preferred by producers in recent years. Another issue is whether the net profit and agricultural income obtained are sufficient to meet the livelihood and needs of the farmer family. Some values of Manisa pomegranate production are given in the graphs below.

Table 1. Number of fruit pomegranate trees in Manisa province (2004-2018)

Topic	Year	Amount (kg)	Change compared to previous year (%)*	Tendency	Change compared to 2004 (%)*	Tendency
Number of Fruit Producing Trees (Pieces)	2004	35380	100	►	100	►
	2005	36020	2	▲	102	▲
	2006	37640	4	▲	106	▲
	2007	41990	12	▲	119	▲
	2008	42510	1	▲	120	▲
	2009	43010	1	▲	122	▲
	2010	73810	72	▲	209	▲
	2011	91660	24	▲	259	▲
	2012	98619	8	▲	279	▲
	2013	231198	134	▲	653	▲
	2014	243756	5	▲	689	▲
	2015	232890	-4	▼	658	▲
	2016	230781	-1	▼	652	▲
	2017	220126	-5	▼	622	▲
	2018	207496	-6	▼	586	▲

Source: Turkstat (2018) Access Date: 17.04.2019

* The index for the year 2004 was taken as 100

Table 2. Number of pomegranate trees not producing fruits (2015-2018)

Topic	Year	Amount (kg)	Change compared to previous year (%)*	Tendency	Change compared to 2004 (%)*	Tendency
	2015	63430	-19	▼	1164	▲
	2016	61966	-2	▼	1137	▲
	2017	42879	-31	▼	787	▲
	2018	37482	-13	▼	688	▲

Source: Turkstat (2018) Access Date: 17.04.2019

* The index for the year 2004 was taken as 100

Table3. Manisa province pomegranate production area (2004-2018)

Topic	Year	Area (da)	Change compared to previous year (%)*	Tendency	Change compared to 2004 (%)*	Tendency
Production area (da)	2004	350	100	►	100	►
	2005	360	3	▲	103	▲
	2006	545	51	▲	156	▲
	2007	1060	94	▲	303	▲
	2008	2308	118	▲	659	▲
	2009	2660	15	▲	760	▲
	2010	4026	51	▲	1150	▲
	2011	4396	9	▲	1256	▲
	2012	4413	0	▲	1261	▲
	2013	5479	24	▲	1565	▲
	2014	5568	2	▲	1591	▲
	2015	5355	-4	▼	1530	▲
	2016	5276	-1	▼	1507	▲
	2017	4638	-12	▼	1325	▲
	2018	4078	-12	▼	1165	▲

Source: Turkstat (2018) Access Date: 17.04.2019

* The index for the year 2004 was taken as 100

Table 4. Pomegranate yield in Manisa province (2004-2018)

Topic	Year	Amount (kg)	Change compared to previous year (%)*	Tendency	Change compared to 2004 (%)*	Tendency
Yield per fruit producing tree	2004	22	100	►	100	►
	2005	22	0	►	100	►
	2006	25	14	▲	114	▲
	2007	18	-28	▼	82	▼
	2008	21	17	▲	95	▼
	2009	23	10	▲	105	▲
	2010	19	-17	▼	86	▼
	2011	19	0	►	86	▼
	2012	18	-5	▼	82	▼
	2013	25	39	▲	114	▲
	2014	25	0	►	114	▲
	2015	24	-4	▼	109	▲
	2016	23	-4	▼	105	▲
	2017	25	9	▲	114	▲
	2018	23	-8	▼	105	▲

Source: Turkstat (2018) Access Date: 17.04.2019
* The index for the year 2004 was taken as 100

Table 5. Pomegranate production amount in Manisa province (2004-2018)

Topic	Year	Amount (tons)	Change compared to previous year (%)*	Tendency	Change compared to 2004 (%)*	Tendency
Production (tons)	2004	775	100	►	100	►
	2005	783	1	▲	101	▲
	2006	931	19	▲	120	▲
	2007	735	-21	▼	95	▼
	2008	876	19	▲	113	▲
	2009	985	12	▲	127	▲
	2010	1410	43	▲	182	▲
	2011	1785	27	▲	230	▲
	2012	1814	2	▲	234	▲
	2013	5673	213	▲	732	▲
	2014	6214	10	▲	802	▲
	2015	5605	-10	▼	723	▲
	2016	5295	-6	▼	683	▲
	2017	5470	3	▲	706	▲
	2018	4864	-11	▼	628	▲

Source: Turkstat (2018) Access Date: 17.04.2019

* The index for the year 2004 was taken as 100

Material and Method

The main material of this research are data obtained by the survey method in 2018. Data were obtained from the pomegranate producers of especially Salihli district, Köprübaşı, Şehzadeler, Akhisar, Gölmarmara and Alaşehir districts of Manisa. Data represented about 77.1% of the pomegranate production area in Manisa, 79.1% of the amount of pomegranate production and about 78% of the number of fruit pomegranate trees. In addition to the information obtained from previous studies on the subject, the data obtained from provincial and district directorates of agriculture were also used as secondary data.

In the research, only pomegranate production activities of the enterprises were examined. In the research, stratified sampling method was used to determine the number of sample enterprises. In this method, a more healthy and detailed study is possible by separating the main mass into homogenous layers with fewer samples (Gunes and Arikan, 1988). The sampling was based on pomegranate production areas of the producers and the following formula based on Neyman distribution was used to find the number of samples. The number of sample enterprises that should be studied with a 5% deviation from the average and 95% confidence level was found 82.

$n = (\sum NhSh)^2 / N^2 D^2 + \sum Nh\ Sh^2$

In the formula above; n is the sample volume, N is the total number of producers, Nh is the number of producers in the layer. D = d / z, and d is the expected deviation from the average, z is standard normal distribution value while Sh^2 is the layer variance.

An analysis of variance was conducted to determine whether the differences between the various elements related to pomegranate production between plants or producers assessed by land groups were significant. The number of pomegranate producers surveyed by land groups is given in Table 6 below.

Research Findings

This research was carried out by creating land groups of pomegranate production gardens at 0-30 da, 31-60 da, 61-100 da and 101 da.

Manisa has made a great leap forward in pomegranate production by establishing new garden facilities especially since 2008. Naturally, it is possible to see the results of these plants from the earliest third year, but normal production starts at the fifth year and the ideal yield year at the ninth or tenth year.

The planting of pomegranate trees in Manisa in 2008 started to give obvious results in 2013 and 2014. The year 2014 was the highest (6214 tons) production. In 2018, production decreased to 4864 tons. This ratio was approximately 1% of Turkey's pomegranate production. The production in 2012 was 1814 tons. One of the aims of this research is to reveal the causes of this rise and fall.

Demographic and general production information

First of all, some demographic and general information of Manisa pomegranate producers are given in Table 7.

The average age of Manisa pomegranate producers is 47.2 and the average of pomegranate production experiences is 12.7 years. The average size of the family is 4.9 people and the average duration of education is 10.1 school years. In the pomegranate production activity, the average number of trees per decare is 51.4 and the average yield is 45.9 kg per decare.

Input Usage Cases of Manisa Pomegranate Production

As can be seen in Table 8, some input usage values of Manisa pomegranate production activity can be seen. Accordingly, the weighted average use of machinery in pomegranate pro-

duction is 2.56 hours per decare. The lowest machine use was observed in enterprises with production area of 101 dacares and above. As the scale of production increases, machine usage time per unit decreases. In terms of machine use, the difference between land groups was found to be statistically significant (p= 0.02) according to variance analysis.

The weighted average labor force usage period in pomegranate production activity is determined as 31.29 hours per decare. Approximately 80% of this period is used during maintenance and harvesting period. The lowest labor force per unit was possible in enterprises of 101 and above. In terms of labor use, the difference between land groups was found to be statistically significant (p= 0.02) according to variance analysis.

As can be seen in Table 8, the average weight of fertilizer use per decare was 55.92 kg. It is seen that the most fertilizer use belongs to the plants smaller than 30 decares.

It can be said that there is a possibility that the use of fertilizer will change inversely in proportion to the plant size to the lowest possible fertilizer utilization level. In terms of fertilizer use, the difference between land groups was found to be statistically significant (p= 0.01) according to variance analysis.

When the level of chemigation use is considered, it is seen that the active ingredient per decare is 6.99 kg. It is seen that the enterprises with the highest amount of chemigation use belong to the enterprises under the size of 30 as in the use of fertilizer. In terms of chemigation use, the difference between land groups was found to be statistically significant (p=0.002) according to variance analysis.

Manisa province pomegranate production cost elements

Table 9 shows the cost elements within the scope of Manisa pomegranate production activity. Accordingly, the total average cost per decare is 1950.4 TL and 66% of this cost is variable and 34% is fixed expenses.

The highest share among the variable expenditures is firstly harvested with 19.6%, then with 14% chemigation and 8.12% fertilizer. The lowest share belongs to marketing activity with 0.64.

In fixed expenses, the highest expense belongs to land rental cost with 14.2% and the lowest expense belongs to management expense with 1.12%.

Manisa province pomegranate production GPV, cost and profitability values

Table 10 shows the GPV, yield, sales price gross and net profit values of Manisa pomegranate production activity. Accordingly, as of 2018, average selling price of pomegranate is 1.14 TL per kg, yield per decare is 2440.6 kg per decare, GPV per decare is 2786.8 TL, average cost is 0.8 TL per kg, gross profit is 1496.8 TL per decare and net profit is 834.52 TL per decare on average. In terms of GPV, the difference between land groups was found to be statistically significant (p= 0.03) according to analysis of variance and the difference between land groups was statistically significant (p= 0.03) in terms of gross profit.

The highest yield per decare is observed in enterprises with 101 and above with 49.1 kg per tree and the cost belongs to large scale enterprises with a return of 0.62 TL/ kg and gross profit of 2187 TL per decare.

Cost-profitability values of pomegranate production in Manisa province in terms of GPV and net profit

Table 11 shows the cost and profitability values of Manisa pomegranate production activities. Accordingly, the GPV value of the machine use per unit is 9.02 TL, the labor force is 5.55 TL, the average total cost is 1.43 TL and the net profit is 0.43 TL. It is seen that the highest net profit belongs to big enterprises with 0.94 TL and 101 and above, the least net profit belongs to enterprises with 0.40 TL and less than 30 da.

Table 6. Production area, production facility and surveyed facility

Pomegranate production area (da)	Production facility in the layer (pcs)	Surveyed facility (pcs)
1-30	875	51
31-60	395	21
61-100	134	8
101- +	6	2
Total	1410	82

Table 7. Some demographic and general production information of Manisa pomegranate production

Age average (year)	Experience (year)	Family average (person)	Male labor force (person)	Average training (academic year)	Number of pomegranate trees (da/trees)	Yield average (kg/tree)
47.2	12.7	4.9	3.59	10.1	51.4	45.9

Table 8. Some input usage values of Manisa pomegranate production

Pomegranate production area (da)	Machine usage (da/h)	Labor use (da/h)	Fertilizer usage (pure-kg/da)	Chemigation use (active sub kg/da)
0.1-30	2.71	32.18	58.39	7.34
31-60	2.44	30.78	53.41	6.82
61-100	1.97	27.41	47.72	5.32
101- +	1.41	21.88	44.64	5.18
Weighted Average	2.56	31.29	55.92	6.99

Table 9. Manisa province pomegranate production cost elements (TL/da)

Cost Elements	Production Area (10-30 da)	Production Area (31-60 da)	Production Area (61-100 da)	Production Area (100-+ da)	Weighted Average	Ratio (%)
Fertilizer	154	161	177	186	158.3	8.12
Chemigation	267	282	291	302	273.6	14
Electricity-Fuel-Water	241	238	232	183	239.1	12.3
Machine Use	72	69	64	56	70.3	3.61
Labor (Excluding harvest)	129	112	101	97	121.4	6.23
Harvest Labor	386	376	372	368	381.8	19.6
packing	30	32	36	41	31.2	1.60
Marketing	11	14	17	19	12.4	0.64
Variable Charges	1290	1284	1290	1252	1288.2	66
Management Share	18	27	31	38	21.8	1.12
Family Labor Share	210	239	164	28	213.0	10.9
Land Rental Cost	276	279	285	296	277.8	14.2
Fixed Capital Interest	124.4	81.9	82.4	64.1	108.2	5.55
Depreciation	33.5	49.1	68.3	92.7	41.4	2.12
Fixed Costs	661.9	676	630.7	518.8	662.3	34
Total Cost	1951.9	1960	1920.7	1770.8	1950.4	100

Table 10. Manisa province pomegranate production, gross production value (GPV), cost and profitability values

Production area (da)	Yield (tree/da)	Yield (kg/da)	Sale cost (TL/kg)	GPV (TL/da)	Cost (TL/kg)	Gross profit (TL/da)	Net Profit (TL/da)
0-30 da	45.4	2419	1.13	2733.5	0.81	1443.47	781.57
31-60 da	46.4	2456	1,16	2849	0.80	1558.96	882.96
61-100 da	47.7	2518	1,16	2920.9	0.76	1630.88	1000.2
101-+ da	49.1	2850	1.22	3477	0.62	2187	1668.2
Weighted Average	45.91	2440.6	1.14	2786.8	0.80	1496.8	834.52

Table 11. Gross production value (GPV) and cost elements of Manisa province pomegranate production

Production Area (da)	GPV/Machine Cost (TL)	GPV/Labor Cost (TL)	GPV/Total Cost (TL)	GPV/Variable Cost (TL)	GPV/Fixed Cost (TL)	Net Profit/Total Cost (TL)
0-30 da	8.73	5.31	1.40	2.12	4.13	0.40
31-60 da	9.28	5.84	1.45	2.22	4.21	0.45
61-100 da	9.87	6.18	1.49	2.26	4.63	0.52
101-+ da	14.55	7.48	1.77	2.78	6.70	0.94
Weighted average	9.02	5.55	1.43	2.16	4.21	0.43

Conclusion

This study held in Manisa where approximately 1% pomegranate production of Turkey's occurs, comparative analysis for profitability and production levels in pomegranate production were intended. Within the scope of the research, findings related to input use, labor, machine use, fertilizer, chemigation and other inputs were considered and the cost of pomegranate production was determined.

As a result of the research, it was found that 2.56 hours/da machine use, 31.29 hours/da labor, 55.92 kg/da fertilizer as pure substance and 6.99 kg/da chemigation including pesticide as active substance. As a result of statistical comparisons between pomegranate width, production regions and education level groups, significant differences were found in pomegranate production area width groups in decare labor and machine use. This finding reveals that as the scale increases, that is, as the size of the garden increases, the mentioned inputs decrease. Similar results were obtained for chemigation use. However, there was no significant difference in the use of input per decare, regionally and according to the producer's level of education.

In the research area, pomegranate production cost per unit area was determined as 1950.4 TL, 66% of which was variable cost and 34% was fixed cost.

In response to these production costs, 2440.6 kg/da pomegranate yield was obtained. The gross sales value calculated by multiplying the average selling price (1.14 TL/kg) and pomegranate yield was calculated as 2786.8 TL/da. The average gross and net profit values were calculated as 1496.8 TL/ da and 834.52 TL/da, respectively. Medicine and machinery costs per decare decrease as the pomegranate grows. This difference was also statistically significant.

The break-even analysis indicated that net profit from pomegranate production in the region's farms and the minimum monthly income required for the livelihood of the farmer's family (at least 3600 TL/month, for 2018) should produce at least 55 tons and more pomegranates. In this case, the pomegranate production area should be at least 25 da or more. It can be said that the efficiency and profitability levels achieved for pomegranate production are not satisfactory and therefore the pomegranate production areas in the region are rapidly decreasing.

Although it seems adequate income derived from pomegranate production in Manisa compared to Turkey agricultural income, alternative products such as Sultani seedless grapes, higher production cost of pomegranate Manisa' Pomegranate trees will be removed and Sultani seedless grape production will continue to transition.

To date, the increase in pomegranate production has been offset by an increase in domestic consumption and exports.

However, in 2018 conditions, it is observed that the saturation of domestic and foreign demand for pomegranate, both for fresh consumption and for processing, has been reached. In this case, the increase in the supply caused a decrease in the prices of pomegranate both in the domestic market and in export prices by half. While the pomegranate export price was USD 1.1 / kg on average in 2010, it decreased to USD 0.52 / kg in 2018 (Turkstat, 2018).

In this respect, it is critical to continue increasing pomegranate exports both freshly and processed, while increasing productivity and lowering costs.

References

Gunes, T. and Arikan, R. (1998) Statistics of Agricultural Economics, Ankara University Faculty of Agriculture Publications, No:1049. [URL]

İnan, İ. H. (2016) Agricultural Economics and Management, İdeal Kültür Yayıncılık. [URL]

Özalp, A. Yılmaz, İ. (2013) Input usage, profitability and productivity analysis of pomegranate production in Antalya province. Journal of Akdeniz University Faculty of Agriculture, 26(1); 19-26. [URL]

Özkan B. Yılmaz, İ. (1999) Cost Calculations for One Year Plants: Current Situation, Problems and Suggestions. Journal of Agricultural Economics 4: 64-80 [URL]

TURKSTAT. Access date: April 17, 2019. [URL]