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5th International Conference on Research of Agricultural and Food Technologies (I-CRAFT'2025)

We wish to begin by expressing our sincerest gratitude for your invaluable contributions to the promotion of Turkiye, both domestically and globally, and for your unwavering support of public diplomacy and research within the vital **agricultural and food sectors**.

It is with great pleasure and pride, made possible by your support, that we announce the successful conclusion of the **5th International Conference on Research of Agricultural and Food Technologies (I-CRAFT'2025)**, held in **GANJA, AZERBAIJAN**, from **October 20-23, 2025**. This event was a fruitful joint organization between **Çukurova University** and the **Azerbaijan State Agricultural University (ADAU)**.

We were deeply honored by the participation of the Minister of Agriculture of Azerbaijan, which underscored the importance of this prestigious organization. The conference successfully brought together stakeholders from universities, producers, manufacturers, and affiliated organizations to foster critical dialogue on the future of our industry.

A total of **136 papers** were submitted to the system, and following a rigorous referee process, **126 scientific papers** were ultimately accepted. With **93 foreign papers** and **44 papers from Turkish participants**, the I-CRAFT'2025 achieved a remarkable **70% international participation rate**, firmly establishing itself as a truly international scientific platform.

To facilitate this significant global collaboration, the **Organizing Committee** covered the travel and accommodation expenses for 14 individuals. Furthermore, the substantial support provided by **TIKA (Turkish Cooperation and Coordination Agency)**, which covered the flight and accommodation costs for 22 academics, was instrumental in maximizing the international reach and impact of our event.

The richness of I-CRAFT'2025's scientific content, the high level of international engagement, and the resulting concrete collaboration outcomes demonstrate that we successfully fulfilled our mission to encourage **innovation in agricultural technologies** for the benefit of all participating nations, especially Turkiye and Azerbaijan.

We once again extend our thanks for your esteemed support that made this successful organization possible, and we respectfully submit this comprehensive report for your information.

Sincerely,

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Characterization of Biochemical and Aromatic Compounds in 'Tainung' Papaya (*Carica Papaya* L.) Cultivar Grown in Türkiye

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Abstract. Papaya (*Carica papaya* L.) is one of the most important fruit species cultivated in tropical and subtropical regions. Although typically a tropical fruit tree, it can also be successfully grown in subtropical areas with favorable microclimatic conditions. The Mediterranean region of Türkiye offers considerable potential for papaya cultivation due to its suitable subtropical climate. Easy cultivation, rapid growth, high adaptability, and short economic return period make papaya a promising alternative crop for expansion in Türkiye. Beyond its nutritional value, papaya is also appreciated for its health-promoting properties. Rich in vitamins A, B, and C, as well as essential minerals, the fruit provides bioactive compounds with antioxidant, anti-inflammatory, anticancer, and digestive regulatory effects, making it valuable for the food, cosmetic, and pharmaceutical industries. This study aimed to investigate the biochemical composition and volatile aroma compounds of the papaya cultivar 'Tainung' grown under the ecological conditions of the Akdeniz district in Mersin (Mediterranean region, Türkiye). The results showed that total antioxidant (DPPH) activity was 39.19%, total phenolic content was 28.09 mg GAE/100 g, and monomeric anthocyanin content was 2.47 mg/L. Four sugars were identified—sucrose, glucose, fructose, and xylose with glucose (36.75%) and fructose (37.99%) being the most abundant. Among organic acids, citric acid (5.67%) and ascorbic acid (0.88%) were dominant. Volatile compound analysis identified a total of 33 components (8 aldehydes, 8 alcohols, 4 esters, 5 acids, and 8 ketones), with acids, aldehydes, and esters as the predominant groups. The most abundant volatiles were butanoic acid (49.72%), acetaldehyde (6.40%), and 2-methoxyethanol acetate (8.66%). These findings indicate that papaya grown under Mediterranean conditions possesses a rich biochemical composition and a distinctive aroma profile. Particularly, its high antioxidant activity, phenolic content, and ascorbic acid levels highlight the potential of papaya as a functional food with notable health benefits. The results provide a scientific basis for promoting papaya cultivation in Türkiye and support its potential use in health-related and industrial applications.

Keywords: Aroma profile, Biochemical composition, *Carica papaya* L, Sugars and organic acids.

1 Introduction

Papaya (*Carica papaya* L.) is a tropical fruit species belonging to the Caricaceae family and is widely cultivated in tropical and subtropical regions due to its nutritional, medicinal, and industrial importance. Originally native to Tropical America, papaya has become an economically significant agricultural commodity in regions such as Africa, Asia, and Latin America [1]. In addition to fresh consumption, papaya is widely utilized in the food industry for the production of jams, beverages, and ice creams

because of its distinctive aroma, vivid color, and high nutritional value [2]. Papaya fruits are characterized by high levels of essential nutrients and bioactive compounds. Rich in vitamin C, β-carotene, lycopene, phenolic compounds, and flavonoids, papaya stands out for its potent antioxidant properties and beneficial effects on human health [1;3]. Antioxidant constituents such as α-tocopherol, ascorbic acid, and flavonoids play a crucial role in alleviating oxidative stress, preventing the damage of biological macromolecules by free radicals, and reducing the risk of chronic diseases [3]. Furthermore, papaya contains several proteolytic enzymes, including papain and chymopapain, which contribute to its anti-inflammatory, anticancer, and antimicrobial properties [1]. The antioxidant capacity of papaya can vary considerably depending on factors such as cultivar, maturity stage, and growing conditions. [3] reported distinct differences in antioxidant activity and total phenolic content among papaya cultivars grown under greenhouse conditions, indicating a strong correlation between phenolic compounds and antioxidant capacity. Similarly, [1] observed significant variations in the mineral, vitamin, and polyphenol contents of newly developed papaya hybrids in Kenya, attributing these differences to genetic and environmental influences. Such compositional variations provide an important foundation for breeding programs aimed at improving nutritional quality and health-promoting traits. Another critical quality determinant in papaya is its aroma profile. The unique taste and fragrance of the fruit directly influence consumer acceptance and marketability. The characteristic papaya aroma results from a complex mixture of volatile compounds, mainly consisting of low-molecular-weight esters, along with alcohols, aldehydes, terpenes, and sulfur compounds. Among these, methyl butanoate and ethyl butanoate are considered the major contributors to the typical fruity and floral aroma of papaya. The composition of volatile compounds varies significantly with cultivar and growing region, which directly affects the sensory quality of the fruit [2]. The improvement of papaya flavor through breeding requires a comprehensive understanding of the biochemical and environmental factors that contribute to flavor profiles, their perceptual mechanisms, and the determination of the optimum ripening stage for consumption. Fruit flavor fundamentally comprises a combination of sugars, organic acids, and volatile compounds [4]. Integrating consumer sensory perception with preferences for sweetness and acidity, together with quantified concentrations and ratios of key volatile compounds, enables the development of predictive tools for identifying and selecting desirable flavor types or profiles. In summary, papaya is a tropical fruit distinguished by its high nutritional value, strong antioxidant potential, and distinctive aromatic characteristics. Understanding the interrelationship between the biochemical composition, antioxidant capacity, and volatile compound profile of papaya is of great importance for the development of superior cultivars with enhanced nutritional and sensory

qualities. A comprehensive evaluation of its phytochemical, nutritional, and aromatic properties provides valuable insights into the functional and economic significance of this species. The present study aims to characterize in detail the biochemical composition and volatile aroma profile of the 'Tainung variety' papaya (*Carica papaya* L.) cultivar grown under greenhouse conditions in Turkey. Within this scope, the fruit juice was analyzed for total phenolic content, antioxidant capacity, organic acid, and sugar contents, while the volatile aroma compounds were qualitatively and quantitatively determined using Gas Chromatography–Mass Spectrometry (GC–MS).

2 Material and Method

2.1 Material

Fruit samples of *Carica papaya* (Tainung variety) were collected from a commercial orchard located in the Akdeniz district of Mersin Province, Türkiye. The experiment was conducted following a completely randomized design (CRD) with three replications. A total of three trees were selected, and three fruits were randomly harvested from each tree at the commercial maturity stage. After harvesting, the fruits were thoroughly washed with distilled water, surface-dried, and the prepared seed samples were subsequently subjected to biochemical and volatile compound analyses.

2.2 Method

Organic Acid Measurement

Organic acids in papaya fruit juice were determined by HPLC analysis developed by [5]. For organic acids extraction, 1 mL of the sample, and 4 mL of 3% metaphosphoric acid were mixed. The mixture was placed in the ultrasonic water bath at 80 °C for 15 min and it was sonicated and centrifuged at 5500 rpm for 15 min. Afterward, the mixture was filtered (Whatman nylon syringe filters, 0.45 µm, 13 mm, diameter) and the HPLC vials were removed. The extract of organic acids was analyzed using a high-performance liquid chromatographic apparatus HPLC (Shimadzu LC 20A VP, Kyoto, Japan) equipped with a UV detector (Shimadzu SPD 20A VP) and we used an 87 H column (5 µm, 300 × 7.8 mm, Transgenomic). As for the operating conditions column temperature, was set at 40 °C; injection volume, 20 µL; detection wavelength, 210 nm; flow rate 0.8 mL/min. and % 0.05 mM sulphuric acid was used as the solvent. Identification of organic acids and determination of peaks is based on the retention times

of peaks and comparison of spectral data according to standards. The identified acids were evaluated according to the relevant standard calibration curves.

Sugar Content Measurement

Glucose, fructose, xylose, and sucrose content in the juice obtained from the harvested papaya were determined by [6,7]. Before analysis, frozen juice samples were thawed at 25 °C 1 mL of juice was added to 4 mL of ultrapure water (Millipore Corp., Bedford, MA, USA). The reaction mixture was placed in an ultrasonic bath and sonicated at 80 °C for 15 min and then centrifuged at 5500 rpm for 15 min and it was filtered before HPLC analysis (Whatman nylon syringe filters, 0.45 µm, 13 mm, diameter). The high-performance liquid chromatographic apparatus (Shimadzu LC 20A VP, Kyoto, Japan) consisted of an in-line degasser, pump, and controller coupled to a Refractive index detector (Shimadzu RID 20A VP) equipped with an automatic injector (20 µL injection volume) interfaced to a PC running Class VP chromatography manager software (Shimadzu, Japan). Separations were performed on a 300 mm × 7.8 mm i.d., 5 µm, reverse-phase Ultrasphere Coregel-87C analytical column (Transgenomic) operating at 70 °C with a flow rate of 0.6 mL min^{-1}. Elution was isocratic ultrapure water. Individual sugars were calculated based on their standards and expressed in % of FW.

Determination of total phenol

Total phenolic content was spectrophotometrically determined using the Folin-Ciocalteu procedure described by [8]. with a slight modification. 50-µl methanol-papaya extract was mixed with 1 mL of the Folin-Cicalteu reagent and 10 mL deionized water. The mixture was kept at room temperature for 10 min then 10 mL of 20% Na2CO3 was added. The mixture held in dark for 2 h before reading at 765 nm wavelength in the spectrophotometer (Thermo Multi Scan Go). The same procedure was applied to gallic acid standards. The total amount of phenolic substance was calculated by using the calibration curve (R2 = 0.9991) prepared with the gallic acid standards [9].

Total monomeric anthocyanin content determination

The pH-differential absorbance method method of [10] was employed to quantify monomeric anthocyanin pigment content of the methanol-papaya extract with in buffers at pH 1.0 (hydrochloric acid–potassium chloride, 0.2 M) and 4.5 (acetate acid–sodium acetate, 1 M). A UV-spectrophotometer

and 1-cm disposable cell were utilized for spectral measurements at 510 and 700 nm. Anthocyanin content was calculated as mg (cyanidin-3-glucoside)/L [10].

Determination of total antioxidant capacity

A spectrophotometric method developed by [11] was employed for the antioxidant activity using the elimination of DPPH (1,1-diphenyl-2-picrylhydrazyl) free radicals. 50-µl-methanol-diluted extract was mixed 3 mL 0.004% (v/v) DPPH solution (Merck). After shaken, the mixture left to stand in the dark for 30 min at room temperature for reaction. Ensuring the desired color formation (from deep violet to light yellow), the mixture was read at 517 nm using UV–VIS spectrophotometer (PerkinElmer, Lamda 5). The blank test was methanol. The amount of the antioxidant activity of papaya extracts (DPPH) was expressed in % (Trolox Equivalent).

Volatile compounds were extracted by solid-phase microextraction (SPME)

Volatile compounds obtained from papaya fruit juice were analyzed using three randomly selected commercially ripe fruits. For each sample, 1 g of homogenized seed tissue was placed in a 20 mL headspace vial, to which 1 mL of $CaCl_2$ solution was added to promote the release of volatile components. The samples were then incubated at 40 °C for 30 minutes to allow equilibration between the sample matrix and the headspace. Following incubation, the volatile compounds were extracted using a solid-phase microextraction (SPME) fiber coated with CAR/PDMS/DVB (gray fiber). The extraction and desorption procedures were performed according to the method described by Polat et al. (2022). The collected volatiles were subsequently analyzed and quantified using a Shimadzu GC-2010 Plus gas chromatography–mass spectrometry (GC–MS) system.

Statistical Analysis

Data were processed using the SPSS statistical package program (version 23.0; SPSS Inc., Chicago, IL, USA). All results were expressed as mean ± standard error (SE) and evaluated by one-way analysis of variance (ANOVA) following the procedure described by [12].

3 Results and Discussion

The **DPPH free radical scavenging activity** (39.19 ± 1.16%) demonstrated a **moderate antioxidant capacity** in papaya fruit. Consistently, the **total phenolic content** (28.09 ± 1.9 mg GAE/100 g) and

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anthocyanin content (2.47 ± 0.29 mg/L) suggest that papaya is a notable source of phenolic compounds (Table 1). Comparable results have been reported for papaya grown in Thailand, where the phenolic content was found to be **54 mg GAE/100 g FW** [13] and for other varieties, where **28 ± 6 mg GAE/100 g FW** was recorded [14]. Moreover, papaya has been identified as one of the **major dietary sources of flavonoids** among the Malaysian population [15]. (Table 1; Figure 1).

Table 1. Shows the results of bioactive compound of papaya fruit

Total Antioxidant (DPPH) (%)	39.19±1.16
Total Phenolic Content (mg/GAE100g)	28.09±1.9
Total Anthocyanin Content (mg/L)	2.47±0.29

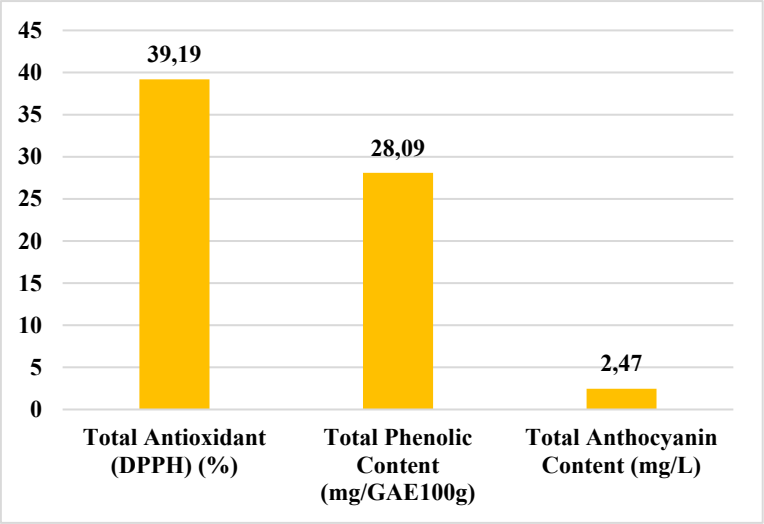

Fig. 1. Contents Antioxidant Capacity (DPPH), Total Phenolic and Anthocyanin of papaya fruit.

Sugars and Organic Acids Composition

This study investigated the chemical composition and antioxidant properties of papaya (*Carica papaya* L.). The results revealed that papaya fruit contains substantial amounts of organic acids and sugars, being particularly rich in citric acid (5.67 ± 0.05%) and ascorbic acid (0.88 ± 0.00%), which are key

contributors to its characteristic flavor and nutritional value. The high levels of fructose (37.99 ± 0.26%) and glucose (36.75 ± 0.12%) further indicate that papaya is an abundant source of natural sugars. (Table 2; Table 3; Figure 2; Figure 3).In conclusion, papaya can be regarded as a functional food due to its high sugar content and considerable levels of ascorbic acid and phenolic compounds. The present findings highlight the nutritional and antioxidant significance of papaya and its potential application as a natural, health-promoting ingredient in the food industry. Supporting this, Im et al. (2016) reported glucose contents of 34.21% and fructose contents of 27.01% in Vietnamese papaya, and glucose contents of 33.26% and fructose contents of 19.81% in the Filipino variety, which are in good agreement with the present study.

Table 2. Shows the results of free organic acid in papaya fruit samples (% FW)

Oxalic Acid	0.02±0.001
Citric Acid	5.67±0.05
Malic Acid	0.26±0.03
Ascorbic Acid	0.88±0.00
Succinic Acid	0.45±0.15
Total acid	6.84±0.63

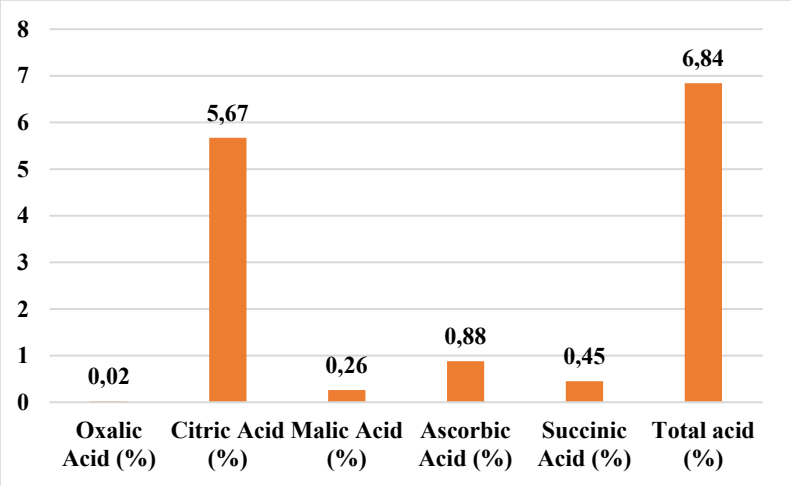

Fig. 2. Individual sugars contents in papaya fruit

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Table 3. Shows the results of free sugars in papaya fruit samples (% FW)

Sucrose (%)	1.76±0.00
Glucose (%)	36.75±0.12
Xylose (%)	1.94±0.02
Fructose (%)	37.99±0.26
Total sugar (%)	78.46±0.29

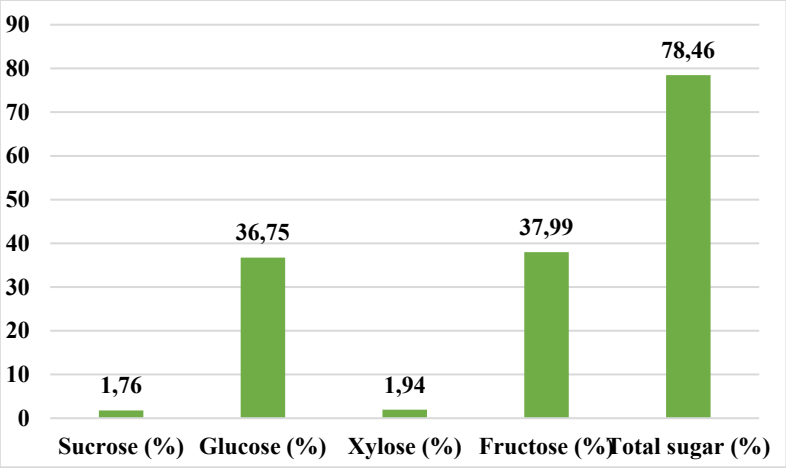

Fig. 3. Individual sugars contents in papaya fruit

Volatile Compound Profile

Headspace Solid-Phase Microextraction (HS-SPME) is a widely applied analytical technique for the qualitative and quantitative determination of volatile compounds, including essential oils and aroma constituents, provided that the target analytes are effectively adsorbed onto the extraction fiber [16] .This method allows rapid, solvent-free extraction of volatiles from the headspace above the sample onto a stationary phase coated on a fused silica fiber [17]. Gas Chromatography (GC), often coupled with Mass Spectrometry (GC–MS), serves as a highly efficient and sensitive analytical platform for profiling plant metabolites and volatile organic compounds [18]. In this study, the volatile aroma compounds of papaya (*Carica papaya* L.) were determined by GC–MS analysis. The results revealed that five major

groups of compounds contribute to the fruit's aroma: aldehydes (13.85%), alcohols (6.10%), esters (14.43%), acids (59.63%), and ketones (5.96%). A total of 33 individual volatile components were identified, including 8 alcohols, 8 aldehydes, 4 esters, 5 acids, and 8 ketones. Among the extraction fibers evaluated, the gray SPME fiber exhibited the highest efficiency in isolating volatile metabolites. Aldehydes, acids, and esters were the predominant contributors to the overall volatile profile. The data indicated that acids represented the dominant group in papaya, with butanoic acid (49.72%) being the most abundant compound. This compound plays a key role in forming the fruit's distinctive sharp and characteristic aroma. In addition, short-chain fatty acids such as acetic acid (4.73%) and hexanoic acid (2.61%) contribute to the typical sour and pungent flavor of papaya. Among aldehydes, acetaldehyde (6.40%) and hexanal (2.86%) were found at the highest concentrations, contributing to the fruit's fresh, green, and fruity aroma. Within the alcohol fraction, 2-methoxyethanol acetate (8.66%) was identified as the major component, known to impart a sweet and floral note to the aroma profile. Although esters (14.43%) and ketones (5.96%) were present in lower proportions, they complement papaya's overall complex aroma structure. In conclusion, the volatile compound profile of papaya fruit exhibits a complex matrix dominated by acids and aldehydes, while esters, alcohols, and ketones provide significant complementary notes. This composition constitutes the chemical foundation that defines papaya's characteristic tropical aroma and serves as an important indicator for assessing sensory quality and ripening (Table 2). The diversity and chemical classes of volatile compounds identified in papaya fruit are comparable to those reported in other tropical fruits. The present findings are consistent with previous studies indicating that papaya aroma mainly consists of esters, alcohols, aldehydes, terpenes, ketones, and acids [19,20,21,22,23]. Among the volatile acids, butanoic acid (48.82%) was again identified as the dominant compound responsible for the characteristic sour and slightly pungent aroma of papaya, while acetic and hexanoic acids contribute to its fermented odor. These results align closely with the findings of [19]. and [2], who also reported acetic and butanoic acids as major contributors to papaya's aroma profile.

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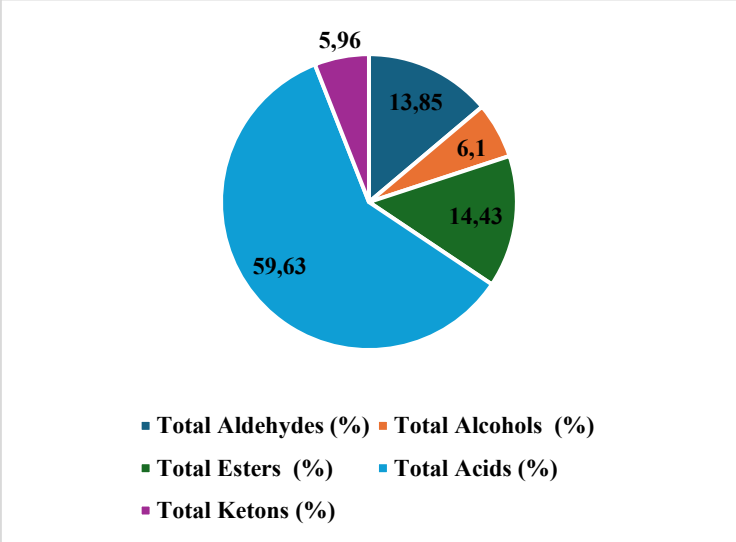

Fig. 4. Volatile composition of papaya fruit (%)

Table 4. Table 4. Volatile compound composition of papaya fruit.

R. Time	Compounds Name	Area %
	Aldehydes	
1.865	Pentanal	1.08
3.112	Hexanal	2.86
0.815	Acetaldehyde	6.40
5.043	Heptanal	0.90
10.074	Nonanal	0.49
17.507	4,5-Epoxypentenal	0.35
10.882	2 Octenal	0.84

11.891	(E, E)-2,4-Heptadienal	0.93
	Total	13.85
	Alcohols	
1.549	Ethanol	2.13
3.907	2-Heptanol	0.83
4.662	1-Penten-3-ol	1.00
6.693	1-Pentanol	0.42
12.719	2-ethyl- 1-Hexanol	0.33
14.377	1-Octanol	0.48
21.594	Benzenemethanol	0.47
10.46	2H-Pyranmethanol, tetrahydro-2,5-dimethyl	0.44
	Total	6.1
	Esters	
1.28	Acetic acid, ethyl ester	1.52
1.032	2-methoxy- acetate	8.66
9.261	Formic acid, hexyl ester	0.35
30.95	1,2-Benzenedicarboxylic acid, diethyl ester	3.90
	Total	14.43

	Acids	
16.266	Butanoic acid	49.72
12.032	Acetic acid	4.73
25.537	Octanoic acid	1.65
21.161	Hexanoic acid	2.61
27.579	Nonanoic acid	0.92
	Total	59.63
	Ketons	
0.294	Butane 2-methyl	2.17
0.507	2-Propanone	0.28
2.295	1-Penten-3-one	0.43
7.859	4-Butoxy-2-butanone	1.42
8.098	2-(1,1-dimethylethyl)-5-methyl-, (2s-cis)-1,3-Dioxan-4-one	0.50
8.71	6-Methyl-5-Hepten-2-One	1.27
20.991	(E)- 5,9-Undecadien-2-One, 6,10-Dimethyl	0.52
26.671	Gamma Decalactone	0.37
	Total	5.96

4 Conclusion

This study comprehensively evaluated the biochemical composition, antioxidant capacity, and volatile compound profile of papaya (*Carica papaya* L.). The results revealed that papaya fruit is particularly rich in natural sugars (fructose and glucose), citric and ascorbic acids, and phenolic compounds, all of which contribute to its nutritional and functional value. Moderate antioxidant activity, as indicated by DPPH radical scavenging and total phenolic content, highlights papaya's potential as a natural source of bioactive compounds beneficial to human health. Volatile analysis demonstrated that acids and aldehydes, particularly butanoic acid, acetaldehyde, and hexanal, are the major contributors to the fruit's distinctive tropical aroma, while esters and alcohols provide complementary floral and fruity notes. These findings confirm that papaya possesses a complex and characteristic aroma profile comparable to other tropical fruits. Overall, papaya can be considered a valuable functional food with high nutritional quality and notable antioxidant potential. Its biochemical and aromatic properties suggest broad applicability in the food, nutraceutical, and flavor industries as a source of natural antioxidants and aromatic compounds.

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I-CRAFT AGRICULTURAL and FOOD TECHNOLOGIES

Degradation of Major Classes Of Recalcitrant Chemicals Hindering The Reuse of Wastewater (A Review)

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Abstract. In this recent age of advanced technology and biotechnology, the existing water bodies that serve as a reservoir for lives are experiencing a surge in the occurrence of chemical contaminant. These classes of contaminants were found to include both organic and inorganic, synthetic and natural pollutants of serious environmental concern. Studies revealed that these contaminants were predominantly recalcitrant chemicals that have been grouped as either toxic, hazardous and carcinogenic. There occurrence is not limited to their numbers, types or variety but also a concentration that is alarming, leading to what is known as emerging chemical contaminants (ECC). Sudden increase in the occurrence of chemical contaminants in our water, wetlands, ponds, wastewaters and sludge were due to increasing population, high demand and consumption, while their persistent in the environment is strongly linked to their recalcitrant nature and physiochemical properties. Partial treatment of wastewater results in the accumulation of recalcitrant chemicals while incomplete degradation of recalcitrant chemicals give birth to emerging contaminant (EC). Incorporation of tertiary treatment systems to our conventional wastewater treatment system in combination with advance treatment processes becomes paramount. Advance oxidation treatment (AOP) processes, membrane systems coupled with bioreactors, biodegradations using bacteria and fungi could be used in combination to remove these recalcitrant chemicals from wastewater. Test to proof the safety and safe reuse of these treated wastewaters should also be conducted using model organism to achieve an ecofriendly treated wastewater.

Keywords: degradation, recalcitrant chemicals, wastewater, ecotoxicity, environmentally friendly.

1 Introduction

Recalcitrant chemicals belonging to both major or minor class leave negative impact in our environment. These class of recalcitrant are predominantly synthetic chemical compounds which have emerged due to high production, aimed at meeting the desired demand of the growing population. Sectors of human endeavors that inevitably release recalcitrant chemical into our water and wastewaters include feeding, health and fashion. Feeding is where food dyes are introduced into our food, chemical preservatives, flavors and seasoning chemicals, heavy metals and other carcinogens. Health sector can be divided into two basic unit beauty (aesthetics) personal care products (PCP) and medications where pharmaceutical drugs fit in. Fashion is where dyes are used on fabrics for fashion and for makeups [1]. Synthetic

compounds used in the manufacture of drugs by the pharmaceutical industry to keep fit and to treat undesired health situations in humans and animals are the predominant sources of recalcitrant chemical in the wastewater [2, 3] and persists in the environment. Recalcitrant chemicals used in the food and feed sector and synthesized to take care of plant pest, herbicides and other plant diseases of significance.

Ternes and Joss [4] reported that utilization of pharmaceuticals by humans is estimated at 15g.cap^{-1}. and 50-150g.cap^{-1} in the developing countries and industrialized countries respectively. According to Ajay et al. [5] advancement in healthcare have created a swift change and awareness for the pharmaceutical industry which make the sector one of the most GDP buster of the global economy, generating about $50 billion annually. The unfortunate aspect of the industry is the generation of waste that are mostly chemical byproducts of serious environmental concern weighing up to about 200 tons per year. Animal health treatments in the veterinary sector are reported to have almost equal consumption of pharmaceuticals as in humans [4].

Moreso, improper use of personal care products, mishandling of pharmaceutical waste and improper disposal of expired drugs and steroids, and uncontrolled discharge of wastewater or illegal release of partially or untreated industrial wastewater into water bodies or municipal wastewater treatment plants. The raising concern of personal care products (PCPs) has been linked to its toxicological and ecological impact in the ecosystem. Recent studies have demonstrated their presence in the environment and their faith is questioned. Research conducted by [6, 7, 8] revealed the presence of recalcitrant chemicals of personal care origin at a concentration above environmental allowable limit.

The spectrum of pharmaceutical chemicals in the environment coupled with their unpredicted impact on the ecosystem are the main reason for their growing concern. The vast majority of pharmaceutical chemicals vary from each other due to their design; mode of action target tissues, hence these intentions are guided by their physical and chemical properties. Even with the existing variety of pharmaceutical chemicals more synthetic chemicals are being formulated and designed to meet higher efficiency. Due to high production of pharmaceuticals, acetaminophen (ACT) or paracetamol were reported in the environment at a concentration far above allowable limit [9].

Efforts have been made by scientist to stop accumulation of these recalcitrant chemicals and there persistent in nature by employing various methods of degradation, remediation and mineralization. Most of these recalcitrant chemicals are known to have escaped the primary and secondary treatment

processes of the treatment plants. From among the treatment techniques reported include advance oxidation processes (AOP) [2, 3] bioremediation [10], biodegradation [1] and membrane removal [11].

Recalcitrant chemicals have numerous ways through which they get into wastewater and to the environment. The recalcitrant chemicals gain access to our water through direct domestic usage, while their presence in our wastewater treatment plants is mainly through discharge from the municipal waste. Persistence of recalcitrant chemicals in our environment to the ecosystem occur when the pollutants in question escape the treatment processes that are been carried out in our conventional wastewater treatment plant [12, 13].

Studies have reported reduction of the recalcitrant chemical after passing through wastewater treatment systems. The results achieved from these systems are related to some of the properties of the recalcitrant chemicals that support sorption (adsorption or absorption). Some properties of the recalcitrant chemicals that made sorption possible include physical and chemical properties, polarity, functional groups, charges and others. Even with the above-mentioned properties, recalcitrant chemicals find their ways into the environment, because some conditions need to be meet for a successful sorption to take place and subsequent removal of the recalcitrant from the wastewater.

Most conventional municipal wastewater treatment systems are not equipped or designed to handle recalcitrant chemicals of great concern hence leading to the discharge of the treated wastewater into nearby water bodies rendering the ecosystem loaded with recalcitrant chemicals.

Recalcitrant chemicals of pharmaceutical origin have been included in the group of emerging contaminants due to their dynamic and cryptic health problems impacted on humans and farm animals. These unclear nature of health problems caused by recalcitrant chemicals of both pharmaceutical origin and personal care products have raised issues of great concern in the past two decades. Guedes-Alonso et al. [14] reported the presence of recalcitrant chemical of great concern “priority pollutants” (fluoroquinolones) having a hazardous effect on the ecosystem after escaping from wastewater treatment system. This was the result of a study conducted in Spain in 2013 from the analysis of two wastewater treatment systems after post treatment and more were found to include anti-inflammatory drugs, analgesic and weight regulatory pills [15].

2 Treatment Methods

Most conventional wastewater treatment plants that are designed without tertiary treatment facilities are usually not capable of handling recalcitrant chemicals hence, treated wastewaters containing such pollutants are discharged to receiving water bodies. These water reserves will contain trace amounts of some of the classes of recalcitrant chemicals if not all, hence creating fear for the reuse of wastewater. Guedes-Alonso et al. [14] in their research, while working with a membrane bioreactor reported a result of successful removal of the recalcitrant chemical with great success relative to sorption of the pollutants on an activated sludge at the secondary treatment stage of the conventional wastewater treatment system. Llers et al. [16] reported results with similar success [15, 17].

Miège et al. [18] demonstrated in research comparing some treatment processes for the removal of recalcitrant chemicals of personal care origin and pharmaceutical sources. The results proved that membrane bioreactors and activated sludge coupled with nitrogen treatment gave most promising results with reliable efficiency for the removal of recalcitrant chemicals of both origins [15].

Bioremediation using enzymes extracted from fungi has gained ground and the interesting part are the potentials demonstrated by the extracellular (ligninolytic) enzymes produced by white rot fungi [19, 20] which enabled them to captivate the interest of researchers. Laccase, LiP, MnP HrP are examples of the most reported ligninolytic enzymes that have demonstrated excellent biodegrading and bio-detoxifying results when use to treat industrial wastewater [1, 21,22, 23]. Studies on laccase have been on the leading front since reports on its substrate non-specificity. Laccase enzyme gave excellent results during dye decolorization [1, 19, 24, 25] biodegradation of hazardous compounds and complex micro-pollutants [26] bioremediation of xenobiotic and recalcitrant pollutants [27, 28, 29, 30] bioconversion of fuel (jet fuel) [31] and monomers crosslinking [32, 33].

Advanced oxidation processes (AOPs) are defined as technologies used in environmental management that generate hydroxyl radicals to effectively degrade complex organic pollutants in water and soil, which are often resistant to traditional treatment methods. AOPs are used similarly to other drinking water treatment processes such as membranes, granular activated carbon, air stripping, and biological degradation [3].

3 Detection and Determination of the Recalcitrant Chemical Pollutants

Studies reveal the existence of recalcitrant chemical pollutant in small concentration and in trace amounts, but their negative impact and unpredictive level of danger up to a catastrophic level is what is posing treat to both environment and human health [1, 34]. Tracing these chemical pollutants, detecting them as well as determining their concentrations in different media complex (biotic and a biotics) e.g. solids (sediments and sludge), liquids (leachates and wastewater) and others (living organisms) at different destinations becomes paramount [35].

Therefore, strategic combination of extraction techniques before instrumental analytical becomes key to achieving techniques that are more sensitive, selective, fast, and friendly to the environment. The solubility and mass transfer of any analyte is the primary driving force guiding its successful detection in any matrix [36]. Moreso, advancement in research is able to come up with more classical techniques that bring about advancement in both separation and quantification methods that have become the basics of modern standards methods of sample analysis example liquid-liquid extraction [37] for liquid samples and Soxhlet extraction methods for solid samples [38].

While the liquid-liquid extraction uses liquid, the solid extraction method (soxhlet) employs a very practical method of sample preparation that uses no solvent or a very small amount. Pawliszyn [39] classified the analyte extracting phases into; sorbent, membrane and gas also reporting that most basic steps involve in sample preparation include but not limited to careful sampling and proper homogenization, proper extraction using the right method followed by clean-up and analyte concentration before injecting or sending it to the chromatographic machine for analysis. Future research in the areas of extraction and determination should be improved to target analytes of interest using more sensitive techniques that will bring about ease of handling and less toxic and environmentally friendly.

Advancement in modern analytical instrumentation of high precision have enabled scientist to also improve on the systems and methods of analytical separation and detection techniques. To mention but a few from the advancement are such as GC–MS (Gas chromatography Mass Spectrometry), GC–MS/MS (Gas Chromatography tandem Mass Spectrometry), LC-MS (Liquid Chromatography-Mass Spectrometry) and LC-MS/MS (Liquid Chromatography-tandem Mass Spectrometry) all of which have inbuilt libraries of all the contaminants and their properties which make comparison easy. Technological evolution of instruments with high precision have helped to detect, determine and measure the concentration of recalcitrant chemicals up to nano quantities per liter level present in water and

wastewater [37]. The results of a study conducted between 1999 and 2000 by the United States Geological Survey (USGS) confirmed the presence of recalcitrant chemicals, more than 50 of which are pharmaceutical chemicals in 139 streams across 30 states in the USA [12, 39]

4 Toxicity and Ecofriendly Test

Toxicity analysis is a major analysis that can restore or give room for wastewater reuse by end users. Toxicity test are experiments design to test the impact of chemicals on a set of microorganisms at the list allowable concentration that will impact minimal effect on the environment or the minimum concentration of toxic waste that can allow for a period of time that the environment can take care of it with minimal or no threat. Different reference organisms were used for different test pending on the available test method. Toxicity analysis is practically achieved by exposing some series of organism in a medium which could either be soil or water in other to evaluate the effect of the contaminant at varying concentration. The effect of the chemical contaminant could be monitored in terms of survival of the organism in the contaminated medium relative to a control (free and uncontaminated media). Various other survival activities could be monitored which ae not limited to growth, reproduction and other behavioral patterns. In the case of recalcitrant, toxic and carcinogenic chemicals sometimes hormonal changes and behavioral changes are also put into consideration during toxicity test. Some of the most reported toxicity test include the use of fluorescence bacteria (Vibrio fischeri), model organism such as crustaceans and algae and sometimes E. coli or cell cultures are also employed. Toxicity analysis conducted by [1,34] reported a successful toxicity test result with Microtox toxicity test kits which were based on the monitoring of luminous intensity of Vibrio Fischeri luminescent microorganisms before, after and at intervals during the degradation experiments.

Dauda and Erkurt [1] demonstrated the use of model organisms for testing the toxicity of intermediate compounds and final products generated during degradation of anthraquinone. Toxicity results from research conducted by [1, 40, 41, 42, 43] revealed that the microorganism responded differently to different toxicity levels during the degradation experiments. Toxicity test carried out at different stages of degradation showed an increasing toxicity level prior to reduction then mineralization. Reasons for the increased toxicity were due to the formation of toxic intermediates formed during degradation processes as proven by [34] and during degradation of anthraquinone dye [1]. Hence drawing the attention of researchers towards avoiding partial degradation or incomplete mineralization, as this might result in the production of even more toxic chemical compounds than the original mother compound.

Results from toxicity tests after biodegradation with enzymes and mineralization with advanced oxidation systems revealed that the treated medium containing the chemical pollutants contain a smaller number of intermediate products which implies presence of chemicals of less toxicity to the environment making them environmentally friendly.

5 Conclusions and Future Prospectives

Conventional system for the treatment of polluted water and wastewater from municipalities are not capable of removing recalcitrant chemical of high environmental concern. The presence of recalcitrant chemicals of compounded origins with different characteristic properties ranging from organic to inorganic, natural and synthetic, simple to complex make it very hard for the conventional system to treat efficiently. Therefore, these conventional systems will inevitably discharge the treated wastewater containing these recalcitrant chemicals into the receiving water bodies hence making the water bodies a reservoir for contaminant and recalcitrant chemicals. These array of properties are a reason for including the recalcitrant chemicals into hazardous, carcinogenic, mutagenic, biogenic hormone, endocrine disrupting compounds and many other classes of pollutants including the recently discovered pollutant as emerging contaminants. Moreso, both sludge and activated sludge that are generated from both primary and secondary treatment systems respectively are often loaded with high concentration of recalcitrant chemicals serving as reservoir or sink for the pollutant. Unfortunately, when these sludges are used as fertilizers, they serve as intermediary sink for the recalcitrant chemicals.

The discharge of inadequately treated wastewater in to the nearby wet lands, pond streams and other water bodies in the name of proper disposal of wastewater is the primary source of carcinogen and environmental contamination. Alternatively, the direct use of the same ill-treated wastewater leaded with recalcitrant chemicals will directly or indirectly affect the bioactivities and performance of our agricultural crops. The need for tertiary treatment systems coupled with advanced wastewater treatment technologies becomes a priority if we have to save ourselves and the environment.

No single independent treatment system is perfect for the removal of recalcitrant chemicals, but researches have proven that a combination of the advance systems will give the perfect result [6, 7, 8]. Researches have demonstrated the combination of membrane bioreactors and systems that uses both

oxidative and reductive processes were found effective for the removal of a number recalcitrant chemical compound [17, 18].

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Determination of Aroma Compounds in Frozen and Fresh of Arnavutköy Strawberry Samples Using Two Different Spme Methods

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Abstract. The strawberry (*Fragaria* × *ananassa*) is classified among berry fruits, and its cultivation as a horticultural crop has a long historical background. Historical records indicate that a strawberry variety introduced from Istanbul to Ereğli many years ago was initially referred to as Arnavutköy and later became known as the Ottoman strawberry. Subsequently, this variety was hybridized with local landraces, particularly those referred to as Karaçilek, resulting in the development of new cultivars. The Arnavutköy strawberry is characterized by relatively small fruit size yet possesses a notably rich aromatic profile. Aroma is recognized as one of the most critical quality attributes of strawberries, with volatile compounds (VOCs) playing a pivotal role in determining consumer perception, acceptance, and overall preference. The identification of key volatile metabolites that confer the distinctive sensory attributes of the fruit is of considerable importance, as these compounds contribute to its fundamental sensory identity and uniqueness. In the present study, volatile composition analyses of fresh and frozen fruits of the Arnavutköy strawberry cultivar were conducted using gas chromatography–mass spectrometry (GC–MS) with using two distinct SPME fibers

Keywords: Arnavutköy, Strawberry, aroma, volatile compounds

1 Introduction

The strawberry, with its unique aroma, is one of the most popular fruits worldwide. Aroma is one of the most important determinants of strawberry (*Fragaria* × *ananassa* Duch.) flavour, directly influencing consumer preference and commercial quality. The characteristic aroma of strawberries arises from a highly complex mixture of volatile organic compounds (VOCs), which include esters, furanones, terpenoids, aldehydes, alcohols, and sulfur-containing compounds (Schwieterman et al., 2014; Ulrich et al., 2024). To date, more than 350 VOCs have been identified in strawberries, although only a limited number exhibit significant odour activity due to their low perception thresholds (Pérez et al., 2021). Among them, esters such as ethyl butanoate, ethyl hexanoate, and methyl butanoate contribute fruity and sweet notes and are recognized as the predominant aroma-active group in ripe fruits (Wang & Seymour, 2017). Furanones, particularly 2,5-dimethyl-4-hydroxy-3(2H)-furanone (furaneol) and 2,5-

dimethyl-4-methoxy-3(2H)-furanone (mesifuran), provide sweet, caramel-like nuances and increase markedly during ripening (Ulrich et al., 2024). Terpenoids such as linalool and nerolidol impart floral and citrus-like notes, whereas aldehydes and alcohols, including hexanal, trans-2-hexenal, and 1-hexanol, are responsible for fresh, green aromas (Pérez et al., 2021). Despite their low concentrations, sulfur volatiles such as methanethiol can exert a strong sensory effect due to their very low odour thresholds (Sánchez-Sevilla et al., 2017). The biosynthesis and relative abundance of these VOCs are strongly influenced by genotype, fruit maturity, and postharvest conditions, all of which determine the overall flavour quality perceived by consumers (Ulrich et al., 2024). In the present study, volatile composition analyses of fresh and frozen fruits of the Arnavutköy strawberry cultivar were conducted using gas chromatography–mass spectrometry (GC–MS) with using two distinct SPME fibers.

2 Material and Method

2.1 Material

In this study, frozen and fresh samples of the Arnavutköy strawberry variety were used. The Arnavutköy variety is an aromatic, aromatic, and biotic stress-resistant local variety. It is preferred in breeding programs in Turkey due to its tolerance to Phytophthora infestans (Kepenek 2016).

2.2 Method

2.3 The determination of Volatile Compounds

Volatile compounds were extracted using solid phase microextraction (SPME). For each sample, 1 g of homogenized strawberry was placed in a headspace vial with 1 mL of $CaCl_2$ and incubated for 30 minutes at 40 °C. A 85 µm CAR/PDMS (carboxen/polydimethylsiloxane; light blue) SPME fiber (Supelco Co., Bellefonte, PA, USA) was used for extraction. The adsorbed volatile compounds were analyzed using a Shimadzu GC-2010 Plus GC-MS system (Shimadzu Corporation, Kyoto, Japan) equipped with an HP-Innowax column (30 m × 0.25 mm i.d., 0.25 µm film thickness) and helium as the carrier gas. The GC oven temperature was initially held at 40 °C, then increased to 260 °C at a rate of 5 °C/min, and maintained at 260 °C for 40 minutes. Compound identification was based on library searches using Wiley, NIST, and Flavour databases. Relative percentages were calculated from the total ion chromatograms using Shimadzu's GC-MS Postrun Analysis software (GC-MS-QP2010, Japan).

Tentative compound identification was performed by comparing mass spectra with those in the NIST08 library (Kafkas et al., 2018b).

3 Results and Discussion

Aroma is a very important parameter in strawberry quality. Therefore, there are many studies related to aroma. In a study conducted within the borders of Aydın, aroma analyses were performed using GC-MS on Florida Fortuna, Rubygem, and Sabrina strawberry varieties. Upon examining the results, it was reported that, according to the aroma composition analysis results obtained using GC-MS, 16, 18, and 20 volatile compounds were identified in the Florida Fortuna, Sabrina, and Rubygem varieties, respectively, and that esters, which generally give fruit scent, were observed to be the active compounds in all strawberries (Görgüç, et al., 2019). Volatile compounds in strawberries are responsible for their aroma and contribute to the flavor of fresh strawberries. These compounds constitute only 0.01-0.001% of the fruit's fresh weight. However, they have a significant effect on strawberry quality (Buttery, 1981). Fresh strawberries produce numerous volatile compounds; among these, esters are the most abundant in terms of both quantity and quality. Research indicates that there are 131 different esters in strawberry aroma (Latrasse, 1991). In addition to esters, aldehydes (Schreier, 1980) and furanones (Larsen and Poll, 1992) account for 50% of strawberry volatiles among other compound classes. Only a small fraction of the hundreds of volatile compounds produced by fresh strawberries contribute to the fruit's aroma and taste. The characteristic aroma is a mixture of a number of volatile compounds; no single “character effect” compound is responsible for the strawberry aroma. A compound's contribution to the aroma depends on its odor threshold and concentration in the fruit (Forney, et al., 2000; Kafkas, et al., 2018a). In another study conducted, the enantiomeric ratios of 8 volatile compounds (methyl 2-methylbutanoate, ethyl 2-methylbutanoate, 2-methylbutanoic acid, linalool, α-ionone, γ-decalactone, γ-undecalactone, δ-dodecalactone) in 7 strawberry aromas and 14 strawberry syrups from 6 strawberry varieties. The volatile compounds identified at the end of the study, γ-Decalactone (enantiomeric ratios; 100/0, 100/0, 99/1, 99/1, 100/0, and 99/1 in strawberries; 100/0 in 3 natural aromas, 100/0 in natural flavored syrup) were found to be the most important indicator of synthetic and natural strawberry flavor. This was followed by ethyl 2-methylbutanoate (enantiomeric ratios: 0/100, 5/95, 5/95 in 3 natural aromas, 0/100 in natural flavored syrup), and the study indicated that the best resolution was in α-ionone (RS>4). Furthermore, in the investigation of the enantiomeric ratios of 7 chiral (compound molecules containing

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two hydroxyl groups) volatile compounds and uniquely selected strawberry samples, the use of the CP-Chirasil-Dex CB chiral column was found to offer more reliable possibilities for the determination of natural strawberry aroma in complex matrices (Průchová et al., 2022). In our study, aroma components were detected in fresh and frozen Arnavutköy strawberries using two different SPME cartridges, red and gray. The results obtained from the analysis of frozen samples using the red SPME are presented in Table 1 and Figure 1.

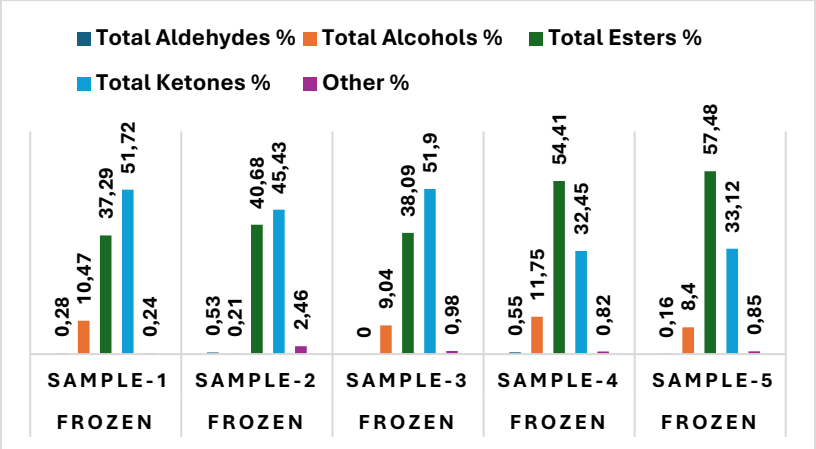

Fig. 5. Determination of Volatile Compounds of Frozen Arnavutköy Strawberries with using red SPME (%)

According to this analysis, aroma components were found in the following ranges: total aldehydes (0.16-0.55%), total alcohols (0.21-11.75%), total esters (37.29 - 57.48%), total ketones (32.45-51.90%), and other components (0.24-0.98%). The results obtained from the analysis of fresh Arnavutköy samples using gray SPME are presented in Table 2 and Figure 2.

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Table 1. Determination of Volatile Compounds of Frozen Arnavutköy Strawberries with using red

Compound name	Frozen Sample-1	Frozen Sample-2	Frozen Sample-3	Frozen Sample-4	Frozen Sample-5
Aldehydes(-al)					
Nonanal	0.1	0.34	-	0.19	-
Undecanal	0.18	0.19	-	0.22	0.16
Farnesal	-	-	-	0.14	-
Total	**0.28**	**0.53**	-	**0.55**	**0.16**
Alcohols(-ol)					
Nonan-2-ol	0.13	-	0.12	0.13	-
2-Tridecanol	10.07	10.68	8.61	10.62	8.07
Phenol, 2-methoxy-4-(2-propenyl)	0.14	-	-	0.34	0.18
1-Dodecanol	0.13	-	-	-	-
1-Decanol				0.22	0.15
beta.-Citronellol	-	0.21	0.31	0.44	-
Total	**10.47**	**0.21**	**9.04**	**11.75**	**8.4**
Esters(-ate)					
Octanoate <ethyl->	0.35	0.18	0.23	0.13	0.13
Propanoic acid, 2-methyl-, 2-methylpropyl ester	0.91			-	
Octanoic acid, methyl ester	0.13			-	
acetic acid, hexyl ester	0.47	0.18	0.18	-	
Acetic acid, phenylmethyl ester	-	-	0.11	-	
acetic acid, 2-ethylhexyl ester	3.09	2.11	2.48	1.09	1.08
decanoic acid, methyl ester	0.26	0.35	0.3	0.39	0.43
decanoic acid, ethyl ester	0.25	0.25	0.31	0.47	0.56
Acetic acid, dodecyl ester	-	-	0.2	0.36	0.39
acetic acid, decyl ester	1.27	1.3	1.37	1.74	1.94
Acetic acid, nonyl ester	-	-	0.09	-	
dodecanoic acid, methyl ester	0.62	2.13	1.15	1.72	1.76
dodecanoic acid, ethyl ester	0.47	0.95	0.8	1.09	1.41
butanoic acid, 2-octyl ester	0.32	0.42	0.27	1.35	1.58
capronate <ethyl->	0.32			-	
Propanoic acid, 2-octyl ester	-	-	-	-	0.11
2-octanol, acetate	0.43	0.41	0.49	0.34	0.37
butyl decanoate	0.13	0.45			
myrtenylacetate	0.1	0.14	0.24		
methylanthranilate	2.1	1.46	0.95	0.6	0.41
cinnamyl acetate	0.56	0.85	0.74	1.42	1.04
Tridecyl acetate	0.13				
Undecyl acetate	-	-	0.12		
2-Heptadecanol, acetate	24.82	27.64	26.57	41.52	42.52
trans-2-Dodecen-1-ol, acetate	0.39	0.78			
(z)-3-phenyl-2-propenoic acid, methyl ester	-	0.16	0.13	0.15	0.09
trans-2-Dodecen-1-ol, acetate	-	-	0.6		
5-Tetradecen-1-ol, acetate, (Z)-	-	0.15	0.12	1.11	1.18
n-Capric acid n-heptyl ester	-	-	0.31	0.35	0.47
ıso butyl decanoate	-	-	-	-	0.14
Cyclooctanol, acetate	-	-	-	-	1.14
Caprylate <hexyl->	0.17	0.26	0.19	-	-
lauric acid, n-octyl ester	-	0.23	0.14	-	-
Hexanoic acid, 3-tridecyl ester	-	-	-	0.34	0.44
Hexanoic acid, octyl ester	-	-	-	0.24	0.29
Total	37.29	**40.68**	**38.09**	**54.41**	**57.48**
Ketons(one)					
2-Undecanone	40.72	31.22	41.02	21.46	21.47
2-Heptanone	0.27	-	-		
2-Nonanone	0.93	0.42	0.6	0.28	0.22
2-Pentadecanone	0.71	1.96	0.99	1.52	1.95
Ketone, 1-cyclohexen-1-yl phenyl	-	-	-	0.34	0.31
.gamma. decalactone				0.23	-
Farnesene <(E,E)-, alpha->	0.12	-	-	-	-
2(3H)-Furanone, 5-hexyldihydro-	0.35	-	-	-	-
2-Tridecanone	8.62	11.83	9.29	8.62	9.17
Total	**51.72**	**45.43**	**51.9**	**32.45**	**33.12**
Other					
Dodecanoic acid	0.12	-	-	-	-
Pentadecanoic acid	-	-	0.11	0.14	-
Tetradecane	0.12	-	-	-	-
4-octylbutan-4-olide	-	0.2	0.11	-	-
4-hexylbutan-4-olide	-	0.59	0.39	-	0.13
dodecanoic acid	-	0.13	-	-	-
pentan-1,3-diol diisobutyrate, 2,2,4-trimethyl-	-	0.15	0.09	-	-
benzene, methyl-	-	1.18	-	-	-
.beta. elemene	-	0.21	0.18	-	0.11
2-Hexadecen-1-ol, 3,7,11,15-tetramethyl	-	-	-	0.32	0.41
Cytidine	-	-	-	0.22	-
Palmitic acid	-	-	-	0.14	0.2
2-Methoxy-6-vinylnaphthalene	-	-	0.1	-	-
Total	**0.24**	**2.46**	**0.98**	**0.82**	**0.85**

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Table 5. Volatile Compounds of Fresh Arnavutköy Strawberries with using gray SPME (%)

Compound name	Fresh Sample 1	Fresh Sample 2	Fresh Sample 3	Fresh Sample 4	Fresh Sample 5
Aldehydes(-al)					
Undec-8-enal <cis->	2.62	2.3	-	1.16	-
Hexanal	-	0.95	-	-	-
Furfural	-	-	-	-	1.35
Nonanal	2.39	4.21	0.68	1.05	-
Dec-2(E)-enal	-	-	-	1.2	-
Deca-2(E),4(E)-dienal	-	-	0.8	-	-
Propanal, 2-oxo-	-	-	-	-	0.39
Octanal	-	2.11	-	-	-
2-Decenal, (E)-	-	2.42	0.77	-	-
Hydroxy methyl furfural	-	1.84	-	-	-
2-Dodecenal	-	-	1.01	-	-
5 methyl furfural	-	-	-	-	0.54
furan-2,5-dicarboxaldehyde	-	-	-	-	0.63
2-Furancarboxaldehyde, 5-(hydroxymethyl)-	14.99	-	18.62	4.06	50.4
Total Aldehydes	**20**	**13.83**	**21.88**	**7.47**	**53.31**
Alcohols(-ol)					
2-Tridecanol	3.44	4.12	3.51	2.94	0.77
Phenol, 4,4'-(1-methylethylidene)bis-	-	7.86	2.41	4.68	0.45
Syringol <4-methyl->	-	-	-	-	0.39
2-Furanmethanol	-	-	-	-	0.62
Phenol, 2-methoxy-4-(2-propenyl)	0.21	-	-	-	-
Silanediol, dimethyl-	7.49	8.85	-	5.04	-
Total Alcohols	**19.14**	**20.83**	**5.92**	**12.66**	**2.13**
Esters(-ate)					
methylanthranilate	2.7	3.8	-	-	-
Silanediol, dimethyl-, ester	-	-	7.79	-	-
(E)-Ethyl Undec-2-enoate	-	-	-	-	0.89
Cinnamyl acetate <(E)->	-	1.18	1.93	1.42	-
5-Tetradecen-1-ol, acetate, (Z)-	-	-	0.72	3.1	-
1,2,3-Propanetriol, monoacetate	-	-	-	-	1.1
Caproate <methyl->	-	-	-	1.1	
Caprylic acetate	-	-	-	-	1.05
2-Heptadecanol, acetate	-	-	-	-	2.18
Laurate <ethyl->	-	-	-	1	-
2-Propenoic acid, methyl ester	-	-	-	-	0.39
Butyric acid, 3-tetradecyl ester	-	-	0.47	-	-
trans-2-Dodecen-1-ol, acetate	-	-	3.03	-	-
Benzoic acid, 2-amino-, methyl ester	-	-	4.66	1.67	-
Dodecanoic acid, methyl ester	-	-	0.43	-	-
Undecyl acetate	-	-	0.65	-	-
methyl-2-furoate	-	-	-	-	1.25
2-Heptadecanol, acetate	6.97	15.12	11.31	13.62	-
2-Hexen-1-ol, acetate, (E)-	-	1	0.56	-	-
Acetic acid, decyl ester	-	1.18	-	-	-
Acetic acid, 2-ethylhexyl ester	-	1.99	0.63	1.86	-
Acetic acid, hexyl ester	-	1.51	0.62	-	-
Total Esters	**9.67**	**25.78**	**32.59**	**23.77**	**6.86**
Ketons(one)					
2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	1.65		2.28	-	13.27
2-acetyl-2-hydroxy-, gamma.-butyrolactone	-	-	-	-	0.66
2-Pentadecanone (CAS)	-	-	0.95	1.63	-
2-Undecanone	15.51	23.56	13.48	24.72	3.99
Tetradecalactone <delta->	-	-	-	1.12	-
Nonadecane	-	-	-	1.24	-
2-Nonanone	-	-	0.44	-	-
Methyl undecyl ketone	-	2.61	-	-	-
2-Tridecanone	2	-	2.56	4.01	0.75
Total Ketones	**19.16**	**26.17**	**19.61**	**32.72**	**18.67**
Other					
Pentadecanoic acid	0.24	4.84	2.43	3.46	1.74
Stearyltrimethylammonium chloride	-	2.06	-	-	-
Cetrimonium Bromide	-	-	0.54	-	-
2-Propenoic acid, 3-phenyl-	-	-	0.53	-	-
2-Propanone, 1-hydroxy-	-	-	-	-	0.53
Nonanoic acid	-	-	0.53	-	
Acetic acid	-	-	-	-	5.04
2-Hexadecen-1-ol, 3,7,11,15-tetramethyl	-	-	1.83	-	
Guanosine	-	-	-	-	2.88
Caprylic acid <4-methyl->	-	-	-	-	0.95
Formic acid	-	-	-	-	1.32
9-Octadecenoic acid, (E)-	-	-	-	-	0.38
2-Propenamide, 2-methyl-N-phenyl-	-	1.52	0.43	1.6	-
D-Allose	-	-	-	-	0.46
2-Propenoic acid, 3-phenyl-	-	-	-	-	0.65
acetic acid	3.93	-	6.74	2.2	-
4h-pyran-4-one, 3-hydroxy-2-methyl-	-	-	-	-	1.6
d-Mannose	-	-	-	-	1.88
Hexanoic acid	1.76	-	-	-	-
N,N-Dimethyl-Pentadecylamine	16.29	-	-	-	-
Dodecanamide, N,N-bis(2-hydroxyethyl)-	-	-	0.68	-	-
Benzene, methyl-	-	3.72	-	1.19	-
Dodecanoic acid	-	-	-	1.89	-
Methane, tetranitro-	-	-	0.32	-	-
heptadecene-(8)-carbonic acid-(1)	-	1.25	-	-	-
2-Propenoic acid, 3-phenyl-	1.8	-	-	-	-
Total	**32.02**	**13.39**	**20.03**	**10.34**	**17.43**

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Accordingly, total aldehydes (7.47-53.31%), total alcohols (2.13-20.83%), total esters (6.86-32.59%), total ketones (18.67-32.72%), and other compounds (10.34-32.02%).

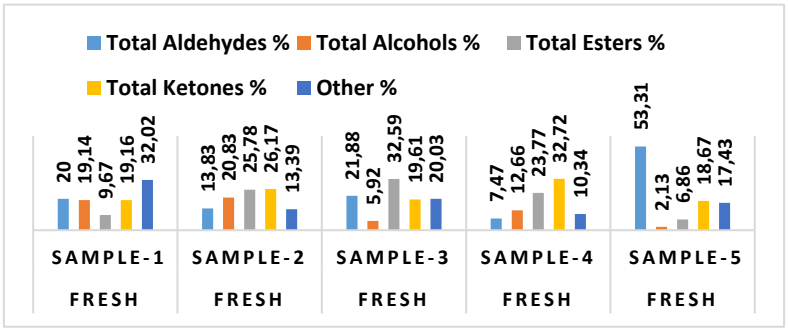

Fig. 6. Determination of Volatile Compounds of Fresh Arnavutköy Strawberries with using Gray SPME (%)

4 Conclusion

According to the results of this study, the highest total aldehyde content in fresh Arnavutköy fruits was detected in Sample-5 using gray SPME, at 53.31%. In addition, the highest total alcohol and total ester ratios in fresh Arnavutköy fruits were measured as 20.83% and 25.78% in Sample-2 using gray SPME. Furthermore, the highest total ketone ratio was found to be 20.03% in Sample-3. In the analysis performed with red SPME on samples of the frozen Arnavutköy strawberry variety, the highest ester ratio was detected in Sample-5 at 57.48%. While the highest ketone ratio was found in Sample-3 at 51.9%, the highest aldehyde was observed at 0.55% and alcohol ratios at 11.75% in Sample-4. This study will shed light on more comprehensive studies involving other Arnavutköy species.

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Determination of Biochemical and Volatile Properties of Papaya (*Carica Papaya* L.) Seeds

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Abstract .edicinal plants have long been utilized as natural prophylactic and therapeutic agents due to their abundance of bioactive compounds, easy accessibility, and relatively low toxicity. Currently, the demand for natural therapeutic agents is increasing as they are incorporated into both foods and pharmaceuticals as alternatives to synthetic compounds. Papaya (*Carica papaya* L.), belonging to the Caricaceae family, is an important plant cultivated in tropical and subtropical regions. While papaya fruit is widely consumed, its seeds are often discarded as waste, despite being rich in essential micronutrients and secondary metabolites with significant therapeutic potential. This study aimed to evaluate the biochemical composition, sensory characteristics, and volatile compounds of papaya seeds cultivated in Türkiye. The results revealed that total antioxidant (DPPH) activity was 50.75%, and total phenolic content was 81.96 mg GAE/100 g. Four sugars (sucrose, glucose, fructose, and xylose) were identified, with glucose (1860.92 mg/100 g) and fructose (1684.45 mg/100 g) being the most abundant. Volatile compound analysis identified 30 compounds (3 aldehydes, 4 alcohols, 8 esters, 6 acids, 8 terpenes, and 1 ketone), with acids, esters, and aldehydes as the predominant groups. The major volatiles were benzaldehyde (35.74%), hexanoic acid (5.79%), and hexanoic acid, hexyl ester (5.37%).

Keywords: Bioactive compounds, Papaya seed, sugars, volatile compounds profile

1 Introduction

Carica papaya L., belonging to the family Caricaceae, has long been utilized in traditional medicine for the treatment of various ailments (Mello et al., 2008). Papaya is a widely cultivated tropical fruit grown across Central America, South Asia, and Africa, and is valued both for domestic consumption and for export markets (Ikram et al., 2015). Although originally native to southern Mexico, the species is now cultivated throughout the tropical and subtropical regions of the world and ranks as the third most important tropical fruit crop globally (Ghaffarilaleh et al., 2019). The papaya plant is medium-sized and capable of bearing fruit year-round. Ripe papaya fruits are typically consumed fresh or processed into products such as juice, jam, or wine (Ikram et al., 2015). However, both processing and fresh consumption generate substantial amounts of by-products, particularly seeds and peels. These residues

are known to possess considerable nutritional and functional potential and can be valorized into high-value bioproducts, thus contributing to waste minimization and environmental sustainability.

Almost all parts of the papaya plant including the fruit, flowers, leaves, seeds, roots, and latex have been reported to exhibit medicinal properties. Numerous studies have demonstrated the biological activities of different plant parts, attributing antioxidant capacity mainly to polyphenols, carotenoids, and antioxidant vitamins such as vitamins C and E. Antioxidants are molecules capable of significantly delaying or preventing oxidation even at low concentrations (Halliwell et al., 1995). Their biological functions include reducing DNA damage, decreasing lipid peroxidation, enhancing immune response, and inhibiting carcinogenic cell transformations (Gropper et al., 2009). Phenolic compounds, in particular, are recognized as key bioactive phytochemicals contributing to health-promoting properties (Cao et al., 1996). Several studies have reported a strong correlation between total phenolic content and antioxidant activity in various seeds, fruits, and vegetables (Yang et al., 2009).

While the seeds of many fruits are inedible and typically discarded (Kothari & Seshadri, 2010), papaya seeds are non-toxic and can be consumed as a dietary supplement or used as a spice owing to their sharp, pepper-like flavor. Chemically, papaya seeds contain considerable amounts of protein (28–44%), crude fiber (22–32%), and lipids (approximately 28%) in defatted and non-defatted samples (Marfo et al., 1986). Several studies have shown that papaya seeds are rich in phytochemicals such as flavonoids, phenols, saponins, tannins, steroids, and terpenoids, which contribute to their potent antioxidant capacity (Gadzama et al., 2016; Olagunju et al., 2009; Salla et al., 2016; Zhou et al., 2011). Moreover, papaya seed extracts have been reported to exhibit various pharmacological activities, including hypolipidemic, nephroprotective, anticholesterolemic, antihelminthic, anti-amoebic, antiulcerogenic, antiparasitic, and antibacterial effects (Aravind et al., 2013; Gadzama et al., 2016; Nwangwa & Ekhoye, 2013).

Despite their valuable composition, papaya seeds are generally not consumed, and over 20% of the total fruit biomass (including seeds and peels) is discarded as waste (Pathak et al., 2018), contributing to environmental pollution (Senrayan & Venkatachalam, 2018). Papaya seeds contain approximately 30% oil, rich in palmitic, stearic, oleic, and linoleic acids, along with tocopherols and carotenoids, which

offer both nutritional and functional benefits (Anwar et al., 2018; Malacrida et al., 2012). These by-products have been utilized as raw materials for biodiesel production, dietary additives, and fermentation substrates (Pathak et al., 2018; Senrayan & Venkatachalam, 2018). Additionally, papaya seeds are rich in bioactive compounds such as carpaine, glucotropaeolin, benzyl isothiocyanate (BITC), caricin, and the enzyme myrosinase (Vij & Prashar, 2015). These naturally occurring phytochemicals including flavonoids, carotenoids, alkaloids, tannins, tocopherols, phytosterols, and phenolic compounds represent valuable sources of antioxidant agents (Alotaibi et al., 2017). Consequently, papaya seeds have attracted increasing attention as promising natural resources for the development of functional foods, nutraceuticals, and food additives (Pathak et al., 2018).

Aroma is a critical sensory attribute influenced by complex mixtures of low-molecular-weight volatile compounds. The volatile profiles of papaya fruit have been extensively characterized across various cultivars (Balbontín et al., 2010; Pino, 2014; Rossetto et al., 2008). The distinctive aroma of papaya is primarily attributed to esters and alcohols, although more than 300 volatile compounds have been identified in the fruit (Withopf et al., 1997). The qualitative and quantitative composition of these volatiles varies significantly among cultivars (Balbontín et al., 2007).

Papaya is a relatively new fruit crop in Türkiye and has shown promising adaptability to the country's southern regions (Güneş & Gubbuk, 2012). Nevertheless, limited scientific information is available regarding the biochemical and aromatic composition of papaya fruits cultivated under Turkish conditions. Therefore, the present study aimed to characterize the volatile compounds, organic acids, sugars, total phenolic content, and antioxidant capacity of the Tainung papaya variety grown in the Mersin province of Türkiye.

2 Material and Method

2.1 Material

Fruit samples of *Carica papaya* L (Tainung variety) were collected from a commercial orchard located in the Akdeniz district of Mersin Province, Türkiye. The experiment was conducted following a

completely randomized design (CRD) with three replications. A total of three trees were selected, and three fruits were randomly harvested from each tree at the commercial maturity stage. After harvesting, the fruits were washed thoroughly with water and surface dried. The seeds were manually separated from the pulp, cleaned, and processed using traditional methods. The prepared seed samples were then subjected to biochemical and volatile compound analyses.

2.2 Method

2.3 Determination of total phenol

The total phenolic content was determined colorimetrically using Folin Ciocalteu's reagent (Spanos and Wrolstad, 1990). Results were described as mg of gallic acid equivalent/100 g of weight (mg/ GAE 100 g).

2.4 Determination of total antioxidant capacity

Total antioxidants capacity was determined using the DPPH (1,1-diphenyl 2 picrylhydrazyl) method proposed by Brand Williams (1995) with slight modifications. DPPH was prepared fresh at 0.06 µM. The mixture was blended for 1 minute before being kept in the dark for 30 minutes at room temperature. Then, 1950 µL DPPH- was added to 50 µL banana sample. Absorbance of mixture was measured at 515 nm. Radical scavenging activity %DPPH inhibition was calculated using the following equation:

%Inhibition = 100 × [(Abs blank (t = 30)) − (Abs sample)]/[(Abs blank (t = 30))].

2.5 Total monomeric anthocyanin content determination

For total anthocyanin analysis, the method described by Giusti and Wrolstad, (2001) was used with some modifications. Total anthocyanin determination was read at 510 nm and 700 nm in a spectrophotometer and calculation was made according to the following formula;

Absorbance = X = (510 nm pH 1 - 700 nm pH 1) - (510 nm pH 4.5 - 700 nm pH 4.5)

Anthocyanin content (mg/L) = (absorbance/ 29600 x 1) x 1000 x 445 x 20

2.6 Individual sugar determination

Sugars were extracted individually following the procedure described by Mikulic-Petkovsek et al. (2016). Homogenized, unfrozen fruit samples were placed in a test tube containing 4 mL of bi-distilled water, at 1 g. The mixture was continuously mixed on a vortex mixer at room temperature for thirty minutes. After extraction, the samples were centrifuged at 9000 x g for ten minutes at 4°C to obtain the supernatant and then filtered into vials using cellulose membranes. The extracts were stored at -20°C for further investigation. The high-performance liquid chromatography (HPLC) system was equipped with a refractive index detector (Shimadzu RID 20A VP, Kyoto, Japan), an in-line degasser, a pump, a controller, and a 20 µL injection volume automatic injector. Chromatographic separation was performed using a reverse-phase Ultrasphere Coregel-87 C analytical column (300 mm × 7.8 mm i.d., 5 µm, Transgenomic) at a flow rate of 0.6 mL/min, heated to 70 °C, and with the mobile phase being ultrapure water. Data acquisition and processing were performed with Shimadzu Class VP chromatography management software (Kyoto, Japan). Concentrations of individual sugars are reported as percentages of fresh weight (FW) determined using original standards.

2.7 Volatile compounds were extracted by solid-phase microextraction (SPME)

Volatile compounds obtained from papaya seed juice were analyzed using three randomly selected commercially ripe fruits. For each sample, 1 g of homogenized seed tissue was placed in a 20 mL headspace vial, to which 1 mL of $CaCl_2$ solution was added to promote the release of volatile components. The samples were then incubated at 40 °C for 30 minutes to allow equilibration between the sample matrix and the headspace. Following incubation, the volatile compounds were extracted using a solid-phase microextraction (SPME) fiber coated with CAR/PDMS/DVB (gray fiber). The extraction and desorption procedures were performed according to the method described by Polat et al. (2022). The collected volatiles were subsequently analyzed and quantified using a Shimadzu GC-2010 Plus gas chromatography–mass spectrometry (GC–MS) system.

2.8 Statistical Analysis

Data were processed using the SPSS statistical package program (version 23.0; SPSS Inc., Chicago, IL, USA). All results were expressed as mean ± standard error (SE) and evaluated by one-way analysis of variance (ANOVA) following the procedure described by Steel et al. (1997).

3 Results and Discussion

The DPPH assay is one of the most widely used methods for evaluating the antioxidant capacity of plant extracts. It is based on the reduction of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical by physiologically active compounds with antioxidant potential, including tocopherols, ascorbates, flavonoids, aromatic amines, Maillard-type browning products, and peptides. Upon accepting an electron or hydrogen atom from these compounds, the deep violet color of DPPH fades, and the degree of discoloration can be quantified spectrophotometrically. This change reflects the hydrogen-donating ability of the sample, which is a direct indicator of its radical scavenging activity (Re et al., 1991).

Among phytochemicals, phenolics and polyphenols have attracted significant attention because of their diverse physiological functions, including antioxidant, antimutagenic, and antitumor activities (Othman et al., 2007). Plant phenolics, abundant in fruits and vegetables, are recognized for their free radical scavenging abilities, which may have important health-promoting implications (Dziedzic & Hudson, 1983; Lopez-Velez et al., 2003;Govindarajan et al., 2007).Within this group, anthocyanins represent a major class of flavonoids responsible for the cyanic coloration observed in many flowers, fruits, and leaves of angiosperms, with hues ranging from salmon pink to dark blue (Andersen et al., 2006). In recent years, interest in anthocyanins has increased considerably due to their potential role as nutritional supplements. Regular dietary intake of anthocyanins from fruits, vegetables, wines, jams, and preserves has been linked to a reduced risk of chronic diseases such as cancer, cardiovascular disorders, diabetes mellitus, hypertension, cataracts, viral infections, and Alzheimer’s disease. Their strong antioxidant capacity underlies their classification as important nutraceuticals, with a potential preventive role in diseases associated with oxidative stress (Middleton et al., 2000). Studies on the phytochemical characterization of plan products are always a challenge, as many groups of compounds can be present,

in different forms and in diverse concentrations, specifically in a free form or associated to other structures from the cell walls (Oliveira-Alves et al., 2017).

3.1 Antioxidant activity (DPPH Radical Scavenging Assay)

In the present study, the antioxidant capacity of papaya seed extract was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The inhibition percentage was determined as 50.75% (Table 1; Figure 1). Maisarah et al. (2013) reported considerable variation in the antioxidant activities of different papaya plant parts, ranging from 58% to 91%. The order of antioxidant activity was found to be: unripe fruit (90.67%) > young leaves (90.01%) > ripe fruit (88.12%) > seeds (58.97%). Similarly, Kothari and Seshadri (2010) observed that the total antioxidant activity of various C. papaya seed extracts ranged between 123.40% and 1681% (expressed per gram of dry extract), with the highest activity recorded in aqueous extracts. In contrast, hexane extracts exhibited comparatively lower antioxidant potential, suggesting that the polarity of the extraction solvent significantly influences the recovery of antioxidant compounds.

3.2 Total phenolic content (TPC)

The total phenolic content (TPC) of papaya seed extract was determined using the Folin–Ciocalteu method, with gallic acid as the standard. Results were expressed as mg gallic acid equivalents (GAE) per gram of fruit sample. The TPC value for papaya seed extract was 81.96 ± 1.52 mg GAE/g (Table 1; Figure 1).

Ávila et al. (2020) reported that free phenolic fractions exhibit higher physiological potential, including greater antioxidant capacity, compared to bound phenolics. In their study, the highest TPC values were 77.91 mg GAE/100 g (seed 3, free fraction) and 46.19 mg GAE/100 g (seed 3, bound fraction). Similarly, the TPC of methanolic extracts of *Carica papaya* seeds was reported as 62.27 mg GAE/100 g. Kothari and Seshadri (2010) observed that the total phenolic content of different C. papaya seed extracts ranged between 23.51 and 528.29 mg GAE/g of dry extract, with the highest TPC recorded in chloroform–methanol extracts. Acetone extracts, on the other hand, appeared to be less efficient in phenolic

compound extraction. Maisarah et al. (2013) reported significant variation in TPC among different parts of the papaya plant, ranging from 30.32 ± 6.90 to 424.89 ± 0.22 mg GAE/100 g dry weight. The distribution of TPC followed the order: young leaves > unripe fruit > ripe fruit > seeds. Pawpaw (2016) demonstrated that extracts from unripe papaya seeds contained higher levels of total phenolics (131.00 mg GAE/100 g) compared to papaya peels (126.75 mg GAE/100 g).

3.3 Total Anthocyanin Content

In the present study, the total anthocyanin content (TAC) of papaya seed was determined as 1.45 ± 0.43 mg/L, expressed as cyanidin-3-glucoside equivalents (Table 1; Figure 1). Phytochemicals such as phenolics and flavonoids are well known for their health-promoting effects, and among these, anthocyanins represent one of the most biologically active subclasses of flavonoids. Their vivid pigmentation and potent antioxidant activity not only contribute to the aesthetic and sensory qualities of plant-derived foods but also play a crucial role in mitigating oxidative stress and reducing the risk of chronic diseases (Kumar & Pandey, 2013). Maisarah et al. (2013) reported that the distribution of phenolics and anthocyanins significantly varied among different papaya organs, with the highest levels detected in young leaves, followed by unripe fruits, ripe fruits, and seeds. Similarly, Ávila et al. (2020) emphasized that free phenolic fractions of C. papaya seeds exhibited greater antioxidant potential than bound phenolics, highlighting the importance of extractable compounds in the seed matrix. Furthermore, Siddiqua et al. (2010) recorded comparable antioxidant and phenolic levels in papaya seed extracts, suggesting that even though seeds are a by-product of fruit processing, they can be a valuable source of bioactive compounds with functional properties. These findings are consistent with the current results, which indicate that papaya seeds, while containing relatively lower anthocyanin concentrations compared to fruit tissues, still exhibit measurable antioxidant potential due to their phenolic composition.

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Table 6. Shows the results of bioactive compound of papaya seed

Total Antioxidant (DPPH) (%)	50.75±1.52
Total Phenolic Content (mg GAE/100 g)	81.96±3.94
Total Anthocyanin Content (mg/L)	1.45±0.43

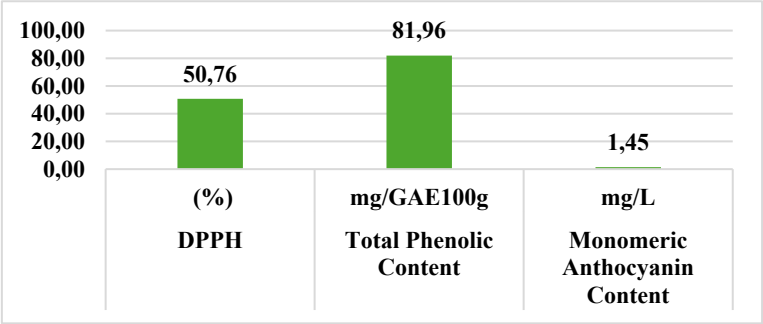

Fig. 7. Contents Antioxidant Capacity (DPPH), Total Phenolic and Anthocyanin of papaya seed.

3.4 Sugars Composition

In the present study, four major sugars were identified in papaya seed extracts glucose, fructose, sucrose, and xylose with glucose and fructose being the predominant components (Table 2; Figure 2). Among these, glucose (1.86%) was the most abundant, followed by fructose (1.68%), sucrose (0.095%), and xylose (0.079%), resulting in a total sugar content of 3.72%. These results align with earlier findings by Marfo et al. (1986), who reported the presence of sucrose (1.77%), xylose (0.77%), glucose (0.11%), and fructose (0.09%) in papaya seeds, confirming that simple sugars constitute an important component of papaya seed composition. Similarly, Maisarah et al. (2013) observed that the sugar concentration in papaya fruits varied significantly among plant parts and maturity stages, with the highest levels typically found in ripe pulp followed by unripe fruit and seeds. Ikram et al. (2015) also reported that the ratio of

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glucose to fructose strongly influences the sensory sweetness and flavor characteristics of papaya. The relatively higher glucose and fructose concentrations observed in the present study may be attributed to both varietal differences (Tainung cultivar) and environmental factors such as temperature and sunlight exposure during fruit development in the Mersin region. Sugars are not only essential for flavor development but also serve as precursors for volatile compound formation through Maillard and fermentation reactions, thus influencing both the aromatic and nutritional quality of the seeds.

Table 7. Shows the results of free sugars in papaya seed samples (% FW)

Sucrose	0.10±0.00
Glucose	1.86±0.00
Xylose	0.08±0.00
Fruktose	1.68±0.01
Total sugar	3.72±0.01

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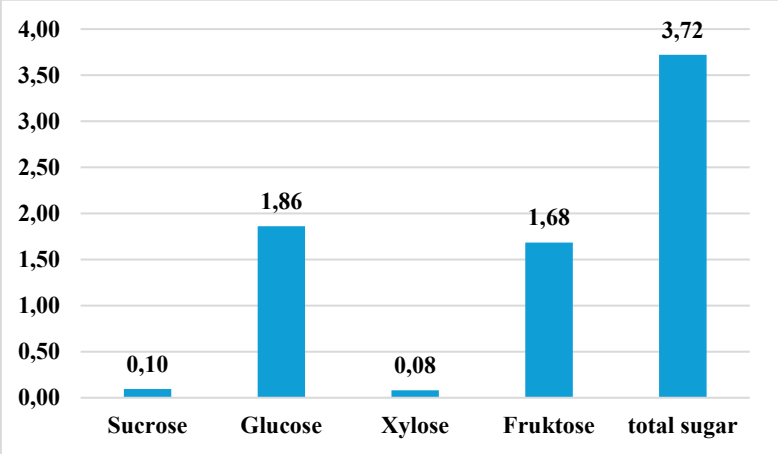

Fig. 8. Individual sugars contents in papaya seed (% FW)

3.5 Volatile Compound Profile

Headspace Solid-Phase Microextraction (HS-SPME) is a widely applied analytical technique for the qualitative and quantitative determination of volatile compounds, including essential oils and aroma constituents, provided that the target analytes are effectively adsorbed onto the extraction fiber (Toci et al., 2012). This technique enables rapid and solvent-free extraction of volatiles from the headspace above the sample onto a stationary phase coated on a fused silica fiber (Roberts et al., 2000). Gas Chromatography (GC), often coupled with Mass Spectrometry (GC-MS), serves as a highly efficient and sensitive analytical platform for profiling plant metabolites and volatile organic compounds (Sgorbini et al., 2006). In the present study, the volatile composition of papaya (*Carica papaya* L.) seeds was analyzed using the gray SPME method. The main classes of volatiles identified were aldehydes (40.04%), alcohols (8.32%), esters (16.61%), ketones (6.28%), acids (19.73%), and terpenes (8.82%) (Table 3; Figure 3). A total of 30 individual volatile components were detected, including 4 alcohols, 3 aldehydes, 8 esters, 6 acids, 1 ketone, and 8 terpenes (Table 3). Among the extraction techniques evaluated, the gray SPME method exhibited the highest efficiency in isolating volatile metabolites. Aldehydes, acids, and esters were found to be the dominant contributors to the volatile profile. Benzaldehyde was identified as the most abundant aldehyde in papaya seeds, accounting for 35.74% of

the total volatile composition. This compound is known for its characteristic almond-like aroma, which plays a key role in defining the overall fragrance profile of plant-derived materials. Beyond its sensory relevance, benzaldehyde serves as an important industrial precursor used in the synthesis of dyes, perfumes, and various pharmaceutical compounds. Its high abundance in papaya seeds suggests a significant contribution to their aroma and may reflect underlying metabolic pathways involved in aromatic aldehyde biosynthesis. Among the esters, hexanoic acid, hexyl ester (5.37%) and 1,2-benzenedicarboxylic acid, diethyl ester (4.98%) was the most prominent. In the terpene group, tetradecane (1.75%) and hexadecane (1.68%) were the major constituents, while 1-hexanol, 2-ethyl- (4.88%) represented the dominant alcohol. The major acids detected were hexanoic acid (5.79%) and acetic acid (4.18%) (Table 3). Hexanoic acid (C6H12O2) is a straight-chain saturated fatty acid that functions as both a plant and human metabolite and serves as the conjugate acid of hexanoate. This medium-chain fatty acid contributes to the characteristic odor profile of plant-derived materials and has broad industrial relevance, being used in the production of food additives, flavoring agents, pharmaceuticals, perfumes, lubricants, and friction modifiers (de Araújo Cavalcante et al., 2017; San-Valero et al., 2020). The diversity and chemical classes of volatile compounds identified in papaya seeds were comparable to those reported for papaya fruit and other tropical species. The present findings are consistent with previous studies demonstrating that the aroma profile of papaya is primarily composed of esters, alcohols, aldehydes, terpenes, ketones, and acids (Flath et al., 1983; Pino et al., 2003; Ayala-Zavala et al., 2004; Yahia et al., 2011). These compound groups play key roles in defining the distinctive fruity, floral, and sweet sensory characteristics associated with papaya and related tropical fruits. Overall, the results of this study confirm that aldehydes and esters are the predominant contributors to the characteristic aroma profile of papaya seeds. Aldehydes, particularly benzaldehyde, impart sweet, nutty, and almond-like notes, whereas esters contribute fruity and floral tones both of which are essential for the sensory quality of tropical fruits (Pino, 2014; Balbontín et al., 2010). The predominance of these volatiles suggests that papaya seeds possess notable aromatic and functional potential, which could be further explored for applications in flavor formulation, natural fragrance development, and nutraceutical product innovation.

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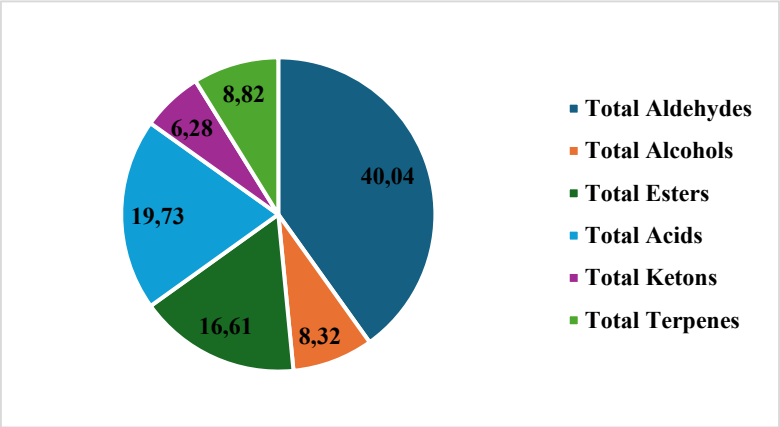

Fig. 9. Volatile composition of papaya seed (%)

Table 8. Volatile Compounds of papaya seed with SPME grey fiber (DVB/CAR/PDMS)

R.time	Compounds Name	Area %
	Aldehydes	
0.967	3-D-4-methyl-2-pentanol	3.35
10.063	Nonanal	0.95
13.206	Benzaldehyde	35.74
	Total	40.04
	Alcohols	
14.367	1-Octanol	0.87
1.558	Ethanol	1.98

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12.718	2-ethyl 1-Hexanol	4.88
21.592	Benzenemethanol	0.59
	Total	8.32
	Esters	
6.182	Hexanoic acid, ethyl ester	1.45
10.679	Butanoıc Acıd, Hexyl Ester	1.62
12.198	Acetic acid, 2-ethylhexyl ester	0.62
15.461	Hexanoic acid, hexyl ester	5.37
18.238	Acetic acid, phenylmethyl ester	0.84
19.045	Benzoic acid, 2-hydroxy- methyl ester	0.88
27.774	Thiocyanic acid, phenylmethyl ester	0.85
30.953	1,2-Benzenedicarboxylic acid, diethyl ester	4.98
	Total	16.61
	Acids	
12.008	Acetic acid	4.18
16.287	Butanoic acid	2.94

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21.157	Hexanoic acid	5.79
27.579	Nonanoic acid	2.65
25.671	Octanoic acid	2.86
32.203	Benzoic acid	1.31
	Total	19.73
	Ketons	
26.710	2(3H)-Furanone, 5-hexyldihydro	6.28
	Total	6.28
	Terpenes	
1.928	Decane	0.98
3.298	Undecane	1.34
5.302	Hendecane	0.76
10.221	Tetradecane	1.75
13.080	Benzene	0.76
15.166	Hexadecane	1.68
18.116	Naphthalene	0.87
20.319	Benzene, 1-methoxy-4-(1-propenyl)	0.68

Total	8.82

4 Conclusion

The present study provides the first comprehensive evaluation of the biochemical composition and volatile profile of papaya (*Carica papaya* L.) seeds cultivated in Türkiye. The findings revealed that papaya seeds possess a considerable amount of total phenolic compounds (81.96 mg GAE/100 g) and moderate antioxidant activity (50.75%), indicating their potential as a natural source of antioxidants. Furthermore, four major sugars glucose, fructose, sucrose, and xylose were identified, with glucose and fructose being the predominant components, contributing to the overall sweetness and potential functional value of the seeds. The volatile compound analysis identified 30 individual components, predominantly aldehydes, esters, and acids, with benzaldehyde, hexanoic acid, and hexanoic acid hexyl ester as the most abundant compounds. These volatiles are likely responsible for the characteristic aroma profile of papaya seeds and may play a significant role in their sensory and functional properties. Overall, the biochemical and volatile profiles obtained in this study suggest that papaya seeds, which are often discarded as by-products, represent a valuable source of natural antioxidants, aromatic compounds, and nutraceutical ingredients. Future studies should focus on exploring the bioavailability, stability, and potential industrial applications of these compounds in food formulation, pharmaceuticals, and cosmetics. Additionally, further comparative analyses among papaya cultivars grown under different agroecological conditions in Türkiye would provide deeper insights into their compositional variability and potential for commercial utilization.

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Determination of Fatty Acid Composition And Olive Oil Quality Parameters of the Gemlik Olive Variety Grown in Adiyaman

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Abstract. Adıyaman province has a climate and soil structure that is extremely favorable for olive cultivation due to its ecological characteristics. The climate of Adıyaman Province is generally characterized by dry and hot summers and mild and rainy winters in the south, and dry and cool summers and rainy and cold winters in the north. For this reason, Adıyaman Province serves as a bridge between the Eastern Anatolia and Mediterranean regions. The average annual rainfall is around 715.1 mm. According to 2024 TÜİK data, the area planted with table olive varieties in Adıyaman is 11,186 decares, while the area planted with oil olive varieties is 31,269 decares. The annual production of table olives in the province is 2,237 tons, while the production of oil olives is 5,101 tons. Adıyaman produces approximately 5% of Türkiye's table olive production and approximately 20% of Türkiye's oil olive production. The dominant olive variety grown for oil in Adıyaman is Gemlik. Olive oils produced in Adıyaman are distinguished from other olive oils by their unique aroma, color, and chemical properties. For this reason, the olive oils produced in the province are candidates for geographical indication as "Adıyaman Olive Oil." In this study, the sensory characteristics, fatty acid composition, and other quality parameters of oils obtained from the Gemlik olive variety grown in Adıyaman province were examined. It was determined that Adıyaman olive oil had a fruitiness score of 4.4±0.2, a bitterness score of 2.6±0.1, and a pungency score of 3.8±0.1, and that there were no sensory characteristics indicating low quality or deterioration in the oil. Additionally, the free fatty acid content of the examined olive oils was found to be 0.49±0.01%, the refractive index was 1.4683, peroxide content of 8.8±0.3 meq/kg, and UV-specific absorbance of K232=1.952, K270=0.1615, and ΔK=0.003. On the other hand, a total of 11 different fatty acids, including oleic acid, palmitic acid, and linoleic acid, were detected in the olive oils. In the olive oils examined, the oleic acid content was found to be 73.81 ± 0.19%, the palmitic acid content was 13.32 ± 0.52%, and the linoleic acid content was 6.41 ± 0.85%.

Keywords: Adıyaman, olive oil, sensory analysis, fatty acid composition, oleic acid

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1 Introduction

The olive is an important plant species, valued both for the oil obtained from its fruits and for the direct consumption of the fruits themselves, and is regarded as a source of healing with positive contributions to human health. In particular, due to its content of health-promoting phytochemicals, the global demand for olives and olive products has been steadily increasing. Worldwide, olives are predominantly cultivated in regions characterized by a Mediterranean climate or in countries along the Mediterranean coast. Owing to its climate and soil structure, Türkiye is one of the countries with ecological superiority and advantage in olive cultivation. Accordingly, Türkiye is recognized as one of the major producers of olives and olive oil worldwide. Based on FAO statistics, the country's total olive production amounted to 1,520,000 tons in 2023 (FAO, 2025). Of this total, 68% was comprised of olives designated for oil production (TUIK, 2024). In 2023, olive production across Türkiye decreased by 51% compared to the previous year. In contrast, table olive exports increased by 14.7% in the same year. The main export destinations were Iraq, Romania, and Germany. On the other hand, 98% of Türkiye's table olive imports originated from Syria. In terms of yield, the average yield per tree was 18 kg in 2022, but this value reduced to 9 kg in 2023. Table olive production is most concentrated in the Aegean Region. Between 2019 and 2023, olive oil production, yield levels, and changes in foreign trade volume in Türkiye were analyzed using graphical data. In 2019, the area cultivated for table olives was 6,450 thousand decares, and by 2024 it had reached 6,698,906 thousand decares. This increase indicates the continued interest in olive cultivation in Türkiye. However, total olive production has shown a fluctuating trend over the years. In table olive production, Türkiye is followed by Spain and Egypt. On a global scale, table olive production has generally remained stable compared to the previous year (Ministry of Agriculture and Forestry, 2024). Türkiye's olive oil imports rose from 26 thousand tons in 2019 to 63 thousand tons in 2023. Nevertheless, the most noteworthy development has occurred in the export sector. In 2023, olive oil exports reached 182 thousand tons, representing nearly a threefold increase compared to previous years (Figure 1). This trend underscores Türkiye's enhanced competitiveness in international markets

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and reflects the strategic orientation of production surpluses toward foreign markets (Özözen, 2024; FAO, 2025; TÜİK, 2024).

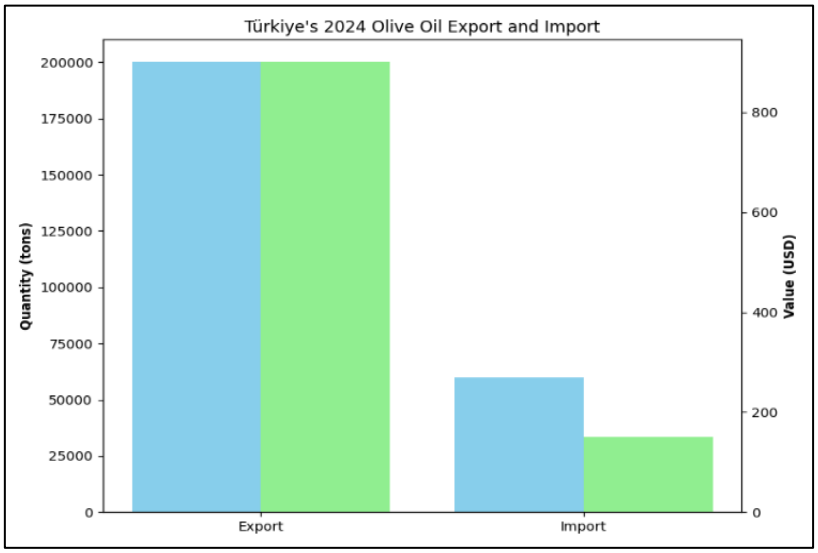

Figure 1. Türkiye's olive oil export and import volumes (FAO, 2025; TÜİK, 2024)

Considering the export markets, policies, and global trends of 2023, it is evident that Türkiye's olive oil sector demonstrated remarkable progress in terms of both production and exports. Due to high yields, a contraction in global supply, and increasing foreign demand, Türkiye has emerged as one of the leading countries in the global olive oil market. In this context, Türkiye's olive oil exports in 2023 amounted to approximately 182 thousand tons. As shown in Figure 2, this amount represents a significant increase compared to previous years. The main reasons behind this rise are estimated to be the 80% increase in olive oil production during the 2022–2023 season, reaching 422 thousand tons, and the global decline in production. In particular, drought and yield losses in traditional producer countries such as Spain and Italy positioned Türkiye as an alternative supplier. In terms of major export markets, Spain accounted for the largest share of Türkiye's olive oil exports with 48.9%, followed by the United States with 13.3%,

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Italy with 7.4%, Saudi Arabia with 3.9%, and Japan with 2.9%. The remaining 23.6% of exports were directed to other countries (Figure 2). This distribution demonstrates that Türkiye has become a strong player not only in European markets but also in American and Asian markets.

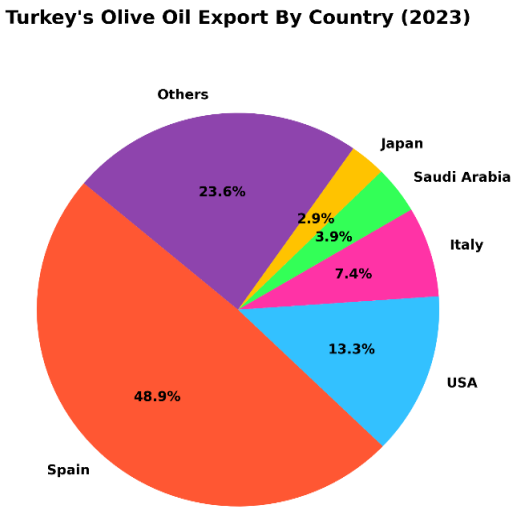

Figure 2. Distribution of Türkiye's olive oil exports by countries in 2023 (FAO, 2025; TÜİK, 2024)

Spain's rationale for purchasing olive oil in bulk from Türkiye can be explained by its re-export strategy, as well as its use of these products in its domestic market. Exports to distant markets like the US and Japan are the most significant indicator of the growing recognition of Turkish olive oil in the global market. A temporary restriction was imposed on bulk and barreled olive oil exports in 2023 to control price increases in the domestic market. It was determined that a three-month restriction was implemented in August of that year, during which time exports of packaged products were prioritized.

Following these restrictions, exports were reopened; however, it was noted that a more controlled export policy was adopted with consideration for domestic market balance. Meanwhile, in 2023, various supports and incentives were provided to olive oil producers by the Ministry of Agriculture and Forestry. These incentives and supports ensured the sustainability of production and played a significant role in

increasing export capacity. Nevertheless, global trends and Türkiye's position indicate that world olive oil production decreased by 12.5% in the 2022/23 season. However, global trends and Türkiye's position led to a 12.5% decline in world olive oil production in the 2022/23 season. This reduction caused a sharp increase in global prices, with olive oil prices rising by 102% in Türkiye, 84% in Spain, and 58% in Italy. Owing to increased production, global supply shortages, and strategic market diversification, Türkiye is expected to strengthen its position in the global olive oil trade. In the coming years, through branding, packaging, and quality-oriented production strategies, Türkiye is anticipated to make its competitive position in olive oil production more sustainable (Ministry of Agriculture and Forestry, 2024; Özözen, 2024; FAO, 2025; TÜİK, 2024). The Southern Aegean Region (especially Aydın, Muğla, and İzmir) stands out as the region with the highest potential for olive oil production in Türkiye by 2025 (Figure 3). This region leads Türkiye in terms of both production volume and quality. In Türkiye, regions such as the Southern Aegean Region (Muğla, Aydın, İzmir), the Marmara Region (Balıkesir, Bursa), the Gulf Region (Çanakkale, Edremit), the Gediz Region (around Manisa), and the Coastal and Eastern Mediterranean Region (Antalya, Mersin, Hatay) are highly suitable for olive cultivation in terms of their climate, soil structure, and traditional production culture. By 2025, the Southern Aegean Region (especially Aydın, Muğla, and İzmir) stands out as the region with the highest potential for olive oil production in Türkiye. This region leads Türkiye in both production volume and quality. While the Aegean and Marmara regions lead the way in this area, olive and olive oil production has also gained significant momentum in Southeastern Anatolia in recent years. This has been achieved thanks to both the emergence of new production areas due to climate change and regional development policies. Furthermore, Southeastern Anatolia's climate, particularly its location within the Mediterranean climate transition zone, allows for the cultivation of many fruit species, including olives, in addition to citrus fruits.

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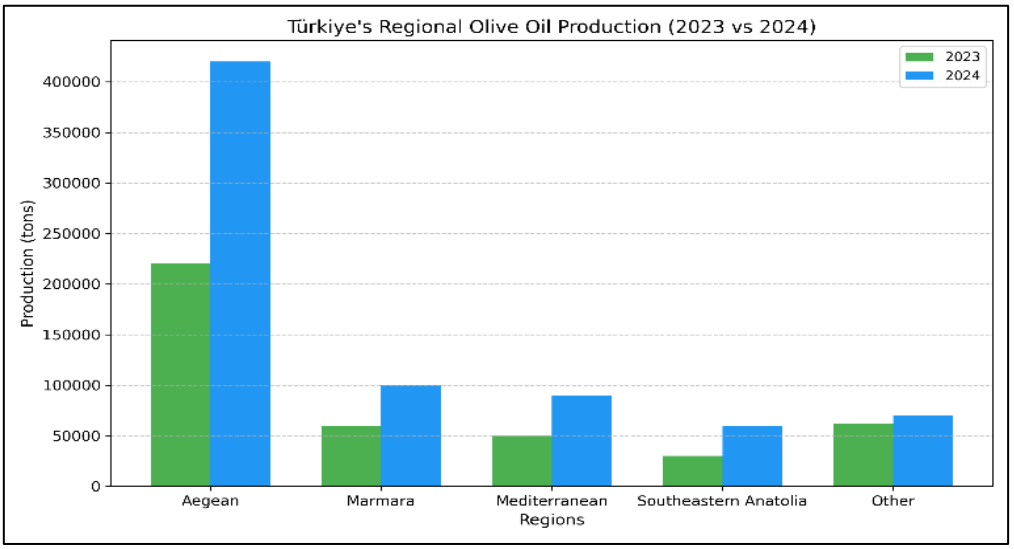

Figure 3. Olive oil production by region in Türkiye (TÜİK, 2024, Ministry of Agriculture and Forestry Reports, 2024)

Olive oil varieties commonly grown in Türkiye are preferred for their high yields and quality in olive oil production. The Ayvalık (Edremit) variety is Türkiye's most well-known olive oil variety. Other prominent olive oil varieties include Memecik, Gemlik, Erkence, and Uslu. Furthermore, the olive varieties most suited to the climate conditions of Southeastern Anatolia are those originating in this region, such as Halhalı (Derik Halhalı), Nizip Yağlık, and Kilis Yağlık (Sakar and Ünver, 2011). Some producers in the region with irrigation facilities and land suitable for mechanized harvesting have also begun to prefer Arbequina, an oil olive variety originating in Spain. The Arbequina olive variety is an oil olive variety whose planting area has rapidly increased worldwide in recent years. This variety is particularly notable for its suitability for super-dense planting systems and its early yield (Atmaca and Ülger, 2017). However, its most significant disadvantage is that, due to its shallow root system, it is not as drought-resistant as native varieties (Gemlik, Halhalı, Nizip Yağlık, and Kilis Yağlık, etc.) (Ay, 2018; Özkul, 2018; Mete et al., 2019; Şahin and Şeker, 2022). Orchards established in the region must be irrigated. Due to the topographic structure of olive growing areas in Adıyaman province and the limited irrigation opportunities, Gemlik is the preferred olive variety for oil production throughout the province.

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Among the provinces leading olive oil production in Türkiye are Aydın, Muğla, İzmir, Balıkesir, and Manisa (Figure 4). In the Southeastern Anatolia Region, the province of Adıyaman has recently shown an increasing trend in olive and olive oil production. According to TÜİK (2024), olive cultivation in Adıyaman covers a total area of 42,455 decares. In terms of cultivated area, Besni district ranks first with 13,950 decares, followed by Kâhta district with 12,520 decares and the Central district with 10,750 decares. The olive cultivation areas in other districts of Adıyaman range between 460 and 1,780 decares. In Adıyaman, olive production decreased from 5,750 tons in 2022 to 4,102 tons in 2023, representing a 29% decline, and then increased to 7,338 tons in 2024, marking a 179% rise. This increase can be attributed both to the annual expansion of areas cultivated with oil olive varieties in the province and to yield fluctuations caused by alternate bearing, which has not been fully mitigated in olive cultivation. Olive oil holds significant economic and cultural importance in Türkiye's agricultural production. According to TÜİK (2024), olive oil production reached approximately 1,040,000 tons, reflecting a remarkable 146.7% increase in line with the growth in olive production.

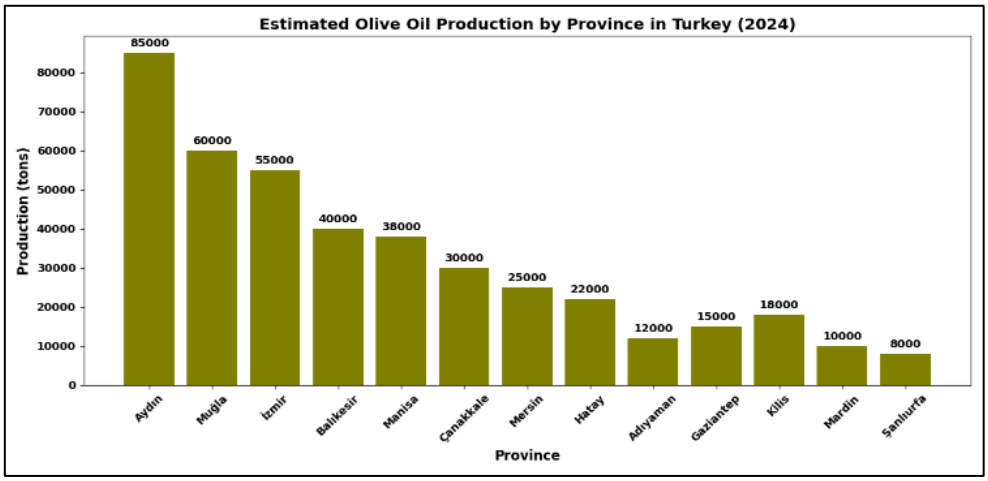

Figure 4. Olive oil production by province in Türkiye (TÜİK, Ministry of Agriculture and Forestry Reports, 2024)

Different analytical methods are employed to determine the quality parameters of olive oil. One such method, sensory analysis, is a scientific approach used to perceive, measure, monitor, and interpret the characteristics of foods as detected through auditory, tactile, olfactory, and visual senses. In chemical analysis methods, the chemical composition of olive oil is examined to obtain information about its quality and purity. The most assessed criteria in these analyses are free fatty acid content, peroxide value, and total phenolic compounds (Özkaya et al., 2010). On the other hand, the fatty acid composition of olive oil is also considered among the evaluated parameters, as it provides significant indicators regarding both the quality of the oil and its storage conditions. From a sensory perspective, extra natural olive oil exhibits a distinctive characteristic profile, attributable to the presence of more than one hundred flavor and aroma compounds. This profile is influenced by numerous factors, including the olive cultivar, ecological conditions, geographical origin, tree nutritional status, seasonal variations, processing techniques, fruit maturity stage, harvest time, storage conditions, and several other determinants. In Adıyaman, a Southeastern Anatolian province, olive groves are rapidly expanding, particularly in the Kahta and Besni districts. Production has increased by 2024, and boutique olive oil production has begun through local cooperatives. However, the lack of modern pressing facilities and limited marketing channels make commercialization of this production difficult. The Nizip district of Gaziantep is known throughout Türkiye for its geographically indicated "Nizip Olive Oil." In the province, olive oil production increased in 2024, serving both domestic consumption and export markets. Although olive oil factories in Nizip contribute to the regional economy, producers are still not sufficiently competitive in the export of packaged and branded products. Kilis Province is distinguished by the “Kilis Oil Olive” cultivar, which is notable for its high oil content and aromatic profile. Olive production in Kilis also increased in 2024. However, cultivation in the province is predominantly carried out by small family farms and cooperatives. Although the quality is high, branding and access to international markets remain limited. In Mardin, olive production increased significantly in 2024, largely associated with the expansion of olive groves in the Derik district. Olive and olive oil production in the province is primarily directed toward domestic consumption. If modern agricultural techniques are more widely adopted, the region has the potential to become a significant production center. In Şanlıurfa, olive production has increased in recent years; however, the production volume still falls

considerably short of its potential. Olive cultivation areas have been expanding in nearly all districts, particularly in Birecik, Bozova, Hilvan, and Halfeti. On the other hand, insufficient irrigation infrastructure and limited producer knowledge remain among the key challenges facing olive cultivation in the province. The sectoral problems of olive cultivation in Southeastern Anatolia can be summarized as follows. The inadequacy of modern pressing and storage facilities reduces the quality and yield of olive oil. The increasing effects of arid climate conditions and the lack of irrigation infrastructure limit olive tree yields. Most of the product is sold in bulk, with almost no packaged or branded products. Producers lack knowledge of modern agricultural techniques, quality control, and organic production. Cooperatives are not sufficiently institutionalized and have limited financial and managerial capacity. To solve these problems, it is necessary to establish at least one modern pressing and storage facility in each province, develop special irrigation projects for olive groves, organize quality, hygiene, organic production and marketing training for producers, provide financial support to cooperatives and increase their managerial capacity, increase the promotion of geographically indicated products, create regional brands, and carry out R&D projects in collaboration with local universities. The Southeastern Anatolia Region has the potential to become one of Türkiye's emerging regions for olive oil production. The provinces of Adıyaman, Gaziantep, Kilis, Mardin, and Şanlıurfa are both climatic and culturally suitable for this type of production. However, transforming this potential into sustainable success requires strategic investments in infrastructure, education, marketing, and institutionalization (Korkmaz and Ak, 2018; Sakar, 2018; Süygün and Can, 2025). Adıyaman is a province that aims to make progress in olive cultivation. It aims to leverage its potential and geographical advantages in marketing olive oil produced by processing olives grown in the province. Indeed, the olive oils produced in Adıyaman possess qualities that will attract both regional and national demand. In this respect, it is a candidate for a geographical indication. This study, conducted to determine the quality characteristics of Adıyaman olive oil, explored the sensory and chemical properties of olive oils obtained from the Gemlik olive variety grown in Adıyaman province.

2 Material and Method

This study used olive oil samples obtained through cold pressing from the Gemlik olive variety, a widely cultivated olive oil variety used in the production of table olives and olive oil in Adıyaman province. Two 1-liter olive oil samples were collected from three different olive oil processing plants in the province, each stored in lacquered cans. One liter of each olive oil sample was transported to the Adıyaman University Central Laboratory for chemical analysis. The remaining one liter was sent to the İzmir Olive Research Institute for sensory analysis.

2.1 Sensorial Analysis

The olive oil samples for sensory evaluation were presented to the panelists in appropriate standard tasting glasses. Approximately 12.8–14.6 g (14–16 ml) of olive oil samples were placed in the tasting glass, covered with a watch glass, and allowed to stand. The olive oil samples were then placed on a heater set at 28ºC ± 2ºC and heated. Because morning hours are the best time to taste olive oil, tasting sessions took place between 10:00 and 12:00 in the morning. The tasting laboratory was air-conditioned to maintain a constant temperature and good hygrometric conditions. Before the tasting analysis, the room temperature was set at a constant 20–25ºC. Tasting analyses were conducted with a minimum of eight tasters, and the results were then statistically evaluated and tabulated by the panel leader (Arucu, 2013).

2.2 Chemical Analysis

For the determination of free fatty acids in the olive oils studied, 5 g oil samples were taken and dissolved by adding 50 ml of a 1:1 ethyl alcohol-diethyl ether mixture. A few drops of phenolphthalein were added to the prepared samples and titrated with a 0.1 N ethyl alcohol-potassium hydroxide (KOH) solution until a permanent pink color was obtained. The results were expressed as oleic acid (%). (AOCS, 2017).

For peroxide determination, 10 ml of chloroform was added to 2 g of oil sample, mixed thoroughly, and the oil sample was dissolved. 15 ml of glacial acetic acid (CH_3COOH) and 1 ml of saturated potassium iodide (KI) solution were added sequentially. The samples were homogenized for 1 min and then kept in the dark for 5 min. 75 ml of distilled water was added to the samples, followed by a few drops of 1% starch solution, and a titration was performed with 0.01 N sodium thiosulfate ($Na_2S_2O_3$) solution. The analysis results were expressed as milliequivalents/gram (meq) of active oxygen per kg of oil (AOCS, 2017). The refractive indices of the olive oil samples were determined according to Nas et al. (2001), and the results are presented for 20 °C.

The specific absorbances of the olive oils examined within the scope of the study were measured at 232 nm and 270 nm wavelengths with a spectrophotometer (Shimadzu UV-1205) and were obtained by calculating the absorbance at a concentration of 1 g/100 ml (IOOC, 2001). ΔK values were then calculated (Çelik et al., 2021).

2.3 Determination of Fatty Acid Composition

For the determination of fatty acid composition of the oliveoils, fatty acid methyl esters were prepared (Bligh et al., 1959). Briefly, 0.1 g of oliveoil was mixed with 10 mL of n-hexan and 0.5 mL KOH (0.2 N, in methanol). Then, the ensemble was centrifugated at 500 rpm for 10 min and hold at least two hours in dark place. Then, the clear upper phase was passed through the 0.45 µm PTFE filter and transferred to the vials (Tanacı, 2015). A GC-2010 Plus gas chromatograph (Shimadzu Corporation, Kyoto, Japan) equipped with flame ionization detector (FID) and a TR-CN100 (Teknokroma, Barcelona, Spain) capillary column (100 m x 0.25 mm x 0.20 µm) was used for analyses. Hydrogen was used as the carrier gas and the flow rate was controlled at 30 ml min^{-1} . The detector temperature was kept at 260 °C and the injection amount of the sample was 2.0 µL. The initial temperature procedure was 140 °C (6 min), then raised to 200 °C at 3 °C per min, and reached at 240 °C for 20 min. The peaks were identified with Supelco 37 component FAME mix (Sigma–Aldrich). The results were expressed as percentage (Bligh et al., 1959; Tanacı, 2015).

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3 Results and Discussion

The results of the sensory and chemical analyses obtained from the examined olive oil samples are presented in Table 1. According to the findings, the unique characteristics of the olive oil obtained from the Gemlik olive variety grown in Adıyaman are noteworthy. Generally, the fatty acid composition of olive oil is most affected by environmental conditions. Due to this ecological advantage of Adıyaman province, Adıyaman olive oil is particularly characterized by its low free fatty acidity, balanced aroma, desirable sensory properties, and bright color. In sensory tests, Adıyaman olive oil was scored as 4.4±0.2 for fruitiness (green), 2.6±0.1 for bitterness, and 3.8±0.1 for pungency. Furthermore, considering the scores of other sensory quality parameters examined (heat, moldiness, vinegaryness, etc.), it is understood that the handling and storage conditions of the olives before production, the pressing conditions, and the storage and storage conditions after production were all adequate.

Studies have shown that aromas do not influence the fruitiness of olive oils, while biophenols influence bitterness and pungency. The Memecik variety has a higher pungency value, while the Gemlik variety has lower bitterness and pungency than other varieties. Furthermore, it has been reported that malaxation temperature is insignificant on phenol content in Gemlik olive oils at different temperatures and holding times. However, it was found that malaxation temperature was significant at 30 minutes, and malaxation temperature was not significant on fruitiness. However, a 60-minute holding time was significant, and neither temperature nor duration of malaxation were significant on bitterness.

Table 1. Some Sensory Properties of Adıyaman Olive Oil

Sensory Properties	
Fruitiness	4.4±0.2 - Green
Bitterness	2.6±0.1

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Pungency-Burning Sensation	3.8±0.1
Fusty-Muddy Sediment	0
Musty-Humid-Earthy	0
Winey-Vinegary-Acid-Sour	0
Wet Wood	0
Rancid-Stale	0
Other	0

It has been determined that both the temperature and the duration of malaxation are not significant for the pungency value in sensory analysis (Nebioğlu, 2020). The quality of olive oil is significantly influenced by geographical factors such as proximity to the sea or inland areas, topography, latitude, longitude, and climatic conditions including precipitation and winds. Therefore, differences in olive oil quality are observed among regions. In Türkiye, olive varieties grown in the Southeastern Anatolia region have a high oil content in the fruit. This increases both the quality and the commercial value of the olive oil produced in the region. Climatic factors have a strong impact on the ripening time of olives and the chemical composition of the oil in the fruit. Consequently, they considerably affect the natural antioxidants, phenols, tocopherols, and the oxidative stability of the oil (Özdoğan and Tunalıoğlu, 2017). In olive oil samples obtained from the Gemlik variety, no defects such as fusty/muddy sediment, musty/humid, winey-vinegary, metallic, or rancid were perceived by the panelists. In the study, the fruitiness value of Gemlik olive oil samples was determined by the panelists to range between 2.90 and 4.02. According to these values, it was concluded that the oil obtained from the Gemlik variety is classified as extra natural olive oil based on the criteria of the Turkish Food Codex (Zeytin et al., 2008).

Considering all studies, when compared with the sensory analysis results of Adıyaman olive oil, it is evident that Adıyaman olive oil possesses superior qualities according to the Turkish Food Codex criteria. Furthermore, the chemical properties of Adıyaman olive oil were also found to meet the standards required for extra natural olive oils. Indeed, the average values obtained from the analyzed oils were as follows: free fatty acidity 0.49 ± 0.01%, refractive index 1.4683, peroxide content 8.8 ± 0.3 meq/kg, specific UV absorbance K232 = 1.952, K270 = 0.1615, and ΔK = 0.003 (Table 2).

Table 2. Some Chemical Properties of Adıyaman Olive Oil

Chemical Properties	
Free Fatty Acid (%)	0.49±0.01
Refractive Index	1.4683
Peroxide Value (meq/kg)	8.8±0.3
Specific Absorbance in UV	K232=1.952
	K270=0.1615
	ΔK=0.003

Table 3 presents the fatty acid composition of Adıyaman olive oil. Accordingly, 11 different fatty acids were identified in the olive oils. Previous studies by various researchers have determined that olive oils may contain more than 60 fatty acids. However, our study examined only 37 fatty acids, and 11 of these were identified in the olive oils. The fatty acids we identified in the olive oils are as follows: Palmitic acid (C16:0), Palmitoleic acid (C16:1), Heptadecanoic acid (C17:0), Stearic acid (C18:0), Oleic acid

(C18:1n9c), Linoleic acid (C18:2n6c), Arachidic acid (C20:0), Eicosenoic acid (C20:1n9), Gamma-linolenic acid (C18:3n3), Behenic acid (C22:0) and Lignoceric acid (C24:0).

Among the fatty acids detected in olive oils, the most abundant fatty acid was oleic acid with a percentage of 73.81 ± 0.19%. This was followed by palmitic acid (13.32 ± 0.52%), linoleic acid (6.41 ± 0.85%), stearic acid (3.30 ± 0.05%), and palmitoleic acid (1.40 ± 0.28%). On the other hand, the percentages of other fatty acids detected in the oils were quite low. Indeed, arachidonic acid was determined as 0.46 ± 0.01%, eicosenoic acid as 0.26 ± 0.03%, gamma-linolenic acid as 0.69 ± 0.02%, behenic acid as 0.12 ± 0.00%, and lignoceric acid as 0.09 ± 0.01%.

Table 3. Fatty Acid Composition of Adıyaman Olive Oil

Fatty Acids	Content in Oil (%)
Palmitic acid (C16:0)	13.32 ± 0.52
Palmitoleic acid (C16:1)	1.40 ± 0.28
Heptadecanoic acid (C17:0)	0.15 ± 0.03
Stearic acid (C18:0)	3.30 ± 0.05
Oleic acid (C18:1n9c)	73.81 ± 0.19
Linoleic acid (C18:2n6c)	6.41 ± 0.85
Arachidic acid (C20:0)	0.46 ± 0.01
Eicosenoic acid (C20:1n9)	0.26 ± 0.03

Gamma-linolenic acid (C18:3n3)	0.69 ± 0.02
Behenic acid (C22:0)	0.12 ± 0.00
Lignoceric acid (C24:0)	0.09 ± 0.01

Studies have shown that the most important characteristic used in characterizing olive oils is their fatty acid composition, and that olive oils are characterized by their high oleic acid content (Erinç and Kıralan, 2008). A study on the fatty acid composition of the Gemlik olive variety found that the oleic acid content in their fatty acid composition ranged from 62.12% to 63.89%. It was also reported that this fatty acid was followed by palmitic acid (14.51-14.83%), linoleic acid (11.46-12.56%), stearic acid (3.78-4.65%), and linolenic acid (0.11-0.15%). In a study titled "Obtainment of New Olive Varieties by Hybridization" at the Atatürk Horticulture Central Research Institute, the composition of fatty acids in the oils of GE122, GE123 and GE124 types obtained by hybridizing Gemlik and Edincik Su varieties was investigated. Of these olive types that can be registered for oil production, it has been reported that there is no difference in linoleic acid content between GE123 and GE124, but the linoleic acid content of GE122 differs from that of other candidate varieties. On the other hand, when the candidate varieties were compared according to maturity index, it was determined that the oleic acid content ranged from 74.63% to 77.57% at the 3rd maturity index, while the oleic acid content ranged from 71.20% to 73.34% at the 5th maturity index (Özdemir et al., 2016; Didin et al., 2021). In another study conducted on the determination of the quality characteristics and aroma composition fatty acid composition of Gemlik olive variety fruit grown in Adana, which can be processed as both table olive and oil, it was determined that oleic acid had the highest proportion with 66.1% among the fatty acids, followed by palmitic acid and linoleic acid, respectively. In addition, 46 aroma compounds were identified in the fruit of Gemlik olive variety and their total amount was found to be 2681.29 µg/kg. It was stated that the groups with the highest number and amount of compounds were higher alcohols, volatile acids, aldehydes, ketones and volatile phenols. While the aroma compound group found at the highest rate was higher alcohols

(44.61%), the most dominant compounds in the composition were determined to be 4-hydroxy-4-methyl-2-pentanone, 2-butoxyethanol and (E,E)-α-farnesene, respectively (Koyuncu and Cabaroğlu, 2020). In the light of the given literature information; It was observed that the findings obtained from our study were parallel to the findings of previous researchers.

4 Conclusion

Sensory analyses indicate that Adıyaman olive oil's fruitiness (4.4±0.2), bitterness (2.6±0.1), and pungency (3.8±0.1) scores indicate high quality in accordance with the Turkish Food Codex criteria. No negative sensory characteristics (moldy, damp, winey, etc.) were observed in the olive oils examined. Chemical analyses indicate that the olive oil's free fatty acidity (0.49%), peroxide value (8.8 meq/kg), and UV absorbance values (K232=1.952; K270=0.1615; ΔK=0.003) meet the quality standards. The fatty acid composition has the desired balanced distribution in olive oils. Indeed, when the fatty acids in olive oils are ranked proportionally, they are oleic acid (73.81%), palmitic acid (13.32%), and linoleic acid (6.41%). This composition presents a positive profile in terms of olive oil's high nutritional value and oxidative stability. Furthermore, the lower oleic acid content in the fatty acid composition of one of the olive oil production facilities compared to the other facilities suggests that the olive oils from this facility are subject to some storage problems.

Adıyaman's ecological conditions (climate, geographic structure, distance from the sea, etc.) positively affect the quality of the oils obtained from olives grown in the province. The characteristics of olive oil obtained from Gemlik olives, unique to the Adıyaman region, are valuable for establishing a regional brand. Therefore, it is necessary to support local producers and accelerate geographical indication studies. To increase fruitiness, a malaxation time of 60 minutes is recommended in pressing facilities. Integrating this time into production processes can improve the quality of olive oil production. Furthermore, training programs should be organized at regular intervals to enable panelists to take a more active role in sensory analyses in sensory training programs, thus making quality control processes more reliable. Considering the fatty acid composition, the high oleic acid content once again demonstrates that olive oil is a highly valuable food for health. Regular monitoring of the fatty acid

composition during the production process is recommended. Adıyaman's climatic and geographical advantages directly affect olive oil quality. Therefore, it is important that agricultural activities in the region be carried out in accordance with sustainability principles. In conclusion, this study; This study is significant as it is the first to identify the characteristics of Adıyaman olive oil and promote it to a wider audience. The findings will also inform future studies aimed at determining olive oil quality parameters.

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Effects of Some Natural Organic Additives *in Vitro* Plant Propagation

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Abstract. Tissue culture is a technique that enables the rapid and large-scale multiplication of plants using any parts of them under aseptic conditions. Its success depends on various factors, including the plant species and cultivar, type and age of the explant, culture conditions, and the composition of the culture medium. Determination of specific nutritional requirements of plants can be challenging in tissue culture studies which makes the optimization of culture media components essential. However, the high cost of some medium components has driven the search for alternative, low-cost organic additives that do not compromise plantlet quality. Numerous studies have investigated the effects of organic growth additives on plant development. Commonly used complex organic additives are plant extracts such as coconut water (CW), banana extracts, and a variety of fruit juices; casein hydrolysate (CH), yeast extract (YE) etc. These additives provide natural carbon sources and are rich in vitamins, phenolic compounds, fiber, hormones, proteins, lipids, and minerals. Previous studies have demonstrated that modification of medium composition with natural complex additives can induce cell division, stimulate callus formation, and support both rooting and shoot development. Optimum concentration of these additives is crucial for promoting plant or cell growth and development. This review will focus on the effects of supplementing culture media with coconut water, banana homogenate, and casein hydrolysate as organic additives on *in vitro* plant growth and development. Understanding the effects of these natural additives may prove valuable for plant species that have not yet been investigated in this respect, offering a foundation for future research.

Keywords: tissue culture, organic additives, *in vitro* propagation

1 Introduction

Plant tissue culture is a biotechnological technique that enables the propagation of plants from any explant tissue containing meristematic cells, cultivated under aseptic conditions on a nutrient culture

medium. This approach offers wide-ranging applications, including: (1) the large-scale clonal plantlet propagation (micropropagation); (2) the production of disease-free plants through meristem culture; (3) the genetic conservation of endangered and rare species; (4) crop improvement via somaclonal variation and mutation breeding; (5) valuable secondary metabolites production for pharmaceutical and industrial purposes; (6) the creation and regeneration of genetically modified plants through genetic transformation techniques; (7) the generation of haploid and dihaploid plants via anther and microspore culture; (8) somatic hybridization through protoplast fusion; (9) long-term germplasm preservation using cryopreservation methods; and (10) virus elimination to produce pathogen-free planting material [1]. This technique also offers an effective application for meeting demand for planting material and afforestation programs to tackle impacts of global climate change and human activities on agriculture-forest-livestock systems [2]. Success of plant tissue culture studies depends on varying factors including plant species and even genotype; types of explants; age and health of the donor plant; physical culture conditions such as temperature, humidity and light; culture media composition etc. Since this technique can be genotype-specific depending on the species used, optimization of the culture medium composition is essential for successful large-scale plant propagation [3].

The culture medium typically consists of macronutrients and micronutrients essential for plant growth, vitamins and minerals that support metabolic functions, amino acids that promote cell division and differentiation, sugars serving as energy sources, gelling agents to solidify the medium, and plant growth regulators (PGRs) that govern morphogenesis and organ development [4]. PGRs are crucial components of the culture medium. They have various effects on plant growth and development depending on their types and concentration. Since plant growth regulators (PGRs) can be effective even in small amounts, determining their optimum concentrations will be beneficial for large-scale micropropagation studies, for enhancing secondary metabolite production in pharmaceutical applications. Although tissue culture techniques have many applications as mentioned above, components of culture medium are expensive. So, it is required for the development of inexpensive options for low-cost tissue culture technology. For this purpose, many studies have been conducted to determine the effect of cost-effective organic additive plant extracts on plant growth and development, ensuring that the quality of the produced plants is not

compromised [5]. A variety of complex organic additives are used as supplements in plant tissue culture media, such as natural extracts (coconut water, corn extract, potato extract banana extract), fruit juices (tomato, orange, papaya), protein hydrolysates (casein hydrolysate, peptone, yeast extract) which provide essential nutrients and natural growth regulators to enhance in vitro growth and morphogenesis [6] These additives contain natural carbon sources and are rich in vitamins, phenolic compounds, fiber, hormones, proteins, lipids, and minerals [7].

This review will examine the effects of organic additives such as coconut water (CW), banana homogenate, and casein hydrolysate on in vitro plant growth and development. Understanding their effects could contribute to future research by providing a scientific basis for plant species that have not yet been investigated in this respect.

2 Some Organic Growth Additives to enhance *in vitro* Culture Techniques

Culture media generally contains basal components (including a carbon and energy source, inorganic salts, vitamins, PGRs) and optional (organic nitrogen compounds, organic acids, and kind of complex natural extracts) components. Optional components can be supplemented into medium depending on purpose of study to enhance *in vitro* plant growth and development. Moreover, types of organic nutrient and their concentrations are critical depending on specific needs of species or tissues and even genotype [4,8].

2.1 Effect of Coconut Water (CW) as an Organic Additive on in vitro Plant Growth and Development

Coconut water is called "Fluid of Life" since it is rich in amino acids, nutrients, minerals, essential electrolytes, vitamins and phytohormones and is low in sugars and calories [9]. It acts as plant hormone sources (auxin and cytokinin), containing compounds such as indole-3-acetic acid (IAA), kinetin, and zeatin which enhance cell division in the roots and shoot systems, adventitious root development, and micropropagation [10].

Many studies have been conducted using coconut water as an organic additive into culture medium to enhance seed germination, shoot multiplication and elongation, and root induction. Although orchids produce numerous seeds, their small size and lack of nutritional reserves result in a low rate of natural reproduction. Furthermore, the seeds of some species have hard seed coats, which can make germination difficult. The addition of coconut water to the nutrient medium showed a markedly positive effect on in vitro seed germination, protocorm formation, and seedling development of Cypripedium macranthos Sw., compared with birch sap, maple sap, banana powder, and peptone [11].There are many studies conducted using endangered and endemic epiphyte orchid species on addition of coconut water on medium and resulted with higher seed germination and protocorm proliferation [12 13; 14] . De Stefano et al. (2022) observed similar result on night scented orchid seeds germination, two times more than control medium [5]. Being a natural carbon source, coconut water has also been determined to facilitate seed germination due to containing amino acids, vitamins, minerals and various organic ions [15].

Peixe et al. (2007) reported that coconut water and BAP can replace zeatin for olive micropropagation [16]. Rate of shoot multiplication enhanced with addition of coconut water to medium, resulting in a cost-effective medium formulation. Study on Asparagus officinalis micropropagation, 20% (v/v) coconut water became the most effective organic additive for shoot induction and root formation in the in vitro plantlets. Moreover, the longest shoot and root lengths were also observed on the same medium. [17]. Conversely, some studies have shown that higher concentrations of CW delayed the development of purple coneflower [18], inhibited shoot elongation, and reduced the shoot length of Dragon Fruit trees [19]. Therefore, there is a need to determine optimal concentrations for different plant species, standardize its use, and further investigate its mechanisms of action. Such studies would facilitate the broader integration of coconut water into plant biotechnology applications.

2.2 Effect of Banana Homogenate (BH) as an Organic Additive on *in vitro* Plant Growth and Development

Bananas are one of the most commonly consumed fruits due to their low cost and high nutrient content containing carbohydrates, proteins, a variety of minerals (Na, Fe, K, Ca, Mg, Ma, Zn) and vitamins (A,

B1, B2, B3, B6, pantothenic acid, folic acid and ascorbic acid) [20, 21]. It contains natural auxins and gibberellins, which act as plant growth regulators in vitro studies. It has been used as an organic additive and energy sources for in vitro previous studies to support the plant growth and development especially for heterotrophic plants during the early stages of in vitro cultivation [22].

There are various studies in literature conducted using banana homogenate as an organic additive in medium resulting in enhanced seed germination [23]; protocorm formation and regeneration [24]; improved shoot and root elongation supported shoot length and root growth [25]. Gansau et al. (2016) investigated effect of different types of organic additives (coconut water, tomato juice, banana pulp and peptone) on protocorm proliferation and development for Dendrobium lowii. The highest growth index was obtained from 25 g/L banana pulp treatment, where 100% of the protocorms developed into shoots [26]. Islam et al. (2015) found that effect of different concentration of banana homogenate varied on the Dendrobium sp. var. Sonia protocorm in terms of PLBs multiplication and plantlet regeneration. While 1/2 MS medium supplemented with 100 ml L-1 of banana homogenate was the best for protocorm like body multiplication, shoot and root regeneration was observed from 25 ml L-1 BH treatment [24]. It was considered that excessive BH may inhibit cell growth and development due to high concentration of sugars or calcium or sodium. Lee et al. (2022) also reported that increasing the amount of BH in the medium led to similar effects, inhibiting the growth and development of Ficus carica cv. Japanese BTM 6 [27].

The highest number of shoots and leaves, as well as the greatest increase in shoot height, was observed in plants cultured on medium supplemented with 10 g/L BH among the four treatments tested. Banana homogenate is one of the most common organic additives in vitro studies. But efficiency of organic additives varies species worked on or even genotype and concentration of additives. The determination of the optimum and efficient concentration of banana homogenate incorporated into the culture medium may contribute to supported plant growth and development and a reduced input cost resulted from plant growth regulators.

2.3 Effect of Casein Hydrolysate (CH) as an Organic Additive on *in vitro* Plant Growth and Development

Casein hydrolysate (CH) is an organic compound containing low molecular weight proteins, amino acids, vitamins, calcium, phosphate, which enhances plant growth by providing a source of reduced nitrogen [28]. Additionally, plant cells can efficiently metabolize and utilize nitrogen from organic sources compared to inorganic sources, highlighting CH as an effective source of nitrogen [29].

Several researches have conducted to show how CH effected the distinct plant species in terms of somatic embryogenesis [30], callus induction [28,31] seed germination and seedling growth [32], shoot regeneration and proliferation [33,34]. Ramakrishnan et al. (2013) found that frequency of callus induction and embryogenic callus formation in Allium cepa L. enhanced with addition of glycine, proline, and casein hydrolysate [31]. Effect of different organic additive also tested were on Barhi Date Palm (Phoenix dactylifera L.) for somatic embryo formation and shoot regeneration. It is determined that casein hydrolysate addition (5.0 g/L and 2.5 g/L) induced secondary somatic embryo formation and enhanced the plantlet regeneration, respectively. However, yeast extraction addition found that not effective as well as coconut milk (30%) and CH (2.5g/l) in term of readings in all assessed concentrations and plantlet regeneration [30]. A study work on okra Abelmoschus esculentus genotype (CoBhH1) reported by Daniel et al. (2018) that CH addition into MS medium supplemented with different type of auxins (2,4-D, NAA) and L-glutamine induced the somatic embryogenesis and improved new plantlet formation from cotyledonary leaf explants. Also, regenerated plants examined by ISSR analysis were determined as morphologically similar to the parent plant [35]. According to Al-Asadi et al. (2024), combination of CH and dicamba (DIC) enhanced the callus development (4.0 mg/L (DIC) + 1.0 g/L CH), shoot proliferation (4.0 mg/L DIC + 0.5 g/L CH), and biochemical properties of the Barhee date variety [36]. In similar study, Amer et al. (2017) searched on two Egyptian rice cultivars to determine the effect of different concentrations of tryptophan, glutamine, and casein hydrolysate separately. They found that addition of CH encouraged in both callus induction (300 mg/L for both cultivars) and shoot regeneration (100 and 200 mg/L) [28]. There are also various studies conducted to determine effect of casein alone or combined with different types of auxin and cytokines in terms of shoot induction and

multiplication. Samiei et al. (2021) stated that CH addition (600 mg/l) into media containing a constant amount of (BAP) and (NAA) promoted shoot proliferation (173%) whereas silver nitrate (100 mg/l) resulted in the formation of the longest (2.5 cm length) and highest quality shoots on in vitro propagation of Rosa canina. [33]. In similar study, Georgieva et al. (2025) reported that addition of CH into medium enriched with BAP and IBA combination increased the shoot length and multiplication of black raspberry cv. Cumberland [34]. Moreover, it is reported that BAP (2.5 mg/L) combined with 600 mg/L CH resulted in the highest root length on Red Barangan banana [37]. As for concerning seed germination and seedling growth, Borbolla-Pérez et al. (2024) established an in vitro asymbiotic germination protocol for Vanilla planifolia seeds due to their low germination rate. They tested different types of medium, different concentrations of zeatin and CH, and their combinations. They observed the highest viability and vigorous seedling growth (74.5%) with 1/4 MS supplemented with 500 mg/L casein hydrolysate, whereas the combination of zeatin with casein hydrolysate (CH = 500 mg/L; zeatin = 0.50 mg/L) resulted in 53% callus formation [32]. However, some researchers found that high amount of casein hydrolysate (200–500 mg/l) usage resulted in shoot tip necrosis and vitrification [35], as well as growth retardation [38,39] in some plant species which means that of casein hydrolyte addition into medium varies to species, even genotype and purpose of study. That's why more studies should be conducted to determine optimum concentration of casein hydrolysate used for plant growth and development each plant species.

3 Conclusions

Natural organic additives contain carbohydrates, proteins, a variety of minerals and vitamins, essential electrolytes and phytohormones for plant growth and development. Various studies have conducted using different kind of organic additives improved callus induction, somatic embryogenesis, enhanced shoot length and proliferation and root formation. Including those organic compounds into plant tissue culture studies could be sustainable and cost-efficient approach. But it is requisite to determine the optimum concentration of organic additives in the medium, depending on species, genotype, type of

explant, and plant species requirements, to standardize their application in tissue culture protocols and to conduct further studies to better understand how they influence cellular and developmental processes.

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I-CRAFT AGRICULTURAL and FOOD TECHNOLOGIES

Frost and Drought Events in Çukurova's Agricultural Production: Impacts and Strategic Recommendations for Future Resilience

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Abstract. All agricultural activities are highly dependent on climatic conditions. Various adverse consequences of climate change—such as shifts in precipitation regimes, rising temperatures, meteorological and hydrological droughts, and an increased frequency of natural disasters—can significantly affect agricultural productivity, growth rates, and food security. Frost and drought events, which are among the most critical risks to agricultural production, represent natural hazards with economic, social, and environmental implications. These events occur when temperatures fall below or rise above the tolerance thresholds of crops, increasingly as a result of climate change. In the 2025 growing season, farmers in Türkiye, as in many other parts of the world, were confronted with a range of extreme weather and climate phenomena. This paper presents recommendations for measures and practices that can be adopted to reduce the economic impacts of future frost and drought events during agricultural production periods.

Keywords: Cukurova, Drought, Agricultural Frost, Agriculture, Climate Change.

1 Introduction

All agricultural activities are largely dependent on climatic conditions. Various adverse consequences of climate change including changes in precipitation regimes, rising temperatures, meteorological and hydrological droughts, and natural disasters—can exert significant negative impacts on agricultural productivity, growth rates, and food security.

Among the most critical risks in agricultural production are frost and drought events. These are natural phenomena with economic, social, and environmental repercussions, typically triggered when

temperatures fall below or rise above crop tolerance thresholds as a result of climate change. Unlike frost, drought events are also strongly influenced by the absence of precipitation and the reduction of irrigation water availability, in addition to temperature anomalies [1]. This study outlines recommendations for measures and management practices aimed at mitigating the economic impacts of future frost and drought events that may occur during agricultural production periods.

Cukurova is one of Türkiye's most important agricultural production centers, representing approximately 5% of the nation's total arable and cultivable land and hosting one of the most fertile plains in the World [2]. The region contributes nearly 10% of the country's total agricultural production value and supports a wide diversity of crops. However, this diversity requires different agronomic strategies due to varying crop temperature requirements and frost tolerance levels, making agricultural production management in the region complex. In the 2025 growing season, the region experienced reduced precipitation and extreme temperature fluctuations, leading to widespread drought and frost events across Cukurova.

As a result of drought, yields of major field crops such as cereals and oilseed sunflower were severely reduced. Concurrently, frost events caused extensive damage to citrus orchards (particularly lemon and orange), as well as apricot, cherry, and peach/nectarine orchards, leading not only to lower yields but also to the removal of some severely damaged orchards.

Consistent with IPCC AR6 WGII, climate change is already negatively affecting agricultural productivity in low- to mid-latitudes via heat and water-stress interactions and increasing variability. Regional strategies proposed here (phenology-aware frost protection, drought-smart irrigation, cultivar choice) align with adaptation pathways recommended for agrifood systems [13].

For annual crops such as cereals and oilseed sunflower, yield losses are confined to the affected production season; assuming no recurrence of extreme drought or frost in subsequent years, economic impacts are short-term. In contrast, perennial fruit trees are more vulnerable: frost damage often leads

to reduced yields for several following years, and the re-establishment of uprooted orchards delays their contribution to the agricultural economy.

2 Frost and Drought

2.1 Agricultural Frost and Frost Events in Türkiye

Agricultural frost refers to a natural event that occurs when air temperature falls below or approaches 0 °C, reaching levels that can damage crops [3]. It becomes a critical meteorological hazard when it causes injury to plants in agricultural fields following a frost episode. Frost events are typically classified according to temperature: light frost between 0 °C and −2.2 °C, moderate frost between −2.2 °C and −4.4 °C, and severe frost below −4.4 °C. When nighttime temperatures drop below freezing, the leaves, fruits, and buds of plants can suffer serious injury, reducing crop yields and causing economic losses for farmers.

Agricultural frost can harm many crop species, with fruit trees, vegetables, and flowering plants among the most affected. Key factors that contribute to frost damage include sub-zero air temperatures, humid conditions (which exacerbate damage at low temperatures), and wind (which accelerates temperature drops). The impacts of agricultural frost manifest through yield losses, increased production costs, and negative consequences for economic sustainability. Harvest reduction, yield decline, plant stress, and ultimately economic losses typically follow frost episodes. Moreover, frost events are not limited to a single production season; they can disrupt regional economies by affecting multiple crops simultaneously.

During the 2025 production year, farmers across Türkiye encountered a variety of extreme weather and climate events. Drought, floods, hailstorms, excessive rainfall, and cold spells affected agricultural production and farmer incomes at different times of the season. This complex risk landscape places considerable pressure on both producers and consumers in the agri-food sector, which is already struggling to manage seasonal and long-term uncertainties. Recent frost events have highlighted the critical role of climate in maintaining the continuity of agricultural production in Türkiye.

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Agricultural frost events are most frequent in Eastern Anatolia, Central Anatolia, and the highlands of the Black Sea region, where winter temperatures can drop to −20 °C. Such events typically occur during autumn (October–November), winter (December–February), and spring (March–April). Sudden temperature drops in spring can also cause frost damage in both highland and lowland areas.

Although frost events occur at different times and locations each year, during the 2025 production season Türkiye experienced three major and widespread frost episodes in February, March, and April. While the February and March events were relatively limited in impact, the severe frost between April 10 and 13 affected crops nationwide.

In April, temperatures fell below freezing in many regions, and initial assessments indicated that at least 36 provinces and a wide variety of crops were affected. Key damage included apricot, cherry, and apple orchards in Central Anatolia; vineyards in the Aegean and Thrace; hazelnut in the Black Sea region; citrus (especially lemon) and watermelon in Çukurova; and almond and quince orchards in Eastern Anatolia.

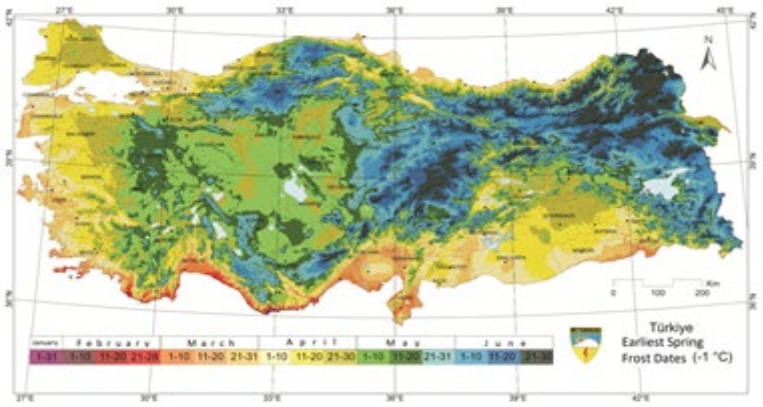

Fig. 10. Map of the latest frost dates in Türkiye (−1 °C)

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2.2 Agricultural Frost in Cukurova

In Cukurova and Adana, severe agricultural frost events that occurred in February, March, and April of 2025 caused significant damage to agricultural crops. The frost episodes seriously affected watermelon, lemon, and other citrus crops, leading to leaf and flower burn, followed by defoliation and flower drop. Citrus trees—particularly lemon trees—shed their leaves and flowers extensively. Observations indicated that, after pruning, some trees attempted to re-sprout leaves and initiate flowering again.

However, it became clear that these trees would either fail to produce fruit during the current season or produce only limited yields. In some areas, yield reductions are also expected in the following production year due to the cumulative physiological stress caused by the frost events.

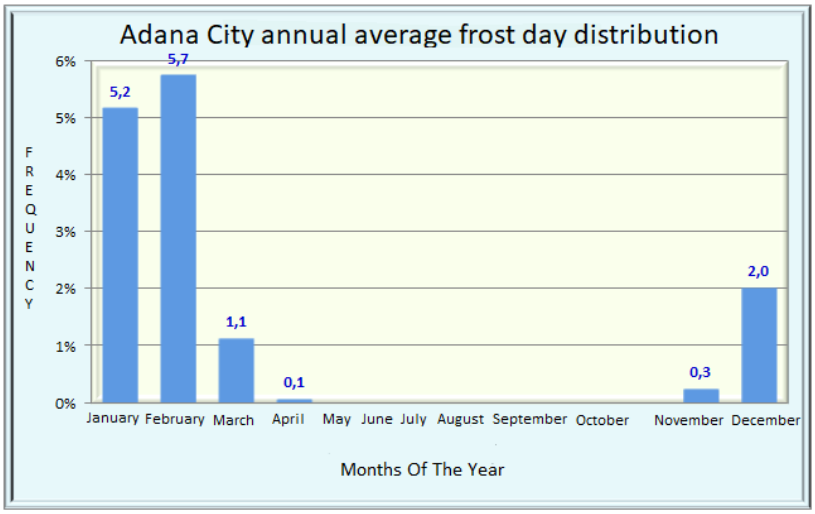

Fig. 11. Annual average frost distribution in Adana province.

Although April exhibits the lowest frost frequency in Adana, the risk to agriculture remains high because many perennial crops are at their most sensitive phenophases (full bloom/fruit set). With warming winters reducing accumulated winter chill, irregular flowering and asynchronous budbreak can further heighten vulnerability. This underscores the need for regional frost-risk calendars linked to phenology rather than climatology alone [11]. As can be seen from the graph, April has the lowest frequency of

frost events in Adana Province considered the heart of the fertile Cukurova region, which encompasses approximately 1.6 million hectares of total agricultural land with an occurrence rate of only 0.1%. Despite this, agricultural areas in Adana, where around 40,000 families rely on farming for their livelihoods, were severely affected by the agricultural frost event that occurred in April. This episode may mark the beginning of difficult times for both the national and household economies. In the coming years, it will be crucial to enhance economic productivity by conducting research and development to breed frost-resistant crop varieties and by developing technological solutions for frost mitigation.

Recent evidence shows that warming advances spring phenology (budburst/flowering) without a commensurate advancement of the last spring frost, thereby increasing exposure to late-spring frost—especially in fruit trees and vineyards. This phenological mismatch has been documented across Europe and East Asia and is relevant for Cukurova's citrus, stone fruit, and grape systems where early flowering is common under warm winters. Strategic responses include selecting later-blooming cultivars, managing rest break with chill-aware models, and adjusting pruning to delay phenophases in high-risk years [7, 8, 9].

Critical damage temperatures for citrus indicate that lemon buds/blossoms are injured near −2.8 °C, small lemon fruit near −1.4 to −0.8 °C, and green oranges near −1.9 to −1.4 °C. These thresholds help set action temperatures for protection. Among active methods, under-tree microsprinklers can protect young trees during radiation frosts by releasing latent heat as ice forms; however, their effectiveness declines in advective (windy) freezes, and fruit protection is limited. System layout (nozzle placement on the N–NW side; adequate discharge) and real-time decision tools improve outcomes. For mixed-age orchards, combining microsprinklers with wind machines or heaters is recommended where inversions are strong [10].

2.3 Agricultural Drought and Drought in Türkiye

Drought can be broadly defined as a natural climatic phenomenon that arises from temporary imbalances in atmospheric moisture, leading to water scarcity in a given region. It can occur in virtually any climate

zone from arid to humid regions although areas with arid climates are generally more vulnerable due to chronic moisture deficits and highly variable precipitation patterns [4]. Among extreme events, drought typically develops slowly, persists for long periods, and is one of the least predictable atmospheric hazards, while its impacts can be extensive.

Drought should be characterized using complementary indices that capture atmospheric demand as well as precipitation. The Standardized Precipitation Index (SPI) quantifies precipitation anomalies across time scales and is widely used operationally; the Standardized Precipitation-Evapotranspiration Index (SPEI) extends SPI by incorporating potential evapotranspiration, making it sensitive to warming-driven increases in atmospheric water demand. Utilizing SPI/SPEI concurrently enables discrimination of meteorological, agricultural, and hydrological drought linkages in Cukurova's irrigation-dependent systems [12].

Drought should not be viewed merely as a physical or natural phenomenon. Because human societies and economic activities are highly dependent on water resources, drought has wide-ranging social, environmental, and economic consequences. Prolonged dry conditions reduce atmospheric and soil moisture, deplete vegetation, forests, and water resources, and ultimately give rise to serious environmental, economic, and social challenges.

Drought is essentially a law of nature: when atmospheric moisture availability declines temporarily, it causes water shortages that negatively affect water resources, agriculture, and all living organisms. It is a slow-onset natural disaster, but its impacts are profound and far-reaching [5].

As illustrated in the map below, large parts of Türkiye are currently experiencing severe drought conditions. With the exception of parts of the Black Sea, Marmara, and Aegean regions, drought has been observed across most of the country.

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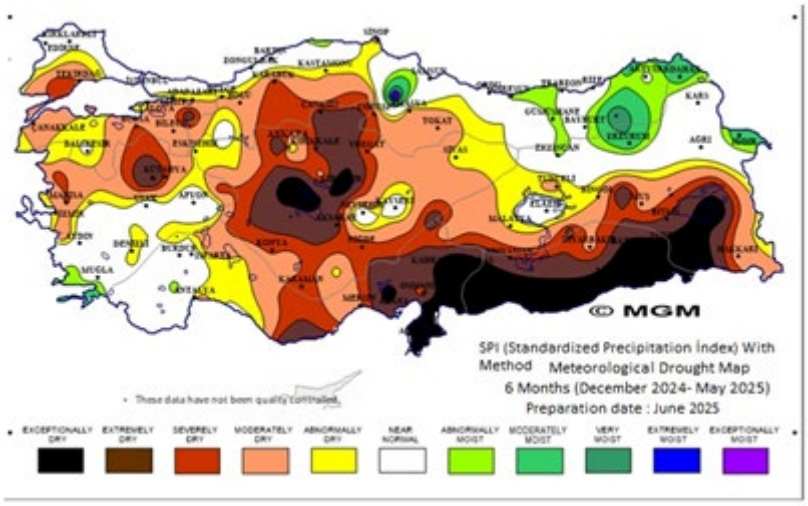

Fig. 12. Six-month drought map of Türkiye (December 2024 – May 2025)

The six-month national drought assessment by the Turkish State Meteorological Service (MGM) for December 2024–May 2025 depicts extensive areas under severe to extreme drought classifications, consistent with the observed rainfall deficits in Cukurova. Embedding SPI/SPEI maps from MGM into the regional analysis would strengthen attribution of yield losses to combined precipitation shortfalls and high evaporative demand.

2.4 Agricultural Drought in Cukurova

In recent years, drought has caused severe yield losses in many agricultural crops across Türkiye. Reservoir water levels have dropped below critical thresholds, raising serious concerns about the country's future water security. If this trend continues and no effective measures are taken, Türkiye risks becoming a water-scarce country in the near future.

For example, according to meteorological data from the TİGEM Cukurova Agricultural Enterprise weather station, total precipitation between October 2024 and June 2025 was recorded at 226 mm, compared to the ten-year average of approximately 551 mm [6]. This clearly indicates the severity of the drought experienced in the Cukurova region. Similarly, the average wheat yield at TİGEM Cukurova

over the past decade has been 455 kg per decare, but in 2025 yields dropped dramatically to 180 kg per decare.

Due to these conditions, approximately 10,000 decares of cereal fields in Cukurova were abandoned for the 2025 production season because they were not suitable for harvesting. The same situation applies to sunflower fields in rainfed areas, where significant yield losses are expected.

Given the TİGEM station totals (226 mm vs. ~551 mm 10-yr mean, Oct 2024–Jun 2025), anomalies of this magnitude would typically correspond to “severe–extreme” SPI classes at seasonal scales in many Mediterranean sites, implying substantial soil-moisture deficits during critical growth stages. To quantify expected yield penalties and evaluate mitigation, AquaCrop can be used to simulate yield response to water and to compare rainfed, supplemental, and deficit-irrigation scenarios for cereals and oilseed crops in Cukurova conditions.

3 Conclusions

For field crops, frost events can result in severe yield reductions or even total crop failure within the affected production year. In orchards, frost can cause tissue damage, shoot dieback, and abnormal sprouting, leading to further yield reductions over the following one to two years.

The agricultural impacts of severe frost events should be systematically assessed through “Evaluation and Mitigation of Agricultural Frost Impacts” consultation and coordination meetings to develop appropriate planning and response strategies. Additionally, climate change is causing earlier flowering in fruit trees, increasing their vulnerability to late frost damage. Therefore, cultivar and species selection, as well as orchard establishment planning, should be adjusted accordingly.

Although rising temperatures associated with climate change may initially lead to earlier harvest dates, inadequate winter chilling can later cause harvest delays and yield declines. When early ripening coincides with drought stress, agricultural losses may become severe, resulting in substantial economic impacts.

To address drought, comprehensive plans must be developed for the sustainable management of existing water resources. Excessive water consumption should be avoided, and modern pressurized irrigation systems that provide more uniform application while consuming less water should be promoted.

Society must also learn to use water resources more consciously. Drought and water scarcity policies should be strengthened, and farmers must be educated on efficient water use practices.

Moreover, national frost and drought maps should be updated to reflect changing climatic conditions, and regional cropping patterns should be revised accordingly. Farmers need to be supported and guided to adapt to these new cropping systems. This is not only a national necessity but a global one. For the Turkic world and beyond, paying close attention to these issues is essential for advancing agricultural production and sustaining agriculture-based economies.

Adaptation should prioritize pressurized irrigation with precise scheduling and consider regulated/deficit irrigation where agronomically safe. Evidence shows that well-designed deficit irrigation regimes can maintain economic yields while markedly improving water productivity, especially in perennial systems; in annuals, AquaCrop-guided scheduling helps target most sensitive growth stages to avoid disproportionate penalties.

Declaration of Competing Interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported.

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Kefir As a Living Food: Fermentation, Function, and Health

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Abstract. Milk kefir and water kefir are the two main varieties of kefir, a fermented beverage recognized for its probiotic qualities and health advantages. People in the mountainous area between Europe and Asia have been consuming kefir milk for thousands of years. The process of making milk kefir involves fermenting milk (often from cows, goats, or sheep) with milk kefir grains, which are symbiotic cultures of yeasts and lactic acid bacteria. A tangy, creamy beverage full of vitamins, probiotics, and bioactive components is the end product. By fermenting sugar water or fruit juice with water kefir grains, which have a distinct microbial population suited to a non-dairy environment, water kefir, on the other hand, is a dairy-free substitute. In addition to providing probiotic advantages, this results in a mildly carbonated, sweet-tart beverage that is appropriate for vegetarians and others who are lactose intolerant. Both varieties of kefir support intestinal health. These grains are inhabited by a varied symbiotic ecosystem of yeasts, lactic acid bacteria, acetic acid bacteria, and other microorganisms. In kefir grains, the most prevalent bacteria are lactic acid bacterial taxa, such as Lactobacillus, Lactococcus, Leuconostoc, and Streptococcus. These grains also frequently contain yeast species such as Candida, Saccharomyces, Kluyveromyces, Kazachstania, and Pichia. Kefir drinks have been linked to several significant health benefits, including as improved lactose digestion, anti-carcinogenic, anti-hypertensive, and anti-diabetic properties, among others. In addition, kefir supports intestinal health by keeping the intestinal flora in balance. All of these health benefits are brought about by the kefir bacteria, their interactions, and the metabolic products they produce throughout the fermentation process. Thus, this review aims to provide information on fermentation, function, and health benefits of kefir.

Keywords: Kefir, Fermentation, Health benefits.

1 Introduction

Kefir has health-promoting properties, making it one of the most popular functional foods. According to [1] kefir is a fermented carbonated beverage with a low alcohol content and an acidic character that is produced by the proto cooperation of bacteria and yeasts that settle in a substrate matrix with milk or water. Water and milk kefirs are generated by usage of distinct gelatinous particles that harbor probiotic microorganisms, referred to as water kefir grains and milk kefir grains. These two beverages, fermented

from these grains, exhibit differing physical, chemical, and microbiological properties [2]. Kefir is categorized as a natural probiotic beverage by FAO (Food and Agriculture Organization) and WHO (the World Health Organization) because of its microbial composition, which includes multiple lactic acid bacteria (LAB) species with known health benefits. The primary microorganisms found in kefir are typically yeasts, acetic acid bacteria, and LAB, despite their varying microbiological compositions [3]. The type of milk used, fermentation circumstances, geographical origin of the grains, and processing techniques are some of the variables that affect the microbial diversity and composition of kefir grains [4].

Lactic acid bacterial taxa, including Lactobacillus, Lactococcus, Leuconostoc, and Streptococcus, are the most common bacteria found in kefir grains. Furthermore, Kluyveromyces, Kazachstania, Candida, Saccharomyces, and Pichia are frequent yeast species that are present in these grains [5]. Notably, local production methods and the unique properties of the fermentation substrate can have a substantial effect on the predominance of these microorganisms. Metabolites such as ethanol, carbon dioxide, organic acids, and bioactive peptides that are created during the fermentation of kefir are essential in determining the drink's sensory and functional characteristics. Kefir's pH is lowered by organic acids like lactic and acetic acid, which also have antibacterial properties that prevent the growth of infections. The drink's unique texture and effervescent quality are caused by carbon dioxide and ethanol, byproducts of the yeast's metabolic activities. Bacteria break down milk proteins to produce bioactive peptides, which have antibacterial, immunomodulatory, and antioxidant properties and may be able to control the body's inflammatory response [6]. In addition, antimicrobial, antimutagenic, anticancer, antioxidant, immune system-stimulating, cholesterol-lowering, and anti-apoptotic properties are just a few of the many health advantages of kefir [7]. Thus, this review aims to provide uptodate information on fermentation, function, and health benefits of kefir.

1.1 Fermentation

Due to the growing interest in healthy nutrition and its numerous benefits, fermentation, a method of food preservation that has been utilized for centuries, has recently drawn more attention. Due to its cheap

energy cost and capacity to maintain and improve the product's qualities, this metabolic process is priceless [8]. It is well recognized that this technique may improve food items' sensory qualities, add functional qualities, and increase their nutritional value. Food items' nutritional content, acceptability, and sensory qualities can all be enhanced by the safe process of fermentation. A common method for creating fermented foods and food additives is microbial fermentation. Nowadays, a variety of additional products are made using microbial fermentation in addition to fermented meals like cheese, wine, and beer [9].

Traditionally, milk kefir and water kefir are made from distinct gelatinous particles called "milk kefir grains" and "water kefir grains" that contain probiotics. The two unique fermented drinks made from these grains have different microbiological compositions, as well as diverse physical and chemical properties. Both milk kefir and water kefir possess functional characteristics. Water kefir can be a highly essential source of probiotics, prebiotics, and antioxidants for vegans and those who are allergic to or intolerant to dairy products, even as milk kefir offers substantial quantities of protein along with these nutrients. Because of their possible health advantages, both of these grains are significant [2].

Fig. 13. Characteristics of milk and water kefir grains [2]

Table 9. Comparison of milk and water kefir grains (adapted by [10] and [2]).

Water kefir	Milk Kefir
-Made with water kefir grains	-Prepared with milk kefir grains

-Uses a sucrose solution enriched with dried fruits or fruit extracts as the medium	-The main medium is milk derived from bovine animals (such as cow or goat)
-Able to ferment a broader range of substrates	-Capable of fermenting a more limited variety of substrates
-Grains appear translucent, gelatinous, and comparatively fragile	-Grains are opaque, usually white or cream, and comparatively tougher
-The main exopolysaccharide of the grains is α-glucan	-The key exopolysaccharide in the grains is kefiran
-Strains of acetic acid bacteria occur more frequently	-Acetic acid–producing bacteria occur less frequently
-Saccharomyces species are the predominant yeasts	-Saccharomyces yeasts represent only a small fraction of the microbiota
-Lactococcus bacteria are scarcely detected	-Lactococcus species are generally more prevalent
-Candida yeasts are rarely encountered	-Candida yeasts tend to be found more often
-Appropriate for people who follow a vegan diet or are lactose intolerant	-Unsuitable for people with lactose intolerance or those following a vegan diet

Inoculating milk with kefir grains is the standard process for making milk kefir. Kefir grains consist of lactic acid bacteria (LAB), acetic acid bacteria, yeasts, and the matrix of protein and polysaccharide arising from their metabolic processes. The fermentation process of kefir is significantly impacted by the wide variety of bacterial species found in kefir grains. The most frequently co-occurring genus

include Lactobacillus, Lactococcus, Leuconostoc, Acetobacter, Bifidobacterium, Streptococcus, Enterobacter, and Acinetobacter. In addition, the most frequently co-occurring yeasts genera are Kazachstania, Saccharomyces, Kluyveromyces, Dipodascaceae, and Dekkera. Whole, semi-skimmed, or skimmed pasteurized milk from goats, cows, camels, sheep, or buffalo can be used to make kefir. The most popular is kefir made from cow's milk. As a starting culture, the kefir grains can be introduced to the fermentation substrate. Following fermentation, the grains are filtered out of the fermented milk. Following grain separation, kefir can be consumed right once or stored in the refrigerator for later use. Alcoholic fermentation causes CO2, ethanol, and vitamin B complex to build up during the cooling phase. Because of this maturing stage, the product has less lactose, which makes it suitable for ingestion by those with diabetes and lactose intolerance. Kefir's nutritional composition varies greatly and is affected by the kind of milk, the origin of the grains used, the fermentation period and temperature, and storage conditions [11].

Water kefir, often known as aqua kefir or sugary kefir, is created from several special probiotics that contain water kefir grains, which are gelatinous grains. Water kefir grains consist of a polysaccharide matrix (mainly dextran and to a lesser extent levan) in which microorganisms are embedded. Grains include lactic acid bacteria, acetic acid bacteria, yeasts and occasionally bifidobacteria [12].

The comparisons of milk kefir and water kefir were made comprehensively by [2] and [10] (Table 1). It was reported that water kefir and milk kefir are different symbiotic systems. First, each grain is made up of a different matrix of polysaccharides: the heteropolysaccharide glucogalactan, called kefiran, which is made by Lb. kefiranofaciens, and the homopolysaccharide α1,6-glucan, which is made by Lactobacillus hilgardii in the case of water kefir grains. Second, even though milk kefir grains may grow in non-dairy substrates, the disaccharide that bacteria digest is different; lactose for milk kefir and sucrose for water kefir [13]. Milk kefir grains can occasionally be produced in plant-based "milk," but they need milk or whey-based medium. Water kefir grains need solutions made of vegetables, fruits, or cereals that provide enough fermentable fructose or sucrose [14]. Thirdly, the species present in the two types of grains are different. Table 1 shows the comparision of water kefir and milk kefir. Guzel-Seydim et al. [2] reported that the microbiological composition, chemical characteristics, and even color of milk

kefir grains and water kefir grains differ significantly, with milk kefir having a greater nutritious value than water kefir.

There are wide variations in the microbial diversity of kefir reported in the literature. Lynch et al. [10] indicated that bacteria such as lactic acid bacteria (Lactococcus, Lactobacillus, Bifidobacteria, Leuconostoc, Oenococcus and Pediococcus), acetic acid bacteria (Gluconobacter, Gluconacetobacter and Acetobacter), and other bacteria (Zymomonas) have been found in water kefir grains. They also have yeast such as Kluyveromyces, Saccharomyces, Hanseniaspora, Dekkera, Torulaspora, Kazachstania, Zygotorulaspora, Pichia, and Yarrowia. Ouyang et al. [15] reported that water kefir grains from Lincang in China were primarily were dominated by members of Acetobacter, Phenylobacterium, Lactobacillus, and Sediminibacterium and fungi that were members of Issatchenkia, Kodamaea and Saccharomyces.

While milk kefir grain contains around 65% to 80% lactobacilli, 5–25% lactococci and Leuconostoc spp., and 10–15% yeasts, milk kefir itself has a microbial makeup of roughly 80% lactococci and Leuconostoc spp., 10%–15% yeasts, and 5–10% lactobacilli [16]. Lacticaseibacillus paracasei, Lactobacillus kefiranofaciens, Lactobacillus acidophilus, Lactiplantibacillus plantarum, and Lactobacillus delbrueckii subsp. bulgaricus are the primary bacterial species present in kefir grains [1]. In both milk and water kefir, lactic acid bacteria execute a lactic fermentation, which increases the fermented beverage's acidity and viscosity; acetic acid bacteria give fermented milk a more pronounced sour flavor; and yeast ethanol and CO2 enhance the product's flavor. The kefir microbes have probiotic potential because they can produce antagonistic substances like organic acids and bacteriocins, which prevent pathogenic bacteria from adhering to the intestinal mucosa and improve gut health [1]. They also show high resistance to the low pH and bile salts in the gastrointestinal tract.

1.2 Health Benefits of Kefir

The gut microbiota is a virtual organ system that is crucial for preserving health and wellbeing. The varied microbiota and the large range of bioactive chemicals created during fermentation are responsible

for these effects. Figure 1 shows a schematic layout of the possible positive impacts of kefir on human physiology and health.

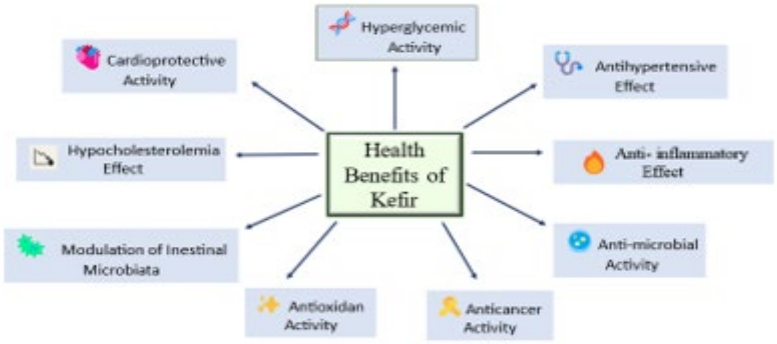

Fig. 14. A schematic layout of the possible positive impacts of kefir on human health.

Kefir-derived LAB has been demonstrated to have positive effects on intestinal microbiota (improved digestion and gut health), inflammation, type 2 diabetes, anti-carcinogenic effects, cholesterol levels, cardiovascular diseases, hypertension and immunomodulatory activities [11, 17, 18, 19, 20]. Furthermore, recent research has shown encouraging outcomes regarding antiviral effects, notably in the instance of COVID-19 [21]. But in order to produce a consistent end product, time requires the creation of a standard kefir production procedure.

2 Conclusion

Because of their high probiotic content, milk kefir and water kefir are both beneficial fermented drinks with many health advantages. Water kefir is a great dairy-free substitute that is appropriate for people who are lactose intolerant or on a vegan diet, even though milk kefir offers a more nutrient-dense choice with greater amounts of protein, calcium, and vitamins. Both promote digestive health, strengthen the

immune system, and improve general well-being despite variations in their microbial compositions and substrates.

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Nutritional Benefits and Health Risks of Seafood Consumption

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Abstract. Around the world, seafood especially fish is an important part of people's diets because of the presence of both macronutrients (proteins, lipids and ash) and micronutrients (vitamins and minerals). These are key nutrients that support general health and wellbeing. Numerous marine species, including fish, shellfish, and crustaceans, are distinguished by its particular nutritional characteristics. Many nutritionists and health experts support consuming more seafood because of its high-quality protein, low fats, and essential micronutrients like vitamin D, iodine, and selenium. The polyunsaturated omega-3 fatty acids found in seafood, especially EPA and DHA, are necessary for brain development, cardiovascular health, and inflammation reduction. In addition, their regular intake has been shown to help with weight control, cognitive development in children, and lowering the risk of high blood pressure, inflammatory and neurodegenerative diseases. Despite its benefits, seafood consumption also poses certain risks. Environmental contamination introduces hazardous compounds such as heavy metals, pesticides, polycyclic aromatic hydrocarbons (PAHs), and microplastics into marine ecosystems. These pollutants can accumulate in seafood and potentially threaten human health. This review discusses the benefits and risks of eating seafood and the main points to consider when choosing and consuming it.

Keywords: Seafood, Nutrition, Health Benefits, Health Risks.

1 Introduction

Concerns about nutrition may arise as the world's population grows, and fish is a significant source of animal protein. The rise globally in consumption of fish shows that the health benefits of its consumption are well-established both scientifically and nutritionally [1,2]. It also means that fisheries and aquaculture will continue to play a crucial role in meeting the global population's demand for animal protein, with aquaculture being the dominant supplier. However, in terms of their share of the total animal protein supply, Norway (22.6%), Portugal (20.6%), and Spain (18.0%) were the top three

European countries that consumed fish and seafood, while Turkey (surprisingly) had the lowest consumption rate (3.2%, despite being the EU's largest producer of marine finfish aquaculture). [3].

Atherosclerosis, diabetes mellitus, stroke, coronary heart disease, and several kinds of cancer are among the causes of mortality that are unmistakably linked to dietary habits and lifestyle choices [4, 5]. With regard to dietary habits, a proper balance of nutrient intake is essential for maintaining health and avoiding lifestyle-related diseases. Eating seafood offers a variety of nutrients, including protein, vitamins, minerals, and the polyunsaturated long chain omega-3 fatty acids EPA (eicosapentanoic acid) and DHA (docosahexaenoic acid). In general, seafood is high in protein and unsaturated fats, low in calories, low in saturated fats and cholesterol and rich in minerals. Nutrients especially DHA and EPA, vitamin D, vitamin B12, iron (Fe), calcium (Ca), zinc (Zn), phosphorus (P), selenium (Se), fluorine (F), iodine (I) are found in fish and other seafood and are linked to a number of positive health impacts [6].

Seafood, particularly fish, have more free amino acids, non-saturated fatty acids, less connective tissues, and higher enzyme activities than other muscle products [6]. Thus, seafoods are perishable products, resulting in quality deterioration, such as lipid oxidation, protein degradation, and changes in fish taste, texture, and odor [7, 8]. These factors lead to seafood having a limited shelf life, which lowers customer acceptance. Thus, these products need to be processed and preserved properly to preserve their quality and safety. Moreover, it can also pose health risks if contaminated seafood with harmful substances like heavy metals, bacteria, viruses, or toxins [9]. Consuming contaminated seafood may lead to foodborne illnesses, allergic reactions, or long-term health problems. This review discusses the nutritional benefits and potential risks of seafood consumption, along with key considerations for its selection and safe consumption.Subsequent paragraphs, however, are indented.

1.1 The Chemical Composition of Fish

Fish proximate composition is crucial for understanding their nutritional profile and serves as an indicator for evaluating their quality, physiological state, and nutritional status [10]. A considerable amount of work has been carried out about the chemical analyses of fish species such as protein, fat,

moisture, and ash levels, which is important from a variety of perspectives for producers, customers, and scientists. Such a research helps to better understand physiological condition of fish, nutritional value as well as how to process and preserve it. Fish is mostly composed of 66%–81% moisture, 16%–21% protein, 1.2%–1.5% mineral, 0.2%–25% fat, and 0%–0.5% carbohydrate [11]. In general, carbohydrates and non-protein compounds are usually ignored during analysis, since they make up a little portion of wet mass, usually thought to be insignificant (<0.5%) [12]. Various factors, including eating behaviors, food composition, rate of feeding, sex, age, size, habitats, genetics, season, and migration, may influence the chemical composition of fish species [13].

1.2 Proteins

The protein levels of raw finfish flesh range from 17% to 22%. Fish proteins have a relatively high nutritional value due to their advantageous essential amino acid composition. All of the necessary amino acids, especially methionine and lysine, are abundant in fish proteins [14]. It contains less fat than red meat and offers easily digestible protein with high biological value that is essential for the body's growth and development, for the maintenance and repair of damaged tissues, and for the synthesis of hormones and enzymes needed for many body functions. Three different types of proteins are usually found in fish muscle: 30% to 35% of the total protein level is made up of sarcoplasmic or enzymatic proteins (globulin, albumin, and enzymes); 60% to 65% is made up of myofibrillar or contractile proteins (actin, tropomyosin, myosin, and actomyosin); and 3% to 5% is made up of stroma or connective tissue proteins (collagen) [15]. Fish muscles are more digestible than other animal proteins because they include lower quantities of connective tissue. Fish muscle also typically contains 10–40% more non-protein nitrogen than that of terrestrial animals. This nitrogen content includes amino acids, small peptides, trimethylamine oxide (TMAO), trimethylamine, creatine, creatinine, and nucleotides [16].

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1.3 Lipid

Fat level of fish ranges from 0.2 to 25%. Marine fish are commonly classified according to the fat content of their fillets and grouped as lean (<2% fat), low fat (2%–4% fat), medium (4–8% fat), and high fat (> 8% fat) [17]. Lean fish, such as sole, typically have whitish flesh, whereas species with moderate fat content (e.g., cod, haddock, halibut, and pollock) display white to off-white flesh. In contrast, high-fat fish, including herring, sardine, anchovy, and salmon, generally is pigmented such as yellow, pink, or greyish [14]. Fish fat content varies greatly among species and is affected by a number of biotic and abiotic parameters, including season, water temperature, sex, location in body, pH, age salinity, reproductive cycle, type and quantity of food available [18, 10].

Seafood contain polyunsaturated fatty acids (PUFAs), especially EPA and DHA, which are essential for proper growth of children and also reduce the occurrence of cardiovascular disease [19]. It has been found that the concentration of EPA and DHA fatty acids is generally higher in case of marine fishes [20]. Fats also contribute to energy supply and help with adequate absorption of vitamins A, D, E, and K [21].

Many people are interested in omega-3 long-chain polyunsaturated fatty acids (PUFAs), or n-3 LC-PUFAs, because of their distinct structure and biological functions. Omega-3 fatty acids are essential and need to be taken from diet. Fatty acids such as alpha-linolenic acid (ALA) (18:3n-3) and linoleic acid (LA) (18:2n-6) are known as essential fatty acids since these substances cannot be generated by humans [22]. Due to their plant-based synthesis, LA and ALA are mostly present in high concentrations in plant-based foods. For instance, LA is abundant in a variety of seeds, nuts, and plant oils [23]. For the synthesis of additional PUFAs, such as arachidonic acid (AA) (C20:4, n-6), docosahexaenoic acid (DHA) (C22:6, n-3), and eicosapentaenoic acid (EPA) (C20:5, n-3), the essential fatty acids LA and ALA are required.

Long-chain omega-3 PUFAs are produced by a sequence of enzymatic processes that are part of the metabolic route for the production of omega-3 PUFAs from ALA to DHA (Figure 1). It is ALA that initiates this metabolic process. An essential function of the enzymes desaturase and elongase is to

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convert dietary fatty acids into EPA-DHA. Enzymes that are desaturase help add double bonds to the metabolic pathway, whereas those that are elongase help add carbon atoms. The conversion of ALA to stearidonic acid is aided by the extra double bond that desaturase enzymes contribute. To create eicosatetraenoic acid (20:4n-3), elongase enzymes then add two carbon atoms to stearidonic acid. Extended EPA produces docosapentaenoic acid (DPA, 22:5n-3), whereas desaturation of DPA produces DHA (22:6n-3) [24]. The human body can only convert ALA to DHA to a very little extent, and the conversion rate from EPA to DHA is very low less than 1% [5]. For the early development of the brain and eyes, DHA is essential [25]. The plant omega-3 polyunsaturated fatty acid ALA's primary functions include regulating the conversion of LA to AA and serving as a substrate for the production of EPA [23]. The main sources of EPA and DHA are marine fatty fish species such as mackerel, herring, anchovy, sardines [26]. Additionally, fortified foods are thought to be a rich source of omega-3 fatty acids [27].

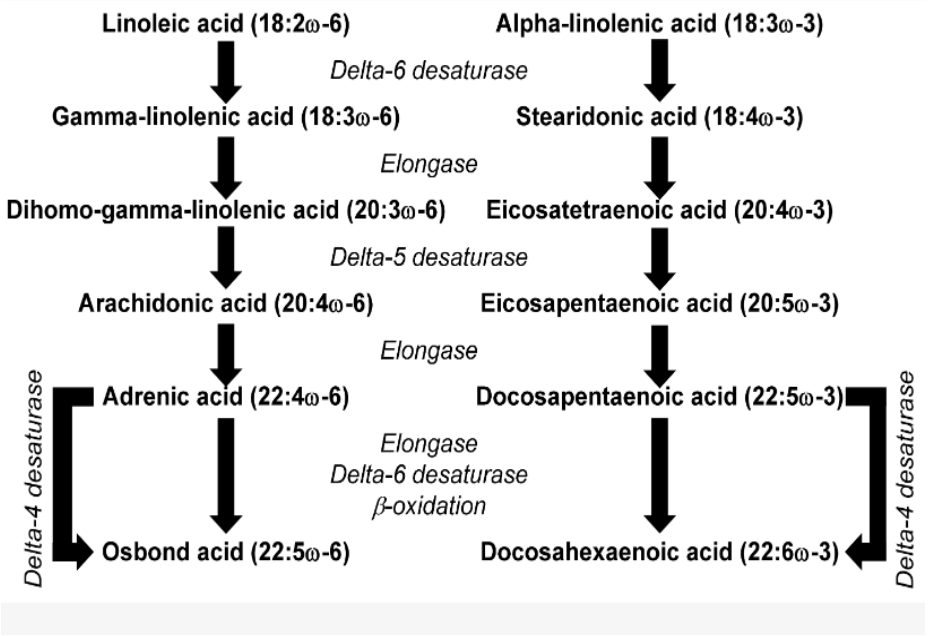

Fig. 15. Metabolic pathway of omega-3 and omega 6 polyunsaturated fatty acids [24, 23].

1.4 Moisture

The moisture content of the majority of fish species typically ranges from 60% to 80%. Analyzing the moisture content of a fish is one of the first and most fundamental steps in determining the nutritional value of its complete body. Food's moisture content is a great way to determine how many calories, protein, and fat it contains. Fish with a lower moisture content is richer in fat and protein and has a greater density of calories [10]. According to [28], moisture content serves as a reliable indicator of the relative levels of energy, lipids, and proteins in fish, showing an inverse relationship with these parameters: the lower the water content, the higher the energy density and the greater the amounts of proteins and lipids.

1.5 Minerals

The detection of minerals in fish is linked to ash. Fish's mineral composition reflects their entire inorganic content, and the best way to determine it is to measure the fish's ash content first. The residue that remains after the fish sample has been completely ashed is called ash. After all of the organic material has been burned up, this inorganic residue is left behind. Ash level alters from 0.5% to 5% of total fish body weight [10]. Raw marine fish muscle and invertebrates contain between 0.6 to 1.5% wet weight of minerals such sodium, potassium, calcium, magnesium, and phosphorus, as well as microelements like selenium, fluorine, iodine, cobalt, manganese, and molybdenum [14]. According to [29], a variety of elements, including species, food, seasons, salinity, geographic location, and environmental factors including temperature, are in charge of causing changes in the mineral concentration of fish and shellfish. Furthermore, it is known that the minerals and trace elements that comprise the overall ash contents are influenced by a variety of other factors, including eating habits, the environment, migration ecology, and even the ability to thrive in the same habitat [30].

1.6 Vitamins

Vitamins provide essential organic components for enzymes catalyzing a number of metabolic reactions in the human body. Seafood and fish products have vitamins including vitamin D, vitamin A, vitamin

C, vitamin E, vitamin B12, choline and folic acid [31]. Since many communities are concerned about vitamin D deficiency, eating fish can also help prevent symptoms like rickets and osteomalacia. Compared to meat or poultry, fish have a higher vitamin E concentration, which varies by species and tissue (dark muscle has higher levels). Depending on food, season, age, and size, the amount varies from 0.1 mg/100 g in certain wild fish species to 3–4 mg/100 g in cultured fish [32].

1.7 Health Benefits of Seafood Consumption

The beneficial effects of regular consumption of fish and seafood are supported by a significant number of studies. According to epidemiological research and clinical trials, consuming enough omega-3 fatty acids and maintaining a healthy ratio of omega-6 to omega-3 may lower the risk of blood pressure, inflammation, cardiovascular disease, and several forms of cancer (Figure 2). In addition to the beneficial effects of omega-3 fatty acids on the heart, their regular intake has been shown to help with weight control, cognitive development in children, and lowering the risk of high blood pressure, coronary heart disease, and stroke, as well as depression, and inflammatory diseases like rheumatoid arthritis [33, 34, 35]. In addition, Parkinson's disease and Alzheimer's disease are two types of neurodegenerative diseases associated with aging in the central nervous system. Numerous studies have shown that fish has a potential dietary therapy for the treatment of these diseases [36, 37, 38]. Omega-3 PUFAs (EPA + DHA) have promising effects on COVID-19 patients, according to recent data [39]. Accordingly, the Food and Agriculture Organization (FAO) of the United Nations (UN) and the World Health Organization (WHO) advise consuming one to two servings of seafood per week [40]. Specifically, the recommended daily intake of n-3 PUFAs varies by country and age group; it typically falls between 250 and 500 mg/day (equal to at least two servings of fish per week), and it is greater in newborns and pregnant and nursing women [41, 42].

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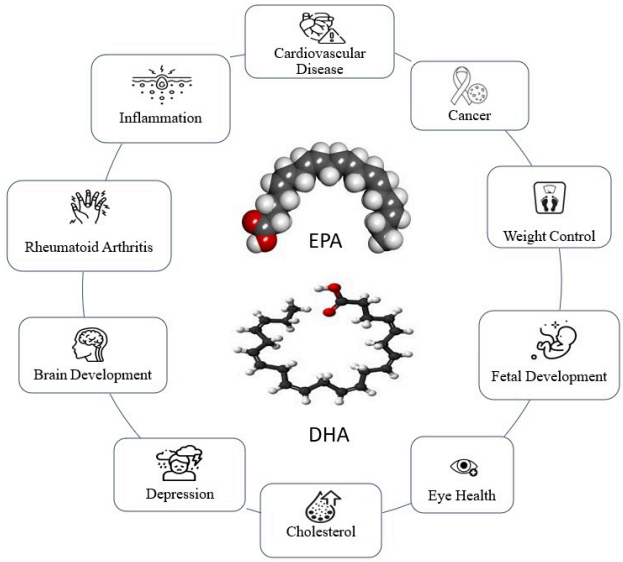

Fig. 16. The beneficial effects of regular consumption of omega 3 fatty acids.

1.8 Health Risks of Seafood Consumption

The health concerns of seafood must be taken into account despite their high nutritional content since they absorb pollutants and chemicals from their surroundings. Due to an increase in both natural and man-made activities, aquatic ecosystems have been continually subjected to heavy metal pollution in recent years [43]. Anthropogenic activities are generally acknowledged as the main contributors of heavy metals in aquatic systems. These activities include crop cultivation, erosion from agricultural areas, and the discharge of household and industrial trash. Heavy metals penetrate these systems, dissolve in the water, and easily build up in the organs of aquatic creatures, including fish, before entering the bodies of people who eat the contaminated fish [44]. Since very few trace metals are necessary for living things, these metals are divided into essential and non-essential categories. They can, however, be toxic to species at higher concentrations [45]. Toxic metals found in seafood, including copper (Co), cadmium (Cd), chromium (Cr), nickel (Ni), arsenic (As), lead (Pb), zinc (Zn), and mercury (Hg), can harm the human body by causing neurological disorders, kidney and liver damage, circulatory system issues, congenital abnormalities, immune system and reproductive system changes, and an

increased risk of cancer [46, 47, 48, 49, 50]. A certain range of cellular concentrations of Cu, Mn, Fe, Zn, and Ni are necessary for regular human cellular processes. In the aquatic food chain, fish are at the top. Therefore, determining the levels of heavy metals in fish will not only help us comprehend the pollution caused by pollutants, but it will also help us identify the potential health risk that comes with eating finfish.

Another powerful contaminant in aquatic waterbodies is pesticides that are chemical compounds, either natural or synthetic, that are toxic in nature and commonly used to control various pests, insects, weeds, and pathogens to improve the yield quantity and quality. As a result, they have detrimental effects on aquatic organisms. The World Health Organization (WHO) divided pesticides into four groups based on their level of toxicity: extremely hazardous, highly hazardous, moderately hazardous, and slightly hazardous [51]. Around the world, a wide variety of pesticide groups, including insecticides, herbicides, bactericides, larvicides, and fungicides, are widely utilized. The primary source of pesticides in aquatic environments is agricultural runoff, which poses a major risk to aquatic animals and human health due to their accumulation in fish and hence destroy the food chain [52]. The sustainability of the environment and human health are both significantly impacted by the rising use of pesticides worldwide. The extensive use of pesticides contaminates the air, water, and soil, affecting ecosystem services and biodiversity. Furthermore, there are long-term health hazards associated with pesticide residues in food and the environment, including as cancer, endocrine disruption, neurological diseases, cognitive dysfunction, and cancer [53, 54].

In addition to pesticides, seafood has been reported to contain various industrial chemicals, including polychlorinated biphenyls (PCBs), used in electrical and hydraulic equipment, and polybrominated diphenyl ethers (PBDEs), employed as flame retardants in industrial and consumer products. Polycyclic aromatic hydrocarbons (PAHs), unlike purely anthropogenic contaminants, originate from both natural and human-related sources, arising as byproducts of incomplete combustion during industrial processes, vehicle emissions, and cooking methods such as grilling or smoking, as well as from natural events like wildfires [55].

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Microplastics (MPs) with a diameter of less than 5 mm are produced when plastic trash breaks down through physical, chemical, and biological processes [56]. MPs, which are common in aquatic ecosystems, may be found in surface waters from the Arctic to Antarctica, as well as in beaches, deep sea sediments, and coastal sediments [57]. With the rise of plastic garbage, MPs have become a serious environmental issue. MPs have been shown in earlier research to have a variety of harmful impacts on aquatic organisms such as fish, benthic animals, zooplankton, phytoplankton [58, 59]. MPs have the ability to absorb dangerous contaminants from the environment, including pesticides, heavy metals, and persistent organic pollutants (POPs) [60]. The release of these toxins into the bodies of seafoods after ingestion can cause the toxins to bioaccumulate and biomagnify in the food chain. Because MPs may enter the human food chain through fish, there may be health problems, including developmental abnormalities, reproductive issues, and the risk of cancer [61]. The presence of MP in fresh and processed fish products from the German retail market was examined by Süssmann et al. [62]. 130 products in all, including canned, frozen, smoked, marinated, and fresh seafood, were examined. In seafood products, 97% of the MP found were less than 150 μm. Plastics were present in just 16% of the products under examination in quantities higher than those detectable by pyrolysis-gas chromatography-mass spectrometry, allowing for their identification and quantification. Polypropylene, polyethylene terephthalate, or polystyrene made up the identified MP. An estimated 16,500 particles of MP were consumed annually per person in Germany from seafood consumption. The highest MP contents were observed in both fresh and canned products. The study found a correlation between food contact materials and higher MP prevalence in seafood.

Biogenic amines (BAs) may pose a danger to people. They have low molecular weight, physiologically active substances and found naturally in a large range of foods. Bacterial activity or enzymatic activities aid in the decarboxylation of amino acids, which creates BAs in food products [63]. There are two amines among BA's that have the most toxic effects: histamine and tyramine. High histamine levels are observed in fish belonging to the Scombridae and Scomberesocidae families, such as mackerel, tuna, bonito, and bluefish etc. European Standard – Commission Regulation (EC) 1441/2007 applies only to fish products and allows histamine levels of 100–200 mg/kg in unprocessed fish [64]. The US Food and

Drug Administration (FDA) recommends lower histamine levels of up to 50 mg/kg in fish and fish products [65]. The amount of histamine in food products is not subject to any particular regulations. "Scombroid poisoning" or "histamine poisoning" are terms used to describe the reaction to histamine toxicity. Tongue tingling, rash, vomiting, diarrhea, burning feeling, headache and lightheadedness, nausea, blood pressure decrease, vasodilation, cerebral bleeding, palpitations, or trouble breathing are the most typical signs of a high histamine consumption. The effects of histamine poisoning appear a few hours after histamine consumption, but may also manifest several days after consumption [66]. Consequently, monitoring and managing histamine content in seafood is crucial for ensuring both product quality and consumer safety.

To reduce the health risks associated with seafood consumption, the following points should be taken into consideration:

- Prefer seafood caught from clean and less polluted waters which greatly reduces the risk of heavy metal and microplastic contamination.
- Prefer farmed fish from certified sustainable aquaculture systems, which are subject to stricter monitoring of feed and water quality.
- Buy seafood from reliable sources that follow safety regulations and ensure that the seafood is fresh and have no off odor/flavor.
- Choose pelagic species (such as sardines, anchovies, or mackerel) since they generally accumulate fewer pollutants than bottom-dwelling fish.
- Diversify fish consumption by eating different species instead of relying on a single type which lowers the risk of long-term exposure to specific contaminants.
- Ensure proper handling, and maintain the cold chain during storage of seafood products to minimize microbial and chemical risks.
- Cook seafood thoroughly using methods such as steaming, baking, or grilling. This will eliminate harmful bacteria and parasites while minimizing the formation of PAHs.
- Stay updated on local authority about contamination levels in seafood and which species can be consumed in which season.

- Limit the consumption of raw or undercooked seafood (such as sushi, oysters, or clams) to reduce the risk of foodborne infections.
- To further reduce exposure, remove the skin and internal organs, which tend to accumulate certain pollutants.
- Pay attention to portion size and frequency when consuming seafood, and establish a balance between nutritional value and contamination risks.

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Ochratoxin a in Maize: Origin, Occurrence, and Health Implications, Albania Case

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Abstract. Mycotoxins are toxic secondary metabolites produced by fungi that frequently contaminate cereal grains, compromising food safety. Among the most concerning are aflatoxins and ochratoxin A (OTA), primarily produced by Aspergillus and Penicillium species. OTA contamination typically occurs under warm, humid conditions, especially during poor post-harvest storage. Albania's maize-growing regions—Lushnja, Fieri, Korça, Shkodra, and Elbasan—are climatically predisposed to such risks. Although OTA is not the most prevalent mycotoxin in Albanian maize, recent findings confirm its presence in samples linked to inadequate storage. OTA is nephrotoxic and has been associated with renal damage and Balkan Endemic Nephropathy. While the OTA incidence on Albanian maize is relatively low, the risk assessment indicate high concentration levels for OTA in positive contaminated samples. Poor storage and limited monitoring elevate the crop vulnerability. These findings underscore the need for systematic testing and improved post-harvest practices to mitigate contamination and protect public health.

Keywords: Mycotoxins, Ochratoxin A, Maize, Albania, Food And Feed Safety, Nephrotoxic.

1 Introduction

Maize and other cereal grains are vital to the global food supply, serving as staples for billions of people and essential components of animal feed. However, these crops increasingly are contaminated by mycotoxins, toxic secondary metabolites produced by fungi under stress conditions. They are a diverse and ubiquitous group of fungal compounds specifically associated with the precipitation of deleterious effects in humans and animals (D'Mello, 2003; Neme and Mohamed, 2017). Their presence in food and feed chain has raised food safety and security concerns globally. Common foods susceptible to mycotoxins contamination include grains such as maize, sorghum, millet, wheat, and rice as well as peanut/groundnut.

Mycotoxin contamination is associated by huge economic impact globally, manifested as loss of human and animal health and life, increased healthcare costs, reduced livestock production, disposal costs of contaminated foods and feeds, research investment, and regulatory programs aimed at reducing or avoiding mycotoxins from products (Zain, 2011; Tahiri et al., 2025).

Viewed globally, food safety is regularly compromised by the presence of mycotoxins occurring in cereal grains, nuts, fruit and green coffee beans. Animals exposure to mycotoxin contaminated feed will bring their metabolites in foods of animal origin, e.g. milk, eggs, etc (Kabak, Dobson, & Var, 2006).

One of mycotoxins being in focus of human and animal toxicity has been Ochratoxin A. First evidence on Ochratoxin A toxicity in humans was reported in '70s of last century, when OTA exposure has been associated with renal damage and Balkan Endemic Nephropathy (Pavlović, 2013).

1.1 Origin, chemistry, and toxicity of main mycotoxins

The mycotoxins of major concern in human and animal health are produced by *Aspergillus*, *Penicillium*, *Fusarium, Alternaria* and *Claviceps*, genera. (D'Mello, 2003).

In foods and feedstocks most important mycotoxins produced by Aspergillus genus are aflatoxins, mainly by *Aspergillus flavus* and *A. parasiticus*; Ochratoxin A (OTA) by *Aspergillus ochraceus* and related species. Sterigmatocystin, by *A. versicolor*, and cyclopiazonic acid, by *A. flavus* (Pitt & Hocking, 2022).

Over 80 species from the genus Penicillium are producers of most important mycotoxins related to human exposure including: OTA, citrinin, cyclopiazonic acid, patulin (Pitt & Hocking, 2022).

The discovery of toxigenic strains of the fungus *A. ochraceus* Wilh., led to the isolation and structure elucidation of ochratoxin A (Van der Merwe et al, 1965). OTA is produced by Aspergillus and Penicillium species. The economic impact of this mycotoxin is significant because it is present in a wide range of food products. In particular, *P. verrucosum* is responsible for OTA contamination of cereal-

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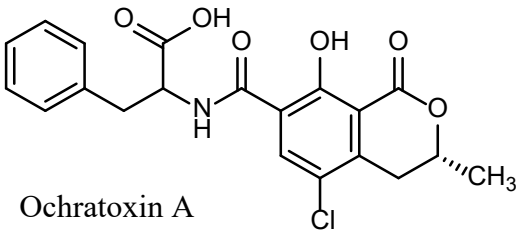
based products, *P. nordicum* is responsible for the contamination of some dried foods, *A. ochraceus, A. carbonarius, A. westerdijkiae,* and *A. steynii* are responsible for the contamination of coffee beans, cocoa, pepper, and dried fruits. Whereas the contamination of grapes, grape juice, raisins, cider, and wine is mainly attributed to *A. carbonarius* and, to a lesser extent, to *A. niger* species "together".

Chemically, OTA is a structure built from a dihydroisocoumarin ring linked to an L-phenylalanine unit (Gallo *et al*., 2012).



Ochratoxin A

1.2 Kinetics and biotransformation of ochratoxin A in organism

Main route of OTA exposure is through gastrointestinal tract. The metabolism of OTA is unclear, while biotransformation occurs mostly in the liver and kidneys. OTA molecules accumulate in the blood, liver, and kidneys. Due to its strong binding affinity to blood proteins, OTA has a longer half-life in blood than tissues (Kőszegi & Poór 2016). Urine and feces are essential excretion routes for plasma mycotoxin removal in all species, while milk is a major excretion route in mammals (Yanfei et al., 2018). Following the intestinal absorption, OTA molecules interacts with serum proteins. According to protein binding affinity and extent, serum half-life varies greatly among species, mostly albumin, over 99%, allowing passive absorption in its non-ionic state (Ringot, Chango, Schneider, Larondelle, 2006). This largely explains its long biological half-life. OTA has cumulative toxicity and quick absorption and sluggish elimination. Bile acid recirculation helps OTA molecules enter the circulatory system from the intestines and redistribute into other tissues (Kőszegi & Poór 2016).

Animals absorb different amounts of OTA. Thus, 66% in pigs, 56% in rodents, and 40% in birds. The tissue distribution in pigs, rodents, poultry, and goats is kidney > liver > muscle > fat (Huff & Hamilton, 1979).

Grain products include Ochratoxin B (OTB), the dechlorinated derivative of OTA. Because there is no chlorine atom to influence structural dissociation, this toxin is 10 times less lethal than OTA (Harris and Mantle, 2001).

1.3 Combination Effect over Mycotoxins toxicity

Natural toxicoses are often caused by exposure to mixtures of mycotoxins present in food products. This combination can lead to additive, synergistic or antagonistic toxicity effects (Smith, Madec, Coton, Hymery, 2016).

The combination effect of OTA and OTB have been shown to be additive. Combined effects of OTA and citrinin have shown synergistic effects with respect to nephrotoxicity in poultry and pigs. Another combined effect is shown with OTA and penicillic acid, that synergistically increase mortality in poultry and rodents, renal lesions in fish.

Being most important mycotoxins, the combined effects of OTA and AFB1, have been in focus. Co-exposure of animals to OTA and FB1 has shown pathological signs of toxicity, for example pulmonary edema, kidney and liver lesions, a synergism in cytotoxic effects between FB1 and OTA in cytotoxicity has been observed (Singh & Kumari, 2022).

Information regarding the combined effects of OTA and zearalenone (ZEA) indicate for antagonistic interaction effects. OTA and trichothecenes often exhibit additive or synergistic toxicity, especially in kidney, liver, and immune tissues. This is due to overlapping mechanisms such as oxidative stress, inhibition of protein synthesis, and disruption of cellular signaling (Kolawole, Siri-Anusornsak, Petchkongkaew, Elliott, 2024).

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1.4 Mold growth and mycotoxin production influencing factors in grains

The crops of both cool temperate and hot tropical regions can be affected by ochratoxin because they are produced by different Aspergillus and Penicillium species (Altomare, Logrieco, & Gallo, 2021).

Fungi are the primary cause of spoilage in stored maize and can cause detrimental changes in appearance, quantity, and quality of stored grain, thereby reducing the end-use value of maize for food, feed, and biofuels (Channaiah & Maier, 2014).The fungi can produce mycotoxins while maize is in the field, during processing, transportation, and storage (FAO, 2011). Aspergillus and Fusarium species can infect maize pre-harvest, and mycotoxin contamination can increase if storage conditions are poorly managed (Chulze, 2010). The Penicillium toxins in maize occur mainly during storage and when the harvest is delayed producing penicillic acid and OTA (Pitt, Taniwaki, & Cole, 2013).

The factors affecting grain contamination include biological factors (susceptible crop), environmental factors (temperature, moisture availability, humidity, mechanical injury, and insect/bird damage), harvesting (crop maturity, temperature, moisture, and handling), storage (structure, conditions, moisture, and temperature), handling and processing (Figure 2) (Milani, 2013).

Their occurrence may start in different stages of crop production, starting from the field, harvesting, handling, storage, and processing. In overall, is established scientifically, that DON, ZEN, and fumonisins contaminate the grains at the field/or pre-harvest stage, while aflatoxins and OTA are mostly occurring during storage due to improper postharvest handling (Neme & Mohammed, 2017).

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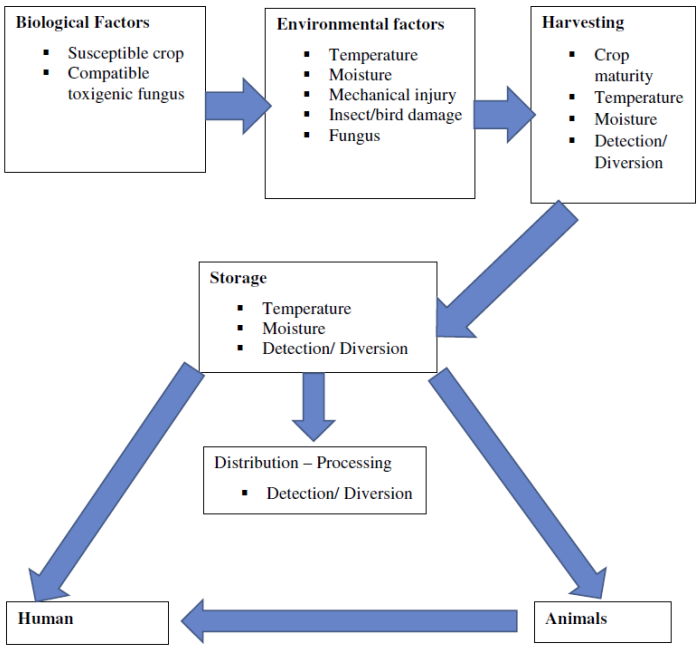

Fig. 17. Factors affecting mycotoxin occurrence in human food and animal feed chains. (Adapted from Neme and Mohammed, 2017).

The temperature and moisture content of the grain or commodity are the most critical factors favoring fungal growth and mycotoxin production. Relative humidity is another factor influencing the moisture content of stored grain resulting in water available for mold growth and subsequent mycotoxin production. The optimum temperature and water activity for mycotoxin production in grains are presented in Table 1.

Table 10. Optimum temperatures and water activity for mycotoxin production.

Mycotoxins	**Temperature (°C)**	**Water activity**
Aflatoxin	33	0.99

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Ochratoxin	25-30	0.98
Fumonisin	15-30	0.9-0.995
Zearalenone	25	0.96
Deoxynivalenol	26-30	0.995
Citrinin	20-30	0.75-0.85

Adapted from Milani (2013).

Insect infestation is another factor that promotes fungal inoculation and subsequent mycotoxin contamination in several ways of grain (Abbas *et al*., 2013). Control of storage insects through sorting out damaged grain will reduce the risks to invasion by storage molds, including *A. flavus* (Medina, Rodriguez, Magan, 2014).

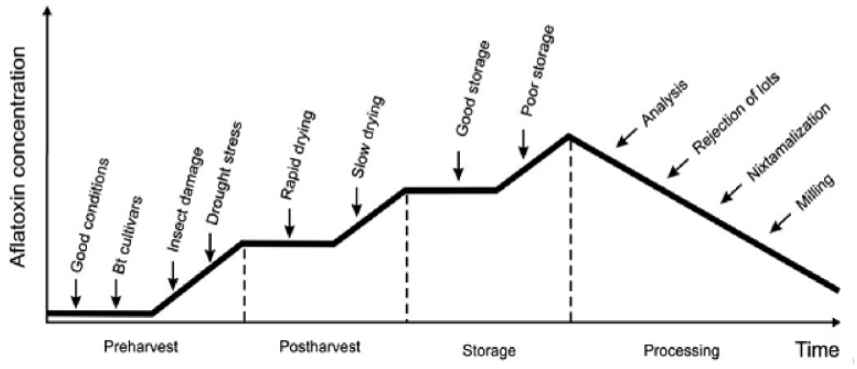

Fig. 18. Aflatoxin formation and reduction in maize. (Adapted from Pitt et al. 2013)

1.4 Postharvest mitigation strategies on mycotoxin contamination

The main postharvest factors for grain mycotoxin contamination are mechanical injury, insect infestation, time of harvesting, drying method, types of storage structure and conditions, handling and processing.

An integrated system management approach is important to mitigate the problem (Fumagalli et al., 2021). Several pre- and postharvest mitigation strategies have been developed to prevent the growth of fungi as well as to decontaminate and detoxify food, contaminated by mycotoxin (Kabak, Dobson, Var, 2006). Pre-harvest methods include using resistant varieties, field management, crop rotation, use of biological and chemical agents, harvest management (Adegoke & Letuma, 2013).

Postharvest interventions include rapid and proper drying, proper transportation and packaging, sorting, cleaning, drying, smoking, postharvest insect control, and the use of pesticides as storage protectants (Hell & Mutegi, 2011; Wild et al., 2015). Good storage conditions, use preserving agents and irradiation prevent mycotoxin contamination after harvesting (Adegoke & Letuma, 2013). Effective postharvest management of stored commodities requires clear monitoring criteria and effective implementation in relation to abiotic and biotic factors, hygiene to ensure that mycotoxin contamination is minimized (Magan & Aldred, 2007).

A control program for mycotoxins from field to table must incorporate HACCP principles, necessitating a comprehensive understanding of the interactions between toxigenic fungi and agricultural crops, on-farm cultivation and harvesting methods, and the processing of foods for human consumption. Furthermore, it is essential to consider commercial and trade channels, including the preservation and distribution of food to consumers (Richard, 2007).

1.5 Harvesting

Postharvest strategies for preventing mycotoxin contamination in stored grains begin at harvest. The timing of harvest greatly affects the extent of mycotoxin contamination. Delayed harvest significantly

increased the level aflatoxin in maize, result in poor quality seed due to mold infections and subsequent aflatoxin contamination of the seeds/pods. Mycotoxin content increases with delayed harvest coupled with rain precipitation (Channaiah, 2011).

Freshly harvested cereals should be cleaned to remove damaged kernels and other foreign matter. Strategies such, when 10% or more of the ears have 10-20% mold damage, the field should be scheduled for the earliest possible harvest (Munkvold *et al*., 2019). Avoiding mechanical damage and grain contact with soil at harvesting stage also minimize contamination of fungal infection.

1.6 Storage conditions

Grains are subjected to quality loss during storage. In developing countries, inadequate storage practices account for 20-50% of crop losses (Kumar & Kalita, 2017). The quality deterioration in stored grains is caused mainly by a natural process which breaks down organic matter through either physical/chemical processes or biological processes which contained nutrients and energy are used by other life forms. Store fungi include all species of *Aspergillus*, *Fusarium*, and *Penicillium* (Atanda et al., 2011). Cereal grains are particularly susceptible to grow by Aspergilli in storage environments where the main toxigenic species are *A. flavus* and *A. parasiticus* for aflatoxins, and *Penicillium verrucosum* is the main producer in cereals for OTA (Kabak et al., 2006).

During storage, the temperature and relative humidity of the grains are the main mitigation strategies to minimize the growth of fungi. Moisture control is the main critical one for prevention of mycotoxins in grains.

The following moisture contents are considered safe during storage: 14% for maize (Channaiah, 2011). During storage, once the grain a_w drops below a certain value the mycotoxin production will stop. Sweeney and Dobson (1998) found aflatoxins can be produced at a_w values ranging from 0.95 to 0.99 with a minimum aw value of 0.82 for *A. flavus*, while the minimum a_w for OTA production is 0.80.

Generally, provided grain is stored at a moisture content equivalent to $a_w = 0.70$ no spoilage will occur (Medina, Rodriguez, Magan, 2014).

Another important parameters to prevent the growth of molds is temperature. Ideally, grain should be cooled after drying and maintained at 1ºC-4ºC for the duration of storage, while during the summer months the grain temperature can be maintained between 10 ºC and 15 ºC (Munkvold et al., 2019). At low or cold temperature, fungal contaminants not killed, but their growth and metabolism are minimal.

Aflatoxins are produced at temperatures ranging from 12 to 40 ºC (Sweeney & Dobson, 1998), while OTA production by *P. verrucosum* occurs between 10 - 25 ºC (Olsen et al., 2003). *A. flavus* has an unusually high tolerance to heat, compared with other fungi; it thrives in temperatures approaching 37.8 ºC and even higher (Medina et al., 2014).

1.7 OTA contamination of maize harvested in Albania

In a study presenting the OTA contamination in maize harvested in a two year period 201402015, resulted that 71% of samples were contaminated with aflatoxins, of them 36% over the threshold for AFB1 as per EU regulation (EU 2023/915). Maximum values: 3550 µg/kg (AFB1), 4822 µg/kg for total Aflatoxins. The OTA was detected in maize samples surpassed the maximum permissible limit of 5 µg/kg, with overall incidence of 7.0%. The OTA concentration in contaminated samples was minimum (187 µg/kg) to maximum (488 µg/kg), resulting in mean value of 336 µg/kg (Topi et al., 2023).

Risk assessment indicate that all positive samples surpassed the maximum allowable level for OTA in unprocessed cereals (5 µg/kg as per EU Regulation No. 2023/915).

Comparative analysis at both regional and international levels reveals that the Fier and Lushnja regions exhibited the greatest concentrations of AFB1, attributable to the Mediterranean environment and heavy maize cultivation. In comparison to adjacent nations (Serbia, Croatia, Romania), Albania had elevated contamination levels in maize, particularly in 2014 (Topi et al., 2017; Topi et al., 2019; Topi et al., 2021; Mato et al., 2024; Topi et al., 2024).

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Occurrence of OTA in maize (2014–2015) indicate that the detected amounts are exceedingly elevated, posing a substantial risk to public health, particularly if the maize is utilized for human food or animal feed. Comparative analysis by region indicate that western regions present high concern, mainly do to the climate factors, such as high temperatures, and humidity. Maize sample from the Kruja region was found 333 µg/kg of OTA, while maize sample collected from Lushnja, 187 µg/kg (Topi et al., 2023).

OTA mycotoxin was not identified in the other regions (Elbasan and Korça). Study of mycotoxin co-occurrence is of high relevance since the interaction between OTA and aflatoxins (AFs) may produce synergistic effects, particularly on the immune system and liver metabolism.

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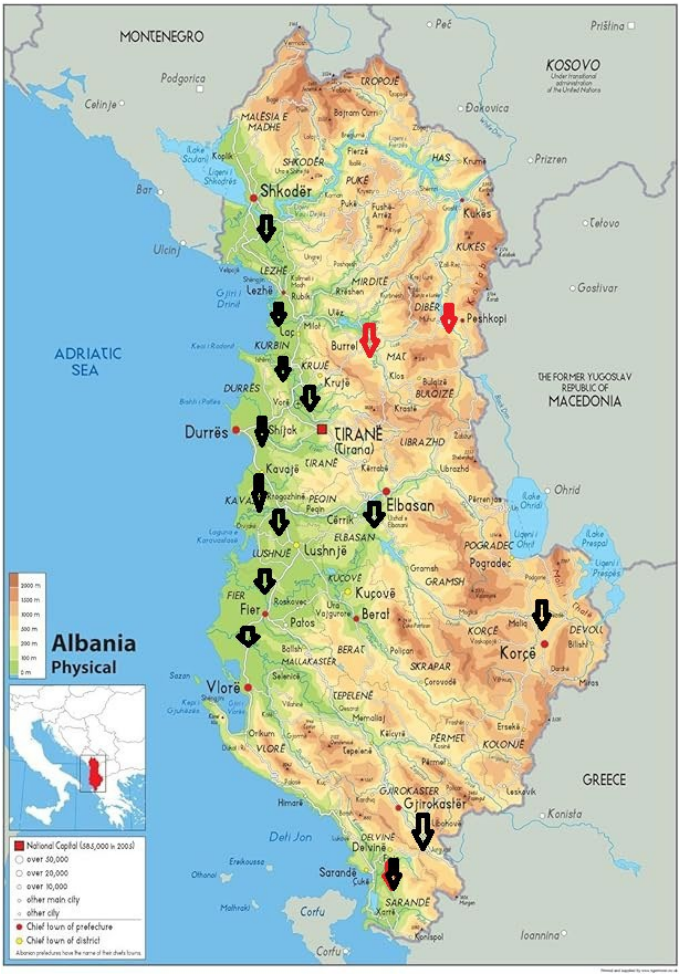

Fig. 19. Main maize producing areas of Albania.

2 Conclusion

Despite the low incidence of ochratoxin A (OTA) in Albanian corn samples (7%), all positive cases exceeded EU safety limits, with concentrations reaching up to 488 µg/kg. This highlights a serious food safety concern, especially in regions with warm, humid climates. OTA contamination is often linked to

poor post-harvest practices. Preventive measures — such as timely harvesting, proper drying, sanitation, and aeration — are essential. Additionally, grain processing and the use of natural fungistats can help reduce mycotoxin levels. The co-occurrence of OTA with aflatoxins suggests possible synergistic toxicity, warranting further investigation.

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I-CRAFT AGRICULTURAL and FOOD TECHNOLOGIES

Protoplast Fusion as a Breeding Tool for Berries: A Review of Prospects and Current Limitations

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Abstract. Berries of all different species, such as (Vitis, Fragaria, Vaccinium), are known for their abundance and diversity of health benefits and economic importance. However, their traditional breeding and production are hindered by long generation cycles and limited gene pools, which slow and limit the growth rate of new varieties that have been developed. The fusion technique allows the transfer and enhancement of important plant traits, including increased disease resistance, environmental stress tolerance, and improved fruit characteristics. Although research on berry crops is still in its infancy, proven research on grapes, strawberries, and others indicates the usefulness of this technique for enhancing genetic diversity. Nevertheless, major challenges continue, particularly in protoplast differentiation, protoplast fusion, regeneration efficiency, and selection of stable mixtures. The integration of emerging tools such as genome editing, and analysis is expected to increase the effectiveness of protoplast fusion. In short, this technology will achieve significant advances in berry genetics, poised to become a cornerstone of both basic research and applied breeding programs.

Keywords: Protoplast fusion, somatic hybridization, berry crops, breeding, plant biotechnology

1 Introduction

Berries and varieties (strawberries, raspberries, blackberries, blueberries, etc.) are a valuable fruit group in terms of agroeconomics are far ahead because of their unique taste, impact on human health, and growth in the global market. Berries are rich in fiber, vitamins, and minerals, enriched with bioactive compounds, especially anthocyanins and antioxidant and anti-inflammatory properties [1]. Research shows that eating these fruits is beneficial for cardiovascular health, supports cognitive function, and offers protection against certain cancers [2; 3]. The volume of consumers in the global berry market,

which has a fast-moving market such as fresh, frozen, and processed products, is expected to hit a high level in the coming years [4].

Plant breeding is one of the methods of creating and selecting the best plant varieties and incorporating superior plant phenotypes in the development of improved crop varieties. In these ways, they meet the needs of consumers and farmers. The main objective of plant breeding is to improve and increase yield, nutritional quality, and other characteristics useful in daily life [5]. Traditional plant breeding methods are the dynamo of agricultural development, so new products with better quality and more specialized have been developed and continue to be developed. Biotechnology and genetic engineering work by introducing natural genes and then carrying desired traits and giving them to another plant so that the genes can be hybridized and improved [6]. In recent years, research and biotechnological tools have emerged in the breeding and development of new plants. The term protoplast was introduced by Heinstein to describe the components in a plant cell with totipotency [7]. Plant cell protoplasts are hybridized in biotechnology [8], through which this technique can overcome obstacles associated with distant hybridization, infertility or the presence of male and female flowers, thus allowing the creation of new species and valuable wild resources [9]. Additionally, protoplasts are able to directly take up organelle DNA, viruses, plasmids, and so on. protoplast isolation and protoplast dissection, which serve as valuable tools for studying gene function in plants. In woody plants, protoplast hybridization techniques are less commonly used, and although efforts continue, the lack of an efficient system for protoplast isolation has not yet evolved [10]. Protoplasts can be used as efficient receptors for transient transformation and serve as important tools for gene function and manipulation. However, records for the protoplast transformation system in blueberry are not significant [11]. The protoplast transformation system is capable of directly transporting genetic material in a geometrically ordered manner. Due to the absence of a cell wall, two protoplasts are more easily connected by chemicals. Recently, direct delivery of ribonucleoprotein complexes consisting of Cas9 and guide RNA (gRNA) has been used in protoplast-based DNA-free genome editing systems in several plants[12;13;14]. To obtain cell protoplasts involves a purification and separation process; specific enzymes are used to break down cell walls and separate protoplasts from other plant cells [15].

Breeding studies in strawberry are mainly conducted for yield improvement, disease and pest resistance, and tolerance to adverse environmental conditions [16]. Breeding studies in strawberries focus on improving fruit shape and size, color, flavor and aroma, shelf life and storage [17]. In crops such as strawberries, due to demand, much work is being done to create new generations that can adapt to changing environmental conditions and market demands [18]. This method, which is based on crossing plants within the same or closely related species, may limit genetic diversity. Biotechnological methods, on the other hand, allow for faster growth of stress-tolerant crops by directly manipulating plant genes or introducing new traits from other species. Biotechnological tools such as genetic engineering, breeding, and gene editing can expedite the breeding process significantly.

2 Key Challenges of Traditional Berry Breeding

Plant breeding is a method of creating, selecting, and stabilizing superior plant phenotypes to develop improved crop varieties that meet the needs of farmers and consumers. The main objectives of plant breeding are to improve yield, nutritional value, and other profitable traits [5].

Traditional methods of plant breeding are the source of the development of new varieties of plants and the foundation of agriculture. Biotechnology and genetic engineering are faster ways to select and cross plants with desired traits. The basic concepts of traditional plant breeding are selection, hybridization, breeding, and backcrossing [6]. Berry varieties face significant barriers in the application of traditional breeding methods due to their high genetic heterogeneity and complex biological systems. These barriers slow the process of developing new varieties, limiting their ability to respond to increasing global demand.

2.1 Genetic and Biological Barriers

The efficiency of breeding programs is primarily affected by natural constraints arising from the underlying genetic and biological structure of plants. The length of the life cycle of the grapefruit, which takes several months or years for a new generation to form and process

Slows reproduction [19]. Furthermore, such hybrids are often unevenly matched and confound undesirable traits and pose a threat to the genes of the new generation (e.g., disease resistance and high-quality of the fruits)

Better methods are needed for stringent selection to accommodate the desired genotype [20]. Furthermore, different levels of ploidy also pose a significant barrier. For example, cultivated strawberries (Fragaria × ananassa) are octoploid (8n), whereas many strawberry cultivars are tetraploid (4n) or hexaploid (6n).

Successful fusion of different numbers of chromosomes is not easy and sometimes leads to infertility or abnormally developed offspring [21; 22].

2.2 Reproductive Barriers

Genetic systems that regulate reproduction are an effective barrier to breeding many berry species due to their incompatibility, which complicates the development of pure lines and requires careful pollinator selection in breeding programs to ensure fertility [23]. Interspecies incompatibilities sometimes also create barriers between close species, such as root growth arrest, zygote failure, or hybrid embryo abortion that prevent the transfer of valuable genes (eg, disease resistance, abiotic stress tolerance) from wild relatives to cultivated species [24].

2.3 Practical and Operational Challenges

A number of practical obstacles have slowed reproduction including selection of good parent plants, control of crossing and fertilization, seed harvesting, shuttle planting, long-term evaluation for genotype approval generally takes about 10-15 years [25]. In addition,plants are heavily influenced by environmental conditions (soil, climate and irrigation). Validation of new genotypes requires cultivation trials in different areas over a period of several years increasing the breeding time [24]. Finally, modern commercial varieties are often derived from a narrow genetic pool, making them vulnerable to unexpected stresses such as emerging diseases or climate change. While the key lies in increasing

genetic diversity within the wild gene pool, the breeding challenges described above severely limit access to this resource [19]. Mukherjee and Gantait (2024) stated that biotechnological progress of strawberry research and breeding has advanced significantly, supporting micropropagation, genetic improvement, germplasm conservation, and added value. Major techniques include *in vitro* stem and bud regeneration, callus culture, somatic embryogenesis, protoplast culture, artificial seed formation and cryopreservation.

3 Protoplast Fusion Technology: Basic Principles and Methodology

Protoplast fusion means mixing the protoplasts of two plant species after breaking the cell wall using chemicals and enzymes. This technique aims to overcome barriers to reproduction and create a new plant that is genetically different [26]. The process consists of four basic phases: isolation, integration, selection, and regeneration.

3.1 Protoplast Isolation and Culture

Protoplasts are active plant cells that are able to grow outside the cell wall and are alive. This structure transfers plant genetic material faster and is considered the foundation of biotechnology in modern science [27].

Isolation Method

Light selection: Young tissues are actively dividing so into

External probes are implanted and callus cultures are used. Good and easy cell selection Breaking down the cell wall directly affects protoplast removal and replication.

Enzyme digestion: The tissue is incubated for several hours in a solution containing a mixture of cell wall-degrading enzymes, such as cellulase and macerozyme (pectinase). Enzyme concentration and incubation time are important parameters.

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1. Thermal stability: Because protoplasts are wallless and unprotected, they are temperature sensitive. Mannitol or sorbitol, a heating agent, is added to the medium to prevent protoplast rupture.

2. Purification: After digestion, the mixture is filtered through a mesh and centrifuged to remove cell debris and undigested tissue. The purified protoplast suspension is ready to be used for direct fusion or transformation [28]

3.2 Fusion Methods and Working Mechanisms

Two basic coupling methods are widely used:

1. Chemical Fusion (PEG Method):

Mechanism: Polyethylene glycol (PEG) physically binds to cell membranes and induces a transient membrane-modifying reaction. PEG bridges the gap, causing the lipid bilayer to rearrange.

Advantages: Easily available and inexpensive does not require technique. Disadvantages: Due to the release of toxins, the ability of protoplasts to aggregate is reduced [28].

2. Electric Fusion (Electrofusion):

Mechanism: This method consists of two stages:

Alignment (pearl chain): An alternating electric field (AC) applied to the protoplast suspension arranges it in a manner called “pearl chain”.- Fusion: Short, high voltage pulses of direct current (DC) are applied to the aligned cells. This pressure creates temporary and microscopic pores in the cell membrane, causing the cells to contact through the pores when they come into contact and stick together to form a single hybrid cell.

Advantages: High fusion efficiency, controlled environment, lack of chemical toxicity, and process repeatability.

Disadvantages: Requires a specialized and expensive electrofusion device. Parameters such as voltage and pulse duration must be optimized for each species.

Exploring Protoplast Culture: Media, Species, and Experimental Perspectives

Plant nutrient medium, hormones and nutrient balance play an important role in the quality of protoplast division and formation in the most important nutrient medium for protoplast growth (Murashige and Skoog media, Kao and Michayluk media, Gamborg B5 media, Nitsch media) [29]. Within plant media energy sources such as sugars and temperature regulators mannitol and sorbitol affect (high rate of division, colony formation, and microcallus formation) Initial osmolarity should be similar to the enzyme solution and decrease gradually as division progresses [30]. (Auxins, cytokinins, gibberellins) Other supplements such as (vitamins and minerals) that are effective in the growth and proliferation of clones formed from protoplast fusion [31]. Additional supplements, such as antioxidants, antibiotics, amino acids and vitamins, can be added to the growth medium to promote protoplast growth and microcallus formation [32].

3.3 Post-Fusion Process: Hybrid Selection and Regeneration

Hybrid selection: When protoplasts are cultivated into the nutrient medium it is necessary to separate the integrated protoplasts from the simple protoplasts of unintegrated individuals For the selection of hybrids various methods are used:1.

Selective media: Some strains selected as parents are sensitive to lack of antibiotics or certain nutrients, so only mixed cells can be grown and selected

Isolation of fluorescence-activated cells: Protoplasts labeled with different fluorescent dyes (e.g., FDA, calcofluorescent white) can be manually selected under flow cytometry or fluorescence microscopy.

This allows physical separation of mixed cells (they carry both fluorescent dyes) and is a very effective method [26].

Regeneration: Selected hybrid cells are cultured for plant regeneration. This is the most challenging step.

1. Callose formation: Mixed cells are added back in media containing appropriate auxin and cytokinins for callus formation.

2. Organic formation: Callus can be activated by stimulating plant growth to become a full-fledged plant.

3. Whole plant formation: Whole plants consisting of roots, stems, and leaves formed as a result of protoplast mixture can be transferred to pots under controlled conditions [33].

4 Conclusion and Future Perspectives

This review shows that the combination of two protoplasts can overcome sexual incompatibility, a major biological obstacle to berry plant diversification.

The protoplast fusion technique is a complementary method to traditional plant breeding that accelerates genetic diversification through gene transfer between different plants, induction of cytoplasmic male sterility, and polyploidy engineering. Future approaches should focus on combining protoplast fusion with Other advanced biotechnologies.

CRISPR-Protoplast Synergy: Gene Editing Technology Compatible with Protoplast Integration Systems. After the genes are integrated, CRISPR-Cas9 directly modifies the protoplasts, which is an opportunity to control the desired trait gene while clearing the background of the unwanted gene [34].

In conclusion, protoplast fusions should be considered an evolving technology in berries breeding. With interdisciplinary collaboration (cell biology, supplementary genetics, bioinformatics) and continuous

advances in biotechnology, this technique is highly likely to overcome current limitations and usher in a new era in berry fruit breeding.

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Risks of Biogenic Amines Accumulations From Probiotics

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Abstract. Probiotics, defined as live bacteria that provide health benefits when taken in sufficient quantities, are commonly used in functional foods owing to their functions in pathogen suppression, immunological modulation, and gut barrier enhancement. Nevertheless, their ability to generate biogenic amines (BAs) during fermentation poses significant safety concerns. BAs like histamine, tyramine, putrescine, and cadaverine are produced primarily by microbial decarboxylation of amino acids and are found in fermented dairy, meat, vegetable, and beverage products. While BAs play an important role in physiological processes, excessive consumption has been related to negative consequences such as histamine intolerance, hypertensive crises, migraines, gastrointestinal discomfort, and possible carcinogenicity due to interactions with nitrites. BA formation is influenced by raw material quality, microbial strain diversity, and environmental factors like pH, temperature, and salt content. Some lactic acid bacteria and Enterococcus strains are remarkable high producers, while others show little or no activity, emphasizing the necessity of strain-specific evaluation. Mitigation strategies include carefully selecting non-BA-producing strains, following strict hygiene measures, optimising fermentation settings, and using starter cultures capable of decomposing BA. Emerging omics methods offer significant capabilities for tracking BA-related genes, proteins, and metabolites, making probiotic treatments safer. Besides, consumer awareness and regulatory control are critical, as toxicologically substantial BA levels may not result in sensory deterioration. Moreover, assuring the safety of probiotic foods necessitates a balanced risk-benefit analysis that includes technological controls, genetic screening, and rapid detection techniques. By solving these issues, the probiotic sector can maintain innovation while also protecting public health.

Keywords: Probiotics; Biogenic amines; Fermented foods; Food safety

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1 Introduction

1.1 . Overview of Probiotics

Probiotics, meaning "for life," are defined as Live microbes which confer a health benefit to their host when administered in adequate amounts by the World Health Organisation (WHO) [1]. The most frequently employed probiotics are Lactobacillus, Bifidobacterium, Enterococcus, Lactococcus, and Streptococcus [2]. Probiotics are microorganisms that directly or indirectly affect human health. Fighting pathogens, stimulating the immune system, and protecting and improving the intestinal epithelial barrier are among the most fundamental effects of probiotics [3]. Probiotics prevent pathogens from adhering to intestinal epithelial cells and inhibit their growth by competing with them for nutrients. They also suppress pathogen growth by producing antimicrobial substances (defensins, bacteriocins, and/or hydrogen peroxide) and by lowering the pH of the organic acids they produce [4]. Probiotics can regulate the immune system by directly interacting with the intestines through the molecules they produce or by utilising their cell structure components [5]. Microbe-Associated Molecular Structures (MAMPs), such as peptidoglycan, lipopolysaccharide, teichoic acid, lipoteichoic acid, bacterial DNA, exopolysaccharide, and flagella, interact with receptors (pattern recognition receptors, PRRs) that recognise these structures in the immune system, activating the innate and adaptive immune systems [6, 7]. The epithelial barrier is damaged by toxins produced by pathogens and pro-inflammatory cytokines produced by the immune system against pathogens, increasing the permeability of epithelial cells. Thus, pathogens and unwanted metabolites cross the epithelial barrier and enter the bloodstream. Probiotics help ensure the stability of epithelial cells by activating various signalling pathways and triggering mechanisms such as preventing programmed cell death, producing defensins, strengthening the bonds between epithelial cells, and increasing mucus secretion [8].

1.2 What are Biogenic Amines?

Biogenic amines (BAs) are a class of structurally varied, basic nitrogen-containing compounds that are prevalent in numerous food products. These compounds are particularly common in items produced through fermentation, such as cheese, wine, and beer, in addition to being found in fish, meats, and other

derived products [9]. BAs are called biogenic because they are formed as a result of the activity of living organisms. The cleavage of the carboxyl radical of amino acids forms amines. This process is called decarboxylation, and the enzyme responsible is called decarboxylase. The enzymes responsible for decarboxylation can be produced by both microorganisms and are present in animal and plant tissues [10]. Endogenously, biogenic amines are low-molecular-weight organic compounds produced by tissues. Exogenously, they are antinutritional factors resulting from the decarboxylase activity of fermenting microorganisms in processed foods [11]. Endogenous amines, which play essential roles as neurotransmitters in the body, include catecholamines (dopamine, epinephrine, and norepinephrine), indolamines (serotonin, melatonin, and 5-hydroxytryptamine), and histamines. The name of exogenous amines comes from the amino acid from which they originated [12, 9].

1.3 Importance of Studying Their Risks

Biogenic amines are considered fundamental precursors for the biosynthesis of essential macromolecules, including proteins, hormones, and nucleic acids. Certain polyamines, such as putrescine, spermidine, and spermine, are particularly critical for maintaining intestinal function and regulating healthy metabolic processes [11, 12]. While vital for biological activity, the excessive intake of these compounds from dietary sources can elicit a range of adverse systemic effects. These psychoactive and vasoactive substances, including histamine, tyramine, and tryptamine, are known to influence blood pressure and neurological function. For instance, histamine, a potent vasoactive amine, can induce vasodilation and subsequent hypotension, leading to symptoms such as headaches, flushing, and oedema. The ingestion of more than 8 mg of histamine is capable of causing acute intoxication [9, 12].

The body’s ability to manage biogenic amine levels relies on the activity of detoxifying enzymes like diamine oxidase (DAO) and monoamine oxidase (MAO) [13,10, 14]. Impaired function of these enzymes, whether due to genetic predispositions, gastrointestinal pathologies, or pharmacological inhibition, can lead to amine intolerance and heightened toxicity. Furthermore, some amines, such as putrescine and cadaverine, can competitively inhibit the oxidation of histamine, thereby exacerbating its

toxic effects [10]. Beyond histamine, other biogenic amines pose distinct health risks. The vasoconstrictive properties of tyramine, tryptamine, and phenylethylamine can contribute to hypertension. Notably, the consumption of tyramine-rich foods by individuals on MAO inhibitor therapy may precipitate a hypertensive crisis or trigger migraine attacks [9, 13]. In the central nervous system, biogenic amines are crucial for neurotransmission. A deficiency in cerebral putrescine has been implicated in the pathophysiology of depression. Similarly, imbalances in serotonin levels are linked to eating disorders, and MAO-inhibiting medications are widely utilized to modulate these levels for the treatment of depression and anxiety disorders [10]. Conversely, certain polyamines exhibit potential carcinogenic properties. Putrescine, cadaverine, and spermidine are capable of reacting with nitrites to generate carcinogenic compounds [10, 15]. In high concentrations, polyamines can also induce programmed cell death (apoptosis) and suppress cellular proliferation, with high-dose putrescine specifically linked to these effects through increased nitric oxide synthesis and direct binding to carcinogens [9, 15]. Agmatine is a biogenic amine with diverse pharmacological effects. It is noted for its nephroprotective benefits through an increase in glomerular filtration rate and its role in glucose homeostasis. Interestingly, research indicates that agmatine levels are elevated in individuals with schizophrenia compared to healthy controls [10, 11].

2 Biogenic Amines: Formation and Types

Biogenic amines are formed from the breakdown of proteins primarily through enzymatic pathways such as the decarboxylation of amino acids, as well as the amination and transamination of other organic compounds [10, 16]. BAs are classified according to the number of amine groups they contain and their chemical structure. According to the first classification system, these compounds can be divided into monoamines (e.g., tyramine, dopamine, norepinephrine, histamine, and serotonin), diamines (e.g., putrescine and cadaverine), and polyamines (e.g., spermine and spermidine). Alternatively, they are classified according to their chemical structure as aliphatic (e.g., putrescine, cadaverine), aromatic (e.g., tyramine, phenylethylamine), and heterocyclic (e.g., histamine, serotonin) compounds [9, 11, 17, 18].

2.1 Mechanisms of Biogenic Amine Production

The formation of biogenic amines is a consequence of the microbial decarboxylation of amino acids, a process that is catalyzed by specific decarboxylase enzymes found in various bacterial strains [10, 11, 16]. The microorganisms responsible for this enzymatic activity include both Gram-negative and Gram-positive species, such as those from the *Enterobacteriaceae*, *Lactobacillaceae*, and *Staphylococcus* families [19]. The accumulation of these compounds is a multifaceted process determined by three primary groups of factors. Intrinsic factors: These relate to the raw material's composition, including its pH and the availability of free amino acids. Extrinsic factors: These encompass the conditions under which the food is processed and stored, such as temperature, storage duration, and the type of processing (e.g., cooking, fermentation). Microbiological factors: This involves the presence and activity of specific microbial strains that possess decarboxylase capabilities. Consequently, the establishment of proper sanitary conditions during food production and handling is critical, as it serves to control the growth and proliferation of these amine-producing microbial strains [11, 20].

The most critical process in the formation of biogenic amines is the secondary changes that result from the decarboxylation of amino acids. This process occurs due to either tissue- or microbe-derived decarboxylase enzymes. In foods, decarboxylation caused by microbial degradation is more common [18]. Specifically, in fermented foods, amine formation happens in three main ways. The amination of aldehydes and ketones, the secondary conversion of certain amino acids through microbial decarboxylation, and the hydrolysis of nitrogen-containing components, such as nitrogenous compounds and their degradation products [10, 11, 20]. Common amino acids preceding BAs and the BAs they produce are histidine (histamine), tyrosine (tyramine), phenylalanine (phenylethylamine), tryptophan (tryptamine), ornithine and agmatine (putrescine), and lysine (cadavarin). Among these, the single-step decarboxylation of histidine, tyrosine, tryptophan, lysine, and phenylalanine can directly form the corresponding amine. Putrescine is a diamine that can be produced by decarboxylation of ornithine or deamination of agmatine. Spermine and spermidine are polyamines formed by the sequential addition of aminopropyl groups to putrescine [10, 11, 21].

2.2 Common Biogenic Amines in Probiotic Fermentation

Biogenic amines play a vital role in numerous biological processes, including cell membrane stability, immune system function, and the prevention of some chronic illnesses. They contribute to essential cellular activities like the synthesis of nucleic acids and proteins. Additionally, certain biogenic amines act as signaling molecules and mediators in the body. For example, some are involved in growth regulation (spermine, spermidine, and cadaverine), neural communication (serotonin), and inflammatory responses (histamine and tyramine) [10].

Histamine is a natural compound in the body, synthesised from the amino acid histidine with the help of the enzyme histidine decarboxylase and pyridoxal phosphate. The amount and location of histamine differ significantly among the tissues of all vertebrates. This compound has several essential functions. It acts as a neurotransmitter and regulates vascular permeability. It's also involved in controlling body temperature, stomach acid levels (pH), stomach volume, and brain activity. Additionally, it is a key player in triggering allergic reactions [22].

Tryptamine is a monoamine alkaloid formed when the enzyme aromatic L-amino acid decarboxylase acts on the amino acid tryptophan. This compound is found in trace amounts in the brains of mammals, where it can act as a neurotransmitter or neuromodulator and has been shown to increase blood pressure. Additionally, tryptamine is present in plants, fungi, and other animals [10].

In a similar fashion, tyramine is produced from the amino acid tyrosine via the enzyme tyrosine decarboxylase. While commonly found in fermented foods, it can also be present in fresh and processed seafood. Specific bacteria, such as Lactobacillus spp. and Enterococcus spp., are known to be tyramine producers [10, 22]. Tyramine also has a strong antioxidant effect due to the amine and hydroxyl groups in its structure [23]. The health implications of tyramine are significant, especially for individuals on monoamine oxidase inhibitors (MAOIs), as its consumption can lead to hypertensive crises. In some fermented fish products, tyramine levels can reach as high as 200 mg/kg, posing a health risk if consumed in large amounts [22, 24]. Humans, as well as certain fungi, bacteria, and a variety of plant and animal species, can produce phenylethylamine from the aromatic amino acid phenylalanine. This

synthesis is carried out by the enzyme aromatic L-amino acid decarboxylase [10]. In the human central nervous system, phenylethylamine acts as a neurotransmitter, helping to regulate blood pressure and remove norepinephrine. However, high levels of this compound can cause adverse health effects, with symptoms including hypertension, and cerebral hemorrhage [16, 25]. Consuming phenylethylamine in the presence of tyramine has been linked to several adverse health effects. This combination is known to trigger migraine attacks in susceptible individuals and can also lead to an increase in blood pressure [26].

The most common cellular polyamines, putrecine, spermidine, spermine, and cadaverine, play a role in bacterial cell growth and proliferation. These compounds possess a polycationic structure that allows them to easily attach to anions within the cell. In addition to their role in cell growth, polyamines are also vital for regulating nucleic acids, aiding in protein synthesis, and maintaining the stability of cell membranes [15].

Both putrescine and cadaverine are biogenic amines that signal food spoilage. Putrescine is derived from the decarboxylation of the amino acid ornithine, a reaction catalyzed by ornithine decarboxylase. It can also be synthesized from arginine via the agmatine and carbamoylputrescine pathways. Cadaverine, on the other hand, is formed from the decarboxylation of lysine [10].

These two amines are often found in decaying food, particularly seafood, and are considered reliable indicators of decomposition. While not directly toxic, they can potentiate the effects of histamine by blocking the enzymes that normally break it down. Shim et al. found that putrescine and cadaverine levels in various fish species varied significantly based on storage conditions and duration, with ranges of 10–300 mg/kg and 20–500 mg/kg, respectively. Beyond their role in spoilage, putrescine, which is produced by bacteria and fungi, also serves as a precursor for spermidine and spermine, and is involved in essential biological processes like cell growth, cell division, and tumorigenesis [22, 27].

Spermidine synthase is the enzyme responsible for synthesizing spermidine from putrescine. This compound is a vital precursor for other polyamines, including spermine and its structural variant,

thermospermine. Spermidine plays a critical part in many key biological functions. It helps maintain membrane potential and regulates both intracellular pH and cell volume. Furthermore, as a polyamine involved in cellular metabolism, spermidine contributes to the inhibition of neuronal nitric oxide synthase and supports the development of intestinal tissues [10].

Spermine, a polyamine found in all eukaryotic cells, is formed from its precursor, spermidine, through the enzyme spermine synthase. Although its precursor amino acid is ornithine, it's not a direct conversion. Spermine is present in a wide range of organisms and tissues due to its vital role in cellular metabolism. It contributes to the development of intestinal tissues and helps stabilize the helical structure of viruses [28].

Agmatine is produced from the amino acid arginine through the action of the enzyme arginine decarboxylase. It plays a key role in polyamine metabolism, as it's converted to putrescine via the agmatine enzyme. This compound has several important functions. It helps regulate the synthesis of nitric oxide and is involved in the activity of matrix metalloproteinases and other enzymes that lead to the production of H_2O_2 [10, 28].

In humans, putrescine, cadaverine, and agmatine can hinder the breakdown of histamine, which in turn raises its toxicity levels. Furthermore, spermine, spermidine, and putrescine demonstrate antioxidant properties. These compounds are capable of preventing the oxidation of polyunsaturated fatty acids, with their effectiveness being directly proportional to their amine content [10, 29].

2.3 Factors Influencing Their Production

Several factors influence biogenic amine formation in fermented foods during processing, such as raw material quality, microbial cultures, and environmental conditions like pH, temperature, and fermentation time [26].

Since the level of biogenic amines (BAs) in food is influenced by sanitation and can fluctuate throughout its preparation and storage, measuring these compounds offers a reliable way to assess food quality. In

their natural state, BAs are already present in raw ingredients such as grapes, fresh milk, and uncooked meats. For instance, using unsanitary meat for sausage production is known to severely inhibit the activity of the beneficial bacteria *Lactobacillus sakei* CTC494, a strain that lacks amino acid decarboxylase. Furthermore, significant quantities of spermidine, putrescine, and cadaverine have been shown to accumulate in the skins and seeds of grapes as part of the winemaking process [16, 30].

The quantity and variety of amines in food depend on the product's composition and the presence of microorganisms. Many bacteria from the Enterobacteriaceae family, along with species of *Pediococcus, Enterococcus,* and *Lactobacillus,* are known to contribute to biogenic amine (BA) formation. Several microbial groups have been found to have decarboxylase activity. Specifically, bacteria like *Lactobacilli, Pseudomonads, Enterobacteriaceae*, and *Enterococci* found in meat products are known to possess this ability. While most yeasts can produce significant amounts of cadaverine and putrescine, only a few—such as *Debaryomyces hansenii* and *Yarrowia lipolytica* isolated from cheese—can also produce histamine and tyramine [31]. For seafood, particular bacterial species have been confirmed as histamine producers. These include *Staphylococcus xylosus* from salted anchovies, *Morganella morganii, Hafnia alvei*, and *Klebsiella pneumoniae* from tuna, and *Aeromonas hydrophila* from mackerel. Furthermore, researchers like Pessione et al. have identified *Lactobacillus sp.* 30a and *Lactobacillus sp.* w53 from wine as producers of histamine, putrescine, and cadaverine [9, 21, 31]. In yoghurt, the formation of BAs, particularly tyramine and histamine, is primarily attributed to Streptococcus thermophilus. A number of studies have also shown that histamine-producing bacteria exist in fermented soybean products [31].

Amine formation in foods is a complex process influenced by a combination of factors, including temperature, pH, and salt content. Amine production accelerates under specific temperature conditions. Research indicates that the optimal temperature range for the activity of amine-forming microorganisms is 20-37°C. For instance, *Morganella morganii* in fish produces the most histamine at 25°C. Similarly, *Carnobacterium divergens* generates more tyramine in meat-fat mixtures at 25°C compared to 15°C. Studies have also shown that higher storage or processing temperatures lead to increased biogenic amine (BA) formation in various food products, including carp meat [29, 31, 32]. The pH level also plays a

crucial role. The ideal pH for the decarboxylation activity responsible for amine formation is around 5.0. However, the relationship is not always straightforward; in wine production, for example, a higher pH can lead to increased biogenic amine accumulation. In fermented sausages, a slow or insufficient drop in pH during the initial stages of ripening has been linked to higher histamine levels. Additionally, some bacteria, like *Carnobacterium divergens*, produce more tyramine at a pH of 5.3 than at 4.9. Conversely, high salt concentrations can inhibit this process. When salt content exceeds 5%, the formation of biogenic amines is significantly reduced [19, 29, 32].

3 Probiotic Strains and Biogenic Amine Production

3.1 Lactic Acid Bacteria and Fermented Foods

Several species of Lactic Acid Bacteria (LAB) are recognised as safe by food safety authorities around the world. They are considered beneficial bacteria that enhance food safety and quality and have been part of traditional diets for centuries. LAB create lactic acid during fermentation by breaking down sugars like lactose and fructose. Found naturally in plants, food, and animal digestive systems, these microorganisms are among the most researched [33]. They are central to food fermentation and have been used for millennia in a process called lacto-fermentation [34]. Although the term "lactic" comes from the Latin word for milk, this fermentation isn't limited to dairy; it also occurs in foods like pickles, sauerkraut, kimchi, and sourdough bread. The resulting lactic acid gives these foods their characteristic sour flavour and acts as a preservative. Many LAB species are considered safe by global food authorities and are valued for improving both food safety and quality [35].

LAB are highly adaptable microbes that can thrive with or without oxygen. However, their fermentation metabolism is only triggered when oxygen is limited [36]. These bacteria are fundamental to the fermentation of many foods, transforming everyday ingredients like milk, grains, vegetables, and meat into distinct, tangy products such as yoghurt, sourdough bread, kimchi, and cured sausages. LAB can occur naturally in foods under the right conditions, or specific "starter cultures" can be added to achieve a desired outcome [37].

LAB enhances food quality in several key ways. They act as a natural preservative by lowering the food's pH, which creates an acidic environment that prevents harmful bacteria from growing and extends shelf life. They are also responsible for the unique flavor and texture of fermented foods; for instance, they give sourdough its signature tang and yogurt its creamy consistency by producing exopolysaccharides. Furthermore, some LAB species improve food safety by creating antimicrobial compounds that inhibit the growth of dangerous pathogens [35, 36]. LAB also has applications in agriculture, medicine, cosmetics, and the production of eco-friendly plastics (Raman et al. 2022).

The lactic acid produced by LAB is a key component in a wide variety of foods. These include fermented dairy products like yoghurt, cheese, and kefir; vegetables and fruits such as cabbage, cucumber, and mangoes; and grains found in products like sourdough bread and miso paste. They are also essential in fermenting beverages like kombucha and boza, as well as cured meats and fish [36].

3.2 Variability Among Strains

Lactobacilli are considered significant BA producers in various fermented foods, including meat, cheese, and beverages. While their role is generally beneficial, some strains can accumulate high levels of BA, which can be a food safety concern [19].

In fermented sausages, *Lactobacillus curvatus* and *Lactobacillus sakei* are the predominant species. Most *L. curvatus* strains are known to be tyramine producers. Other species, such as *L. paracasei, L. brevis,* and *L. plantarum*, have also been identified as BA producers in meat [19, 39].

Lactobacilli are responsible for BA accumulation in many cheeses. Certain strains of *L. buchneri, L. parabuchneri,* and *L. helveticus* can produce high levels of histamine even under refrigerated conditions. *L. brevis* and *L. curvatus* strains in cheese are also known to produce tyramine [19, 40].

In wine and cider, Lactobacillus species such as *L. brevis, L. hilgardii*, and *L. rhamnosus* are responsible for the accumulation of BAs, particularly histamine and tyramine. Production is influenced by conditions such as pH and the presence of specific decarboxylase-positive strains [19].

Some LAB strains that cause spoilage in beer, such as *L. brevis, Lactobacillus lindneri*, and *Lactobacillus paracollinoides*, have been identified as sources of BAs. These bacteria can produce tyramine, ornithine, and histamine as a metabolic strategy for survival in acidic and nutrient-poor environments. *Lactobacillus rossiae* is one of the putrescine-producing bacteria in sourdough. This strain utilises a specific metabolic pathway to produce putrescine, which serves as a biochemical defence mechanism and helps it survive in acidic conditions [19, 41].

The ability to produce BAs is highly strain-dependent, even among the same species. It is often linked to the presence of specific genes such as hdc (for histamine) and tdc (for tyramine). Some strains can acquire the genes necessary for BA production through horizontal gene transfer [19].

The *Enterococcus* genus has a dual reputation in food science. While not formally designated as "Generally Regarded As Safe" (GRAS) or included on the Qualified Presumption of Safety (QPS) list, some species are widely used in traditional food fermentation, particularly in cheeses and dry sausages. They are valued for their ability to thrive in challenging conditions like high salt and low pH, and for their contribution to flavor through proteolytic and lipolytic activities. Certain strains even show promise as probiotics and can produce bacteriocins that inhibit harmful bacteria [19, 39].

However, their presence is also a significant concern due to their strong potential for producing biogenic amines (BAs), especially tyramine. This capability is not universal across the genus but is highly dependent on the specific strain. For example, species like *E. faecium, E. faecalis*, and *E. durans* are frequently identified as tyramine producers in cheese, meat, and wine. The production of BAs is not just a random occurrence; it's a biochemical defense mechanism that helps the bacteria survive in harsh, acidic environments by regulating their internal pH [18, 19].

Research shows that the presence of the gene clusters responsible for BA production, such as the tdc gene for tyramine, is common in many Enterococcus strains. However, simply having the gene does not guarantee BA production. The actual accumulation of these compounds depends on the gene's expression, which is influenced by environmental factors like pH, temperature, and salt concentration.

For instance, some studies indicate that a lower pH and salt stress can actually upregulate the expression of the genes that lead to increased tyramine production. This variability underscores the importance of a case-by-case evaluation of each Enterococcus strain used in food production to ensure safety [19].

Bifidobacteria are a key group of bacteria, largely found in the human gut, that play a significant role in human health and nutrition. They are notable for their ability to synthesise crucial vitamins, including riboflavin, thiamine, vitamin B6, and vitamin K, along with other important bioactive compounds like folic acid, niacin, and pyridoxine. The presence of these bacteria in fermented milk products enhances their nutritional profile, making them rich sources of free amino acids and vitamins [42].

While some Bifidobacterium strains have the potential to produce BA, their ability to do so is generally more limited than that of other fermentative microorganisms such as LAB. These strains produce small amounts of cadaverine and tyramine [43].

3.3 Case Studies from Fermented Dairy, Vegetables, and Beverages

BA synthesis in fermented foods is an enzymatic process that primarily begins with the hydrolysis of proteins. This first step, carried out by microbial proteases, releases free amino acids, which then serve as precursors for the formation of BA. As previously mentioned, the most common and critical pathway for this conversion is amino acid decarboxylation, where microbial enzymes act on amino acid substrates. Less common pathways for BA production include amination and transamination of ketones and aldehydes [26, 44].

Besides histamine, which has been the most extensively researched, tyramine, phenylethylamine, tryptamine, putrescine, cadaverine, spermidine, and spermine are the BAs detected in fermented fish that pose a risk. Among these, histamine, putrescine, and cadaverine levels typically rise as fish begins to spoil, while spermine and spermidine concentrations simultaneously decrease [31]. Bacteria identified as BA producers in fermented fish include Enterobacteriaceae and Lactobacillus species. Methods such as ensuring the use of hygienic raw materials, optimising processing and storage conditions, and using effective starter cultures are used to prevent or reduce BA formation in fermented fish [16].

Dairy products, especially aged cheeses, are a significant source of BA. Among these, the cheese reaction, caused by tyramine produced in high concentrations in cheese, is a foodborne illness. Furthermore, cheese is second only to fermented fish in terms of histamine production. The synthesis of these compounds is attributed to various microorganisms [9, 19]. Many of the starter and adjunct cultures intentionally used in cheesemaking and the dairy industry are known to produce BA. These include lactic acid bacteria from various genera, such as *Lactobacillus, Pediococcus, Enterococcus, Leuconostoc, Lactococcus*, and Streptococcus. Some yeast species, such as *Debaryomyces hansenii, Yarrowia lipolytica, Pichia jadinii*, and *Geotrichum candidum*, also play a role in this process [45].

Polyamines play a fundamental role in plant physiology, where they are integral to a wide range of cellular processes. These compounds are essential for functions like cell division and differentiation, the biosynthesis of nucleic acids and proteins, and maintaining membrane stability. They also contribute to a plant's ability to cope with environmental stress and delay senescence [46].

The distribution of these compounds is not uniform across all produce. Fruits and their juices, for example, are particularly rich in putrescine, while green vegetables contain higher concentrations of spermidine. While other aromatic amines, such as tyramine, are less common than polyamines, they can still accumulate to exceptionally high levels in certain vegetables, where they are believed to act as a defence mechanism against insects and herbivores [46, 47].

Fermentation has long been employed as a preservation technique for fruits and vegetables across various cultures. This traditional method is applied to a wide array of produce, from staple items like white and red cabbage (sauerkraut) to a more diverse range including broccoli, cauliflower, Brussels sprouts, peppers, carrots, beets, and tomatoes [26, 48]. The final concentration of biogenic amines (BAs) in these products is not uniform, as a confluence of variables determines it. The specific properties of the raw material, climatic conditions, and agricultural practices all play a role, as do the particular processing and storage parameters applied during fermentation [48, 49, 50].

4 Mitigation Strategies

Although biogenic amines play a role in human health, some can lead to negative health effects when present in high concentrations. These adverse reactions can also occur if the body's metabolic processes are impaired due to genetic factors, certain illnesses, or the use of medications that inhibit amine oxidase enzymes.

4.1 Selection of Non-BA-Producing Strains

Bacterial growth and fermentation processes can lead to the formation of amino acid derivatives known as biogenic amines (BAs), which can have various effects on human physiology and well-being [21]. When consumed in high concentrations, these compounds can pose a health risk, leading to symptoms like headaches, heart palpitations, and gastrointestinal distress [19]. The level of toxicity is influenced by the specific type of BA, individual sensitivity, and the co-consumption of substances like alcohol or monoamine oxidase inhibitors, which impair the body’s ability to detoxify these compounds [19, 51].

Histamine and tyramine are considered the most dangerous BAs due to the severity of their symptoms. Histamine, often found in fish like tuna and sardines, can trigger "scombroid fish poisoning," an allergic-like reaction. Tyramine, commonly linked to the "cheese reaction," can cause migraines and other severe cardiovascular and respiratory issues. Other BAs, including putrescine and cadaverine, can also cause toxicity and enhance the effects of histamine and tyramine by interfering with the enzymes that break them down [19].

Despite these risks, there is no global legal standard for BAs in food, with the key exception of a specified maximum histamine level in certain fish products in the European Union [19, 52].

BA contamination can come from various sources, including spoilage bacteria like *Enterobacteriaceae*. However, LAB, a group of Gram-positive microorganisms central to food fermentation, are considered particularly efficient producers of tyramine and other BAs like histamine, putrescine, and cadaverine [25].

Given that BA production varies greatly between different bacterial strains, the development of safe probiotic products hinges on careful strain selection. This involves a comprehensive in vitro screening process where bacteria are tested for their ability to produce BAs under various food-like conditions. Crucially, genetic analysis using techniques like genome sequencing is employed to verify the absence of the genes—such as tdc (tyrosine decarboxylase), hdc (histidine decarboxylase), odc (ornithine decarboxylase) ve ldc (lysine decarboxylase)—that are responsible for BA synthesis. This rigorous approach allows for the creation of a detailed safety profile for each probiotic strain, ensuring they are both beneficial and safe for human consumption [53].

4.2 Technological Approaches in Fermentation Control

The BA profile and amount in fermented products vary depending on the microorganism population and environmental conditions. The most fundamental and first step in controlling biogenic amine formation is ensuring the initial quality of the raw material and the hygienic conditions of the production process. A large portion of BAs found in foods is produced by decarboxylase-positive microorganisms, either naturally occurring or introduced during the production process. The presence of contaminating bacteria capable of producing BAs, particularly Enterobacteriaceae, can cause rapid and uncontrolled amine accumulation from the beginning of fermentation. This severely limits the effectiveness of subsequent technological control methods. Even the use of commercial starter cultures may be insufficient to address this high level of contamination, increasing the risk of undesirable BA levels in the final product. Therefore, the most effective strategy for BA control begins at the source and necessitates the selection of high-quality raw materials and the implementation of strict hygiene standards [29].

The physical and chemical conditions of fermentation processes are the most critical parameters directly affecting BA formation. These parameters work synergistically to determine microorganism growth and decarboxylase enzyme activity. Temperature: While temperatures near optimal growth values can boost cell metabolism and proliferation, which often increases the production of BAs due to a higher cell count, a large number of decarboxylating cells alone doesn't guarantee a high final amount of Bas [11, 29]. Studies using *E. faecalis* EF37 in a model system showed that increasing temperature from 16°C to

44°C corresponded to faster growth and more rapid and intense tyramine accumulation. The highest activity of a pure commercial tyrosine decarboxylase extracted from *E. faecalis* was found at a temperature between 30 and 37°C. In contrast, the optimum temperature of a tyrosine decarboxylase obtained from *L. brevis* was 50°C. However, it was rapidly inactivated at higher temperatures, and the enzyme's activity at the optimum temperature decreased rapidly during storage at 50°C for one hour [29, 54, 55].

There are limited reports on the relationship between temperature and other BAs (putrescine, cadaverine, and tryptamine). Generally, the accumulation of BAs, including cadaverine and putrescine, increased with temperature [29]. pH: Decarboxylation is a cellular response to acidic conditions; therefore, numerous studies have investigated the relationship between pH and BA accumulation. The effect of pH varies depending on whether the focus is on the activity of a pure enzyme or the decarboxylase activity of living cells. Regardless of the specific focus, low pH has been widely demonstrated to trigger the transcription of genes in many decarboxylase clusters, thereby increasing the ability of cells to withstand acidic stress [29].

The pH range in which amino acid decarboxylase enzymes exhibit optimal activity is between 5.0 and 6.5. Therefore, lowering the pH below this range during the production process, particularly to below 4.0, inhibits the growth and enzyme activity of BA-producing microorganisms, thereby suppressing amine formation [25].

Salt Concentration: Elevated salt levels typically lead to lower biogenic amine (BA) accumulation in food products, primarily by hindering the metabolic processes of decarboxylating microbes. Gram-negative bacteria are particularly susceptible to this effect, showing greater inhibition from increased salt concentrations compared to Gram-positive bacteria. This approach to controlling BAs, however, conflicts with the current health-conscious trend of reducing sodium chloride in food. Different bacterial species exhibit varied responses to salt concentration, resulting in inconsistent effects on biogenic amine (BA) production [29].

The influence of salt on histidine decarboxylase activity in Streptococcus thermophilus is not uniform across different contexts: it acts differently on whole, living cells than on a cell-free extract. While a 2.5% salt concentration virtually stopped histamine formation in the living cells, the enzyme's activity in the extract was unaffected up to 5% NaCl. Furthermore, even when the NaCl level reached 20–30%, the decarboxylase in the extract still maintained some function, though it started to decrease after 5% [29, 56].

Conversely, a *Tetragenococcus muriaticus* strain, isolated from fish sauce, exhibited maximum histamine generation later in its exponential growth phase, specifically when salt levels were between 5–7% NaCl. This strain is known for its ability to sustain histidine decarboxylase activity even in a 20% salt environment [29, 56]. Furthermore, during the fermentation of sauerkraut, the overall amine content was greater in products with 1.5% NaCl compared to those with 0.5% NaCl for both *Lactobacillus plantarum* and *Leuconostoc mesenteroides* [50]. These observations suggest a complex relationship where, despite salt's potential to slow down bacterial growth, rising NaCl levels can sometimes directly increase certain biogenic amines. This phenomenon is often attributed to the critical function of Na+ ions in the sodium/proton antiport system, a mechanism that helps cells cope with stress by actively pumping H+ ions out [29].

Several established methods exist for reducing biogenic amine (BA) accumulation in foods, ranging from processing techniques to ingredient additions [31].

Modified Atmosphere Packaging (MAP) utilises carbon dioxide to significantly extend a food's shelf life by suppressing the growth of bacteria that form histamine [57]. Additionally, incorporating preservatives and additives into food has been shown to effectively curb BA production [31]. Applying high hydrostatic pressure is an effective way to lower both the bacterial count and the resulting BA levels in both raw ingredients and finished food items [24].

Irradiation is another physical method used for BA reduction, with reports showing that gamma irradiation successfully lowers histamine, tyramine, cadaverine, and putrescine in fish, such as blue jack

mackerel, dramatically reduces histamine in Bonito, and effectively controls BA formation in products like soybean paste [31, 59]. Smoking also proves beneficial; smoked samples consistently show lower levels of histamine, tyramine, cadaverine, putrescine, spermine, and spermidine compared to non-smoked samples. The smoking process inherently creates aseptic qualities in the product, thereby inhibiting the growth of amine-decarboxylating bacteria and reducing BA concentration [31]. The choice of starter culture plays a critical role in controlling BA formation. While some strains produce BAs, others have "negative" decarboxylase activity or contain enzymes capable of oxidising and breaking down existing biogenic amines in the food. The strategic use of selected starter cultures has successfully prevented the buildup of BA in various fermented products, including sausage, wine, fish sauce, and cheese [29, 31, 60]. Another technique for lowering biogenic amines (BAs) in food involves using oxidising agents to break down the BAs. Several microorganisms are recognised for their ability to oxidise these compounds, including *Natrinema gari*, *Vergibacillus sp SK33, Micrococcus varians, Lactobacillus sakei, Lactobacillus curvatus, Staphylococcus xylosus,* and *Brevibacterium linen* [31].

4.3 Consumer Awareness and Labeling

Food safety remains a top concern for both consumers and global health organisations. The World Health Organisation (WHO) estimates that more than 200 diseases are foodborne, and that the majority of the worldwide population will experience a foodborne illness at some point. Because it is difficult to establish a definitive causal link between food contamination and related illness or death, the true incidence of these diseases is likely underestimated. Given that food contamination can occur at any stage of the global supply chain (from production and distribution to preparation and consumption), every stakeholder, from producer to consumer, bears the responsibility for ensuring the safety of the food supply [61, 62, 63].

BA control and monitoring are critical not only for toxicological and public health reasons, but also because they can serve as valuable indicators of food quality and acceptability. Managing food quality, encompassing safety, nutrition, availability, convenience, integrity, and freshness, is a fundamental component of ensuring food safety [62, 63].

BAs have been widely used as quality indices in various food matrices, including meat, fish, and wines, indicating freshness and/or spoilage levels and helping to control food and beverage processing processes [62]. Traditional BA-based indices, such as the one developed by Mietz and Karmas, have been used to assess fish decomposition. This index is based on the correlation between increasing putrescine, cadaverine, and histamine levels and decreasing spermidine and spermine levels during storage. Scores below 1 indicate good quality, scores between 1 and 10 indicate tolerable quality, and scores above 10 indicate product decomposition [62, 64]. However, this traditional index has proven less effective for products such as cheese, meat, and meat products because it does not account for tyramine, the predominant BA in these foods. To address this issue, an alternative Biogenic Amine Index (BAI) has been proposed for meat, defined as the sum of the concentrations of putrescine, cadaverine, histamine, and tyramine. BAI < 5 mg/kg indicates good quality, fresh meat; 5-20 mg/kg indicates acceptable quality meat showing the first signs of spoilage; 20-50 mg/kg indicates poor quality meat; and >50 mg/kg indicates spoiled meat [62]. The effectiveness of BAs as a quality index is highly product-dependent, influenced by factors such as the nature of the product (e.g., fresh, canned, fermented). BA indices have demonstrated higher reliability in fresh and heat-treated meat products than in fermented products. This inconsistency is attributed mainly to the significant variability in BA concentrations in fermented foods. Numerous processing variables, such as ripening, fermentation, yeast cultures, and additives, influence this variability. Consequently, developing a universally reliable BA index to predict product quality is a complex task [62, 63]. A significant concern is that foods containing toxicologically unacceptable levels of some BAs (e.g., histamine or tyramine) may appear organoleptically "normal." For example, in products like tuna and salmon, histamine levels that pose a health risk may not be detectable by consumers' senses before consumption. This critical disconnect between sensory perception and chemical safety highlights the need for rigorous external oversight of these compounds [62]. Consequently, a comprehensive understanding of the aetiology and control mechanisms of foodborne illness is essential for effective prevention [62, 63].

5 Future Perspectives

5.1 Advances in Omics Approaches

The study of biological systems has been revolutionised by "Omics" technologies, defined as methodologies for the large-scale investigation and quantification of data representing the complete composition and function of a biological system at a specific molecular level. For decades, four core omics disciplines, genomics, transcriptomics, proteomics, and metabolomics, have significantly advanced biological research [65, 66]. Genomics permits the decoding of the entire genetic complement of probiotic microorganisms. This capability is essential for identifying key genes that confer tolerance to the harsh conditions of the gastrointestinal tract, thereby improving their survival within the host. Furthermore, comparative genomics allows for the analysis of evolutionary relationships among strains, their connection to the gut microbiome, and the detection of gene acquisition or loss via horizontal gene transfer [65, 67]. Transcriptomics is widely employed to investigate host-microbe interactions, specifically focusing on elucidating how probiotics modulate the host's immune responses through changes in gene expression patterns [65, 68]. Proteomics provides comprehensive identification of critical proteins involved in the interactions between probiotics and their environment, including the intestinal tract and nutrient sources within food. A unique strength of proteomics is its ability to identify post-translational modifications (e.g., methylation, phosphorylation, or glycosylation), which can profoundly alter protein function and are frequently missed by other analytical techniques [65]. Metabolomics centers on the analysis of bioactive compounds secreted by probiotic organisms that are directly responsible for conferring health benefits to the host [65, 67]. The synergistic integration of these four major omics platforms, genomics, transcriptomics, proteomics, and metabolomics, forms the basis of Pro-biomics. This combined approach facilitates a holistic and comprehensive analysis of probiotic organisms, fundamentally deepening our understanding of their function, mechanism of action, and potential therapeutic applications [65].

5.2 Risk-Benefit Assessment of Probiotics in Functional Foods

Functional foods are traditionally understood as natural or modified food products that contain biologically active compounds (known or unknown) and that provide a clinically confirmed health benefit when consumed in defined, effective, and non-toxic amounts [69, 70]. This benefit is particularly relevant to the prevention, management, or treatment of chronic diseases [71]. Common characteristics that distinguish functional foods from standard dietary components are their consumption methods and biological effects. One of their characteristics is their dietary integration. They are incorporated into the regular diet but can also be offered in concentrated forms such as capsules or other nutritional supplements. Their key distinction lies in their capacity to beneficially influence one or more targeted physiological functions beyond the simple provision of essential nutrients [70]. This beneficial effect is likely to be associated with an improvement in overall health and well-being, or a documented reduction in disease risk. Functional foods represent a category of dietary interventions that essentially aim to manage health through targeted biological effects proactively [70, 72].

5.3 Research Gaps and Emerging Solutions

The assessment of food quality, freshness, and hygienic status fundamentally relies on accurately quantifying Biogenic Amines (BAs). Although conventional separation techniques such as High-Performance Liquid Chromatography (HPLC), Gas Chromatography (GC), and Capillary Zone Electrophoresis (CZE) are routinely employed for BA detection, they present significant practical drawbacks [73]. These methods are inherently costly, requiring access to centralised laboratories and expertise from highly trained personnel. Current analytical challenges include the necessity of optimising derivatisation reagent levels and, critically, minimising analytical time. Complex food matrices often necessitate extended chromatographic separations to effectively resolve BAs from interfering amino acids [74].

The complex matrix of food materials remains a major impediment to accurate BA quantification. While techniques like sample cleanup, preconcentration, and the use of internal standards can mitigate the "matrix effect" and allow for ultra-trace detection, these preparatory steps contribute to the overall time

consumption. Crucially, standard laboratory-based analytical methods cannot be performed outside the laboratory, resulting in a significant time lag between sample collection, analysis, and result reporting. This delay is a severe disadvantage, as it precludes timely intervention in cases of food spoilage where initial amino acid degradation may already be detectable. Consequently, there is an urgent demand for alternative methodologies that deliver rapid, reliable, cost-effective, and user-friendly devices suitable for on-site, in-field use [75].

To address these shortcomings, the development of an automated, green pre-concentration method for BAs is projected to establish a new gold standard for the food industry. Such a development would represent a significant achievement in creating an ideal "green" analytical methodology for BA detection within complex food systems. Future regulations, particularly concerning fermented products, will undoubtedly require rapid and robust analytical techniques to ensure compliance and public safety [73].

6 Conclusion

The incorporation of probiotics into functional foods has shown significant health benefits, particularly in improving gut health, modifying immunological responses, and adding to general well-being. However, the ability of several probiotic and fermentative microorganism to create biogenic amines (BAs) poses a significant food safety risk. Histamine, tyramine, putrescine, and cadaverine, among others, are not only necessary for physiological functions but, when present in high concentrations, can cause adverse effects ranging from headaches and gastrointestinal distress to hypertensive crises, allergic-like reactions, and carcinogenic outcomes. The complexity of BA production, which is influenced by strain variability, environmental parameters like pH, temperature, and salt, and raw material quality, demonstrates the multifactorial nature of this risk.

Mitigation solutions must be proactive and multidisciplinary. The selection of non-BA-producing probiotic bacteria, confirmed by genetic screening for decarboxylase genes, is critical. Complementary technological solutions, such as strict hygiene, process optimization, and the use of BA-degrading starting cultures, can further reduce risks. Advances in omics technologies provide potential methods for identifying, monitoring, and regulating BA-related pathways, allowing for more precise risk-benefit

assessments of probiotic applications. Additionally, advances in rapid, on-site BA detection tools will be critical in maintaining product safety and facilitating regulatory supervision.

Consequently, while probiotics remain valuable in functional foods, their safe application necessitates balancing known benefits with the potential risks of BA accumulation. A comprehensive, science-driven approach (covering microbiological, technological, analytical, and regulatory perspectives) will be required to ensure that probiotic-based foods remain safe, effective, and health-enhancing dietary components.

Table 11. Common Probiotic Strains Associated with Biogenic Amine Production and Their Occurrence in Various Fermented Foods

Probiotic Genus/Species	**Biogenic Amine(s) Produced**	**Fermented Food Source**	**Key Reference(s)**
Lactobacillus curvatus	Tyramine, Histamine	Fermented Sausages, Cheese	Holck et al. 2017; Barbieri et al. 2019
Lactobacillus buchneri, L. parabuchneri, L. helveticus	Histamine	Cheeses (aged)	Diaz et al. 2018; Barbieri et al. 2019
Lactobacillus brevis	Histamine, Tyramine	Wine, Cider, Cheese, Beer	Barbieri et al. 2019
Lactobacillus rossiae	Putrescine	Sourdough	Xu et al. 2020; Barbieri et al. 2019
Enterococcus faecium, E. faecalis, E. durans	Tyramine (High Producers)	Cheese, Dry Sausages, Wine	Natrella et al. 2024; Barbieri et al. 2019
Streptococcus thermophilus	Tyramine, Histamine	Yogurt	Doeun et al. 2017
Bifidobacterium spp.	Cadaverine, Tyramine (Low amounts)	Fermented Milk Products	Lorencová et al. 2012

Enterobacteriaceae spp. (Spoilage)	Histamine, Tyramine, Putrescine, Cadaverine	Fermented Fish, Meat	Ding et al. 2024; Barbieri et al. 2019

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Table 12. Types of Biogenic Amines, Their Precursor Amino Acids, and Associated Health Effects

Biogenic Amine	Precursor Amino Acid	Classification	Associated Health Effects (Exogenous Intake)	Reference(s)
Histamine	Histidine	Heterocyclic Monoamine	Vasoactive effects: Vasodilation, hypotension, headaches, flushing, allergic-like reactions ("scombroid poisoning").	(Durak-Dados et al. 2020; Sudo 2019)
Tyramine	Tyrosine	Aromatic Monoamine	Vasoconstrictive effects: Can trigger hypertensive crisis and migraines, especially in individuals on MAOIs ("cheese reaction").	(Kettner et al. 2020; Durak-Dados et al. 2020)
Putrescine	Ornithine, Agmatine	Aliphatic Diamine	Inhibits DAO, potentiating Histamine toxicity. Precursor for Spermidine/Spermine. Potential carcinogen with nitrites.	(Erdag et al. 2019; Nair et al. 2025)
Cadaverine	Lysine	Aliphatic Diamine	Inhibits DAO, potentiating Histamine toxicity. Indicator of food spoilage. Potential carcinogen with nitrites.	(Erdag et al. 2019; Shim et al. 2022)
Phenylethylamine	Phenylalanine	Aromatic Monoamine	Vasoconstrictive: Hypertension, cerebral hemorrhage. Linked to triggering migraines in susceptible individuals.	(Ding et al. 2024; Visciano et al. 2020)

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Abbreviations: DAO: Diamine Oxidase, MAOIs: MAO Inhibitors

Table 13. Health Risks Associated with Biogenic Amines: Intolerance, Toxicity, and Regulatory Guidance

Risk Component	Description/Mechanism	Toxicological Thresholds & Guidance	Reference(s)
Intolerance Mechanism	Impaired degradation of BAs, often due to low activity of detoxifying enzymes DAO or MAO, caused by genetic factors, GI pathologies, or pharmacological inhibition.	Individual tolerance varies significantly, complicating risk assessment. The body's ability to detoxify is a critical factor.	(Kettner et al. 2020; Erdag et al. 2019)
Acute Toxicity Thresholds	The ingestion of more than 8 mg of Histamine is capable of causing acute intoxication. Tyramine-rich foods pose a severe risk to individuals on MAO inhibitor therapy.	Histamine in Fish (EU): Specified maximum levels exist for certain fish products (e.g., tuna) but no single global legal standard for BAs in all foods.	(Durak-Dados et al. 2020; EFSA 2011; Barbieri et al. 2019)
Chronic/Long-term Risks	Certain polyamines (Putrescine, Cadaverine, Spermidine) can react with nitrites to generate carcinogenic compounds. High concentrations of polyamines can also suppress cellular proliferation and induce apoptosis.	Quality Indices (BAI): Used as a proxy for safety/freshness. Meat: BAI (Good Quality); (Spoiled).	(Nair et al. 2025; Ruiz-Capillas and Herrero 2019)
Drug Interactions	MAOIs: Medications used for depression/anxiety. Consumption of Tyramine-rich foods can precipitate a life-threatening Hypertensive Crisis due to blocked MAO activity.	Alcohol: Impairs the body's ability to detoxify BAs, potentially exacerbating symptoms.	(Kettner et al. 2020; Barbieri et al. 2019)

Abbreviations: BA; Biogenic Amine, DAO: Diamine Oxidase, MAO: Monoamine Oxidase, MAOIs: MAO Inhibitors, GI: Gastrointestinal

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Table 14. Strategies for Mitigating Biogenic Amine Formation in Probiotic Foods

Strategy Type	Approach/Technique	Mechanism of Action	Reference(s)
Genetic/Strain Control	Non-BA-Producing Strain Selection	Rigorous in vitro screening and genetic analysis (checking for genes) to create a detailed safety profile before application.	(Fentie et al. 2024; Barbieri et al. 2019)
Technological Control (Raw Material)	Strict Hygiene & Quality Control	Selecting high-quality, uncontaminated raw materials; this is the fundamental step to control amine-producing microbial strains (e.g., *Enterobacteriaceae*).	(Gardini et al. 2016; Özogul et al. 2018)
Technological Control (Fermentation)	Optimization of Process Parameters	Rapidly lowering pH below (inhibits decarboxylase growth/activity). Controlling temperature to avoid the optimal range for amine production.	(Visciano et al. 2020; Gardini et al. 2016)
Technological Control (Post-Processing)	Physical/Chemical Methods	MAP (suppresses histamine formers). Irradiation (Gamma rays reduce BAs in fish/soybean paste). Smoking (creates aseptic qualities, inhibiting bacteria). Oxidizing Agents (break down existing BAs).	(Doeun et al. 2017; Ozoğul et al. 2006)
Biological Control	Use of Specific Starter Cultures	Strategic use of strains that either lack decarboxylase activity or contain enzymes capable of oxidizing and breaking down existing BAs in the food matrix (BA-degrading strains).	(Gardini et al. 2016; Doeun et al. 2017)
Future/Analytical Focus	Omics & On-Site Detection	Integrating Genomics/Metabolomics for holistic BA monitoring. Developing rapid, cost-effective, on-site analytical devices to overcome the time lag of traditional lab methods (HPLC/GC).	(Verma et al. 2025; Ahmad et al. 2019)

Abbreviations: BA; Biogenic Amine, MAP: Modified Atmosphere Packaging, HPLC: High-Performance Liquid Chromatography, GC: Gas Chromatography

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Sustainability Assessment of Natural Fibers Used in the Textile Industry: A Literature Review

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Abstract. The textile industry uses natural or synthetic fibers, or both, as raw materials. Global climate change affects the production potential of natural fibers by increasing pressure on air, soil, and water resources. Consequently, the production of these fibers, which the sector requires, faces significant sustainability challenges. This literature review comparatively examines the natural fibers commonly used in the textile sector—cotton, jute, flax, and wool—based on environmental, economic, and social sustainability criteria. Each fiber's production process was evaluated, along with factors such as water consumption, chemical input requirements, carbon and water footprints, biodegradability, soil health impacts, and production conditions. The findings indicate that the sustainability performance of fibers varies significantly depending on fiber type, production method, and geographical conditions. This review aims to assess the impact of natural fibers on sustainable fashion and circular textile systems and to provide guidance for future research and industrial applications.

Keywords: Natural Fibers, Global Climate Change, Water Footprint, Carbon Footprint

1 Introduction

Textile industry, which meets the most important basic need in human life after food and water (Madhav et al., 2018; Mostafizur Rahman et al., 2023), covers an integrated process that includes a series of complex stages such as yarn production using natural and/or artificial fibers, weaving, knitting, washing, bleaching, dyeing-printing, finishing and apparel (Turkes et al., 2024). The textile industry, which provides employment to tens of millions of people worldwide (Harsanto et al., 2023), produces products with various properties for use in agriculture, automotive, construction, geotextile, industrial, medical, sports, etc. fields (Sun et al., 2018; Roy et al., 2020; Harsanto et al., 2023). As one of the largest manufacturing industries in the world economy, the sector is expected to reach a turnover of 2.25 trillion

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US dollars in 2025 (De Felice et al., 2025). The textile and apparel industries cause a range of environmental problems, including chemical use, high water/energy consumption, air pollution, solid waste, and odor generation at every stage of production, from the cultivation of raw materials to the disposal of finished products (Islam et al., 2022). Fiber production accounts for approximately 12% of the total environmental impact of textile products (Sigaard and Laitala, 2023).

The United Nations Environment Programme (UNEP)'s sustainable development goals, which provide global guidance and cooperation to address significant environmental and socio-economic challenges, such as achieving sustainable development for the world, combating climate change, reversing environmental degradation, and managing resource use for a growing population, and to implement transformations and actions on the ground, provide a path to follow to shape a sustainable future for the world (Cordella et al., 2023).

Considering the textile sector's employment, economic scale, and environmental impacts, evaluating resource use in production is crucial for the sector's sustainability. The main raw materials of the sector are natural and synthetic fibers (Nayak et al., 2023). Global synthetic and natural fiber production increased from 57 million tons in 2000 to 111 million tons in 2020. It is expected to reach 145 million tons by 2030 (Periyasamy and Tehrani-Bagha, 2022). The declining global reserves of petroleum, the raw material for synthetic fibers, its exorbitant prices, and its environmental impact are accelerating the shift towards green products as a fiber source (Dhir, 2022). Rising environmental concerns and the depletion of petroleum resources are increasing the importance of natural fibers and encouraging researchers and industries to use sustainable fibers instead of traditional synthetic fibers (Ahmed and Mondal, 2021). Natural fibers are primarily obtained from plants and animals. Additionally, natural mineral fibers with specialized uses are considered in this category, but their use is limited (Jabbar and Shaker, 2016). The classification of some natural fibers of plant and animal origin used in the textile industry is presented in Table 1 (Jabbar and Shaker, 2016; Nayak et. al., 2023).

Table 15. Classification of natural fibers

Natural Fibres

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From Plants				From Animals	
Seed Fibres	**Leaf Fibres**	**Grass Fibres**	**Bast Fibres**	**Wool and Animal Hair**	**Silk Fibres**
Cotton	Sisal	Bagasse	Flax	Sheep	Bombyx
Coir	Pineapple	Bamboo	Jute	Alpaca	Eri
Oil Palm	Banana	Canary	Hemp	Angora	Muga
		Corn	Ramie	Camel	Mulberry
		Sabai	Sisal	Cashmere	Tussar
				Rabbit	Spider Silk
				Yak	

Natural fibers are used in apparel and technical textiles across various sectors. However, they also exhibit negative characteristics that limit their application in textiles, including their hydrophilic structure, swelling due to water absorption, increased susceptibility to microbial attack, poor mechanical strength, lower color fastness, and non-uniform distribution compared with synthetic fibers (Ahmed and Mondal, 2021). Furthermore, the use of plant-based natural fibers such as cotton, linen, sisal, hemp, jute, and pineapple is more environmentally friendly than synthetic fibers due to their renewability, biodegradability, better specific properties, non-corrosiveness, natural availability, and photosynthetic activity during cultivation (Nayak et al., 2023). These characteristics necessitate consideration of the limitations of natural fibers as well as the opportunities and risks they present for environmental sustainability.

This study aims to examine the role of natural fibers in sustainable fashion and circular textile systems and to assess their environmental advantages and disadvantages. In this context, the production processes for cotton, linen, jute, and wool, the most commonly used natural fibers in textiles, were evaluated on the basis of data from the literature with respect to parameters such as water and energy consumption, levels of chemical use, carbon and water footprints, biodegradability, soil health impacts,

and renewability. The study employed a comparative analysis method, and the contributions of different fiber types to sustainable fashion and technical textile applications were discussed from the perspective of environmental compatibility, product life cycle, and circular economy.

2 Material and Method

An effective literature review provides a solid foundation for the development of knowledge. It supports the development of a theoretical framework, reveals intensively studied areas, and clarifies topics requiring further research (Hussain and Wahab, 2018; Leite et al., 2019). When a topic is approached and logically integrated on the basis of the reviews and findings from previous studies, further progress can be made in that field (Yıldız, 2022). In this study, the environmental impacts and sustainability dimensions of the production processes for natural fibers, the basic raw materials of the textile industry, were assessed using environmental metrics. Environmental metrics are quantitative measurements used to assess the environmental impact of human activities (Zarea et al., 2019; Mirmoradi et al., 2025). These metrics help track trends, evaluate performance, and guide decision-making processes toward more sustainable practices and policies (Alishah et al., 2019; Mirmoradi et al., 2025). Monitoring these environmental metrics can help identify areas for improvement, set reduction targets, and monitor progress toward sustainability goals (Mirmoradi et al., 2025). In this study, carbon footprint and water footprint, widely used environmental metrics that reflect the impact of human activities on the planet, were used to assess the sustainability of fibers. Furthermore, various environmental metrics used in the literature on the sustainability of natural fibers are included for informational purposes. This study investigates the sustainability of natural fibers and uses data from the literature. For this purpose, 157 articles published in online databases and high-impact research journals were searched, and after reviewing their abstracts, information was collected from 68 research articles and several reports and book chapters. The keywords used to search the databases were as follows: textile, textile fiber, cotton, linen, jute, wool, water footprint, carbon footprint, sustainability in agriculture, agricultural inputs for natural fibers, waste minimization in agriculture, and pollution prevention in agriculture.

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3 Results and Discussion

3.1 . Global Status of Fiber Use

According to Textile Exchange's latest Materials Market Report, global fiber production reached an all-time high of 124 million tons in 2023. Natural fibers constitute approximately one-quarter of this amount. The percentage distribution of global fiber production from the 2024 Textile Exchange Materials Market Report is shown in Figure 1, and the fiber production and forecasts for 1975–2030 are presented in Figure 2 (Textile Exchange Materials Market Report, 2024).

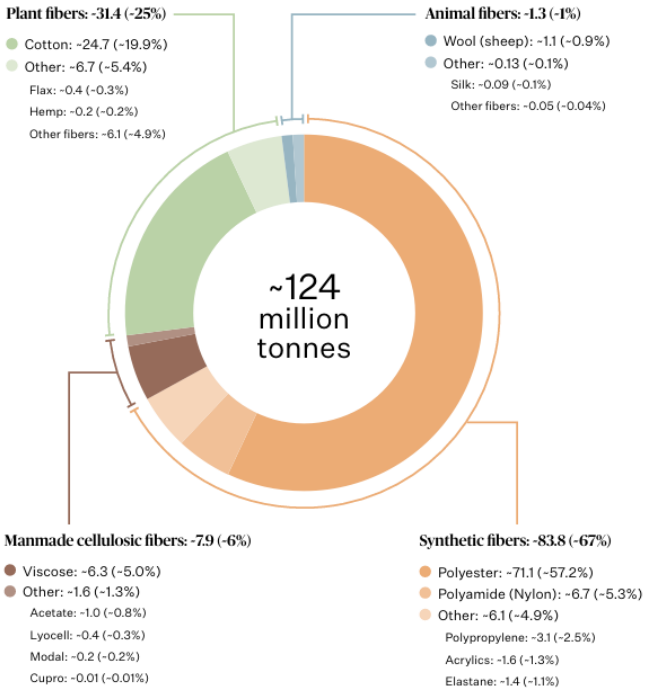

Fig. 20. Global fiber production in 2023 (in million tonnes and % of global fiber production)

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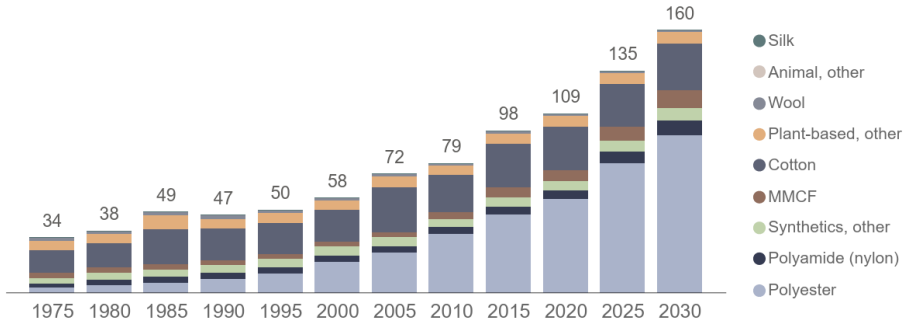

Fig. 21. Global fiber production (million tonnes)

An examination of Figures 1 and 2 reveals that cotton has by far the largest share among natural fibers, accounting for approximately 20% of total global fiber production. Other plant fibers, such as flax, jute, hemp, and sisal, account for approximately 2–3% of the total, while animal fibers, such as wool, silk, and mohair, account for approximately 1%. These fibers play a significant role in textile and technical textile applications owing to their properties, such as comfort, moisture absorption, breathability, and biodegradability. The production of natural fibers is affected by environmental factors such as climate conditions, agricultural productivity, water use, and carbon emissions, and these factors impact the environment (Gonzalez et al., 2023), necessitating their evaluation from both an economic and an ecological perspective.

3.2 Environmental Sustainability Assessment of the Natural Fibers Considered in the Study

Considering the data in Figures 1 and 2, this study evaluated the sustainability and environmental impacts of natural plant fibers such as cotton, linen, and jute, and animal fibers such as wool, all used in the textile industry. General information on these fibers is provided below under various headings.

Cotton

Cotton (Gossypium spp.), although botanically a perennial plant, is generally grown as an annual in agricultural production and is the world's most important source of fiber. Cultivated primarily for fiber production, cotton also contributes to animal feed through the oil obtained from its seeds and the oil

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cake remaining after extraction (Constable and Bange, 2015; Huang et al., 2022). The production cycle of cotton is 150–180 days (Dristy et al., 2024). It is estimated that cotton, cultivated on an area of 31.92 × 10^6 ha in approximately 80 subtropical and tropical countries, has an annual turnover of US$5.68 billion (Vitale et al., 2024). In areas with irrigation facilities, Cotton yield averages 800 kg/ha and increases by 10–20 kg/ha annually (Constable and Bange, 2015). The cumulative energy requirements for producing 1 kg of cotton fiber (growing) and 1 kg of textile (yarn) are 68.5 MJ and 368 MJ, respectively (La Rosa and Grammatikos, 2019). Cotton accounts for one-fourth of the global fiber market (Yu and Yang, 2025). Approximately 25 million tons of cotton are produced annually worldwide. The top ten cotton-producing countries are India, China, the United States, Pakistan, Brazil, Australia, Uzbekistan, Türkiye, Turkmenistan, and Burkina Faso (Khan et al., 2020). The harvesting of some traditional natural fibers, such as cotton, requires substantial amounts of water and pesticides (Nayak et al., 2023), thereby necessitating an assessment of the environmental impacts of cotton production. Given the socioeconomic value of cotton, it has been reported that it can help reduce extreme poverty (SDG 1) and achieve the Zero Hunger target (SDG 2) of the UNEP 2030 Sustainable Development Goals (SDGs) (Kang et al., 2023). The results of the literature review on the environmental impacts of cotton production are presented in Table 2.

Table 16. Environmental impacts of cotton production

Cultivation Area	Green Water Footprint (m^3/ton)	Blue Water Footprint (m^3/ton)	Total Water Footprint (m^3/ton)	Ecological Footprint (m^2·anual/ton)	Carbon Footprint	Source
Türkiye	205	1,641	1,846		715	Muratoğlu (2024) Yu and Yang (2025)
Greece	~320	~1,700	2,020			Tsakmakis et al. (2018)
Kazakhstan	962	1,462	2,424			Chapagain et al. (2006) Yu and Yang (2025)
Kyrgyzstan	665	2,384	3,049			
Tajikistan	388	5,858	6,246			

Turkmenistan	191	6,875	7,066		971	
Uzbekistan	255	4,171	4,426		1,021	
USA	1,673	576	2,249		904	Chapagain et al. (2006) Yu and Yang (2025)
Brazil	2,575	46	2,621		693	
Benin					303	
Pakistan	1,054	3,860	4914		1,366	
India	6,512	2,150	8662		1,080	
China	1,258	760	2018		747	
Egypt		2,171.8			2,622.6	Mehmeti et al. (2024)
		2,070		31,320	2,950	Korol et al. (2020)

As shown in Table 2, the impacts of cotton grown in different regions, such as the water and carbon footprints, vary. Therefore, planning for the sustainability of cotton production should be conducted on a global scale while considering local resources. The ecological footprint assessed in the study by Korol et al. (2020) estimates the amount of biologically productive land and water surface required to compensate for the resources consumed for consumption, development, treatment of some waste, storage of other waste, and absorption of emissions from fossil fuel and nuclear energy consumption. Thus, the impact of cotton on productive land and water surface per unit of production can also be quantitatively assessed. The values of 1 ton of cotton textile production in the environmental impact categories of Water Resource Depletion, Acidification, and Eutrophication were reported as 1,736 m^3 water eq, 165 kgSO_2 eq, and 70.84 kg PO_4 eq, respectively (La Rosa and Grammatikos, 2019).

Flax.

Flax (*Linum usitatissimum L.)* is an economically important fiber crop cultivated for both its long, cellulose-rich fibers and its seeds. Archaeological findings from the Neolithic period reveal the use of flax fiber in early cultures. It is also known that the plant was cultivated in Egypt approximately 6,000

years ago (Chabi et al., 2023). It has also been reported that flax was used as a wound dressing in ancient times (Gębarowski et al., 2022). Flaxseed contains 37–42% oil and is classified as a drying oil due to its high alpha-linolenic acid content. It is beneficial to human health due to its content of omega-3 fatty acids, lignans, and fiber (Zare et al., 2021). Flax fibers are an important raw material for industrial applications such as textiles, paper, and composite materials (Huang et al., 2021). Whether the primary selection criterion in flax breeding is based on seed oil content or stem fiber yield remains a matter of debate (Chabi et al., 2023). The flax production cycle is 90–120 days (Fenart et al., 2010). Currently, flaxseed is produced primarily in Eastern Europe, Central Asia, and North America, and the main producers are the Russian Federation, Kazakhstan, Canada, and China. Cultivated areas in Eastern Europe increased by 74.6% between 2011 and 2021. The highest yields are achieved in France (1.9 tons/ha), representing a threefold increase since the early 1970s. Europe is the world leader in flax fiber production, with France as the leading producer. The highest quality fiber is produced in an area extending between France and the Netherlands due to the mild climate and the availability of well-drained land. The area under fiber flax cultivation is increasing annually in Europe. Because every part of the flax plant can be used in many areas, such as animal feed, oil production, textiles, paper, heating sources, chipboard production, etc., flax is a waste-free product (Moyse et al., 2023). The energy efficiency of flax fiber production was found to be the lowest among those of jute, kenaf, and sunn hemp fiber production. It has also been reported to consume more chemical fertilizers, diesel fuel, pesticides, and seed energy than other fibers. Fertilization and fiber processing are estimated to be the largest contributors to greenhouse gas emissions (Singh et al., 2018).

A literature review on the environmental impacts of flax production found that the green, blue, and total water footprints are 2,637, 443 and 3,481 m^3/ton, respectively (Mekonnen and Hoekstra, 2011). Carbon footprint has been calculated at different values in different studies as 520 kg CO_2eq/ton (Singh et al., 2018), 1,100 kg CO_2eq/ton (Dissanayake et al., 2009).

Jute.

Jute (*Corchorus* spp.) is a herbaceous, deciduous, perennial plant that generally grows up to 1.5 meters tall. Jute plants cultivated for fiber can grow up to 4 meters tall (Rahman and Rahman, 2024). The jute production cycle is 110–120 days (Ahmed et al., 2023). Jute fibers have been used throughout history for the production of rope, clothing, and sacks (Jarin et al., 2024). Today, they are used across various fields, including the textile industry, packaging materials, geotextiles, composite materials, paper production, food, medicine, and construction (Rahman and Rahman, 2024). Jute is known as the "golden fiber" due to its color and significant material value, and it is the most important and cheapest textile fiber after cotton (Gangarde et al., 2024). India, Bangladesh, China, Myanmar, Nepal, and Thailand are the primary jute producers in the world (Islam and Alauddin, 2012). India is the largest producer of jute, and with technological advances, jute productivity has increased from 1.04 tonnes/ha to 2.56 tonnes/ha since independence (Kar et al., 2023). The cultivation phase of jute fiber production has the most significant environmental impact. This impact becomes particularly evident when assessed in terms of carbon footprint. Fertilizer and pesticide use in the production process accounts for approximately 23% of the total environmental impact. This stage, which typically involves the highest level of mechanization, uses electrically powered machinery and equipment to process the fibers. Furthermore, because irrigation is included in the cultivation phase, the cultivation phase is the largest contributor to the total water footprint of jute production. In contrast, the transportation process, which does not require specialized equipment, has the lowest environmental impact (Korol et al., 2020).

A literature review on the environmental impacts of jute production has yielded impact assessments in different categories. In the water footprint assessment, the blue water footprint was reported as 1,550 m^3/ton and the ecological footprint as 4,730 m^2·annual/ton (Korol et al., 2020). The carbon footprint was calculated to be 565 kg CO2eq per ton (Singh et al., 2018). The cumulative energy requirements for producing 1 kg of jute fiber (cultivation) and 1 kg of jute textile (yarn) are 29.55 and 97 MJ, respectively. The values in the environmental impact categories of Water Resource Depletion, Acidification, and Eutrophication were reported as 188 m^3 water eq/ton, 41 kgSO_2 eq/ton, and 14.93 kg PO_4eq/ton, respectively (La Rosa and Grammatikos, 2019).

Wool (Sheep).

Wool is a proteinaceous fiber sheared from sheep. Wool fibers have unique biological, physical, and chemical properties and are becoming increasingly important as demand for natural products increases (Zhou et al., 2024). Wool is a renewable resource, and the average sheep produces 2.3 to 3.6 kg of raw wool per year (Koyuncu, 2024). 4.5 kg of wool is equivalent to more than 10 meters of fabric. This amount of fabric is sufficient to make six sweaters; three suits and trousers; or a large sofa cover (International Wool Textile Organization, 2024). It is valued for its insulation, moisture-buffering properties, flame resistance, and biodegradability (Corscadden et al., 2014). These properties stem from its unique fiber architecture and specialized protein composition, which distinguish it from other natural and synthetic fibers. According to data from the International Wool Textile Organization (IWTO), the number of sheep is reported to have reached 1.296 billion head in 2022, with greasy wool production reaching 1977.3 million kilograms and clean wool production reaching 1051.2 million kilograms (Zhou et al., 2025). China has the largest sheep population. Other leading sheep producers include India, Australia, Mongolia, Türkiye, the United Kingdom, South Africa, and New Zealand (International Wool Textile Organization, 2024).

A literature review of the environmental impacts of wool production yielded assessments across various categories. Research indicates that preparing each ton of wool fiber consumes 2.3–2.5 tons of water (Duan, 2010; Li et al., 2022). Various studies have reported that the carbon footprint of wool is between 10 and 70 kg CO2eq/kg (Brock et al., 2013; Wiedemann et al., 2015; Wiedemann et al., 2016; Dougherty et al., 2018; Bech et al., 2019; Bianco et al., 2023). The cumulative energy required to produce 1 kg of belly wool fiber is 91.8 MJ. The values in the Water Resource Depletion, Acidification, and Eutrophication environmental impact categories have been reported to be 12.7 m^3 water eq, 1.59 mol H^+ eq, and 0.0171 kg PO4eq, respectively (Bianco et al., 2023).

3.3 Discussion and General Evaluation

Natural fibers play a central role in the textile industry's sustainability transformation due to their renewable origins, biodegradability, and lower carbon footprint than that of synthetic fibers. According to life cycle assessment (LCA) studies, bast fibers such as flax, hemp, and jute typically have 20–50% lower carbon footprints than those of synthetic fibers such as polyester or nylon (Singh et al., 2018). On the other hand, despite being noted for its durability and low production costs, polyester contributes significantly to terrestrial ecotoxicity and microplastic pollution. Recycled polyester fibers have been reported to release approximately 2.3 times as many microplastics as virgin polyester fibers due to thermal degradation during the recycling process (Özkan and Gündoğdu, 2021).

While the environmental performance of natural fibers is lower than that of synthetic/polyester fibers, it is not uniform; it varies significantly depending on agricultural inputs, regional growing conditions, and processing technologies.

Among bast fibers, jute offers significant environmental advantages over conventional cotton. Jute cultivation requires significantly less irrigation and fewer chemical inputs and achieves lower values in environmental impact categories such as human toxicity, eutrophication, and acidification (Bhalla et al., 2018; Gonzalez et al., 2023). Similarly, flax stands out as a sustainable alternative, requiring less water and energy in production processes while providing higher yields per hectare (Moazzem et al., 2021). To better utilize the agricultural and nutritional potential of flax, brown-seeded genotypes are recommended for cultivation under water-stress conditions (Zare et al., 2021). Because the environmental burden of flax production is particularly concentrated during the agricultural phase, fertilizer and pesticide use, as well as fuel consumption for agricultural machinery, accounts for a large portion of these impacts.

Although cotton is one of the most important textile crops globally, it contributes significantly to the depletion of freshwater resources and to greenhouse gas emissions due to its dependence on intensive irrigation and nitrogen fertilizers (Yu and Yang, 2025). Studies show that modern irrigation technologies, such as drip irrigation, can reduce the total water footprint by 5–12% compared to

traditional sprinkler systems (Tsakmakis et al., 2018). Similarly, increasing nitrogen use efficiency and using renewable energy in agricultural equipment can significantly reduce the carbon footprint of cotton production. Ammonium nitrate production and its use for fertilizing plants has been reported to be the most significant contributor to global warming potential (Le Duigou et al., 2011). However, both the heavy reliance on herbicides and pesticides and the environmental risks associated with genetically modified (GM) cotton still pose significant sustainability challenges (Vitale et al., 2024).

Wool, an animal-derived fiber, has higher environmental impacts per unit of fiber than those of plant fibers. This is particularly due to processes associated with animal production, such as feed production, methane emissions, and pasture use.

Therefore, sustainability assessment should not only consider the carbon and water footprints but also focus on factors that occur throughout the life cycle, such as biodegradability, recyclability, and social and ethical impacts. As emphasized by Sandin et al. (2019), there is no “inherently sustainable or unsustainable” fiber type; sustainability depends on context-specific management styles and decisions made throughout the life cycle. New materials, such as bio-based fibers derived from citrus pulp, mushrooms, or industrial waste, have the potential to expand the range of sustainable textiles; however, the lack of standardized LCA data on this topic hinders comparative analyses.

4 Conclusion

This review has revealed that natural fibers generally have lower carbon and water impacts than synthetic fibers; however, their sustainability performance is closely linked to cultivation methods, input intensity, and processing. Among plant fibers, flax, hemp, and jute have the most positive environmental profile, while traditional cotton and silk have the most negative impacts due to their high resource use and environmental burden. Methods such as precision agriculture practices, use of organic fertilizers, integration of renewable energy, and environmentally friendly flax processing technologies (e.g., bioretting) can significantly reduce the environmental footprint of natural fiber production.

However, achieving sustainability in the textile industry is not possible solely through correct fiber selection. True sustainability requires a holistic lifecycle approach encompassing all stages from raw material procurement to the end of the product's lifecycle. Increasing product durability, developing recycling systems, and making decisions based on accurate environmental data are key elements of this transformation. Consequently, the transition to sustainability in the textile industry requires both technological innovations and systemic transformations throughout the supply chain, placing natural fibers at the center of a circular and low-impact textile economy.

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I-CRAFT AGRICULTURAL and FOOD TECHNOLOGIES

Table, Dried and Wine Grape Production and Potential in Southeastern Anatolia

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Abstract. In this study, structural changes in grape production in the Southeastern Anatolia Region and throughout Türkiye were analyzed based on data from 2004, 2014, and 2024. All grape varieties commercially cultivated in Southeastern Anatolia were considered in the study. A significant decline in vineyard areas has been observed across the Southeastern Anatolia Region between 2004 and 2024. For example, vineyards for seedless raisins decreased from 6,250 decares in 2004 to 150 decares in 2024, while production decreased from 4,750 tons to 90 tons. Similarly, table grape vineyards decreased from 12,830 decares to 4,300 decares, and production volume peaked at 7,644 tons in 2014 before declining to 4,850 tons in 2024. Despite the decline in vineyard areas in the region, there have been significant increases in productivity. The yield per decare for wine grapes rose from 184 kg in 2004 to 560 kg in 2024, more than tripling. Similarly, productivity for dried seedless grapes increased from 345 kg/da to 569 kg/da. When evaluated at the provincial level, there has been a notable increase in wine grape production in Adıyaman. The production area, which was 100 decares in 2014, increased to 1,500 decares in 2024, while the production volume increased from 75 tons to 750 tons. In Gaziantep, the area dedicated to dried seedless grapes has undergone a substantial decline, from 121,830 decares to 48,081 decares. However, it has been determined that the production amount has undergone an increase from 28,920 tons to 42,307 tons, a development that can be attributed to the enhancement of grape yield per unit area. In contrast, it is observed that a significant proportion of the total vineyard areas in Şanlıurfa (95%), Siirt (50%), and Gaziantep (44%) have been lost. In particular, the production of dried seedless grapes in Şanlıurfa fell from 31,054 tons in 2004 to only 815 tons in 2024. A similar trend is observed across Türkiye. The area under dried seedless grapes decreased from 750,000 decares in 2004 to 491,612 decares in 2024, while production fell from 350,000 tons to 317,053 tons. Although there has been a partial increase in yield (from 529 kg/da to 682 kg/da) in the cultivation of wine grape varieties across the country, the decline in vineyard areas has negatively affected the production of wine grape varieties. In this context, the aim of the study is to determine the potential for grape production for different commercial purposes in the region, identify structural changes in regional viticulture, and establish future production strategies. In conclusion, it has been determined that vineyard cultivation in the Southeastern Anatolia Region is undergoing a complex transformation process in which the decline in production areas and partial increases in productivity are intertwined. In this situation, the need to restructure regional agricultural policies and revise production strategies with a focus on productivity is considered to be of paramount importance.

Keywords: Grape production trends, Agricultural productivity analysis, Southeastern Anatolia agriculture, Cultivation area reduction, Turkish viticulture sector

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1 Introduction

Viticulture in Anatolia has a deep-rooted history dating back to prehistoric times. Findings from the Hittite period, such as a solid gold wine jug and goblet dating to around 3000 B.C. and exhibited in the Museum of Anatolian Civilizations (Deliorman et al., 2011), provide strong evidence of the ancient presence of the grapevine in Anatolia. For example, the Hittite hieroglyphs on the rock reliefs of Konya Ereğli İvriz, dating back to the 8th century BC, contain the following words: "I am Warpalawas, the ruler and hero King of Tuwana. When I was a prince in the palace, I planted these vines. May Tarhunzas grant them abundance and prosperity." (Dilay, 2018) reveal the ancient history of viticulture in the region. While depictions of vines, grapes, and wine are frequently seen in Hittite works, the texts also mention the words vine (tuwarsa), grape (geštın), dried grape (geštın hád.du.a), and wine (wiyana) (Kiracı et al., 2024). According to the provisions of the law, viticulture was one of the fundamental sources of the Hittite economy (Ünar, 2019). Furthermore, ancient historians accept that Dionysus, the protector of vineyards in Greek mythology, came to Greece from Anatolia. In this context, the geography of Anatolia stands out as one of the oldest agricultural centers in human history due to its historical and economic importance in terms of viticulture (Sağlam and Çalkan Sağlam, 2018). Similarly, the Southeastern Anatolia Region, located in northern Mesopotamia, is one of the places where agriculture first began and has a long history of viticulture. Archaeological excavations indicate that viticulture in the region (Nevali Çori, Çayönü Höyüğü, Titriş Höyük, etc.) dates back to as early as 7000 BCE (Öz, 2011; Mertol and Keskin, 2022). Grape seeds, industrial wine production facilities, and wine storage containers have been found in ancient settlements, particularly around Şanlıurfa, Diyarbakır, Mardin, Adıyaman, and Gaziantep. In ancient times, during the Assyrian, Sumerian, and Hittite periods, grapes were consumed as fresh fruit and also used to make wine, vinegar, and dried fruit. During the Roman and Byzantine periods, viticulture became more systematic and developed into a commercial activity. So much so that coins with grapevine motifs were minted during the Byzantine period. During the Ottoman period, viticulture became one of the primary sources of income for the rural population in southeastern Anatolia. Small vineyards were established in the courtyards of homes, and traditional food products such as grape molasses, pestil, bastık, and sucuk were produced from grapes (Oraman, 1965).

The Southeastern Anatolia Region's climate, in terms of geographic and climatic suitability, is highly suitable for viticulture. Hot, dry summers and mild winters allow vineyards to produce high yields. The region's soil structure, low humidity, and long sunshine hours enhance both the quality and longevity of the grapes. The provinces of Gaziantep, Şanlıurfa, Mardin, Adıyaman, Diyarbakır, and Batman, in particular, are still areas where viticulture is intensively practiced. Gaziantep, Diyarbakır, and Mardin are among the top ten grape-producing provinces in Türkiye (Metin and Gündüz, 2024). Various researchers have identified numerous local grape varieties in the region (Kısakürek, 1950; Gürsöz, 1993; Kaplan, 1994; Karataş, 2005; Karataş et al., 2016a). Some of these are; Abderi, Ağek, Asuri, Azezi, Bağılti, Bakari, Balma, Bastık Kabcığı, Belbezık, Belülük, Benitaht, Besni, Bizdok, Boğazkere, Cibin, Çiloreş, Deyvani, Dımışkı, Dökülgen, Eşgar, Habo, Hasani, Hatun Parmağı, Hazirani, Horoz Karası, Hönüsü, Karakurutma, Karfoki, Karik, Kızıl Banki, Kilis Karası, Kohar, Mazrone, Mevji, Mikeri, Muhammedi, Tahannebi, Tayfi, Tilgören, Tümbo, Vanki, Reşek, and Zeyti. Many synonyms and different types of these varieties are frequently encountered in the region (Polat, 2016; Yalçın, 2021; Koyuncu, 2025). On the other hand, some of these varieties have been registered as standard grape varieties (Çelik, 2002). While some of these varieties are suitable for multi-purpose commercial use, others are only suitable for one type of commercial use (Karataş et al., 2010). However, some varieties are also used in small family businesses for the production of various food products and wine using traditional methods. Considering its economic and social contributions, viticulture is more than just an agricultural activity in the Southeastern Anatolia Region; it is one of the cornerstones of rural development. Grape production provides income to thousands of families, both directly and indirectly. In particular, small family businesses secure their livelihoods by selling grape products in local markets. Value-added products such as grape molasses, dried fruit rolls, grape paste, and walnut-stuffed sausage occupy an important place in both the domestic market and exports. In recent years, with the establishment of cooperatives and support for women producers, viticulture has become more organized and sustainable. The active participation of women in the production process has had a positive impact on the region both economically and socially. In addition, grapes and grape products play an important role in cultural promotion through gastronomic tourism and local festivals.

In terms of the current state of viticulture and future prospects, viticulture in Southeastern Anatolia is being modernized with the support of modern agricultural techniques in an effort to make it more efficient (Odabaşıoğlu, 2020). Innovations such as drip irrigation systems, organic farming practices, and methods of combating diseases and pests are increasing the productivity of producers. In addition, the increase in the number of geographically indicated products obtained through the processing of grapes and raisins (Adıyaman Besni Grapes, Antep Karası Dry Grapes, Gaziantep Koruk Ekşisi, Diyarbakır Boğazkere Grapes, Ömerli Karfoki Grapes, Midyat Zeynebi Grapes, etc.) is raising the brand value of the region's grape varieties. Thanks to projects supported by the Ministry of Agriculture and Forestry and local administrations, young farmers are encouraged to take up viticulture, thereby helping to prevent rural migration. Collaborations with universities and research institutes are of great importance for the preservation and development of grape varieties unique to the region. Considering the diversity and uses of seeded grape varieties, the Southeastern Anatolia Region has unique potential for the cultivation of seeded grape varieties due to its climatic and soil characteristics. On the other hand, despite having some local seedless grape varieties such as Hönüsü, the region does not have the same potential for seedless grape production. The grape varieties cultivated for table use in the region are generally characterized by their large, sweet berries and sparse clusters. Among table grapes with seeds, varieties such as Horoz Karası, Tahannebi, and Hatun Parmağı, which are unique to the region, are popular among consumers for their distinctive flavors. These grapes are consumed fresh, used in breakfast dishes, or incorporated into various desserts. Seedless table grapes, on the other hand, offer significant convenience for families with children and consumers who prefer not to remove seeds. These varieties are preferred in salads, fruit platters, and light snacks. Dried grape cultivation also plays an important role in the region's economy. The hot and dry summers of Southeastern Anatolia provide ideal conditions for grapes to dry naturally. Indeed, Besni, one of Türkiye's most important seedless dried grape varieties, is grown in Adıyaman and dried in the same location, marketed as a geographically indicated product. The Boğazkere grape variety, renowned worldwide and valued for winemaking, originates from the province of Diyarbakır in the region and is widely cultivated in the province's vineyards. Varieties such as Şire (Bastık Kabarcığı), Mazrone, and Azezi are grape varieties with high sugar content that are preferred especially in the production of pestil and pekmez. Dried grapes occupy

an important place in both local markets and international trade thanks to their long shelf life and nutritional value. Indeed, Türkiye is one of the world's leading producers of both seedless and seeded dried grapes. Nearly all of Türkiye's grape export revenue comes from dried grape exports, and the seeded dried grapes produced in the region hold an important place in this market. Wine grape cultivation is another important area that increases the region's potential. These ancient lands of Anatolia, which have been the center of wine production since ancient times, enable the production of high-quality wines with grapes that have different terroir characteristics (Anlı, 2006; Atilla, 2006; Çelik et al., 2000; Doğer, 2004). Some local grape varieties in the region, such as Antep Karası and Mazrone, produce distinctive and aromatic wines when used in winemaking. Çelik et al. (2005), reported that the region's arid areas, with their cool nights during the hot and dry summer growing season, provide sufficient sugar accumulation in the grapes (20–23% in white varieties, 22–25% in black varieties), high acidity, and aromatic and tannin content for the production of quality wine. The province of Mardin has great potential for marketing traditional Syrian wines (Demiray, 2022). In recent years, boutique wine producers have also become increasingly interested in the region, and the potential of local varieties has begun to be explored using modern winemaking techniques. This situation also contributes to the development of the region in terms of wine tourism. The aim of this study is to determine the current status and potential of viticulture in the Southeastern Anatolia Region and to establish a future grape production strategy for the region. Research and genetic resource studies conducted to preserve and develop the grape diversity of the Southeastern Anatolia Region are of critical importance for passing on the region's rich viticultural heritage to future generations. Adapting farmers in the region to modern production techniques and diversifying value-added products will further enhance the Southeast Anatolia Region's grape cultivation potential. With this diversity and historical legacy, the region will continue to strengthen its position as one of Türkiye's key viticulture centers.

2 Material and Method

The study was conducted using data from the Turkish Statistical Institute (TÜİK), data from the Provincial and District Directorates of the Ministry of Agriculture and Forestry, Annual Reports of the

Ministry of Agriculture and Forestry, reports from producer associations and development agencies, scientific research on grape varieties grown in the Southeastern Anatolia Region, results reports from various symposiums, conferences, and workshops, as well as scientific articles and papers. The metadata obtained from production data has been collected and presented in tables. All these data have been evaluated and interpreted in line with current literature. In addition, stakeholder, peer, and producer opinions were also considered within the scope of the study, and assessments and recommendations were made by revealing the potential and structural characteristics of viticulture in the Southeastern Anatolia region.

3 Result and Discussion

Table 1 presents a comparative analysis of table grape cultivation in the Southeastern Anatolia Region between 2004, 2014, and 2024, compared to the rest of Türkiye. Upon examining the data in Table 1, it is evident that there has been a significant decline in the vineyard areas dedicated to table grape cultivation across Türkiye. The total area, which was 2,650,000 decares in 2004, decreased to 2,298,938 decares in 2014 and further to 1,601,920 decares in 2024. This situation shows that there have been fundamental changes in Türkiye 's agricultural policies and farmers' production preferences. A similar trend has been observed in vineyards where table grape varieties are grown in the Southeastern Anatolia Region. The total planting area of grapes cultivated for this purpose in the region decreased from 708,390 decares in 2004 to 621,608 decares in 2014 and further to 559,706 decares in 2024. However, despite this decline, it has been determined that the region's share of vineyards producing table grapes in Türkiye has increased from 27% to 35%. This situation stems from a 40% decrease in the area of vineyards producing table grapes with seeds across the country over the last 20 years. On the other hand, vineyard areas where table grape varieties are cultivated in the provinces of Kilis, Şanlıurfa, Adıyaman, and Siirt have decreased by 95%, 89%, 46%, and 52%, respectively, between 2004 and 2024. In the region, where gardens are mostly planted with pistachios, olives, and similar fruit types, producers plant vines between rows until the main fruit type reaches maturity. This allows producers to earn a modest income from their gardens during the few unproductive years, as well as to produce the grapes and grape

products necessary for their family consumption. When fruit trees reach their productive age, however, the care practices applied to the main crop are not applied to the vines, so the vines often become neglected, lose their productivity, and are subsequently uprooted. Additionally, vines are among the first plants to be affected by rapid urbanization. Unlike other cultivated fruit types, vines require more frequent monitoring and care, so vineyards have traditionally been established near residential areas. Due to population growth and economic development, vineyard areas in expanding cities are prioritized for urbanization and construction, leading to the removal of vineyards. On the other hand, there are also examples of an increase in vineyards where table grape varieties such as Mardin and Şırnak are grown. The planting area in Mardin province increased from 114,450 decares in 2004 to 173,768 decares in 2024, representing a 51% increase, which also reveals that different provinces in the region have adopted different agricultural strategies and have different cultivation conditions. Indeed, in Şırnak, the planting area for table grape varieties increased significantly from 2,530 decares to 24,623 decares. Despite the decline in agricultural land due to urban sprawl and investments in industrial development in the Southeastern Anatolia region and throughout Türkiye, the improvement in grape yield per unit area has led to a 24% increase in the production of table grape varieties in the Southeastern Anatolia region and only a 6% decrease in Türkiye. As shown in Table 1, the average yield of table grapes in Türkiye increased from 566 kg/da in 2004 to 688 kg/da in 2014 and 880 kg/da in 2024. This situation indicates that modern viticulture techniques have begun to be applied across the country, priority has been given to cultivating more productive grape varieties with high adaptability to viticulture regions, and the amount of product obtained per unit area has increased due to seasonal changes in climatic conditions. A similar conclusion can be drawn for the Southeastern Anatolia Region. Indeed, both nationwide and in the region, there has been an approximately 56% increase in yield per unit area in areas where table grape varieties are grown. However, the decline in productivity in some provinces may be due to producers adopting innovations more slowly than other producers in the country, continuing to produce mainly with local varieties, not viewing viticulture as their primary source of income, or the region's ecology being more negatively affected by global climate change in recent years compared to other production regions. Indeed, some survey studies conducted in the region (Uyak et al., 2011; Yıldırım and Onay, 2012; Çakır et al., 2014a; Çakır et al., 2014b; Çakır et al., 2015; Çakır et al., 2017a; Çakır et

al., 2017b; Gazioğlu Şensoy et al., 2020; Ünal, 2019; Anonymous, 2021; Yalçın et al., 2021) have yielded findings consistent with our observations. When the region is evaluated on a provincial basis, the first striking finding is the increase in yield in Siirt province. In Siirt province, the yield of table grapes was 119 kg/da in 2004, while in 2024, this figure reached 1,819 kg/da. In Gaziantep province, grape yield increased more than threefold, from 294 kg/da in 2004 to 987 kg/da in 2024. Another province where a steady increase (22%) in the amount of table grapes with seeds obtained per unit area was observed is Diyarbakır. However, there are also some issues in Diyarbakır viticulture. According to Karataş et al. (2016b), producers do not engage in conscious and effective control of diseases and pests in their vineyards, there is no standardization in terms of planting density and training methods, irrigation is not practiced, soil cultivation and fertilization are carried out at a very limited level, summer pruning and canopy management are unknown and therefore not practiced, and producers lack sufficient information about grants and support programs, making it difficult for them to access these resources. Despite the decline in planting areas across Türkiye, total production volumes have been largely maintained thanks to increases in yield. Indeed, grape production, which was 1,500,000 tons in 2004, rose to 1,580,585 tons in 2014, but fell to 1,409,156 tons in 2024. Semerci et al. (2015) reported that between 1990 and 2013, vineyard areas in Türkiye decreased by 19.2%, but grape production increased by 14.6%. The researchers listed the problems they identified in Turkish viticulture as follows: low yields, the lack of an effective role played by producer cooperatives in the marketing of grapes and the absence of market regulation, the lack of organization among producers, the absence of a support system specific to viticulture, quality and standardization problems in grape products, and a lack of product diversity. Çelik (2018) states that, according to official statistics, Türkiye 's total vineyard area decreased by 10.3% between 2007 and 2016, while grape production increased by 11%. The researcher also reported that, despite significant declines in some years, total fresh grape production in Türkiye has remained above 4 million tons since 2009. The researcher reported that during the same period, table grape production increased by 4.1%, while raisin production saw a significant increase of 26.2%, and wine/grape juice production decreased by 2%. In addition, it is stated that, according to the average for the past ten years, 52.1% of the grapes produced in Türkiye are consumed as table grapes, 36.6% as dried grapes, and 11.3% as wine/grape juice grapes. In 2024, the shares of table, raisin, and wine/grape

juice grapes in total grape production were 52.7%, 36.4%, and 10.9%, respectively (TÜİK, 2025). This situation shows that grape variety selection in Türkiye is still being carried out for the same commercial purposes.

Table 17. Changes in Production Areas and Quantities of Table Grapes (TÜİK, 2025)

As a result, despite a decline in the area planted with table grape varieties, the Southeastern

Provinces	Production area (da)			Yield (kg/da)			Production Quantity (ton)		
	2004	2014	2024	2004	2014	2024	2004	2014	2024
Adıyaman	83.200	76.975	45.262	220	692	581	18.273	53.256	26.316
Batman	28.550	47.068	32.938	628	344	400	17.925	16.181	13.176
Diyarbakır	176.730	148.540	143.607	541	564	658	95.568	83.747	94.544
Gaziantep	99.850	103.042	101.043	294	544	987	29.359	56.021	99.721
Kilis	67.040	3.769	3.550	125	330	500	8.375	1.243	1.776
Mardin	114.450	134.708	173.768	660	458	455	75.560	61.644	79.115
Siirt	54.000	25.575	25.811	119	577	1.819	6.440	14.755	46.948
Şanlıurfa	82.040	65.826	9.104	648	591	688	53.163	38.898	6.267
Şırnak	2.530	16.105	24.623	596	766	422	1.509	12.341	10.391
Güneydoğu	708.390	621.608	559.706	432	544	676	306.172	338.086	378.254
Türkiye	2.650.000	2.298.938	1.601.920	566	688	880	1.500.000	1.580.585	1.409.156

Anatolia Region has managed to increase its total production volume, particularly due to increased productivity. Indeed, total table grape production in the Southeastern Anatolia Region increased from 306,172 tons in 2004 to 338,086 tons in 2014 and 378,254 tons in 2024. This situation may indicate a transition to more intensive and efficient methods in grape cultivation in the region. However, when evaluating this aspect, the differences between provinces are quite striking. While some provinces have experienced significant losses in terms of area and production, others have stood out with increases in productivity and production volume. Despite the continuous decline in planting area across Türkiye, the relative preservation of total production thanks to increased productivity reflects technological advances and adaptability in the agricultural sector. In the future, the sustainability of these trends will depend on factors such as climate change, water resource management, and farmer support. Therefore, it is crucial to continue detailed analyses of regional ecological conditions (Alsancak Sırlı et al., 2015) and for each province to develop its own unique strategies and implement appropriate policies (Baykul et al., 2018).

In the Southeastern Anatolia region, between 2004 and 2024, the area under cultivation for seedless table grape production decreased by approximately 66%, and production volume decreased by 9.5% (Table 2). Additionally, the region's share of national production has also declined over the years. There are many reasons why the production of seedless table grape varieties is not widespread in the region. The most important of these is that temperatures can occasionally exceed 40°C during the summer months, which negatively affects berry development, thereby reducing yield and grape quality. Large-scale dry viticulture is practiced in the Southeastern Anatolia region, meaning that the vineyards are not irrigated. It is quite difficult to successfully produce seedless grape varieties, which are less tolerant to high temperatures and drought than seeded grape varieties, in this ecology and under these cultivation conditions. In particular, hot winds blowing during the early stages of berry formation cause a significant portion of the berries on the clusters to dry out. The lack of widespread adoption of high-stem training systems that keep the clusters in the shade in the region, coupled with the continued preference for local training methods in vineyards, is another factor negatively impacting the success of seedless grape cultivation. For this reason, the cultivation of seedless table grape varieties is almost non-existent in provinces such as Kilis, Mardin, Siirt, and Şanlıurfa, located in the Southeastern Anatolia region. A similar situation applies to seedless raisin grape varieties. When examining the total table seedless grape production in the Southeastern Anatolia region (4,850 tons), it is seen that the main contribution to the region comes from production in the province of Gaziantep. On the other hand, as of 2024, table seedless grape production is also carried out on a small scale in the provinces of Adıyaman and Batman. However, in terms of grape yield per unit area, only Gaziantep province (1,180 kg/da) achieves a value close to the Turkish average (1,343 kg/da), while productivity in the other two provinces where production takes place remains at very low levels. In Gaziantep province, the productivity of seedless table grape production was 400 kg/da in 2004, reached 1,000 kg/da in 2014, and reached 1,180 kg/da in 2024. This situation indicates that production continues in smaller areas using modern techniques and efficient methods, rather than in large vineyards where traditional methods are used. On the other hand, thanks to the increasing number of irrigation projects in the region in recent years and the support and grants provided for the installation of high-stem training systems, it is likely that there will be a partial increase in the cultivation of seedless table grape varieties in the near future. In contrast, in the

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production of seedless table grape varieties across Türkiye, it can be seen that the yield per unit area increased from 2004 to 2014, rising from 1,143 kg/da to 1,724 kg/da. This upward trend later reversed, and by 2024, yields had decreased to 1,343 kg/da, marking a significant decline. This situation indicates that there have been some fluctuations in productivity in the cultivation of seedless table grape varieties across Türkiye. On the other hand, unlike in the Southeast, it can be said that production has increased in some regions where viticulture is practiced, relative to the total area.

Table 18. Changes in Production Areas and Quantities of Seedless Table Grapes (TÜİK, 2025)

	Production area (da)			Yield (kg/da)			Production Quantity (ton)		
	2004	2014	2024	2004	2014	2024	2004	2014	2024
Adıyaman	-	302	200	-	712	445	-	215	89
Batman	-	80	100	-	550	400	-	44	40
Diyarbakır	-	5.650	-	-	599		-	3.385	-
Gaziantep	12.000	4.000	4.000	400	1.000	1.180	4.800	4.000	4.721
Kilis	-	-	-	-	-	-	-	-	-
Mardin	-	-	-	-	-	-	-	-	-
Siirt	-	-	-	-	-	-	-	-	-
Şanlıurfa	-	-	-	-	-	-	-	-	-
Şırnak	830	-	-	677	-	-	562	-	-
Güneydoğu	12.830	10.032	4.300	418	762	1128	5.362	7.644	4.850
Türkiye	350.000	340.077	310.314	1.143	1.724	1.343	400.000	586.164	416.759

As a result, it can be said that a strategic change has taken place in the cultivation of seedless table grape varieties in the Southeastern Anatolia Region. While moving away from large areas and old production models, productivity has increased in small areas. However, this increase in productivity has not been sufficient to compensate for the decrease in area, resulting in a significant decline in the total production volume of table grape varieties in the region. This situation suggests that farmers in the region are attempting to adapt to factors such as economic conditions, water resources, climate change, or market demand, but these efforts have not yet sufficiently reflected in the total production. In Türkiye, although the vineyard area covered by grape varieties cultivated for this type of consumption has decreased by 11%, production volumes have remained stable. This shows that the change in the Southeast is based on regional and specific reasons. Previous researchers have emphasized that within the important provinces

for vineyard area and grape production in the Southeast Anatolia region, Mardin, Gaziantep, Kilis, and Adıyaman are important provinces for dried grape production (Söylemezoğlu et al., 2025), while Gaziantep, Mardin, and Diyarbakır are important provinces for table grape production (Anonymous, 2021; Gürsöz, 1993; Kiracı et al., 2024). Across Türkiye, the provinces that stand out in wine grape production are Denizli, Tokat, Nevşehir, Elâzığ, Kilis, Çanakkale, and Tekirdağ (Kiracı et al., 2024). On the other hand, the provinces of Siirt, Diyarbakır, and Adıyaman hold an important position in organic grape production within the country (Söylemezoğlu et al., 2025). Table 3 presents data on the cultivation of wine grape varieties in the Southeastern Anatolia Region between 2004, 2014, and 2024, broken down by province and for Türkiye. Based on these data, it can be seen that no wine grapes were produced in Batman province in any of the three years under review. Among the reasons for this are the lack of demand for wine consumption due to the socio-cultural behaviors and religious beliefs of the people living in Batman Province, the absence of wine production facilities, and similar factors. In Adıyaman province, the area planted with wine grape varieties increased significantly from 100 decares in 2014 to 1,500 decares in 2024. However, the yield per unit area of wine grape varieties in the province decreased from 750 kg/da in 2014 to 500 kg/da in 2024. However, thanks to the increase in cultivated areas, production has increased tenfold. In recent years, Mardin province has emerged as a rapidly growing player in wine grape production. Although there has been development in this area in the provinces of Diyarbakır, Adıyaman, and Siirt, Mardin is ahead of other provinces in terms of marketing thanks to its long-standing Syriac wine culture. However, the grape yield per unit of vineyard area is quite low, not only compared to the world average but also compared to the national average. The reasons for this include the continued cultivation of local varieties, the insufficient spread of advanced training systems in the province, the use of traditional methods during pruning, ongoing issues in technical areas such as fertilization and irrigation, inadequate and improper measures against diseases and pests, and the lack of knowledge and implementation of practices such as canopy management and summer pruning. Despite high temperatures and droughts during the later stages of the vegetation period, the lack of use of shade covers, the failure to select rootstocks suitable for the variety, and similar issues can be listed as reasons. In the provinces of Diyarbakır and Kilis, it has been observed that the area of vineyards planted with wine grape varieties has fluctuated over the years. Today, Kilis is the province

with the highest wine grape production in the region, yielding 11,730 tons of grapes from 73,980 decares of land in 2004. Although the area of vineyards cultivated with wine grapes in the province reached 130,114 decares and the production amount reached 49,859 tons in 2014, the vineyard area later decreased to 62,258 decares and the production amount decreased to 36,217 tons. Nevertheless, the increase in yield in the wine grape vineyards of Kilis province over the last 20 years (364%) is quite remarkable. Kilis province was home to wineries in the early years of the republic (Tokuçoğlu, 2012). Local and standard grape varieties suitable for wine production are frequently found in the vineyards of the province. However, the wine grapes produced in this province today are sold and processed in wineries in different provinces. Diyarbakır province is still home to small-scale wineries today. Wine grape production in Diyarbakır has followed a similar trend to that in Kilis province. From 2004 to 2024, vineyard areas were determined to be 8,000 da, 18,620 da, and 9,280 da, respectively. In parallel, wine grape production in the province was recorded as 700 tons, 13,052 tons, and 6,526 tons. However, the grape yield per unit area in Diyarbakır has seen a significant increase, nearly eightfold over the past 20 years. The increase in production volume and yield in Diyarbakır province is largely related to the compatibility of the locally grown wine grape varieties Boğazkere and Öküzgözü, which are internationally recognized and in increasing demand, with the regional climate and their widespread cultivation in the region's vineyards. However, Çelik and colleagues (2005) emphasized that the unplanned spread of the Boğazkere variety in the province's vineyards, using outdated techniques and mostly unvaccinated saplings, is cause for concern for the future of viticulture in the region. In the provinces of Gaziantep and Şanlıurfa, wine grape vineyards are gradually decreasing. In particular, wine grape production in Şanlıurfa has almost come to a complete halt. Among the reasons for this situation are: the preference for alternative crops that generate more income, increasingly frequent and prolonged periods of extreme heat, the lack of sufficient market and demand, and similar factors. On the other hand, wine grape vineyards still exist to a significant extent in Gaziantep, but the downward trend is a cause for concern. Nevertheless, in 2024, the amount of wine grapes produced per unit area increased by 3.45 times compared to 2004, suggesting that both new and established producers are applying cultural practices correctly and on time, as well as adopting new techniques that enhance productivity. While there was no wine grape cultivation in Siirt province in 2004 and 2014, by 2024, it was observed

that products were obtained from the newly established vineyards. Indeed, today in Siirt Province, 2,630 tons of wine grapes are produced annually from 1,461 decares of vineyard area. With a yield of 1,800 kg/da per decare, Siirt Province has a productivity that is well above the regional and even national average. This situation may be attributed to the establishment of new wine grape vineyards in Siirt Province in accordance with modern viticulture practices, the careful approach to cultivation, adherence to terroir rules, the selection of grape varieties with high adaptability to the region, or similar factors. The production method in this province should be closely monitored, and successful practices should be disseminated to other provinces in the region.

The Southeastern Anatolia Region accounts for 27% of Türkiye's total vineyard area and approximately 18% of total grape production. When considering only vineyards where wine grape varieties are cultivated, the Southeastern Anatolia Region accounts for 25% of the country's vineyard area and 21% of production volume. In this regard, the region holds a significant position within Turkish viticulture (Turkish Statistical Institute, 2025). On the other hand, wine grape production accounts for approximately 11% of total grape production in Türkiye. However, not all of these grapes are used for wine production; a significant portion is used in the production of fruit juice, vinegar, fruit leather, grape molasses, walnut sausage, köfter, and similar food products. Some sources report that only 2-3% of the grapes produced in Türkiye are used in wine production. This situation is also clearly evident when comparing Türkiye 's position in the global grape production rankings and its grape production volume with its position in wine production and wine production values (OIV, 2025). Although wine grape areas have decreased across Türkiye over the past 20 years, grape yields per unit area have continued to increase. On the other hand, the production dynamics of wine grapes differ greatly from those of table and raisin grapes, both across Türkiye and in the Southeastern Anatolia region (Polat et al., 2018). When considering the entire Southeast Anatolia region in terms of wine grape cultivation, it has been observed that, despite fluctuations in vineyard areas and production volumes over the past 20 years, grape yields per unit area have increased. This increase in yield has offset the decline in vineyard areas, and by 2024, wine grape production in the region has reached 2.4 times the level of 2004. However, the yield obtained

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from vineyards where wine grape varieties are grown in the region (560 kg/da) is still below the Turkish average (682 kg/da).

Table 19. Changes in Wine Grape Production Areas and Quantities (TÜİK, 2025)

	Production area (da)			Yield (kg/da)			Production Quantity (ton)		
	2004	**2014**	**2024**	**2004**	**2014**	**2024**	**2004**	**2014**	**2024**
Adıyaman	-	100	1.500	-	750	500		75	750
Batman	-	-	-	-	-	-	-	-	-
Diyarbakır	8.000	18.620	9.280	88	701	703	700	13.052	6.526
Gaziantep	53.480	9.560	9.535	217	566	749	11.594	5.414	7.140
Kilis	73.980	130.114	62.528	159	383	579	11.730	49.859	36.217
Mardin	2.060	63.757	55.820	485	428	451	1.000	27.264	25.184
Siirt	-	-	1.461	-	-	1.800	-	-	2.630
Şanlıurfa	40.920	1.827	30	191	883	500	7.835	1.613	15
Şırnak	50	-	70	720	-	400	36	-	28
Güneydoğu	178.490	223.978	140.224	184	434	560	32.895	97.277	78.490
Türkiye	700.000	687.512	558.243	529	647	682	370.000	445.127	380.738

Table 4 provides detailed data on the cultivation of “dried seedless grape” varieties in the provinces of the Southeastern Anatolia region. In Siirt province, it is observed that no enclosed vineyard areas have been established for the cultivation of dried seedless grape varieties since 2004, and that vineyard activities for this commercial purpose have not been continued in the province. In Adıyaman province, both the vineyard areas (from 15,100 da in 2004 to 39,939 da in 2024) and the production quantities (from 4,830 tons in 2004 to 25,668 tons in 2024) have shown a steady and significant increase over the years. In the vineyards where dried seedless grape varieties are cultivated in the province, productivity has doubled by 2024 compared to 2004. Particularly, consumer demand for the Besni grape variety, which has been granted a geographical indication, ensures that production in the province is maintained and even increased. As of 2024, Mardin province is the province with the highest production of dried seedless grape varieties in Southeastern Anatolia. It has maintained its leadership in this field since 2004. The Karfoki grape variety, which is grown in Mardin province and has a geographical indication, has a very special place in this production. Between 2004 and 2024, the vineyard area where dried grape varieties are grown in Mardin province increased by 1.8 times, while the production volume increased

by 1.4 times. However, the grape yield per unit vineyard area has decreased from 558 kg/da to 450 kg/da over the years. When considering data on grape cultivation for other commercial purposes in Mardin Province, a general decline in yield is evident across the province's vineyards. The most likely reason for this situation is that the local grape varieties grown in the province are struggling to cope with the stress caused by the temperature increases resulting from climate change. Gaziantep, which ranked first in the region in terms of vineyard area for dried seedless grape varieties in 2004 (121,830 da), lost a significant portion of these vineyards by 2014 (48,399 da). In subsequent years, vineyards growing dried grape varieties have been largely preserved. On the other hand, productivity increased 2.4 times between 2004 and 2014, and 1.4 times between 2014 and 2024. It has been determined that this increase in yield has compensated for the decline in vineyard area, enabling production to exceed the 2004 level of 28,920 tons and reach 42,307 tons in 2024. Accordingly, Gaziantep serves as a successful example of increasing production through productivity despite a decline in vineyard area. Between 2004 and 2024, Kilis province has set an important example by steadily increasing both its vineyard area (1.7 times), productivity (3.7 times), and total production volume (6.3 times) in terms of the cultivation of raisin grape varieties. It can be said that Kilis is an important and growing player in the cultivation of raisin grapes. Batman province, despite becoming an important producer in 2014 in terms of vineyard area and production volume for dried seedless grape varieties (25,785 da and 14,420 tons), has been unable to maintain this production model. In 2024, due to both the reduction in vineyard areas and the decline in productivity, the province's grape production fell to 7,141 tons per year. Between 2004 and 2014, Diyarbakır largely preserved the vineyard areas where dried seedless grape varieties were grown and even increased grape production from 2,973 tons to 17,663 tons thanks to increased productivity during this period. However, between 2014 and 2024, production was abandoned in nearly all of the vineyards where these varieties were grown, resulting in a decline in production volume to 841 tons. This change means that producers in Diyarbakır have largely abandoned the production of dried grape varieties with seeds. The reason for this may be that producers prefer alternative product patterns. Despite being one of the top three provinces in southeastern Anatolia for raisin grape cultivation between 2004 and 2014, Şanlıurfa has lost nearly all (97.5%) of its vineyard areas by 2024. There are several reasons for this change in Şanlıurfa. These include: the dismantling of vineyards due to urbanization; the end of vineyard

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cultivation, which was carried out as intercropping, due to the fruiting of trees in orchards of higher-income fruit types such as pistachios and olives; the shift toward crops that generate higher income, and the decline in vineyard productivity due to bureaucratic issues related to irrigation, in addition to rising temperatures and drought (Bekişli et al., 2015; Polat et al., 2018; Özel and Eser, 2021). Eser (2019) found that in areas where viticulture is practiced as intercropping among other fruit trees, different training systems are not used, and production continues with traditional training methods. On the other hand, the decline of vineyards in Şanlıurfa province has also led to the complete loss of a portion of the province's local grapevine genetic resources. Some grape varieties identified by Gürsöz (1993) are no longer found in vineyards today. In Şırnak province, the production of dried seedless grape varieties has shown sharp declines and increases over the past 20 years, with an annual production of 2,922 tons on 5,955 hectares as of 2024.

Table 20. Changes in Production Areas and Quantities of Dried Grapes with Seeds (TÜİK, 2025)

	Production area (da)			Yield (kg/da)			Production Quantity (ton)		
	2004	2014	2024	2004	2014	2024	2004	2014	2024
Adıyaman	15.100	29.296	39.939	320	693	643	4.830	20.297	25.668
Batman	2.200	25.785	17.850	220	559	400	485	14.420	7.141
Diyarbakır	26.800	23.285	1.028	111	759	818	2.973	17.663	841
Gaziantep	121.830	48.399	48.081	237	621	880	28.920	30.073	42.307
Kilis	31.520	33.405	53.908	159	330	585	5.020	11.013	31.552
Mardin	72.260	145.800	128.104	558	477	450	40.305	69.534	57.605
Siirt	-	-	-	-	-	-	-	-	-
Şanlıurfa	69.420	47.885	1.747	447	707	467	31.054	33.869	815
Şırnak	8.500	92	5.955	758	304	491	6.439	28	2.922
Güneydoğu	347.630	353.947	296.612	345	556	569	120.026	196.897	168.851
Türkiye	750.000	628.137	491.612	467	681	645	350.000	427.533	317.053

The Southeastern Anatolia Region is Türkiye's most important region in terms of the area and production volume of raisin grape cultivation (Ünal and Soltekin, 2018). Indeed, as of 2024, the region accounts for 60% of the vineyards where dried seedless grape varieties are cultivated in Türkiye, and 53% of the

total harvest comes from this region. The total vineyard area dedicated to this purpose in the region was 347,630 hectares in 2004, but this area has decreased to 296,612 hectares by 2024. The major losses of vineyards, particularly in Diyarbakır, Gaziantep, and Şanlıurfa, are the main reasons for this situation. On the other hand, the average grape yield obtained from vineyards where dried grape varieties are grown in the region has steadily increased from 345 kg/da to 569 kg/da over the past 20 years. The continuous upward trend in vineyard productivity in the region is largely attributed to productivity increases in Gaziantep, Kilis, Adıyaman, and Diyarbakır. However, the average productivity value in the region remains below the national average (645 kg/da). The amount of dried seedless grape varieties grown in vineyards in the region was 120,026 tons in 2004, but it jumped significantly to 196,897 tons in 2014, only to decline to 168,851 tons in 2024. Across Türkiye, the area of vineyards where dried seedless grape varieties are cultivated decreased significantly and continuously from 750,000 da in 2004 to 628,137 da in 2014 and 491,612 da in 2024. The grape yield per unit vineyard area has increased from 467 kg/da (2004) to 645 kg/da (2024). However, the increase in yield has not been able to compensate for the decline in vineyard areas dedicated to dried seedless grape varieties across the country, resulting in a production decrease of approximately 10% over the past 20 years, dropping to 317,053 tons. Nevertheless, the increase in grape yield per unit vineyard area is encouraging both at the regional and national levels. This situation indicates that more modern agricultural techniques are beginning to be adopted, that there is an increasing trend toward grape varieties that are highly adaptable to the ecological conditions of the regions where they are grown and are highly productive, and that the varieties most suitable for the commercial purposes of the region are being selected. When examining the data in Table 5, it is observed that in the last 20 years (from 2004 to 2024), no vineyards for drying seedless grape varieties have been established in the provinces of Adıyaman, Gaziantep, Kilis, Mardin, Siirt, and Şanlıurfa, nor were any previously present. The primary reasons for this situation include: the fact that viticulture in these provinces is primarily carried out without irrigation, the absence of ecological conditions suitable for the cultivation of seedless raisin grape varieties in these provinces, the lack of productive and high-quality varieties among local varieties that could be cultivated for this purpose, and similar factors. In addition, the fact that the Aegean Region, where the production of seedless raisin varieties is widespread in Türkiye, has a global brand value also causes other regions to

avoid competition. Indeed, Türkiye is the most important player in the global raisin market with a 31.6% share of exports thanks to the export of seedless raisins produced in the Aegean Region. Although vineyards for the cultivation of these varieties have been established from time to time in the provinces of Şırnak, Batman, and Diyarbakır, as of 2024, the total amount of grapes obtained from seedless raisin grape varieties in Southeastern Anatolia is only 90 tons. The total vineyard area where seedless raisin grapes are grown across Türkiye has remained almost the same over the past 20 years, despite some declines. Production volume, however, has increased due to the rise in the amount of grapes produced per unit of vineyard area. As of 2024, Türkiye produces a total of 944,294 tons of seedless raisin grapes annually across 760,761 hectares of vineyards. This production volume represents the amount of fresh grapes obtained from these varieties. Türkiye has a higher productivity in the cultivation of both table seedless and dried seedless grape varieties compared to other varieties. This situation stems from the productivity of these varieties, the ecological advantages of the regions where they are intensively cultivated, the high level of awareness among producers, and the application of modern viticulture techniques in these regions. The Southeastern Anatolia Region, however, does not possess these advantages. This situation indicates that the trend in Southeastern Anatolia is based on regional and specific reasons, while the importance of seedless raisin grape varieties is maintained throughout Türkiye.

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Table 21. Changes in Production Areas and Quantities of Seedless Grapes for Drying (TÜİK, 2025)

	Production area (da)			Yield (kg/da)			Production Quantity (ton)		
	2004	2014	2024	2004	2014	2024	2004	2014	2024
Adıyaman	-	-	-	-	-	-	-	-	-
Batman	-	250	30	-	308	400	-	77	12
Diyarbakır	-	1.700	-	-	154	-	-	261	-
Gaziantep	-	-	-	-	-	-	-	-	-
Kilis	-	-	-	-	-	-	-	-	-
Mardin	-	-	-	-	-	-	-	-	-
Siirt	-	-	-	-	-	-	-	-	-
Şanlıurfa	-	-	-	-	-	-	-	-	-
Şırnak	6.250	-	120	760	-	650	4.750	-	78
Güneydoğu	6.250	1.950	150	760	173	600	4.750	338	90
Türkiye	750.000	716.265	760.761	1.173	1.586	1.241	880.000	1.135.947	944.294

4 Conclusion

In this study, the dynamics of grape production in the Southeastern Anatolia Region and Türkiye between 2004, 2014, and 2024 were analyzed based on the principle of grouping grapes according to their commercial evaluation classes. The findings clearly show that a significant transformation has taken place in the viticulture sector at the regional and national levels. During this transformation process, a sharp decline in vineyard areas was observed in the Southeastern Anatolia Region, while there was an increase in the amount of product obtained from unit vineyard areas. Indeed, the total area under viticulture in the region decreased from 1,235,590 da in 2004 to 1,000,992 da in 2024. On the other hand, the production amount increased from 469,205 tons in 2004 to 630,535 tons in 2024. The increase in grape production in the region was achieved through increased yields in vineyards. A similar situation has occurred throughout Türkiye. While the total vineyard area in Türkiye was 5,200,000 da in 2004, it decreased to 3,722,850 da in 2024. Although the production volume fluctuates according to seasonal climatic changes, it is largely maintained. The decline in vineyard areas both across Türkiye and in the Southeastern Anatolia Region indicates that producers have shifted toward the cultivation of different, high-yield crops. The findings suggest that producers continuing cultivation in the remaining vineyard areas have adopted more modern agricultural techniques, appropriate variety/rootstock selection, and likely improved water management. It is evident that a multidimensional transformation has taken place

in grape production in the Southeastern Anatolia Region. The transformation in viticulture in the Southeastern Anatolia Region has not been limited to a reduction in area and an increase in yield, but has also resulted in the purpose of grape production being shaped in line with market demands and, possibly, the varieties grown changing accordingly.

In this regard, it can be said that the provinces in the region have begun to develop their own viticulture strategies. Indeed, in provinces where viticulture is practiced, production for certain commercial purposes has been discontinued, but there has been a focus on the production of grape varieties that have increased yields and generated satisfactory income. Among the most prominent examples of this situation are: Diyarbakır and Şanlıurfa's withdrawal from the production of dried seedless grape varieties, Adıyaman and Kilis's increase in the production of wine and dried seedless grape varieties, Şırnak's increase in the production of table seedless grape varieties, and Siirt's shift toward wine grape cultivation in addition to its existing table grape vineyards. A more in-depth examination of the driving forces behind this transformation and its future potential is necessary. As a result, it is evident that structural changes are taking place in the viticulture sector across Türkiye and that new strategies are needed for viticulture regions in terms of sustainability. Grape production in the Southeastern Anatolia Region is undergoing a complex transformation process characterized by a significant increase in yield per unit area, accompanied by a serious decline in acreage. This situation suggests that farmers are reevaluating their production preferences and shifting toward more efficient but often smaller-scale production due to various factors such as climate change, limited water resources, changes in market demands, and agricultural policies. While some provinces in the region are withdrawing from grape production, provinces such as Gaziantep, Adıyaman, and Kilis are emerging as models focused on efficiency or growth in grape production for specific commercial purposes. Similar trends across Türkiye underscore the importance of strategic planning, technological adaptation, and policies that take regional differences into account across the entire viticulture sector.

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5 Suggestions

5.1 Promoting Efficiency-Focused Agricultural Policies

Despite the decline in fruit-growing areas in the Southeastern Anatolia Region, the increase in efficiency demonstrates the effectiveness of modern agricultural techniques. Therefore, efficiency-focused production models should be promoted by increasing training programs, technical support, and incentives for farmers in the region.

Promotion of Drought- and Climate-Resistant Varieties and Rootstocks: Considering that climatic factors are behind the reduction in acreage, the development and dissemination of drought-resistant grape varieties and rootstocks is of great importance.

Focus on Value-Added Products: The increase in wine grape production demonstrates that value-added products can contribute to the regional economy. In this regard, investments should be encouraged in areas such as wine production, organic dried products, and geographically indicated table grapes.

Strategic Planning that Takes Regional Differences into Account: The success stories of provinces such as Gaziantep, Adıyaman, and Kilis demonstrate the need to develop different strategies according to each province's potential. Therefore, province-based agricultural policies should be created, and support should be tailored to these differences.

Strengthening Producer Cooperatives: In order to increase productivity and marketing power in shrinking production areas, producer unions and cooperatives should be supported, and financing, training, and marketing support should be provided to farmers through these structures.

Data-Driven Agriculture and Digitalization: Agricultural data collection systems, satellite-supported monitoring, and digital agriculture applications should be widespread to ensure more informed production decisions. This will increase both productivity and sustainability.

Development of a National Grape Strategy: Since similar structural transformations are taking place throughout Türkiye, a comprehensive national grape strategy should be developed; this strategy should cover production, processing, marketing, and export dimensions.

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I-CRAFT AGRICULTURAL and FOOD TECHNOLOGIES

Utilization Of Rumen Boluses in Nutritional and Health Management of Ruminants

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Abstract. This review discusses the mechanisms of action, areas of application and effects on performance of rumen boluses used in different physiological periods of ruminants. Maximum productivity in farm animals, the protection of animal health and welfare and the sustainability of production are closely related to their adequate and balanced feeding in the different physiological periods as well as effective disease management. In recent years, the use of rumen boluses has become a practical method of supporting animal nutrition to achieve these goals. Rumen boluses are structures that are placed in the rumen and have a long-term release. They allow the controlled administration of mineral and vitamin supplements as well as pharmacological agents. Thanks to their sustained-release structures, they provide the necessary nutrients and medicines that animals need in a controlled manner during various physiological periods such as the transition period, pregnancy and lactation. Thus, they provide positive contribution to economic production by preventing metabolic diseases, improving reproductive performance, promoting immunity and the growth and development performance of the offspring. As a result, in light of the finding from the literature, it is suggested that rumen boluses can be used for both nutritional support and therapeutic purposes and can be evaluated as part of a total herd management strategy.

Keywords: Sheep, Goat, Rumen bolus, Prepartum, Postpartum.

1 Introduction

The transition period in ruminants, encompassing approximately prepartum three weeks and postpartum four weeks, is a critical period characterized by intense metabolic and hormonal changes (Drackley, 1999). During this period, increased energy and nutrient requirements, particularly when combined with the high metabolic demands of parturition and lactation, make animals more susceptible to metabolic disorders (Overton and Waldron, 2004). Inadequate nutrition or unbalanced rations can lead to metabolic disorders like hypocalcemia, ketosis and fatty liver. These diseases negatively impact not only animal

health but also milk yield, fertility, and the immune system, leading to significant economic losses (Grummer, 1995). Various nutritional supplements are used in transition management to support metabolic balance and prevent nutrient deficiencies during these periods. Boluses, one of these, provide nutritional support when the animal needs it by releasing elements such as calcium, magnesium, phosphorus, selenium, and vitamins in a controlled manner (Mulligan et al., 2006). Bolus applications are particularly effective in preventing mineral imbalances such as hypocalcemia, which occurs after parturition, and also offer a protective effect against ketosis and other energy metabolism disorders (Martinez et al., 2016). Recent studies have shown that bolus applications have positive effects not only on metabolic health but also on performance criteria such as the immune system, milk yield, offspring growth, and fertility (Zhao et al., 2022). In this context, evaluating the effects of bolus applications in small ruminants during different physiological periods is becoming important for both animal health and productivity, as well as for breeder economics.

This review examines the effects of rumen bolus utilisation as a nutritional supplement and medication in ruminants on especially metabolic diseases in the transition period, milk yield, the immune system, the digestive system, fertility and offspring growth and development.

1.1 Effects of rumen bolus use on metabolic diseases

In ruminants, the increased nutritional requirements during parturition and lactation lead to the occurrence of various metabolic disorders. Metabolic problems such as fatty liver syndrome, ketosis, hypocalcemia, hypomagnesia and rumen acidosis, which are frequently encountered in livestock enterprises, can significantly affect animal health, product quality and business economy. The occurrence of these diseases is often due to an inadequate supply of energy, minerals and vitamins. In recent years, nutritional supplements in bolus form have become increasingly important in the prevention and treatment of these problems. Boluses, with their controlled-release ingredients, help to maintain metabolic balance and provide an effective solution for both disease prevention and treatment support. Studies on the use of boluses in metabolic disorders are presented below.

In the study conducted by Martinez et al. (2016), the effects of oral calcium bolus supplementation on the health status and milk yield of Holstein dairy cows during the early lactation period (transition period) after parturition were evaluated. A total of 450 cows were randomly assigned to three different treatment groups of 150 animals each, which received oral calcium bolus at different times after parturition. The treatment groups were as follows: a control group, a group that received two boluses of calcium immediately after calving and on the first postpartum day, and a group that received two oral boluses of calcium on the second and fourth postpartum days. It was reported that calcium supplementation had no effect on milk yield in the first month after calving. In multiparous cows, calcium supplementation had a positive effect on milk production in the first 30 days of lactation in high-producing animals. However, in cows with lower milk yields, it did not bring any significant benefit and had a negative effect on economic results. Consequently, it was recommended that oral calcium supplementation after calving should only be used in high-producing cows with multiple births. However, the researchers explained that it should not be used in cows with only one birth, as it may have negative effects on reproduction and offer no benefits for reproductive performance.

Another study was conducted to evaluate the efficacy of bolus administration in the context of treatment protocols adapted to clinical signs in small ruminants diagnosed with rumen acidosis (26 sheep and 16 goats showing symptoms of acidosis) (Tufani et al., 2013). In the animals that received apples, cooked rice, beets and chapatti (Indian bread) along with their ration, a total of 42 animals were diagnosed with rumen acidosis. These animals were divided into three treatment groups according to clinical severity (Group I = mild, Group II = moderate, Group III = severe). The distribution of sheep and goats in each group was as follows: Group I = 18 small ruminants (11 sheep, 7 goats); Group II = 16 small ruminants (10 sheep, 6 goats); and Group III = 8 small ruminants (5 sheep, 3 goats). For the treatment of acidosis, the animals in group I received oral sodium bicarbonate in combination with a bolus preparation called “Rumentas". The animals in group II received oral sodium bicarbonate with the bolus preparation "Rumentas" plus parenteral fluid therapy and high-dose vitamin B1. The animals in group III received only parenteral fluid therapy and high-dose vitamin B1. In addition, all groups received antihistamines as supplementary therapy. The use of the bolus preparation was reported to

contribute significantly to the control of clinical signs, especially in mild to moderate acidosis, by supporting rumen motility and stabilizing systemic parameters. After treatment, the vast majority of animals recovered completely, with only two cases of mortality reported, one animal each from groups II and III. The results suggest that bolus preparations can serve as an effective supportive treatment method for rumen acidosis. In the study by Aliarabi et al. (2017), 18 Markhoz goats were divided into two treatment groups. One group received a slow-release bolus containing selenium and zinc, while the other group served as a control and received no additional supplementation. In the goats receiving the bolus, significant improvements in immune system parameters were observed, plasma selenium levels increased and a reduction in markers of metabolic stress was reported.

Based on the studies presented above, it can be concluded that oral bolus administration can have significant effects on various clinical and production parameters in both cattle and small ruminants. However, the effectiveness of these measures depends on the physiological condition of the animal, the severity of the disease, the stage of lactation and the individual production potential. In particular, the administration of calcium bolus has been shown to be beneficial in increasing milk yield in multiparous and high-yielding cows, while bolus preparations for the treatment of rumen acidosis have helped to maintain rumen function in mild to moderate cases. In addition, mineral-containing boluses have shown positive effects on the immune system and have played a role in stabilizing metabolic parameters such as rumen pH. However, it should be emphasized that indiscriminate or general application of boluses to all animals may not be economically or physiologically viable. Therefore, targeted and selective use based on the individual condition of the animal is crucial.

1.2 Effects of rumen bolus use on fertility

The reproductive performance of ruminants is one of the most important parameters that directly influence the economic structure and sustainability of livestock farms. Among the environmental factors that influence fertility, nutrition is the most important determinant. In small ruminants, there are three nutritionally critical periods: the pre-mating phase (flushing), early lactation (peak milk yield) and the last third of gestation, when fetal development is at its most intense. Deficiencies in trace elements such

as zinc, selenium, copper, cobalt and iodine in this periods can lead to reproductive disorders such as irregular oestrus cycles, embryonic mortality, reduced pregnancy rates and dystocia (León-Cruz et al., 2020).

In a study conducted on high-producing Holstein cows exposed to heat stress, the effects of a bolus of trace elements and vitamins on reproductive and lactation performance were investigated (Khorsandi et al., 2016). In the group receiving the bolus, a reduction in the number of "open days" was observed, along with a significant increase in cumulative pregnancy rates up to the fifth insemination and improvements in the chemical composition of the milk. In addition, positive changes in milk quality indicators, such as a lower somatic cell count and a higher fat content of the milk, were also observed. In another study conducted in India on Kankrej cattle, the effects of an 80 g mineral bolus given immediately after calving on reproductive parameters were investigated up to 140 days after parturition (Naikoo et al., 2020). The results showed that only one cow in the bolus-treated group became pregnant at first insemination, and although plasma levels of progesterone, cholesterol and triglycerides fluctuated over time, no statistically significant differences were found between the bolus-treated group and the control group. In a study conducted on Kangal sheep, the effects of vitamin and mineral supplementation on reproductive performance were compared between injectable and bolus forms (Takcı et al., 2023). The study found that the bolus administered 40–45 days prior to estrus synchronization had no significant effect on reproductive performance indicators such as estrus rate, pregnancy rate, lambing rate, embryonic mortality rate, and fertility. However, the dystocia rate was significantly lower in the group that received the bolus.

In another study by Kendall et al. (2000) conducted on eight-month-old male lambs, the effect of bolus supplementation on reproductive performance was investigated. In Ram breed lambs administered a bolus named Zincosel, which contains zinc, cobalt, and selenium, increases in sperm motility, percentage of live sperm, and membrane integrity were observed. Additionally, an increase in glutathione peroxidase (GSH-Px) enzyme activity and positive effects on sperm membrane health were reported. Similarly, in another study where Hampshire and Suffolk rams were given a bolus containing 500 mg of selenium, significant improvements were noted in sperm motility, volume, concentration,

and viability, along with an increase in GSH-Px enzyme activity (Carrillo-Nieto et al., 2018). Mineral boluses are known to contribute not only to fertility but also to immune function and the control of parasitic infections. In a study conducted on Spanish goats, copper-containing boluses were found to be effective against Haemonchus contortus infection, significantly reducing parasite egg counts (Burke & Miller, 2006).

Studies investigating the general effects of nutrition on reproduction reported that inadequate mineral intake negatively affects the hypothalamic-pituitary-ovarian axis, disrupts GnRH secretion and suppresses the oestrus cycle (Assan et al., 2025). A common finding of all these studies is that intraruminal bolus administration is an effective method of correcting mineral deficiencies. However, numerous factors including animal species, age, breed, lactation status, bolus composition, timing of administration and environmental conditions can directly influence the results of such interventions. It is therefore crucial to develop targeted formulations rather than adopting a "one size fits all" approach. Controlled-release mineral boluses have the potential to improve both male and female fertility parameters in ruminants, either directly or indirectly. However, knowing the most appropriate bolus type, delivery method and timing for each species and breed will contribute to more economically efficient production.

1.3 Effects of rumen bolus use on the growth and developmental characteristics of the offspring

The growth and development of newborn offspring in animal production are closely linked to the timely and sufficient intake of essential nutrients. In this context, intraruminal bolus administration has significant effects on early growth rate, rumen development and immune system support in young animals.

In a study conducted on Mehraban sheep, the effects of a bolus of slow-release minerals (P, Mg, Zn, Co, I, Se) and a selenium injection administered approximately six weeks before parturition were evaluated in terms of biochemical parameters and offspring development (Aliarabi and Fadayifar, 2016). For this purpose, 105 sheep were divided into three treatment groups: The first group received a mineral-

containing bolus, the second group received a selenium injection and the third group received no treatment and served as a control. Blood samples taken before and after birth were analyzed for GSH-Px, alkaline phosphatase (ALP) and vitamin B12 levels. In addition, the live weights of the lambs at birth and weaning were recorded. At the end of the study, it was found that GSH-Px activity increased in both the mineral bolus group and the selenium injection group before birth; however, this effect only persisted in the bolus group after birth. Administration of the mineral bolus significantly increased vitamin B12 and ALP levels in both periods. In addition, the lambs in the mineral bolus group showed significantly higher live weights and daily body weight gains, as well as a lower mortality rate and a lower incidence of white muscle disease. Similarly, the studies by Kendall et al. (1997) and Kendall et al. (2000) also reported that the administration of mineral boluses before birth had positive and lasting effects on the health of the ewes and the development of the lambs.

In a study examining the effects of slow-release selenium and sulfamethazine boluses in kids, a significant decrease in the incidence of parasite infections was found (Diaz-Sanchez et al., 2020). It was also found that bolus administration significantly improved growth performance for a certain period of time. This finding suggests that the effectiveness of mineral bolus applications in terms of performance outcomes may vary depending on the active ingredient, dosage and timing of administration. In a study conducted on Saanen goats, the in vitro release kinetics and field performance of continuous-release boluses were evaluated (Çomak et al., 2024). Field trials showed that the group receiving bolus treatment had a higher rate of multiple births and the offspring of goats that gave birth to twins and triplets had significantly higher body weight gain compared to the control group. The researchers concluded that long-acting boluses can have positive effects on reproductive performance as well as on the growth and development of the kids. In Afshar sheep, boluses containing trace elements have been reported to improve plasma mineral concentrations, increase fertility rates and reduce infertility, all of which have a positive effect on the health of the offspring (Abdollahi et al., 2015).

In summary, rumen boluses containing various trace elements, vitamins and immune-supporting substances have a significant effect on the growth and development of the offspring. Especially in pregnant breeding animals, nutrient requirements increase in terms of both quantity and variety in the

last three weeks of the prenatal period due to the rapid development of the fetus and the start of milk synthesis in the mammary glands. Therefore, the administration of bolus supplements in the rumen 3–4 weeks before weaning can provide significant benefits for the health of the maternal animal and newborn. Offspring that complete their prenatal development adequately and are born at a higher birth weight tend to have better postnatal growth and development. The controlled and sustained release of minerals helps to maintain the continuity of important metabolic processes, particularly those involving key elements such as copper, zinc and cobalt, thus supporting animal health and improving growth performance. In addition, the inclusion of antiparasitic agents in the bolus contributes to a reduction in the parasite load, which indirectly leads to better growth results. To optimize the positive effects of rumen bolus technology on offspring growth, improvements in formulation and physical design are required. This includes the development of dissolution profiles tailored to specific animal species and growth stages, as well as the adaptation of manufacturing processes to field conditions. Future research focusing on these areas will help to improve the efficiency of bolus applications and support the development of sustainable growth promotion strategies in animal husbandry.

1.4 Effects of rumen bolus use on lactation efficiency and composition

In small ruminants, milk yield and milk composition during lactation are influenced by various factors such as nutrition, metabolic balance and adequate micronutrient intake. In this context, mineral and nutrient supplements administered in bolus form are often used to support the overall health of the animals and improve both the quantity and quality of milk. Recent studies have shown that the administration of slow-release bolus supplements, particularly in late gestation, can have significant effects on milk production and milk components.

Rashnoo et al (2020) reported in their study on goats that the administration of slow-release boluses containing selenium (Se) and iodine (I) during the late gestation period resulted in a significant increase in milk yield, milk fat percentage and overall milk chemical composition. In addition, a significant increase in Se and I concentrations in the milk was observed. The kids of the selenium-fed goats also showed higher daily live weight gains and weaning weights compared to the control group. Another

study investigated the effects of an oral calcium bolus administered during parturition on blood calcium, magnesium and phosphorus levels as well as milk yield and chemical composition during early lactation (Alhelo and Serbester, 2024). The study concluded that daily and total milk yield increased by 9.6% and 9.3% respectively during the first 28 days of lactation. However, no significant differences in milk composition or total lactation performance were found between the cows receiving the calcium bolus and those in the control group.

Similarly, a study conducted on 75 Italian Holstein cows investigated the effects of a bolus containing a herbal supplement. The bolus, which contained Echinacea purpurea, Silybum marianum, L-carnitine and vitamin E, was reported to positively influence energy metabolism, immune response and liver health factors that indirectly contributed to an increase in milk production (Esposito et al., 2024). The use of selenium (Se) bolus in small ruminants has also had a positive effect on milk yield and composition (Rashnoo et al., 2020). Selenium is a trace element involved in the synthesis of antioxidant enzymes such as glutathione peroxidase. Studies using organic, inorganic and nano-forms of selenium supplementation have reported an increase in milk yield as well as improvements in fat and protein content of milk (Amin et al., 2022). In particular, the use of Se yeast (organic selenium) was found to increase the fat and protein content of milk while reducing the number of somatic cells (Reczyńska et al., 2019). Rashnoo et al. (2020) also reported that the administration of a selenium-containing bolus during gestation in 40 dairy goats led to a significant increase in milk yield after birth as well as in the fat and protein content of the milk. In addition, selenium and iodine concentrations increased in the milk of the animals aplied bolus, which was associated with improved serum antioxidant levels in the offspring.

In a study conducted by Pirestani et al. (2011), the effects of slow-release mineral and vitamin boluses compared to feed supplements were investigated in relation to milk yield, composition and udder immune system in dairy cows. Sixty Holstein cows were used in the study and randomly divided into two treatment groups of 30 animals each: one group received a mineral-enriched bolus, while the other group received daily minerals in their feed for six months. The results showed no significant differences in milk yield between the groups. However, remarkable improvements in milk composition were

observed in the bolus-treated group. In addition, the somatic cell count (SCC) was lower in the bolus group, indicating a positive influence on udder health. From an immunological point of view, bolus administration increased the level of immunity-related proteins in the milk. Thus, although both treatment groups performed similarly in terms of milk yield, the bolus was shown to have positive effects on milk composition, udder health and immune function. In particular, the decrease in SCC indicates a lower risk of mastitis. In addition, the authors emphasized that long-acting sustained-release boluses can optimize mineral release and prevent daily fluctuations in mineral intake commonly observed with feed-based supplementation.

In a four-year study making in grazing cattle, the effects of long-acting boluses containing copper (Cu), selenium (Se) and cobalt (Co) on performance and reproductive traits were investigated (Sprinkle et al., 2021). In the study, two treatment groups that received bolus supplementation at different times were compared with a control group, with a total of 924 cows consisting of Hereford and crossbred cows being examined. At the end of the study, the use of boluses showed no significant effects on body condition scores or milk yield. However, positive effects on calf birth weight, weaning weight and calving interval were observed. It was also reported that bolus supplementation could compensate for mineral deficiencies in the pastures and possibly improve productivity. The researchers emphasized that long-acting mineral boluses have the potential to increase herd productivity, especially in areas where trace mineral deficiencies are prevalent. Kachuee et al (2019) investigated the effects of supplementation with organic, inorganic and selenium nanoparticles administered during late gestation in Khalkhali goats on selenium, zinc, copper and iron concentrations and on the transfer of these trace elements to the offspring via the placenta, colostrum and milk. The results showed that supplementing the diet of pregnant goats with various forms of selenium including sodium selenite (SS), selenomethionine (SM) and selenium nanoparticles (SN) resulted in increased Se levels in whole blood and serum compared to the control group. Differences were observed in the transplacental transfer capacity of the various Se forms. For example, the goats that received SM had higher whole blood and serum Se concentrations in their kids than those that received SS or SN. The researchers also found that supplementation with SN failed to increase Se concentrations in newborn kids. In addition, colostral Se concentrations were higher

in the SM group than in the SS and SN groups, suggesting that selenomethionine passed into the milk at the fourth week of lactation at a higher rate than sodium selenite and Se nanoparticles.

The effects of plant-based boluses on milk yield have also been demonstrated in various studies. For example, the use of Galactin Vet Bolus, a plant-based product, resulted in a significant increase in milk production in Holstein × Jersey crossbred cows (Ravikumar and Bhagwat, 2008). This increase was particularly evident in cows in the later stages of lactation, with some animals showing an increase in milk yield of more than 20. In addition to milk yield, studies on the chemical composition of milk have shown that mineral boluses can affect calcium metabolism, which may indirectly affect milk composition. However, most studies have found only limited direct effects on milk composition. For example, calcium-containing boluses have been shown to increase serum calcium levels after birth, thereby reducing the risk of hypocalcemia. However, the direct effects of such interventions on milk yield and composition have not been clearly demonstrated (Jahani-Moghadam et al., 2018). In addition, a study by Baig and Bhagwat (2009) reported that the herbal bolus Galactin Vet has positive effects on milk yield and contributes to an improvement in milk composition, particularly in terms of fat and non-fat content (SNF). The preparation contains herbal substances such as Leptadenia reticulata, Asparagus racemosus and Withania somnifera, which are known to have galactopoietic properties. The researchers pointed out that these effects are mediated by the stimulation of prolactin secretion, which in turn promotes milk synthesis.

In general, slow-release boluses containing minerals or phytonutrients administered during late gestation or early lactation have been shown to increase milk production and positively influence the chemical composition of milk in ruminants. These effects can be attributed to the active ingredients in the boluses, which may influence the endocrine system, support metabolic balance and improve antioxidant defense mechanisms.

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1.5 Effects of rumen bolus use on the immune system and digestion

Boluses used as feed additives for ruminants have been developed to meet physiological needs, maintain metabolic balance and support the immune system. These bolus applications, which contain vitamins, minerals, probiotics and other bioactive compounds, exert direct effects in the gastrointestinal system through controlled release. As a result, they improve digestive efficiency and contribute to the development of immune resistance against disease. Studies investigating the effects of rumen bolus on the immune and digestive systems are presented below.

In a study investigating the effects of boluses with trace elements containing zinc, cobalt and selenium on the immune system, 34 Suffolk cross lambs were used at three months of age (Kendall et al., 2012). Each bolus weighed approximately 33 g and contained 15.1% zinc, 0.52% cobalt and 0.15% selenium. During the trial period, the lambs grazed for 63 days without additional feed or mineral supplementation and were later moved to fresh pasture and slaughtered at either 86 or 121 days of age. Bolus administration resulted in a significant increase in erythrocyte glutathione peroxidase (eGSH-Px) activity and vitamin B12 levels. These results indicate a strengthening of the immune system, especially compared to the control group, which showed signs of cobalt and selenium deficiency. Although plasma zinc levels decreased over time in both groups, they remained higher in the bolus-treated group. No differences were found in the zinc concentrations in the liver. However, due to the suppressive effect of zinc on copper absorption, liver copper levels were lower in the bolus group. Regarding growth performance, daily live weight gains between day 42 and 63 were significantly higher in the lambs receiving the bolus than in the control group a result that can be attributed to the cobalt deficiency. In addition, increased IgG levels in the lambs treated with the bolus indicated an improved immune response. In conclusion, the administration of trace element bolus especially in grazing systems where mineral availability is limited supports immune function and improves growth performance in lambs.

In a study of 105 pregnant Mehraban ewes about six weeks before lambing, the efficacy of selenium and vitamin E injections was compared with that of slow-release boluses containing zinc, selenium and cobalt (Aliarabi & Fadayifar, 2013). At the end of the study, it was reported that the treatment with slow-

release boluses resulted in significantly higher levels of antioxidant enzyme activity, plasma selenium and vitamin B12 in the pregnant ewes than the inorganic Se and vitamin E injections. In addition, the lambs of the bolus-treated ewes showed better growth performance and survival rate. In another study, 20 out of 100 Alpine goat kids naturally infected with Eimeria, weaned, showing signs of diarrhea and not previously treated prophylactically or therapeutically were selected to evaluate the effects of an intraruminal bolus containing sodium sulfametazine (SM) and selenium (Se) (Díaz-Sánchez et al., 2020). In conclusion, The bolus with 4 g SM and 90 mg Se led to a significantly lower load of Eimeria spp. in kids with an average body weight of 13.7 kg. Rose et al (2012) conducted a study with 38 Holstein–Friesian cows and administered a bolus of iodine, selenium and cobalt in the rumen approximately 57 days before calving. The results showed no significant effect of bolus administration on colostral IgG uptake in calves. However, a positive correlation was found between plasma T_3 concentration at 24 hours of age and IgG absorption and a negative correlation between plasma T_4 concentration at one hour of age and IgG transfer. These results suggest that complex interactions between thyroid hormones particularly T_3 and T_4 may play a role in modulating IgG uptake in neonates.

In a study conducted by Chaleshtori et al. (2021) using 80 Lori-Bakhtiari ewes, the effects of slow-release copper boluses administered during the late gestation period on lamb development were evaluated. Despite maternal serum copper levels remaining within physiological norms, lambs born to the bolus-treated ewes exhibited significantly higher weaning weights, daily body weight gains, serum copper, ceruloplasmin levels, and hematological parameters (hematocrit, hemoglobin concentration, and red blood cell count) compared to those in the control group. These findings indicate that intraruminal slow-release copper boluses given in late pregnancy can positively impact growth performance and specific hematological measures in lambs. Mineral deficiencies particularly of trace elements such as copper, selenium, and cobalt can adversely affect immune, growth, and reproductive performance in ruminants. To investigate whether soluble glass-form boluses could support these element levels, two field trials were conducted in grazing yaks (Tibetan cattle) (Liu Zongping, 2007). In these studies, 100 yaks were involved, with half of the animals receiving commercial boluses containing copper, cobalt, and selenium. Biochemical analyses of blood samples showed that yaks applied bolus had significantly

higher serum ceruloplasmin, vitamin B_{12}, and erythrocyte glutathione peroxidase activities. Additionally, their serum selenium and copper concentrations were significantly elevated compared to controls, although zinc and cobalt concentrations were similar between the treatment groups. In another investigation, slow-release boluses containing Se, Cu, Zn, Co, and Mn were administered approximately 60 days before parturition to Naemi ewes and their offspring (Abdelrahman et al., 2017). Significant increases were observed in mineral levels in both blood and colostrum. Specifically, bolus-treated ewes exhibited higher blood levels of calcium, zinc, cobalt, and selenium, along with increased inorganic matter in colostrum. In their lambs, phosphorus, cobalt, and selenium concentrations were elevated. Moreover, bolus-treated ewes demonstrated higher total protein and cholesterol levels, while their lambs had reduced levels of glucose, urea, and triglycerides. The offspring of ewes applied bolus also displayed markedly higher live weights at 30 and 60 days post-birth compared to control group. In addition, the researchers noted that trace mineral supplementation via bolus contributed positively to both milk composition and colostrum quality.

A review of previous studies shows that slow-release boluses containing trace elements, vitamins and bioactive compounds administered to ruminants have significant and multifaceted positive effects on animal health, immune function, metabolic balance and growth performance. Bolus applications in particular are seen to be an effective way to correct mineral deficiencies, improve antioxidant defenses and support digestive health in grazing animals, pregnant females or young ruminants. Boluses containing selenium, zinc, copper, cobalt and vitamin B_{12} have been reported to increase concentrations of minerals in plasma and liver, boost immune defenses and improve various hematological parameters. In addition, these applications have been shown to have a positive effect on offspring development, colostrum quality and postnatal growth performance. Overall, bolus supplementation in the rumen is recommended as a strategic approach to support healthy growth, immune strength and productivity in ruminants, especially in populations at risk of trace element deficiencies.

1.6 Effects of rumen bolus use as a medicine on animal health

In recent years, the increasing use of bolus-form drug applications for improving animal health and achieving optimal productivity has emerged as an effective strategy. These pharmaceutical boluses are typically administered orally and are formulated as dense tablets designed to remain in the rumen for extended periods, offering controlled release of active ingredients. Their use reduces the need for frequent or repeated drug administration, thereby minimizing animal stress and labor costs. In small ruminants such as sheep and goats, boluses containing minerals, vitamins, or drugs are widely used for nutritional supplementation, immune support, and control of parasitic diseases. Thanks to the anatomical structure of the ruminoreticular system, these high-density boluses can remain in the gastrointestinal tract for long durations and maintain prolonged efficacy (Ramteke et al., 2014).

The use of slow-release boluses containing zinc, selenium, and cobalt has been reported to improve growth performance and blood biochemical parameters in Markhoz goat kids (Aliarabi et al., 2017). The same study found significant increases in daily live weight gain, blood concentrations of vitamin B12 and zinc, alkaline phosphatase activity, and glutathione peroxidase levels in the bolus-treated group. Furthermore, an increase in the thyroid hormone T3 was observed, indicating the bolus's supportive role in metabolic activities. In grazing-based small ruminant systems, where mineral deficiencies are common, administering such mineral supplements in bolus form strengthens the immune system, enhances disease resistance, and positively impacts reproductive and growth performance. Gutiérrez-Blanco (2006) evaluated the effects of a slow-release intraruminal sulfamethazine bolus on parasite excretion in feces and live weight gain in Pelibuey lambs naturally infected with Eimeria spp. The study concluded that the parasite count in feces significantly decreased in bolus-treated lambs and that growth performance improved, particularly under grazing conditions. Thus, the controlled-release properties of the bolus enabled long-lasting antiparasitic activity from a single dose. However, in lambs reared only under entansive conditions, performance parameters did not show significant improvement, highlighting the importance of evaluating bolus efficacy in the context of environmental factors.

From a pharmaceutical perspective, bolus formulations are designed to remain in the ruminoreticular compartment for extended periods due to their high density, during which they continuously release active ingredients to maintain therapeutic effects. However, there are technical challenges associated with bolus manufacturing. The requirement to include high concentrations of active compounds limits the use of binders, diluents, and excipients, potentially affecting bolus stability and release profiles. Additionally, the application equipment (bolus gun) and techniques must be appropriate for the species and administered with care. When evaluated based on existing scientific data, the use of boluses in ruminants offers significant advantages for both animal health and farm productivity. Boluses used for correcting nutritional deficiencies or for disease prevention and treatment contribute to animal welfare and enhance production profitability due to their prolonged effects. However, achieving these benefits requires careful consideration of factors such as bolus formulation, method of administration, dosage, and target species. Studies in the literature have demonstrated that bolus applications improve metabolic health indicators and are effective in combating parasitic infections. In this context, bolus technology can be regarded as a strategic tool for sustainable production and effective herd health management in ruminant livestock (Gutiérrez et al., 2006; Ramteke et al., 2014; Aliarabi et al., 2016).

2 Conclusion

Based on the studies, it can be said that rumen boluses are an innovative and effective tools for optimizing nutrition and health management in ruminants. In particular, boluses, which ensure a sustained and controlled release of minerals and vitamins, allow the continuous coverage of the physiological needs of ruminants. In addition, the use of boluses containing antiparasitic and probiotic agents offers significant benefits in maintaining animal health. Studies have shown that rumen boluses can have positive effects on live weight gain, milk yield, immune status and overall animal performance. However, it should be noted that the effectiveness of bolus administration may depend on factors such as bolus composition, method of administration and the age and species of the animal. Therefore, the following recommendations are suggested:

Bolus formulations should be specific to the animal species (cattle, sheep, goats), age and physiological status (gestation, lactation, growth period) and boluses with appropriate content and release profiles should be selected.

Rumen boluses containing antiparasitic agents can be considered as an alternative strategy for parasite control, especially in large herds, as they are easy to use and accurately dosed. Especially in regions with high seasonal parasite infestations, long-acting antiparasitic bolus formulations can be an alternative to the widespread use of medication.

There is a need for more controlled studies on the long-term effects of boluses on rumen microflora, variations in animal response due to genetic differences and interactions with different feed rations. Therefore, further field studies are needed to evaluate the effects and performance of rumen boluses under different environmental conditions and in different ruminant species.

Finally, a detailed analysis of the economic impact of bolus applications in animal production will provide decision makers with concrete data to support the wider adoption of this technology in practice.

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I-CRAFT AGRICULTURAL and FOOD TECHNOLOGIES

Investigation of the Relationships Between Body Weight, Body Measurements and Testicular Measurements in Saanen Kids

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Abstract. This study was conducted to investigate the relationships between body weight, body measurements and testicular measurements in Saanen kids. For this purpose, 45 male Saanen kids obtained from goats with synchronized births were used. Body weight (BW), body measurements such as withers height (WH), rump height (RH), body length (BL), chest depth (CD), chest girth (CG) and testicular measurements such as testicular diameter (TD), testicular length (TL), scrotal circumference (SC), scrotal length (SL), scrotal volume (SV), testicular volume (TV) were measured in the kids in the first 3 months after birth. The overall mean values of WH, RH, BL, CD, CG, TD, TL, SC, SL, SV, TV and BW of the Saanen kids were 48.19±0.44 cm, 47.58±0.54 cm, 45.02±0.45 cm, 17.69±0.23 cm, 47.30±0.54 cm, 2.01±0.05 cm, 3.95±0.09 cm, 9.70±0.27 cm, 56.25±1.37 cm, 43.33±2.38 cm^3, 17.34±1.41 cm^3 and 11.46±0.25 kg, respectively. The mean values for body weight, body measurements and testicular measurements differed significantly between all months ($p < 0.01$). The correlation coefficient between SC and TV was highest ($p < 0.01$) except for month 1. In the first month, the correlation coefficient between WH and RH was the highest, the correlation coefficient between SC and TV was the second highest ($p < 0.01$).

Keywords: Body measurement, Testicular measurement, Saanen, Correlation.

1 Introduction

Saanen goats are among the most preferred dairy goat breeds in the world due to their high milk yield and their pronounced adaptability to different environmental conditions (Akdag et al., 2011). In addition to milk production, the reproductive performance of the males also plays a decisive role in the productivity of the herd and the sustainability of genetic progress. Especially in high-yielding dairy breeds such as the Saanen, early evaluation of growth and reproductive traits in male kids is of great importance for the success of breeding programs. In this context, morphological traits such as body weight and body measurements are important indicators that reflect the animal's general state of development and health. On the other hand, testicular measurements are widely used for the selection

of future superior male goats (Fonseca et al., 2021). Determining the relationships between body measurements and testicular traits can enable the early identification of individuals with high reproductive potential. Such information allows producers to identify superior males at an early age without incurring the cost of keeping unproductive animals. In addition, the amount of the hormone testosterone produced in the testes affects both the quality and quantity of semen depending on testicular size (Gofur et al., 2014) and also aids to development of muscular and skeletal system (Peralta et al., 1994). Therefore, the amount of testosterone produced in relation to testicular size is crucial for healthy growth and development in kids and for ensuring sustainable reproductive performance later in life. However, there are few studies on this topic and there is a need for comprehensive data analysis, particularly in Saanen kids. Correlation analysis is needed to examine the relationships between body and testicular measurements of Saanen kids. The correlation coefficient found in the correlation analysis measures the direction and strength of the relationship between two measurements (Schober et al., 2018).

The aim of this study was to evaluate the relationships between live weight and body measurements such as withers height, rump height, body length, chest depth and chest circumference, and testicular traits such as testicular diameter and length, scrotal circumference and length, scrotal volume and testicular volume in male Saanen offspring.The findings are intended to provide a scientific basis for early breeding selection in dairy goat breeding. The correlation analysis of body measurements and testicular measurements in Saanen kids can provide valuable information for genetic selection, nutritional management and the development of breeding strategies.

2 Material and Method

The present study was conducted in the eastern Mediterranean region, where subtropical climatic conditions prevail. The province of Adana, where the study was conducted, is a province in the Mediterranean region located at 37º north latitude and 35º east longitude and 40 meters above sea level. Summers in the region are hot and dry, while winters are warm and rainy. The annual average humidity is 66% and the prevailing winds come from the north and south. Due to the differences in pressure throughout the seasons, the winds can blow from both directions. However, in summer they generally blow from the south and southwest and in winter from the north.

The present study was conducted on 45 male kid obtained from Saanen goats raised in Cukurova University, Faculty of Agriculture, Dairy Goat Breeding Research and Application Unit, Adana, Türkiye. The kids used for the experiment were weaned according to standard farm routines. Accordingly, the kids were fed a sufficient amount of colostrum in the first few days after birth. They then remained with their dams until they were one month old and began to consume roughage and concentrates from the second week onwards. When the kids were one month old, they were separated from their dams during the day and reunited with them overnight after the evening milking. At the age of 1.5 months, the kids were then allowed to stay with their dams for two hours after both the morning and evening milking. When the kids were three months old, they were completely weaned. After the 2nd weeks to kids were fed with concentrated feed having 16% HP and 2400 kcal/kg energy content and alfalfa hay having about 14% HP content as roughage.

After birth, the body weight, body measurements (withers height, rump height, body length, chest depth, chest girth) and testicular measurements (testicular diameter, testicular length, scrotal circumference, scrotal length, scrotal volume and testicular volume) of the kids were determined at one, two and three months of age. Accordingly, the body weight (BW) of the animals was determined at one, two, and three months of age by individual weighing with a digital scale (TEM, EKO-600) with an accuracy of 50 g on the same day. The withers height, rump height, body length and chest depth of the kids were measured with a measuring stick, and the chest girth was measured with a measuring tape. The testicular diameter and testicular length of the kids were measured with a caliper, scrotal circumference and scrotal length were measured with a tape and scrotal volume was measured with a measuring container. Testicular volume of kids were determined according to Godfrey et al. (1998). Body measurements and other testicular measurements were taken as follow.

Withers height (WH, cm): The vertical distance between the highest point of the withers and the ground level.

Rump height (RH, cm): The vertical distance between the highest point of the rump and the ground level.

Body length (BL, cm): The body length was determined by measuring the distance between the tip of the shoulder and the hip bone tip.

Chest depth (CD, cm): The vertical distance between the highest point of the withers and the sternum.

Chest girth (CG, cm): The length of a 360° circumference measured from the back of the withers, above the sternum.

Testicular diameter (TD, cm): The testicular diameter was determined by measuring the widest part of each testicle.

Testicular length (TL, cm): Testicular length was determined by measuring the length between the tip of the testis and the epididymis.

Scrotal circumference (SC, cm): Scrotal circumference was determined by measuring the circumference of a pair of testicles at the widest point.

Scrotal length (SL, cm): Scrotal length was determined by measuring the distance from the point where the scrotum joins the inguinal region to the tip.

Scrotal volume (SV, cm^3): The scrotal volume was determined by measuring the amount of water displaced by the testis from a measuring container.

Testicular volume (TV, cm^3): 0.0396 × (average testis length) × (scrotal circumference)².

Data collected were analyzed using SPSS 25 V. Descriptive statistics (Means ± SE) of body weight, body measurements, and testicular measurements taken in the first 3 months after birth were given. The differences between the three months were analyzed using the Friedman test. Where statistically significant differences were observed, pairwise comparisons were conducted using the Wilcoxon signed-rank test. The significance threshold was adjusted using the Bonferroni correction to control for Type I error in multiple comparisons (α/3). Relationships between the body weight, body measurements and testicular measurements were calculated by Spearman's rank correlation coefficient.

3 Results and Discussion

Descriptive statistics (Means ± SE) of body weight, body measurements and testicular measurements have been presented in different months in Table 1. The normality assumption for the three repeated measurements was assessed using the Shapiro–Wilk test. Since the assumption was violated in at least one of the month ($p < 0.05$ or $p < 0.01$), the non-parametric Friedman test was employed instead of

repeated measures ANOVA to evaluate differences across the three months. The results indicated a statistically significant difference among the months for all measurements ($p < 0.01$).

Table 22. Body measurements, testicular measurements and body weight of Saanen kids according to months.

Variables	Month 1	Month 2	Month 3	Overall
BW	7.53±0.12	11.49±0.31	15.37±0.33	11.46±0.25
WH	43.80±0.35	48.37±0.49	52.41±0.49	48.19±0.44
RH	43.84±0.38	47.52±0.63	51.40±0.63	47.58±0.54
BL	41.59±0.45	43.94±0.45	49.53±0.45	45.02±0.45
CD	15.63±0.19	17.59±0.25	19.86±0.25	17.69±0.23
CG	43.69±0.44	46.38±0.59	51.84±0.59	47.30±0.54
TD	1.49±0.04	2.12±0.06	2.44±0.06	2.01±0.05
TL	3.16±0.07	4.15±0.11	4.54±0.11	3.95±0.09
SC	7.42±0.17	10.46±0.33	11.23±0.31	9.70±0.27
SL	49.20±1.17	55.43±1.42	64.14±1.52	56.25±1.37
SV	12.17±0.86	40.65±2.98	77.19±3.31	43.33±2.38
TV	7.32±0.49	19.97±1.76	24.73±1.98	17.34±1.41

BW: body weight, WH: withers height, RH: rump height, BL: body leght, CD: chest depth, CG: chest girth, TD: testicular diameter, TL: testicular length, SC: scrotal circumference, SL: scrotal length, SV: scrotal valume, TV: testicular volume.

As a result of Wilcoxon signed-rank test with Bonferroni correction post hoc test, a statistically significant difference was found between the 1st month and the 2nd and 3rd month, as well as between the 2nd month and the 3rd month in terms of body weight, all body measurements and all testis measurements ($p < 0.0167$). All means increased progressively from Month 1 to Month 3.

Changes in body weights, body measurements and testicular measurements in Saanen kids during the first 3 months are given in Figure 1, 2 and 3.

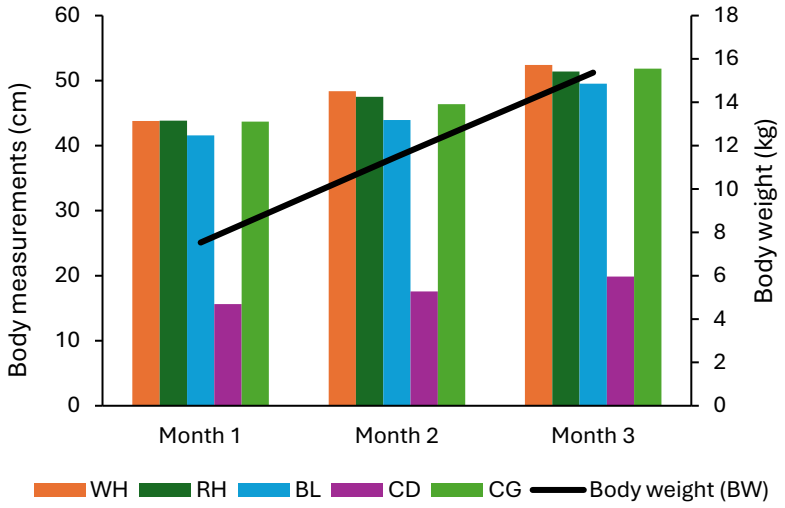

Fig. 22. Changes in body weights (kg) and body measurements (cm) in Saanen kids during the first 3 months.

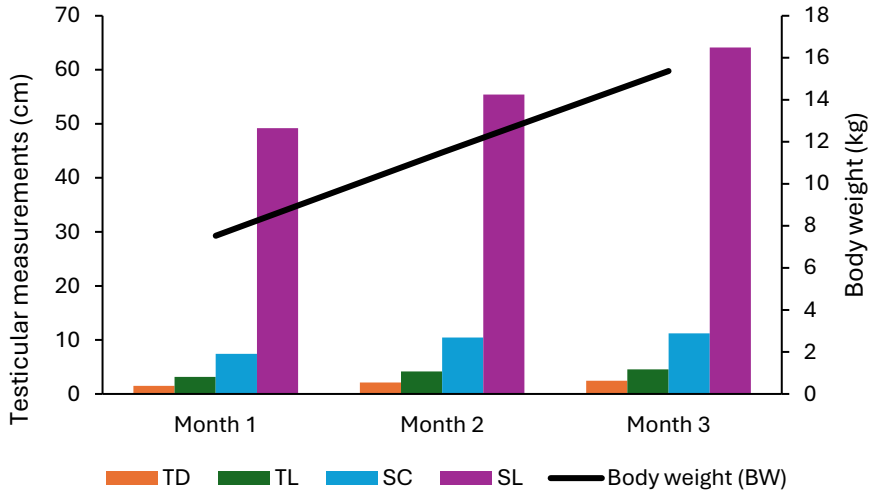

Fig. 23. Fig. **2.** Changes in body weights (kg) and testicular measurements (cm) in Saanen kids during the first 3 months.

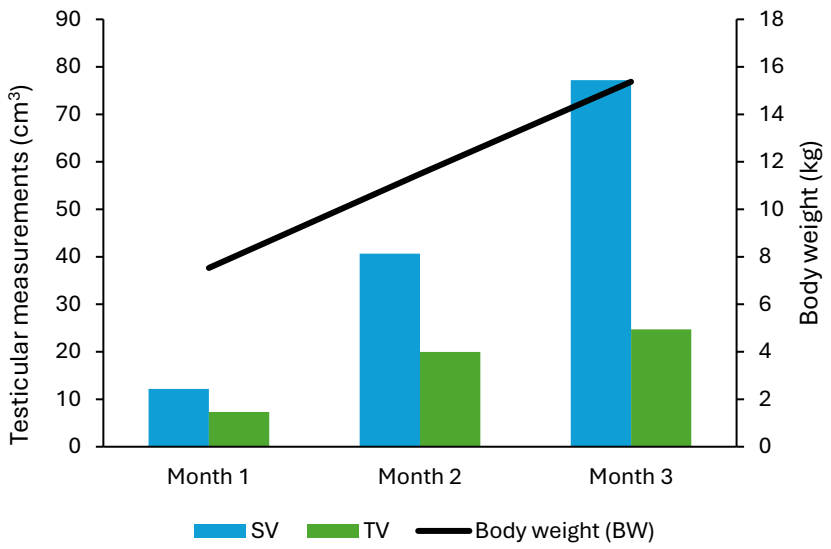

Fig. 24. Changes in body weights (kg) and testicular measurements (cm^3) in Saanen kids during the first 3 months.

The relationships between body weights, body and testicular measurements of Saanen kids at month 1 in Table 2. Due to the non-normal distribution of some variables, Spearman's rank correlation coefficient was used to assess the relationships between variables (Kornbrot, 2005; 2014).

When the 1rd month measurements are analyzed, the correlation coefficient between WH and RH (r = 0.97) was the highest. The correlation coefficient between SC and TV (r = 0.95) was the second highest. In addition, except for the correlations between BW and WH, RH, BL, CD, CG, SC, SL, SV, TV and between BL and SV, all other correlations among the variables were statistically significant ($p < 0.05$ or $p < 0.01$).

Table 23. Correlations coefficients (r) between body measurements, testicular measurements and body weights (Month 1).

Variable	BW	WH	RH	BL	CD	CG	TD	TL	SC	SL	SV	TV
BW (kg)	1	0.23^{NS}	0.19^{NS}	0.12^{NS}	0.14^{NS}	0.20^{NS}	0.31*	0.33*	0.18^{NS}	0.27^{NS}	0.21^{NS}	0.24^{NS}
WH (cm)		1	**0.97****	0.61**	0.79**	0.81**	0.63**	0.59**	0.62**	0.55**	0.39**	0.62**
RH (cm)			1	0.57**	0.77**	0.83**	0.64**	0.60**	0.61**	0.56**	0.37*	0.62**
BL (cm)				1	0.62**	0.56**	0.53**	0.49**	0.59**	0.55**	0.28^{NS}	0.60**
CD (cm)					1	0.84**	0.65**	0.59**	0.60**	0.60**	0.46**	0.60**
CG (cm)						1	0.71**	0.73**	0.77**	0.66**	0.57**	0.78**
TD (cm)							1	0.71**	0.69**	0.61**	0.54**	0.74**
TL (cm)								1	0.72**	0.73**	0.51**	0.88**
SC (cm)									1	0.69**	0.53**	0.95**

SL (cm)	1	0.44**	0.73**
SV (cm^3)		1	0.56**
TV (cm^3)			1

BW: body weight, WH: withers height, RH: rump height, BL: body leght, CD: chest depth, CG: chest girth, TD: testicular diameter, TL: testicular length, SC: scrotal circumference, SL: scrotal length, SV: scrotal valume, TV: testicular volume. NS: $P > 0.05$, *: $p < 0.05$, **: $p < 0.01$, marked correlation is the highest correlation.

In Table 2, the lower or statistically not significant correlations between body weight and some testicular and body measurements at one month can be explained by the kids' early stages of anatomical and physiological development. Because testes are relatively small at this stage, testosterone production, which affects the musculoskeletal system and testicular size, is lower than at later ages. However, these correlations were observed to strengthen with the increase in testosterone production due to testicular development in the second and third months. Similar results were reported by Koyuncu et al. (2005), who stated that the relationships between body and testicular variables become more pronounced as development progresses. The relationships between body weights, body and testicular measurements of Saanen kids at month 2 in Table 3.

Table 24. Correlations coefficients (r) between body measurements, testicular measurements and body weights (Month 2).

Variable	BW	WH	RH	BL	CD	CG	TD	TL	SC	SL	SV	TV
BW (kg)	1	0.73**	0.69**	0.48**	0.68**	0.79**	0.72**	0.76**	0.65**	0.65**	0.58**	0.71**
WH (cm)		1	0.87**	0.58**	0.71**	0.81**	0.72**	0.67**	0.72**	0.57**	0.60**	0.74**
RH (cm)			1	0.58**	0.72**	0.80**	0.71**	0.71**	0.70**	0.58**	0.62**	0.72**
BL (cm)				1	0.66**	0.71**	0.65**	0.61**	0.61**	0.57**	0.51**	0.62**
CD (cm)					1	0.88**	0.83**	0.80**	0.69**	0.68**	0.58**	0.75**
CG (cm)						1	0.85**	0.85**	0.77**	0.73**	0.68**	0.81**
TD (cm)							1	0.90**	0.81**	0.83**	0.68**	0.86**
TL (cm)								1	0.86**	0.90**	0.70**	0.92**
SC (cm)									1	0.78**	0.79**	**0.98****
SL (cm)										1	0.74**	0.83**
SV (cm^3)											1	0.77**
TV (cm^3)												1

BW: body weight, WH: withers height, RH: rump height, BL: body leght, CD: chest depth, CG: chest girth, TD: testicular diameter, TL: testicular length, SC: scrotal circumference, SL: scrotal length, SV: scrotal valume, TV: testicular volume. **: $p < 0.01$, marked correlation is the highest correlation

When the 2nd month measurements are analyzed, the correlation coefficient between SC and TV ($r = 0.98$) was the highest and this correlation was statistically significant ($p < 0.01$). Moreover, comparing Table 2 and Table 3, there have been increases in the correlation between many variables.

Selection for fertility in sheep can be done effectively by identifying correlated traits in young rams, such as testicular size (Land and Carr, 1975). In addition, Dyrmundsson (1973) emphasized that body weight is a more reliable indicator of the onset of puberty than chronological age. Testicular size has been shown to have a significant positive correlation with ejaculate volume, sperm concentration and viability, while it is negatively associated with the proportion of abnormal sperm. These relationships have been confirmed in various livestock species such as cattle, goats and pigs (Condorelli et al., 2013; Jacyno et al., 2015; Almaguer et al., 2017; Hagiya et al., 2018). In addition to the effect on male reproductive performance, testicular size also influences the annual number of litters produced (Serdar et al., 2021). Yadav et al. (2019) reported a significant positive correlation between scrotal circumference and testicular volume, sperm motility and overall sperm movement in buffalo bulls. These results emphasize testicular development as one of the most effective indicators for the evaluation of male fertility. In the present study, body weight and morphometric traits, including testicular dimensions, were found to be positively and often highly significantly correlated in male kids. In particular, the strong correlation between scrotal circumference (SC) and testicular volume (TV) ($r = 0.98$, $p < 0.01$) emphasizes the potential use of these parameters in the selection of prepubertal male breeding. This result supports previous studies demonstrating the importance of testicular size as an important predictor of male reproductive ability.

The relationships between body weights, body and testicular measurements of Saanen kids at month 3 in Table 4. When the 3rd month measurements are analyzed, the correlation coefficient between SC and TV was the highest ($r = 0.98$, $p < 0.01$). The correlation coefficient between CD and CG and between TL and TV was the second highest ($r = 0.95$, $p < 0.01$) (Table 4). Moreover, comparing Table 3 and Table 4, the correlations between the variables did not major change.

Table 25. Correlations coefficients (r) between body measurements, testicular measurements and body weights (Month 3).

Variable	BW	WH	RH	BL	CD	CG	TD	TL	SC	SL	SV	TV
BW (kg)	1	0.60**	0.59**	0.45**	0.59**	0.71**	0.63**	0.67**	0.61**	0.56**	0.60**	0.65**
WH (cm)		1	0.87**	0.58**	0.71**	0.81**	0.69**	0.66**	0.70**	0.54**	0.56**	0.71**
RH (cm)			1	0.58**	0.72**	0.80**	0.66**	0.66**	0.68**	0.52**	0.55**	0.69**
BL (cm)				1	0.66**	0.71**	0.66**	0.61**	0.61**	0.53**	0.49**	0.61**
CD (cm)					1	0.88**	0.72**	0.72**	0.66**	0.63**	0.52**	0.69**
CG (cm)						1	0.78**	0.79**	0.76**	0.69**	0.65**	0.78**
TD (cm)							1	0.87**	0.84**	0.82**	0.74**	0.86**
TL (cm)								1	0.90**	0.90**	0.74**	0.95**
SC (cm)									1	0.83**	0.83**	**0.98****
SL (cm)										1	0.75**	0.86**
SV (cm^3)											1	0.80**
TV (cm^3)												1

BW: body weight, WH: withers height, RH: rump height, BL: body leght, CD: chest depth, CG: chest girth, TD: testicular diameter, TL: testicular length, SC: scrotal circumference, SL: scrotal length, SV: scrotal valume, TV: testicular volume. **: $p < 0.01$, marked correlation is the highest correlation

Androgens such as testosterone promote muscle development, bone growth and metabolic activity in males (Clarke et al., 2012; Rizk et al., 2023). These hormonal effects lead to higher growth rates, higher feed efficiency and greater muscle development. High correlations were found between measurements such as testicular length (TL), testicular diameter (TD), and testicular volume (TV) and structural body measurements such as chest girth (CG), body length (BL), and chest depth (CD). This suggests that body development and reproductive system development proceed in parallel. Therefore, overall growth performance may be an important determinant of potential reproductive success. Furthermore, previous studies have clearly demonstrated that testicular volume and scrotal volume are highly correlated with sperm production and testosterone levels (Marire et al., 1991; Chen et al., 2024). Therefore, testicular measurements are parameters that reflect not only morphological but also functional efficiency. The results of our study support these approaches.

Table 26. Correlations coefficients (r) between body measurements, testicular measurements and body weights (Overall).

Variable	BW	WH	RH	BL	CD	CG	TD	TL	SC	SL	SV	TV
BW (kg)	1	0.69**	0.66**	0.45**	0.63**	0.76**	0.70**	0.73**	0.65**	0.64**	0.61**	0.69**
WH (cm)		1	0.89**	0.64**	0.78**	0.86**	0.76**	0.72**	0.77**	0.65**	0.67**	0.76**
RH (cm)			1	0.61**	0.74**	0.83**	0.75**	0.74**	0.77**	0.65**	0.66**	0.75**

BL (cm)	1	0.71**	0.71**	0.70**	0.62**	0.66**	0.65**	0.55**	0.66**
CD (cm)		1	0.89**	0.77**	0.74**	0.72**	0.69**	0.62**	0.74**
CG (cm)			1	0.79**	0.84**	0.80**	0.75**	0.73**	0.83**
TD (cm)				1	0.83**	0.82**	0.81**	0.73**	0.83**
TL (cm)					1	0.91**	0.91**	0.77**	0.95**
SC (cm)						1	0.86**	0.81**	**0.98****
SL (cm)							1	0.77**	0.89**
SV (cm^3)								1	0.81**
TV (cm^3)									1

BW: body weight, WH: withers height, RH: rump height, BL: body leght, CD: chest depth, CG: chest girth, TD: testicular diameter, TL: testicular length, SC: scrotal circumference, SL: scrotal length, SV: scrotal valume, TV: testicular volume. **:p<0.01, marked correlation is the highest correlation

When 3 months together measurements are analysed, the correlation coefficient between SC and TV was the highest ($r = 0.98$, $p < 0.01$). The correlation coefficient between TL and TV was the second highest ($r = 0.95$, $p < 0.01$). In general, the high correlations detected between testicular and body measurements provide important contributions to the definition of reliable biometric indicators that can be used in early age breeding selection in dairy goat breeds.

4 Conclusion

This study found that body weight, morphological body measurements and testicular measurements were found to be statistically significantly different in male Saanen kids in the first three months after birth. The highest values for all variables were recorded in the third month.

The data obtained strong positive correlation between scrotal circumference (SC) and testicular volume (TV), which reached its highest value in overall ($r = 0.98$, $p < 0.01$). The correlations between testicular length (TL) and testicular volume (TV), testicular length (TL) and scrotal circumference (SC), testicular length (TL) and scrotal volume (SL) as well as between chest depth (CD) and chest girth (CG), withers height (WH) and rump height (RH), scrotal length (SL) and testicular volume (TV) were also high in overall.

In the first month, correlations between body weight and some testicular traits were limited, and some were not statistically significant. This could be due to the incomplete anatomical development of the

kid. However, these correlations became stronger in the second and third months, and the relationship between body weight and testicular development became clearer. This suggests that early growth performance may be closely related to reproductive ability.

Overall, the strong correlation between scrotal circumference (SC) and testicular volume (TV) suggests that this measurement can be used as a practical and reliable indicator for breeding selection. Morphological body measurements and testicular examinations at an early age can facilitate the selection of males with high reproductive potential and thus contribute to more effective and targeted genetic breeding programs in dairy goat herds. Therefore, further evaluation of parameters such as hormone levels and semen quality in future studies in addition to the measurements considered in this study is crucial to confirm these results.

Disclosure of Interests. The authors have no competing interests to declare that are relevant to the content of this article.

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I-CRAFT AGRICULTURAL and FOOD TECHNOLOGIES

Mesenchymal Stem Cells and Hippocampal Neurogenesis in CNS Disorders: Paracrine and Cellular Mechanisms

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Abstract. Adult hippocampal neurogenesis plays a pivotal role in cognitive function, memory consolidation, and neural plasticity. However, its decline in neurodegenerative and neuroinflammatory conditions such as Alzheimer's disease, Parkinson's disease, traumatic brain injury (TBI), and multiple sclerosis (MS) necessitates the development of regenerative therapies. Mesenchymal stem cells (MSCs) and their derivatives have emerged as promising agents in this regard, due to their unique capacity for immunomodulation, neuroprotection, and paracrine signaling. This review explores the cellular and acellular mechanisms by which MSCs enhance hippocampal neurogenesis, focusing on critical signaling pathways including BDNF, PI3K/AKT, ERK/CREB, and Wnt. We examine preclinical and clinical evidence supporting the efficacy of MSCs, neural stem cells derived from MSCs (MSC-NSCs), MSC-derived extracellular vesicles (MSC-Exos), and genetically modified MSCs. The therapeutic relevance of MSCs is further highlighted in organoid and iPSC-based models, illustrating their translational potential. We also discuss emerging strategies to overcome limitations in MSC survival, homing, and differentiation capacity. Overall, this review underscores the multifaceted role of MSCs in targeting hippocampal neurogenesis and offers insight into future directions for stem cell-based interventions in central nervous system disorders.

Keyword: Stem cell, neurogenesis, neuroplasticity

1 Introduction

Throughout life, the brain continuously adapts and evolves in response to both environmental and internal stimuli. This plasticity underlies critical cognitive processes such as learning and memory. Neuroplasticity—encompassing both the formation of new neurons (neurogenesis) and the remodeling of synaptic connections—constitutes a fundamental mechanism that enables this adaptability. Among

the neurogenic regions of the adult brain, the hippocampus stands out as a key site where new neurons are generated and integrated into existing circuits, playing an essential role in cognitive function [1].

However, aging, stress, trauma, and neurodegenerative diseases can impair neurogenesis and synaptic plasticity, leading to cognitive decline [2]. In recent years, stem cell-based approaches have gained considerable attention for their potential to restore neural function. The regenerative, immunomodulatory, and trophic effects of stem cells—particularly mesenchymal stem cells (MSCs)—are being investigated for their capacity to enhance hippocampal neurogenesis and counteract cognitive impairment [3,4].

Given their dual paracrine and cellular mechanisms of action, MSCs and their derivatives represent a promising therapeutic strategy in central nervous system (CNS) disorders. Understanding the pathways through which they influence neurogenesis and synaptic plasticity may offer new avenues for preserving and enhancing learning and memory. This review aims to provide a comprehensive overview of the impact of MSCs on hippocampal neurogenesis, highlighting their role in neurorestoration across various CNS pathologies.

2 The Role of The Brain in Learning and Memory Processes

The nervous system processes environmental stimuli, enabling organisms to learn and adapt. Learning involves acquiring new information, while memory refers to its storage and retrieval. Both functions critically depend on neuroplasticity—the brain's ability to reorganize its synaptic connections by strengthening frequently used pathways and weakening less-used ones, consistent with Hebb’s principle of “neurons that fire together, wire together” [5-6]. Long-term potentiation (LTP) underlies the strengthening of synapses during learning and is considered a cellular mechanism for memory encoding [7].

Newly acquired information is initially held in short-term memory. If deemed relevant or rehearsed, it is transferred into long-term memory through mechanisms largely supported by temporal lobe structures. The hippocampus plays a central role in this process, particularly in the consolidation of information and in supporting spatial and contextual learning [8,9].

While neuroimaging studies indicate the involvement of other brain areas such as the prefrontal cortex and amygdala in learning and memory, the hippocampus stands out due to its unique capability for adult neurogenesis, which is essential for the formation of new memories [10].

In summary, learning and memory are dynamic processes driven by neuroplasticity, with the hippocampus acting as a crucial hub for these adaptive neural mechanisms.

3 The Role of the Hippocampus in Neuroplasticity and Neurogenesis

The hippocampus, a major structure of the limbic system located in the medial temporal lobe, plays a pivotal role in cognitive functions including learning, memory formation, and spatial navigation. It is particularly important for processing information, consolidating memory, and transforming short-term memories into long-term ones [11]. Anatomically, the hippocampus is divided into three main subregions: CA1, CA3, and the dentate gyrus (DG). These areas are organized into a trisynaptic circuit that facilitates communication and plasticity. The dentate gyrus is particularly notable as one of the few regions in the adult brain where neurogenesis persists throughout life.

In contrast to embryonic development, adult neurogenesis occurs mainly in the subgranular zone (SGZ) of the dentate gyrus, where neural stem cells generate new granule neurons. These newborn neurons integrate into existing hippocampal circuits through processes including migration, maturation, synapse formation (synaptogenesis), and functional incorporation, contributing to learning, mood regulation, and memory [12].

Numerous intrinsic and extrinsic factors regulate this process. Positive modulators include environmental enrichment, physical exercise (notably treadmill running), and cognitive stimulation, all of which have been shown to enhance neurogenesis. Conversely, chronic stress, elevated glucocorticoid levels, aging, and neurodegenerative diseases are detrimental to hippocampal neurogenesis [1, 13]

Among the cellular correlates of hippocampal plasticity, long-term potentiation (LTP) stands out as a hallmark mechanism. LTP reflects a sustained increase in synaptic efficacy and plays a vital role in learning and memory. Notably, adult-born neurons actively participate in LTP, enabling greater adaptability and relearning capacity [10]. In tandem, long-term depression (LTD) serves as a balancing

mechanism by weakening synaptic strength, which is critical for refining neural circuits, removing redundant connections, and updating stored information [14]. The interaction between LTP and LTD enhances the flexibility of hippocampal networks, particularly during the integration of newly generated neurons, where they undergo activity-dependent synaptic remodeling [15].

Beyond its role in cognitive processing, the hippocampus represents a strategic target for regenerative therapies. Interventions involving stem cells or neurotrophic factors aim to modulate hippocampal plasticity and neurogenesis. Such strategies have shown promise in improving cognitive function and slowing cognitive decline, especially in age-related and neurodegenerative disorders such as Alzheimer's [16].

3.1 Neuroplasticity and Neurogenesis

Neuroplasticity refers to the brain's remarkable ability to structurally and functionally reorganize in response to internal and external stimuli [17]. This adaptive process involves the strengthening, weakening, or rewiring of synaptic connections, thereby facilitating learning, memory formation, and functional recovery following injury or disease [18,19].

Adult neurogenesis, defined as the generation of new neurons in the mature brain, predominantly occurs in two regions: the subgranular zone (SGZ) of the hippocampal dentate gyrus and the subventricular zone (SVZ) of the lateral ventricles. As a critical component of structural neuroplasticity, adult neurogenesis plays a central role in maintaining cognitive flexibility and emotional regulation [1].

Both neurogenesis and synaptic plasticity are modulated by a broad range of intrinsic and extrinsic factors. Positive stimuli such as physical activity, enriched environments, and certain pharmacological agents have been shown to enhance these processes. In contrast, aging, chronic stress, and neurodegenerative conditions are associated with a decline in neurogenic activity and plasticity [20, 21].

Further sections will provide an in-depth discussion of the molecular pathways underlying neurogenesis—such as brain-derived neurotrophic factor (BDNF) signaling and the PI3K/AKT pathway—and explore how mesenchymal stem cells (MSCs) may influence these mechanisms through paracrine and cellular effects.

3.2 Stem Cells and Types

Stem cells are undifferentiated cells capable of self-renewal, proliferation, and differentiation into specialized cell types under appropriate conditions [22]. Based on their developmental potential and source, stem cells are classified into several categories.

Embryonic stem cells (ESCs) are pluripotent cells derived from the inner cell mass of the blastocyst. They can differentiate into all cell types originating from the ectoderm, mesoderm, and endoderm layers. This pluripotency means that ESCs can give rise to virtually any cell type in the body, making them extremely valuable for regenerative medicine and developmental studies. However, ESCs do not have the capacity to form extraembryonic tissues such as the placenta, which distinguishes them from totipotent cells.

Totipotent cells, such as the zygote and early-stage blastomeres, possess the most extensive differentiation potential. They can form all embryonic and extraembryonic tissues, meaning they have the capacity to generate a complete organism. This totipotency is generally restricted to the earliest stages of embryogenesis [23,24].

In contrast, progenitor cells are typically unipotent or have limited multipotent capacity, meaning they are generally restricted to differentiating into a single or a few related cell types. These cells serve as intermediate precursors in the differentiation hierarchy and are crucial for tissue maintenance and repair but lack the extensive plasticity of stem cells.

Multipotent adult stem cells reside in various tissues such as bone marrow, skin, and brain, and exhibit a more restricted differentiation capacity compared to pluripotent stem cells. Among them, hematopoietic stem cells (HSCs) are multipotent and can generate all blood cell types—including erythrocytes, leukocytes, and thrombocytes—thus maintaining the hematopoietic system throughout life [25]. These cells are fundamental for hematopoiesis and have been widely used clinically in bone marrow transplantation. Another major population of adult stem cells is mesenchymal stem cells (MSCs), also referred to as mesenchymal stromal cells. MSCs are multipotent cells primarily isolated from bone marrow but also found in adipose tissue and umbilical cord blood. They can differentiate into osteoblasts, chondrocytes, and adipocytes [26]. Besides their differentiation potential, MSCs have important paracrine and immunomodulatory functions, which make them promising candidates for regenerative therapies, including those targeting neurogenesis and neuroplasticity.

In addition, induced pluripotent stem cells (iPSCs) represent a groundbreaking class of stem cells generated via genetic reprogramming of somatic adult cells. By introducing four key transcription factors—Oct4, Sox2, Klf4, and c-Myc—into differentiated cells, researchers have successfully reprogrammed these cells back to a pluripotent state similar to ESCs [27,28]. This reprogramming endows iPSCs with the capacity for indefinite self-renewal and differentiation into various cell types in vitro. Importantly, iPSC technology overcomes ethical issues related to ESCs and allows the generation of patient-specific cells, making them invaluable for personalized regenerative medicine and disease modeling. As highlighted by Sances et al. (2016), iPSCs enable the generation of neuronal cells from individual patients, overcoming species-specific limitations inherent in animal models and paving the way for tailored therapeutic strategies [29].

Table 1. Classification of Stem Cells by Potency, Source, and Clinical Utility

Stem Cell Type	Source	Potency	Differentiation Capacity	Clinical / Research Use	References
Totipotent Stem Cells	Zygote, first few embryonic cells	Totipotent	All embryonic + extraembryonic tissues	Early embryonic development research	Wagers & Weissman, 2004
Embryonic Stem Cells (ESCs)	Inner cell mass of blastocyst	Pluripotent	All cell types from ectoderm, mesoderm, endoderm	Regenerative medicine, tissue modeling, ethical concerns	Weissman, 2000; Thomson, 1998 [30]
Induced Pluripotent Stem Cells (iPSCs)	Reprogrammed somatic cells	Pluripotent	Similar to ESCs	Personalized therapy, disease modeling, neuroregeneration	Takahashi & Yamanaka, 2006
Multipotent Stem Cells	Fetal and adult tissues	Multipotent	Multiple cell types within one germ layer	Tissue repair, immunomodulation	Dykstra, B., et al., 2007

Mesenchymal Stem Cells (MSCs)	Bone marrow, adipose tissue, umbilical cord	Multipotent	Osteoblasts, adipocytes, chondrocytes	CNS regeneration, paracrine support, anti-inflammatory effects	Bianco P, 2014
Hematopoietic Stem Cells (HSCs)	Bone marrow, peripheral blood	Multipotent	All blood cell lineages	Hematologic disorders, bone marrow transplants	Dykstra, B., et al., 2007
Progenitor / Unipotent Cells	Tissue-specific niches	Unipotent	One specific cell type	Local tissue maintenance, limited regeneration	Wagers & Weissman, 2004

3.3 Areas of Use of Stem Cells

The regenerative capacity of vital organs such as the brain, liver, and heart is quite limited. However, stem cells offer promising options for repairing damaged tissues and organs due to their ability to divide and differentiate [31]. Hematopoietic stem cells (HSCs) are widely used in treating hematological diseases, especially leukemias [32].

Stem cells also have broad applications in orthopedic injuries, skin diseases, burns, immune deficiencies, certain cancers, and autoimmune diseases. As such, stem cell therapies are central to regenerative medicine [33].

Mesenchymal stem cells (MSCs) are being investigated for the treatment of various degenerative diseases due to their differentiation potential into connective tissue cells and their anti-inflammatory and immunomodulatory effects. Neurological disorders represent a key area of MSC application. Experimental and clinical studies are exploring MSC therapies in Parkinson's disease, Alzheimer's disease, spinal cord injuries, stroke, multiple sclerosis, traumatic brain injury, cerebral palsy, and other nervous system diseases.

Clinically, stem cells are also utilized in orthopedic injuries, skin disorders, infertility, autoimmune diseases, burn healing, certain cancers, and immune deficiencies, reinforcing their central role in regenerative medicine [31].

3.4 Effects of Stem Cells on Neurogenesis Mechanisms

Mechanisms of Action of MSCs

MSCs exert their neurogenic effects through the activation of multiple molecular signaling pathways that are crucial for neurogenesis and synaptic plasticity. Among these pathways, brain-derived neurotrophic factor (BDNF) plays a pivotal role in promoting neuronal survival, growth, and functional connectivity. Additionally, the PI3K/AKT pathway is essential for cell proliferation and survival, while Wnt signaling is critically involved in neural development and hippocampal neurogenesis. The ERK-CREB signaling axis further supports learning processes by facilitating synaptic plasticity and memory consolidation. Through the modulation of these signaling cascades, MSCs confer a variety of beneficial effects including neurogenesis, angiogenesis, anti-inflammatory actions, and neuroprotection [34-36].

Recent studies have demonstrated that MSCs can enhance synaptic plasticity by modulating the inflammatory milieu. For example, Brown and colleagues (2021) reported that neural stem cells (NSCs) derived from primitive human MSCs ameliorated symptoms and promoted neurogenesis in a murine model of experimental autoimmune encephalomyelitis (EAE), an established model of multiple sclerosis (MS). These NSCs exhibited superior efficacy compared to MSCs by migrating to the central nervous system, increasing anti-inflammatory responses, and normalizing the balance of regulatory T cells (Treg) and Th17 cells. Furthermore, NSC treatment enhanced myelination, upregulated neuroprotective gene expression, and suppressed inflammation and astrogliosis-related genes, suggesting that neurogenesis is promoted primarily via BDNF and fibroblast growth factor (FGF) signaling pathways [37].

Similarly, Jiménez-Acosta et al. (2023) emphasized the therapeutic potential of MSCs in neurological disorders such as Parkinson’s disease, Alzheimer’s disease, and spinal cord injuries. Their ability to mitigate inflammation, prevent neuronal apoptosis, and promote neurogenesis positions MSCs as

promising agents in regenerative neurology. Moreover, patient-derived MSCs serve as valuable models for studying disease pathogenesis and therapeutic responses [38].

In the context of stroke recovery, Zhang et al. (2022) demonstrated that MSC treatment reduces inflammation, stimulates angiogenesis and neurogenesis, and decreases infarct volume. These effects were largely attributed to the release of trophic factors and extracellular vehicles (EVs) from MSCs, which facilitate repair mechanisms in damaged brain tissue [39].

Heng et al. (2022) highlighted the neurogenic potential of dental and oral stem cells derived from the neural crest. While these cells are promising candidates for neuroregenerative therapies due to their origin, they also tend toward osteo-odontogenic differentiation. To maximize their neurogenic capacity, recreating the native neural electrical microenvironment using electroactive or electroconductive scaffolds is critical. However, research on such scaffolds remains limited, especially in dental and oral stem cells, necessitating further studies for clinical translation [40].

In addition to cellular therapies, MSC-derived extracellular vesicles (MSC-Exos) have emerged as potent non-cellular therapeutic agents. Harrell et al. (2021) underscored that these vesicles, enriched with microRNAs, growth factors, and anti-inflammatory cytokines, exert neuroprotective, immunomodulatory, and neurogenic effects. Experimental models have shown that MSC-Exos can ameliorate cognitive deficits, promote neovascularization, and prevent neuronal loss, highlighting their potential in treating neurocognitive disorders [41].

Skok (2021) further discussed the role of MSC-derived vesicles in ameliorating cognitive dysfunction associated with neuroinflammation. This study emphasized the importance of α7 nicotinic acetylcholine receptors and paracrine signaling mechanisms of MSCs in restoring neural function. The author also noted that enhancing MSC migration, survival, and trophic factor production could improve therapeutic efficacy [42].

The effects of intranasally administered human MSC-derived extracellular vesicles (hMSC-EVs) were examined by Kodali et al. (2023) in a traumatic brain injury (TBI) model. A single dose of EVs restored neurogenesis in the subgranular zone-granular cell layer (SGZ-GCL) to healthy levels and reduced synaptic loss. These benefits were mechanistically linked to the reactivation of the BDNF-ERK-CREB signaling pathway, with sustained elevation of BDNF during the chronic phase post-injury [35].

In a related study, Fu et al. (2024) reported that exosomes secreted by human adipose-derived MSCs (hADSCs), enriched with long non-coding RNA IFNG-AS1, improved autism spectrum disorder (ASD)-like behaviors and enhanced neurogenesis in BTBR mouse models. These exosomes activated the miR-21a-3p/PI3K/AKT signaling pathway, highlighting their therapeutic potential as a non-cellular strategy for ASD [43].

Genetic modification of MSCs has also been investigated to augment their therapeutic efficacy. Choi et al. (2022) demonstrated that BDNF-overexpressing MSCs (BDNF-eMSCs) markedly attenuated hippocampal damage, glial activation, and neuronal loss after TBI. Repeated administration of BDNF-eMSCs reduced lesion size and significantly improved neurological and cognitive outcomes, confirming that targeted genetic modification enhances neurogenesis and brain repair [44].

Another innovative approach involves the use of cerebral organoids derived from human embryonic stem cells (hESCs). Kim et al. (2022) transplanted 8-week cultured human cerebral organoids (hCOs) into mice with mild TBI, resulting in reduced neuronal loss, enhanced angiogenesis, and increased neurogenesis in the dentate gyrus and subventricular zone. These findings suggest that hCO transplantation supports functional recovery by reconstructing damaged cortical structures and promoting neuroregeneration [45].

Furthermore, Kot et al. (2022) reviewed the synergistic effects of pharmacological and cellular therapies on adult neurogenesis. They noted that MSCs and antidepressants both stimulate hippocampal neurogenesis through modulation of the Wnt signaling pathway, supporting structural and functional brain plasticity. The review also highlighted the translational challenges of MSC-based therapies, advocating for further optimization and clinical validation [46].

Long-term studies on bone marrow stromal cells (BMSCs) by Mahmood et al. (2006) revealed that higher doses of intravenously administered BMSCs significantly improved functional outcomes and increased BDNF levels in a rat TBI model, suggesting dose-dependent neurogenic and neuroprotective effects [47].

Additionally, Song et al. (2020) explored the transplantation of dopaminergic progenitors derived from human induced pluripotent stem cells (hiPSCs) for Parkinson’s disease. The protocol produced cells with robust dopaminergic characteristics that safely improved motor functions in rodent models, providing a promising platform for autologous cell-based therapy [48].

In summary, an expanding body of literature supports the neuroregenerative potential of stem cells, particularly MSCs and their derivatives, across diverse neurological disease models. These cells enhance neurogenesis through both direct cellular effects and paracrine mechanisms, modulate inflammatory responses, and support functional recovery. Key molecular mediators include BDNF, PI3K/AKT, Wnt, and ERK-CREB signaling pathways. The paracrine activity of MSCs in neurogenic niches such as the hippocampus presents a promising avenue for therapeutic interventions targeting age-related cognitive decline and post-traumatic brain repair.

4 Conclusion

The hippocampus is central to neuroplasticity and neurogenesis, serving as a critical hub for cognitive processes. Extensive research indicates that mesenchymal stem cells, particularly in the hippocampus, enhance neurogenesis and synaptic plasticity, with potential to improve cognitive functions. Stem cell-based therapies represent a promising frontier in the treatment of neurodegenerative diseases and brain injuries, offering innovative strategies to restore neural function and support regeneration.

Disclosure of Interests

The authors have no competing interests to declare that are relevant to the content of this article

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I-CRAFT AGRICULTURAL and FOOD TECHNOLOGIES

The Effect of Hatching Egg Disinfection on Egg Weight, Hatching Traits, And Chick Quality in Pekin Ducks

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Abstract. This study investigated the effect of egg disinfection before incubation on egg weight, hatching traits, and chick quality in pekin ducks. The egg weight loss (Ew-L) between embryonic day (ED) 0-25 was 9.31±0.42% and 8.70±0.24% in groups A and B, respectively (P=0.333). The hatchability of fertile eggs (HFE) was 51.65±5.22% in Group A and 55.77±5.22% in Group B (P=0.633), while the embryonic mortality (EM) was 51.29±6.71% and 44.23±6.71% in Groups A and B, respectively (P=0.535). The chick weight at hatch (CWH) was 47.69±0.90 g in Group A and 47.99±1.07 g in Group B (P=0.833), while the chick yield (CY) was 63.26±1.35% in Group A and 64.65±0.78% in Group B (P=0.467). The chick length was significantly higher in Group B than in Group A (P=0.050). The current study suggests that the disinfection of soiled hatching eggs of ducks could improve hatchability, chick length, chick weight, and chick yield at hatch and reduce embryonic mortality without any negative effect on egg weight loss.

Keywords: Ducks, Disinfection, Eggs, Egg Weight Loss, Hatchability, Incubation

1 Introduction

Due to increasing levels of income and standard of living, the demand for poultry products, especially eggs, has improved tremendously [1], which has increased the search for the production of other alternative poultry species to support the total poultry egg [2] and meat consumption. Pekin ducks have been identified as one of the alternative poultry species with low production and maintenance costs but a high egg and meat nutritive value [3]. Ducks as an alternative poultry species are reared in many parts of the world however, production is mostly concentrated in Asia [4] and in deed [5] reported that in Southeast Asia, duck meat is the second most consumed poultry meat. Compared to chicken eggs, duck eggs have been reported to have higher protein, energy, carbohydrate, vitamins, iron, and sodium content [6]. Several authors have also reported duck eggs as a good source of protein and other nutrients, and are regarded as food with high nutritional quality [7, 8]. Moreover, a higher amount of total essential and non-essential amino acids in duck meat compared to chicken meat was reported [5].

In many parts of the world, duck breeders are mostly reared in free-range/litter or non-cage housing systems due to their high space and welfare requirements, with female ducks mostly laying eggs on the floor.

Additionally, ducks are waterfowl and turn to perform different highly motivated water-related behaviors, including preening, dabbling, head dipping, and sieving. In free-range/litter systems with water pools, ducks mostly wet/soil the litter materials with water droppings from their bodies, and eggs end up being soiled with mud, leading to several microbial contaminations of the eggshells and the internal constituents of the eggs. Indeed, in our farm (Çukurova University Poultry Research Unit, Adana, Türkiye), we realized that ducks reared in a litter system with nipple drinkers still exhibited severe bathing behavior, causing the research unit (pens) to be flooded with water.

In the current study, the eggs were collected in a free-range system, with the majority soiled with mud. Because of the nature of ducks and their breeding environments, the disinfection of duck hatching eggs before incubation is very crucial for embryonic development, hatching, and post-hatch performance, and indeed [9] reported that duck eggs have lower hatchability due to the higher number of soiled eggs. The disinfection of hatching eggs is crucial for the reduction of the bacterial load in order to prevent hatching problems and issues associated with chick performance [10].

Therefore, this study investigated the effect of egg disinfection before incubation on egg weight, hatching traits, and chick quality in Pekin ducks.

2 Materials and Methods

Ethical Statement

This study was conducted under the guidelines for animal experiments of the Ministry of Food, Agriculture and Livestock, Türkiye. Approval was granted by the animal experiments local ethics committee.

Animal Material, Experimental Groups and Incubation Conditions

A total of 120 hatching eggs of Pekin duck breeders at 60 week of age (wk) were used in this study. The eggs were first weighed using a scale of 0.1g precision and divided into 2 groups. The groups consisted Group A (undisinfected eggs) and Group B (Eggs disinfected prior to incubation). The fumigation process was carried out by mixing 14 ml of formaldehyde with 7 g of potassium permanganate which later spread through diffusion in gaseous form. The eggs remained in the chamber of containment with the diffused gas for approximately 25 minutes.

The eggs were incubated under standard conditions (37.8 ºC and 70% humidity) between embryonic age (ED) 0 and 24. At ED 25, candling was conducted and fertile eggs were transferred to the hatcher with a temperature and humidity of 37.5 ºC and 70%, respectively.

Evaluation of Egg Weight Loss, Hatchability, Embryonic Mortality, Chick weight, Chick Yield, and Chick Quality

At ED 7, 14, 20, and 25 all the eggs were weighed again using a scale with a precision of 0.1g. The egg weight loss (EwL%) at different ED was evaluated by subtracting the weight of the eggs at a specific ED from the weight of the eggs before incubation, dividing it by the weight of the eggs before incubation, and multiplying by 100%. The egg EwL% was evaluated using the formula below.

$$EwL\% = \frac{Egg\ weight\ before\ incubation - Egg\ weight\ at\ a\ specific\ ED}{Egg\ weight\ before\ incubation} \times 100 \quad (1)$$

At hatch, all the ducklings were weighed using a scale with 0.1 g precision, and all the unhatched eggs were broken to actually confirm embryos died. The hatchability of fertile eggs (HFE), embryonic mortality (EM), and chick yield (CY) were evaluated using the formula below. Selected chick quality traits (activity, eyes, legs, and appearance) from Tona et al. (2003) were evaluated using 17 and 15 ducklings from Group A and B, respectively. The duckling length was measured from the beak to the finger of the middle toe of the chicks using a ruler/rule placed on a square table. The egg HFE, EM, and CY were evaluated using the formula below.

$$\text{HFE\%}=\frac{Number\ of\ hatched\ chicks}{Total\ number\ of\ fertile\ eggs}\times 100 \quad (1)$$

$$\text{EM\%}=\frac{Number\ of\ dead\ embryos}{Total\ number\ of\ fertile\ eggs}\times 100 \quad (3) \quad (2)$$

$$\text{CY\%}=\frac{Average\ weight\ of\ hatched\ chicks\ in\ a\ specific\ group}{Average\ weight\ of\ eggs\ before\ incubation\ for\ that\ group}\times 100 \quad (2)$$

Statistical Analysis

The normality test and test of homogeneity of the data were conducted using Shapiro-Wilk and Levene's tests, respectively. It was confirmed that the data showed normal distribution. After confirming the normality of the data, the analysis of variance, Student t-test, was applied to the data. The *p-value* was set at $P \leq 0.05$. The statistical software package JMP 18 (SAS, 2017) was used for data analysis.

3 Results and Discussion

The effect of egg disinfection before incubation on EW (g) and Ew-L (%) is presented in Tables 1 and 2, respectively. The EW and the Ew-L at different ED did not significantly vary between the two experimental groups ($P>0.05$) and it could be possible that the disinfection of eggs with formaldehyde did not influence the eggshell conductance, the mechanism involved in the control of moisture loss between the egg and its environment. Our findings confirm the results of previous studies [11, 12] that have also reported that eggs disinfected before incubation did not significantly differ in terms of moisture loss or Ew-L compared to the control eggs (undisinfected). Other authors [13] also reported that while different disinfectants had no effect on EW at ED 18, the Ew-L after incubation was lowest in eggs disinfected with propolis. The differences in results could be related to the type of disinfectant used, the method of disinfection, or the degree of egg contamination.

Table 27. The effect of egg disinfection before incubation on egg weight (g) Group A (undisinfected eggs, control), Group B (Disinfected eggs), EWBI (Egg weight before incubation), EW (Egg weight), ED (Embryonic day).

Group	Ew-L (%)			
	ED0-7	ED0-14	ED0-20	ED0-25
A	2.99±0.13	5.74±1.05	7.73±0.74	9.31±0.42
B	3.07±0.07	5.59±0.61	7.79±0.42	8.70±0.24
P-value	0.620	0.910	0.953	0.333

Table 28. The effect of egg disinfection before incubation on egg weight loss (%)

Group	Ew-L (%)			
	ED0-7	ED0-14	ED0-20	ED0-25
A	2.99±0.13	5.74±1.05	7.73±0.74	9.31±0.42
B	3.07±0.07	5.59±0.61	7.79±0.42	8.70±0.24
P-value	0.620	0.910	0.953	0.333

Group A (undisinfected eggs, control), Group B (Disinfected eggs), Ew-L (Egg weight loss), ED (Embryonic day).

The effect of egg disinfection before incubation on HFE, EM, CWH, and CY is given in Table 3. The HFE, EM, CWH, and CY did not significantly vary between the two experimental groups in the present study (P>0.05), and it could be possible the degree of pathogenic contamination of eggs was not detrimental enough to influence embryonic growth and development and the mechanisms involved in hatching processes of chicks. Our findings agree with the results of [14], who reported no significant effect of duck egg disinfection on hatchability, EM, and CWH. It was also reported lower EM and higher HFE in duck eggs disinfected before incubation compared to undisinfected eggs; however, the CWH did not significantly vary among the groups [11]. Clean duck eggs disinfected before incubation did not significantly vary in terms of hatchability compared to the control eggs; however, soiled eggs disinfected before incubation had higher hatchability compared to soiled undisinfected eggs [9]. In geese, significantly higher EM and lower hatchability in dirty eggs compared to treated eggs before incubation were identified [15]. In addition, higher CY in chicken eggs disinfected with propolis was reported by [16]. The differences in results could be related to the type of disinfectant used, the method of disinfection, or the degree of egg contamination.

Table 29. The effect of egg disinfection before incubation on HFE, EM, CWH, and CY

Group	Hatching traits			
	HFE	EM	CWH	CY
A	51.65±5.22	51.29±6.71	47.69±0.90	63.26±1.35
B	55.77±5.22	44.23±6.71	47.99±1.07	64.65±0.78
P-value	0.633	0.535	0.833	0.467

Group A (undisinfected eggs, control), Group B (Disinfected eggs), HFE (Hatchability of fertile eggs), EM (Embryonic mortality), CWH (Chick weight at hatch), CY (Chick yield).

The effect of egg disinfection before incubation on chick quality score is presented in Table 4. The duckling activity, appearance, eyes, and leg scores did not significantly vary between the experimental groups (P>0.05), and our findings agree with the results of [17], who also reported that the number of saleable chicks from disinfected eggs did not significantly differ from the number of saleable chicks from undisinfected eggs. Our findings contradict the results of [18], who identified the highest number of saleable chicks from eggs disinfected using 30% hydrogen peroxide vapor.

However, in the present study, the duckling length at hatch was significantly higher in Group B than in Group A (P=0.05), and we speculated that the disinfection of eggs with formaldehyde might have prevented the negative effect of microorganisms/pathogens on progenitor cell specification, cell migration, epithelial-to-mesenchymal transition, and differentiation and maturation of chondrocytes, the mechanism involved in bone development in poultry, leading to higher skeletal or body length in ducklings from the disinfected eggs.

Table 30. The effect of egg disinfection before incubation on chick quality score

Group	Chick length (cm)	Activity	Appearance	Eyes	Legs
A	20.17±0.26[b]	2.47±0.71	9.18±0.73	15.53±0.34	16.00
B	20.94±0.28[a]	1.60±0.75	8.53±0.77	16.00±0.37	16.00
P-value	0.050*	0.405	0.548	0.356	-

Group A (undisinfected eggs, control), Group B (Disinfected eggs).

4 Conclusion

The current study suggests that the disinfection of soiled hatching eggs of ducks could improve hatchability, chick length, chick weight, and chick yield at hatch and reduce embryonic mortality without any negative effect on egg weight loss.

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I-CRAFT AGRICULTURAL and FOOD TECHNOLOGIES

The Effect of Egg Storage Positioning on Hatching, Growth Performance, Carcass, And Welfare Traits in Broiler Chickens

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Abstract. This study investigated the effect of egg storage positioning on hatching, growth, carcass, and welfare traits in broiler chickens. A total 90 eggs of broiler breeders at 33 wk were used. The experimental groups consisted of eggs stored with either the broad end-up (BEU) or the narrow end-up (NEU) for 7 d. Each group consisted of with 45 eggs. The average temperature and humidity of the storage room was 20 ° C and 70%, respectively. The average egg weight before storage was 59.49±0.43 (g) and 61.00±0.43 (g) for BEU and NEU, respectively. However, the mean values for the egg weight after storage was 59.10±0.42 (g) for BEU and 60.53±0.42 (g) for NEU. The hatchability of fertile eggs was 97.56 % for NEU and 97.67 % for BEU. While the hatchability of set eggs was 93.33% for BEU and 88.89% for NEU. The FI, FCR, BW, BWG, TI and NOI did not significantly vary between the groups (*P>0.05*). While the CCY was significantly higher in BEU, the SP-W was significantly higher in NEU *(P<0.05).* The BR nearly reached a significant level, higher in BEU than in NEU (*P=0.057*). The temperature of the footpad nearly reached a significant level, higher in BEU compared to NEU (*P=0.058*). Storing eggs with the NEU may have the potential to reduce stress and fear in broiler chickens.

Keywords: Broiler, Storage, Growth, Carcass, Welfare

1 Introduction

The global rise in demand for chicken meat and eggs [1, 2] has led to an exponential expansion in the growing or production of layer chickens for eggs and broiler chickens for meat. The lack of religious censure and the low cost of chicken meat and eggs are the main causes of the increased demand and consumption [3, 4, 5]. Furthermore, [6] noted that rising incomes and standards of living have led to a huge growth in the demand for poultry goods, particularly eggs. Breeder firms have expanded their capacity, increasing the quantity of hatching eggs produced daily, monthly, and annually in order to guarantee a consistent supply of chicken meat and eggs.

Chicks are hatched in batches, and hatching eggs are stored for a specific amount of time because many hatcheries have limited capacity that are lower than the quantity of hatching eggs they receive [7]. Commercial hatcheries frequently store eggs before incubation, and it is well recognized that hatching traits are influenced by storage temperature, humidity, turning, and storage length [8]. Numerous techniques have been used to reduce the detrimental effects of storage on the quality of eggs, the development of the embryo, hatching, and the performance of broiler chickens after hatching. Nevertheless, there hasn't been enough use of storing hatching eggs with the narrow end facing up.

It is common practice to keep both table and hatching eggs with the broad end facing up with the growing embryo underneath the pores (air cells). Because the broad-end of eggs contains the biggest and highest pores, moisture evaporates from it more quickly. Egg quality, embryonic development, hatching procedures, and post-hatch chick quality attributes may all be impacted by extreme dehydration of the interior components of eggs. The storing of eggs with the broad-end or narrow-end up or in a horizontal posture has produced conflicting findings, despite the paucity of available literature. For example, hatching eggs stored horizontally, with the narrow-end up, or broad-end up did not significantly differ in weight before incubation and at ED 21 [9]. Additionally, [10] found that the weight of the eggs after storage was similar between eggs stored with the narrow-end up and those that were stored broad-end up. However, [11] found that chicken eggs stored with the broad end up lost more weight at 7 and 14 days of storage than those stored with the narrow end up.

Therefore, this study investigated the effect of egg storage positioning on hatching, growth performance, carcass, and welfare traits in broiler chickens.

1.1 Animal material, experimental groups and incubation conditions

A total of 90 hatching eggs of broiler breeders at 33 weeks of age (wk) were used in this study. The eggs were first weighed using a scale of 0.1g precision and divided into 2 groups. The groups consisted Group A (Eggs stored with the broad-end up, normal) and Group B (Eggs stored with the narrow-end up). The eggs of the various groups were stored for 7 days (d) with an average temperature and humidity of 18 ^{o}C and 75%. The egg weight before storage was 59.49±0.43 g and 61.00±0.43 g for groups A and B, respectively. After storage, the eggs were incubated under standard conditions (37.8 ^{o}C and 65% humidity) between ED 0 and 18. At ED 18, candling was conducted and fertile eggs were transferred to the hatcher with a temperature and humidity of 37.5 ^{o}C and 70%, respectively.

At hatch, all the chicks were weighed using a scale with 0.1 g precision, and all the unhatched eggs were broken to actually confirm embryos died. The hatchability of set eggs (HSE), hatchability of fertile eggs (HFE), and embryonic mortality (EM) were evaluated using the formula below.

$$\text{HSE\%}=\frac{Number\ of\ hatched\ chicks}{Total\ number\ of\ eggs\ set}\times 100 \quad (1)$$

$$\text{HFE\%}=\frac{Number\ of\ hatched\ chicks}{Total\ number\ of\ fertile\ eggs}\times 100 \quad (2)$$

$$\text{EM\%}=\frac{Number\ of\ dead\ embryos}{Total\ number\ of\ fertile\ eggs}\times 100 \quad (3)$$

1.2 Post-hatch housing conditions and feeding

The chicks from the various experimental groups were reared for six wk in a litter system with 3 replicates per group each having 10 chicks. Feed and water were provided *ad libitum*. At 1 wk of age, the brooding temperature was 30-33ºC. The temperature was gradually reduced to 22-24 ºC between 2 and 6 wk of age. The photoperiodic lightning used was 21 hours of light and 3 hours of darkness. The composition of the diet used in this study is given in Table 1.

Table 1. Nutritional composition of the diet used in this experiment

Composition	Feed type		
	Starter diet (1-11 d)	Grower diet (12-24 d)	Finisher diet (28-42 d)
Lysine (%)	1.44	1.15	1.05
Methionine (%)	0.56	0.47	0.47
Metabolic energy (kcal/kg)	3000	3200	3200
Vitamin D3 (IU/kg)	2000	2000	2000
Vitamin A (IU/kg)	10,000	10,000	10,000
Crude protein (%)	23	19.5	19.5

Crude cellulose (%)	3.6	3.6	3.07
Crude fat (%)	5.4	7.2	7.2
Crude ash (%)	6.3	5.3	5.3
Calcium (%)	3.96	0.78	0.78
Phosphorus (%)	0.48	0.39	0.39
Sodium (%)	0. 16	0.16	0.16
Manganese (mg/kg)	80	80	80
Zinc (mg/kg)	60	60	60
Cobalt (mg/kg)	0.5	0.5	0.5
Selenium (mg/kg)	0.15	0.15	0.15
Iodine (mg/kg)	2	2	2
Iron [iron sulphate monohydrate] (mg/kg)	60	60	60
Copper [copper sulphate pentahydrate] (mg/kg)	5	5	5

1.3 Evaluation of performance, stress, fear, and carcass parameters

The body weight (BW) was measured weekly using a scale with a precision of 0.1 g. The feed intake (FI) was measured by subtracting the leftover feed at the end of a particular week from the feed given at the beginning of that week. The weekly body weight gain (BWG), feed conversion ratio (FCR), and FI were evaluated using the formula below.

$$BWG = (\text{B}ody\ weight\ of\ subsequent\ weeks\ -\ body\ weight\ of\ previous\ weeks) \quad (4)$$

$$FI = (Feed\ given\ at\ the\ beginning\ of\ a\ particular\ week - feed\ left\ at\ the\ end\ of\ that\ week) \quad (5)$$

$$FCR = \frac{Feed\ intake\ (g)}{Body\ weight\ gain\ (g)} \quad (6)$$

At 5 wk of age, the footpad, beak, comb, and metatarsal temperature of 4 chickens per treatment was identified using a WOHLER ST-D2 (±0.3ºC) infrared thermometer under post-hatch heat stress conditions (30±1 ºC, 30%RH). The cloacal temperature was identified by inserting an MT101 clinical thermometer (±0.1 ºC) 3cm inside the cloacal for 20-30s [5].

In this trial, the measure of fear was tonic immobility (TI). For TI responses, a total of 12 birds from each group were examined. The hens were held on their backs on a table by the experimenter to induce tonic immobility. The bird's head was closed for 15 seconds with the left hand while a little force was applied to the chest with the right. The experimenter held the birds upside-down for 15 seconds while applying light pressure to the sternum. Then, gently and delicately, the experimenter took their hand away from the bird. If the bird stayed immobile after the 15s restraint was initiated, the TI duration was recorded from that moment using a stopwatch until the bird righted itself. Following five unsuccessful attempts at 15-second restraint, the birds' TI was reported as zero (0). 300s was the maximum TI duration. A separate chamber inside the manufacturing plant was used for the tonic immobility test.

At 6 wk of age, chickens whose live weights were close to the average live weight of each replicate were slaughtered. At slaughter, the weight of the hot carcass, heart, spleen, gizzard, and liver were determined using a scale with 0.0 1 g precision. The hot carcass was then stored in a refrigerator at +4 ºC for 24 hours. After the 24-hour cold storage, the weight of the cold carcass, abdominal fat, and carcass parts (breast, thigh, wings, and back) were identified with a scale of 0.0 1 g precision. The cold and hot carcass yield were evaluated according to the formula below [12, 13].

$$\text{Hot carcass yield } (\%) = \frac{Hot\ carcass\ weight\ (g)}{Slaughter\ weight\ (g)} \times 100 \quad (7)$$

$$\text{Cold carcass yield } (\%) = \frac{Cold\ carcass\ weight\ (g)}{Slaughter\ weight\ (g)} \times 100 \quad (8)$$

1.4 Statistical analysis

The normality test and test of homogeneity of the data were conducted using Shapiro-Wilk and Levene's tests, respectively. It was confirmed that the data showed normal distribution. After confirming the normality of the data, the analysis of variance (ANOVA), independent samples t-test was applied to the data. The *p-value* was set at $P \leq 0.05$. The statistical software package JMP 18 (SAS, 2017) was used for data analysis.

2 Results and Discussion

The effect of egg storage position of on egg weight, hatchability, embryonic mortality, and chick weight are presented in Table 2. The EW-AS and at ED 18 was significantly higher in Group B than in Group A ($P<0.05$) and it was speculated that the storage of eggs with the broad-end up might have increased the eggshell conductance or accelerated the stimulation of the mechanism involved in the gaseous exchange between the egg and its environment. This contradict the findings of [9], who reported that hatching eggs of chukar partridge (Alectoris chukar) stored with broad-end up, narrow-end up or in a horizontal position did not significantly vary in terms of egg weight before incubation and at ED 21. In addition, [10] **and** [14] also reported that eggs stored with either the broad or the narrow-end up did not vary in terms of EW-AS. The difference in results could be related to the specie, hen age, or duration of storage. The Ew-L did not significantly differ between the groups which aligns with the findings of [9]. We therefore speculate that the storage duration or position in the current study was not detrimental enough to cause significant changes in Ew-L between the groups.

The HSE and HFE was higher in in Group A than in Group B and we speculate that the storage of eggs with the narrow-end up might have negatively influenced the mechanisms, hormones or genes involved hatching processes of chicks. Our results contradict the findings of [9] and [14] who reported higher hatchability in eggs stored with the narrow-end up compared to those stored with the broad-end up.

The EM was higher in Group B indicating that the storage of eggs with the narrow-end up might have facilitated or increased the apoptotic death of embryonic cells leading to higher EM. This contradicts the findings of [9] and [14] who reported lower EM in eggs stored with the narrow-end up compared to those stored with the broad-end up.

The chick weight at hatch did not significantly vary between the experimental groups and we speculate that the storage of eggs at different positions could not influence embryonic or satellites cells, the primary responsible for muscle growth development however, this result disagree with the findings of [15] who significantly identified higher chick weight at hatch in chicken eggs stored with narrow-end up.

The effect of egg storage positioning on growth performance traits in broiler chickens is presented in Table 3. This study is the first to examine the effect of egg storage position on BW, BWG, FI, and FCR however, the performance traits examined did not significantly vary between the experimental groups

(*P>0.05*). It is possible that the different storage positions or the duration of storage did not have any significant effect on of embryonic development, intestinal development and health and other factors responsible for the control of voluntary feed intake in birds after hatch.

The effect of egg storage positioning on carcass and internal organs in broiler chickens Table 4. The CCY was significantly higher in Group A than in Group B (*P<0.05*) which probably due to the higher cold carcass weight of the birds in that group. However, the SP-W was significantly higher in Group B than in Group A (*P<0.05*) indicating the storage of eggs with the narrow-end up might have facilitated or enhanced the activity of the cells, hormones, genes or mechanisms involved in the growth and development of immune organs. The remaining carcass and organ traits did not significantly vary between the experimental groups (*P>0.05*).

The effect of egg storage positioning on stress and fear responses in broiler chickens is given in Table 5. The breast, metatarsal, footpad, wattle, comb, rectum, duration of tonic immobility, and number of inductions did not significantly vary between the experimental groups (*P>0.05*) indicating that the egg storage position did not alter the mechanisms involves in the activation of fear and stress in chickens.

Table 2. The effect of egg storage position of on egg weight, hatchability, embryonic mortality, and chick weight at hatch

Groups	EW-AS (g)	EW-ED 18 (g)	Ew-L (ED 0-18) (%)	HSE (%)	HFE (%)	EM (%)	Chick weight (g)
A	59.10±0.42	54.29±0.40	8.67±0.16	93.33	97.67	Şub.33	43.35±0.35
B	60.53±0.42	56.00±0.41	8.73±0.16	88.89	97.56	Şub.44	43.97±0.35
P-value	0.019*	0.004*	0.781	-	-	-	0.209

Group A (Eggs stored with the broad-end up, normal), Group B (Eggs stored with the narrow-end up), EW (Egg weight), AS (After storage), EWL (Egg weight loss), HSE (Hatchability of set eggs), HFE (Hatchability of fertile eggs), EM (Embryonic mortality), ED (Embryonic day).

Table 3. The effect of egg storage positioning on growth performance traits in broiler chickens

	Groups	Age of broilers (wk) 1	2	3	4	5	6
BW (g)	A	173.93±2.12	459.77±3.10	950.10±51.51	1525.17±24.72	2451.27±38.96	3145.72±54.87
	B	174.34±2.12	462.03±3.10	935.17±15.51	1520.73±24.72	2403.17±38.96	3115.93±56.87
	P-value	0.868	0.607	0.499	0.899	0.382	0.708
	A	130.55±4.90	285.87±10.79	490.33±10.83	575.07±12.83	926.10±27.86	696.07±25.66

BWG (g)	B	130.46±4.90	287.60±10.79	473.13±10.83	585.57±12.83	901.80±27.86	686.93±25.66
	P-value	0.990	0.915	0.324	0.594	0.571	0.814
	A	1802.00±29.56	5955.00±155.43	6786.67±114.01	9000	13246.67±333.65	11336.67±371.75
FI (g)	B	1824.67±29.56	5702.67±155.43	6477.67±114.01	9000	12779.67±333.65	10955.67±371.75
	P-value	0.616	0.315	0.128	-	0.378	0.509
	A	1.38±0.06	2.09±0.01	1.38±0.03	1.57±0.04	1.43±0.06	1.69±0.15
FCR	B	1.40±0.06	2.00±0.01	1.37±0.03	1.54±0.04	1.42±0.06	1.67±0.15
	P-value	0.818	0.552	0.743	0.532	0.881	0.952

Group A (Eggs stored with the broad-end up, normal), Group B (Eggs stored with the narrow-end up), BW (Body weight), BWG (Body weight gain), FI (Feed intake), FCR (Feed conversion ratio), wk (week).

Table 4. The effect of egg storage positioning on carcass and internal organs in broiler chickens

Groups	SW (g)	HCW (g)	HCY (%)	CCW (g)	CCY (%)	TH-W (g)	BR (g)	WG-W (g)	NK-W (g)
A	3213.33 ± 41.42	2566.56 ± 34.09	79.89 ± 0.27	2518.25 ± 33.67	78.38 ± 0.27	668.06 ± 14.27	1194.63 ± 19.36	198.85 ± Şub.54	181.56 ± Haz.15
B	3216.31 ± 41.42	2545.69 ± 34.09	79.34 ± 0.27	2480.38± 33.67	77.33 ± 0.27	672.50 ± 14.27	1140.44 ± 19.36	198.13 ± Şub.37	191.81 ± Haz.15
P-value	0.959	0.668	0.158	0.433	0.009*	0.827	0.057	0.839	0.248

Groups	BK-W (g)	HT-W (g)	L-W (g)	G-W (g)	SP-W (g)	B-W (g)	PRO (g)	AF-W (g)
A	257.13 ± May.89	16.50 ± 0.77	57.16 ± Oca.95	52.09 ± Şub.63	Şub.66 ± 0.20	May.24 ± 0.41	Eyl.99 ± 0.48	27.60 ± Oca.63
B	258.81 ± May.89	15.88 ± 0.82	59.89 ± Oca.83	55.12 ± Şub.47	Mar.33 ± 0.19	May.35 ± 0.39	Eki.39 ± 0.43	27.26 ± Oca.68
P-value	0.841	0.585	0.317	0.407	0.024*	0.854	0.539	0.885

Group A (Eggs stored with the broad-end up, normal), Group B (Eggs stored with the narrow-end up), SW (Slaughter weight), HCW (Hot carcass weight), HCY (Hot carcass yield), CCY (Cold carcass yield), TH-W (Thig weight), BR (Breast weight), WG-W (Wing weight), NK-W (Neck weight), BK-W (Back weight), HT-W (Heart weight), L-W (Liver weight), G-W (Gizzard weight), SP-W (Spleen weight), B-W (Bursa Fabricus), PRO (proventriculus)AF-W (Abdominal fat weight).

Table 5. The effect of egg storage positioning on stress and fear responses in broiler chickens

Group	Breast (°C)	Metatarsal (°C)	Footpad (°C)	Wattle (°C)	Comb (°C)	Rectum (°C)	No Induction (minute)	TI Duration (minute)
A	36.89±0.11	37.32±0.17	36.46±0.18	36.46±0.20	35.87±0.15	41.10±0.01	1.50±0.14	3.53±0.43
B	37.09±0.11	37.48±0.17	35.98±0.18	36.18±0.20	35.81±0.15	41.22±0.01	1.18±0.14	2.80±0.43
P value	0.217	0.479	0.058	0.331	0.777	0.306	0.107	0.234

Group A (Eggs stored with the broad-end up, normal), Group B (Eggs stored with the narrow-end up).

3 Conclusion

In the current study, it was concluded that the storage of eggs with the narrow or broad-end up could be beneficial for egg weight, immune organs or carcass traits without any negative effect on performance and welfare traits.

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Disclosure of Interests. The authors have no competing interests to declare that are relevant to the content of this article.

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