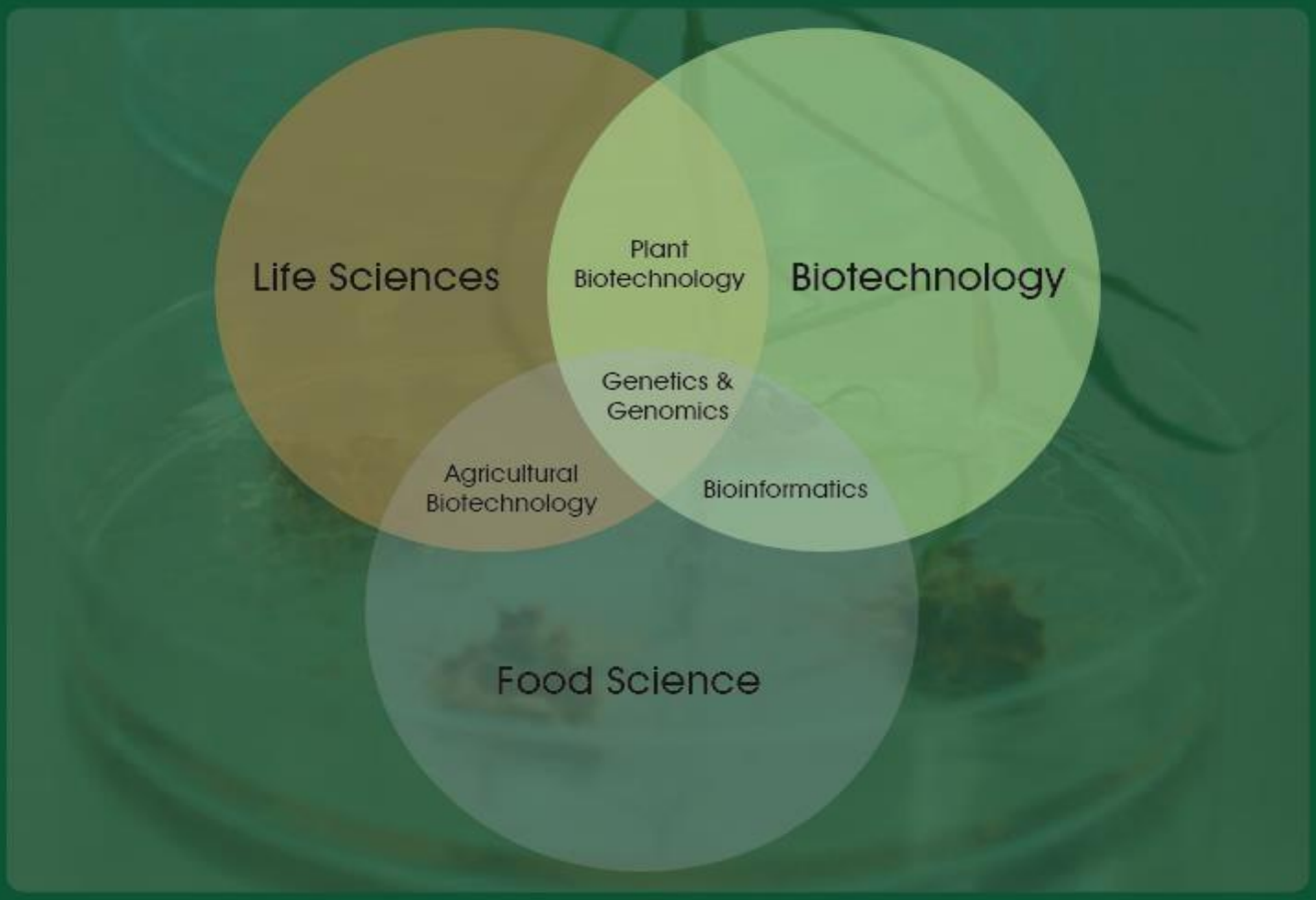
International Journal of Life Sciences and Biotechnology

e-ISSN:2651-4621



Volume/Cilt:7 **2024** **Issue / Sayı:2**

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Yazilarin yasal ve hukuki sorumluluğu yazarlara aittir. Tüm haklari saklidir.
Derginin hiçbir bölümü, yazili ön izin olmaksizin ve dergi adina referans gösterilmeden herhangi bir formatta çoğaltilamaz veya kullanilamaz.

From The Editor;

Dear Readers and Authors,

As “İnternational Journal of Life Sciences and Biotechnology”, we are pleased and honored to present the 19th issue of the journal. "İnternational Journal of Life Sciences and Biotechnology" is an international double peer-reviewed open access academic journal published on the basis of research- development and code of practice.

The aims of this journal are to contribute in theoretical and practical applications in relevant researchers of Life Sciences, Biology, Biotechnology, Bioengineering, Agricultural Sciences, Food Biotechnology and Genetics institutions and organizations in Turkey, and to publish solution based papers depending on the principle of impartiality and scientific ethics principles, focusing on innovative and added value work, discussing the current and future.

With these thoughts, We are especially thankful to academicians honoring with the articles, valuable scientists involved in editorial boards and reviewers for their contributions to the evaluation processes with through their opinions/ideas/contributions/criticisms in the second issue of 2024 "İnternational Journal of Life Sciences and Biotechnology". Hope to see you in the next issue…

15. 08. 2024
Editor in Chief
Prof. Dr. Ali Aslan

Editörden;

Değerli okurlar ve yazarlar,

“İnternational Journal of Life Sciences and Biotechnology” olarak dergimizin on dokuzuncu sayısını yayın hayatına sunmaktan mutluluk ve onur duyuyoruz. “İnternational Journal of Life Sciences and Biotechnology” dergisi araştırma- geliştirme ve uygulama ilkeleri baz alınarak yayınlanan uluslararası hakemli açık erişimli akademik bir elektronik dergidir.

“İnternational Journal of Life Sciences and Biotechnology” dergisi Yaşam Bilimleri, Biyoloji, Biyoteknoloji, Biyomühendislik, Ziraat Bilimleri, Gıda Biyoteknolojisi ve Genetik alanlarındaki ilgili araştırmacılara, kurum ve kuruluşlara teorik ve pratik uygulamalarda katkı sağlamayı, tarafsızlık ve bilim etiği ilkelerine bağlı kalarak çözüm temelli, yenilikçi ve katma değeri olan çalışmalara odaklanan, günceli ve geleceği tartışan çalışmaların yayınlanmasını hedeflemektedir.

Bu düşüncelerle 2024 yılı ikinci sayısını yayınladığımız “Internatıonal Journal of Lıfe Sciences and Biotechnology” dergisini, makaleleri ile onurlandıran akademisyenlere, Fikir / Görüş / Öneri / Katkı ve Eleştirileri ile değerlendirme süreçlerine katkılarından dolayı hakem ve yayın kurullarında yer alan kıymetli bilim insanlarına yürekten teşekkür ediyoruz. Bir sonraki sayıda görüşmek ümidiyle…

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Contents/ İçindekiler

Research Articles/ Araştırma Makaleleri

Review Articles / Derleme makaleleri

International Journal of Life Sciences and Biotechnology
Bozkurt, et al., Determination of Fatty Acid Composition of *Silurus triostegus* Heckel, 1843 and *Arabibarbus grypus* (Heckel, 1843) Species Living in Ilısu Dam Lake (Batman/TÜRKİYE). International Journal of Life Sciences and Biotechnology, 2024 7(2): p. 74-79
https://doi.org/10.38001/ijlsb.1415281
Araştırma Makale/ Research article

Determination of Fatty Acid Composition of *Silurus triostegus* Heckel, 1843 and *Arabibarbus grypus* (Heckel, 1843) Species Living in Ilısu Dam Lake (Batman/TÜRKİYE)

Ramazan Bozkurt[1], Arslan Yusuf Yüksel[2], Muhammed Yaşar Dörtbudak[3], Arif Parmaksiz[4*]

ABSTRACT
In this study, fatty acid compounds in the muscle tissues of the economically important Mesopotamian catfish (*Silurus triostegus*, Heckel, 1843) and Shabut (*Arabibarbus grypus*, (Heckel, 1843)), which live in the Ilısu Dam Lake on the Tigris River, were investigated. For fatty acid analysis, muscle tissues were taken from the samples obtained from local fishermen, placed in tubes and lipids were obtained by applying chloroform/methanol method. The methylation steps of fatty acids in the samples were performed in accordance with TS EN ISO 12966:2 (TS EN ISO 12966-2., 2017) method. The total fatty acid content of saturated fatty acids (SFA) value was detected as 27,97 ± 0,26 in *A. grypus*, 32.46 ± 0.37 in *S. triostegus*, The value of Monounsaturated Fatty Acids (MUFA) varied between 42.63 ± 0.6% in *A. grypus* and 41.5 ± 0.51% in *S. triostegus*. Total polyunsaturated polyunsaturated acids (PUFA) ranged between 21.83 ± 1.49 for *A. grypus* and 25.82 ± 1.94 for *S. triostegus*. MUFA values in all samples were higher than SFA and PUFA values for both fish species. Docosa Hexaenoic Acid (DHA) and Eicosa Pentaenoic Acid (EPA) are commonly consumed omega-3 types. *A. grypus* and *S. triostegus* are within the recommended limits for EPA (C20:5n3) and DHA (C22:6n3). For *A. grypus*, ω3/ω6 values were (1,06); for *S. triostegus* ω3/ω6 values were (1.38). When the ω3/ω6 values of both fish species were analysed for human health, it was determined that the ω3/ω6 values of omega fatty acids *A. grypus* and *S. triostegus* were above 1. As a result information on the composition of 20 types of fatty acids and ω3/ω6 amounts were obtained in muscle tissue of *A. grypus* and *S. triostegus* species living in Ilısu Dam Lake and most preferred by local people. The levels of fat and its components in these species, which may cause different problems when accumulated in the body as a result of changing consumption habits, were indicated in the study.

ARTICLE HISTORY
Received
5 January 2024
Accepted
20 February 2024

KEYWORDS
Fatty acid composition,
Arabibarbus grypus,
Silurus triostegus,
Ilısu dam lake,
MUFA,
PUFA,
ω3/ω6

Introduction

The rapid increase in the world population leads to a continuous increase in food demand [1]. While terrestrial resources are insufficient with this increase, fish foods play an important role as an alternative food source [1]. With their high nutritional quality, fish are becoming an integral part of the human diet. Especially in developing countries, fisheries and aquaculture play a critical role in providing both food and income [1,2,3]. Fish is one of the most natural sources of protein, fatty acids, vitamins and minerals, which are essential components for the healthy growth and functioning of the body metabolism [4].
Omega-3 (ω-3) fatty acids are one of the important nutrients that should be taken into the body for a healthy life and protection against diseases. These are fatty acids that are essential for the body and cannot be produced in the human body. Therefore, fish is important to get omega-3 fatty acids [5]. However,

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preferences and changes in dietary habits have increased the imbalance in the ratio of omega-6 to omega-3, especially by increasing the consumption of omega-6 fatty acids and decreasing the consumption of omega-3 [6]. Within the scope of the Southeastern Anatolia Project, a series of dams and hydroelectric power plants were built on the Tigris and Euphrates Rivers. Ilısu Dam is a part of Turkey's Southeastern Anatolia Project (GAP) and is a dam located on the Tigris River. There are many fish species in the Tigris River and the Ilısu Dam Lake built on it. It is located in the main river bed, 65 km above the border of Syria and Iraq. Among these species are Mesopotamian Catfish (*Silurus triostegus*) and Shabut (*Arabibarbus grypus*), which have high economic value and are frequently fishing by fishermen. Since these species are found in the Tigris and Euphrates basins, they contribute significantly to the local economy [7]. These two species belong to the Cyprinidae family and live in the Euphrates and Tigris river systems [8].

Fish play an important role in the human diet and nutrients, especially omega-3 fatty acids from seafood, are vital for our health. However, changes in dietary habits can lead to health problems by increasing the imbalance in the ratio of omega-6 to omega-3. Therefore, a more detailed evaluation of the nutritional quality of fish is needed, especially for species living in regions such as the Tigris River and Ilısu Dam Lake. Such research may help people to maintain a healthier and more balanced diet. In this study, it was aimed to determine the nutritional value in terms of fatty acid content by taking muscle samples of *S. triostegus* and *A. grypus* species living in Ilısu Dam Lake, fishing and trading by fishermen.

Material and Methods

The fish samples in the study were purchased by fishermen from fishermen fishing at the Ilisu Dam (Tigris) in February 2022. The fish caught were *A. grypus* (Shabut) and *S. triostegus* (Mesopotamian spring). Fish were taken randomly.

Material

The fish in the study were caught at the Ilısu Dam (Tigris) in February 2022 and purchased from fishermen in Hasankeyf (Batman). *A. grypus and S. triostegus* fishes in the study are economically important species that are consumed by the local people. Fish samples were selected randomly. For fatty acid analysis of the fish samples, muscle tissues were taken from each of the fish samples and placed in tubes.

Methods

Analysis of fatty acids

The lipids of each fish sample were extracted with chloroform/methanol (2:1 v/v) according to the method [9]. The methylation steps of fatty acids in the samples were carried out in accordance with the method of TS EN ISO 12966:2 [10]. For the analysis of fish samples, 100 mg of oil sample was weighed in a 10 ml screw cap test tube. Approximately 2 ml of isooctane and 100 µl of 0.2 M methanolic KOH solution were added. The solutions in the tubes were mixed using a vortex device. Mixing of the solutions in the tubes took 1 minute. 2 ml of 40% NaCl solution was added to the test tube and mixed with vortex again. The isooctane phase in the study was placed in a flask. Then approximately 1 g of sodium hydrogen sulphate was added and shaken and stirred. After a period of approximately 30 minutes, approximately 1 µL of the sample was taken from the top and syringed into gas chromatography (GC) kits.

Gas chromatography

After the necessary laboratory procedures, the samples taken from the dorsal muscles of the fish were syringed into the GC (Thermo type Trace GC form Gas Cromatography) device for analysis. The fatty acids in the fish samples were analysed by Thermo type Trace GC model with FID sensor. A 60 m HP-88 capillary column was used to analyse the samples. The sensor and injection block temperatures were regulated at 280 °C and 250 °C. The column was subjected to alternating temperatures. Firstly, it was kept at a temperature of 50 ºC for about 2 minutes. Then it was increased to 180 ºC with an increase of 20 ºC/min. It was then immediately increased to 230 ºC with an increase of 5 ºC/min. This temperature programme was maintained for approximately 5,5 min. The amount of syringing (injection) in the experiment was 1 µL and the division scale was programmed as 1/50.

Analysing the data

Twenty types of fatty acids were analysed in Ilısu Dam Lake. In the calculations made in the studies, "mean ± standard error" values are used only to determine certain characteristics of a group (such as age, height, length, weight); if it is desired to learn the difference in a relationship between different groups, the "mean ± standard error" form of calculation of the values obtained becomes more meaningful [11]. The data obtained from the study were transferred to the table as the mean ± standard error of the gas chromatographic assay data in the form of percentage area (%). The determination of the differences between the data between the

fish groups was determined by statistical evaluation (One-Way ANOVA- SPSS) at significance levels of $p<0,05$ according to the significance level of 0,05 (Table 2).

Table 1 Mean weight (g)±SD and mean length (mm)±SD values of the two fish species examined in the study (SE: Standart Error).

Species	**Species name**	**Number of samples**	**Mean weight (g) ± SE**	**Average standard length (cm)± SE**
Shabut	*A. grypus*	12	520.67± 367	420±95,54
Mesopotamian catfish	*S. triostegus*	12	605.17±436.62	472.31±135

Results

Fish meat, which has an important place in the human diet, is a good source of fatty acids. Fatty acid composition of fish may vary depending on many reasons such as fish species and geographical conditions. In this study, fatty acid types and ratios of the two fish species living in Ilısu Dam Lake and consumed most by people were determined and shown in (Table 2).

In terms of total fatty acids in the fish samples, the fatty acid ratio in S. triostegus (Catfish) species (99.78 %) was higher than *A. grypus* (Shabut) species (92.43 %). When A. grypus iω3/ω6 ratios in Ilısu Dam Lake and Atatürk Dam Lake [12]. Were compared, they were between 2.4± 0.1 - 4.8± 0.1. Since Ilısu Dam Lake has just started to fill up, these values were found to be low. When the proportional amounts of lipid values in fish are compared with each other, ω-3/ω-6 ratio is preferred. The ω-3/ω-6 ratios of fatty acids in fish vary between 1-5. Considering the WHO/FAO recommendation, the most realistic diet and ω3/ω6 ratio should be 5:1 or slightly less [12,13]. SFA, MUFA and PUFA fatty acid values (%) of *A. grypus* are shown in (Figure 1).

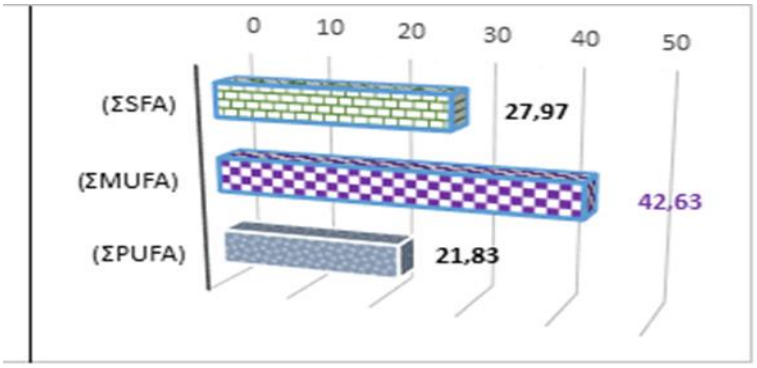

Fig 1 Total fatty acid values of *A. grypus* (%)

When total fatty acid values of *A. grypus* were analysed, the highest value was found in total MUFAs (42,63 %) and the lowest value was found in total PUFAs (21,83 %). SFA, MUFA and PUFA fatty acid values (%) of *S. triostegus* are given in (Figure 2).

When the total fatty acid values of *S.triostegus* were analysed in Figure 2, the highest value was recorded as total MUFA (41,5 %) and the lowest value was recorded as total PUFA (25,82 %). For fish samples, monounsaturated fatty acids (MUFA) were the highest fatty acid group in all samples analysed, followed by saturated fatty acids (SFA) and polyunsaturated fatty acids (PUFA). It is that people's own bodies cannot produce and must obtain from outside Omega-3 fatty acids in the form of alpha-linolenic acid (C18:3n3; ALA), eicosapentaenoic acid (C20:5n3; EPA) and docosahexaenoic acid (C22:6n3; DHA) are called essential or basic fatty acids (TYAs). ALA (C18:3n3), one of the Omega-3 fatty acids taken from outside with food, is then converted into EPA and DHA by our body [15]. It is beneficial for people to consume Omega-3 oils in order to lead a healthier life. Vigorous and adult people can get at least 0.5-1 g of omega-3 per day by consuming fish at least twice a week [16]. Total saturated fatty acid (ΣSFA) values in *A. grypus*

(Shabut) were found to be 27.97 ± 0.26 in our study in the Ilısu Dam Lake. These data were found as 31.07±0.70 in Atatürk Dam Lake [17].

Table 2 Fatty acid ratios (%) in muscle tissues of *A. grypus* and *S. triostegus* individuals*

Fatty Acids	***A. grypus, Mean ± S.E***	***S. triostegus, Mean ± S.E***
(C12:0) Lauric Acid	0,16 ± 0,1[a]	0,44 ± 0,13[a]
(C14:0) Myristic Acid	2,35 ± 0,46[a]	2,11 ± 0,21[a]
(C15:0) Pentadecanoic Acid	0,35 ± 0,4[a]	0,67 ± 0,06[b]
(C16:0) Palmitic Acid	19,61 ± 0,59 [a]	20,21 ± 1,36[b]
(C17:0) Heptadecanoic Acid	0,47 ± 0,51[a]	0,82 ± 0,10[b]
(C18:0) Stearic Acid	4,29 ± 0,74[a]	7,05 ± 0,37[b]
(C20:0) Arachidic Acid	0,39 ± 0,2[a]	0,5 ± 0,07[b]
(C22:0) Behenic Acid	0,35 ± 0,21[a]	0,66 ± 0,09[b]
Total Saturated Fatty Acid (ΣSFA)**	27,97 ± 0,26	32,46 ± 0,37
(C14:1) Myristoleic Acid	0,23 ± 0,16[a]	0,31 ± 0,06[b]
(C16:1) Palmitoleic Acid	5,29 ± 0,93[a]	6,69 ± 0,74[b]
(C17:1) cis-10-Heptadecenoic Acid	0,5 ± 0,64[a]	0,73 ± 0,06[b]
(C18:1n9c) Oleic Acid	35,5 ± 0,94[a]	32,1 ± 1,26[b]
(C20:1n9) cis-11-Eicosenoic Acid	0,76 ± 0,34[a]	0,9 ± 0,08[a]
(C24:1n9 Nervonic Acid)	0,35 ± 0,43[a]	0,77 ± 0,06[b]
Total Monounsaturated Fatty Acid (ΣMUFA)**	**42,63 ± 0,6**	41,5 ± **0,51**
(C18:3n3) A-Linolenic Acid	3,01 ± 0,79[a]	4,27 ± 1,01[b]
(C20:5n3) cis-5,8,11,14,17-Eicosapentaenoic Acid (EPA)	2,37 ± 0,7[a]	3,24 ± 0,26[b]
(C22:6n3) cis-4,7,10,13,16,19-Docosahexaenoic Acid (DHA)	5,85 ± 1,42[a]	7,42 ± 0,71[b]
Σ ω-3	**11,23** ± 1,13	**14,93** ± 1,31
(C18:2n6c) Linoleic Acid	8,15 ± 0,7[a]	7,01 ± 0,83[b]
(C20:2) cis-11,14-Eicosadienoic Acid	0,25 ± 0,14[a]	0,37 ± 0,50[b]
(C20:4n6) Arachidonic Acid	2,2 ± 0,85[a]	3,51 ± 0,23[b]
Σ ω-6	10,6 ± 0,57[a]	10,89 ± 0,37[a]
Total Polyunsaturated Fatty Acid (ΣPUFA)**	21,83 ± 1,50	25,82 ± 1,95
ω-3/ω-6	1,06	1,38
EPA+ DHA	8,22	10,66
PUFA/SFA	**0,79**	0,80
Σ Fatty Acid	92,43	99,78

* The data identified with the same letters in each row are not different from each other at the P>0.05 probability level.
** (ΣSFA): Total Saturated Fatty Acid; ΣMUFA: Total Monounsaturated Fatty Acid; ΣPUFA: Total Polyunsaturated Fatty Acid

Total monounsaturated fatty acids (ΣMUFA) data in *S. triostegus* were found as 32.46 ± 0.37. These data were found as 21,86 in Tigris River [17].The total monounsaturated fatty acid (ΣMUFA) data of *A. grypus* and *S. triostegus* were 42.63 + 0.6 and 41.5 + 0.51, respectively. In this study in Ilısu Reservoir, total monounsaturated was detected in *A. grypus* and *S. triostegus*. The total monounsaturated fatty acids (ΣMUFA) data for *A. grypus* (Shabut) were 42.63 + 0.6 and 41.5 + 0.51 for *S. triostegus*. The ΣMUFA values for *A. grypus* were found to be 39.96±0.18 in Atatürk Reservoir [17]. In another study in Ataturk Reservoir, it was found between 35.2±0.2 - 44.2±0.0, The (ΣMUFA) values in *S. triostegus* were 41.5 ± 0.51. [12]. In a study in Tigris River, they found between 37 - 38.71 [18]; in a study in Atatürk Dam Lake, they found between 23.01 ± 1.2 - 37.21 ± 1.43 [7].

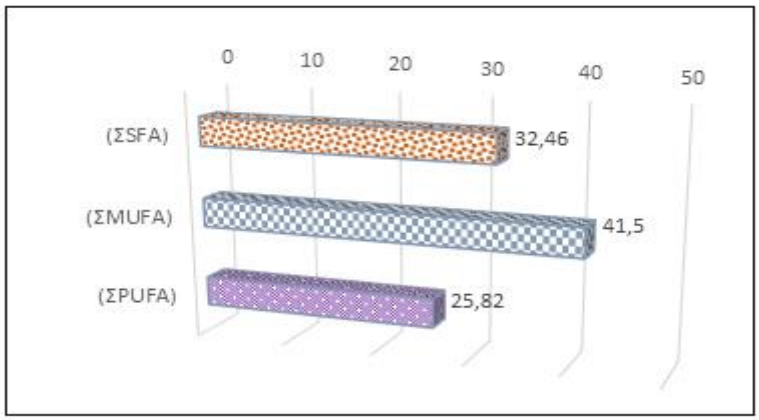

Fig 2 Total fatty acid values (%) of *S. triostegus*

The Σ PUFA values of polyunsaturated fatty acids were determined as 21.83 ± 1.50 for *A. grypus* and 25.82 ± 1.95 for *S. triostegus*, respectively. Σ PUFA values for *A. grypus* were found as 28,85 ± 0,68 in Atatürk Dam Lake [7]. Σ PUFA data for *S. triostegus* were found as 16, 94 in Tigris River [19].

Conclusion and Discussion

In the study, total monounsaturated fatty acids (MUFA) values in the muscle tissues of both fish samples were the highest. These fatty acids were followed by saturated (SFA) and polyunsaturated fatty acids (PUFA) values, respectively. The EPA value (C20:5n3) in *A. grypus* was 2.37 ± 0.7 and 3.24 ± 0.26 in *S. triostegus*. The DHA value (C22:6n3) in *A. grypus* was 5.85 ± 1.42 and 7.42 ± 0.71 in *S. triostegus*.
Oleic acid values in both fish (C18:1n9c) were the highest (*A. grypus*: 35,5 ± 0,94; *S. triostegus* 32,1 ± 1,26). The highest values (C16:0) of palmitic acid (*A. grypus*: 19,61 ± 0,59; *S. triostegus* 20,21 ± 1,36) followed the Oleic acid data in *A. grypus* and *S. triostegus* species. PUFA/SFA ratios were 0.79 for *A. grypus* and 0.80 for *S. triostegus.*
Total Σ ω3 values were 11.23 ± 1.13 in *A. grypus*, 14.93 ± 1.31 in *S. triostegus*; Σ ω6 values were 10.6 ± 0.57 (*A. grypus*), 10.89 ± 0.37 (*S. triostegus*). Total fatty acids (Σ Fatty Acid) values in the samples were 2.43 in *A. grypus* and 99.78 in *S. triostegus*. The ω3/ω6 values were 1,06 in *A. grypus* and 1,38 in *S. triostegus*. In terms of ω3/ω6, *A. grypus* and *S. triostegus* were 1.06 and 1.38, respectively. When the ω3/ω6 data of both fishes were analysed, it was seen that *S. triostegus* Omega-3 fatty acid ratio was slightly higher than *A. grypus*. Although the ratio of ω3/ω6 Omega-3 fatty acids in both samples was slightly above 1, both fish (*A. grypus* and *S. triostegus*) species can be recommended as an important food source for human nutrition.
The DHA and EPA amounts of both species in this study in Ilısu Dam Lake were found to be close to each other. The amount of PUFA in S. triostegus was higher than *A. grypus.* According to these results, S.triostegus is a better source of Omega-3 than *A. grypus* species in terms of ω3. Polyunsaturated fatty acids (PUFA) constitute the ω3 sources of seafood and especially fish. Although the amounts of alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in this group vary among fish species, they are still the most important sources of human nutrition. Alpha-linolenic acid (ALA) is a plant-based essential omega-3 source that should be consumed with food. In addition, EPA and DHA, which humans cannot synthesise, are easier to meet from fish. Omega-3, which the body cannot get enough and has many health benefits, should be taken in sufficient amounts in the form of some plants, fish, some algae varieties and fish oils [20,21,22].
As a result of this study, information on the composition of 20 types of fatty acids and ω3/ω6 amounts of *Arabibarbus grypus* and *Silurus triostegus*, which live in Ilısu Reservoir and are consumed in the region, were determined.

Funding
The author did not receive support from any organization for the submitted work.

Data Availability statement
The author confirms that the data supporting this study are cited in the article.

Compliance with ethical standards

Conflict of interest / Çıkar çatışması
The author declare no conflict of interest.

Ethical standards
The study is proper with ethical standards.

Authors' contributions
During the study, The formulation of the idea or hypothesis for the manuscript, the supervision and responsibility for the organisation and progress of the manuscript was carried out by Ramazan Bozkurt, the organisation and reporting of the data by Arif Parmaksız, the explanation and presentation of the findings by Muhammed Yaşar Dörtbudak, the drafting of the manuscript by Arslan Yusuf Yüksel and the evaluation of the manuscript before submission not only in terms of spelling and grammar but also in terms of intellectual content by all authors.

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International Journal of Life Sciences and Biotechnology
Mogol G., Gultakti I.E., Akarsu M., Mikro-Kabarcığın Silika ile Kapsüllenerek Mukavemetinin Artırılması. International Journal of Life Sciences and Biotechnology, 2024 7(2): p. 80-87.
https://doi.org/10.38001/ijlsb.1423127
Araştırma Makale/ Research article

Mikro-Kabarcığın Silika ile Kapsüllenerek Mukavemetinin Artırılması

Gizem Mogol[1], Ibrahim Emre Gultakti[1*], Murat Akarsu[1]

ÖZET
Mikro-kabarcıklar (MKlar) tıbbi ultrason görüntülemede yüksek kontrast sağlayabilme yetenekleri ile dikkat çekmektedir. MKlar gaz çekirdeklerinin sıkıştırılması ve kanın ultrasonla etkileşimi sonucu deforme olarak, MKların kabuğundaki büzülme-genleşme hareketleri ile yaygın bir kontrast ajanı olarak kullanılmaktadır. MKların çekirdek ve kapsül yapısı, kabarcığın stabilitesini ve bununla beraber etkinlik süresine etki etmektedir. Literatürdeki çalışmalar, farklı Q değerlerine sahip gaz çekirdek yapılı MKların lipit, protein, polimer ve sürfaktan gibi malzemelerle kapsüllendiğini göstermektedir. Bu kapsülasyon malzemeleri ile oluşturulan MKların ise tıbbi ultrasonik görüntülemede kullanılabilmesi için yeterli yapısal stabiliteye, etkin parçacık boyutuna ve yüksek kontrasta aynı anda sahip olması beklenmektedir. Bu araştırmada ise daha önce literatürde çalışılmamış olan n-pentan (C_5) ve perfloropropan (C_3H_8) gazlarının sol-jel yöntemiyle silika (SiO_2) kapsülasyonu yapılarak, stabil MKlar elde edilmesi ve kontrast ajanı olarak kullanılması hedeflenmiştir. İdeal olarak belirlenen SiO_2-C_5 sistemleri, emülsiyon içinde 7 gün, hava ile etkileşiminde ise 10 dakika boyunca stabilitesini koruduğu görülmüştür. SiO_2-C_3H_8 sistemleri için, çekirdek yapı miktarı olan C_3H_8'in 1,5 kat arttırılması ile ortalama hidrodinamik parçacık boyutunun 5,556 µm'den 1,281 µm'ye düştüğü ve zeta potansiyel değerlerinin sırasıyla -21,2 mV ve -41,9 mV olduğu görülmüştür. İdeal SiO_2-C_3H_8 sisteminin 15 güne kadar emülsiyon içinde, 15 dakika da hava ile etkileşimi sonrasında stabilitesini koruduğu görülmüştür.

MAKALE GEÇMİŞİ
Geliş
21 Ocak 2024
Kabul
28 Mart 2024

ANAHTAR KELİMELER
Kontrast ajanı,
Mikro kabarcık,
Mikrokapsül,
Silika,
Sol-Jel

Increasing the Strength of the Micro-bubble by Encapsulating with Silica

Microbubbles (MKs) have garnered attention in medical ultrasound imaging due to their ability to provide high contrast. These MKs deform when the gas core is compressed and when they interact with blood in the capillaries under ultrasound exposure. The deformation of MKs, characterized by the contraction-expansion movements of the MKs shell, has made them a widely used contrast agent. The core and shell structure of MKs significantly influence their stability and, consequently, their effectiveness. Studies in the literature have shown that MKs with gas cores having different Q values are encapsulated with materials such as lipids, proteins, polymers, and surfactants. These encapsulated MKs are expected to possess structural stability, effective particle size, and high contrast simultaneously to be suitable for medical ultrasound imaging. In this study, previously unexplored n-pentane (C_5) and perfluoropropane (C_3H_8), were encapsulated within silica (SiO_2) using the sol-gel method to obtain stable MKs for use as contrast agents. The ideally determined SiO_2-C_5 systems have been observed to maintain their stability for 7 days within the emulsion and for 10 minutes in interaction with air. For SiO_2-C_3H_8 systems, increasing the amount of the core structure, C_3H_8, by 1.5 times resulted in a reduction of the average hydrodynamic particle size from 5.556 µm to 1.281 µm, and the zeta potential values were observed to be -21.2 mV and -41.9 mV, respectively. The ideal SiO_2-C_3H_8 system was observed to maintain its stability for up to 15 days within the emulsion, and for 15 minutes after interaction with air.

ARTICLE HISTORY
Received
21 January 2024
Accepted
28 March 2024

KEY WORDS
Contrast agent,
Micro bubble,
Micro capsule,
Silica,
Sol-Gel

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Giriş

Tıbbi çalışmalarda, vücutta etkin ve stabil kontrast ajanlarının geliştirilmesi için, jelatinle kapsüllenmiş mikro kabarcıklar (MKlar) [1], gazla birleştirilmiş lipozomlar [2], sonikasyon ile denatüre olmuş proteinler [3], florlarmış organik bileşikler içeren emülsiyonlar [4] gibi birçok farklı alanda kapsamlı araştırmalar yapılmıştır. Gaz halindeki çekirdeklerin sıkıştırılması sonucu, kılcal damarlar içinden geçen kan ultrason ile hızlıca deforme olabilmektedir. Bu deformasyonlar, ultrasona maruz bırakılan sıkılaştırılmış ve kapsüle edilmiş gaz olan MKların kabuğunda büzülme ve genişlemelere neden olarak, ultrason görüntüleme sırasında daha yüksek kontrast sağlamasına ve hedefe ilaç salınımı veya gen iletimi gibi uygulamalarda kullanılmasına olanak tanımaktadır [5, 6].

MKlar genellikle kullanım alanlarına göre 0,1-10 μm arasında boyutlara sahip olup, son yıllarda ultrason görüntülemede kontrast arttırıcı ajan ve güdümlü ilaç salınımı gibi birçok farklı biyomedikal uygulamalarda kullanılmaktadır [7]. Aynı zamanda, ilaç ve genler için intravenöz O_2 verme ajanı olarak [8], trombolik ajan olarak ve kan-beyin bariyerini aşarak ilaç iletimi için dağıtım aracı olarak da kullanım alanları mevcuttur [9].

MKların biyomedikal uygulamalardaki kullanımları, kabarcık boyutu, biyouyumluluk ve stabilite gibi faktörlere bağlıdır. Özellikle in-vivo kullanımda, MKların çapı tercihen 1-5 μm arasında dar bir boyut aralığında olmalıdır. Ayrıca, MKların stabilitesi ve raf ömrü de endüstriyel üretim açısından önem arz etmektedir. Bu faktörler kabuğun fiziko-kimyasal özelliği ve kapsüllenen gaz ile çevreleyen sulu ortamın taşıma özelliği ile etkileşime girebilmekte ve buna göre değişkenlik gösterebilmektedir [10, 11, 12, 13].

Bir gazın yoğunluğu, çözünürlüğü ve molar hacminden yola çıkarak, MKların stabilitesini Denklen 1' de verilen matematiksel bir ifade ile hesaplanabilmekte ve kabarcık boyutu-hayatta kalma zamanı, gaz molekül ağırlığına göre tahmini Q değeri gibi bulunabilmektedir [11]. Q değeri 5'ten büyükse, X gazından oluşan MKlar, mikro hava kabarcıklarına göre daha kararlı ve çözelti içinde daha uzun süre hayatta kalabilirler. Q değeri ne kadar artarsa kabarcıkları daha kararlıdır ve daha uzun bir şekilde patlamadan stabil kalabilirler. Bu, tıbbi uygulamalarda daha uzun süreli ve iyi bir kontrast sağlamaktadır [14].

$$Q = 4,0 \times 10^{-7} [p_x / c_x \times D_x] \quad (1)$$

Literatürde MKlar lipid, protein, polimer gibi koruyucu bir kabuğun, oksijen, perflorokarbon veya kükürt heksaflorür gibi çeşitli gazları kapsüllemesiyle meydana gelmektedir. Lipid, protein, polimer kapsülasyonları MK yapısına fazla rijitlik kazandırarak, kontrastı ve stabiliteyi düşürme gibi çeşitli dezavantajlar taşımaktadır [11].

Bu çalışma, literatürde çalışılmamış olan yüksek bir Q-değerine sahip perfloropropan ile daha düşük bir Q-değerine sahip n-pentan kullanılarak sentezlenen MKların, silika ile kapsüllenerek, biyomedikal alanda kullanılması amacıyla 1-5 μm aralığında stabil SKMK (silika kapsül mikro kabarcık) emülsiyonlarının oluşturulması hedeflenmiştir.

Materyal ve Metot

Malzemeler ve karakterizasyon

Çekirdek stabilizasyonu için perfloropropan (%99 saf) (C_3F_8) ABCR' den ve n-pentan (%99 saf) (C_5H_{12}) Merck'ten temin edilmiştir. Silis kaynağı tetraetoksisilan (%99 saf) ($C_8H_{20}O_4Si$) Alfa Aesar' dan temin edilmiştir. Sürfaktan olarak tween 85 ($C_{60}H_{108}O_8 \cdot (C_2H_4O)_n$, Merck'ten, capstone FS-30 Dupont'dan, D-sorbitol ($C_6H_{14}O_6$) Sigma Aldrich'den temin edilmiştir. Katalizör olarak kullanılan amonyak (NH_3) (%24 saf) Birpa' dan temin edilmiştir.

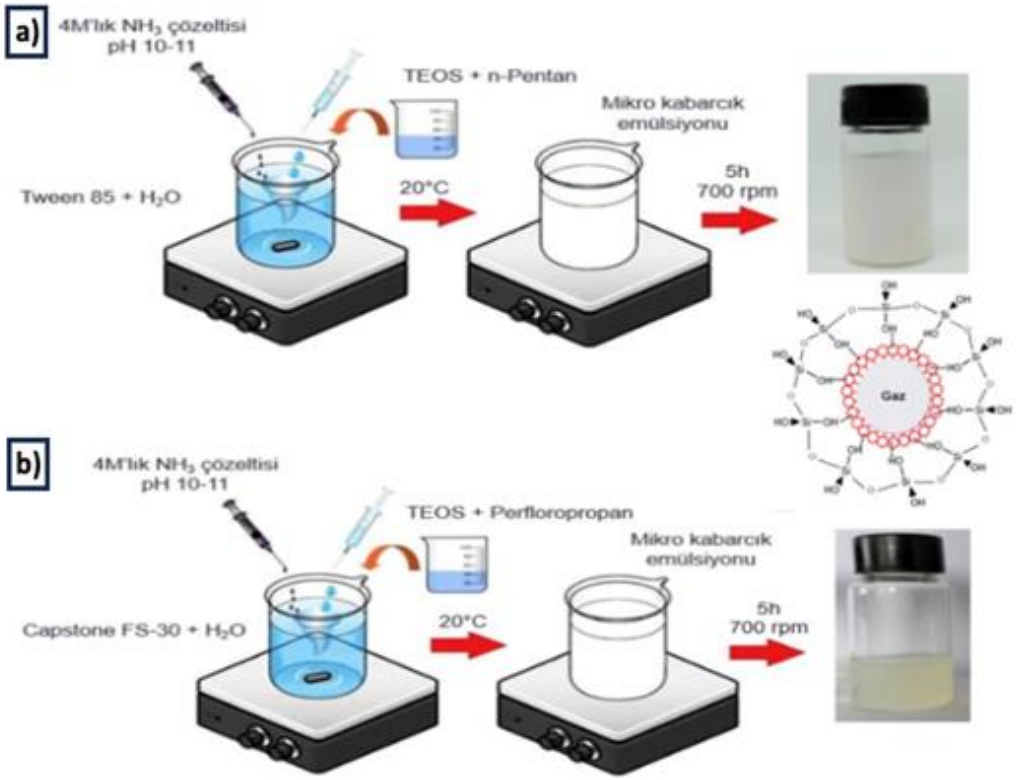
SKMK dispersiyonlarının zeta potansiyelleri ve hidrodinamik çapları Malvern Zetasizer Nano ZS serisi karakterizasyon cihazı ile belirlenmiştir. FT-IR spektrometresiyle, oluşturulan SKMKların yapısındaki fonksiyonel gruplar ve TEOS hidroliz takibi, Perkin Elmer TWO model FT-IR spektrometresiyle 400–4000 cm^{-1} dalga sayısı aralığında sıvı ölçüm aparatı ve ATR tekniğiyle gerçekleştirilmiştir. OLYMPUS-BX41M model mikroskoba bağlı OLYMPUS-DP20 kamera sistemi yardımıyla lam üzerine damlatılan dispersiyonlardan görüntüler alınmış ve mikron boyutlu yapıların partikül çapı ve yapısı incelenmiştir. Dispersiyon pH değerleri XS Instruments pH 60 VioLab pH metre ve CS 201T pH elektrotu ile ölçülmüştür. Zeiss Leo 906E marka geçirimli elektron mikroskobu (TEM) ile 80kV' da sentezlenen SKMKların morfolojisi ve boyutları karakterize edilmiştir.

Metot ve yöntem

T85 ve n-pentan (C_5) sisteminin hazırlanması

Farklı miktarlardaki ultra saf suya farklı miktarlarda T85 sürfaktanı eklenerek 10 dakika boyunca homojenizatörde karıştırılmıştır. İkinci bir kap içerisine 12,72 g TEOS eklenmiş ve karışım halindeyken

üzerine 3 g n-pentan (C_5) (Q değeri = 21-80) kaba hızlıca eklenmiştir. 36,1°C kaynama noktasına sahip olan n-pentan'ın ortamdan uzaklaşmasını önlemek amacıyla buz banyosu kullanılarak sistem sıcaklığı 20°C'ye sabitlenmiş ve kap gaz çıkışı olmayacak şekilde, manyetik karıştırıcıda 700 rpm'de 10 dakika boyunca karıştırılmıştır. Karışım sonunda ikinci kaptaki TEOS ve n-pentan çözeltisi T85 sürfaktanı içeren çözeltiye damla damla (20 µL/dakika) eklenmiştir. Sistem 1 saat boyunca karıştırılmış ve daha sonra farklı miktarlarda katalizör eklenerek 5 saat boyunca 700 rpm'de sol-jel yöntemi ile SiO_2 kapsüllü MK emülsiyonu oluşumu için karıştırılmaya bırakılmıştır (Şekil 1a). İlgili veriler Tablo 1'de verilmiştir. Katalizör olarak kullanılan amonyak çözeltisi (NH_3) %24'lük (d=1,097 g/ml), 4,00 M'lık hazırlanmıştır.



Şekil 1 (a) T85 / C_5, (b) FS-30 / C_3H_8 sistemlerinin hazırlanışının şematik gösterimi
Figure 1 Schematic representation of the preparation of (a) T85 / C5, (b) FS-30 / C3H8 systems

FS-30 ve perfloropropan (C_3F_8) sisteminin hazırlanması

25,77 g ultra saf suya farklı miktarlarda florosürfaktan olan FS-30 eklenerek 10 dakika boyunca homojenizatörde karıştırılmıştır. İkinci bir kap içerisine 4,24 g TEOS eklenmiş ve karışım halindeyken üzerine farklı hacimlerde perfloropropan (C_3F_8) (Q değeri = 1001-10000) enjekte edilerek gaz çıkışı olmayacak şekilde, manyetik karıştırıcıda 700 rpm'de 10 dakika boyunca karıştırılmıştır. Karışım sonunda ikinci kaptaki TEOS ve perfloropropan çözeltisi FS30 sürfaktanı içeren çözeltiye damla damla (20 µL/dakika) eklenmiş ve sistem 1 saat boyunca karıştırılmıştır. Karışıma 1,27 g amonyak katalizörü eklenerek 5 saat boyunca 700 rpm'de karıştırılarak Şekil 1b'de görüldüğü gibi SKMK emülsiyonu oluşumu için karıştırılmaya bırakılmıştır.

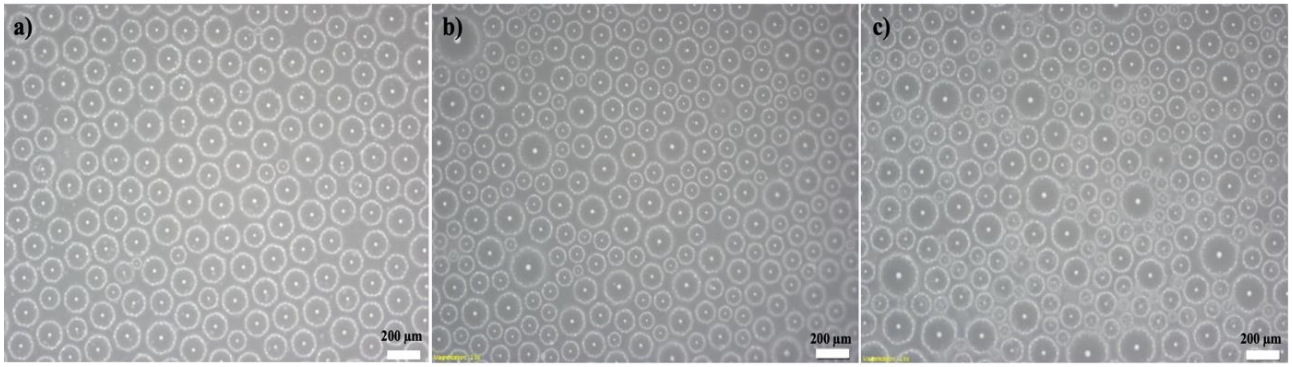
Tablo 1 T85 / C_5 sistemi ve FS-30 / C_3H_8 sistem sentezleri
Table 1 Syntheses of the T85 / C5 system and the FS-30 / C3H8 system

	Kullanılan Kimyasallar					
Sistem Numarası	**T85 (g)**	**H_2O (g)**	**C_5 (g)**	**TEOS (g)**	**SiO_2 (%)**	**NH_3 (4M) (g)**
1A	1,10	79,36	3	12,72	3,67	3,82
1B	1,57	79,89	3	12,72	3,67	3,82
1C	2,36	78,10	3	12,72	3,67	3,82
2A	1,57	223,59	3	12,72	1,5	3,82
2B	1,57	223,59	3	12,72	1,5	2,54
2C	1,57	223,59	3	12,72	1,5	1,90
2D	1,57	223,59	3	12,72	1,5	1,27

	FS30 (g)	H_2O (g)	C_3H_8 (cc)	TEOS (g)	SiO_2 (%)	NH_3 (4M) (g)
3A	4,22	25,77	1	4,24	1,5	1,27
3B	4,22	25,77	1,5	4,24	1,5	1,27

Tartışma

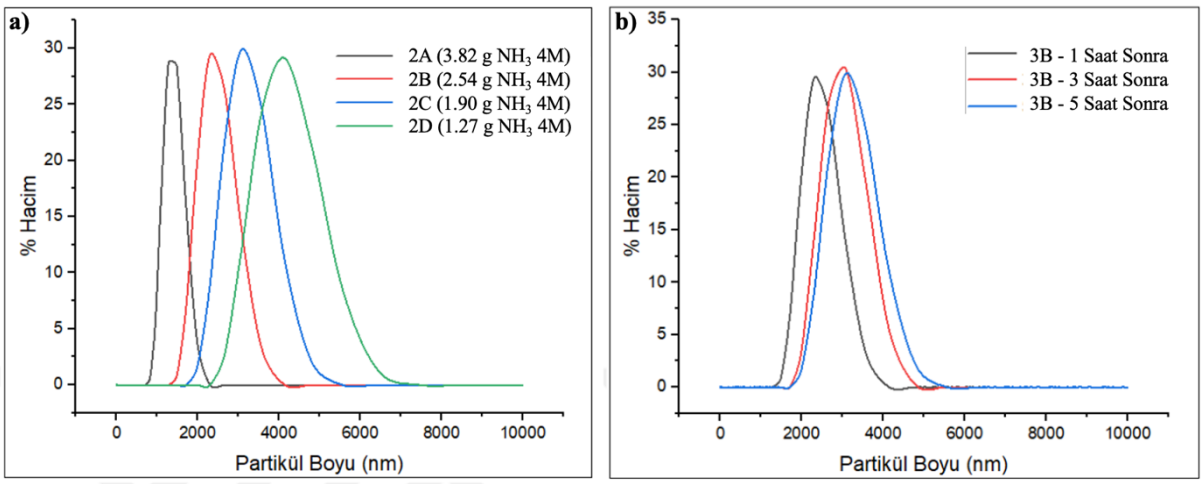
T85 sürfaktan miktarı optimizasyonunda 1A, 1B ve 1C sistemlerine ait optik mikroskop görüntüleri Şekil 2'de gösterilmiştir. Kırılma indisi farkı ile çekirdeği oluşturan n- pentandan, inorganik kabuk yapısını oluşturan SiO_2 yapıların varlığı gözlemlenebilmektedir. Sistem 1'de üretilen tüm SKMKlar düşük stabiliteye sahip olup, MKlar sentezlendikten sonra hava ile etkileşime girerek hızlıca aglomere olmuştur (Şekil 2). SKMKların dispersiyonu, sentezlendikten sonra 7 gün boyunca sabit ve ışık almayan bir ortamda bekletilmiş ve SKMKlar dispersiyon dibine çökerek gözle görülebilir bir aglomerasyona uğramışlardır. En az dip kalıntı olan dispersiyon, 1,57 g (ağırlıkça %10) T85 sürfaktanı eklenmiş 1B sisteminde olduğu belirlenmiştir.



Şekil 2 T85 sürfaktanı ve n-pentan ile hazırlanan sistemlerin optik mikroskop görüntüleri, (a) 1A, (b) 1B, (c) 1C sistemleri

Figure 2 Optical microscope images of systems prepared with T85 surfactant and n-pentane, (a) System 1A, (b) System 1B, (c) System 1C

2A, 2B, 2C ve 2D sistemlerinde NH_3 katalizör miktarının azalmasıyla parçacık boyutunun büyüdüğü gözlemlenmiştir. Şekil 3'te verilen DLS verilerine göre, sırasıyla 3,82 g (pH=11,65), 2,54 g (pH=11,23), 1,90 g (pH=10,89), ve 1,27 g (pH=10,43) olacak şekilde azaltılan NH_3 katalizör miktarı ile hidrodinamik parçacık boyutu sırasıyla 1,281 µm, 2,305 µm, 3,091 µm ve 4,145 µm ölçülmüştür (Şekil 3a). 2A, 2B, 2C ve 2D sistemlerinin sırasıyla zeta potansiyeli; -39,1 mV, -35,8 mV, -30,9 mV, -29,7 mV olarak ölçülmüştür (Tablo 2). Sistem 2'de üretilen tüm parçacıklar stabil olup, 15 gün stabilite testinden sonra herhangi bir dip kalıntı gözlemlenmemiştir. 2A, 2B ve 2C sistemleri stabil silika zeta potansiyeli -30mV'un altında olup en stabil sistem ve en düşük parçacık boyutuna sahip olan sistem olarak seçilmiştir [15, 16].



Şekil 3 (a) 2A, 2B, 2C, 2D sistemlerinin hacimce partikül boyut dağılım grafiği, (b) 3B sisteminin 1. 3. ve 5. Saat sonunda ölçülen hacimce hidrodinamik partikül boyut dağılım grafiği

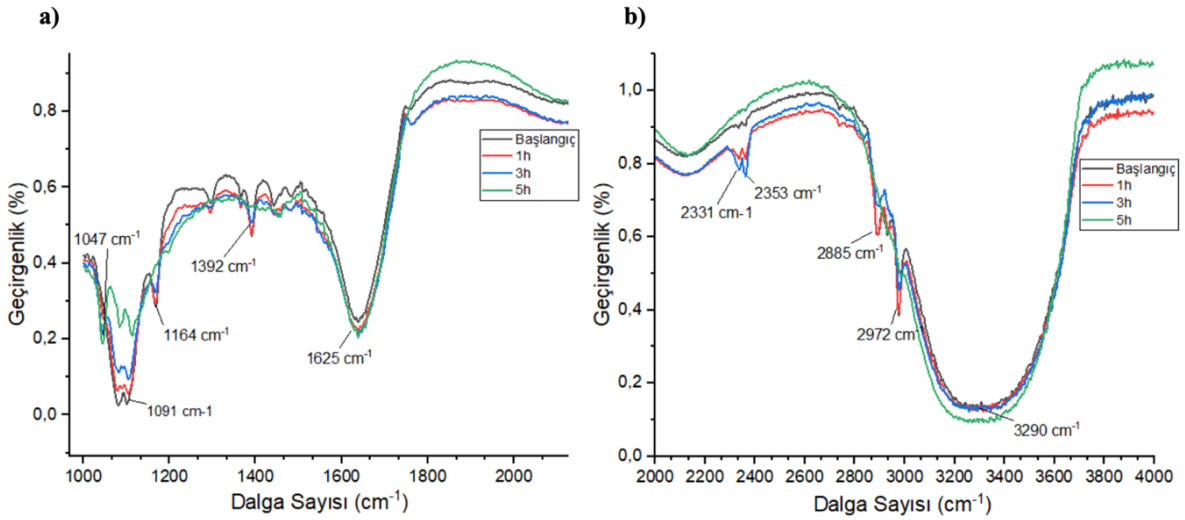
Figure 3 (a) Volumetric particle size distribution graph of the systems 2A, 2B, 2C, 2D, (b) Volumetric hydrodynamic particle size distribution graph measured after 1, 3, and 5 hours for the 3B system

C_3H_8' in miktarları değiştirilerek incelenene 3A ve 3B sistemlerinde, 1 mL C_3H_8 eklenmiş 3A sisteminde homojen bir dağılım olmadığı DLS ile belirlenmiştir. 1. saat, 3. saat ve 5. saat sonunda % hacim miktarlarına göre incelendiğinde, zamanla nano boyuttaki tanecik miktarının azalıp mikro boyuta ulaştığı görülmektedir. 1. saat, 3. saat ve 5. saatlerdeki tanecik boyutu sırasıyla, %5,3'ü 164,2 nm; %24,9'u 5,554 µm, %8,5'u 255 nm; %26'sı 5,560 µm ve %4,5'u 295,3 nm; %29,4'ü 5,556 µm şeklinde olup zeta potansiyeli; -21,2 mV' dur. Şekil 3b'de görülen 1,5 mL C_3H_8 kullanılarak elde edilen 3B sisteminde, SKMKlar dispersiyon içerisinde tekil dağılıma sahip olup, 1. saat, 3. saat ve 5. saatlerdeki tanecik boyutu sırasıyla 2,305 µm, 3,091 µm ve 3,095 µm şeklindedir. 3A sistemine göre daha ideal bir sistem olan 3B dispersiyonun zeta potansiyeli; -4,9 mV olup, SKMK dispersiyonunda 15 günlük stabilite testi sonunda herhangi bir dip kalıntı gözlenmemiştir.

Tablo 2 T85/C_5 sistemi ve FS30/C_3H_8 ile hazırlanan sistemlerinin ortalama hidrodinamik partikül boyutları ve zeta potansiyelleri
Table 2 Average hydrodynamic particle sizes and zeta potentials of the systems prepared with T85/C5 and FS-30/C3H8

Sistem Numarası	Hidrodinamik Partikül Boyutu (µm)	Zeta Potansiyeli (mV)
2A	1,281	-39,1
2B	2,305	-35,8
2C	3,091	-30,9
2D	4,145	-29,7
3A	5,556	-21,2
3B	3,095	-41,9

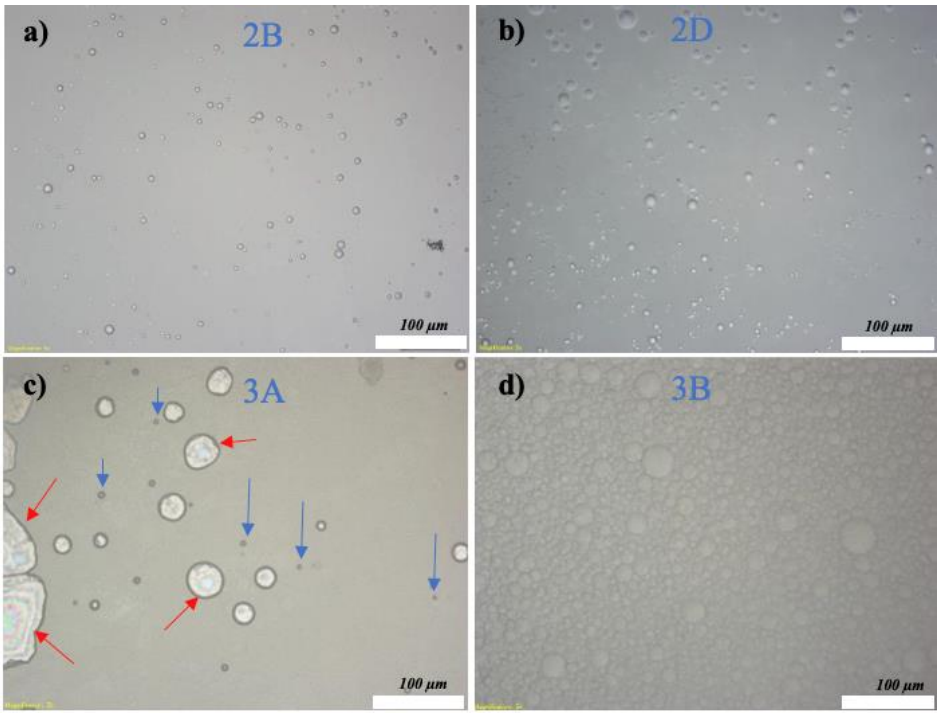
Bazik karakterli amonyak (NH_3) katalizörüne ek olarak, 4M asetik asit (CH_3COOH) katalizörü ile de farklı parametreler denenmiş olup, asit katalizli yapılan tüm sistemlerde jelleşme olduğu gözlemlenmiştir.
2A sistemi olan SiO_2 çeperli C_5 çekirdek yapılı SKMKların, başlangıç (taze sentez), 1. 3. ve 5. saat sonra FT-IR spektrumu çekilmiştir. TEOS hidrolizi sonucunda oluşan tetrahidroksisilan yapısı, kondenzasyon reaksiyonları ile SiO_2 çeper yapısını meydana getirmektedir. TEOS hidrolizi, 1091 cm^{-1} (Si-O asimetrik gerilmesi) bandındaki pik şiddetinin reaksiyon süresi boyunca azalması ile anlaşılmaktadır. Şekil 4'te görülen FT-IR spektrumları incelendiğinde, reaksiyon süresinin artırılmasıyla 1047 cm^{-1}'deki Si-O-Si pik şiddetinde bir artış gözlemektedir [17]. 1164 cm^{-1}'de görülen pik T85 yapısında bulunan C-O asimetrik gerilmesinden [16], 1392 cm^{-1}'deki pik simetrik C-H eğilmesinden, 2885 cm^{-1}'deki pik -CH_2 asimetrik C-H gerilmesinden, 2972 cm^{-1}' deki pik –CH_3'e ait asimetrik C-H gerilmesinden kaynaklanmaktadır. 1635 cm^{-1}'deki pik T85 yapısında bulunan karbonil grubuna ait C=O asimetrik gerilmesinden ve ortamda bulunan NH_3 katalizörüne ait N-H eğilmesinden, 3290 cm^{-1}'deki pik ise H_2O'daki –OH asimetrik gerilmelerinden kaynaklanmaktadır [19, 20].



Şekil 4 2A sisteminin başlangıç (taze sentez), 1, 3 ve 5 saat sonraki FT-IR spektrumu; (a) 1000-2000 cm^{-1}, (b) 2000-4000 cm^{-1}
Figure 4 FT-IR spectrum of the 2A system at the initial (fresh synthesis) after 1, 3, and 5 hours; (a) 1000-2000 cm^{-1}, (b) 2000-4000 cm^{-1}

Ölçümlerin devamlılığı ve stabilizasyon için SKMK dispersiyonu hava almayan kaplarda muhafaza edilmiştir. 10 dakikayı geçen sürelerde ise hava ile etkileşime girerek SKMKlar patlamıştır. Hava almayan kapalı plastik bir kap içerisinde muhafaza edildiğinde ise 2. ve 3. sistemlerdeki SKMK dispersiyonlarının

hepsi, 7 günden fazla stabilitesini korumaktadır. Şekil 5'te optik mikroskobu ile alınan tüm ölçümlerde SKMK dispersiyonu lam üzerinde kuruma ve hava ile etkileşim sürecine girdiğinden dolayı partikül boyutları DLS verilerine göre büyük gözlemlenmiştir. 2A, 2B, 2C ve 3B sistemleri en stabil sistemlerdir. Ancak bu sistemlerde bile dispersiyon içerisinden alınan numunelerde aglomerasyon kinetikleri ile genleşen C_5 ve C_3H_8 zamanla partikül boyutlarının büyümesine neden olmaktadır [21, 22].



Şekil 5: T85/C_5 ile hazırlanan SKMKlara ait optik mikroskobu görüntüleri, (a) 2B sistemi, (b) 2D sistemi; FS-30/C_3H_8 ile hazırlanan SKMKlara ait optik mikroskobu görüntüleri, (c) 3A sistemi, (d) 3B sistemi
Figure 5 Optical microscope images of SMKs prepared with T85/C5, (a) System 2B, (b) System 2D; optical microscope images of SMKs prepared with FS-30/C3H8, (c) System 3A, (d) System 3B

Şekil 5'te verilen optik mikroskobu görüntüleri incelendiğinde, çekirdek yapısı n-pentan ve çeperi silika olan 2 numaralı sistemlerde kırılma indisi farkı sebebiyle çeperler net olarak optik mikroskobunda görülmektedir. 2B, 2C ve 2D sistemlerindeki NH_3 konsantrasyonunun giderek azaltılması, SKMKların partikül boyutunu arttığı görülmüştür [23] (Şekil 5a, 5b, 5c).
Şekil 5d, 5e ve 5f'de literatürde Q değeri 1001-10.000 arasında olan ve yüksek stabilite sağladığı bilinen C_3F_8 çekirdek yapılı 3 numaralı sistemlerin optik mikroskobu görüntüleri verilmiştir. Şekil 5d'de 3A sistemine ait optik mikroskop görüntülerinde mavi oklar ile gösterilen ~5 µm ± 2 µm yapıların yanı sıra kırmızı ok ile gösterilen küresel ve küresel olmayan daha büyük boyutlarda sahip agregasyonların olduğu görülmektedir. Homojen olmayan bu SKMKlar hava ile etkileşime girdikten sonra çeperlerden bozulmalar başlamaktadır. Şekil 5e'de 3B sistemine ait optik mikroskop görüntüsü incelendiğinde, SKMKların 3A sistemine göre daha homojen boyutlarda olduğu görülmektedir. Bu sistemdeki SKMKlar hava ile etkileştikten sonra 15 dakika boyunca stabil halde kalabilmektedir.

Sonuç

Bu araştırmada, ultrason kontrast ajanı olarak tıbbi ultrason görüntülemede kullanılması amacı ile SKMKlar sentezlenmiştir. Literatürde daha önce çalışılmamış çekirdek/kapsül malzemesi sistemleri olarak, Q değeri 21-80 olan n-pentan (C_5) ve Q değeri 1001-10000 olan perfloropropan(C_3F_8), sırasıyla Tween 85 (T85) ve Capstone FS-30 sürfaktanları kullanılarak sol-jel yöntemi ile silika kapsülasyonu sağlanmıştır. İki farklı sistem üzerinde yapılan optimizasyon çalışmaları ile ideal sürfaktan miktarının C_5 sistemleri için ağırlıkça %10 olduğu saptanmıştır. Ayrıca NH_3 katalizörünün ~3 kat arttırılması ile ortalama hidrodinamik partikül boyutunun 4,145 µm'den 1,281 µm'ye düştüğü ve zeta potansiyel değerinin ise sırasıyla -29,7 mV ve -39,1 mV olduğu görülmüştür. İdeal olarak belirlenen sistemlerin 7 gün boyunca emülsiyonda stabil olarak kaldığı ve hava ile etkileşimi esnasında ise 10 dakikaya kadar stabil kaldığı gözlemlenmiştir. C_3H_8 sistemlerinde C_3H_8 miktarının 1,5 kat arttırılması ile zeta potansiyel değerleri sırasıyla -21,2 mV ve -41,9 mV olup, ortalama hidrodinamik parçacık boyutunun ise 5,556 µm'den 3,095 µm'ye düştüğü belirlenmiştir. İdeal C_3H_8

sistemi ile oluşturulan SKMKların 15 güne kadar emülsiyon içerisinde, 15 dakika da hava ile etkileşimi sonunda stabilitesini koruduğu gözlemlenmiştir.

Abbreviations/Kısaltmalar

MK: Mikro kabarcık; MKs: Mikro kabarcıklar; SKMK: Silica kapsül mikro kabarcık; SKMKs: Silica kapsül mikro kabarcıklar, T85: Polyoxyethylenesorbitan trioleate; FS-30: Capstone FS-30 floro surfactant

Acknowledgments / Teşekkürler

We would like to thank the administrators and staff of Akdeniz Universit for their valuable support.

Akdeniz Üniversitesi yönetimi ve personeline bu çalışmadaki değerli destekleri için teşekkür ederiz.

Funding / Fon desteği

This research was funded by Akdeniz University Scientific Research Project Coordination Unite with the project number FYL-2021-5550.

Bu araştırmanın fonu, Akdeniz Üniversitesi Bilimsel Araştırma Projeleri Koordinasyon Birimi tarafından FYL-2021-5550 proje numarası ile desteklenmiştir.

Data Availability statement / Veri Kullanılabilirliği bildirimi

The author confirms that the data supporting this study are cited in the article.

Yazar, bu çalışmayı destekleyen verilere makalede atıfta bulunulduğunu onaylamaktadır.

Compliance with ethical standards / Etik standartlara uyum

Conflict of interest / Çıkar çatışması

The author declare no conflict of interest.

Yazar herhangi bir çıkar çatışması beyan etmemektedir.

Ethical standards / Etik standartlar

The study is proper with ethical standards.

Çalışma etik standartlara uygundur.

Authors' contributions / Yazar katkıları

This research paper is derived from Gizem Moğol's master's thesis, for which she conducted the research and experiments. İbrahim Emre Gültaktı assisted in the research and co-authored the paper with Murat Akarsu.

Bu araştırma makalesi, Gizem Moğol'un yüksek lisans tezinden türetilmiş olup araştırma ve deneylerini yapmıştır. İbrahim Emre Gültaktı araştırmalarda yardımda bulunmuş olup Murat Akarsu ile beraber makalenin yazarıdır.

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International Journal of Life Sciences and Biotechnology
Önder, A., Davutlar, G., Ay, M., and Cömert Önder, F. Virtual Screening, Molecular Docking, and Molecular Dynamics Simulation Studies on Potential Phytochemicals as Sphingosine Kinase 1 Inhibitors for Cancer Therapy. Journal of Life Sciences and Biotechnology, 2024 7(2): p. 88-101.
https://doi.org/10.38001/ijlsb.1199393
Araştırma Makale/ Research article

Virtual Screening, Molecular Docking, and Molecular Dynamics Simulation Studies on Potential Phytochemicals as Sphingosine Kinase 1 Inhibitors for Cancer Therapy

Alper Önder[1], Gülce Davutlar[2], Mehmet Ay[1], Ferah Cömert Önder[3]*

ABSTRACT
Sphingosine kinases (SphKs) as lipid kinases catalyze the phosphorylation of sphingosine (Sph) to sphingosine-1-phosphate (S1P). Targeting the S1P signaling pathway is a significant strategy for many human diseases. Herein, we evaluated main prenylated bioactive components of a medicinal plant and performed a virtual screening study with flavonoid compounds and then, molecular docking and molecular dynamics (MD) simulation for the targeted cancer therapy. *In silico* ADMET and drug-likeness results were determined by BIOVIA Discovery Studio (DS). Molecular docking and molecular dynamics (MD) simulations were carried out by using Glide/SP and Desmond of Maestro with the filtered ligands. Glide/SP docking results showed higher binding affinity with xanthohumol (XN), 8-prenylnaringenin (8-PN), and neobavaisoflavone against SphK1. Three hits displayed strong hydrogen binding between the specific amino acid residues of targeting SphK1. There were no significant structural changes between SphK1-XN and SphK1-neobavaisoflavone complexes during 200 ns MD simulation analysis performed by GROMACS. Root-mean square deviation (RMSD) average values of XN- and neobavaisoflavone-protein complexes were compared to free SphK1 and were found as 0.2626 nm, 0.2589 nm, and 0.2508 nm, respectively. As a result, XN and 8-PN, and neobavaisoflavone have been determined as potential inhibitor candidates of SphK1 to examine for further *in vitro* and *in vivo* studies.

ARTICLE HISTORY
Received
06 March 2024
Accepted
31 May 2024

KEY WORDS
Sphingosine kinase,
virtual screening,
cancer,
molecular docking,
MD simulation

Introduction

Sphingolipids are lipid signaling molecules that contribute to the regulation of cellular processes [1]. Sphingomyelin metabolites are ceramide (Cer), sphingosine (Sph) and sphingosine-1-phosphate (S1P) and play an important role in the development of diseases such as cancer [2]. Ceramide and sphingosine act as pro-apoptotic molecules involved in cell cycle arrest and inducing apoptosis [3]. Moreover, increasing the level of ceramide or sphingosine with the change of this balance may provide a therapeutic intervention in cancer [4]. Sphingosine-1-phosphate (S1P), that interconvertible opposite effect with other metabolites creates sphingolipid rheostat, plays a role in cell proliferation, cell survival, apoptosis, angiogenesis, and inflammation. Inhibition of SphK1 activity or decreasing the level of S1P may be effective in human disease such as cancer [5]. According to previously reported studies, an upregulation of SphK1 has been reported in various cancer types such as lung, brain, breast, prostate etc.. [6-9]. SphK1 also possesses a role in human cancer cells to certain chemotherapeutic drugs [10]. It was reported that there was a collaboration between overexpression of SphK1 in malignant tissues [11]. Moreover, signaling of S1P/SphK1 is associated with a wide variety of metabolic and inflammatory diseases such as diabetes, pulmonary fibrosis, Alzheimer's disease, obesity, rheumatoid arthritis, and sepsis [11]. These findings indicate that SphK1 is an interesting drug target for the discovery of new molecules for SphK1-related diseases.

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Due to the remarkable role in cancer and other human diseases, the researchers have been interested in developing new therapeutics for SphK1 drug targets [12]. Up to date, although many therapeutics have been developed and investigated by *in vitro* and *in vivo* studies. There is still need to determine newly potential candidates due to the limited usage of synthetic compounds because of side effects or non-bioavailability. Natural compounds are used to discover specific inhibitors for drug targets with their no or lower side effects. Furthermore, many naturally occurring compounds isolated from plants have a broad-spectrum for their biological activities such as anticancer, anti-inflammatory etc.. Thus, it is thought that natural compounds are potential candidates to be used in further studies [13]. In recent years, scientists have tried to understand *in vitro* and *in vivo* activity of phytochemicals used in traditional medicine [14]. Since the effects on medicinal plants have been also reported, it is very important to further develop and research studies with these medicinal plants for the prevention or treatment of diseases [15]. These literature findings can provide the basis for the design of new drugs for the treatment of important diseases such as cancer, diabetes, and Alzheimer's disease [16].

Humulus lupulus L. (hops) plant has been used for medicinal purposes since ancient times [16,17]. Hop cones contain a lot of secondary metabolites and the main compounds including bitter acids, flavonoids, terpenes, and chalcones have been found in female inflorescences of hops [17-19]. It has been reported that the prenylated flavonoids from hop cones such as xanthohumol (XN), iso-xanthohumol (IXN), and 8-prenylnaringenin (8-PN) has various biological activities including cardiovascular, anticancer, antiinflammatory, antioxidant, and pro-apoptotic effects etc. [18-21]. Furthermore, it has been reported that bitter acids including isohumulones obtained from hop cones significantly reduce the level of cholesterol in the blood [22].

Herein, the aim of this study is to investigate the main prenylated components of hop by the computational studies containing molecular docking and molecular dynamics (MD) simulation and ADMET prediction as potential candidates against SphK1. Furthermore, a flavonoid compound library was evaluated by virtual screening, molecular docking, and MD simulation studies to determine their binding affinities for targeting protein. Molecular docking and MD simulation results show that for the first time XN and neobavaisoflavone are two hits that may be useful for future studies in inhibiting SphK1 especially in cancer treatments. The workflow for this study is shown in Figure 1.

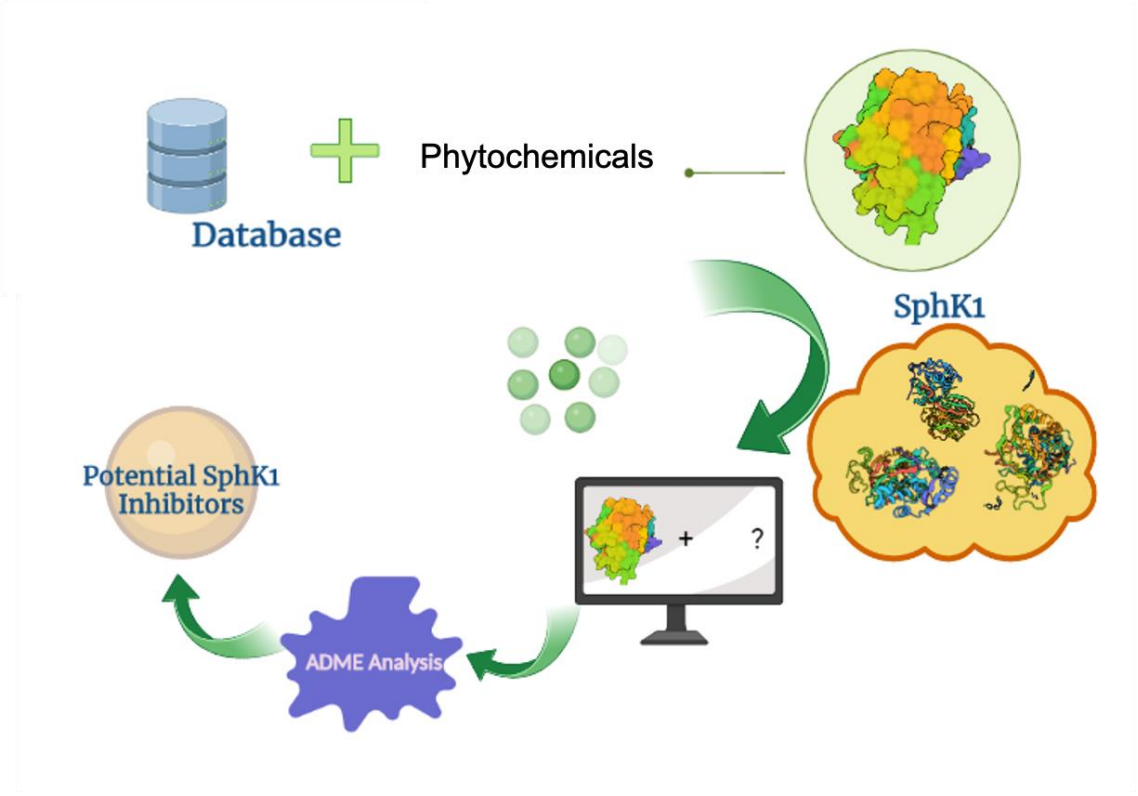

Fig 1 A representative workflow for this study.

Material and Methods

Protein and ligand preparation

The crystal structures of SphK1 were downloaded from the Protein Data Bank (PDB) (3VZB, 3VZC, 4V24) (https://www.rcsb.org/). According to our previously reported study, the calculation parameters were used [23]. Default settings of Maestro were used. Protein preparation wizard and Prime module of Maestro were used to prepare the target protein before docking (Schrödinger Release 2021-4, LLC, New York, NY). PROPKA was used to add hydrogen atoms [24]. Hop components were downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov/). Downloaded ligands were prepared by using the LipPrep module of Maestro. The ionization state was calculated at neutral pH by using Epik [25]. OPLS2005 force field was used and 0.3 Å RMSD was considered.

Molecular docking

Glide/SP (Standard Precision) method was used to determine the binding pose and molecular interactions between the protein-ligand complex [26]. Default settings of flexible ligand docking were kept as 5000 poses per ligand, and best 400 poses per ligand for energy minimization. The Grid box was formed by using co-crystal ligand [12, 23].

Structure-based virtual screening

A flavonoid compound library was downloaded from Selleckchem (https://www.selleckchem.com/). Firstly, ADMET descriptors and Filter by Lipinski and Veber Rule (Ro5) modules of BIOVIA Discovery Studio (DS) were used for the filtration of ligands, respectively [27]. Aqueous solubility [28], brain barrier penetration [29], CYP2D6 binding [30], hepatotoxicity [31], intestinal absorption [32], and plasma protein binding [33] were selected in ADMET Descriptors module of DS. Throughout the Filter by Lipinski and Veber Rules module of DS, hydrogen bond donors, acceptors, molecular weight, and AlogP values were 5, 10, 500, and 5, respectively. According to Veber rule, rotatable bonds, polar surface area, hydrogen bond donors and acceptors were 10, 140, and 12, respectively. Then, the resulting 136 compounds were prepared by using the LigPrep module of Maestro for molecular docking study and prepared candidates were docked to SphK1 target protein.

Molecular dynamics (MD) simulation

The top docking poses of SphK1-XN and SphK1-neobavaisoflavone obtained following docking were prepared by using Chimera software for MD simulation [34]. Molecular topology files were formed by accessing www.swissparam.ch [35] MD simulation analysis of protein and protein-ligand complexes were carried out by GROMACS 2023.1 using CHARMM force field parameters [36-38]. TIP3P water model was used for each protein [39]. The simulation box was neutralized by using Na+ and Cl- ions. The energy minimization was performed for each MD simulation system. The temperature of each system was gradually increased from 0 K to 300 K during the equilibration period at constant volume, pressure (1 atm), and temperature (300 K) under periodic boundary conditions. It was carried out for 200 ns at molecular mechanics level on free SphK1, SphK1-XN, and SphK1-neobavachin. The resulting trajectories were saved for further analysis using GROMACS' internal utilities. The output files (.xvg) were analyzed by XMGRACE software [40,41].

Results

Molecular docking study of hop flavonoids

To understand the binding pattern between phloroglucinol core structure, prenyl groups and target protein, a molecular docking study was performed with major bioactive prenylated components of *H. lupulus* L.. Hop prenylated bioactive components including XN, IXN, xanthogalenol, desmethylxanthohumol (DesXN), 6-prenylnaringenin (6-PN), 8-PN, and bitter acids such as humulone, lupulone, cohumulone, and colupulone were docked against target protein (3VZB). These results showed that XN, 8-PN, and IXN had the highest binding scores as -8.904 kcal/mol, -8.582 kcal/mol, and -8.148 kcal/mol, respectively. In addition, XN derivatives, DesXN and xanthogalenol displayed lower binding scores as -7.927 kcal/mol and -7.889 kcal/mol, respectively. Cohumulone is one of the alpha acids of hops and is calculated for its binding energy as -5.978 kcal/mol. The calculated binding scores of major prenylated components of hop cones were given in Table 1.

Furthermore, according to the interactions between ligand and target protein, these ligands showed the expected interactions such as H-binding and pi-alkyl with important amino acid residues such as Asp178, Phe288, Phe173, Met306, and Leu299 of Sph binding of SphK1. In general, the ligands interacted with the substrate-binding Asp178 residue of SphK1. Strong hydrogen bonding between hydroxyl groups of ligands

and Asp178 was observed. A detailed binding pattern of these compounds with SphK1 is illustrated in Figure 2 and 3. As a result, hop components XN and 8-PN could be potential SphK1 inhibitors.

Table 1 Glide/SP docking scores of major prenylated bioactive components of hop cones against SphK1

No	Ligand	Glide/SP (kcal/mol) (3VZB)	Ligand	Glide/SP (kcal/mol) (4V24)
1	XN	-8.904	8-PN	-9.507
2	8-PN	-8.582	XN	-8.906
3	IXN	-8.148	IXN	-8.366
4	DesXN	-7.889	Cohumulone	-7.750
5	Xanthogalenol	-7.331	Adhumulone	-7.690
6	Cohumulone	-5.978	DesXN	-7.545
7	6-PN	-5.406	6-PN	-7.290

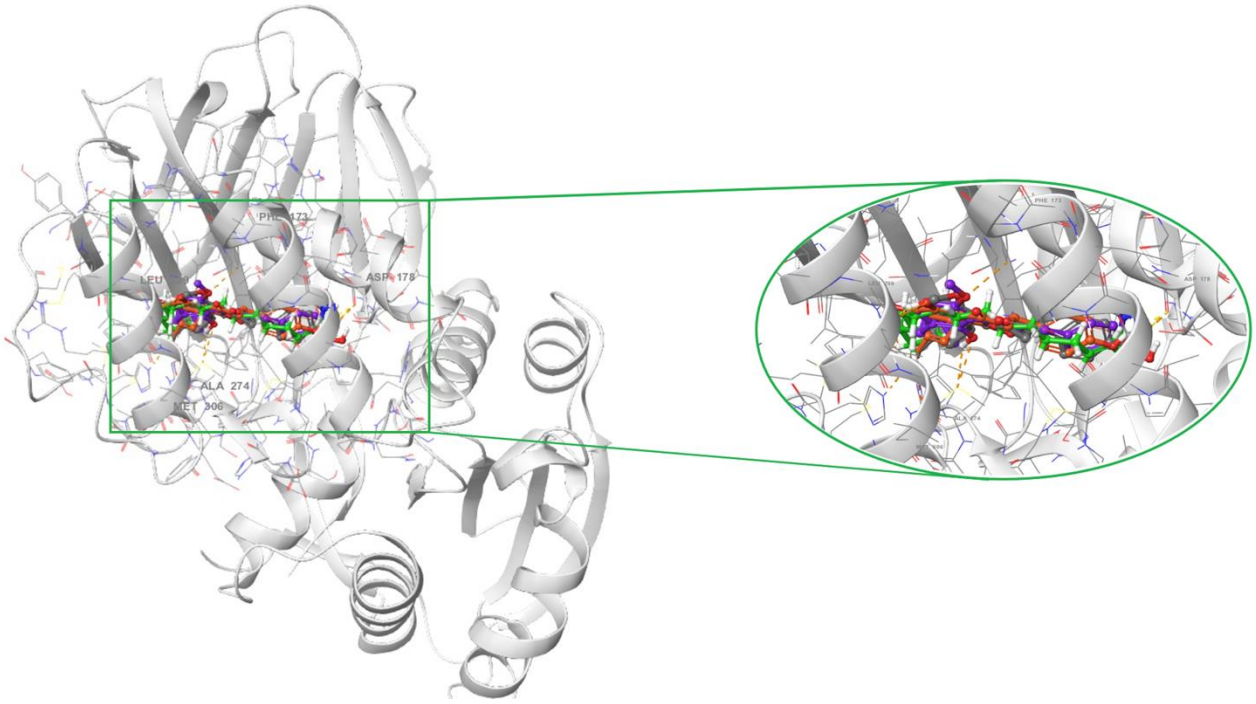

Fig 2 Binding pattern of XN, 8-PN, and IXN (pdb: 3VZB). Compounds were given as colored in magenda, orange, and grey, respectively. Key residues were labeled.

When hop prenylated components were docked against protein (pdb: 4V24), 8-PN and XN displayed the highest binding scores as -9.507 kcal/mol and -8.906 kcal/mol, respectively, followed by IXN (-8.366 kcal/mol) and cohumulone (-7.750 kcal/mol). Adhumulone (-7.690 kcal/mol) is another alpha acid that exhibits a similar score like cohumulone. In conclusion, among *H. lupulus* L. components, XN and 8-PN displayed the best binding scores against SphK1. Glide/SP docking scores were given in Table 1. Top docking poses of hit ligands are shown in the binding pocket of protein in Figure 4. Binding mode prediction of 8-PN and known inhibitor SKI-II is given in Figure 5.

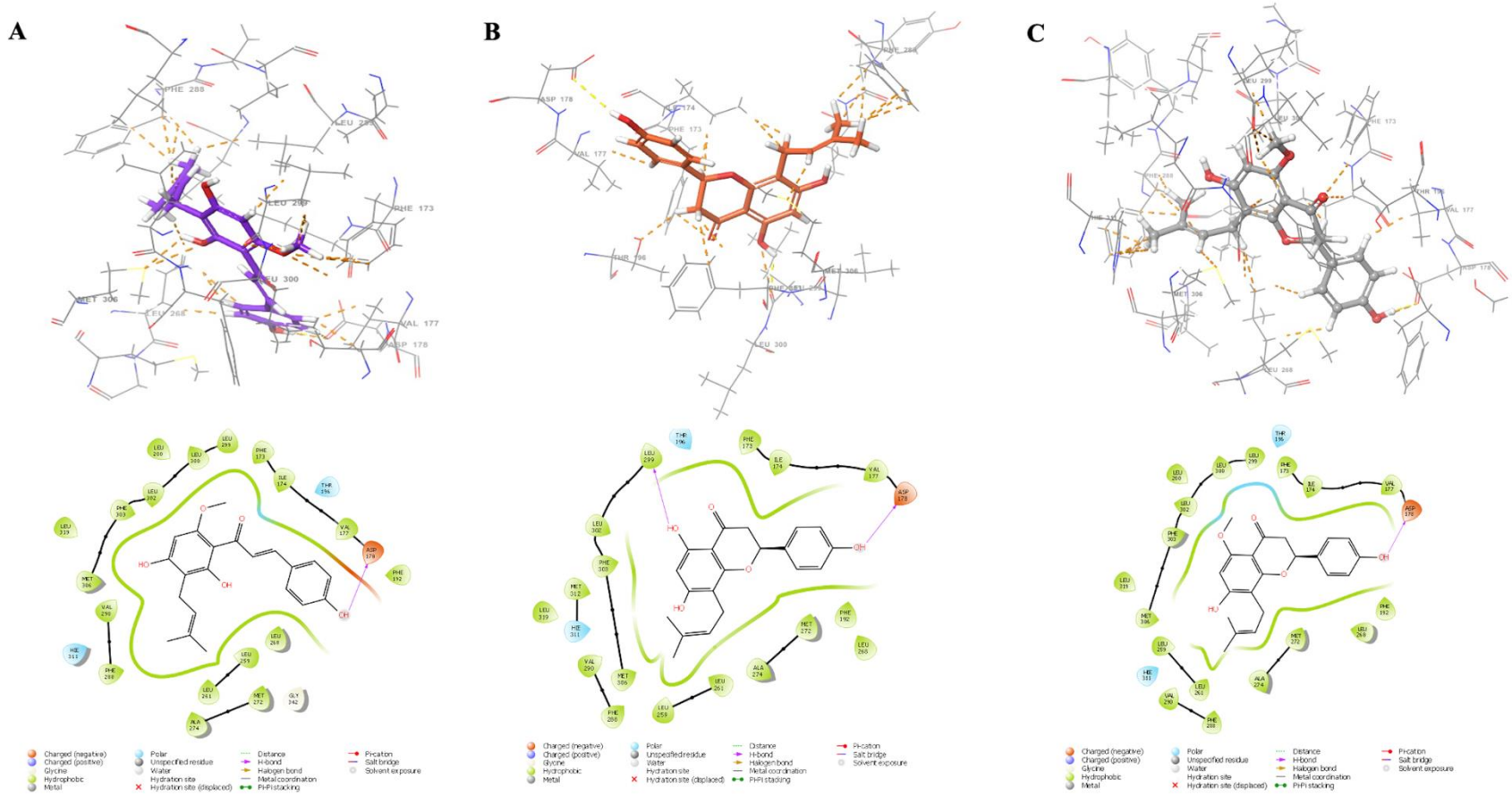

Fig 3 Binding pattern and 2D interaction diagram of XN (A), 8-PN (B), and IXN (C) (pdb: 3VZB).

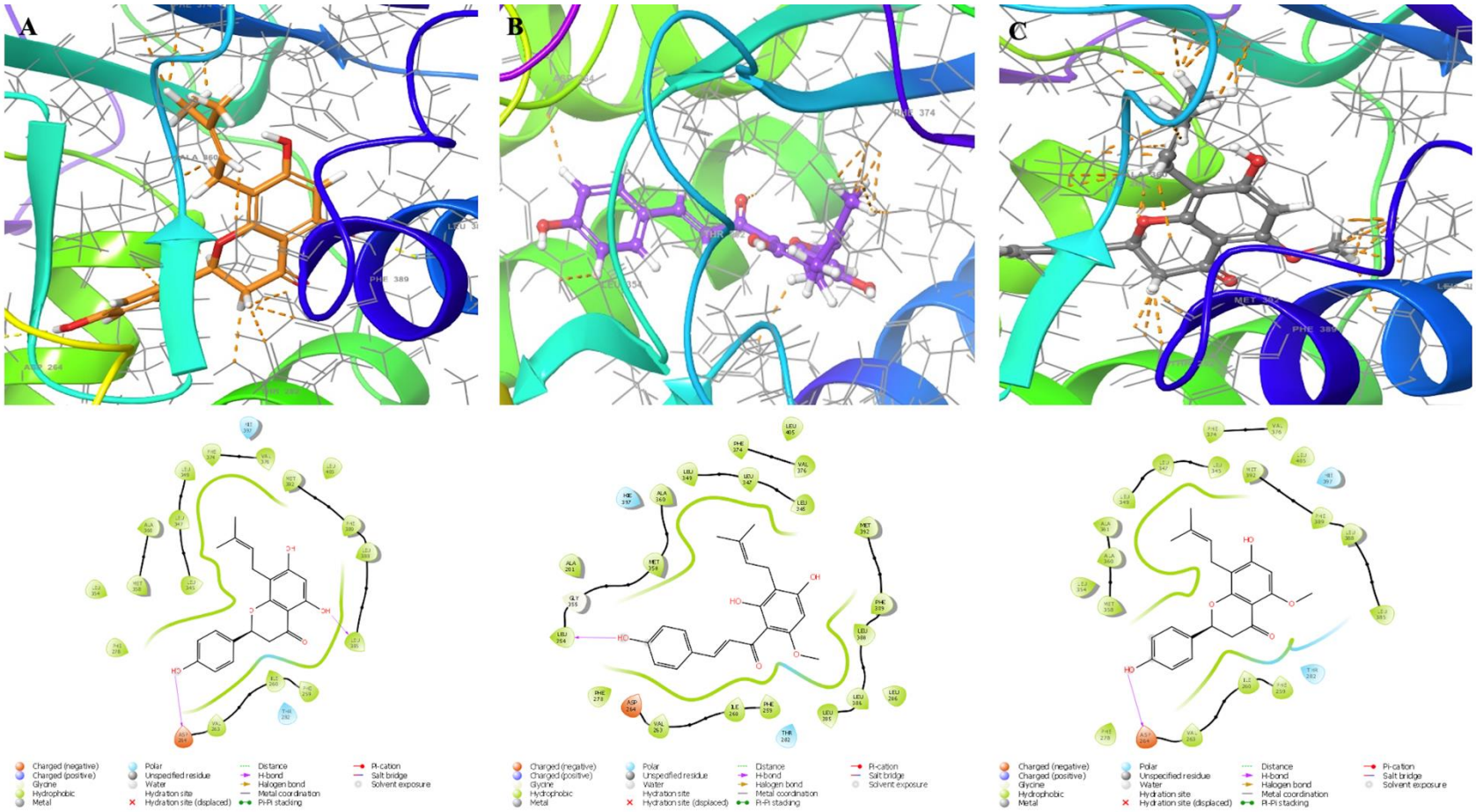

Fig 4 Binding pattern and 2D interaction diagram of 8-PN (A), XN (B), and IXN (C) (pdb: 4V24).

Structure based virtual screening

To understand the potential role of major prenylated flavonoids of *H. lupulus* L. against SphK1, we have thought to analyze flavonoid-based natural compounds. For this purpose, a structure-based virtual screening was performed by using a flavonoid compound library of Selleckchem. Following ADMET and Ro5 analysis in DS, filtered 136 compounds were docked against SphK1 by Glide/SP method. Throughout these flavonoid compounds, it is indicated that the selected hits with high binding scores were given in Table 2.

Table 2 Glide/SP docking scores of selected naturally occurring flavonoid compounds against SphK1 (pdb: 3VZB).

No	Ligand	Glide/SP (kcal/mol)	No	Ligand	Glide/SP (kcal/mol)
1	Neobavaisoflavone	-9.650	8	Genistein	-8.998
2	Licochalcone A	-9.609	9	Daidzein	-8.994
3	Corylin	-9.252	10	XN	-8.904
4	Tectorigenin	-9.161	11	Naringenin	-8.419
5	Equol	-9.108	12	Luteolin	-8.352
6	alpha-Naphthoflavone	-9.089	13	Taxifolin	-8.348
7	Glycitein	-9.019	14	Quercetin	-8.328

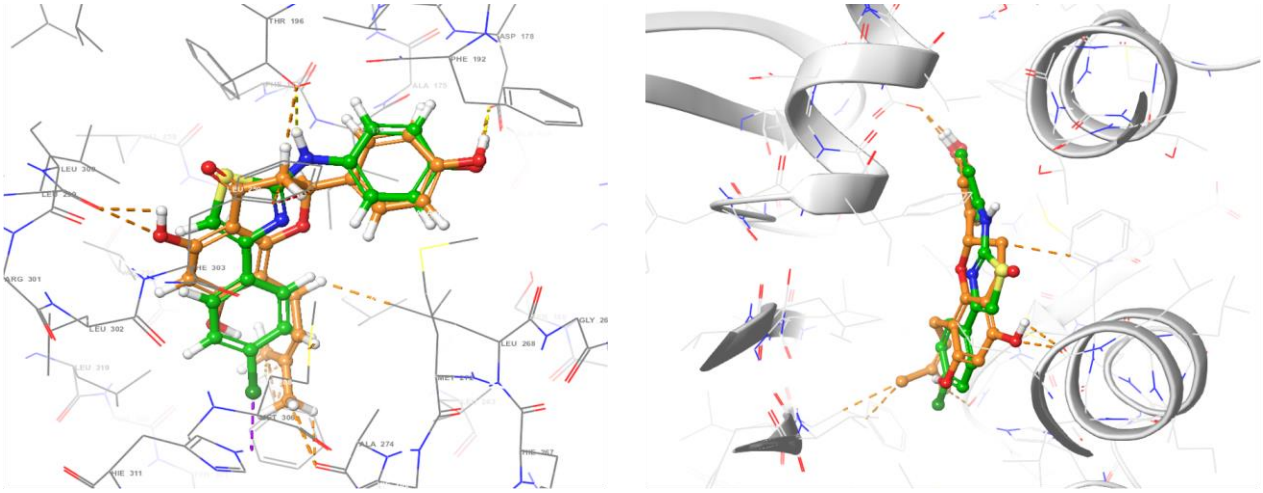

Fig 5 Binding pattern of 8-PN (orange) and SKI-II (green) (pdb: 3VZC).

Among a series of screened flavonoid compounds (pdb: 3VZC), the docking score was found for 2',3',4'-trihydroxy flavone (2D08) as -9.910 kcal/mol and also, isobavachin, which is a 8-prenylated flavone (-9.761 kcal/mol) exhibited higher binding score than quercetin. The interactions were observed between main residues such as Asp178, Phe303, Thr196, and Ile174. Other important naturally occurring compound genkwanin (especially known as apigenin derivative) is a O-methylated flavone has the highest docking score as -9.288 kcal/mol with H-binding between Asp178 and hydroxyl group and pi-pi stacking between Phe303 and phenyl ring. It was followed by butein is a tetrahydroxy chalcone compound (-9.288 kcal/mol) with the interactions between important residues such as Asp178 and 3,4-dihydroxy groups on phenyl ring. Fisetin is a flavonol, known as another dietary antioxidant, exhibits a good binding score (-9.074 kcal/mol) against SphK1 like quercetin and taxifolin (also called as dihydroquercetin) (-8.925 kcal/mol).

On the other hand, daidzein, taxifolin, luteolin, naringenin, and quercetin significantly placed in the substrate binding pocket of SphK1. The binding scores of neobavaisoflavone and licochalcone were calculated with high binding scores as -9.650 kcal/mol and -9.609 kcal/mol, respectively. The main interactions were observed at amino acid residues including Asp178 and Leu299 with hydroxyl groups of chromen-4-one core structure and phenyl ring, respectively. The interactions such as H-binding of licochalcone A with the high docking score have been identified between Asp178 and 4-hydroxy phenyl. Thus, both compounds displayed H-bond interaction with the substrate binding site of target protein. The binding pattern against target protein was given in Figure 6.

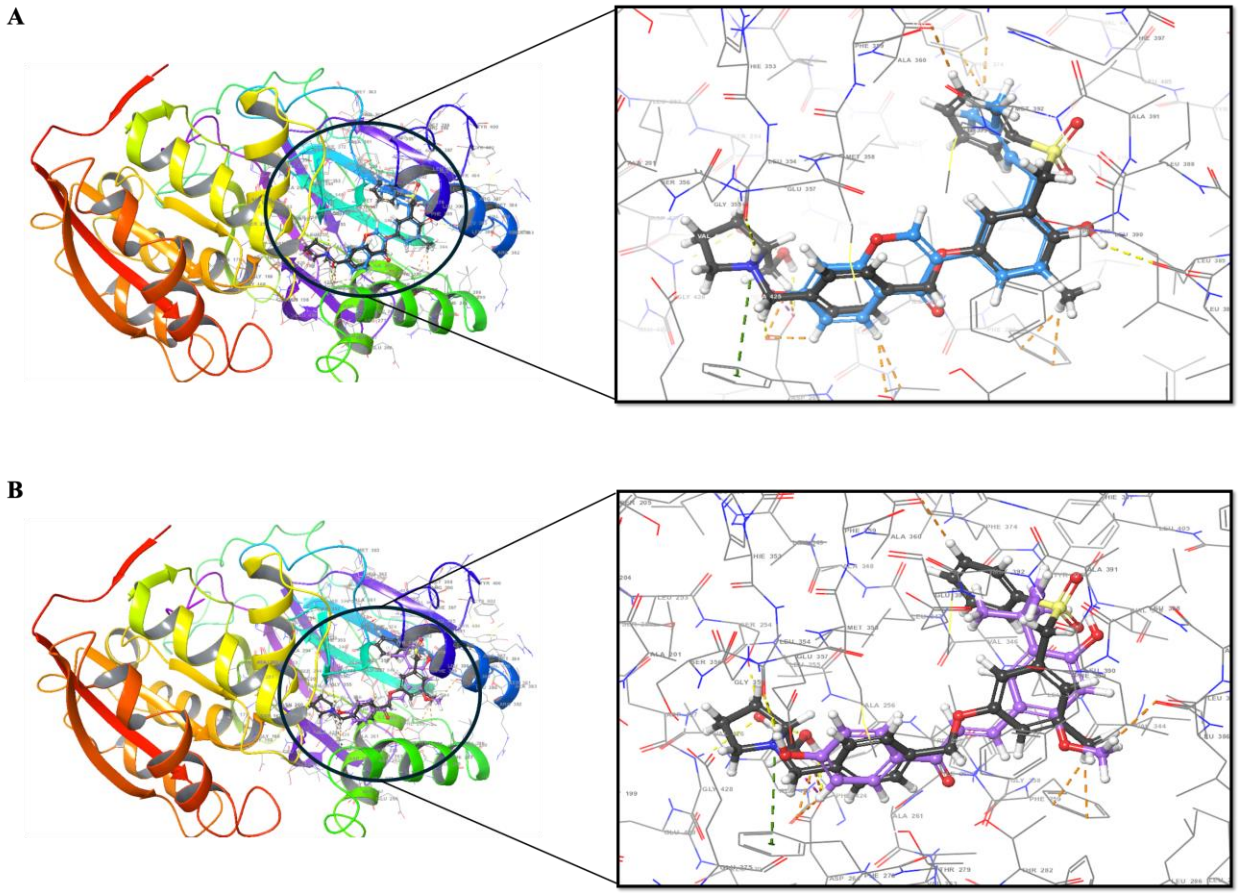

Fig 6 Binding pattern of neobavaisoflavone (blue) and PF543 (dark grey) (A) and licochalcone A (lilac) and PF543 (dark grey) (B).

Neobavaisoflavone exhibited strong H-bond interactions between amino acid residues Asp264 and Leu385 with 7-hydroxy group of chromen-4-one and 4-hydroxy substituted phenyl ring. Whereas 7-hydroxy group of substituted chromen-4-one core structure of daidzein interacted with Leu385. In addition, 4-hydroxyphenyl of licochalcone A showed strong H-bond with Leu354. These flavonoids and chalcone compounds may be revealed as potential SphK1 inhibitors in place of known inhibitor PF543. Besides, XN was found with the same binding pattern as known inhibitor PF543. Consequently, it is clear that hydroxyl groups of flavonoids contribute to the inhibitor activity with strong H-binding for targeted SphK1. In addition, van der Waals interactions have a role for binding the pattern of the flavonoids to the target protein with alkyl groups.

Molecular Dynamics (MD) Simulation

MD simulations were performed to understand protein-ligand complex. When XN and neobavaisoflavone were bond to the target protein, the structural behavior of SphK1 was evaluated with MD trajectories analysis. It appears that the structural conformation is better stabilized by XN. As is known, the structural deviation and conformational changes of a protein was determined with the calculations of root-mean square deviation (RMSD). Herein, RMSD average values of XN- and neobavaisoflavone-protein complex were compared to free SphK1 and were found as 0.2626 ± 0.026 nm, 0.2589 ± 0.029 nm, and 0.2508 ± 0.033 nm, respectively. Thus, it is though that there have been no significant changes in RMSD values (Figure 7a). Root mean square fluctuation (RMSF) shows that structural flexibility, the average fluctuation of each residue with the binding of ligand (Figure 7b). Radius of gyration (Rg) is given for the analyze the conformational changes and stability of the protein. The average Rg was found as approximately 2.035 ± 0.010 nm, 2.040 ± 0.012 nm, and 2.051 ± 0.009 nm for free SphK1 and SphK1-XN and SphK1-Neo, respectively. It shows that there is no significant structural changes during the analysis (Figure 7c). Solvent Accessible Surface Area (SASA) has displayed the average values 156.86 ± 3.08 nm^2 and 164.91 ± 2.72 nm^2, and 156.40 ± 2.44 nm^2 for free SphK1, SphK1-XN, and SphK1-Neo, respectively. Although neobavaisoflavone was no structural changes in SphK1, XN had a little change compared to free SphK1 (Figure 7d).

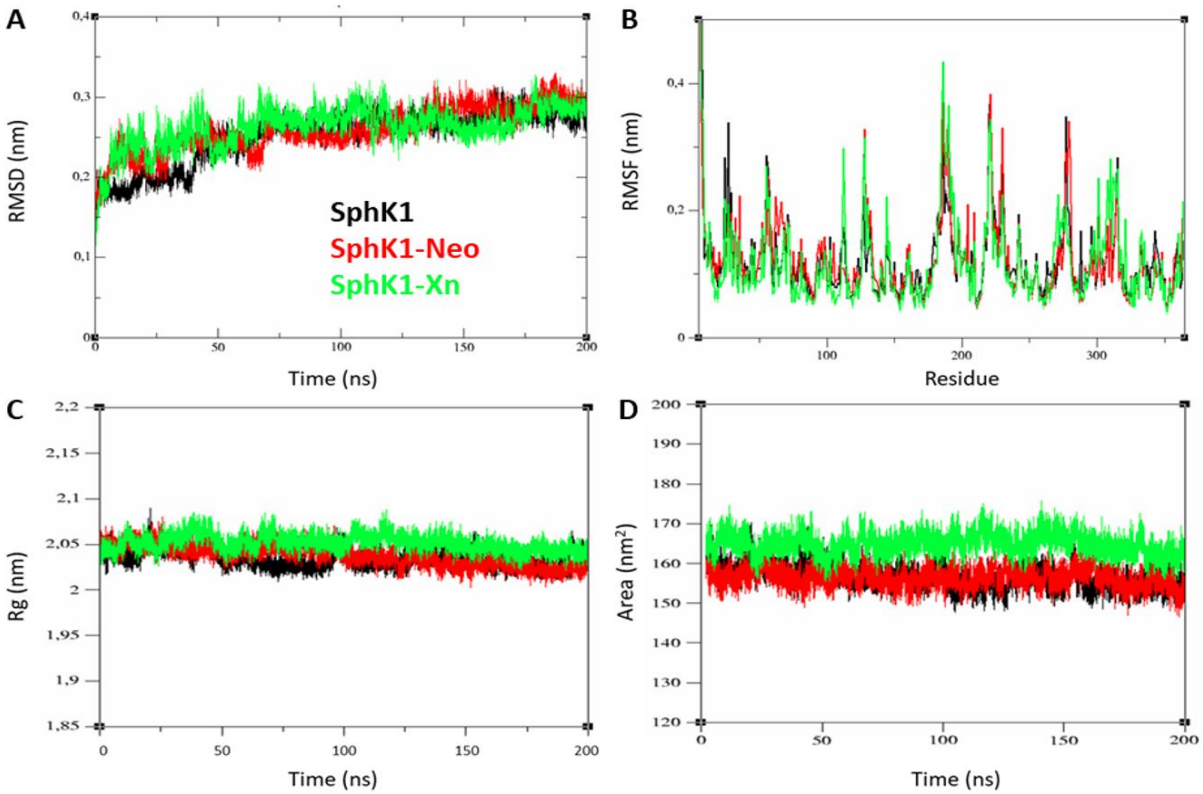

Fig 7 Structural dynamics of SphK1 before and after XN and Neobavaisoflavone complex. (A) RMSD plot of SphK1. (B) RMSF plot of SphK1. (C) Radius of gyration (Rg). (D) SASA plot of SphK1. The values were obtained from the 200 ns MD simulation. Black, red, and green represent values for free SphK1, SphK1-Neobavaisoflavone, and SphK1-XN complex, respectively.

ADMET, drug likeness and toxicity prediction

The molecular weight (MW) was filtered to a value less than 500 g/mol. *In silico* pharmacokinetic properties including ADMET descriptors, drug similarity and ADMET solubility, CYP2D6 binding, hepatotoxicity, blood brain barrier (BBB) penetration, plasma protein binding (PPB), intestinal absorption, MW, and number of H acceptors and donors are given in Table 3 and 4. According to these

results, ADMET solubility level was found in the range of 2 and 3. Thus, these values indicate that all compounds are soluble in water. The ligands have a good human intestinal absorption level between 0 and 1, indicating that all selected compounds will be well absorbed by the gut. The blood brain-barrier permeability of all compounds was evaluated, and it was observed that the compounds had values between 1-4. Predictive inhibitors of CYP450 enzymes (3A4, 2D6 and 2C9) are important for drug metabolism and do not implicate all compounds as inhibitors of CYP450 enzymes. It was found that all compounds did not show hepatotoxicity. The plasma protein binding level of all compounds ranged from 0.00 to 0.957.

Conclusion and Discussion

Absorption, distribution, metabolism, and excretion are investigated of ADME properties that are of great importance for drug development strategy to gain information about the potential of success of candidate therapeutic molecules [42]. Flavonoid compounds were analyzed with their ADMET properties including toxicity prediction and Ro5, and the resulting compounds were screened against SphK1 [43]. The number of hydrogen bond donors and acceptors [44] which is an important molecular identifier and serves to predict human oral bioavailability, and too many H-bond donors and acceptors negatively affect the permeability of compounds. H-bond acceptors and donors must be less than or equal to 10 and 5, respectively. All compound numbers of hydrogen bond acceptors and donors do not violate Ro5. In conclusion, this study contributed to the identification of the best flavonoids and bioactive components of *H. lupulus* L. regarding pharmacokinetic properties and the compounds could be used as potential drug targets. Throughout the reported studies, bioactive natural products were identified as potential SphK1 inhibitors. ZINC database library was filtered by using Lipinski's rule, ADMET, carcinogenicity, and PAINS. In a previously reported study, quercetin was investigated against SphK1 target by molecular docking study [41]. It was reported that its binding affinity was calculated as -8.2 kcal/mol. Thus, the interactions of quercetin with amino acid residues of target protein have been revealed as Asp178, Thr196, Ile174, Phe303, and Met306 [41]. As a result, a flavonoid quercetin was determined as a potent inhibitor of SphK1 due to the direct interaction with the substrate binding pocket in a reported study [41]. Consequently, the researchers suggested that dietary phytochemicals could be new therapeutics to develop SphK1 inhibitors [41]. Selected hits exhibited the high binding scores toward SphK1 in the range of -9.65 kcal/mol and -8.33 kcal/mol. However, the binding affinities of SphK1 inhibitors SKI-II and PF543 were calculated as -9.1 and -8.8 and 9.90 kcal/mol, respectively, in the previously reported studies [45,46]. According to each subtype of a flavonoid compound, has a hydroxyflavone core structure substituted with hydroxyl groups, binding scores show similarity with a flavonoid quercetin (-8.328 kcal/mol) with the interactions of specific amino acid residues such as Asp178, Thr196, Leu268, and Ile174. High binding affinities were determined in the range of -12.2 kcal/mol and -11.8 kcal/mol. Among these compounds, substituted naphthalene carbamate and substituted phenyl have been reported as potential inhibitor candidates [45]. In the other study, filtered by drug likeness and ADMET properties ligand-based virtual screening was applied to determine hit candidates by using various databases [46]. According to molecular docking study, hit compounds were determined with the highest binding energies compared to known inhibitor PF543. Hit1 compound was reported at the interactions with active site residues such as Phe259, Leu354, Asp254, and Ile260 by two conventional H-bonds compared to known inhibitors [46]. The interactions were observed between the 4-hydroxy group and phenyl ring bound to residues Asp178 and Phe303 of substrate binding pocket with H-bond and pi-pi stacking, respectively.

Table 3 ADMET Profiles of hop plant components against SphK1 (pdb: 3VZB).

No	Ligand	MW	ADMET solubility level[a]	BBB level[b]	Absorption level[c]	CYP2D6[d]	Hepatotoxicity[e]	PPB level[f]	H Acceptor	H Donor
1	XN	427.921	2	1	0	0	0	0	5	0
2	8-PN	270.28	3	2	0	0	0.001	0.859	4	1
3	IXN	338.397	2	1	0	0	0.004	0.157	4	2
4	DesXN	254.238	3	1	0	0.575	0.985	0.799	4	2
5	Xanthogalenol	284.263	3	2	0	0.008	0.738	0.957	5	2
6	Cohumulone	322.355	2	3	0	0.016	0.003	0.736	4	2
7	6-PN	254.238	3	1	0	0.041	0.048	0.298	4	2

[a]Aqueous-solubility level: 0 (extremely low); 1 (very low, but possible); 2 (low); 3 (good). [b]Blood Brain Barrier level: 0 (very high penetrant); 1 (high); 2 (medium); 3 (low); 4 (undefined). [e]Human-intestinal absorption level: 0 (good); 1 (moderate); 2 (poor); 3 (very poor). [d]Cytochrome P450 2D6 level: 0 (non-inhibitor); 1 (inhibitor). [e]Hepatotoxicity: 0 (nontoxic); 1 (toxic). [f]Plasma Protein Binding: 0 (absorbent weak); 1 (absorbent strong).

Table 4 ADMET Profiles of selected flavonoid compounds against SphK1 (pdb: 3VZB).

No	Ligand	MW	ADMET solubility level[a]	BBB level[b]	Absorption level[c]	CYP2D6[d]	Hepatotoxicity[e]	PPB level[f]	H Acceptor	H Donor
1	Neobavaisoflavone	322.36	2	1	0	0.016	0.003	0.736	4	2
2	Licochalcone A	338.40	2	1	0	0	0.004	0.157	4	2
3	Corylin	320.34	2	1	0	0.007	0.423	0.188	4	1
4	Tectorigenin	300.26	3	3	0	0.007	0.691	0.882	6	3
5	Equol	242.27	3	1	0	0.005	0.578	0.872	3	2
7	Glycitein	284.26	3	3	0	0.008	0.738	0.957	5	2
8	Genistein	270.24	3	3	0	0.666	0.989	0.805	4	3
9	Daidzein	254.24	3	2	0	0.575	0.985	0.799	5	2
10	XN	354.40	2	4	0	0	0.000	0.026	5	3
11	Taxifolin	304.25	3	4	1	0	0.014	0.098	7	5
12	Luteolin	286.24	3	4	0	0.618	0.526	0.324	6	4
13	Quercetin	302.24	3	4	1	0.431	0.964	0.611	7	5
14	Naringenin	272.25	3	3	0	0	0.034	0.685	5	3

Herein, flavonoids including luteolin, daidzein, naringenin, and kaempferol were identified as potential SphK1 inhibitors in place of known SKI-II that is a lipid substrate competitive inhibitor [47]. According to structural changes of SphK1-quercetin complex, RMSD values were reported as 0.37 nm and 0.38 nm for SphK1 free and SphK1-quercetin complex, respectively. It was reported that the structure of SphK1 was stabilized with the binding of quercetin. In addition, a little conformational change in SphK1 was observed at Rg and SASA values for free SphK1 and SphK1-quercetin were given as 1.96 nm and 2.03 nm, and 145.61 nm^2 and 153.09 nm^2, respectively [44]. One of the reported MD simulation analyses shows that identified by structure-based virtual screening two natural compounds from the ZINC database can include potential scaffolds for further studies. The results of RMSD, RMSF, Rg, and SASA obtained by using GROMACS for free SphK1 and two ligands reported between 0.30-0.28 nm, 0.13-0.11 nm, 1.96-2.00 nm, and 145.61-147.05 nm^2, respectively [45]. In another study, MD simulation analysis was performed by SphK1, SphK1-PF543, and hits. *In silico* analysis indicated that the identified compounds had strong inhibitor potential to be used in the discovery and development of new SphK1 inhibitor candidates [12]. As a result, inhibition of SphKs using small molecules is an important strategy for cancer treatments and others. Due to its remarkable role in cancer progression, metastasis, and other diseases, SphK1 has emerged as a new therapeutic target to combat these diseases and develop effective therapeutics.

Herein, we identified some potential prenylated flavonoid compounds against SphK1. XN and prenylnaringenin compounds are the main components of *H. lupulus* L. medicinal plant. These compounds are widely used in biological activity studies such as cancer and inflammation-related metabolic pathways [48-50]. This study also indicates that these prenylated bioactive compounds can be potential for targeted SphK1. These results were supported by the structure-based virtual screening study by using a flavonoid compound library. Our findings have shown that prenylated flavonoid compounds with hydroxyl groups may be potential inhibitor candidates to be used in SphK1-targeted drug discovery and development. *In vitro* studies are recommended.

Funding
This study was supported by Çanakkale Onsekiz Mart University, the Scientific Research Coordination Unit (Project number TSA-2021-3729). The website biorender.com was utilized for creating the workflow.

Data Availability statement
The authors confirm that the data supporting this study are cited in the article.

Compliance with ethical standards

Conflict of interest
The authors declare no conflict of interest.

Ethical standards
The study is proper with ethical standards.

Authors' contributions
Concept- A.O., M.A., F.C.O.; Design- A.O., M.A., F.C.O.; Data Collection-Analysis-Writing- A.O., G.D., M.A., F.C.O.

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International Journal of Life Sciences and Biotechnology
Uzun, F. et al., Mera Tescillerindeki Anlaşmazlıkların Giderilmesinde Referans Alınabilecek Bazı Uygulamaların İrdelenmesi (Kastamonu, İncesu Köyü Merası Örneği). International Journal of Life Sciences and Biotechnology, 2024 7(2): p. 102-111.
https://doi.org/10.38001/ijlsb.1439685
Araştırma Makale/ Research article

Mera Tescillerindeki Anlaşmazlıkların Giderilmesinde Referans Alınabilecek Bazı Uygulamaların İrdelenmesi (Kastamonu, İncesu Köyü Merası Örneği)

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ÖZET
Mera kanunu çerçevesinde gerçekleştirilen meraların tespit, tahdit ve tescil çalışmalarında Tarım İl Müdürlüğü ve Orman Müdürlüğü arasında bir takım uyuşmazlıklar meydana gelebilmektedir. Bu uyuşmazlıkların giderilmesinde, genel olarak Orman Müdürlüğü'nün yönlendirmesiyle memleket haritaları dikkate alınmaktadır. Ancak sadece bu haritalar baz alınarak varılan kararlar, kamuoyunu tam olarak tatmin etmemektedir. Bu çalışma kapsamında, öncelikle İncesu köyü merasının bir kısmının memleket haritaları gerekçe gösterilerek orman olarak tescil edilme süreci irdelenmiş, akabinde loop metodu ile meranın vejetasyon etüdü yapılmıştır. Bitki örtüsünün belirlenmesine yönelik 2023 yılında yürütülen vejetasyon etüdü çalışmasında, alanın % 73.59'u otsu, kalan % 26.41'inin ise çalı ve ağaçlardan oluştuğu görülmüştür. Alanın bitki örtüsünün bu haliyle "Orta" kalitede mera vasfı taşıdığı belirlenmiştir. Diğer yandan, Avrupa Çevre Ajansı" tarafından belirlenen "Arazi Örtüsü/Kullanımı Sınıflandırması" ve Orman Genel Müdürlüğü'nün E-Harita Uygulaması verilerine göre de, alandaki otsu tabakanın bu gün olduğu gibi geçmişte de çalı-ağaç katına göre çok daha fazla oranda toprak yüzeyini örttüğü tespit edilmiştir. Bütün veriler dikkate alındığında, çalışılan alanın ormandan daha ziyade mera olarak kayıt altına alınmasının daha uygun olacağı anlaşılmıştır.

MAKALE GEÇMİŞİ
Geliş
19 Şubat 2024
Kabul
16 Nisan 2024

ANAHTAR KELİMELER
Mera kanunu,
orman kanunu,
mera durumu,
memleket haritaları,
coğrafi bilgi sistemi

Examination of Practices that can be taken as a Reference in Elimination of Disputes in Rangeland Registrations (Kastamonu, İncesu Village Rangeland Example)

ABSTRACT
In determining and registering rangelands within the scope of the Rangeland Law, some disputes may arise between the Provincial Directorate of Agriculture and the Forestry Directorate. In resolving these disputes, country maps are generally taken into consideration with the guidance of the Forestry Directorate. However, decisions based only on these maps do not fully satisfy the public. Within the scope of this study, firstly, the process of registering a part of the rangeland of İncesu village as forest based on the country maps was examined, and then the vegetation survey of the rangeland was carried out with the loop method.In the vegetation survey study to determine the vegetation cover, it was seen that 73.59% of the area was herbaceous and the remaining 26.41% consisted of shrubs and trees. It has been determined that the vegetation of the area has the characteristics of "medium" quality rangeland. On the other hand, according to the "Land Cover/Use Classification" determined by the European Environment Agency and the E-Map Application data of the General Directorate of Forestry, the herbaceous layer in the area covers the soil surface to a much greater extent than the shrub-tree layer today and in the past. Considering all the data, it was understood that it would be more appropriate to register the studied area as pasture rather than forest.

ARTICLE HİSTORY
Received
16 February 2024
Accepted
16 April 2024

KEY WORDS
Rangeland law,
forest law,
rangeland condition,
country map,
geographic information system

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Giriş

Mülga Köy Hizmetleri Kurumu'nun 1970 yılı kayıtlarında çayır ve mera alanları 21.698.400 ha olarak geçmektedir [1]. Sonraki yıllarda yapılan 1991 Tarım Sayımında 12.377.600 ha [2], 2001 TÜİK sayımında ise 14.616.687 olarak güncellenmiştir [1]. Mera Kanunu çerçevesinde mera, yaylak, kışlakların tespiti çalışmalarına ait ilk veriler 1999 yılı itibarıyla alınmaya başlanmış, 2023 yılı itibarıyla 13.147.201 ha'a ulaşmıştır [1]. Bu rakamlara göre çayır ve mera alanları 1970 yılından bu yana % 39.41 oranında azalmıştır. Orman Bakanlığı'nın olmadığı süreçte, mera olarak sınıflandırılan çalılık alanlar 1969 yılında Orman Bakanlığı'nın kurulmasıyla orman-fundalık sınırları içerisine alınmıştır. Bu süreçte niteliklerinde hiçbir değişiklik olmayan bu alanlar, sadece arazi sınıflamasından ileri gelen bir değişiklikle mera olmaktan çıkarılmıştır. Mera alanlarında kısa sürede görülen bu büyük azalma yanında, o günden bu yana mera alanlarının azar azar orman kayıtlarına alındığı istatistiklerde görülmektedir. Orman Genel Müdürlüğünün verilerine göre ülkemizin orman varlığı 1973 yılı itibarıyla 20.199.296 ha iken, 2022 yılı itibarıyla 23.245.000 ha'a ulaşmıştır [3]. İstatistiki veriler ormanlık alanlarda 50 yıl içinde 3.045.704 ha'lık bir artış meydana geldiğini göstermektedir.

Ülkemiz orman alanında saptanan bu artışta mera alanlarını etkileyen 2 temel uygulamadan söz etmek mümkündür. Bunlardan ilki erozyona maruz kalan ve ıslahının mümkün olmadığı belirlenen mera alanlarında toprak muhafaza amaçlı ağaçlandırma çalışmaları nedeniyle Mera Kanunu'nun 3. Bölüm 14. Maddesi (Değişik:3/7.2005-5403/27 md.) ve Mülga Gıda Tarım ve Hayvancılık Bakanlığı ile Orman ve Su İşleri Bakanlığı arasında ağaçlandırma seferberliği kapsamında yapılacak ortak çalışmalara ilişkin 17.01.2012 tarihli protokol gereğince tahsis amacı değişikliği yapılarak bir kısım mera alanlarının vasfının değiştirilerek orman kayıtlarına geçmesidir [4]. İkinci olarak, kadimden beri mera olarak kullanılmakla birlikte görevli kişilere bildirimde ihmal edilen ve hatta bazılarının Osmanlıca evrakları olduğu halde okunamadığından mera olarak kayıtlara alınmada eksik kalınan mera alanlarının Orman Genel Müdürlüğü'nce orman olarak kayıtlara geçirildiği göze çarpmaktadır [5, 6, 7, 8].

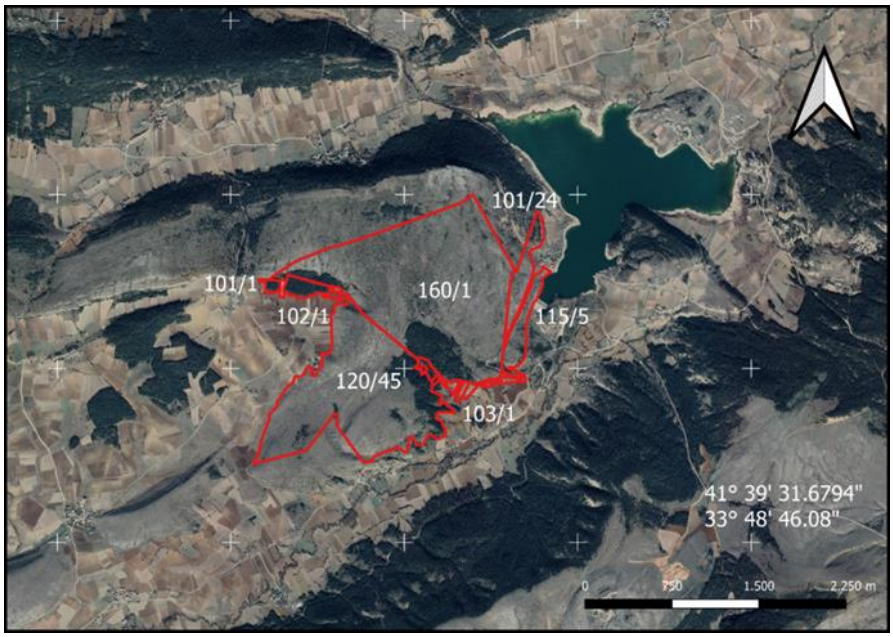
Her ne kadar 4342 sayılı Mera Kanunu'nun meraların hukuki durumlarının ifade edildiği 2. Bölüm, 4. Maddesinde mera, yaylak ve kışlaklar ile ilgili davalarda zaman aşımı uygulanamaz prensibinden hareketle, meraların süreklilik arz eden bir şekilde orman sınırları içerisine alınmasının önüne geçilmesi yönünde mahkemeler kanalıyla hukuki çabalar harcansa da yukarıda da ifade edildiği gibi bu kayıpların önüne geçilemediği görülmektedir. Mahkemelere intikal etmiş davalarda, meralara oranla kamuoyunun daha çok ilgisine mazhar olan ormanların lehine olacak şekilde pozitif ayrımcılığın da bunda etkisi olduğu söylenilebilir.

Sonuç olarak son 50 yıllık süreçte mera alanları şu ya da bu sebeplerle -istisnalar hariç- sürekli orman alanlarına dahil edilmiş ve oldukça yüksek bir kayıp yaşamıştır. Bunun en büyük sebebi orman varlığı kadar bir alan kaplayan meralarımızın üst yönetici erk tarafından yakın zamanlara kadar öneminin anlaşılamamış olması ve bu alanlar ile ilgili uzunca bir süre kanuni düzenleme ve kamu teşkilatlanmasının gerçekleştirilmeyişidir. Yaklaşık olarak ormanlar ile aynı büyüklükte olan meralar hakkında kapsamlı bir kanun ancak 1998 yılında çıkartılabilmiştir. Kanun çerçevesinde 2018 yılına kadar Tarım Teşkilatı içerisinde Bitkisel Üretim Şubesi'ne bağlı, Mera Birimi şeklinde temsil edilirken, 2018 yılında Çayır, *Mera* ve Yem Bitkileri *Şube Müdürlüğü olarak teşkilatlanabilmiştir.* Ormanlar ise mera biriminden 29 yıl öncesinde Bakanlık seviyesinde sahiplenilen bir varlığımız olmuştur. Gelinen noktada azalan mera varlığı ve hayvan ırklarındaki dönüşüm gibi daha başka sebeplerle mera hayvancılığı sürekli gerilemiş, hayvansal üretimde istenilen gelişim sağlanamamıştır. Sürekli artan hayvansal ürün talebinin, yurtdışından hayvan ithal edilerek karşılanması yoluna gidilmiştir [9]. Problemin çözümünde teknik ve sosyal çözüm önerileri bir araya getirilip sağlıklı bir yol bulunamayınca da kayda değer bir başarı sağlanamamıştır. Hayvansal ürünlerde üretim artışının sağlanmasında çözüm üretilmesi gereken en öncelikli hususun, yeterli miktarda ot üretimi olduğu gerçeği bilinse de, çözüme yönelik çalışmalar çeşitli sebeplerle akamete uğramıştır.

Bu çalışmanın amacı, kadimden beri mera olarak kullanılan dolayısıyla mera olarak tespiti yapılan, ancak memleket haritaları gerekçe gösterilerek orman olarak tescil edilen İncesu köyü sınırları içerisinde yer alan alanın, bitkisel varlığı, uydu görüntüleri ve mera hukuku bakımından irdelenerek yapılan işlemin teknik ve hukuki dayanakları değerlendirilmiştir.

Materyal ve Metot

Çalışılan alan, İncesu köyü sınırları içerisinde Kürüz Geçesi mevkiindedir. Alanın tapu alanı: 1720.3 da, Ada/parsel numarası: 160/1 ve Pafta numarası: E31.C.06.A'dır. Alanın coğrafi konumu Şekil 1'de verilmiştir [10].



Şekil 1 Çalışılan alanın uydu görüntüsü
Figure 1 Satellite image of the studied area

Yarı nemli iklim sınıfına giren Kastamonu ilinin [11]; uzun yıllara ait ortalama yıllık yağış toplamı 483.8 mm, ortalama sıcaklık değeri ise 9.9 ºC'dir [12]. İlin kuzeyi nemli, Seydiler ilçesi'nin de yer aldığı güneyi ise yarı nemli iklime sahiptir.

Seydiler ilçesinde yazlar sıcak ve az bulutlu ve kışlar dondurucu soğuk, karlı ve parçalı bulutludur. Yıl içerisinde sıcaklık normalde -5 ile 25 °C arasında değişiklik göstermekte, nadiren -13 °C'nin altına düşmekte ve 30 °C'nin üzerine çıkmaktadır.

2023 yılı itibarıyla alandaki bitki örtüsünün hâlihazırdaki durumunu belirlemeyi amaçlayan bu çalışmada ilk olarak modifiye edilmiş tekerlekli lup (halka) metodu kullanılarak alandaki bitki türleri ve bunların oranları belirlenmiştir. Çalışma, hâkim bitkilerin çiçeklenme evresinde "yaprak alanı" esas alınarak yürütülmüştür [13]. Ölçüm hatları meranın eğim yönüne paralel olarak seçilerek meranın her eğim derecesindeki vejetasyonu temsil edecek şekilde 6 lup hattında 600 noktada yapılmıştır. Bitki teşhisinde [14] ve [15]'den faydalanılmıştır. Okuma neticesinde tespit edilen bitki türleri azalıcılar, çoğalıcılar ve istilacılar olmak üzere 3 sınıfa ayrılmışlardır. Tespit edilen bitkilerden azalıcıların ve çoğalıcılar (çoğalıcılar botanik kompozisyonda % 20'nin altında oldukları için)'ın oranlarının toplam değeri dikkate alınarak meranın "mera durumu sınıfı" belirlenmiştir. Bitki örtüsünün toprağı kaplama oranı, vejetasyon etüdü sırasında bitkiye rastlanan nokta sayısının ölçülen toplam nokta sayısına oranlanması ile belirlenmiştir [16]. Okunan her bir bitki türüne ait değerler, toplam bitki sayısına oranlanarak türlerin botanik kompozisyondaki oranları tespit edilmiştir. Meraların durum ve sağlık sınıflamaları, [17] ve [18] tarafından ifade edilen kriterlere göre yapılmıştır (Tablo 1).

Alandaki bitki örtüsünü belirlemeye yönelik çalışmada faydalanılan ikinci yöntem, Tüm dünyada arazi sınıflandırması hususunda otorite olarak kabul edilen Corine (Coordination of Information on the Environment - Çevresel Bilginin Koordinasyonu) sistemi ile Avrupa Çevre Ajansı tarafından Türkiye için uydu görüntüleri üzerinden bilgisayar destekli görsel yorumlama metodu ile üretilen arazi örtüsü/kullanımı sınıflandırmasıdır [19]. Çalışmada kullanılan üçüncü yöntem ise Orman Genel Müdürlüğü'nün E-Harita uygulamasıdır [20].

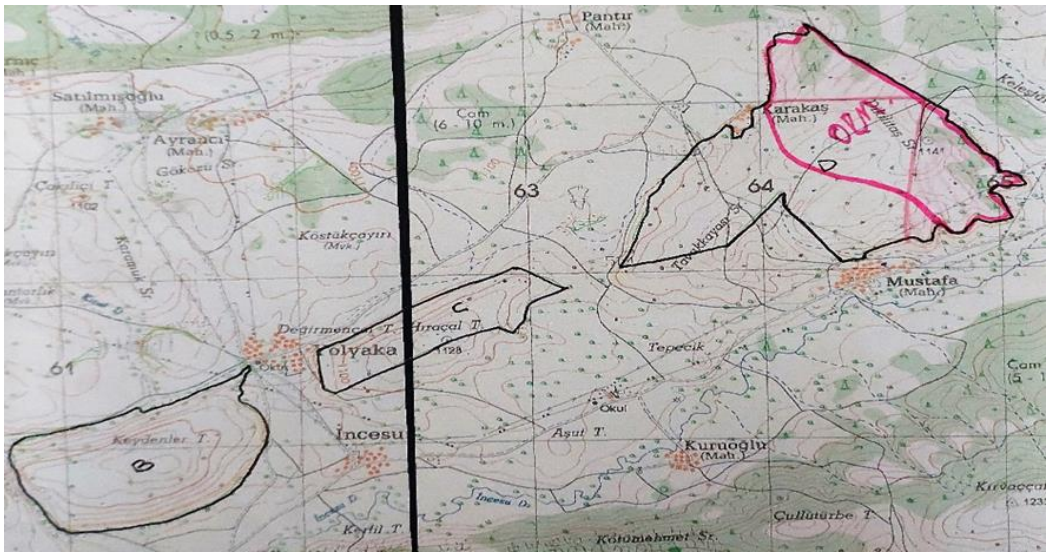
Tablo 1 Mera durumu ve bitki örtüsünün toprağı kaplama derecesi
Table 1 Rangeland condition and soil coverage degree of vegetation

Azalıcı + çoğalıcı bitki türlerinin oranı (%)	Mera durumu sınıfı	Bitki örtüsünün toprağı kaplama oranı (%)	Mera sağlık sınıfı
76-100	Çok İyi	>70	Sağlıklı
51-75	İyi	55-70	Riskli
26-50	Orta	<55	Sorunlu
0-25	Zayıf		

Bulgular ve Tartışma

Çalışma alanının hemen kuzeyi ve kısmen kuzeydoğusu tapulama harici alan, doğusunda Devrekâni ilçesine bağlı Fakılar köyüne ait 101/24 (112.18 da), 115/5 (111.80 da) ve 115/32 (5.44 da) ada/parsel numaralı mera alanları, güneyinde İncesu köyüne ait muhtelif büyüklüklerde tarla alanları ve güneybatısında kısmen işlenilmeyen tarla alanları bulunmaktadır. Alanın batıda büyük sınırını Seydiler ilçesinin Yolyaka köyüne ait 1216.31 da'lık 120/45 ada/parsel numaralı mera, kalan batı sınırının bir kısmını tarla ve çok küçük bir kısmını da 2 parsel halinde 63.99 da'lık orman alanı oluşturmaktadır. Şekil 1'den de görülebileceği üzere alanın en uzun sınırını sırasıyla tapulama harici alan, meralar, tarlalar ve en son olarak ta ormanlık alan oluşturmaktadır.

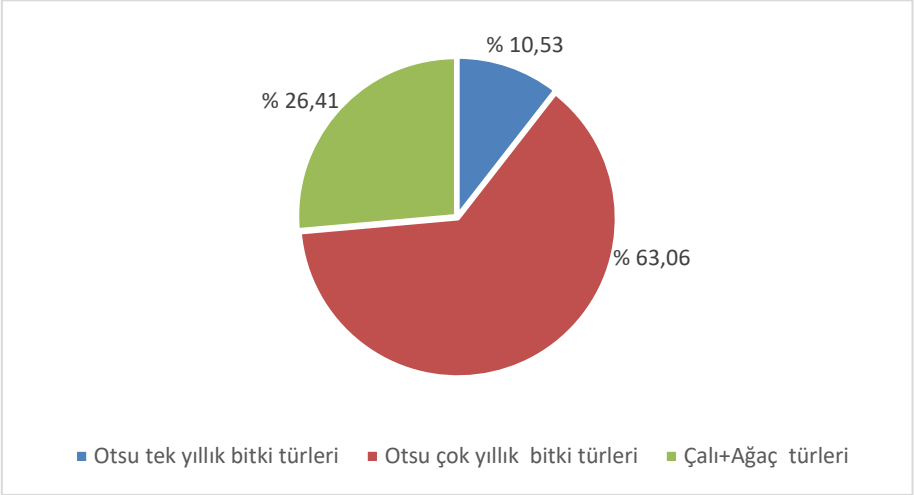
Kastamonu İl Tarım ve Orman Müdürlüğü personelinin sahada yaptığı çalışmada 160/1 ada/parsel numaralı bahse konu çalışma alanı, ilk olarak "Mera" olarak tespit edilmiştir [21]. Tespitin, Mera Kanunu'nun 2. Bölüm, 5/a maddesi uyarınca "Kadimden beri mera, yaylak ve kışlak olarak kullanılan yerler ile aynı amaçla kullanılmak üzere köy veya belediyelere tahsis ya da terkedilen yerler" mera, yaylak ve kışlak olarak köylere tahsis edilmesi hükmüne uygun olduğu görülmektedir. Ancak buradaki "Tespit" mera kanununun 2. Bölüm, 5/b yani "Devletin hüküm ve tasarrufunda veya hazinenin mülkiyetinde bulunan arazilerden etüt sonucu mera, yaylak ve kışlak olarak yararlanılabileceği anlaşılan yerlerin bu amaçla tahsisinin uygun olacağı maddesine dayandırılarak gerçekleştirilmiştir [21]. Devam eden süreçte Mera Kanunu'nun 9. Maddesi gereğince "Orman tahsisi kesinleşen ve kadastrosu yapılan yerlerde mera, yaylak ve kışlakların kesinleşmiş sınırları dikkate alınması" gerekliliği ifade edilmiştir. Bu nedenle Kastamonu Orman Bölge Müdürlüğü'nden görüş istenmiştir. Orman Müdürlüğü de mera tespiti çalışmalarında mera olarak tespit edilen ve "memleket haritasında" [22], (Şekil 2) bahse konu 160/1 no'lu parseli de (D) içeren B, C, D, E, F numaralı parseller olarak ifade edilen alanlar için Küre Orman İşletme Müdürlüğü önce parsellerin "kesinleşmiş orman sınırları" içerisinde kaldığını belirtmişlerdir [23]. Sonraki yazışmalarda aynı kurum kendini tekzip etmiş, orman kadastrosu olmadığından orman sınırlarının kesinleşmediğini, memleket haritalarını dayanak göstererek alanın orman olduğunu iddia etmiştir [24]. En nihayet konu mera komisyonunda görüşülmüş; B, C, E parsellerinin orman olmakla hiçbir alakasının olmadığı, F diye bir parselin ise hiç mevcut olmadığı, bu nedenle Küre Orman İşletme Müdürlüğü'nün itirazının "Geçersiz" olduğuna, "D parselinin de" niteliğinin yeniden belirlenmesine karar vermiştir [25]. Kastamonu İl Tarım ve Orman Müdürlüğü, Çayır Mera ve Yem Bitkileri Şube Müdürlüğü'nün düzenlemiş olduğu evrakta Pafta numarası: E31-c-06-A olarak kaydedilen bu alanın 5/a kaynaklı, yani "Kadimden beri mera alanı olarak kullanılan alan" olarak kayda almıştır [26]. Ancak alan bu haliyle tescil için tapuya kaydedilmemiştir. En nihayetinde alanın 1216.30 da'lık kısmı "mera" (Ada/parsel:120/45) 1720.30 da'lık kısmı ise "orman" olarak kayıtlara geçmiştir [10]. Yukarıda da ifade edildiği gibi kararın gerekçesini "Kadimden beri mera, yaylak …" diye başlayan 4342 sayılı mera kanununun 5/a maddesi değil de, kanun maddesi olarak veya bu tür anlaşmazlıklarda tespit çalışmalarında delil olarak kabul edilebilecek bir kaynak olarak adı geçmeyen "memleket haritaları" oluşturmuştur. Dolayısıyla verilen bu kararda, kanun hükümleri açısından açık bir uyarsızlık görülmektedir.



Şekil 2 Kastamonu Orman Müdürlüğü'nün ormanlık alan olarak nitelediği bazı parsellere delil olarak sunduğu 1/25000 ölçekli memleket haritası
Figure 2 1/25000 scale map of the country presented by Kastamonu Forestry Directorate as evidence for some parcels that it defines as forest areas

Vejetasyon etüdü verilerine göre bitki örtüsünü oluşturan türler

Yapılan vejetasyon etüdü çalışmasından elde edilen verilere göre alanın toprak yüzeyi % 73.59 oranında 41 farklı otsu, % 26.41 oranında ise çalı-ağaç türleri ile örtülüdür (Şekil 3 ve Tablo 2,3).



Şekil 3 Mera vejetasyonunda yer alan bitki türlerinin oransal dağılımı (%)
Figure 3 Proportional distribution of plant species in rangeland vegetation (%)

Vejetasyonunda belirlenen bitki türlerinin kalite derecelerine göre dağılımına göre; 5 adedi (% 10.56) azalıcı, 6 adedi (% 18.64) çoğalıcı, 11 adedi çalı-ağaçsı (% 26.48) ve 29 adedi otsu (% 44.32) olmak üzere 40 adedinin istilacı türlerden oluştuğu görülmüştür. Tablo 1'de verilen sınıflamaya göre bu alan "Orta" kalitede mera durumunu ifade eden bitki örtüsüne sahiptir.

Çalışılan alanda bitki örtüsü içerisindeki oranları itibarıyla öne çıkan azalıcı türler sırasıyla *Medicago falcata* (% 5.06), *Koeleria cristata* (% 3.02) ve *Sanguisorba minör* (% 1.04) olurken, çoğalıcı türler ise sırasıyla, *Festuca ovina* (% 9.00), *Carex acuta* (% 6.16) ve *Poa bulbosa* (% 1.58) olmuştur. Alanda tespit edilen otsu yabancı otlar içerisinde en fazla rastlanılanları *Galium album* (% 4.34), *Calamintha grandiflora* (% 3.79) ve *Astragalus fragrans* (% 3.67)'tır.

Alanın bitki örtüsünün toprağı kaplama oranı % 90.21'dir. Tablo 1'deki sınıflama değerlerine göre bitki örtüsü bakımından alan "Sağlıklı" sınıfta yer almıştır. Buna göre alanın yeterince bitki örtüsü ile kaplı olduğu, herhangi bir erozyon riski altında olmadığı söylenilebilir.

Tablo 2 Vejetasyondaki azalıcı ve çoğalıcı bitki türlerinin ömür uzunlukları ve oranları (%)
Table 2 Lifespan of decreaser and increaser plant species in the vegetation and their proportions (%)

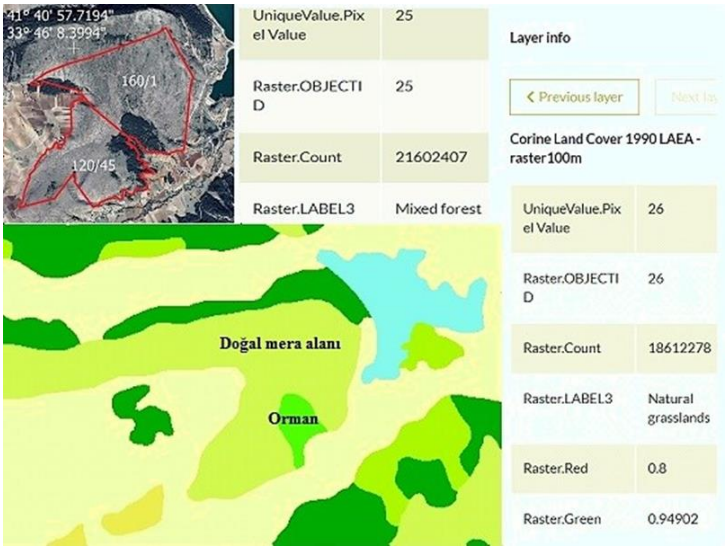
Türler	Bitki Ömrü	%	Türler	Bitki Ömrü	%
Azalıcılar / Decreasers					
Medicago falcata	aÇY	5.06	Trifolium repens	ÇY	0.86
Koeleria cristata	ÇY	3.02	Onobrychis armena	ÇY	0.58
Sanguisorba minör	ÇY	1.04			
Toplam / Total					**10.56**
Çoğalıcılar / Increasers					
Festuca ovina	ÇY	9.00	Teucrium polium	ÇY	0.88
Carex acuta	ÇY	6.16	Brachypodium sylvaticum	ÇY	0.73
Poa bulbosa	ÇY	1.58	Coronilla orientalis	ÇY	0.29
Toplam / Total					**18.64**
Genel toplam / Grand total					**29.20**
aÇY: Çok yıllık otsu bitki					

Tablo 3 Vejetasyondaki istilacı bitki türlerinin ömür uzunlukları ve oranları (%)
Table 3 Lifespan of invader plant species in the vegetation and their proportions (%)

Türler	Bitki Ömrü	%	Türler	Bitki Ömrü	%
İstilacılar / İnvaders					
Galium album	aÇY	4.34	Ononis spinosa	ÇY	0.86
Calamintha grandiflora	ÇY	3.79	Bellis perennis	ÇY	0.68
Astragalus fragrans	ÇY	3.67	Myosotis alpestris	ÇY	0.68
Filipendula vulgaris	ÇY	3.01	Plantago lagopus	TY	0.68
Bromus japonicus	bTY	2.96	Medicago rigidula	TY	0.55
Achillea biebersteinii	ÇY	2.73	Parentucellia latifolia	TY	0.55
Pilosella hoppeana	ÇY	2.66	Trifolium resupinatum	TY	0.55
Astragalus alyssoides	ÇY	2.36	Salvia viridis	TY	0.47
Globularia orientalis	ÇY	2.02	Scorzonera hieraciifolia	ÇY	0.47
Potentilla recta	ÇY	1.53	Cirsium sipyleum	cİY	0.41
Centaurea depressa	TY	1.51	Euphrasia roskoviana	TY	0.41
Veronica elmaliensis	ÇY	1.41	Polygonum arenarium	TY	0.25
Teucrium chamaedrys	ÇY	1.39			
Sedum album	ÇY	1.23	Genista albida (Çalı)		4.07
Fumaria officinalis	TY	1.17	Crataegus monogyna (Çalı)		4.88
Centaurea sessilis	TY	1.02	Juniperus communis (Ağaç)		0.74
Taraxacum aleppicum	ÇY	0.96	Diğer Çalı-Ağaçlar		16.79
Toplam / Total					**70.80**
Azalıcı+Çoğalıcı+İstilacı					**100.0**
aÇY: Çok yıllık otsu bitki, bTY: Tek yıllık otsu bitki, cİY: İki yıllık otsu bitki					

Uydu görüntülerinden yararlanılarak alanın bitki örtüsünün belirlenmesi

Alanın; 1) 2023 yılına ait uydu görüntüleri (Şekil 1), 2) "Avrupa Çevre Ajansı" tarafından belirlenen "Arazi Örtüsü/Kullanımı Sınıflandırmasına" göre uydu görüntüleri üzerinden bilgisayar destekli görsel yorumlama metodu ile üretilen veriler (Şekil 4); [27] ve 3) Orman Genel Müdürlüğü'nün E-Harita Uygulaması (Şekil 5); [20] incelendiğinde, alandaki otsu tabakanın geçmişte de, bugün de çalı-ağaç katına göre çok daha fazla oranda toprak yüzeyini örttüğü açık bir şekilde görülmektedir. Bunun anlamı köydeki yaşlı insanların da ifade ettikleri gibi alanın geçmişi de meradır. Buna göre alan sosyolojik sebeplerle ve orman kayıtlarına geçtikten sonra başta keçi otlatmanın azalması ve günümüz itibarıyla keçi varlığının hiç kalmaması gibi otlatma düzenindeki değişiklikler, otsu bitki örtüsünün hâkim olduğu bu alanda tabiatın kendi sistematiği içerisinde gelişen süksesyonal süreç nedeniyle her geçen gün otsu tabakanın aleyhine, çalı-ağaç tabakasının lehine değişimin olduğu akla en yakın teoridir. Aynı şekilde yakın gelecekte çalı ve ağaçların daha da artacağı ve alanın ormana dönüşeceği açıktır.



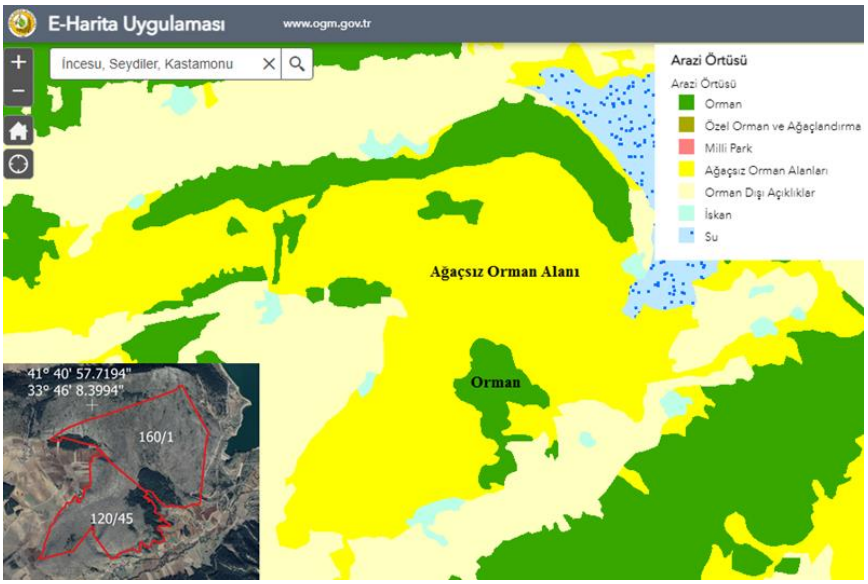
Şekil 4 Corine haritasına göre çalışma alanının bitki örtüsünün niteliği (Mixed forest= Karışık orman, Natural grassland = Doğal mera)

Figure 4. Characteristics of the vegetation of the study area according to the Corine map (Mixed forest= Karışık orman, Natural grassland = Doğal mera)

Yarı-nemli ve yaklaşık yıllık ortalama yağışı 483 mm [12] olan alanın iklim değerleri buna imkân sağlayabilecek asgari nitelikleri taşımaktadır. Aslında otlatmanın bir şekilde azaltıldığı mera alanları ve hatta tarla alanları dahi eğer ki işlenmeyip kendi haline bırakılırsa yukarıda ifade edilen nedenle bu alanların tamamı orman olacaktır [13, 28, 29]. Bu doğal süreç nedeniyle kadimden beri mera olarak kullanılan bu alanın orman kayıtlarına geçirilmesi teknik anlamda doğru değildir. Nitekim Zonguldak İl Mera Komisyonu'nun Yenidoğancılar köyü 152 ada/45 numaralı parsel için kadim mera alanları için zaman aşımı uygulanamayacağına, zemindeki bitki örtüsünde zamanla değişiklik olsa da bu değişikliğin o yerin kadim vasfını kaybettirdiği anlamına gelmediğine 8 lehte ve 1 aleyhte (Orman Bölge Müdürlüğü temsilcisi) oy ile karar vermiştir [7].

Çalışılan alan, geçmişte orman vasfını taşımış olsa, geçmişe yönelik görüntülerden alanda bugünkü halinden daha fazla oranda ağaç varlığına sahip olması gerekliliği vardır. Çünkü tabiatta sürekli olarak gerçekleşen bitki süksesyonunda değişimin yönü, otsu bitki örtüsünden ağaçların gittikçe varlığını artırdığı odunsu bitki yapısına evrilmesi şeklindedir [13, 30, 31]. Nemli-mezotermal iklim kuşağında yer alan Bartın ilinde toprak işlemek suretiyle tamamen otsu bitki türleri kullanarak tesis edilen yapay meranın, 7-8 yıllık bir süreçte bitki örtüsünün % 48.47 oranında yeniden *Rubus, Crataegus, Smilax, Prunus, Rosa, Ulmus, Carpinus, Berberis* gibi çalı-ağaçların olduğu bir yapıya evrildiği ifade edilmiştir [31].

Aynı ilin 14 farklı doğal köy merasında yapılan bir başka çalışmada, vejetasyonların % 12.5 ile % 100'ünü çalı-ağaçların oluşturduğu belirlenmiştir [30] Araştırıcılar, mera vejetasyonlarındaki çalı-ağaçların oransal olarak varlıklarını artırmalarına en önemli sebep olarak ta çalışılan köylerdeki küçükbaş sayısının toplam hayvan varlığının sadece % 1.16'sını, bunun içinde de keçilerin koyunlardan çok daha az oranda olmalarını göstermişlerdir.



Şekil 5 Orman Genel Müdürlüğü'nün E-Harita Uygulamasında alanın bitki örtüsünün niteliği
Figure 5 Characteristics of the vegetation of the area in the E-Map Application of the General Directorate of Forestry

Bilindiği üzere keçiler çalı ve ağaçların vejetasyonlardaki artışlarını engelleyerek meraların mera olarak kalmasını sağlamakta, süksesyonal gelişim ile ormana dönüşmesini engelleyen bir işlev görmektedir [32]. Ancak özellikle 1990'lı yıllarda keçiler, en başta Orman Bakanlığı ve ağaç severler, bu hayvanların ormanlara zarar verdiğini ve varlıklarının azaltılması gerektiği yönünde kamuoyu oluşturmuşlar ve bunda da başarılı olarak meralardaki çalı-ağaç varlığının artmasının önünü açmışlardır [33, 34, 35].

Mera Hukuku'na göre alanın niteliği

Mera Kanunu'nun 5/a maddesi gereğince "Kadimden beri mera, yaylak ve kışlak olarak kullanılan yerler aynı amaçla kullanılmak üzere tahsis edilmelidir". Ancak özellikle bir vesikaya dayanmayan, kadimden beri mera olarak kullanılan alanların mera olarak tespit ve tescilinde buradakine benzer birçok aksaklıklar yaşanmaktadır [6, 36]. Şekil 1 dikkatle incelendiğinde, çalışılan alanın hemen kuzey tarafı tapulama harici alan, onun bir kuzeyi de orman olarak çok daha önceleri kayda geçmiştir. Bu alanın orman kayıtlarına alınmaması, çalışma yapılan yıllarda alanın orman vasfı taşımadığı gerçeğini ifade etmektedir. Orman kadastrosu yapılırken orman olarak tescil edilmesi için yeterli vasıfları taşımayan bu ve benzeri alanların otsu bitki örtüsüne süksesyon neticesinde dâhil olan bir kısım çalı ve ağaçlar veya memleket haritalarının gerekçe gösterilerek –ki bu alanda bitki örtüsünün ancak çeyreği oranındadır- Mera Kanununun 5/a maddesini dikkate almayıp mera olarak tesciline engel olunması bilimsel ve hukuki gerçeklerle uyumlu olmadığı söylenilebilir.

Mera Şube Müdürlüğü'nün hayvan sayıları ve mera alanları ile ekilen yem bitkilerine ait envanter çalışmalarından elde edilen verilere göre, İncesu köyünde hayvanların ihtiyaç duyduğu kaliteli kaba yemin ancak üçte birlik kısmı karşılanabilmektedir [37]. Eksik miktar, başta tahıl samanları olmak üzere düşük kaliteli tarla bitkileri hasat artıklarından karşılanmaya çalışılmaktadır. Bahsi geçen düşük kaliteli kaba yemlerin besleme açısından eksikliği ise pahalı fabrika yemleri ile telafisi de hayvansal ürünlerin maliyetini artırmaktadır. Bu durum hem üretici ve hem de tüketici açısından ekonomik ve sosyal problemlere sebep olabilmektedir.

Sonuç

1) Gerek mera şubesi teknik ekibi ve gerekse bizzat tarafımızca sahada yapılan teknik çalışmalardan elde edilen vejetasyon etüdü sonuçları, 2) Corine, Arazi örtüsü/kullanımı sınıflandırması, 3) Orman Genel Müdürlüğü sitesindeki E-harita görüntüleri ve 4) Kadim mera olgusunun hukuki hükmü dikkate alındığında, çalışılan alanın ormandan ziyade mera niteliği taşıdığı görülmektedir. Hal böyleyken alanın, Mera Kanunu'nun, 2/4. Maddesi'nde, mera, yaylak ve kışlaklar; "…amacı dışında kullanılamaz, zaman aşımı uygulanamaz, sınırları daraltılamaz" ifadesi gereği hukuki statüsünün yeniden gözden geçirilmesi, yerinde olacaktır. Gelecekte bu türlü uyuşmazlıkların giderilmesinde sadece memleket haritaları ile yetinilmeyip, çalışmada ifade edilen kaynaklardan maksimum istifade edecek şekilde bir düzenleme yapılmasına ihtiyaç vardır. Aksi halde ülkemiz mera alanlarının orman sınırları içerisine dâhil edilmeleri

suretiyle yaşanan mera alanı kayıpları mütemadiyen devam edecektir. Mera alanlarında yaşanan bu kayıplar da mera hayvancılığının kısıtlanması ve bu iş kolunun akamete uğramasına neden olacaktır. Unutulmamalıdır ki meralar da ormanlar gibi ülkemizin değerleridir ve en az ormanlar kadar -her anlamda- önemlidir, vazgeçilmezdir, biri ötekinden daha kıymetli değildir.

Teşekkür / Acknowledgements) ve Fon desteği / Funding
Çalışma alanına ulaşım ve verilerin elde edilmesindeki destekleri için, Kastamonu İl Tarım ve Orman Müdürlüğü'ne teşekkür ederiz.
We would like to thank Kastamonu Provincial Directorate of Agriculture and Forestry for their support in accessing the study area and obtaining data.

Yazar katkıları / Authors' contributions
Makalede adı geçen tüm yazarlar makaleye eşit oranda katkı yapmışlardır. Tüm yazarlar makaleyi incelemiş ve onaylamışlardır.
All authors mentioned in the article contributed equally to the article. All authors reviewed and approved the manuscript.

Veri ve materyalin elde edilebilirliği / Availability of data and material
Herhangi bir veri talebi için lütfen ilgili yazarla iletişime geçin.
Please contact the corresponding author for any data request.

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International Journal of Life Sciences and Biotechnology
Turgunbaeva, M. and A. Aygun, Kırgız Milli Ala Arça Parka Ait Yerli Çeşitler Araştırma İstasyonunda Yetişen Albenisi Yüksek Bazı Elma (*Malus x domestica* Borkh.) Çeşitlerinin Bazı Meyve Özellikleri. International Journal of Life Sciences and Biotechnology, 20247(2): p. 112-119.
https://doi.org/10.38001/ijlsb.1480394
Araştırma Makale/ Research article

Kırgız Milli Ala Arça Parka Ait Yerli Çeşitler Araştırma İstasyonunda Yetişen Albenisi Yüksek Bazı Elma (*Malus x domestica* Borkh.) Çeşitlerinin Bazı Meyve Özellikleri

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ÖZET
Bu araştırma, Kırgızistan'nın Bişkek şehri içerisinde bulunan farklı yerlerden daha önceden toplanarak kurulmuş yerel elma çeşitlerine ait koleksiyon bahçesinde yetiştirilen bazı elma çeşitlerinin meyve özelliklerinin belirlenmesi amacıyla yürütülmüştür. Çalışmada toplam 18 adet yerel elma çeşidi incelenmiştir. Elma çeşitlerinde meyve ağırlığı 42.28-261.25 g, meyve boyu 25.39-73.31 mm, meyve eni 30.58-94.19 mm, meyve kalınlığı 31.41-91.36 mm, sap çukuru derinliği 5.22-17.58 mm, sap çukuru eni 9.88-26.42 mm, sap çukuru genişliği 8.59-31.80 mm, sap uzunluğu 13.32-32.21 mm, sap kalınlığı 1.16-9.47 mm, çiçek çukuru derinliği 5.75-28.69 mm, çiçek çukuru genişliği 8.73-22.61 mm, çiçek çukuru eni 4.14-17.46 mm, çekirdek sayısı 3.22-8.60 adet, meyve eti sertliği 2.51-11.35 kg/cm², suda çözünebilir kuru madde miktarı %3.48-16.75, pH 3.02 ile 7.40, titre edilebilir asitlik miktarı %0.21-1.86 aralığında bulunmuştur. Bu sonuçlar bölgede farklı lokasyonlardan daha önce toplanan elmalarda özellik bakımından farklılıklar olduğunu ortaya koymasının yanında bu çeşitlerin piyasada talep edilebileceği fikrini doğurmuştur. Diğer yandan bu genetik kaynakları oluşturan bahçenin yaşlanması ve bakımsız olması yüzünden bu çeşitler yok olma tehlikesi altındadır.

ARTICLE HISTORY
Received
08 Mayıs 2024
Accepted
08 Ağustos 2024

ANAHTAR KELİMELER
Malus x domestica Borkh.,
Kırgızistan,
pomoloji,
astlik,
SÇKM

Some Fruit Characteristics of Some High Albenic Apple (*Malus x domestica* Borkh.) Varieties Grown in Kyrgyz National Ala Archa Park Local Varieties Research Station

ABSTRACT
This study was carried out to determine the fruit characteristics of some apple cultivars grown in the collection orchard of local apple cultivars previously collected from different places in Bishkek city of Kyrgyzstan. A total of 18 local apple cultivars were examined in the study. Fruit weight 42.28-261.25 g, fruit length 25.39-73.31 mm, fruit width 30.58-94.19 mm, fruit thickness 31.41-91.36 mm, stem pit depth 5.22-17.58 mm, stem pit width 9.88-26.42 mm, stem pit width 8.59-31.80 mm, stem length 13.32-32.21 mm, stem thickness 1.16-9. 47 mm, flower pit depth 5.75-28.69 mm, flower pit width 8.73-22.61 mm, flower pit width 4.14-17.46 mm, number of seeds 3.22-8.60, fruit flesh hardness 2.51-11.35 kg/cm², water soluble dry matter 3.48-16.75%, pH 3.02 to 7.40, titratable acidity 0.21-1.86%. These results showed that there were differences in the characteristics of the apples collected from different locations in the region, and it gave rise to the idea that these varieties could be demanded in the market. On the other hand, these varieties are under the danger of extinction due to the aging and neglect of the orchard that constitutes these genetic resources.

ARTICLE HISTORY
Received
08 May 2024
Accepted
08 August 2024

KEY WORDS
Malus x domestica Borkh.,
Kyrgyzstan,
pomology,
acidity,
WSDM

Giriş

Milattan öncesine dayanan elma kültürü, yüksek adaptasyon kabiliyeti ve çeşit sayısının fazlalığı nedeniyle, ılıman iklim meyve türleri arasında en çok tüketilen türdür. Orijini, Anadolu da dahil olmak üzere Güney Kafkasya'ya kadar uzanan bir coğrafyayı içerir. Bugün Dünyanın birçok ülkesinde yaklaşık 100 milyon ton elma üretilmektedir [1]. *Malus x domestica* Borkh dünyanın farklı ülkelerinden gelen 7500'den fazla çeşidi

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içerir, ancak sadece bu çeşitler içerisinde 20 civarında çeşit bahçecilik endüstrisinde yaygın olarak yetiştirilmektedir [2]. Elma (*Malus x domestica* Borkh.) taze meyve tüketiminin yanı sıra, gıda endüstrisinde ve biyolojik olarak aktif bileşiklerin ve diğer ikincil metabolitlerin kaynağı olarak yaygın olarak kullanılmaktadır [3,4]. Günümüzde dünya üzerinde pek çok bölgede geniş elma plantasyonları bulunmaktadır. Birçok çeşit, belirli koşullar altında yetiştirme için istenen özelliklere sahiptir, ancak bunlardan sadece birkaç düzinesi ticari olarak dünya çapında yetiştirilmektedir. Her geçen gün bu çeşitlere yenileri ekleniyor ve yeni çeşitler daha iyi özellikler ile piyasaya sürülmektedir [5]. Kırgızistan Cumhuriyeti Orta Asya ülkelerinin kuzeydoğusunda yer almaktadır: kuzeyden, batıdan ve güneyden Bağımsız Devletler Topluluğu CumhuriyetleriKazakistan, Özbekistan, Tacikistan ve doğudan ve güneydoğudan Çin Ulusal Cumhuriyeti ile sınırı vardır. Tiyan-Şan Dağları'nın iç ve kısmen kuzey ve batı kısımlarının yanı sıra 390 12' ve 430 15' Kuzey enlemlerinde ve 690 16' ve 800 18' Doğu boylamlarında Türkistan-Alay dağlarını kaplar. Kırgızistan topraklarındaki iklim, rakıma bağlı olarak büyük yerel sapmalarla birlikte soğuk kış ve sıcak yaz ile karakterize edilen karasal iklime hakimdir [6]. Orta Asya'da Kırgızistan önemli bir elma orijin merkezidir. Ceviz, Antep fıstığı ve badem türlerinin yanı sıra Kırgızistan'ın farklı bölgelerinde önemli elma popülasyonları ve ormanları bulunmaktadır. Ancak bu materyallerde ciddi antropojenik kayıplar vardır ve yakın gelecekte tamamen kaybolabilirler [6]. Kırgızistan'da doğal olarak bulunan üç elma türü vardır. Bunlar *Malus sieversii, Malus kirghisorum* ve *Malus niedzwetzkyana*'dır. Bu türler aynı zamanda kültür elmalarının kaynağı olarak da bilinmektedir [7]. Malus sieversii, Kazakistan, Kırgızistan, Çin, Tacikistan, Özbekistan ve Türkmenistan'ın kuru ve dağlık bölgelerine doğal olarak yayılmıştır. *Malus kirghisorum* genetik olarak *Malus sieversii*'ye yakındır, ancak meyve şekli, rengi ve diğer özellikleri bakımından farklıdır. *Malus niedzwetzkyana* türlerinde, yaprak, çiçek ve meyveler üzerinde pembe-mor pigmentasyonlar vardır. Bu ülkede yetiştirilen yerel çeşitler muhtemelen bu üç türün hibrit kompleksleridir [6]. Dünya elma üretimi 93.144.358 tona ulaşmıştır. Çin 45.983.400 tonluk üretimiyle dünyada ilk sırada yer alırken, onu Türkiye (4.493.264 ton) ve ABD (4.467.206 ton) takip etmektedir [8]. Kırgızistan elma üretimini 2022 yılı verilerine göre 132.102 ton elma üretimi yapılmaktadır. Kişi başı elma üretimi ise 18.87 kilogramdır. Kırgızistan'da yumuşak ve sert çekirdekli meyve üretimi değerlendirildiğinde elma üretimi ilk sırada yer almaktadır [9]. Bu üretimin büyük bir kısmını yerli ve yabani çeşitler oluşturmaktadır. Bu üretim miktarı Dünya sıralamasında önemli bir yer almasa da elma, Kırgızistanda'da toplam tarım üretimi içerisinde önemli bir rol oynamaktadır. Yine de bu üretim miktarı Kırgızistan gibi elmanın anavatanı olan ve ekolojisi elma yetiştiriciliği için çok uygun olan bir ülke için oldukça düşük miktardadır. Üç farklı türün gen merkezi olmasına rağmen istenilen elma üretimi yapılamamaktadır. Bunun sebepleri arasında çeşit sayısının az olması, modern meyvecilikte kullanılan anaçların ülkeye yeni yeni girmesi ve meyvecilik anlayışının tam olarak oluşmaması sayılabilir. Ülke çapında elma ile ilgili farklı çalışmalar yapılmasına rağmen ıslah yönünden bir çalışmaya rastlanılmamıştır. Bu çalışma mevcut popülasyonda üstün özellikli elma çeşitlerini seçmek ve çeşitlerin özelliklerini belirlemek amacıyla yürütülmüştür.

Materyal ve Yöntem

Materyal

Kırgızistan'ın Çüy bölgesindeki Kırgız Milli Ala Arça Parkı Gen merkezinde bulunan elma genetik kaynakları bahçesinde bulunan elma genotipleri içerisinden meyve özelliklerine göre ön plana çıkan 18 adet yerel elma çeşitleri çalışmanın bitkisel materyalini oluşturmaktadır.

Yöntem

Meyve ağırlığı (g) 0.01 g duyarlı hassas terazide ayrı ayrı tartılmış ve meyve boyu, çapı, kalınlığı, çiçek çukuru derinliği, eni ve genişliği ile sap uzunluğu, kalınlığı, sap çukuru genişliği, derinliği ve eni (mm) ölçümleri dijital kumpas yardımıyla, çekirdek sayısı (adet) elle sayılmış ve bu ölçümlerin ortalaması alınarak her çeşit için belirlenmiştir. Meyvelerin kabuk ve et rengi ölçümleri Colorimeter NR200 marka renk ölçüm cihazıyla, her örneğe ait her bir meyvenin üç farklı noktasından ölçüm yapılmıştır. Meyve kabuk ve et rengi parametreleri üç farklı okuma şeklinde L*, a*, b* olarak belirlenerek L* değeri meyve renginin parlaklığındaki değişimi göstermektedir. L*: siyahtan beyaza, a*: kırmızıdan yeşile, b*: sarıdan maviye renk değişimlerini göstermektedir. Meyve eti sertliği her örneğe ait her bir meyvenin ekvatoral bölgesinde 3 farklı kısmında 1 cm^2 büyüklüğündeki kabuğun kaldırılmasından sonra 11.1 mm delici uç çapına sahip basınç ölçerin (penetrometre) meyve etine batırılması ile kg/cm^2 olarak ölçüm yapılmıştır. Meyve suyunda; suda çözünebilir toplam kuru madde (SÇKM) dijital refraktometre ile (%) ve meyve suyu pH'sı pH metre ile titre edilebilir asitlik miktarı ise 0.1 N NaOH ile meyve suyunun titre edilmesi ve harcanan baz miktarına göre malik asit cinsinden hesaplanması ile % olarak belirlenmiştir.

Bulgular ve Tartışma

Gen kaynakları bahçesinde toplanmış elma çeşitleri arasından seçilen 18 adet yerli çeşitlerin meyve özellikleri incelenmiştir. Yetiştirilen elma çeşitlerinin meyve ağırlığı, meyve boyu, meyve eni ve meyve kalınlığı özellikleri Tablo 1'de verilmiştir. Yapılan ölçümler neticesinde çeşitlerin meyve ağırlığı 42.28-261.25 g aralığında değişim göstermiştir. Belirlenen yerel elma çeşitlerinden Çeşit 16 (261.25 g) en fazla meyve ağırlığına sahip olurken Çeşit 1 (162.4 g) ve Çeşit 4 (162.2 g) takip etmiştir. En az meyve ağırlığına sahip olan çeşitler ise Çeşit 12 (65.54 g), Çeşit 13 (63.71 g) ve Çeşit 14 (42.28 g) olarak belirlenmiştir. Elde edilen çeşitlerinin verilerin ortalaması dikkate alındığında meyve boyu en yüksek olanlar Çeşit 16 (73.31 mm), Çeşit 7 (62.4 mm), Çeşit 18 (60.87 mm) ve en düşük değerlere sahip olanlarda Çeşit 14 (25.39 mm), Çeşit 13 (34.54 mm), Çeşit 11 (45.15 mm) arasında değiştiği tespit edilmiştir. Meyve eni bakımından ise en yüksek Çeşit 16 (94.19 mm), Çeşit 1 (74.99 mm) ve Çeşit 7 (74.75 mm), en düşük ise Çeşit 14 (30.58 mm), Çeşit 13 (44.47 mm), Çeşit 17 (54.55 mm) aralığında değişim göstermişler. İncelenen çeşitlerin meyve kalınlığı bakımından değerlendirilenler 31.41-91.36mm aralığındadır. Çeşit 14 hariç tüm elma çeşşitlerinin meyve kalınlıkları 50 mm üzerinde belirlenmiştir. Çeşitlerin çekirdek sayıları incelendiğinde 3.22-8.60 adet aralığında bir dağılıma sahip olduğu görülmüştür.

Tablo 1. Kırgız Milli Ala Arça Parka Ait Yerli Çeşitler Araştırma İstasyonunda Yetişen Elma Çeşitlerinin Pomolojik Özellikleri

Table 1. Pomological Characteristics of Apple Varieties Grown in Kyrgyz National Ala Archa Park Domestic Varieties Research Station

№	Meyve ağırlığı(g)	Meyve boyu(mm)	Meyve eni(mm)	Meyve kalınlığı(mm)	Çekirdek sayısı (adet)	Meyve eti sertliği (kg/cm²)
Çeşit1	162.40	59.61	74.99	72.94	3.22	3.85
Çeşit2	73,56	47.36	55.73	57.95	5.69	5.25
Çeşit3	97.78	51.50	61.38	62.63	6.00	5.01
Çeşit4	162.20	60.15	71.88	75.00	7.30	5.06
Çeşit5	140.10	59.02	69.60	69.80	8.60	7.75
Çeşit6	121.82	57.11	62.87	67.45	5.12	5.89
Çeşit7	155.10	62.4	74.75	76.41	3.28	4.73
Çeşit8	117.11	57.37	60.9	61.68	6.91	4.45
Çeşit9	101.33	55.12	60.83	61.73	6.94	5.71
Çeşit10	121.60	52.18	68.40	69.33	6.90	5.53
Çeşit11	85.91	45.15	61.71	61.44	4.90	5.39
Çeşit12	65.54	46.73	55.64	58.07	3.33	4.12
Çeşit13	63.71	34.54	44.47	51.39	7.94	11.35
Çeşit14	42.28	25.39	30.58	31.41	5.35	2.86
Çeşit15	97.54	51.12	62.09	62.15	8.12	5.56
Çeşit16	261.25	73.31	94.19	91.36	6.50	2.51
Çeşit17	68.40	47.24	54.55	54.26	7.04	5.50
Çeşit18	129.84	60.87	64.94	64.02	5.53	5.17

İncelenen elma çeşitlerinde meyve sap derinliği 5.22 mm ile 17.58 mm arsında değişmektedir. En yüksek değer Çeşit 6 (17.58 mm) en düşük değerler ise Çeşit 13'te (5.22 mm) belirlenmiştir. Meyve sap eni verilerin ortalamasına göre yüksek olanlar Çeşit 16 (26.42 mm), Çeşit 10 (16.49 mm), Çeşit 7 (16.28 mm) ve düşükler değere sahip olanlar ise Çeşit 3 (9.88 mm), Çeşit 14 (10.14 mm), Çeşit 6 (11.86 mm). Çeşitlerin meyve sap genişliği 8.59-31.8 mm arasında değişmektedir. Yine çeşitlerin sap uzunluğu değerleri bakımından 13.32-32.21 mm arasında bir varyasyon belirlenmiştir. Çeşitlerin meyve sap kalınlıkları arasında da büyük farklılıklar bulunmuştur. Nitekim sap kalınlığı 1.16-9.47 mm arasında değişim göstermiştir.

Meyve şeklini belirlemede bir kriter olan çiçek çukuru derinliği ölçümlerinde en yüksek değerlere Çeşit 16 (28.69 mm) sahip olurken, en düşük değere ise Çeşit 9 (5.75 mm) sahip olmuştur. Çiçek çukuru genişliği parametresi incelendiğinde değerlerin 8.73-22.61 mm aralığında değişim gösterdiği görülmüştür. Meyve çiçek çukur eni ise 4.14-17.46 mm arasında değiştiği tespit edilmiştir.

Kırgız milli Ala Arça parka ait yerli çeşitler araştırma istasyonunda yetişen bazı yerel elma çeşitlerin suda çözünebilir kuru madde miktarı, pH ve titre edilebilir asitlik özellikleri Tablo 4'te verilmiştir. İncelenen çeşitlerde SÇKM içeriği %3.48-16.75 arasında değerler içermiştir. SÇKM içeriğinin yüksek olması bakımından öne çıkan çeşitler Çeşit 10 (%16.75), Çeşit 8 (%15.50), Çeşit 9 (%14.60) ve Çeşit 6 (%14.55)

olarak bulunurken, düşük olması bakımından öne çıkan çeşitler ise Çeşit 13 (%3.48), Çeşit 15 (%10.60), Çeşit 16 (%10.87) olarak tespit edilmiştir. Meyve suyu pH'sı 3.02 ile 7.40 arsında değişmektedir. Meyve kalitesi bakımından önemli bir kriter olan titre edilebilir asitlik miktarları %0.21-1.86 aralığında dağılım göstermiştir.

Tablo 2. Kırgız Milli Ala Arça Parka Ait Yerli Çeşitler Araştırma İstasyonunda Yetişen Elma Çeşitlerinin Meyve Sap Özellikleri
Table 2. Fruit Stalk and Flower Pit Characteristics of Apple Varieties Grown in Kyrgyz National Ala Archa Park Native Varieties Research Station

№	Sap çukuru derinliği(mm)	Sap çukuru eni (mm)	Sap çukuru genişliği(mm)	Sap uzunluğu(mm)	Sap kalınlığı(mm)
Çeşit1	10.27	13.67	14.79	24.70	2.39
Çeşit2	7.39	13.91	13.96	24.85	1.85
Çeşit3	8.25	9.88	10.28	23.32	1.62
Çeşit4	8.25	16.20	16.97	16.30	9.47
Çeşit5	8.40	15.12	15.64	17.16	3.15
Çeşit6	17.58	11.86	12.19	23.31	2.25
Çeşit7	10.29	16.28	14.48	16.41	2.66
Çeşit8	7.32	12.08	13.24	25.37	2.33
Çeşit9	9.21	14.12	13.28	22.82	2.03
Çeşit10	8.08	16.49	17.38	18.62	2.21
Çeşit11	6.53	13.62	13.29	16.32	2.33
Çeşit12	9.76	12.61	13.91	29.17	1.85
Çeşit13	5.22	13.74	14.94	16.72	1.81
Çeşit14	5.92	10.14	8.59	21.34	1.16
Çeşit15	8.99	13.36	14.42	14.12	2.26
Çeşit16	15.98	26.42	31.80	13.32	3.18
Çeşit17	8.48	12.50	11.86	20.85	1.65
Çeşit18	7.70	15.82	14.82	32.21	1.66

Elma çeşitlerine ait meyve kabuk rengi ve meyve et rengi değerlerinde de büyük farklılıklar tespit edilmiştir. L değeri- 6.79 ile 68.03 aralığında, a değeri- 18.41 ile 5.17 aralığında, b değeri- 4.28 ile 35.06 aralığında dağılım göstermiştir.

Çalışmada belirlenen yerel çeşitlerin meyve ağırlıkları 42.28-261.25 g olarak değişim göstermiştir (Tablo 1). Bu sonuçlar; Güleryüz ve ark. (1993) Kağızman ilçesinde yetişen elma çeşitlerinin ortalama meyve ağırlıkları 159.0-313.0 g. Miller ve ark. (2004) Amerikan Birleşik Devletleri ve Kanada'da bazı elma çeşitleri ile yürüttükleri çalışmada ortalama meyve ağırlığı 136-300 g. Farrokhi ve ark. (2011) İran (Maşhad) ilinde elmalarda meyve ağırlığı 7.1-81.67 g. Mratinić ve Akšić (2012) Gümüşhane merkez ilçede yetişen bazı standart ve yerel elma çeşitlerinin meyve ağırlığı 80.70- 195.61 g. Kırkaya ve ark. (2014) Ordu ili Perşembe ilçesinde yetiştirilen yerel elmalarda meyve ağırlığı 76.24-247.23 g. Balta ve ark. (2015) Ordu ilinin Kumru ilçesindeki yerel elma çeşitlerinde meyve ağırlığı 71.41-245.99 g. Çoşkun ve ark. (2016) Isparta (Türkiye) şehrinde 5 yerel ve 2 yabancı orijinli elma çeşidinin meyve ağırlığı 96.99 g ile 184.25 g. Öztürk ve ark. (2016) Samsun ekolojik koşullarında MM 106 elma anacı üzerine aşılı 'Cooper 7 SB2'. 'Golden Delicious'. 'Granny Smith', 'Jersey Mac', 'Red Chief', 'Starkrimson Delicious' ve 'Süper Chief' elma çeşitlerinin meyve ağırlığı 112.3-173.9 g. olarak belirlenmişlerdir. Bu değerlerin bizim çalışmamızda bulunan değerlere paralel veya daha düşük olduğu tespit edildi. Bu farklılıklar genotipik farklılıklardan kaynaklanabileceği gibi ekolojik farklılıklardan da kaynaklanabilir.

Meyve boyutları bakımından yapılan değerlendirmede çeşitlerin meyve boyu değerleri 25.39-73.31 mm aralığında belirlenmiştir (Tablo 1). Bu değerler Miller ve ark. (2004) tarafından Amerikan Birleşik Devletleri ve Kanada'da yaptığı çalışmada bulduğu değerlerle (65-80 mm), Farrokhi ve ark. (2011), İran (Maşhad) ilinde elmalarda bulduğu değerlerle (25.13-77.67 mm), Çoşkun ve Aşkın (2016) Isparta şehrinde 5 yerel ve 2 yabancı orijinli elma çeşidinde bulduğu değerlerle (53.93-65.82 mm), Özmen'in Tokat merkez ve ilçelerinde yetişen yerel çeşitlerinde bulduğu değerlerle (47.93-67.23 mm) benzerlik göstermiş ya da daha yüksek bulunmuştur.

Çalışmamızda meyve eni değerleri 30.58-94.19 mm meyve kalınlığı değerleri 31.41- 91.36 mm olarak tespit edilmiştir. Bulunan değerler Farrokhi ve ark. (2011), İran (Maşhad) ilinde elmalarda yapılan araştırmada belirledikleri meyve eni (21.81-68.33 mm) ve meyve kalınlığı (25.13-77.67 mm) değerlerinden, Şenyurt ve

ark. (2015) yaptıkları çalışmalarında belirledikleri meyve eni değerlerinden (57.27-80.77 mm), Kırkaya, Balta ve Kaya (2014) Ordu ili Perşembe ilçesinde yaptıkları araştırmalarda belirledikleri meyve kalınlığı değerlerinden (44.63-73.98 mm), Balta ve ark. (2015) Ordu ilinin Kumru ilçesinde elmalarda yapılan araştırmada belirledikleri meyve kalınlığı değerlerinden (61.01-95.59 mm), Coşkun ve Aşkın (2016) Isparta şehrinde yürüttükleri çalışmalarında belirledikleri meyve eni değerlerinden (64.86-76.56 mm) daha yüksek veya paralellik olarak saptanmıştır. Bu farklılığın başlıca nedeninin genotipten kaynaklandığı kaçınılmazdır. Bu sebepten dolayı incelenen genotiplerin çeşit adayı olması ve ıslah çalışmalarında kullanılabileceği düşüncesini akla getirmektedir.

Tablo 3. Kırgız Milli Ala Arça Parka Ait Yerli Çeşitler Araştırma İstasyonunda Yetişen Elma Çeşitlerinin Meyve Çiçek Çukuru Özellikleri
Table 3. Flower Pit Characteristics of Apple Varieties Grown in Kyrgyz National Ala Archa Park Native Varieties Research Station

№	Çiçek çukuru derinliği (mm)	Çiçek çukuru genişliği (mm)	Çiçek çukuru eni(mm)
Çeşit1	10.19	13.63	13.18
Çeşit2	8.96	13.13	14.25
Çeşit3	9.97	11.37	10.34
Çeşit4	8.18	15.31	16.74
Çeşit5	9.73	17.40	17.46
Çeşit6	10.69	12.96	12.34
Çeşit7	12.00	19.62	14.41
Çeşit8	6.63	19.86	12.80
Çeşit9	5.75	17.43	15.86
Çeşit10	17.38	16.03	8.18
Çeşit11	12.50	12.21	6.14
Çeşit12	13.75	12.94	9.27
Çeşit13	17.23	14.31	7.40
Çeşit14	9.21	8.73	4.14
Çeşit15	14.62	13.19	7.42
Çeşit16	28.69	22.61	24.00
Çeşit17	12.62	12.32	8.59
Çeşit18	18.07	14.61	11.10

Suda çözünebilir kuru madde miktarı çalışmamızda belirlediğimiz yerel çeşitlerde %3.48-16.75 değerleri arasında bulunmuştur (Tablo 4). Bu bulgular, Güleryüz ve ark. (1993), Kağızman ilçesinde yürüttüğü çalışmada SÇKM miktarını %12.35- 14.45, Miller ve ark. (2004) tarafından Amerikan Birleşik Devletleri ve Kanada'da bazı elma çeşitleri ile yürüttükleri çalışmada ortalama SÇKM oranı %12.30-15.6. Mratinić ve Akšić (2012) tarafından Güney Sırbistan'da elmaların SÇKM değerinin %12.55-19.24, Şenyurt ve ark. (2015) yaptıkları çalışmalarında SÇKM %11.50-15.25, Kırkaya ve ark. (2014) Ordu ili Perşembe ilçesinde yetiştirilen yerel elma genotiplerinde yürüttükleri çalışmada SÇKM oranının % 9.01-13.75, Selcen (2017) Tokat merkez ve ilçelerinde yetişen yerel elma genotiplerinde yaptığı çalışmada SÇKM %9.9-16.8, Selma Boyacı (2019) Kırşehir koşullarında MM 106 yarı bodur elma anacı üzerine aşılı Mondial Gala, Red Chief, Golden Delicious, Braeburn ve Granny Smith elma çeşitlerinin performanslarının belirlenmesi amacıyla yaptığı çalışmada SÇKM %11.16- 15.41 değerleri aralığında bulmuştur. Literatürde bildirilen bu değerlerle bizim değerlerimiz benzerlik göstermektedir. Ancak bazı çeşitlerin asitlik değerlerinin düşük olduğu çeşitlerde tespit edilmiştir.

Elma çeşitlerin meyve suyu pH'larının 3.02 ile 7.40 aralığında değiştiği tespit edilmiştir. Belirlenen bu değerler Kırkaya ve ark (2014) Ordu ili Perşembe ilçesinde yetiştirilen yerel elma genotiplerinin pomolojik, fenolojik ve morfolojik özelliklerinin belirlenmesi amacıyla 2010-2011-2012 yıllarında yaptıkları araştırmada pH değerinin 3.16-3.56, Balta ve ark. (2015) Ordu ilinin Kumru ilçesinde elma çeşitlerinde yaptığı çalışmada tespit ettiği pH miktarı 2.83-4.11, Öztürk ve ark. (2016) Samsun ekolojik koşullarında MM 106 elma anacı üzerine aşılı elma çeşitlerinin çeşitleri üzerinde yaptığı çalışmasında ölçtüğü pH miktarı 3.43-4.34, Selcen'nin (2017). Tokat merkez ve ilçelerinde yetişen yerel elma çeşitlerinde yaptığı araştırmasında saptadığı pH miktarı 2.88-5.30, Boyacı'nın (2019) Kırşehir koşullarında yetişen MM 106 yarı bodur elma anacı üzerine aşılı Mondial

Gala, Red Chief, Golden Delicious, Braeburn ve Granny Smith elma çeşitlerinde yapmış olduğu çalışmaların sonucunda belirlediği pH miktarı 3.31-4.03 değerleri ile benzerlik göstermektedir. Ancak en yüksek pH olarak belirlediğimiz Çeşit 13 çeşidi (7.40) literatürde bildirilen değerlerden daha yüksek değer almıştır. Bu durumun çeşit özelliğinden kaynaklandığı söylenebilir.

Tablo 4. Kırgız Milli Ala Arça Parka Ait Yerli Çeşitler Araştırma İstasyonunda Yetişen Elma Çeşitlerinin Bazı Kimyasal Özellikleri
Table 4. Some Chemical Characteristics of Apple Varieties Grown in the Kyrgyz National Ala Archa Park Domestic Varieties Research Station

№	SÇKM(%)	pH	TEA
Çeşit1	10.95	3.41	0.58
Çeşit2	12.15	3.81	0.21
Çeşit3	13.05	3.57	0.48
Çeşit4	13.75	3.73	0.66
Çeşit5	13.05	3.83	0.69
Çeşit6	14.55	3.84	0.34
Çeşit7	12.20	3.92	0.62
Çeşit8	15.50	5.21	0.79
Çeşit9	14.60	3.59	0.53
Çeşit10	16.75	3.27	1.03
Çeşit11	12.00	3.35	0.74
Çeşit12	11.00	3.69	0.23
Çeşit13	3.48	7.40	0.66
Çeşit14	11.15	3.02	1.86
Çeşit15	10.60	3.50	0.85
Çeşit16	10.87	3.26	1.35
Çeşit17	10.90	3.63	0.39
Çeşit18	13.50	3.62	0.55

Titre edilebilir asitlik miktarı bakımından Kırgız Milli Ala Arça parkta yetiştirilen yerel elma çeşitlerinin değerleri %0.21-1.86 aralığında saptanmıştır. Güleryüz ve ark (1993) Kağızman ilçesinde yetişen Banem, Kaburgalı, Matibey, Mirizo, Şah ve Uzun elma çeşitlerinde yapılan araştırmada asit miktarları %0.29-0.44 Miller ve ark. (2004) Amerikan Birleşik Devletleri ve Kanada'da bazı elma çeşitleri üzerinde yürüttükleri çalışmada titre edilebilir asit miktarı %0.39- 0.98, Kırkaya ve ark. (2014) Ordu ili Perşembe ilçesinde yetiştirilen yerel elma genotiplerinin pomolojik, fenolojik ve morfolojik özelliklerinin belirlenmesi amacıyla 2010-2011-2012 yıllarında yürüttükleri çalışmada TEA oranının %0.40-1.64, Balta ve ark. (2015) Ordu ilinin Kumru ilçesinde elmalarda yaptıkları araştırmada titre edilebilir asit miktarı %0.22-2.01, Öztürk ve ark. (2016) Samsunda yaptıkları araştırmalarında titre edilebilir asitlik %0.39-0.90 ve Selcen'in (2017) Tokat merkez ve ilçelerinde yapmış olduğu araştırmada titre edilebilir asit miktarı %0.20-1.41 değerlerden daha yüksek değer almıştır.
Yapılan çalışmalarda tespit edilen değerlerle bizim tespit ettiğimiz değerler hemen hemen aynı değerlerdir. Elma çeşitlerinde meyve kabuk rengi tespitlerinde L değeri- 62.04 ile Çeşit 14 çeşidinde en düşük 127.11 ile Çeşit 10 çeşidinde en yüksek olarak ölçülmüştür (Tablo 5). a değeri ölçümünde ise en yüksek değer 27.19 olarak Çeşit 10 çeşidinde, en düşük değer- 37.3 olarak Çeşit 6 çeşidinde belirlenmiştir. Bir diğer renk değeri olan b'de ise renk dağılımı- 16.32 ile 45.01 (Çeşit 14-Çeşit 18) arasında bulunmuştur (Tablo 5).
Belirlenen bu değerler diğer araştırıcılardan Boyacı'nın (2019) Kırşehir koşullarında MM 106 yarı bodur elma anacı üzerine aşılı Mondial Gala, Red Chief, Golden Delicious, Braeburn ve Granny Smith elma çeşitlerinde belirlediği meyve kabuk rengi L değeri 19.50-78.86. a değeri- 21.01-43.24, b değeri 10.10-51.24 değerleri ile benzerlik göstermemiştir. Meyve kabuk rengi çeşide yetiştirilen ekolojik faktörlere, toprak koşullarına ve uygulanan kültürel uygulamalarına göre farklılık göstermektedir.

Tablo 5. Kırgız Milli Ala Arça Parka Ait Yerli Çeşitler Araştırma İstasyonunda Yetişen Elma Çeşitlerinin Bazı Meyve Renk Özellikleri

Table 5. Some Fruit Color Characteristics of Apple Varieties Grown in the Kyrgyz National Ala Archa Park Domestic Varieties Research Station

№	Meyve Kabuk Rengi			Meyve Et Rengi		
	L	a	b	L	a	b
Çeşit 1	0.82	-5.34	0.86	-0.6	-0.54	-4.28
Çeşit 2	6.29	3.77	5.52	58.75	5.17	35.06
Çeşit 3	-2.8	6.45	-6.67	-1.42	3.51	6.37
Çeşit 4	66.37	1.07	37.83	65.85	-15.34	3.77
Çeşit 5	54.43	-0.97	18.13	33.07	-2.15	3.17
Çeşit 6	48.28	-37.3	40.89	-6.79	5.13	3.58
Çeşit 7	38.8	21.79	22.53	62.46	-10.69	8.53
Çeşit 8	34.51	13.46	13.66	0.47	1.3	0.42
Çeşit 9	28.08	12.14	8.44	76.87	-7.54	24.27
Çeşit 10	127.1	27.19	20.91	-0.51	4	1.8
Çeşit 11	29.84	26.28	11.43	68.03	-17.21	31.61
Çeşit 12	10.41	20.58	11.32	50.15	-18.06	13.06
Çeşit 13	29.13	8.48	6.92	76.2	-18.41	17.97
Çeşit 14	-62	-2.19	-16.32	21.45	-12.97	3.79
Çeşit 15	58.75	3.26	35.64	52.62	2.49	29.22
Çeşit 16	30.58	11.22	9.45	56.17	-14.75	12.8
Çeşit 17	65.57	-8.99	41.01	54.34	-0.1	26.95
Çeşit 18	67.11	1.51	45.01	17.49	-1.77	0.09

Sonuç ve Öneriler

Bu araştırma Kırgızistan'ın Çüy bölgesindeki Kırgız Milli Ala Arça Parkı'nda yer alan elma genotiplerinin pomolojik özelliklerini inceleyerek bu çeşitlerin üretime kazandırılmasının yanında ıslah çalışmaları için potansiyelini değerlendirmeyi amaçlamaktadır. Araştırma sonucunda incelenen 18 adet yerli elma çeşidinde önemli varyasyonlar tespit edilmiştir. Özellikle meyve büyüklüğü, meyve şekli ve meyve rengi bakımından büyük farklılıklar belirlenmiştir.

Çalışmada incelenen çeşitlerin meyve ağırlıkları bakımından ticari olarak yetiştirilen çeşitlerin ağırlıkları aynı zamanda Çeşit 14 dışındaki çeşitlerin tamamı ise meyve boyutları bakımından 1.sınıf meyve boyutlarına yakın veya üzerindedir. Bu çeşitler raf ömürlerinin belirlenmesi, muhafaza özelliklerinin belirlenmesi, hastalık ve zararlılara karşı dayanıklık özelliklerinin belirlenmesi ile birlikte öncelikle Kırgızistan geneline yayılarak elma üretimine kazandırılmalı düşüncesindeyiz. Çalışmada incelenen yerel çeşitler tescil edilerek koruması ve dünya literatürüne kazandırılması gerektiği kanaatindeyiz. Yerel elma çeşitlerinin korunması ve değerlendirilmesi hem ülke ekonomisine hem de tarımsal çeşitliliğe önemli faydalar sağlayacaktır.

Acknowledgments / Teşekkürler
Verilerin elde edilmesindeki destekleri ve bu çalışmanın yürütülmesi esnasında, çalışmalarıma yön veren hocam Ahmet AYGÜN'e teşekkürlerimi sunarım.
I would like to thank my teacher Ahmet AYGUN for his support in obtaining the data and guiding my studies during the conduct of this study.
Funding / Fon desteği
Yazarlar bu çalışma için herhangi bir kuruluştan destek almamıştır.
The author did not receive support from any organization for the submitted work.
Data Availability statement
Yazar, bu çalışmayı destekleyen verilere makalede atıfta bulunulduğunu onaylamaktadır.
The author confirms that the data supporting this study are cited in the article.
Compliance with ethical standards / Etik standartlara uyum
Conflict of interest / Çıkar çatışması
Yazar herhangi bir çıkar çatışması beyan etmemektedir.
The author declare no conflict of interest.
Ethical standards / Etik standartlar
Çalışma etik standartlara uygundur.
The study is proper with ethical standards.
Authors' contributions / Yzar katkıları

Makalede adı geçen tüm yazarlar makaleye eşit oranda katkı yapmışlardır. Tüm yazarlar makaleyi incelemiş ve onaylamışlardır.
All authors mentioned in the article contributed equally to the article. All authors have reviewed and approved the article.

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International Journal of Life Sciences and Biotechnology
Koluman, A., et al., Biorisks Associated with Synthetic Biology: Virulence plasmid Transfer to Probiotic or Starter Cultures. International Journal of Life Sciences and Biotechnology, 2024 7(2): p. 120-132.
https://doi.org/10.38001/ijlsb.1426971
Derleme Makale/ Review article

Biorisks Associated with Synthetic Biology: Virulence plasmid Transfer to Probiotic or Starter Cultures

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ABSTRACT
Synthetic biology holds promise for revolutionizing biotechnology across diverse fields, yet it also presents inherent biorisks that require careful consideration and mitigation strategies. This paper explores the potential biorisks associated with the transfer of virulence plasmids to probiotic or starter cultures within synthetic biology applications. Beginning with an introduction to synthetic biology principles and the significance of probiotics and starter cultures, the discussion delineates the mechanisms and consequences of virulence plasmid transfer, drawing insights from case studies involving bacterial species like Bifidobacterium and Bacillus, as well as yeast probiotics such as Saccharomyces. Notably, the incidents underscore the emergence of antibiotic resistance genes and multidrug resistance, emphasizing the critical need for robust containment measures and genetic safety precautions. Ethical considerations permeate the discourse, advocating for transparency, informed consent, and responsible innovation in synthetic biology. Ultimately, this paper underscores the importance of global collaboration, harmonized regulatory frameworks, and continual evaluation to ensure the safe and ethical utilization of synthetic biology techniques, thus maximizing benefits while minimizing potential risks to human health and the environment.

ARTICLE HISTORY
Received
28 January 2024
Accepted
06 April 2024

KEYWORDS
Synthetic biology,
biorisks,
probiotic,
virulence plasmid

Introduction

Synthetic biology is a rapidly advancing multidisciplinary field that integrates principles from biology, engineering, and computer science to design and construct novel biological systems [1]. The ability to manipulate and engineer biological organisms has led to a wide range of applications, including the development of improved therapeutics, sustainable biofuels, and enhanced agricultural products [2,3,4]. While the potential benefits of synthetic biology are undeniable, it is essential to recognize and address the potential biorisks associated with these technological advancements [5-9].

One significant biorisk in the context of synthetic biology is the transfer of virulence plasmids to probiotic or starter cultures. Probiotics, which consist of beneficial live microorganisms, are consumed to confer health benefits to the host [10]. Similarly, starter cultures are used in various fermentation processes to initiate specific biochemical reactions [11]. The accidental introduction of a virulence plasmids into these modified organisms may result in unintended consequences, leading to enhanced pathogenicity, potential human health risks, and ecological ramifications [12, 13].

It is essential to comprehend and effectively handle the biorisks linked to synthetic biology methods to fully unlock the potential of this field, all while guaranteeing the safety of both human users and the environment. By identifying potential risks and implementing robust risk mitigation strategies, we can foster responsible innovation and safeguard against unintended consequences in the use of synthetic biology for probiotics and starter cultures. The first section will provide a brief overview of synthetic biology, highlighting its key principles and applications, with a specific focus on the engineering of probiotics and starter cultures. The subsequent sections will delve into the risks associated with virulence plasmid transfer, the potential consequences of such transfers, and strategies to mitigate these risks. Furthermore, case studies of previous incidents involving plasmid transfers in synthetic biology applications will be presented, offering insights into lessons learned and their implications for future research. Ethical considerations and the importance of

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effective communication with the public will also be addressed, recognizing the need to strike a balance between the benefits and potential risks of synthetic biology.

This paper aims to examine the potential biorisks associated with the transfer of virulence plasmids to probiotic or starter cultures. It will explore the mechanisms by which such transfers might occur, analyze the consequences and impacts of such events, and propose risk mitigation strategies to ensure the responsible and safe use of synthetic biology in these applications.

Background

Synthetic biology and genetic engineering

Synthetic biology has revolutionized biotechnology, offering new ways to design and construct biological systems with precision and efficiency. It involves the deliberate design of genetic circuits, metabolic pathways, and other biological components to achieve specific functions [14]. Techniques like gene synthesis [15], gene editing (e.g., CRISPR-Cas9) [16, 17], and DNA assembly [18] enable scientists to engineer living organisms with desired traits and capabilities. Genetic engineering is a fundamental aspect of synthetic biology, allowing researchers to manipulate an organism's genetic material [19]. This involves inserting or deleting specific genes or genetic elements to confer desired properties, such as disease resistance or improved product synthesis, to microorganisms [20, 21].

Probiotics and starter cultures

Probiotics are live microorganisms, including bacteria and yeasts, consumed as dietary supplements or added to food products for potential health benefits [9]. These beneficial microbes aim to restore or enhance the balance of gut bacteria, promoting better digestion, immune function, and overall well-being [9]. Probiotics have gained popularity among consumers seeking natural approaches to support gut health [22-24].

Starter cultures, on the other hand, play a crucial role in food and beverage fermentation processes [11, 25]. These microorganisms initiate controlled biochemical reactions, transforming raw ingredients into various products, such as yogurt, cheese, bread, sauerkraut, and alcoholic beverages [26]. Specific starter cultures are chosen for their ability to produce particular enzymes or metabolic products, influencing the final product's taste, texture, and safety [11].

Virulence plasmids and horizontal gene transfer (HGT)

Virulence plasmids are small, circular DNA elements commonly found in pathogenic bacteria [27]. They carry genes responsible for pathogenicity, antibiotic resistance, and other virulence factors. Horizontal Gene Transfer (HGT) is a process where bacteria transfer genetic material, including plasmids, to other microorganisms [28]. HGT occurs through mechanisms like conjugation, where plasmids are directly transferred between bacteria, transduction, where bacteriophages (viruses infecting bacteria) mediate the transfer of genetic material, and transformation, where bacteria take up foreign DNA from their surroundings [29]. When virulence plasmids are transferred to probiotic or starter cultures through HGT, these normally benign microorganisms may acquire harmful genes and traits. This unintended acquisition could lead to the expression of dangerous proteins, compromising the safety and health benefits of these modified organisms [30-32].

The potential biorisks associated with virulence plasmid transfer to probiotic or starter cultures will be explored and discussing the potential consequences and risk mitigation strategies to ensure the responsible use of synthetic biology techniques in these applications will be made.

Biorisks of Virulence plasmid Transfer

The transfer of virulence plasmids to probiotic or starter cultures presents significant biorisks that demand careful consideration and risk mitigation strategies. These risks primarily arise from the potential for horizontal gene transfer (HGT) of virulence genes and pathogenic traits, leading to unintended consequences and posing threats to human health and the environment [33-35]. This section will delve into the specific biorisks associated with virulence plasmid transfer in the context of synthetic biology applications.

Horizontal gene transfer (HGT)

While HGT plays a crucial role in bacterial evolution and adaptation, it becomes a cause for concern when virulence plasmids carrying harmful genetic elements are inadvertently transferred to probiotic or starter cultures. Unlike vertical gene transfer, where genes are passed from parent to offspring [36], HGT enables the rapid dissemination of genetic information across diverse microbial populations [37-40].

In the case of probiotics, HGT of virulence plasmids could potentially transform harmless strains into virulent pathogens. This transformation may result from the expression of virulence factors or antibiotic resistance genes present in the transferred plasmids [41-43]. Similarly, starter cultures that acquire plasmids from pathogens may produce harmful byproducts during food fermentation, compromising food safety and quality [44, 45].

Enhanced pathogenicity

The acquisition of virulence plasmids by probiotic or starter cultures can confer new pathogenic traits or enhance existing ones [46, 47]. Modified microorganisms may gain the ability to produce toxins, evade the host's immune system, or colonize host tissues more effectively [48]. In the context of starter cultures, enhanced pathogenicity may lead to the production of contaminated food products, posing risks to consumers' health [49, 50].

Environmental spread

The release of genetically modified probiotics or starter cultures into the environment, whether intentional or unintentional, can introduce virulence plasmids into ecosystems and other microbial populations. Environmental spread may occur through interactions with other microorganisms, dissemination through waste streams, or survival in soil and water environments [51]. The potential for environmental spread poses broader ecological risks, as virulence plasmids may transfer to other bacteria in the environment. If these transferred plasmids carry antibiotic resistance genes, they could contribute to the development of antibiotic-resistant bacteria, exacerbating the global health crisis of antimicrobial resistance (AMR) [52 - 54]. Next, the potential consequences and impacts of virulence plasmid transfer to probiotic or starter cultures will be discussed, followed by risk mitigation strategies to address these biorisks responsibly and ensure the safe utilization of synthetic biology in these applications.

Consequences And Impact

Understanding these potential outcomes is crucial for developing effective risk mitigation strategies and ensuring responsible applications of synthetic biology in the context of probiotics and starter cultures.

Health risks to human consumers

If probiotics carrying virulence plasmids reach consumers, they may inadvertently introduce harmful traits into the gut microbiota, leading to gastrointestinal infections or exacerbating existing health conditions. Moreover, individuals with compromised immune systems, such as the elderly, young children, and those undergoing medical treatments, may be more susceptible to the adverse effects of ingesting pathogenic probiotics. While lactic acid bacteria are commonly considered safe and typically do not induce illness, sporadic instances of infections have been documented in individuals undergoing antibiotic therapy or experiencing severe immunocompromise [55, 56]. In such cases, conditions such as endocarditis, bacteremia, and localized infections such as abdominal abscesses, pulmonary infections, and peritonitis are among the most frequently reported diseases associated with Lactobacillus spp [57]. In the human digestive tract, enterococci—mostly E. faecalis and E. faecium—are common microorganisms. These species are frequently isolated from human feces, along with Enterococcus durans [58]. Even while enterococci are commonly found in food products, can be used as starter cultures, and may even be advantageous probiotics, they are opportunistic pathogens that frequently cause nosocomial infections. They are intrinsically resistant to low concentrations of many antibiotic drugs, such as tetracycline, aminoglycosides, clindamycin, lincomycin, and beta-lactams [58, 59]. Enterococci have been found to possess a variety of virulence factors [60, 61]. This heightened vulnerability highlights the need for stringent risk assessment and containment measures to prevent the accidental release of modified organisms into the market.

Contamination of food and fermentation processes

The contamination of starter cultures with virulence plasmids can lead to the production of contaminated food items, posing significant risks to consumers. Consumption of such products could result in foodborne illnesses and outbreaks, leading to economic losses for the food industry and public health concerns [61-63].

In addition to direct contamination, the presence of virulence plasmids in starter cultures may alter fermentation processes, affecting product quality and consistency. The unintended expression of pathogenic traits by modified starter cultures could lead to unwanted byproducts, spoilage, or changes in taste and texture [64].

Environmental dissemination and ecological consequences

The escape or intentional release of modified probiotics or starter cultures into the environment raises ecological concerns. If virulence plasmids spread to other microorganisms in natural ecosystems, it could lead to the unintended development of pathogenic strains in environmental niches [65, 66]. This environmental spread may also contribute to the dissemination of antibiotic resistance genes [65, 67], further aggravating the global issue of AMR and compromising the effectiveness of antibiotics in medicine and agriculture. Moreover, the ecological consequences of uncontrolled dissemination are challenging to predict fully. The introduction of genetically modified microorganisms into ecosystems may disrupt existing ecological balances and interactions, potentially leading to unforeseen ecological disturbances and imbalances in microbial communities [68-71].

In the following section, essential risk mitigation strategies will be discussed to address the biorisks associated with virulence plasmid transfer to probiotic or starter cultures. The aim of these strategies is to ensure the responsible and safe use of synthetic biology techniques in these applications while maximizing their potential benefits. Additionally, valuable insights and lessons for future research and risk management will be provided through case studies of previous incidents involving plasmid transfers.

Risk Mitigation Strategies

To address the biorisks associated with the transfer of virulence plasmids to probiotic or starter cultures, a proactive approach to risk mitigation is essential. Employing a combination of stringent containment measures, genetic safety precautions, and comprehensive risk assessments can help ensure the responsible use of synthetic biology techniques in these applications [72, 73].

Containment measures

Stringent containment protocols are critical to prevent the accidental release of modified microorganisms into the environment. These measures involve physical containment and strict adherence to standard operating procedures to minimize the risk of escape or unintentional dissemination [74, 75]. Laboratories and facilities involved in synthetic biology research should follow appropriate biosafety guidelines and operate under the principles of the "containment hierarchy," which involves multiple layers of protection to prevent exposure to hazardous microorganisms [76].

In addition to laboratory containment, the commercial use of genetically modified probiotics or starter cultures should adhere to robust containment measures [77]. This may include the use of closed fermentation systems [78], a controlled environment, and secure waste disposal procedures to prevent the spread of virulence plasmids to the external environment [79, 80].

Robust genetic safety measures

Genetic safety measures can be employed to reduce the likelihood of plasmid transfer and mitigate potential risks associated with virulence plasmids. One such approach is the use of "suicide genes" or "genetic kill switches," which are genetic elements that cause the death or self-destruction of the modified microorganism under specific conditions [51, 81]. Incorporating these safety mechanisms can ensure that the modified organisms have limited survival and proliferation outside controlled environments, providing an added layer of biocontainment [82,83].

Another genetic safety strategy involves the use of "gene containment systems" to limit horizontal gene transfer [84 - 86]. These systems prevent the transfer of engineered genetic elements, such as plasmids, to other microorganisms [84 - 86]. For instance, synthetic biology researchers can design plasmids with built-in safeguards that restrict their transferability or limit their stability outside of the target organism [84].

Comprehensive risk assessment

Before the release or commercialization of any synthetic biology application involving modified probiotics or starter cultures, comprehensive risk assessments must be conducted. Risk assessment processes should evaluate potential biorisks, consider the intended use, and assess the likelihood and severity of unintended consequences [87, 88].

Risk assessment should be an ongoing and iterative process, continually reevaluating and updating strategies as new scientific information and data become available [89]. This dynamic approach ensures that the most current knowledge and best practices are applied to minimize risks effectively.

In the next section, we will explore relevant case studies of previous incidents involving plasmid transfers in synthetic biology applications. By examining these incidents, we can learn from past experiences and identify areas for improvement in risk management and safety practices.

Case Studies

Examining past incidents involving plasmid transfers in synthetic biology applications provides valuable insights into the potential risks and challenges associated with these technologies. Learning from these case studies can help inform future research, risk management, and the development of improved safety measures.

Incidents of plasmid transfer in synthetic biology applications

Conjugation, phage-associated transduction, and transformation all help to reduce mutant genome transfer between bacterial species via integrons or transposons [90, 91]. Human studies have shown that the incidence of HGT is substantially higher in humans, particularly among gut microbiota and ingested probiotics, implying that they serve as reservoirs of resistance genes. Furthermore, resistance in the oral cavity microbiota, particularly among *Streptococci*, and resistance to tetracycline caused by genes such as tet(M), tet(O), tet(Q), and tet(W), are significant examples of AMR [92].

In Bifidobacterium species, the transfer of genetic elements, especially antibiotic resistance genes, is commonly observed. While intrinsic resistance to mupirocin is prevalent among most species due to its role in protein synthesis, resistance to streptomycin can arise from mutations in the rpsL gene in *B.bifidum* and *B.breve*. Some studies suggest that streptomycin resistance may result from chromosomal mutations, rendering it non-transferable [93, 94]. Several tetracycline resistance-associated genes in species of *Bifidobacterium* have been well-characterized, with *B. adolescentis* and *B. longum* demonstrating the transfer of the tet(W) gene under in vitro conditions. [95], though in-vivo transferability warrants further investigation. Similarly to the *Lactobacillus genus, B. breve* and *B. longum* harbor transporters capable of translocating unrelated compounds, leading to multidrug resistance (MDR) [96]. Certain resistance genes identified in other probiotic species, such as *Lactococcus lactis*, can be transferred to other bacteria, exemplified by tetracycline resistance genes being transferred to *Enterococcus faecalis. Bacillus spp*., routinely employed as probiotics, frequently produce toxins, and while some strains lack plasmids harboring resistance genes, others might transfer AMR genes, omitting resistance to ampicillin [97]. Extrachromosomal elements contribute to resistance to macrolide and tetracycline antibiotics among *Bacillus spp*., with studies focusing on resistance induced by the transfer of CFR-related genes, which encode ribosomal methyltransferase, to other bacteria in the intestine. Safety assessments of Bacillus spp. are currently inadequate and necessitate further investigation [98].

In contrast to bacterial strains, research on the AMR of yeast probiotics is limited. However, a study comparing antimicrobial resistance between bacteria and yeasts in a commercial probiotic product found that *Saccharomyces faecalis* and *Saccharomyces boulardii* exhibited resistance to 80% of antibiotics tested [99]. Additionally, *Pichia kudriavzevii* demonstrated intrinsic resistance to benzylpenicillin and vancomycin, suggesting potential transferability between microorganisms [100].

Lessons learned and implications for future research.

Ensuring the safe and responsible use of synthetic biology techniques is of utmost importance, especially when it comes to dealing with potential biorisks like virulence plasmid transfer to probiotic or starter cultures. To achieve this, several crucial aspects need to be considered. Firstly, understanding the genetic makeup of genetically modified microorganisms is vital. Researchers must thoroughly analyze the plasmids and other genetic elements introduced into these organisms to identify any potentially harmful genes or traits [101]. Secondly, comprehensive risk assessment should be an integral part of the entire research and development process. This should start right from the design phase and continue through the application and potential release stages [102]. By identifying potential risks early on, appropriate risk mitigation strategies can be put in place. Implementing biocontainment strategies and genetic safety measures is another crucial step. Robust containment measures and safety precautions can limit the spread of modified organisms, reducing the risk of unintended plasmid transfer. Moreover, incorporating "suicide genes" or "genetic kill switches" can ensure that these organisms have limited survival outside controlled environments, adding an extra layer of safety [50, 80]. Transparency and effective communication with stakeholders are equally important. This includes engaging with the public and regulatory authorities to address concerns and foster a shared understanding of the potential risks and benefits of synthetic biology applications. Post-market surveillance is essential to continuously monitor the behavior of genetically modified microorganisms after commercial release [103]. This allows for the identification of any unforeseen outcomes or evolving risks, enabling timely interventions and updates to safety measures. Emphasizing the principle of precaution is central to ethical decision-making [103]. Even in the absence of complete scientific certainty, taking precautionary measures ensures that potential risks are thoroughly assessed and prioritized. Global collaboration between scientists, policymakers, and regulatory bodies is critical. Working together can lead to harmonized regulatory standards and ethical frameworks, ensuring responsible innovation and safeguarding against biorisks across borders [104]. Public engagement and involvement in decision-making processes are also vital. Including a diverse group of stakeholders in ethical review boards and public consultations helps gather valuable insights and ensures that public concerns and preferences are taken into account when shaping regulations and policies [105]. It should be taken into account that adopting a holistic approach that encompasses genetic characterization, risk assessment, biocontainment strategies, transparent communication, and ethical considerations is key to addressing biorisks associated with synthetic biology applications. By embracing responsible practices, monitoring developments closely, and fostering global collaboration, we can unlock the full potential of synthetic biology while ensuring the safety and well-being of both humans and the environment. These case studies underscore the importance of continual evaluation, collaboration between researchers and policymakers, and a proactive approach to biosecurity and risk management in synthetic biology.

Implications for responsible innovation

Synthetic biology has the potential to revolutionize various industries and contribute to significant advancements in biotechnology. However, ensuring responsible innovation is paramount to prevent

unintended negative consequences. Researchers, policymakers, and stakeholders must work together to strike a balance between embracing the transformative potential of synthetic biology and safeguarding against potential risks.

The ethical considerations surrounding synthetic biology applications involving virulence plasmid transfer will be explored in the following section. Ethical analysis is used to guide decision-making, encourage transparency, and ensure that the development and deployment of these technologies address societal values and concerns.

Ethical Considerations

The responsible development and deployment of synthetic biology applications involving virulence plasmid transfer to probiotic, or starter cultures demand careful ethical scrutiny. Ethical considerations play a pivotal role in guiding decision-making, ensuring the protection of human health, the environment, and societal values. This section explores key ethical considerations associated with these technologies.

Balancing benefits and risks in synthetic biology

As with any technological advancement, synthetic biology offers immense potential benefits, such as improved healthcare, sustainable agriculture, and environmental remediation [106]. However, it is essential to weigh these potential benefits against the associated risks carefully. Ethical decision-making requires a comprehensive analysis of the potential harms and benefits, taking into account both short-term and long-term consequences.

Balancing the risks and benefits of synthetic biology applications involving virulence plasmid transfer requires transparency, scientific evidence, and public engagement [107]. Policymakers, researchers, and industry stakeholders must involve the public in discussions about the applications' potential implications, fostering open dialogue to ensure that societal values and ethical concerns are adequately addressed.

Public perception and communication

Public perception and understanding of synthetic biology are crucial for its acceptance and responsible implementation. Synthetic biology applications, especially those involving genetically modified microorganisms, can raise concerns among the public regarding safety, health, and environmental impacts. Transparent and effective communication about the risks, benefits, and risk mitigation strategies is essential to build public trust and address public concerns.

Engaging with the public through educational programs, public forums, and stakeholder consultations can help foster informed decision-making and ensure that the public's values and preferences are considered in the development and regulation of these technologies [108]. Ethical communication should strive to be accessible, accurate, and free from sensationalism, fostering a constructive and well-informed public debate [109].

Informed consent and responsible use

The ethical use of synthetic biology technologies demands the principle of informed consent. Individuals who may be affected by the applications should have access to clear and accurate information about the potential risks and benefits. In the context of probiotics or starter cultures, consumers should be informed if the product contains genetically modified microorganisms and understand any associated risks [110].

Responsible use also involves adherence to regulatory guidelines and ethical standards. Research institutions, industries, and policymakers must prioritize safety and ethical considerations, ensuring compliance with established biosafety protocols and regulations. This commitment to responsible practices should extend to the entire lifecycle of the technology, from research and development to commercialization and post-market monitoring [111].

Next, recommendations for regulations and policy frameworks will be presented to support the safe and responsible use of synthetic biology techniques involving virulence plasmid transfer to probiotic or starter cultures. These recommendations aim to foster responsible innovation while safeguarding against potential biorisks and ethical concerns.

Global collaboration and responsible innovation

Given the global nature of synthetic biology and the potential for its applications to cross borders, international collaboration is vital for responsible innovation. Global cooperation can facilitate the exchange of best practices, harmonization of regulatory standards, and collective efforts to address shared biosecurity concerns [112, 113].

Responsible innovation in synthetic biology requires a continuous commitment to improving safety measures, updating risk assessments, and addressing emerging challenges. By proactively integrating ethical considerations into research, development, and application, the field can move forward with the potential to benefit humanity while minimizing potential risks [114, 115].

Regulations and Policy Recommendations

To ensure the safe and responsible use of synthetic biology techniques involving virulence plasmid transfer to probiotic or starter cultures, a robust regulatory framework is essential. This section presents key policy recommendations and regulatory guidelines to address the potential biorisks and ethical considerations associated with these applications.

International guidelines for synthetic biology applications

Harmonization of regulations and guidelines at the international level is critical to foster responsible innovation and mitigate global biorisks. Collaborative efforts between governments, scientific organizations, and international bodies can establish uniform safety standards, risk assessment protocols, and ethical frameworks for synthetic biology applications. International guidelines should prioritize the principles of precaution and transparency. Risk assessments should be conducted for each synthetic biology application, with an emphasis on addressing uncertainties and potential unintended consequences [116]. These guidelines can also serve as a foundation for national and regional regulatory frameworks, providing a consistent approach to ensure safety and ethical considerations [117].

The Cartagena Protocol on Biosafety is a key international treaty addressing the safe handling of living modified organisms (LMOs) from modern biotechnology, including synthetic biology [118-120]. It establishes a framework for transboundary regulation to prevent adverse effects on biodiversity and human health during development, handling, transport, and trade [120]. Emphasizing risk assessment, it encourages nations to evaluate potential environmental and human health risks, fostering effective mitigation measures. The protocol promotes information exchange, transparency, and capacity building, particularly in developing countries [120].

Harmonization of biosafety standards

Within each country, harmonization of biosafety standards is essential to promote uniform safety practices and facilitate the international exchange of genetically modified microorganisms [121]. Regulatory agencies should work collaboratively to develop risk assessment methodologies, containment requirements, and reporting systems for synthetic biology applications. Adopting a risk-based approach can help tailor safety measures to the specific characteristics of each application. Researchers, industries, and regulators should actively engage in ongoing dialogue to identify emerging risks, assess their potential impacts, and update safety guidelines accordingly [122].

Encouraging collaboration between scientists and policymakers

Close collaboration between the scientific community and policymakers is vital to inform evidence-based regulations and ensure that policy decisions align with the latest scientific knowledge. Policymakers should actively seek input from scientists and experts in the field of synthetic biology to develop informed and adaptable regulations. Additionally, fostering interdisciplinary collaborations among scientists, engineers, ethicists, and social scientists can promote a holistic understanding of the potential impacts of synthetic biology applications [123]. This interdisciplinary approach enables the consideration of ethical, social, and environmental dimensions, leading to more comprehensive risk assessments and policy development.

Pre-market and post-market surveillance

Comprehensive pre-market risk assessments are essential for evaluating the safety of synthetic biology applications before commercial release. Risk assessments should include rigorous testing of engineered organisms, including their genetic stability, potential for horizontal gene transfer, and potential environmental impacts [116, 124]. Post-market surveillance is equally important to monitor the long-term safety and performance of synthetic biology applications. Tracking the behavior of modified microorganisms in real-world settings can help identify any unexpected outcomes or evolving risks, enabling timely intervention and necessary updates to safety measures [103].

Public engagement and ethical review

Public engagement should be an integral part of the decision-making process concerning synthetic biology applications. Ethical review boards, involving a diverse group of stakeholders, can assess the ethical implications of specific applications and provide guidance on responsible innovation and risk management.

Public consultations can provide valuable feedback on societal values, concerns, and expectations related to synthetic biology applications. This feedback should be taken into account in the development of regulations and policies to ensure alignment with public preferences and values.

In conclusion, robust regulations and policy frameworks are crucial for harnessing the transformative potential of synthetic biology while minimizing potential biorisks and ethical concerns. By promoting international collaboration, harmonizing biosafety standards, fostering interdisciplinary cooperation, and engaging the public, we can navigate the complexities of synthetic biology responsibly and ethically, ensuring a safer and more sustainable future for these innovative technologies.

Conclusion and Discussion

The rapid advancements in synthetic biology have unlocked vast possibilities for biotechnological innovation, with applications ranging from healthcare to agriculture. However, as we venture further into this promising field, it is imperative to approach its applications with a cautious and responsible mindset. The transfer of virulence plasmids to probiotic or starter cultures presents significant biorisks that demand careful consideration and risk mitigation. This paper has provided a comprehensive exploration of the biorisks associated with virulence plasmid transfer in the context of synthetic biology applications. The potential for horizontal gene transfer and enhanced pathogenicity underscores the need for robust containment measures and genetic safety precautions. Understanding the consequences of unintended plasmid transfer on human health, food safety, and the environment highlights the importance of comprehensive risk assessments at all stages of development and deployment. Ethical considerations play a central role in guiding responsible decision-making and policy development. Striking a balance between the benefits and risks of synthetic biology applications involves transparent communication, public engagement, and adherence to ethical principles such as informed consent and responsible use. To ensure the safe and ethical use of synthetic biology techniques, international collaboration is vital for harmonizing regulatory standards and sharing best practices. Policymakers, scientists, and stakeholders must work together to develop and implement robust regulatory frameworks that prioritize safety, accountability, and the public interest. As we move forward in the field of synthetic biology, we must remain vigilant, continually evaluating and improving safety measures, risk assessments, and ethical practices. Lessons learned from previous incidents and case studies serve as invaluable guides for future research and risk management. By embracing responsible innovation and adhering to the highest ethical standards, we can unlock the full potential of synthetic biology while safeguarding human health, protecting the environment, and respecting societal values. The responsible use of synthetic biology techniques involving virulence plasmid transfer to probiotic, or starter cultures will pave the way for a more sustainable, healthier, and safer future for humanity and our planet. By fostering a culture of responsible research and decision-making, we can harness the transformative power of synthetic biology to address pressing challenges and improve lives while minimizing potential risks and ensuring a more secure and sustainable future for generations to come.

Abbreviations

AMR: Antimicrobial resistance, HGT: Horizontal Gene Transfer, LMOs: Living modified organisms, MDR: Multidrug resistance

Acknowledgments

Not applicable.

Funding

The authors did not receive support from any organization for the submitted work.

Data Availability statement

No new data were created or analyzed in this study.

Compliance with ethical standards

Conflict of interest / Çıkar çatışması

The authors declare no conflict of interest.

Ethical standards

The study is proper with ethical standards.

Authors' contributions

All authors contributed equally to this work.

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International Journal of Life Sciences and Biotechnology
Sahin, R. V., Studies of Molecular Marker in Sunflower (*Helianthus annus* L.). International Journal of Life Sciences and Biotechnology, 2024 7(2): p. 133-141.
https://doi.org/10.38001/ijlsb.1436687
Review article

Studies of Molecular Marker in Sunflower (*Helianthus annus* L.)

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ABSTRACT
Helianthus annus L., known as sunflower, is belong to *Asteraceae* family. This family is one of the biggest angiosperm plant families in between dicotyledons and cultivated sunflower is an important oil plant all around the world. Chloroplast DNA analysis reveals that the origin of the genus dates back to 4.75 to 22.7 million years ago. Sunflower seeds contain 18% protein, 15% cellulose, 9% water, 14% minerals and carbohydrates. In general, sunflower seeds contain 35% to 50% oil by weight. The oil content of sunflower had been specified from 36.9% to 50.2%. These features have made sunflower an important plant worldwide. In principle, the breeding of sunflowers aims to improve the oil content and to get a plant resistant to disease. All breeding methods both classical and biotechnological methods try to contribute these aims. With the help of technology DNA markers have provided useful information about polymorphism, genetic relatedness, and diversity. Technology advances in breeding, especially the use of molecular markers, offer new strategies to obtain high-yielding and resistant plants through DNA sequences located at a known location on the chromosome. Sunflower has been a model plant in *Asteraceae* family in molecular marker studies because of its economic importance. Many molecular marker studies have been conducted against biotic and abiotic stress conditions, increase oil content and nutritional value, and water consumption etc. Considering its economic value, current studies on the plant will shed light on future studies and improvements can be observed in many yield criteria such as water consumption, harvest efficiency, resistance to pests.

ARTICLE HISTORY
Received
13 February 2024
Accepted
16 April 2024

KEY WORDS
Helianthus annus L., sunflower, sunflower breeding, molecular markers

Introduction

Helianthus annuus L. is known as sunflower and belongs to the *Asteraceae family*. The family *Asteraceae*, also known as *Compositae*, is one of the largest families of angiosperm plants among the dicotyledons. Approximately 10% of all flowering plants worldwide belong to the *Asteraceae* family, and this large family includes 1620 genera and 23600 species [1, 2]. This family, which has 12 subfamilies, grows mostly in subtropical and temperate climates, especially in meadows, valleys, grassy plains, rolling plateaus, and mountain slopes [1,3,4,5].

This family includes species of economic importance in various fields such as human nutrition, bioenergy production, oil production and floriculture.

Examination of chloroplast DNA [6] reveals that the origin of this genus dates back to 4.75 to 22.7 million years ago. Species within this genus diverged relatively late, approximately 1.7 to 8.2 million years ago [6]. Mostly people prefers the sunflower *H.annus* as an annual plant. Generally speaking, an annual plant, heliotrope is a large spiked plant derived from the shape and figure of the flower often used to represent the sun. The stem of the plant is hard and hairy, and the leaves are broad and coarsely toothed; it also has disc-shaped flower heads [7]. The heads consist of many single flowers which mature into seeds on a receptacle base [8].

While the trunk of the sunflower plant can reach a height of up to 3 meters, the flower head can have dimensions approaching 30 cm in diameter. The sunflower inflorescence is a constricted cluster consisted of various independent sessile florets that all share the identical receptacle which is also known as the capitulum. Sunflowers have shining yellow ray florets on the external surface and yellowish circle florets inner side. Throughout the maturation, sunflowers alter their way towards the sun and then stop once they begin flowering. This movement of head named as heliotropism [9]. On the outermost circle of the head, there are five petals on the leaves. The bouquet is generally golden yellow and sterile. The flowers that cover

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the large disc on the head are called disc florets. Each disc flower on the disc is called a flower. Disc fibres contain the pistil and stamens. Discoid fibres in the outer ring are more common than those in the inner ring [5]. The plate-like shape of the sunflower is concave and convex, sloping towards the ground. While the cup diameter of sunflower plants typically varies between 18 and 25 cm, this range can vary between 5 and 50 cm across all genotypes. In addition, sunflower yields are remarkable. Head diameter is one of the factors that is hugely affected by ecological circumstances, alike plant height [10]. The sunflower tray is not a single flower but consists of 1,000 to 2,000 individual flowers that are mutually attached to the base of the tray. Self-fertile sunflower, pollen activity, and honey bee for fertilization. It needs insects, including [11].

Weather conditions have a significant impact on the development and growth of sunflowers. Sunflowers are native to North America, but they are grown in many different regions around the world, making them adaptable to a variety of climates. Sunflower plants dislike low temperatures and prefer a temperature range of 20-25°C during the day and 15-18°C at night for optimum growth. Too little sunlight will result in stunted growth and florets. Adequate water supply is critical to sunflower growth, especially during the germination and flowering stages. For optimal growth, sunflower prefers well-drained and nutrient-rich soils with a pH value ranging from 6.0 to 7.5. Poor soil quality can cause negative consequences such as slowed growth and shrinkage of buds. [12, 13]. Sunflower has a characteristic root system that spreads both deeply and widely, which provides convenience in terms of water and nutrient intake. Compared with leaf growth, sunflower root growth is faster, and if there are no limiting factors, the root system can grow to a depth of more than 3 meters to reach the water surface. Water and nutrient absorption are closely related to the structure of the root system [14]. Cultivated sunflowers generally bloom in 60-70 days and become physiologically mature in 80-100 days. The total growth period depends on its genetic structure and environmental conditions, ranging from 125-130 days [15].

Sunflower achenes are composed of seeds and shells, and their oil content is 44% higher than of canola and soybeans. Sunflower seeds contain 18% protein, 15% cellulose, 9% water, 14% minerals and carbohydrates [16]. The oil content of sunflowers depends on the variety, growing conditions and harvest time. In general, sunflower seeds contain 35% to 50% oil by weight. Studies have determined that the oil content of sunflower plants varies between 36.9% and 50.2%. [17].

Research has revealed the role of fatty acid composition and levels of compounds such as tocopherols, sterols, and carotenoids, which are important in determining sunflower oil quality. Generally, sunflower oil contains 55-65% linoleic acid and 20-30% oleic acid. The remaining 5-10% consists of palmitic and stearic acids. The sunflower plant is rich in linoleic acid, a polyunsaturated fatty acid, and has also been identified as a source of important nutrients such as calcium, phosphorus, vitamin E and niacin [18, 19]. It is known to decline low-intensity lipids, progress immunity, and assist to avoid against cardiovascular disorder [20].

Considering the widespread use of the sunflower plant in areas such as food source, feed additive, industrial raw material, breeding the plant with biotechnological methods is important in terms of saving time and financial resources.

The aim of this study is to make a general evaluation about the biotechnological methods used in sunflower breeding.

The Breeding of Sunflower

Archaeological studies have determined that sunflowers were first cultivated by American Indians in 4625 BC. [21]. Immediately after the discovery of America, sunflowers were brought to the botanical garden of Madrid by Spanish explorers. [22]. In 1716, after a patent was received in England to extract oil from sunflower seeds, the plant began to be used as an oil plant. The sunflower plant was introduced to Russia by Russian Tsar Peter I, who appreciated its aesthetic value. In 1829, Bokarev from the Belgorod region discovered the method of obtaining oil from sunflower seeds, which paved the way for the plant to be grown in agricultural fields and used in oil production [23]. The scientific studies about sunflower breeding began in 1912. At that time, the Kruglik Plant Breeding and Experimental Research Station was established [24] 1988. In 1932, several sunflower breeding stations were established by the former Soviet Union; These stations were opened first in Krasnodar (VNIIMK), then in Rostov, Kharkov and Odessa. High-yielding and high-oil content sunflower genotypes (Peredovik, VNIIMK 8931, Smena, etc.) developed in these centers have contributed significantly to the spread of sunflower as an oil crop worldwide and inspired the advancement of sunflower production worldwide. Sunflower cultivation was carried out in 1937 in Saskatchewan, Canada, and in 1950 at the Texas experiment station in the United States. [25]

After the historical progress of sunflower breeding has reached 3 phases. Within the scope of these methods, mass selection for variety development, individual selection method for variety development and hybrid development process are applied. Towards the end of the 19th century, sunflower became more widespread

with the mass selection method and became a local variety grown largely in gardens. [26]. An important achievement of mass selection is the creation of varieties resistant to the sunflower moth (*Homoeosoma nebulella* Denis and Schiffermüller) and the leaf fluke (*Orobanche cumana* Wallr.). In the late 19th and early 20th centuries, sunflower production was seriously damaged by these insects and parasite species. These harmful insect and parasite species have greatly threatened sunflower production [27]. Being simple and economical are among the most important advantages of this method. The adequacy of this technique may vary depending on the heritability of the trait, genotype and environment interaction, and gene effects on the selected trait. This method has been reported to be more effective for traits that have high heritability and are controlled by spliced genes. Mass selection did not lead to an increase in sunflower yield; however, improvements were observed in sunflower oil content, earliness, and resistance to pests and diseases [28, 29]. Individual selection has been the most widespread and victorious technique for sunflower diversity formation. Individual selection to preserve seed reserves in sunflower breeding was initiated by V. S. Pustovoit in about the 1920s; hence this selection method is also known as Pustovoit's reserve method [30]. This method is based on the individual selection of the most suitable plants from the pioneer population. The seeds of the plants collected one by one are divided into two and some of them are used for planting purposes and the remaining part is used as spare seeds. Elite individuals of superior varieties, intervaried hybrids and the best results from previous selection cycles are used as the starting population. [26]. The application of heterosis or F1 viability in order to obtain high yield is stated as the main purpose of sunflower cultivation. From a genetic perspective, heterosis usually occurs as a result of inter-allele interaction (dominance and super-dominance) and to a lesser degree depends on the result of inter-allele interaction (epistasis). [26]. The first studies of sunflower heterosis applications were made in the 1940s, and a 60% increase in yield was observed compared to the varieties. [28, 31].

Breeding practices have been going on for more than fifty years to develop sunflower hybrids. In the 1960s, the first sunflower breeding efforts were initiated in Russia with the aim of developing varieties with high oil content [32]. To achieve this goal, cytoplasmic male sterility (CMS) was developed through hybridization studies between *Helianthus petiolaris* and cultivated sunflower [33]. Sunflower is an open-pollinated species with the help of insects and therefore heterogeneity related to genetic and phenotypic diversity may arise from random mating. [34]. The traditional breeding method requires significant space and resources for plant selection. [35]. Breeding work carried out to develop a new sunflower variety can take ten years. [36]. By creating appropriate breeding lines, restrictions arising from heterogeneity in the lines can be eliminated. Double haploid (DH) lines can be obtained by repeated crosses with parent lines containing preferred traits and progeny selection, or by development of haploids followed by chromosome doubling. [37]. Haploid plants cannot complete meiosis and are therefore sterile [38]. Fertility can vary with factors such as chemical or spontaneous chromosome doubling, and as a result of these factors, 100% homozygosity can be observed in a single generation. [38, 39]. The resulting double haploid (DH) line eliminates the need for backcrossing with a fascinating parent line during numerous crossbreeding processes and thus significantly speeds up the creation of true breeding lines. [40, 41]. Additionally, double haploids can be used to accelerate the incorporation of many mutations, advance additional mutagenesis screens, reduce ploidy levels (e.g., tetraploid vs. diploid), create homozygotes for gametophyte-lethal mutations, and reduce reproductive depression associated with self-pollination. [38, 41]. Double haploids can also be used to rapidly generate mapping populations such as chromosome substitution lines [40].

In sunflower breeding uses some haploid induction methods. Parthenogenesis is one of the methods. Some experiments have been conducted about resistance to broomrape, fungus, imidazoline, and downy mildew [42,43, 44]. The other haploid induction method is anther culture. This method has applicated for the fertility restoration [45, 46, 47].

In 1987, Ishino made a discovery while studying genes that are associated with the conversion of alkaline phosphatase's isozyme in E. coli, which led to the development of CRISPR [48]. For the past four decades, the transfer of sunflower plants via Agrobacterium has been on the rise [49, 50, 51, 52, 53, 54]. Studying molecular biology to improve transgenic sunflowers with properties like pest resistance, herbicide resistance, and increased oil yield is crucial. It is also pivotal to explore the ecological effect of these alterations [55]. Furthermore, an investigation study governed a survey that resulted in the development of CAS-3 and CAS-5 mutants with high levels of stearic acid and palmitic acid contents, respectively [56]. CAS-14 mutants resulted in an increase of stearic acid content up to 37% [57].

What is the Molecular Marker?

Molecular markers are used as an important tool in genetic research. They are often used to identify specific regions on chromosomes and are often associated with phenotypic traits. They are of great importance in

plant breeding programs, especially in plant breeding, in identifying and selecting plants with desired genetic characteristics. There are various types of molecular markers and these types are classified according to the type of genetic activity, method of detection, and mode of transmission [58]. For example, depending on the detection method, hybridization-based techniques or polymerase chain reaction (PCR)-based techniques can be used. There are also different modes depending on the transmission mode. These modes may vary depending on how pointers are transferred. This diversity of molecular markers enables more efficient management of genetic resources in plant breeding. [59, 60]. Markers point out polymorphism, which can increase through a chance of nucleotide or mutation in the genome loci [61] and make it possible to define genetic diversities between individual organisms or species [62]. Molecular marker techniques are used in many areas such as genetic mapping, patrilineal tests, detection of mutant genes associated with hereditary diseases, variety identification, marker-assisted breeding in plant breeding, population history, epidemiology, food safety and population studies. Genetic mapping is used to understand genetic relationships and genomic positions between species, while patrilineal tests are useful to determine ancestral links. It is important to monitor mutant genes to detect causes and carriers of inherited diseases. While variety identification helps determine the accuracy of plant varieties, pointer-assisted breeding is used to quickly transfer desired traits. Population history and epidemiology studies use molecular marker techniques to understand disease spread and genetic diversity. In food safety research, it is important for monitoring nutrients and tracking food-borne diseases. [63].

Genetic variety can be evaluated with either biochemical or DNA markers. DNA markers are nucleotide sequences that determine differences between the genomes of various individuals. Polymorphism can be caused by a variety of factors, such as insertions, deletions, point mutations, duplications, and translocations. However, it does not inhibit the activity of certain genes [64, 65]. DNA markers have ensured beneficial knowledge about polymorphism, genetic relatedness, and range [66].

The implementation of molecular markers to support the selection of resistance genes will make easy the refinement of improved germ-plasm. PCR-based genetic markers have been extensively operated in the mapping and analysis of agronomic properties in many crops [67, 68].

Based on different criteria, molecular markers are divided into various categories. These include the type of gene activity (dominant or codominant markers), the method of detection (hybridization-based techniques or polymerase chain reaction-based techniques), and the mode of transmission (inheritance from maternal organelles, inheritance from paternal organelles, biparental nuclear inheritance, or maternal nuclear inheritance) [64, 65, 69].

Molecular Marker Studies in *Helianthus annus* L.

In the *Asteraceae* family, sunflower is a model system for genomic studies because of its importance [33, 70]. Genetic analysis of sunflowers is essential because their germplasm has a wide range of characteristics such as yield, plant height, seed number, sensitivity, and earliness to abiotic and biotic stresses [70, 71]. According to various estimates, the *Helianthus* genus includes 49 to 67 species of annual and perennial herbaceous plants native to North America. [72, 73, 74]. This change in the number of species requires a more comprehensive examination of the speciation of this taxon. Sunflowers contain diploids, tetraploids and hexaploids, and the basic chromosome number is n = 17 [73]. Generally, morphological and hybridological analyses have been used to determine the relationship between sunflower species. [74, 75]. Common sunflower is a diploid crop with 2n=34 chromosomal number and it has 3000 Mb haploid genome [76].

Helianthus genus; contains 51 wild species with useful allelic variation and agronomic traits such as yield, resistance to abiotic and biotic stresses [77]. In their RFLP analysis, Rieseberg and Seiler found that cultivated sunflower genotypes were derived from a single origin during acculturation, that these lines had low allozyme variability and were all characterized by a single cpDNA [10]. There is also 40-50% nucleotide difference between the gene pool populations of cultivated sunflowers and wild sunflowers [78].

Numerous kinds of molecular markers are utilised in fingerprinting sunflowers like RAPD, AFLP, SNPs [79] and SSRs are one of the most frequently used molecular markers, such as phylogenetics, genome mapping fingerprinting, population studies and, genetic polymorphism prediction and marker-assisted selection, due to their many advantages and simplicity, co-dominant inheritance, low cost and high polymorphism reproducibility [65, 80].

The first mixed genetic SSR map spanned 1423 cM and identified 278 single-locus SSR markers, as well as 379 additional markers (public and privately owned). This initial map is currently used as the reference genetic map for sunflower and has subsequently been further enriched with additional SSR markers to investigate three new map populations [81] Heesacker (2008) [82] created more than 2000 SSR from

genomic sequences (gSSR) and EST (EST-SSR) and are now suitable for mapping and genotyping. *Helianthus* maps have been developed thanks to gSSRs, EST-SSRs, INDELs, and TRAPs markers.

The first molecular genetic linkage map for cultivated sunflower was created using randomly amplified polymorphic DNA (RAPD) markers and RFLP [83, 84, 85, 86, 87, 88]. Various genetic linkage maps have been constructed using increased fragment length polymorphisms. [89, 90].

The current progress of several hundred microsatellite markers for sunflowers has evolved the way to the analysis of molecular genetic variation in this crop [91, 92].

In terms of genetic data, Rieseberg and Seiler (1990) [93] indicated that, a large collection of wild and domesticated sunflower lines has been investigated and reported that domesticated ones exhibit reduced allozyme variability, all characterized by a single cpDNA (chloroplast DNA) restriction fragment length polymorphism (RFLP) haplotype. Although this information makes a single domestication origin seem likely, it is far from certain because the domesticated cpDNA haplotype has a geographically widespread distribution and is found at a relatively high frequency (27%) in the wild. In sunflower, the length of the genome contains 3.6 billion A, T, C and G nucleotides distributed among 17 chromosomes, corresponding to an area of 3.6 gigabases. The situation reported as the biggest problem of the sunflower plant is that more than four-thirds of the total genome length consists of long repetitive DNA segments called long terminal repetitive retrotransposons. These pieces of DNA are very similar to each other, making it difficult to determine which pieces belong where [94]. To better understand the evolutionary history of the sunflower's genome, Badouin et al. [95] compared it to several closely (lettuce, artichoke) and more distantly (coffee, grape) related species. They were able to confirm a large increase in previously known genome size, tripling its size.

The first molecular marker studies against abiotic stress resistance in sunflower was conducted in 1996 [96]. Arce et al. (2012) [97] conducted sunflower uncommon transcription factors and miRNAs playing a key role in responses to abiotic stresses. A number of molecular biology techniques have been used to achieve the desired results. Phylogenetic tree structures, Database analysis, screening of genomic DNA libraries, isolation of cDNA clones, expression studies using Western blot and Northern blot, quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR), both functionally consistent and transient plant transformation, concentrated Focused microscopy and microarrays were performed. The findings reveal that transcription factors are proteins that can bind and interact with specific DNA sequences located in the regulatory regions of target genes. To understand the relationship between leaf expansion parameters and the set of AFLP and SSR molecular markers under water stress conditions, the researchers conducted a study in the F2 and F2:3 offspring of two public sunflower lines and an independent F8 recombinant pure line (RIL) population. The findings show that breeding programs may contribute to the development of molecular markers to better adapt new varieties to water stress. [98].

Genomic sequence data for sunflower is now available, allowing the development of new SNP-based markers associated with economically critical traits. These markers must be specifically linked to traits that have been studied in detail in other organisms; such as seed oil content and resistance genes [99, 100]. The biggest goals of sunflower breeding programs, which are expensive and time-consuming, are stated to be the cultivation of early maturing and high-yielding hybrids. Parental selection of potential high-yielding hybrids and their innocence testing can be cited as limitations in breeding heterosis. Advances in molecular technologies have supported this process. Genotyping enables the search for methods of preserving superior genetic resources [101]. A sunflower breeding program has been developed with the use of molecular markers. These markers generally show codominant properties and are known for their highly polymorphic structure. However, it should also be noted that they have a strong association with the trait of interest, can be measured at all growth stages, and have phenotypically neutral properties [102]. Various studies have documented those studies on sunflower highlight the importance of marker-assisted selection (MAS) in the heterosis generation process, estimating genetic diversity, identifying integrated lines, and detecting heterotic patterns [103]. Markers potentially appropriate for MAS have been identified via Quantitative Traits Loci (QTL) mapping of economically important features [103, 104].

Result and Suggestions

Asteraceae family and the sunflower plant have been an important plant group both economically and scientifically for years. For years, studies have been carried out on many parameters such as obtaining the highest yield per unit area, increasing plant nutritional content, and resistance to diseases and pests. Considering the importance of the sunflower plant in terms of both agricultural and biological diversity and soil fertility, it is seen that it is a plant that always maintains its importance. Changing global climate conditions require the application of innovative approaches in plant cultivation. Access to food resources for

the rapidly growing world population is only possible with innovative agricultural practices. For all these reasons, the use of modern breeding methods, biotechnological methods and molecular markers have become indispensable elements in plant breeding. With the development of modern breeding methods, quality and high-yield products can be obtained per unit area and at the same time, our existing resources can be used effectively.

Compliance with ethical standards
Conflict of interest
The author declares no conflict of interest.

Ethical standards
The study is proper with ethical standards.

Authors' contributions
The entire work was written by Rabia Vildan Şahin.

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Examples;

Table 1 Possible effects of genetically modified organisms

Table 2 Methods of tissue culture in tomato plants

Fig. 1 Amount of GMO products in the world

Fig. 2 Countries with the highest number of GM cultivation in the world

Citation Inside the Text

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Example: Potatoes produced on a global scale are used in many basic areas [1, 2, 3]. Fifty percent of primarily produced potatoes are used for fresh consumption, such as baking, frying, boiling [4, 5, 6, 7].

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Kocacaliskan, I. and I. Tailor, Allelopathic effects of walnut leaf extracts and juglone on seed germination and seedling growth. The Journal of Horticultural Science and Biotechnology, 2001. 76 (4): p. 436-440.

Example of an article with 3 authors;

Segura-Aguilar, J., I. Hakman, and J. Rydström, The effect of 5OH-1,4-naphthoquinone on Norway spruce seeds during germination. Plant Physiology, 1992. 100 (4): p. 1955-1961.

Example of articles with 4 or more authors

Arasoglu, T., et al., Synthesis, characterization, and antibacterial activity of juglone encapsulated PLGA nanoparticles. Journal of applied microbiology, 2017. 123 (6): p. 1407-1419.

Example for the book;

Kocalishkan, I., Allelopathy. 2006, Ankara, Turkey: Our Office Press-In Turkish.

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Kaya, Y., F.Z. Huyop, and M.F. Edbeib, Genetic Diversity in Plants, in Advances in Biosciences, F.Z. Huyop and S. Mohammed, Editors. 2019, Penerbit UTM Press. Malaysia: Malaysia. p. 04-24.

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DECLARATION: This work is part of Gülfidan KUYUMCU's MA thesis. Articles on 27-29 September 2017 held in Bayburt in Turkey was presented as a Oral Presentation I. International Organic Agriculture and Biodiversity Symposium

Genetic Analysis Related To Organized Genetic Changes in Potato And Processed Potatoes

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