ISSN Online 2148-015X

ACADEMIC FOOD JOURNAL

AKADEMİK GIDA

Gıda Bilimi ve Teknolojisi Dergisi

https://dergipark.org.tr/tr/pub/akademik-gida Cilt/Volume: 21 Sayı/Number: 2 Nisan - Haziran 2023

ACADEMIC FOOD JOURNAL

A JOURNAL ON FOOD SCIENCE & TECHNOLOGY

SİDAS MEDYA

AKADEMİK GIDA®
ACADEMIC FOOD JOURNAL

Akademik Gıda® dergisi Gıda Bilimi ve Teknolojisi alanında hazırlanmış özgün araştırma ve derleme makalelerin yayınlandığı hakemli bir dergidir. Araştırma Notu ve Editöre Mektup gibi yazılar da yayın için değerlendirilmektedir. Dergi 3 ayda bir basılmakta olup 4 sayıda bir cilt tamamlanmaktadır. Dergide Türkçe veya İngilizce olarak hazırlanmış makaleler yayınlanmaktadır.

AKADEMİK GIDA®
ACADEMIC FOOD JOURNAL

Uluslararası Yayın Kurulu / International Editorial Board

Gıda Mühendisliği / Food Engineering

Name and Surname	Affiliation	City	Country
Cynthia Ditchfield	University of Sao Paolo, Faculty of Animal Science and Food Engineering, Department of Food Engineering	Sao Paolo	Brazil
Arif Hepbaşlı	Yaşar University, Department of Energy Systems Engineering	İzmir	Turkey
Filiz İçier	Ege University, Faculty of Engineering, Food Engineering Department	İzmir	Turkey
Erkan Karacabey	Süleyman Demirel University, Faculty of Engineering, Food Engineering Department	Isparta	Turkey
Sami Gökhan Özkal	Pamukkale University, Faculty of Engineering, Food Engineering Department	Denizli	Turkey
Konstantinos Petrotos	Technological Educational Institute of Larissa, Department of Agricultural Engineering Technologists	Larissa	Greece
Jenny Ruales	Escuela Politécnica Nacional, Departamento de Ciencias de Alimentos y Biotecnología	Quit	Ecuador
Yahya Tülek	Pamukkale University, Faculty of Engineering, Food Engineering Department	Denizli	Turkey

Gıda Kimyası / Food Chemistry

Name and Surname	Affiliation	City	Country
Fahrettin Göğüş	Gaziantep University, Faculty of Engineering, Food Engineering Department	Gaziantep	Turkey
Piotr Koczon	Warsaw University of Life Sciences, Faculty of Food Sciences, Department of Chemistry	Warsaw	Poland
Erdoğan Küçüköner	Süleyman Demirel University, Faculty of Engineering, Food Engineering Department	Isparta	Turkey
Semih Ötleş	Ege University, Faculty of Engineering, Food Engineering Department	İzmir	Turkey
Beraat Özçelik	Istanbul Technical University, Faculty of Chemical and Metallurgical Engineering, Food Engineering Department	İstanbul	Turkey
Osman Sağdıç	Yıldız Technical University, Faculty of Chemical and Metallurgical Engineering, Food Engineering Department	İstanbul	Turkey
Romeo Toledo	Emeritus Professor, University of Georgia, Department of Food Science and Technology	Georgia	USA

Gıda Mikrobiyolojisi & Biyoteknoloji / Food Microbiology & Biotechnology

Name and Surname	Affiliation	City	Country
Iuliana Aprodu	Dunarea de Jos University of Galati, Department of Food Science, Food Engineering and Applied Biotechnology,	Galati	Romania
Muhammet Arıcı	Yıldız Technical University, Faculty of Chemical and Metallurgical Engineering, Food Engineering Department	İstanbul	Turkey
Jurislav Babic	University of Osijek, Faculty of Food Technology	Osijek	Croatia
Oana Emilia Constantin	Dunarea de Jos University of Galati, Department of Food Science, Food Engineering and Applied Biotechnology,	Galati	Romania
İbrahim Çakır	Abant İzzet Baysal University, Faculty of Engineering, Food Engineering Department	Bolu	Turkey
Ahmet Hilmi Çon	Ondokuz Mayıs University, Faculty of Engineering, Food Engineering Department	Samsun	Turkey
Mehmet Yekta Göksungur	Ege University, Faculty of Engineering, Food Engineering Department	İzmir	Turkey
Şebnem Harsa	İzmir Institute of Technology, Food Engineering Department	İzmir	Turkey
Patricia Munsch-Alatossava	Independent Researcher	Helsinki	Finland
Ömer Şimşek	Yıldız Technical University, Faculty of Chemical and Metallurgical Engineering, Food Engineering Department	İstanbul	Turkey
Özgür Tarhan	Uşak University, Faculty of Engineering, Food Engineering Department	Uşak	Turkey

Gıda Analizleri / Food Analysis

Name and Surname	Affiliation	City	Country
Abdullah Akdoğan	Pamukkale University, Faculty of Engineering, Chemical Engineering Department	Denizli	Turkey
İsmail Hakkı Boyacı	Hacettepe University, Faculty of Engineering, Food Engineering Department	Ankara	Turkey
Hale Seçilmiş Canbay	Burdur Mehmet Akif Ersoy University, Faculty of Science and Arts, Chemistry Department	Burdur	Turkey
Mustafa Zafer Özel	Sensient Flavors Ltd.	Milton Keynes	UK

Gıda Ambalajlama / Food Packaging

Name and Surname	Affiliation	City	Country
Zehra Ayhan	Sakarya University, Faculty of Engineering, Food Engineering Department	Sakarya	Turkey
Cengiz Caner	Çanakkale Onsekiz Mart University, Faculty of Engineering, Food Engineering Department	Çanakkale	Turkey
Ayhan Oral	Çanakkale Onsekiz Mart University, Faculty of Science, Department of Chemistry	Çanakkale	Turkey

Süt Teknolojisi / Dairy Technology

Name and Surname	Affiliation	City	Country
Mohamed H. Abd El-Salam	Emeritus Professor, National Research Centre, Department of Dairy Sciences	Cairo	Egypt
Ayşe Sibel Akalın	Ege University, Faculty of Agriculture, Dairy Technology Department	İzmir	Turkey
Meral Kılıç Akyılmaz	Istanbul Technical University, Faculty of Chemical and Metallurgical Engineering, Food Engineering Department	İstanbul	Turkey
Tapani Alatossava	University of Helsinki, Department of Food and Nutrition	Helsinki	Finland
Rajka Bozanic	University of Zagreb, Faculty of Food Technology and Biotechnology, Department of Food Engineering	Zagreb	Croatia
Abdullah Çağlar	Kocaeli University, Faculty of Agriculture and Natural Sciences, Department Of Agricultural Economics	Kocaeli	Turkey
Songül Çakmakçı	Atatürk University, Faculty of Engineering, Food Engineering Department	Erzurum	Turkey
Ali Adnan Hayaloğlu	İnönü University, Faculty of Engineering, Food Engineering Department	Malatya	Turkey
Harun Kesenkaş	Ege University, Faculty of Agriculture, Dairy Technology Department	İzmir	Turkey
Ahmet Küçükçetin	Akdeniz University, Faculty of Engineering, Food Engineering Department	Antalya	Turkey
Barbaros Özer	Ankara University, Faculty of Agriculture/Department of Dairy Technology, Department of Dairy Technology	Ankara	Turkey
Harun Raşit Uysal	Ege University, Faculty of Agriculture, Department of Dairy Technology	İzmir	Turkey
Yonca Yüceer	Çanakkale Onsekiz Mart University, Faculty of Engineering, Food Engineering Department	Çanakkale	Turkey

Yağ Teknolojisi / Oil and Fat Technology

Name and Surname	Affiliation	City	Country
Aydın Yapar	Pamukkale University, Faculty of Engineering, Food Engineering Department	Denizli	Turkey
Emin Yılmaz	Çanakkale Onsekiz Mart University, Faculty of Engineering, Food Engineering Department	Çanakkale	Turkey

Hububat Teknolojisi / Cereal Technology

Name and Surname	Affiliation	City	Country
Hülya Gül	Süleyman Demirel University, Faculty of Engineering, Food Engineering Department	Isparta	Turkey
Fatma Işık	Pamukkale University, Faculty of Engineering, Food Engineering Department	Denizli	Turkey
Ergun Köse	Manisa Celal Bayar University, Faculty of Engineering, Food Engineering Department	Manisa	Turkey
Pichan Prabasankar	CSIR-Central Food Technological Research Institute, Flour Milling Baking and Confectionery Technology Department	Mysuru	India

Et Teknolojisi / Meat Technology

Name and Surname	Affiliation	City	Country
Nesimi Aktaş	Nevşehir Hacı Bektaş Veli University, Faculty of Engineering and Architecture, Food Engineering Department	Nevşehir	Turkey
Haluk Ergezer	Pamukkale University, Faculty of Engineering, Food Engineering Department	Denizli	Turkey
Hüdayi Ercoşkun	Çankırı Karatekin University, Faculty of Engineering, Food Engineering Department	Çankırı	Turkey
Mükerrem Kaya	Atatürk University, Faculty of Engineering, Food Engineering Department	Erzurum	Turkey
Semra Kayaardı	Manisa Celal Bayar University, Faculty of Engineering, Food Engineering Department	Manisa	Turkey
Jung Hoon Lee	Fort Valley State University, College of Agriculture, Family Sciences and Technology	Georgia	USA
Edward Pospiech	Department of Animal Raw Materials, Institute of Meat Technology, Faculty of Food Sciences and Nutrition, Poznan University of Life Sciences,	Poznan	Poland
Fatma Meltem Serdaroğlu	Ege University, Faculty of Engineering, Food Engineering Department	İzmir	Turkey
Kapllan Sulaj	Agricultural University of Tirana, Faculty of Biotechnology and Biotechnology	Tirana	Albania
İsmail Yılmaz	Namık Kemal University, Faculty of Agriculture, Food Engineering Dept.	Tekirdağ	Turkey

Meyve-Sebze Teknolojisi / Fruit and Vegetable Technology

Name and Surname	Affiliation	City	Country
Chockry Barbana	Canadian Food Inspection Agency	Montréal	Canada
Utku Çopur	Uludağ University, Faculty of Agriculture, Food Engineering Department	Bursa	Turkey
Seda Ersus	Ege University, Faculty of Engineering, Food Engineering Department	İzmir	Turkey
Hakan Karaca	Pamukkale University, Faculty of Engineering, Food Engineering Department	Denizli	Turkey
Sebahattin Nas	Pamukkale University, Faculty of Engineering, Food Engineering Department	Denizli	Turkey
Ayhan Topuz	Akdeniz University, Faculty of Engineering, Food Engineering Department	Antalya	Turkey
Yakup Sedat Velioğlu	Ankara University, Faculty of Engineering, Food Engineering Department	Ankara	Turkey
Ünal Rıza Yaman	Tire Kutsan Vocational School, Department of Food Technology	İzmir	Turkey
Oktay Yemiş	Sakarya University, Faculty of Engineering, Food Engineering Department	Sakarya	Turkey
Ufuk Yücel	Ege University, Ege Vocational Training School, Department of Food Technology	İzmir	Turkey

Sağlık, Beslenme, Toksikoloji ve Gıda / Health, Nutrition, Toxicology and Food

Name and Surname	Affiliation	City	Country
Adriana Pavesi Arisseto Bragotto	State University of Campinas, Faculty of Food Engineering	Campinas	Brazil
Gözde Ede	Çankırı Karatekin University, Faculty of Health Sciences, Nutrition and Dietetic Department	Çankırı	Turkey

SİDAS MEDYA
Akademik Gıda® / Academic Food Journal
Online: 2148-015X
https://dergipark.org.tr/tr/pub/akademik-gida

Akademik Gıda 21(2) 2023

AKADEMİK GIDA

ABSTRACTED / INDEXED / LISTED IN

1. Abstracts on Hygiene and Communicable Diseases
2. Academic Index
3. Academic Keys
4. Academic Search Ultimate
5. Academindex
6. Advanced Science Index (ASI)
7. AgBiotech News and Information
8. AgBiotechNet
9. Agricultural Economics Database
10. Agricultural Engineering Abstracts
11. Agroforestry Abstracts
12. Animal Breeding Abstracts
13. Animal Production Database
14. Animal Science Database
15. Asos İndeks
16. Biocontrol News and Information
17. Biofuels Abstracts
18. Botanical Pesticides
19. CAB Abstracts
20. CAB Direct
21. Cite Factor
22. Crop Science Database
23. CrossRef
24. Dairy Science Abstracts
25. Directory of Research Journals Indexing (DRJI)
26. EBSCO - Academic Search Ultimate Database
27. Environmental Impact
28. Environmental Science Database
29. Eurasian Scientific Journal Index
30. EuroPub
31. Field Crop Abstracts
32. Food Science and Technology Abstracts (FSTA)
33. Forest Science Database
34. Global Health
35. Google Scholar
36. Horticultural Science Abstracts
37. Horticultural Science Database
38. Impact Factor Services for International Journals (IFSIJ)
39. International Innovative Journal Impact Factor (IIJIF)
40. International Institute of Organized Research (I2OR)
41. İdeal Online
42. Maize Abstracts
43. MIAR (Information Matrix for the Analysis of Journals)
44. Nutrition Abstracts and Reviews Series A:Human and Experimental
45. Nutrition Abstracts and Reviews Series B: Livestock Feeds and Feeding
46. Nutrition and Food Sciences Database
47. Ornamental Horticulture
48. Parasitology Database
49. Plant Breeding Abstracts
50. Plant Genetic Resources Abstracts
51. Plant Genetics and Breeding Database
52. Plant Protection Database
53. Postharvest Abstracts
54. Potato Abstracts
55. Poultry Abstracts
56. Protozoological Abstracts
57. Review of Agricultural Entomolog
58. Review of Aromatic and Medicinal Plants (RAMP)
59. Review of Medical and Veterinary Entomology
60. Review of Medical and Veterinary Mycology
61. Review of Plant Pathology
62. Rice Abstracts
63. Rural Development Abstracts
64. Science Library Index
65. Scientific Indexing Services (SIS)
66. SCOPUS
67. Seed Abstracts
68. Scilit
69. Soil Science Database
70. Soils and Fertilizers Abstracts
71. Soybean Abstracts
72. Sugar Industry Abstracts
73. Systematic Impact Factor (SIF)
74. The Belt and Road Initiative Reference Source
75. The Turkish Academic Network and Information Centre Life Sciences Database (TÜBİTAK-ULAKBİM Yaşam Bilimleri Veritabanı, TR-DİZİN)
76. Tropical Diseases Bulletin
77. Veterinary Science Database
78. VetMed Resource
79. Weed Abstracts
80. Wheat, Barley and Triticale Abstracts
81. World Agricultural Economics and Rural Sociology Abstracts (WAERSA)

Akademik Gıda 21 (2) (2023)

İÇİNDEKİLER / CONTENTS

Editörden / Editorial

ACADEMIC FOOD JOURNAL
AKADEMİK GIDA
Gıda Bilimi ve Teknolojisi Dergisi

Sahibi
SİDAS MEDYA AJANS TANITIM DANIŞMANLIK LTD. ŞTİ.Adına
İmtiyaz Sahibi ve Yazı İşleri Sorumlusu
Şakir SARIÇAY

Genel Yayın Yönetmeni
Şakir SARIÇAY
info@akademikgida.com
ssaricay@gmail.com

Baş Editör
Prof. Dr. Oğuz GÜRSOY
ogursoy@yahoo.com

Editörler
Prof. Dr. Özer KINIK
Prof. Dr. Ramazan GÖKÇE
Prof. Dr. Yusuf YILMAZ

Reklam Müdürü
Nurcan AKMAN ŞENGÖR

Hukuk Danışmanı
Av. Yrd. Doç. Dr. Murteza AYDEMİR

Abone Sorumlusu
Halil SOLAK

Grafik Tasarım
Sidas Medya Tasarım Grubu

Yönetim Yeri
Fevzipaşa Bulv. Çelik İş Merkezi
No:162 Kat:3 D:302 Çankaya/İZMİR
Tel: 0 232 441 60 01
Fax: 0 232 441 61 06

Üç Ayda Bir Yayınlanan Dergimiz
Basın Meslek İlkelerine Uymaktadır.

Yıl / Cilt: 21
Sayı: 100
Nisan - Mayıs - Haziran 2023
ISSN Print 1304-7582
ISSN Online 2148-015X
Akademik Gıda Dergisi
Bir SİMEDYA GRUP Yayınıdır.

Yayın Türü: Yerel Süreli
Akademik Gıda Dergisi Hakemli Dergidir.

Akademik Gıda dergisinin 21. yayın yılının ikinci sayısında yine sizlerle birlikteyiz. Bu sayımızda 9 araştırma, 1 derleme çalışması ve 1 görüş yazısı olmak üzere toplam 11 makale yer almaktadır.

Makale yazarlarından zaman zaman gelen sorular nedeniyle makale kabulü ile ilgili daha önce yaptığımız bilgilendirmeyi tekrar etmek istiyoruz. Dergimize yayımlanmak üzere gönderilen makalelerin kabulü halen **http://www.academicfoodjournal.com** adresinden yapılmakta olup, DergiPark üzerindeki makale kabul süreçlerini içeren sistem henüz kullanılmamaktadır.

Yazarlarımıza hatırlatmak istediğimiz diğer önemli bir husus 2020 yılından itibaren dergimize gönderilecek makalelerde Etik Kurul izni gerektiren çalışmaların ilgili izni aldıkları ile ilgili bilgi ve belgelerini dergimize (makalelerini dergimize gönderme aşamasında) sunmaları gerekliliğidir. Dergimizin etik hususlarla ilgili detaylı etik beyanına web sayfamızdan **(https://dergipark.org.tr/tr/pub/akademik-gida/page/6477)** ulaşılabilir. Ayrıca dergimizde araştırma makalelerinin ve İngilizce olarak yazılan makalelerin değerlendirme ve yayınlanma sürelerinin diğer makalelere kıyasla oldukça kısa olduğunu yazarlarımıza tekrar hatırlatmak istiyoruz.

Daha fazla ulusal ve uluslararası veri tabanı ve indekste dizinlenmek ve derginin uluslararası düzeyde tanınırlığını arttırmak için çalışmalarımızı sürdürdüğümüzü zaman zaman sizlere iletiyorduk. Bu çalışmalarımız sonucunda dergimizin 15 Şubat 2022 tarihi itibariyle SCOPUS veri tabanına kabul edildiğini sizlerle paylaşmaktan mutluluk duyuyoruz. Dergimizin 2022 yılı sayılarında yer alan makalelere SCOPUS veri tabanı üzerinden ulaşılabilmektedir (Tüm makalelere erişim için Source Title kısmına "Akademik Gida" yazılması gerekmektedir). Dergimizin kalitesini ve uluslararası alanda saygınlığını arttırabilmemiz için etki faktörünün yükseltilmesi başlıca hedeflerimiz arasındadır. Bu nedenle siz değerli bilim insanlarından gerek dergimize ve gerekse diğer ulusal ve uluslararası dergilere gönderdiğiniz makalelerde Akademik Gıda dergisinde yayımlanan makalelere mümkün olduğunca atıf yapmanızı tekrar rica ediyoruz.

Dergimizin yayıncısı Sidas Medya Limited Şirketi'nin 5 Ocak 2023 tarihli kararı uyarınca, 15 Ocak 2023 tarihinden sonra Akademik Gıda dergisine gönderilen Türkçe makaleler için "kabul/red şartına bağlı olmaksızın" yazar/yazarlar tarafından katkı payı olarak 300 TL (KDV Dahil) ödenmesinin uygun görüldüğünü tekrar hatırlatmak istiyoruz. Diğer taraftan İngilizce olarak dergiye gönderilen makalelerden herhangi bir ücret talep edilmeyecektir **(https://dergipark.org.tr/tr/pub/akademik-gida/price-policy).**

Katkılarınızla dergimizin daha iyi noktalara geleceğine yürekten inanıyoruz. Bu sayının oluşmasında katkıda bulunan; çalışmalarını yayımlanmak üzere dergimize gönderen yazarlara ve bu çalışmaları titizlikle değerlendiren yayın kurulu üyelerimiz ve hakemlerimize teşekkürlerimizi sunuyoruz.

Saygılarımızla.

Prof. Dr. Oğuz Gürsoy
Baş Editör

Prof. Dr. Özer Kınık
Prof. Dr. Ramazan Gökçe
Prof. Dr. Yusuf Yılmaz
Editörler

BİLİMSEL ETKİNLİKLER

Türkiye 1. Gıda Mikrobiyolojisi Kongresi

Atatürk Üniversitesi Ziraat Fakültesi Gıda Mühendisliği ile Yıldız Teknik Üniversitesi Gıda Mühendisliği iş birliğinde 13-16 Eylül 2023 tarihleri arasında "Türkiye 1. Gıda Mikrobiyolojisi Kongresi" düzenlenecektir. Kongre ile ilgili bilgilere **https://gidamikro2023.atauni.edu.tr/** adresinden ulaşılabilir.

III. Ulusal Sütçülük Kongresi

İlki 2017 yılında Ankara Üniversitesi Ziraat Fakültesi Süt Teknolojisi Bölümü ev sahipliğinde gerçekleştirilen Ulusal Sütçülük Kongrelerinin üçüncüsü yine Ankara'da 5-6 Ekim 2023 tarihleri arasında "Sürdürülebilirlik perspektifinden süt endüstrisinin geleceği" teması ile gerçekleştirilecektir. Ülkemizde ve dünyada süt bilimi ve teknolojisi alanındaki son gelişmelerin ve ülkemiz sütçülüğünün uluslararası rekabette var olabilmesi için bilim ve teknolojiye dayalı büyüme stratejilerinin öncelikli olarak tartışılacağı kongre ile ilgili bilgilere **https://www.sutkongresi.com.tr/** adresinden ulaşılabilir.

2. Uluslararası Geleneksel Gıda ve Sürdürülebilir Beslenme Sempozyumu

Sürdürülebilirliğe yönelik küresel zorluklarla mücadele etmenin yollarını aramak olmak üzere gıda, beslenme ve gastronomi alanında yapılan araştırma bulgularına, yenilikçi fikirlere ve önerilere ulaşması amacıyla Toros Üniversitesi tarafından 5-6 Ekim 2023 tarihlerinde çevrimiçi (online) olarak gerçekleştirilecek 2. Uluslararası Geleneksel Gıda ve Sürdürülebilir Beslenme Sempozyumu ile ilgili bilgilere **https://food23.toros.edu.tr/** adresinden ulaşılabilir.

International Congress on Food (ICONFOOD'23)

Sivas Cumhuriyet üniversitesi Gıda Çalışmaları Uygulamaları ve Araştırma Merkezi tarafından 16-18 Ekim 2023 tarihleri arasında "ICONFOOD'23 International Congress on Food" isimli kongre gerçekleştirilecektir. Kongre ile ilgili bilgilere **https://iconfood.cumhuriyet.edu.tr/index.php** adresinden ulaşılabilir.

13. Gıda Mühendisliği Kongresi

Gıda sektörü ile ilgili tüm kurum ve kuruluşları bir araya getirerek gıdalara ilişkin bilimsel gelişmelerin ve güncel konuların tartışılması amacıyla TMMOB Gıda Mühendisleri Odası tarafından on üçüncüsü 2-4 Kasım 2023 tarihleri arasında Ankara'da ulusal bir kongre olarak gerçekleştirilecek 13. Gıda Mühendisliği Kongresi ile ilgili bilgilere **www.gidamuhendisligikongresi.org** adresinden ulaşılabilir.

SİDAS MEDYA
Akademik Gıda®
ISSN Online: 2148-015X
https://dergipark.org.tr/tr/pub/akademik-gida

Akademik Gıda 21(2) (2023) 101-111, DOI: 10.24323/akademik-gida.1350664

Research Paper / Araştırma Makalesi

Optimization of Drying Parameters in the Production of Purple Carrot Puree Powder

Bahar Demircan[1], Yakup Sedat Velioglu[2]

Ankara University, Faculty of Engineering, Department of Food Engineering, 06830, Ankara, Turkey

Received (Geliş Tarihi): 26.07.2022, Accepted (Kabul Tarihi): 08.06.2023
Corresponding author (Yazışmalardan Sorumlu Yazar): velioglu@ankara.edu.tr (Y.S. Velioglu)
+90 312 203 3300 /3619 +90 312 317 8711

ABSTRACT

Purple carrots are an important source of phenolic compounds and spray drying is the most advantageous method to make purple carrots more stable. Optimization analysis was carried out to determine the effects of inlet temperature, pump rate, and maltodextrin concentration on the process yield, antioxidant activity, total phenolic and anthocyanin content in the purple carrot puree powder. The optimum drying parameters obtained by maximizing the dependent variables (desirability=0.809) were determined as 16.51% (w/v) maltodextrin concentration, 180.16°C inlet temperature, and 30.39% pump rate. Process yield was 83.64%. Under optimum conditions, the dependent variables were 81.20% DPPH scavenging antioxidant activity, 5332.87 ppm total phenolic content as gallic acid equivalent, and 449.71 ppm total anthocyanin content as cyanidin-3-glucoside, and results indicated that they were preserved at 92.66, 90.43 and 83.79%, respectively.

Keywords: Experimental design, Antioxidant activity, Total phenolic content, Anthocyanin content

Mor Havuç Püresi Tozu Üretiminde Kurutma Parametrelerinin Optimizasyonu

ÖZ

Mor havuç, önemli bir fenolik bileşik kaynağıdır ve mor havucu daha stabil hale getirmek için püskürterek kurutma en avantajlı yöntemdir. Mor havuç püresi tozunda giriş sıcaklığı, pompa hızı ve maltodekstrin konsantrasyonunun proses verimi, antioksidan aktivite, toplam fenolik ve antosiyanin içeriği üzerindeki etkilerini belirlemek için optimizasyon çalışması yapılmıştır. Bağımlı değişkenlerin (arzu edilebilirlik=0.809) maksimize edilmesiyle elde edilen optimum kurutma parametreleri şu şekilde belirlenmiştir: maltodekstrin konsantrasyonu %16.51 (a/h); giriş sıcaklığı 180.16°C, pompa hızı %30.39. Proses verimi %83.64 olarak bulunmuştur. Optimum koşullarda, bağımlı değişkenler şu şekildedir: antioksidan aktivite (DPPH) %81.20; toplam fenolik madde (gallik asit eşdeğeri olarak) 5332.87 ppm; toplam antosiyanin içeriği (siyanidin-3-glukozit olarak) 449.71 ppm. Buna göre optimum koşullarda, incelenen bu parametreler sırasıyla %92.66, %90.43 ve %83.79 oranlarında korunmaktadır.

Anahtar Kelimeler: Deneysel tasarım, Antioksidan aktivite, Toplam fenolik içeriği, Antosiyanin içeriği

INTRODUCTION

Carrot (*Daucus carota*) is a biennial of the *Umbelliferae* family that contains vitamins and dietary fiber and is widely used in daily diet. The consumption rate of purple carrots greatly varies among countries. Turkey is the world's leading purple carrot producing country and its production of purple carrots is constantly increasing due to demand by the pigment and functional food industries [1-3].

Purple carrots with an attractive red-purple color are particularly used in the production of anthocyanin-rich concentrates for the pigment industry [4]. Purple carrot extracts have a wide range of uses as a healthy alternative to synthetic colorants used in fruit juices, candies, ice creams, jams, marmalade, Turkish delights, beverages, sauces, and soup mixes [5].

Purple carrots contain high amounts of, mainly acylated, anthocyanins (45.4- 17400 mg/kg dry matter) and other phenolic acids that are excellent sources of functional compounds [5]. In this context, it has been reported that the purple carrot has approximately 3 times more antioxidant capacity than the orange carrot [6]. Anthocyanins are pigments sensitive to environmental factors, they are highly unstable components and have first-order degradation kinetics, and therefore, they decrease exponentially over time and must be stabilized and protected against adverse conditions. Additionally, purple carrots have high moisture content in fresh or mashed form, so they are susceptible to microbial spoilage in the cold and even under controlled atmosphere storage conditions [7]. To prolong the shelf life, the high moisture content should be reduced using various methods [6]. As a result, the dried product is valued as an important source for many processed ready-made food products.

Various methods are suggested for drying vegetables in the literature, among which the spray drying method, which offers the opportunity to produce a quality product with low water activity, weight, and ease of storage and transportation, stands out due to its advantages. By adjusting spray drying conditions according to raw material properties, both heat-resistant and heat-sensitive components can be sufficiently preserved. The properties of the vegetable powders obtained using this process mainly depend on the air inlet temperature, feed flow rate, and carrier agent concentration [8]. However, when drying vegetables with high organic acid and sugar content with this method, the product sticks to the cyclone walls, decreasing quality, and yield. To eliminate this negativity and to obtain the powder with high efficiency, the most appropriate approach is to use drying aids that can increase the glass transition temperature of the product. Among these substances, maltodextrin (MD) stands out in terms of cost and effectiveness [9].

Response surface methodology (RSM) is a modeling method used to find the optimum values of independent variables within the scope of dependent variables, especially in studies for optimization [10-12]. In this research, the process parameters (air inlet temperature, pump rate, and MD concentration) were optimized by RSM within the scope of the powder obtained by spray-drying purple carrot puree on antioxidant activity, total phenolic, and anthocyanin content.

There is a lack of literature on the production of a high-content powder with elevated antioxidant activity, total phenolic content, and anthocyanin content through the spray drying process of purple carrot puree. Thus, the novelty of this study is manifested in filling this research gap. By examining the impacts of optimized spray drying conditions on the aforementioned parameters, this research seeks to enhance the knowledge regarding the utilization of purple carrot powder as a valuable resource in the realm of processed food products.

MATERIALS and METHODS

Materials

Purple carrot puree (91.07% wb. moisture content, 7.99 °Brix, 6.39 pH, 0.11% titratable acidity, and 2.5 Bostwick) and MD (NutriDex-18, produced by Omnia Starch, Adana, Turkey) with 18-20 dextrose equivalents (DE) used in the research were kindly donated by Tunay Foods, Erzincan, Turkey, and Durukan Confectionery, Ankara, Turkey, respectively. BUCHI B-290 Mini Spray Dryer (BÜCHI Labortechnik AG, Switzerland) with drying gas flow 40 kg/h, evaporation capacity 1 L.H_2O/h, particle-size diameter 1-25 µm, smallest sample 30 mL, collection system cyclone. All of the chemicals were analytical grade and purchased from Sigma-Aldrich.

Preparing the Feed

The mash was first diluted with 1/4 water and filtered to obtain a consistency suitable for feeding into the spray dryer. 100 mL of the prepared solution was taken into glass bottles, MD was added at the rates determined in the trial plan, and mixed at 600 rpm for 10 min (Heidolph MR Hei-Standard), and the MD was completely dissolved. All of the samples were fed into the instrument at room temperature.

Experimental Design

A 3-factor and 3-level Box-Behnken Design consisting of 15 experimental runs with 3 replicates at the center point (Table 1) to be used for powdering purple carrot puree in a spray dryer were created with RSM in Design Expert 11.0 software (Stat-Ease Inc., ABD). The experimental study order was completely randomized. A quadratic Eq. (1) is used to express the dependent variables as a function of the independent variables.

$$Y_k = ß_{k0} + \sum_{i=1}^{n} ß_{ki} x_i + \sum_{i=1}^{n} ß_{kii} x_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} ß_{kij} x_i x_j \qquad (1)$$

where Y_k = dependent variable, Y_1 = Process yield (%), Y_2 = Antioxidant activity, Y_3 = Total phenolic content, Y_4 = Total anthocyanin content; x_1 = concentration of MD, x_2 = inlet air temperature, x_3 = pump rate; β_{ko} was the value of the fitted response at the center point of the design, $\beta_{ki,kii,kij}$ are the regression coefficients, respectively. Statistical significance was determined by analysis of variance (ANOVA) at a 95% confidence level. The adequacy of the model was checked with R^2 values. The desired targets were (Y_1 is within range and

all other responses max) selected for each variable and response.

In the trial plan, MD concentration (15%-35% w/v), inlet temperature (170-190°C), and pump rate (20%-40%) were independent variables; process yield, antioxidant activity, total phenolic substance content, and total anthocyanin content were dependent variables. The constant process parameters in the spray dryer were used as 100% aspirator rate (35 m^3/h) and 40 mm airflow volume (667 L/h).

Analysis of Powder

The following analyses were performed in 3 replications-3 parallel to 15 different purple carrot powders obtained with the trial plan in Table 1. The same analyses were performed on the purple carrot puree feed, and the protection levels of the chemical parameters determined in the final product were revealed.

Extract Preparation for Antioxidant Activity and Total Phenolic Content Analysis

One gram of powder was mixed with 10 mL of distilled water in an orbital shaker (Heidolph Unimax 2010) at 350 rpm for 15 min. At the end of the period, the samples were kept in an ultrasonic water bath (ISOLAB Laborgerate GmbH) at 20°C for 30 min and then centrifuged (Hettich Zentrifugen-Universal 320R) at 5000 rpm at 20°C for 10 min and the supernatants were used as extracts [13].

Total Phenolic Content

Total phenolic content (TPC) in powder extracts was determined using the Folin-Ciocalteu method described by Slinkard and Singleton [14]. After vortexing (Heidolph D-91126) 60 µL of extract, 4.75 mL of distilled water, 300 µL of Folin-Ciocalteu reagent, and 750 µL of 20% (w/v) sodium carbonate mixture, was incubated (Nüve, EN 400) at 40°C for 30 min. At the end of the period, absorbances were read at 765 nm in the UV-Vis spectrophotometer (Shimadzu UV-1602). The total phenolic content of the samples was expressed as ppm gallic acid equivalent (GAE) by using the calibration chart ($y = 0.0011x + 0.0808$, $R^2 = 0.9989$) prepared with 25, 50, 100, 200, 300, 400 and 500 ppm gallic acid standard solutions.

Antioxidant Activity

The antioxidant activity (AOA) was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method determined by Brand-Williams et al [15]. 100 µL of extract and 3.9 mL of $6x10^{-5}$ M DPPH solution were vortexed and after 30 min of incubation in the dark, the absorbances of the samples were read in a UV-Vis spectrophotometer at 515 nm. The DPPH scavenging activity (%) was calculated with Eq. (2).

$$DPPH\ scavenging\ activity\ (\%) = \frac{(Abs_{control} - Abs_{sample})}{Abs_{control}} * 100 \qquad (2)$$

Total Anthocyanin Content

Total anthocyanin content (TAC) was determined according to the method described by Wang and Xu [16]. 0.5 g of powder was diluted in 50 mL of 2 different buffers (0.025 M potassium chloride (pH = 1.0) and 0.4 M sodium acetate (pH = 4.5)) and it was incubated for 2 h in the dark at room temperature. Absorption (A) was measured at 521.5 and 700 nm. The total anthocyanin content was calculated as ppm cyanidin-3-glucoside (Cn-3G) equivalent according to Eq. (3).

$$Total\ anthocyanin\ content\ (ppm) = \frac{A * MW * DF * 1000}{\varepsilon * l} \qquad (3)$$

where $A = (Abs_{@521.5\ nm} - Abs_{@700\ nm})_{pH\ 1.0} - (Abs_{@521.5\ nm} - Abs_{@700\ nm})_{pH\ 4.5}$, MW is the molecular weight of Cn-3G, DF is the dilution factor, 1000 is the conversion factor, ε is the molar extinction coefficient of Cn-3G, and l is the path length (cm).

Process Yield

The yield (PY) of the powder product obtained according to the amount of feed was determined as a percentage on a dry matter basis.

RESULTS and DISCUSSION

Processing purple carrot puree under different spray drying conditions has resulted in different values for the measured dependent variables (PY, AOA, TPC, and TAC). According to the results presented in Table 1, purple carrot puree processed under different drying conditions exhibited variable ranges for PY (82.59% to 97.14%), AOA (76.575% to 83.213%), TPC (2777.58 to 5929.09 ppm GAE), and TAC (279.628 to 461.02 ppm Cn-3G). These findings indicate that different drying methods have diverse effects on the antioxidant activity, total phenolic, and anthocyanin content of purple carrot samples, in comparison with studies reported in the literature. While there are numerous articles in the literature on drying orange carrots or encapsulating their carotenoid content, studies on purple carrots are comparatively limited [17-21].

Janiszewska et al [18] investigated the effect of different drying methods (convective, microwave-convective, infrared-convective, and freeze) on the physical properties of purple carrot puree and found that vacuum drying method preserved the physical properties of the puree the best. Macura et al [19] examined the effect of freeze-drying and air-drying on purple carrots during the storage process. The research results showed that both

freeze-drying and air-drying had similar effects on the carotenoid and anthocyanin content of purple carrots. Both methods were effective in preserving the levels of carotenoids and anthocyanins in purple carrots. In a different study, Wright et al [20] demonstrated that consumption of dried purple carrots led to significant improvements in weight, lipids, blood pressure, body composition, and inflammatory markers. The group consuming dried purple carrots showed weight loss, reduction in fat mass, decrease in lipid levels, and decrease in inflammatory markers. Additionally, Kidoń and Uwineza [21] focused on the potential of dried purple carrots as a source of bioactive compounds in new smoothie products based on pumpkin, banana, and purple carrot. The research findings indicated that adding dried purple carrot to smoothie products increased their bioactive compound content. Dried purple carrots contain high levels of antioxidants, phenolic compounds, and anthocyanins, which provide health benefits. These findings emphasize the significance of selecting appropriate drying methods for preserving the antioxidant activity and nutritional components of purple carrot puree. Furthermore, a study conducted by Uyan et al [22] addresses the impact of the drying process on the antioxidant activity of purple carrots.

Table 1. Design of experiment and results of dependent variables*

Std	Run	MD concentration (%)	Inlet temperature (°C)	Pump rate (%)	PY (%)	AOA (%DPPH scavenging activity)	TPC (ppm GAE)	TAC (ppm Cn-3G)
3	1	15	190	30	86.26	80.36	5929.09	380.26
8	2	35	180	40	88.84	78.40	2959.39	326.26
9	3	25	170	20	91.16	78.11	3865.45	348.78
11	4	25	170	40	86.96	81.77	3762.42	368.96
7	5	15	180	40	82.59	79.26	5592.73	380.35
13	6	25	180	30	84.92	82.87	3904.85	401.36
10	7	25	190	20	94.10	81.38	3650.30	377.06
5	8	15	180	20	89.17	76.57	5923.03	439.26
12	9	25	190	40	80.26	77.87	3816.97	341.26
15	10	25	180	30	84.26	83.21	3413.94	460.03
14	11	25	180	30	85.01	82.92	3777.58	461.02
2	12	35	170	30	92.36	82.06	2844.24	282.26
1	13	15	170	30	84.60	79.65	5556.36	417.93
6	14	35	180	20	97.14	81.38	2877.58	279.62
4	15	35	190	30	89.07	80.26	2777.58	297.66

*: PY: Process Yield, AOA: antioxidant activity, TPC: Total phenolic content, TAC: Total anthocyanin content

The main distinction of this study lies in the direct processing of purple carrot puree using the spray drying method. This approach minimizes the exposure time of the puree to high temperatures, thus reducing the degradation and loss of components through a rapid drying process. Spray drying creates a thin spray layer, increasing the surface area and facilitating the rapid evaporation of water. As a result, valuable components in purple carrot puree, such as anthocyanins and phenolic compounds, are preserved more effectively. Additionally, the spray drying method offers advantages such as short processing time and low energy consumption. This study demonstrates the positive impact of spray drying on the antioxidant activity, phenolic, and anthocyanin content of purple carrot puree. Consequently, spray drying can be considered as a more efficient and effective option for processing purple carrot puree.

To improve the properties of the powder obtained by spray-drying purple carrot puree, the optimization of the processing conditions was carried out with BBD. Significant model terms, including A, B, C, AB, AC, BC, A^2, B^2, and C^2, were identified for each dependent variable (see Eq. 4-7), and the ANOVA results of these quadratic models are presented in Table 2.

Here, the F and p values associated with the models were utilized to determine statistically significant factor effects. The model F-values of PY, AOA, TPC, and TAC indicated that the model was significant. For statistical models, an F value less than 0.05 indicates the importance of the terms, a P value less than 0.05 indicates the significance of the terms, the lack of fit value indicates the suitability of the model, and the R^2, AdjR^2, Pred R^2>0.80 values indicate the statistical significance of the designed model (Table 3).

The second-order polynomial equations obtained from the experiments, along with their respective coefficients, for PY (Eq. 4), AOA (Eq. 5), TPC (Eq. 6), and TAC (Eq. 7) are provided below.

$$Y_1 = 84.73 + 3.10 * A - 0.67 * B - 4.11 * C - 1.24 * AB - 0.43 * AC - 2.41 * BC + 2.33 * A^2 + 1.01 * B^2 + 2.38 * C^2 \quad (4)$$

$$Y_2 = 83.00 + 0.78 * A - 0.21 * B - 0.02 * C - 0.63 * AB - 1.42 * AC - 1{,}79 * BC - 1.65 * A^2 - 0.77 * B^2 - 2.45 * C^2 \quad (5)$$

$$Y_3 = 3698.79 - 1{,}44 * A + 18{,}18 * B - 23{,}11 * C - 11.85 * AB + 103.03 * AC + 67.42 * BC + 571.21 * A^2 + 6.82 * B^2 + 68.18 * C^2 \quad (6)$$

$$Y_4 = 440.80 - 53.99 * A - 2.71 * B - 3.49 * C + 13.27 * AB + 26.39 * AC - 13.99 * BC - 49.45 * A^2 - 46.82 * B^2 - 34.97 * C^2 \quad (7)$$

where, A is the MD concentration, B is the inlet ait temperature, and C is the pump rate.

Optimization criteria A, B, C, and Y_1 "in range"; $Y_{2,\ 3,\ 4}$ were determined at the same level of importance as "maximize". Optimum processing parameters (desirability=0.809) were obtained as 16.5101% MD concentration, 180.155°C inlet temperature and 30.387% pump rate. Thus, these processing conditions were determined as the optimum levels of the independent variables in the experimental plan. Under these conditions, the results of the analysis were determined as 83.6414% PY, AOA value 81.19% DPPH scavenging activity, TPC value 5332.87 ppm GAE, and TAC value 449.714 ppm Cn-3G. Purple carrot puree, the powder produced with this puree under optimum spray drying conditions, and the aqueous and acidic aqueous solutions of this powder are given in Figure 1.

Table 2. ANOVA of quadratic model terms of responses*

	Sum of Squares	df	Mean Square	F-value	p-value	Inference
PY						
Model	285.66	9	31.74	41.58	0.0004	significant
Residual	3.82	5	0.7634			
Lack of Fit	3.48	3	1.16	6.89	0.1293	not significant
Pure Error	0.3365	2	0.1683			
AOA						
Model	58.64	9	6.52	72.52	<0.0001	significant
Residual	0.4492	5	0.0898			
Lack of Fit	0.3828	3	0.1276	3.84	0.2135	not significant
Pure Error	0.0665	2	0.0332			
TPC						
Model	46605.00	9	5178.33	10.77	0.0088	significant
Residual	2403.57	5	480.71			
Lack of Fit	69.30	3	23.10	0.0198	0.9951	not significant
Pure Error	2334.27	2	1167.14			
TAC						
Model	1.798E+07	9	1.998E+06	57.59	0.0002	significant
Residual	1.735E+05	5	34695.62			
Lack of Fit	43670.89	3	14556.96	0.2243	0.8737	not significant
Pure Error	1.816E+07	14				

*: PY: Process Yield, AOA: antioxidant activity, TPC: Total phenolic content, TAC: Total anthocyanin content

Table 3. Model summary statistics*

Dependent variables	Model	R^2 value	Adj R^2	Pred R^2
PY	quadratic	0.9868	0.9631	0.8050
AOA	quadratic	0.9924	0.9787	0.8938
TPC	quadratic	0.9510	0.8627	0.8702
TAC	quadratic	0.9904	0.9732	0.9454

*: PY: Process Yield, AOA: antioxidant activity, TPC: Total phenolic content, TAC: Total anthocyanin content

The final moisture content of the powder produced from purple carrot puree, which initially had a moisture content of 91.07%, has been reduced to 6.01% with optimal spray drying conditions. It can be observed that the moisture content has been reduced by 93.40% through spray drying, and the powdered product has become more stable. The AOA, TPC, and TAC contents in purple carrot puree were determined as 87.63%, 5896.97 ppm, and 536.69 ppm, respectively. These results indicate that under the recommended conditions, they were preserved at 92.66%, 90.43%, and 83.79%, respectively. BBD and comparison percentage results showed that the value ranges used in the research were statistically significant on yield, antioxidant activity, total phenolic, and total anthocyanin content of the purple carrot puree powder.

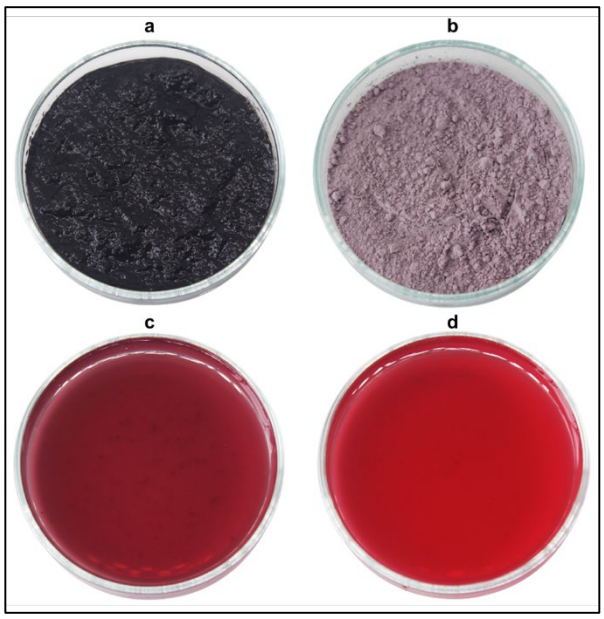


Figure 1. Purple carrot puree (a), purple carrot puree powder (b), rehydrated powder (1%) in distilled water (c), rehydrated powder (1%) in distilled water containing 0.1% acetic acid (d).

In this research, the PY increased and decreased depending on the inlet temperature and other parameters. Temperature is not a factor that can be evaluated on its own. The effect of MD concentration on PY was related to other factors, but in general, PY decreased with increasing MD concentration. The results revealed that, as the pump rate increased, PY generally decreased, but still changed with other factors (Figure 2).

AOA and TPC generally decreased with increasing inlet temperature. Additionally, AOA increased at low temperatures and decreased at high temperatures with the increase of MD concentration (Figure 3). The effect of MD alone on TPC is unclear, however, it is effective along with other parameters. Additionally, AOA and TPC decreased as the pump rate increased (Figure 4).

According to the results, anthocyanin losses increase at high temperatures. It indicates, TAC generally increased as the MD concentration increased and as the pump rate increased, TAC increased and decreased depending on the temperature (Figure 5).

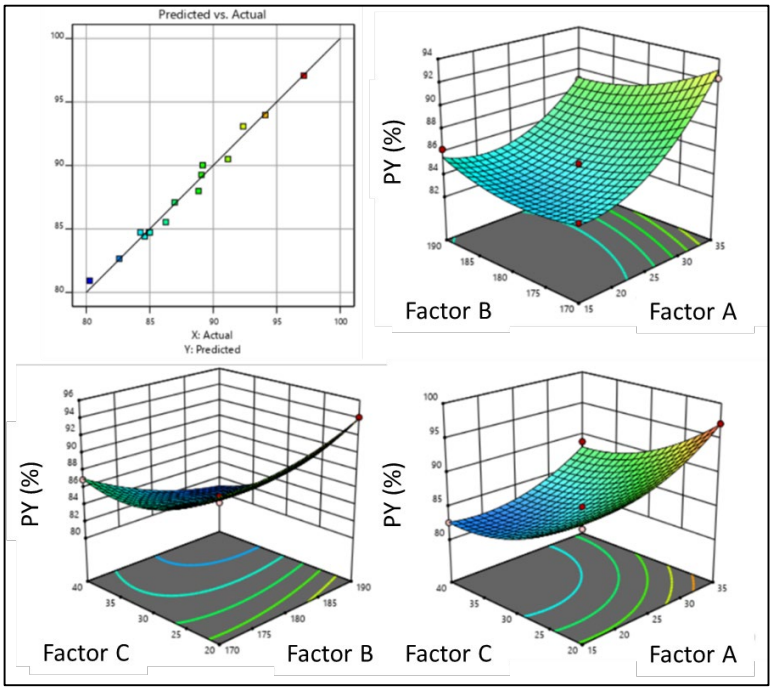


Figure 2. 3D plots of the relevance between the independent variables and process yield (PY)

Effect of Inlet Temperature

Product yield

There are controversial results in the literature on the effect of inlet temperature on product yield in spray drying [23, 24]. To achieve successful drying, it is critical to determine the process yield at different inlet temperatures. Simultaneously, the outlet temperature also has a significant effect on the powder properties. The general judgment on this issue is that the yield increases due to the decrease in the moisture content of the final product because of the high inlet temperature increasing the outlet temperature.

Antioxidant activity and total phenolic content

Polyphenols and other antioxidants can be easily degraded during various heat treatments, including drying. Additionally, losses are likely to occur because of possible oxidation that occurs during drying [25]. The inlet air temperature had a significant effect on the phenolic compounds. At higher inlet temperatures, the total loss of phenolic compounds is generally higher, because of the heat sensitivity of phenolic and other bioactive compounds [26]. About phenomena with this situation, antioxidant activity decreases when phenolics are exposed to high temperatures. According to many researchers, high-temperature spray drying is considered an intense thermal process despite a very short drying time, and the antioxidant activity may decrease after this process. Although the effects of heat treatment on antioxidant activity are controversial in the literature, the general judgment is that the antioxidant activity can be preserved by lowering the spray drying temperature. The opposite situation is explained by the fact that compounds produced during heat treatment have antioxidant properties such as Maillard reaction products [25].

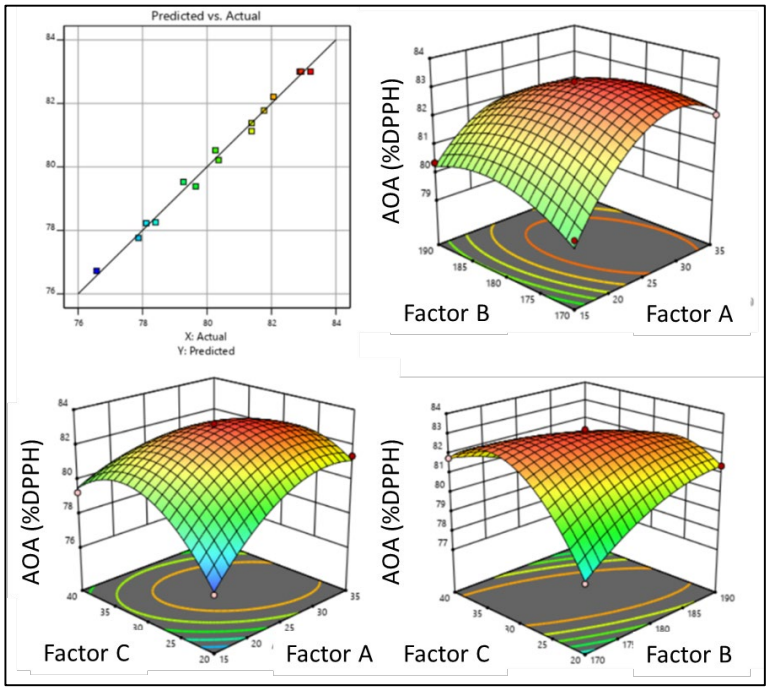


Figure 3. 3D plots of the relevance between the independent variables and antioxidant activity (AOA)

Total anthocyanin content

Pigments are compounds sensitive to thermal processing conditions, and in general, they are damaged by increased temperature in thermal processes. This has been proven on different heat-sensitive compounds such as anthocyanins and various phenolics in different fruit and vegetable powders. Another perspective is based on the high degree of agglomeration occurring due to the higher moisture content of the powders produced at lower inlet air temperatures. The agglomeration process reduces the powder's exposure to oxygen, protecting the anthocyanin pigments from oxidation. A lower exit temperature, on the other hand, causes heat-sensitive compounds such as anthocyanins protected better from heat [27].

Effect of Carrier Agent Concentration

Product yield

Stickiness, which is the most important problem in spray drying of vegetable-based feeds, is due to the presence of sugars (fructose, glucose, sucrose) and organic acids (malic, citric, and tartaric acid) with a very low glass transition temperature (T_g). Feeds rich in these compounds are very difficult to spray dry directly without a carrier agent and it is the key factor in the spray-drying process. To increase T_g in vegetable-based feeds, MD is the most widely used [28-31] and is a successful agent that protects sensitive compounds from environmental factors [3]. As the amount of MD increases, the T_g of the product also increases, so the yield increases. Since the viscosity of the feed is lower

at low MD concentrations, the feed comes into contact with the drying surface longer after atomization, and more sediment is formed on the drying surface, reducing the yield [33]. As these results indicate, a generally applicable fixed carrier concentration cannot be recommended for spray drying [8].

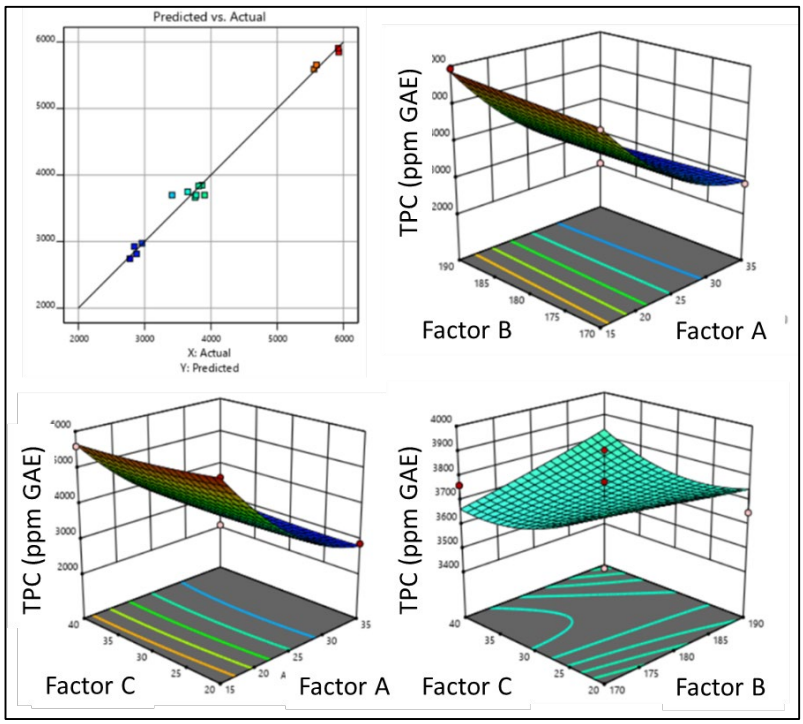


Figure 4. 3D plots of the relevance between the independent variables and total phenolic content (TPC)

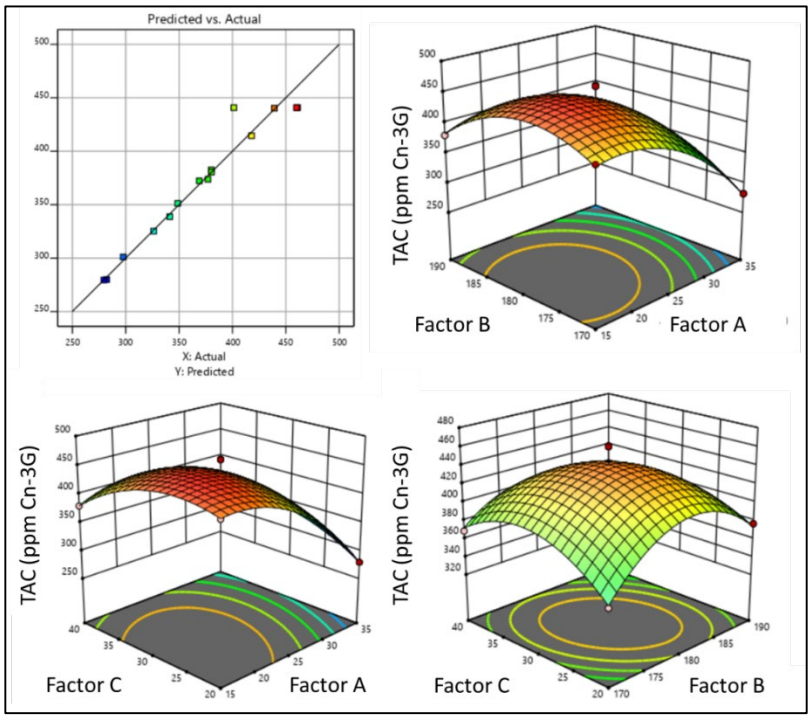


Figure 5. 3D plots of the relevance between the independent variables and total anthocyanin content (TAC)

Antioxidant activity and total phenolic content

In the spray drying process, phenolic compounds are better extracted because of changes in chemical structures, and this increases the total phenolic content. Additionally, the applied heat treatment can also increase the total phenolic content by breaking the glycosidic bonds between aglycone-sugar [34]. MD, a polysaccharide, interacts with phenolics to form complexes that can increase the stability of polyphenols. Thus, the use of higher amounts of carrier agents encapsulates the phenolic compounds better, thus

maintaining the phenolic content and antioxidant activity. The mechanism is that the chemical functional groups in the carrier agents interact with the chemical groups in the feed and affect the stability of the bioactive compounds [35]. However, conflicting results have also been reported in the literature. According to several researchers, as the MD concentration increases, the total phenolic content and antioxidant activity decrease. This situation was explained as the increase in MD concentration, which does not have antioxidant activity on its own, dilutes the feed and decreases the phenolic content and antioxidant activity [36].

Total anthocyanin content

The best way to improve the bioavailability of anthocyanins, which are highly unstable and susceptible to degradation due to their chemical structure, is the encapsulation method. For this purpose, MD is a successful carrier in spray drying to preserve the structure of anthocyanins [7]. The DE value of MD is also critical here; MD with high DE increases anthocyanin and antioxidant content, while MD with low DE contains many long-chain saccharides, creating an oxygen-permeable barrier during microencapsulation, reducing the anthocyanin and antioxidant content [37]. MD with higher DE creates a denser mixture and creates a barrier effect, protecting anthocyanins from oxygen [38]. However, if the MD concentration is too high, the anthocyanin content decreases because as the total solids content increases, the amount of anthocyanin in the feed decreases relatively, that is, the pigments are diluted [36].

Effect of Pump Rate

Product yield

Previous studies reported that an increased feed flow rate has a negative effect on product yield. A higher pump rate means a higher feed flow rate. At a higher feed flow rate, the interaction time between the feed droplets and hot air decreases, and heat and mass transfer occurs less [27]. As a result, because the product becomes difficult to dry, wet particles are formed that adhere to the wall of the drying chamber, so the moisture of the final product increases, and thus the process efficiency decreases.

Antioxidant activity and total phenolic content

Increasing the feed flow rate decreases antioxidant activity and total phenolic content. When the feed is given more slowly, there will be enough time for the heat mass transfer in the drying chamber, so the final product exit temperature will increase and the phenolic components will be damaged. In parallel with this, antioxidant activity decreases [39].

Total anthocyanin content

At a high feed flow rate, higher moisture content and a thin dry layer are formed in the powder product, that is, anthocyanin pigments are damaged because there is no adequate barrier against oxygen. Additionally, at a low pump rate, the product will stay in the drying chamber for a longer time, the final product outlet temperature increases, and this results in decreased anthocyanin content with increasing temperature [40].

CONCLUSION

In case of the relationship between all tested parameters is considered in detail, it was seen that each factor has different effects on the final product. The spray drying process is more advantageous for obtaining functional powder products from purple carrot puree. With this process, purple carrot powder, which has high antioxidant activity, total phenolic, and anthocyanin content, can be used in different industries. In this research, it was found that inlet temperature, carrier agent concentration, and pump rate were effective on tested chemical properties of purple carrot powder in the spray drying process. Finally, the optimum drying conditions were determined as 16,52% MD concentration, 180,16°C inlet temperature, and 30.40% pump rate. With drying under these conditions, the content of purple carrot puree can be preserved above 83%, despite heat treatment.

ACKNOWLEDGMENT

We would like to thank Ankara University Scientific Research Projects (BAP) Unit for their support (Project # 21B0443003).

DECLARATION OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

[1] Kumar, M., Dahuja, A., Sachdev, A., Kaur, C., Varghese, E., Saha, S., Sairam, K.V.S.S. (2019). Valorization of black carrot pomace: microwave assisted extraction of bioactive phytoceuticals and antioxidant activity using box–Behnken design. *Journal of Food Science and Technology*, 56(2), 995-1007.

[2] Bayram, M., Erdogan, S., Esin, Y., Saracoglu, O., Kaya, C. (2014). Farklı siyah havuç miktarlarının şalgam suyunun bileşimine ve duyusal özellikleri üzerine etkisi. *Akademik Gıda*, 12(1), 29-34.

[3] Tanguler, H., Dinc, S.O., Ekenel, G., Aytekin, D. A., Simsek, C., Atakli, H. (2022). Effect of production method and temperature on quality characteristics of shalgam beverages during storage. *Akademik Gıda*, 20(1), 20-29.

[4] Elik, A. (2021). Hot air-assisted radio frequency drying of black carrot pomace: kinetics and product quality. *Innovative Food Science and Emerging Technologies*, 73, 102800.

[5] Agcam, E., Akyıldız, A., Balasubramaniam, V.M. (2017). Optimization of anthocyanins extraction

from black carrot pomace with thermosonication. *Food Chemistry*, 237, 461-470.

[6] Polat, S., Guclu, G., Kelebek, H., Keskin, M., Selli, S. (2022). Comparative Elucidation of colour, volatile and phenolic profiles of black carrot (*Daucus carota* L.) pomace and powders prepared by five different drying methods. *Food Chemistry*, 369, 130941.

[7] Ersus, S., Yurdagel, U. (2007). Microencapsulation of anthocyanin pigments of black carrot (*Daucus carota* L.) by spray drier. *Journal of Food Engineering*, 80(3), 805-812.

[8] Tontul, I., Topuz, A. (2017). Spray-drying of fruit and vegetable juices: effect of drying conditions on the product yield and physical properties. *Trends in Food Science and Technology*, 63, 91-102.

[9] Vardin, H., Yasar, M. (2012). Optimization of pomegranate (*Punica granatum* L.) juice spray-drying as affected by temperature and maltodextrin content. *International Journal of Food Science and Technology*, 47(1), 167-176.

[10] Shavakhi, F., Boo, H.C., Osman, A., Ghazali, H.M. (2012). Effects of enzymatic liquefaction, maltodextrin concentration, and spray-dryer air inlet temperature on pumpkin powder characteristics. *Food and Bioprocess Technology*, 5(7), 2837-2847.

[11] Atacan, K., Yanık, D. K. (2017). Drying blueberry (*Vaccinium corymbosum* L.) juice concentrate by spray dryer: optimization by response surface methodology. *Akademik Gıda*, 15(2), 139-148.

[12] Can, S., Gogus, F., Bozkurt, H. (2022). Optimization of Spray drying encapsulation of bioactive compounds from organic blueberry extract. *Akademik Gıda,* 20(1).1-11.

[13] Gomes, W.F., França, F.R.M., Denadai, M., Andrade, J.K.S., da Silva Oliveira, E.M., de Brito, E.S., Narain, N. (2018). Effect of freeze and spray-drying on physico-chemical characteristics, phenolic compounds and antioxidant activity of papaya pulp. *Journal of Food Science and Technology*, 55(6), 2095-2102.

[14] Slinkard, K., Singleton, V.L. (1977). Total phenol analysis: automation and comparison with manual methods. *American Journal of Enology and Viticulture*, 28(1), 49-55.

[15] Brand-Williams, W., Cuvelier, M.E., Berset, C.L.W.T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food Science and Technology*, 28(1), 25-30.

[16] Wang, W.D., Xu, S.Y. (2007). Degradation kinetics of anthocyanins in blackberry juice and concentrate. *Journal of Food Engineering*, 82(3), 271-275.

[17] Šeregelj, V., Ćetković, G., Čanadanović-Brunet, J., Šaponjac, V. T., Vulić, J., Lević, S., Hidalgo, A. (2021). Encapsulation of carrot waste extract by freeze and spray drying techniques: An optimization study. *LWT-Food Science and Technology*, 138, 110696.

[18] Janiszewska, E., Witrowa-Rajchert, D., Kidoń, M., Czapski, J. (2013). Effect of the applied drying method on the physical properties of purple carrot pomace. *International Agrophysics*, 27(2).

[19] Macura, R., Michalczyk, M., Fiutak, G., Maciejaszek, I. (2019). Effect of freeze-drying and air-drying on the content of carotenoids and anthocyanins in stored purple carrot. *Acta Scientiarum Polonorum Technologia Alimentaria*, 18(2), 135-142.

[20] Wright, O.R., Netzel, G.A., Sakzewski, A.R. (2013). A randomized, double-blind, placebo-controlled trial of the effect of dried purple carrot on body mass, lipids, blood pressure, body composition, and inflammatory markers in overweight and obese adults: the QUENCH trial. *Canadian Journal of Physiology and Pharmacology*, 91(6), 480-488.

[21] Kidoń, M., Uwineza, P.A. (2022). New smoothie products based on pumpkin, banana, and purple carrot as a source of bioactive compounds. *Molecules*, 27(10), 3049.

[22] Uyan, S.E., Baysal, T., Yurdagel, U., El, S.N. (2004). Effects of drying process on antioxidant activity of purple carrots. *Food/Nahrung*, 48(1), 57-60.

[23] Papadakis, S.E., Gardeli, C., Tzia, C. (2006). Spray drying of raisin juice concentrate. *Drying Technology*, 24(2), 173-180.

[24] Bazaria, B., Kumar, P. (2018). Optimization of spray drying parameters for beetroot juice powder using response surface methodology (RSM). *Journal of the Saudi Society of Agricultural Sciences*, 17(4), 408-415.

[25] Samborska, K., Jedlińska, A., Wiktor, A., Derewiaka, D., Wołosiak, R., Matwijczuk, A., Witrowa-Rajchert, D. (2019). The effect of low-temperature spray drying with dehumidified air on phenolic compounds, antioxidant activity, and aroma compounds of rapeseed honey powders. *Food and Bioprocess Technology*, 12(6), 919-932.

[26] Manickavasagan, A., Thangavel, K., Dev, S.R., Delfiya, D.A., Nambi, E., Orsat, V., Raghavan, G.S.V. (2015). Physicochemical characteristics of date powder produced in a pilot-scale spray dryer. *Drying Technology*, 33(9), 1114-1123.

[27] Shishir, M.R.I., Chen, W. (2017). Trends of spray drying: a critical review on drying of fruit and vegetable juices. *Trends in Food Science and Technology*, 65, 49-67.

[28] Jayasundera, M., Adhikari, B., Adhikari, R., Aldred, P. (2011). The effect of protein types and low molecular weight surfactants on spray drying of sugar-rich foods. *Food Hydrocolloids*, 25(3), 459-469.

[29] Jayasundera, M., Adhikari, B., Adhikari, R., Aldred, P. (2011). The effects of proteins and low molecular weight surfactants on spray drying of model sugar-rich foods: powder production and characterisation. *Journal of Food Engineering*, 104(2), 259-271.

[30] Jayasundera, M., Adhikari, B., Howes, T., Aldred, P. (2011). Surface protein coverage and its implications on spray-drying of model sugar-rich foods: solubility, powder production and characterisation. *Food Chemistry*, 128(4), 1003-1016.

[31] Roustapour, O.R., Azad, N.M., Sarshar, M. (2012). Determination of pomegranate juice powder properties produced by a pilot plant spray dryer with a two-fluid nozzle. *Drying Technology*, 30(16), 1906-1917.

[32] Desai, K.G.H., Jin P.H. (2005). Recent developments in microencapsulation of food ingredients. *Drying Technology*, 23(7), 1361-1394.
[33] Igual, M., Ramires, S., Mosquera, L.H., Martínez-Navarrete, N. (2014). Optimization of spray drying conditions for lulo (solanum quitoense l.) pulp. *Powder Technology*, 256, 233-238.
[34] Robert, P., Torres, V., García, P., Vergara, C., Sáenz, C. (2015). The encapsulation of purple cactus pear (*Opuntia ficus-indica*) pulp by using polysaccharide-proteins as encapsulating agents. *LWT-Food Science and Technology*, 60(2), 1039-1045.
[35] Zhang, J., Zhang, C., Chen, X., Quek, S.Y. (2020). Effect of spray drying on phenolic compounds of cranberry juice and their stability during storage. *Journal of Food Engineering*, 269, 109744.
[36] Sánchez-Madrigal, M.Á., Quintero-Ramos, A., Amaya-Guerra, C.A., Meléndez-Pizarro, C.O., Castillo-Hernández, S.L., Aguilera-González, C.J. (2019). Effect of agave fructans as carrier on the encapsulation of blue corn anthocyanins by spray drying. *Foods*, 8(7), 268.
[37] Nayak, C.A., Rastogi, N.K. (2010). Effect of selected additives on microencapsulation of anthocyanin by spray drying. *Drying Technology*, 28(12), 1396-1404.
[38] Ferrari, C.C., Germer, S.P.M., de Aguirre, J.M. (2021). Effects of spray-drying conditions on the physicochemical properties of blackberry powder. *Drying Technology*, 30(2), 154-163.
[39] Bansal, V., Sharma, H.K., Nanda, V. (2014). Optimization of spray drying process parameters for low-fat honey-based milk powder with antioxidant activity. *International Journal of Food Science and Technology*, 49(4), 1196-1202.
[40] Chegini, G.R., Ghobadian, B. (2005). Effect of spray-drying conditions on physical properties of orange juice powder. *Drying Technology*, 23(3), 657-668.

SİDAS MEDYA
Akademik Gıda®
ISSN Online: 2148-015X
https://dergipark.org.tr/tr/pub/akademik-gida

Akademik Gıda 21(2) (2023) 112-118, DOI: 10.24323/akademik-gida.1350684

Research Paper / Araştırma Makalesi

In Vitro Antioxidant and Enzyme Inhibitory Activities of Walnut Male Flowers

Ebru Aydin

Department of Food Engineering, Faculty of Engineering, Suleyman Demirel University, Isparta, Turkey

Received (Geliş Tarihi): 02.06.2023, Accepted (Kabul Tarihi): 24.06.2023
Corresponding author (Yazışmalardan Sorumlu Yazar): ebruaydin@sdu.edu.tr (E. Aydin)
+90 530 099 4462 +90 246 237 0859

ABSTRACT

Walnut (*Juglans regia* L.) male flowers are known for their high phenolic content and associated health benefits, including anti-hypoxic, antihaemolytic, anti-inflammatory, antidepressant, and antioxidant activities. This study represents the first investigation of the inhibitory effects of walnut male flower extract on α-amylase and α-glucosidase enzymes, employing HPAE-PAD (High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection). The inhibitory potential of the extract was compared to that of acarbose, a chemical drug commonly used for this purpose. Furthermore, the antioxidant activity of the extract was also evaluated. The extract demonstrated significant inhibition of α-amylase and α-glucosidase, with half maximal inhibitory concentration (IC_{50}) values of 1.507 mg/mL and 0.803 mg/mL, respectively. In contrast, acarbose exhibited IC_{50} values of 1.031 mg/mL and 0.985 mg/mL for α-amylase and α-glucosidase, respectively. Although the walnut male flower showed greater inhibition of α-glucosidase than acarbose, acarbose exhibited stronger inhibition of α-amylase activity than walnut male flowers. The extract exhibited a DPPH (1,1-Diphenyl-2-picrylhydrazyl radical) free radical scavenging activity, with an IC_{50} value of 19.51 µg/mL. Additionally, the total phenolic content of 277 mg GAE (gallic acid equivalent)/g dry weight (dw) was determined in the extract. These results may highlight the potential of walnut male flowers as a novel enzyme inhibitor for managing type 2 diabetes mellitus. The findings of this study could provide valuable insights for further investigation into the potential applications of walnut male flowers in the food and pharmaceutical industries.

Keywords: Walnut male flowers, α-Amylase, α-Glucosidase, Antioxidant, HPAE-PAD

Ceviz Erkek Çiçeklerinin *In Vitro* Antioksidan ve Enzim İnhibitör Aktiviteleri

ÖZ

Ceviz (*Juglans regia* L.) erkek çiçekleri, yüksek fenolik içeriğine sahip olup, antioksidan, antidepresan, antihipoksik, antienflamatuar ve antihemolitik aktiviteler dahil olmak üzere sağlık üzerine yararlı etkileri ile bilinmektedir. Bu çalışma literatürde ilk defa ceviz erkek çiçeği ekstraktının α-amilaz ve α-glukosidaz enzimleri üzerindeki inhibitör etkilerinin HPAE-PAD (Pulsed Amperometric Detection ile Yüksek Performanslı Anyon Değiştirme Kromatografisi) kullanılarak araştırılmasını temsil etmektedir. Ekstraktın inhibe edici potansiyeli, bu amaç için yaygın olarak kullanılan kimyasal bir ilaç olan akarbozunkiyle karşılaştırıldı. Ayrıca ekstraktın antioksidan aktivitesi de analiz edilmiştir. Ceviz erkek çiçeği ekstraktının IC_{50} değerleri sırasıyla α-amilaz ve α -glukosidaz için 1,507 mg/mL ve 0,803 mg/mL olarak belirlenmiştir. Buna karşılık akarboz için IC_{50} değerleri α -amilaz ve α -glukosidaz için sırasıyla 1.031 mg/mL ve 0.985 mg/mL olarak tespit edilmiştir. Ceviz erkek çiçeği, akarbozdan daha fazla α-glukosidazı durdurucu etki gösterse de, akarbozun ceviz erkek çiçeklerinden daha güçlü α-amilaz aktivitesine sahip olduğu belirlenmiştir. Ceviz erkek çiçeği ekstraktının DPPH serbest radikal yakalama aktivitesi (IC_{50}) 19.51 µg/mL olarak bulunmuştur. Ayrıca toplam fenolik madde miktarı ise 277 mg GAE (gallik asit eşdeğeri)/g kuru madde olarak belirlenmiştir. Bu sonuçlar, ceviz erkek çiçeklerinin tip 2 diyabet hastalığının tedavisinde alternatif bir enzim inhibitörü olarak kullanılabileceğini göstermiştir. Sonuç olarak,

ceviz erkek çiçeklerinin gıda ve ilaç endüstrilerinde potansiyel kullanımına yönelik daha detaylı çalışmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: Ceviz erkek çiçeği, α-Amilaz, α-Glukozidaz, Antioksidant, HPAE-PAD

INTRODUCTION

The global diabetic population reached 536.6 million individuals in 2021, and it is expected to reach 783.2 million by 2045 [1]. In 2021, diabetes and its complications led to approximately 6.7 million reported deaths worldwide, with type 2 diabetes mellitus affecting males at a higher incidence [2]. The World Health Organization (WHO) reported in 2016 that one in eleven individuals currently suffers from diabetes, and this prevalence is anticipated to increase annually [3]. The development of technologies aimed at preventing high blood glucose levels holds promise for reducing these mortality rates. Therefore, there is a growing interest in alternative and natural treatment ways for the management of diabetes.

Juglans regia L., commonly known as walnut, is a tree species indigenous to North America, Europe, and Central Asia [4, 5]. According to the FAOSTAT, the walnut production in-shell is more than 3 million tonnes from a harvested area of 1,106,083 ha worldwide whereas Türkiye produced about 287k tonnes from 141,790 ha [6]. Due to the favourable climate conditions, walnut cultivation is widespread across all regions of Türkiye. The Aegean Region, along with the Mediterranean, Eastern Marmara, and Western Black Sea Regions, stands out as the main areas for walnut production [7]. Although walnut production in many countries focuses on nuts, kernels are widely consumed worldwide as a snack and incorporated into various culinary preparations such as baked goods, salads, breakfast cereals, pasta, and soups [2]. Walnut kernels are also used to extract walnut oil. Extensive research has been conducted on walnut kernels, revealing their high-fat content, including beneficial minerals, proteins, vitamins, phytochemicals, and polyunsaturated fatty acids such as phenolic acids and flavonoids [8-10]. These components offer potential health benefits, including antidiabetic, anti-aging, anti-cancer, anti-inflammatory, and neuroprotective properties. Additionally, various parts of the walnut tree, including leaves, bark, branches, immature green fruit, seed coat, and flowers, contain bioactive compounds with antibiotic, antioxidant, and medicinal properties [8-11]. On the other hand, there are only limited studies about walnut male flowers which is also known as catkins and it was reported that approximately 2000 male flowers per adult walnut tree are present. The major compounds of the walnut male flowers were reported as gallic acid, coumarin, quercetin, polyphenols, flavonoids, sterols, fat, protein, vitamin, and minerals [5, 9]. The phenolic composition of walnut male flowers was reported in different studies. Żurek et al. [10] detected the major phenolics as quercetin 3-*O*-glucoside, quercetin diglucoside and 5-*O*-caffeoylquinic acid. Another recent study also reported the major bioactive compounds of walnut male flowers as follows quercetin, hyperoside, quercitrin, and isoquercitrin [8]. In another study, vanillic acid was found as the main phenolic, and caffeic, ferulic, and chlorogenic acids were also detected [9]. The variation in composition mentioned may be influenced by factors such as the specific species, growing conditions (including soil type, environmental factors during growth, and geographical location), the stage of maturity at the time of harvest, and genetic variations [10, 12]. Walnut male flowers are accepted as a traditional vegetable consumed in specific regions of China and Poland that are employed to prepare infusions, tinctures, liquors, jams, and confectioneries [10]. In addition, in China, it is known as a longevity food due to its various health benefit such as anti-hypoxic, anti-inflammatory, antioxidant, antidepressant, and antihaemolytic activities [9, 10, 13]. Only one study analyzed its potential for regulating the insulin and blood glucose levels of STZ-induced rats [11] whereas there are no studies about the effect of walnut male flowers on digestive enzymes. Acarbose is a pharmaceutical drug prescribed for the treatment of type-2 diabetes and is known for its anti-diabetic properties [12]. It functions by inhibiting the activity of carbohydrate hydrolysis enzymes, specifically α-glucosidase and α-amylase. The phenolic extracts obtained from walnut male flowers have the potential to exhibit an acarbose-like effect.

This study aims to discover the effect of aqueous and methanolic extracts of walnut male flowers from Türkiye on α-glucosidase and human salivary α-amylase using a more sensitive method, HPAE-PAD. Analysis of carbohydrates is available with several methods in the literature. But the detection of carbohydrates with pulsed electrochemical detection at a gold working electrode is a reproducible and sensitive method. Besides, the effects of walnut male flowers extracts are aimed to compare with the chemical drug acarbose.

MATERIALS and METHODS

Plant Material Collection and Extract Preparation

The flowers were collected from trees in Odemis, Izmir, Türkiye in April. The collected material was placed into an ice box and carried to the laboratory within 4-5 h. After that, the walnut male flowers were freeze-dried to remove the moisture and following ground into a powdered form and stored at -20°C until further analysis. The extraction method developed by Żurek et al. [10] was adopted for the methanolic extraction of walnut male flower phenolics using ultrasound (Ivymen, Spain).

Total Phenolic Content (TPC)

The TPC of extract was determined by the Folin & Ciocalteau colorimetric method (T70+UV/VIS spectrophotometer, PG Instruments, UK) [13]. TPC was

expressed as mg of GAE per g of dw. Three replicates were performed for each sample.

DPPH Radical Scavenging Activity

The analysis was conducted using the method developed by Dorman et al. [14]. A mixture consisting of 50 µL of extract, 450 µL of Tris-HCl (hydrochloric acid) buffer (50 mM, pH 7.4), and 1.00 mL of fresh methanolic solution of DPPH (0.10 mM) was vigorously shaken and then placed in a dark place at room temperature (RT) for a duration of 30 minutes. The percentage of DPPH remaining was calculated using the following equation after measuring the absorbance of the samples at 517 nm, as described in equation (1) below.

$$\text{Inhibition \% (DPPH)} = [(Abs_{Control} - Abs_{Sample}) / Abs_{Control}] * 100 \quad (1)$$

The IC_{50} value, which represents the extract concentration required to inhibit 50% of the free radicals, was determined by plotting the DPPH (%) activity and was reported as IC_{50}=µg/mL. All analyses were conducted in triplicate.

Antidiabetic Activity

A buffer solution of 20 mM sodium phosphate and 6.7 mM sodium chloride (pH 6.9) were used to dissolve human salivary α-amylase (S/3160/53 from Fisher Scientific, Loughborough, UK). On the other hand, 10 mM sodium phosphate buffer with pH 7.0 was used to dissolve intestinal acetone rat powder (I1630). Following, both enzyme solutions were vortexed for 30 seconds and then centrifuged at 17,000 x g for 10 minutes. The supernatants were removed to conduct analysis, and these enzyme solutions were prepared freshly before each experiment [15].

Determination of α-Amylase Activity

The developed method by Nyambe-Silavwe et al. [18] was used. 12 g of sodium tartrate was added to 8 mL of 2 M sodium hydroxide and heated until dissolved. It was included to stabilize the colour and protect the product from oxidizing. The DNS (3,5-dinitrosalicylic acid) solution was produced by combining the DNS powder with deionized water (20 mL) before placing the mixture immediately on a heating plate to dissolve. The colorant employed in the α-amylase reaction was DNS. The reducing sugars are produced as a by-product of the hydrolysis of starch by human salivary α-amylase. In an alkaline environment, DNS reacts with the free carbonyl group of the reducing sugars to form 3-amino-5-nitrosalicylic acid, which can be detected at 540 nm. As the reducing sugars are released, DNS altered the colour. DNS and prepared sodium tartrate were combined with deionized water (40 mL) to create the colour reagent solution. 200 mL of the sucrose solution, 50 mL of buffer (pH 7.0-10 mM), 50 mL of plant material, and 200 mL of enzyme solution at various concentrations were combined for the experiment. Following, the mixture was vortexed (10 seconds) and incubated at 37°C (10 min). As it was mentioned by Nyambe-Silavwe and colleagues' study a cartridge (Waters Oasis MAX-003036349A) was applied to eliminate the polyphenol's potential to interfere with colour development, the samples were left for 10 minutes incubation in a boiling water bath to stop the enzyme activity before the addition of colour reagent solution. Following the incubation, to measure the production of reduced sugar amount, a colour reagent solution was added to each sample (1 mL). After that, the samples were immediately placed on ice for cooling down to RT after being placed in a boiling water bath (10 minutes) to prevent enzymic activity. Following this, the samples were transferred to a boiling water bath for 10 minutes again. Then they were placed in vials for electrochemical detection using HPAE-PAD.

Inhibition of α-Glucosidase Enzyme

A previously reported technique was modified to detect the activities of sucrase in an acetone extract of rat intestinal tissues [16] by analysing sugars [sucrose and its products (glucose and fructose)] via a Dionex system running Chromeleon 6.5. Ion-exchange- liquid chromatography (LC) combined with electrochemical detection permits the direct quantification of low-level (pM) carbohydrates below 10,000 Da without the need for derivatization or intensive sample preparation. To optimize the experimental conditions, we initially determined the Michaelis Constant (K_m/V_{max}), which yielded a K_m value of 18 mM and a V_{max} value of 0.09 µmoL sucrose hydrolysis/minute. Subsequently, varying amounts of the enzyme were tested at different concentrations, and the hydrolysis of sucrose was carried out over different time intervals at 37ºC to identify the optimal incubation time and enzyme quantity. For the control sample, a mixture of 18 mM sucrose (200 µL) and 15 mg/mL acetone rat intestinal powder (200 µL) was combined with 100 µL of sodium phosphate buffer, based on preliminary investigations. To evaluate the antidiabetic activity of the extract, the test sample consisted of 100 µL of the extract/solution instead of sodium phosphate buffer. Similarly, for the analysis of antidiabetic activity in walnut male flower extract and acarbose, the test sample contained 100 µL of the respective samples and sodium phosphate buffer. Following this, the samples were incubated at 37ºC for 15 minutes. To stop enzyme activity, 750 µL of acetone was added to the mixtures, which were then immediately vortexed for 10 seconds and cooled down on the ice at room temperature. The acetone was subsequently evaporated using nitrogen gas, and centrifugation was performed once again. Finally, the supernatant was filtered, and the amount of sucrose and its products (glucose and fructose) were determined using HPAE-PAD.

Detection of Carbohydrates with HPAE-PAD Chromatography System

Carbohydrate molecules are often separated using anion-exchange chromatography but considering they

are weak acids, it may be more sensitive to identify them using amperometric detection, due to its specialty to depend on the oxidation of carbohydrates in the presence of sodium hydroxide at the gold electrode. HPAE-PAD (Dionex DX500, Sunnyvale, CA) comprised of a GP40 (gradient pump), PAD (pulsed amperometric detector) system, an LC20 column oven, and electrochemical detectors (ED 40, e.g. gold working, titanium, and silver (reference) electrode). The analytical column for α-glucosidase assay was used as Carbopac PA20 [Dionex, 3×150mm and guard column (3×30mm)], and for human salivary α-amylase assay, Carbopac PA200 (Dionex, 3×250mm) with guard (3×50mm). In addition, the mobile phase was 200 mM sodium hydroxide (NaOH) (flow rate: 0.4 mL/minutes and injection volume: 10 µl). Finally, the elution was achieved using a gradient from 0-30% 200 mM NaOH in 10 minutes, 50% 200 mM NaOH from 10 to 15 minutes, and re-equilibration at 30%, 200 mM NaOH for 15 minutes.

Statistics

The data analysis process was carried out using IBM SPSS Statistics 22. The one-way ANOVA was followed by the Dunnett C test unless the condition was achieved, in which case the Tukey HSD post hoc test was used. If $p \leq 0.05$, differences were regarded as statistically significant unless otherwise stated.

RESULTS and DISCUSSION

TPC and DPPH Radical Scavenging Activity

In Table 1, the assessment of TPC and DPPH activity in walnut male flower extracts is presented, highlighting the relationship with the extraction method employed. In the current study, the TPC content of the walnut male flowers was found 277± 11.08 mg GAE/g dw. In a study, the methanolic extraction of walnut male flowers using ultrasound (UE) was analyzed for its TPC at four different flowering stages. It was detected that its early flowering stage (2.43 g GAE/100g dw) has higher TPC than the later pollen-scattering stage (2.13 g GAE/100g dw) [17]. Zhang et al. [21] also determined the TPC of male flowers using different extraction conditions for UE of methanolic extraction, enzymes assisted-methanolic extraction (EE), and methanolic condensation reflux extraction (ME) and found it as 1351, 1625, and 1236 mg GAE/g extract, respectively. A more recent study also reported the ethanolic extraction of walnut male flowers as 1351 mg GAE/g extract [18]. In another research, the phenolic content of the walnut male flowers was reported as 248 mg GAE/g dw [19]. This research extraction method was adopted for the current study and the results are shown to agree. Muzaffer and Paul [23], Nabavi et al. [24], and Pop et al. [8] conducted studies that yielded comparable results regarding the total phenolic content of walnut male flowers collected from India, Iran, and Romania, respectively, and the TPC of flowers from different countries were determined around 1.45 mg GAE/g dw. In addition, the TPC of various other aerial parts of the walnut tree such as walnut leaves were also examined with the range of 65 mg/g and 194 mg GAE/g dw [20], dry walnut seeds were reported to have a TPC between the range 8.2 to 20.9 mg GAE/g dw [21]. The TPC value reported for the various parts of walnut extract indicates that the male flowers may serve as a rich natural source of polyphenols in comparison to other parts of the walnut tree.

Table 1. Assessment of TPC and DPPH activity in walnut male flower extracts with respect to the extraction method utilized.

Area	Solvent type	Extraction Method	TPC (mg GAE/ g dw)	DPPH (IC_{50} µg/ mL)	References
Türkiye	Methanol	UE	277	19.51	Current study
		UE	1351	51	[22]
China	Methanol	EE	1625	59	[22]
		ME	1236	59	[22]
Poland	Methanol	UE	248	22.34	[19]
India	Methanol	Percolation	129.76	53.95	[23]
Iran	Methanol	Percolation	71.7	674	[24]
Romania	Methanol	UE	1.45	-	[8]

In this research, DPPH free radical scavenging activity demonstrated the lowest IC_{50} value (19.51 ± 1.08 µg/mL) in comparison to the results reported in earlier studies that assessed the antioxidant activity of flowers [10, 21, 24]. Nabavi et al. [24] found the IC_{50} for DPPH radical scavenging activity of extract as 674 µg/mL. While the DPPH radical scavenging capacity of the early flower stages was 84.33%, later pollen-scattering stage were reported as 79.26% [17]. Zhang et al. [21] analyzed the DPPH activity of methanolic extraction using EE, UE, and ME methods and found it as 59 µg/mL, 51 µg/mL, and 59 µg/mL, respectively. Another recent study reported the IC_{50} for DPPH radical scavenging activity of methanolic extraction (22.35 µg/mL) higher than the previously published studies [19]. Based on the available literature, the outcomes may show a strong correlation between the methanol concentration as Żurek et al. [10] used the higher concentration compared to other studies. Similar to TPC, the DPPH radical scavenging activity in the current study and Żurek et al [10] study results are in agreement. Therefore, the results obtained suggest that walnut male flowers possess a strong capacity to remove free radicals, potentially attributed to their rich concentration of bioactive compounds.

Antidiabetic Activity

The inhibition effect of walnut male flower extracts and the chemical drug acarbose, which is used to manage diabetes, on α-glucosidase and α-amylase activity was detected and is presented in Table 2.

According to the literature, the researchers were attracted to analysing the different parts of walnut for their anti-diabetic activity with both *in vitro* and *in vivo* methods. However, this is the first report known about the inhibition of α-glucosidase and human salivary α-amylase by walnut male flowers using a more sensitive method, HPAE-PAD. The results of the current study indicated that the walnut male flower has a higher inhibitory effect for α-glucosidase enzymes than acarbose (Table 1). In addition, the inhibitory effect on α-glucosidase activity was higher than human salivary α-amylase activity for both walnut male flower and acarbose. Also, acarbose indicated a significantly higher inhibitory effect on α-amylase compared to the walnut male flowers. There are several research where the inhibitory effect of bioactive compounds on α-glucosidase activity is higher than the α-amylase activity [12, 27-29]. The activity of polyphenols against α-amylase and α-glucosidase enzymes is influenced by their chemical structure. Changes in functional activity resulting from high binding efficiency may be directly associated with the potential biological activity of these molecules as anti-hyperglycaemic agents [27, 30]. In addition, Ćorković et al. [27] reported that the higher proanthocyanidins content of the extract may increase the inhibitory activity of α-amylase. In a scientific study conducted by Żurek et al. [10], the phenolic components of male flowers from walnut trees were identified using UPLC-PDA-MS/MS (ultra-performance liquid chromatography coupled to photodiode array detection and tandem mass spectrometry). The primary bioactive compounds observed were identified as *O*-flavonol glycosides, with quercetin and kaempferol serving as the aglycones. These compounds were found to be linked with oligosaccharide molecules at the 3- or 7-hydroxyl (OH) position. The higher inhibitory activity for α-glucosidase compared to α-amylase may be attributed to the phenolic content present in walnut male flowers. Based on the obtained results, the findings of the present study are consistent with the literature.

Table 2. Inhibition of digestive enzymes by walnut male flower phenolic extract and acarbose.

Product	IC_{50} (mg/mL)*	
	α-Glucosidase	Human salivary α-amylase
Walnut male flower	0.803 ± 0.01^{x}	1.507 ± 0.08^{x}
Acarbose	0.985 ± 0.01^{y}	1.031 ± 0.05^{y}

*Means that are shared by different superscripts within each column indicate a Tukey's test comparison between the extracts at $p < 0.05$.

In the literature, only one *in vivo* study investigating the anti-diabetic properties of a hydroalcoholic extract derived from walnut male flowers was found [11]. This study focused on streptozotocin (STZ)-induced mice and demonstrated a significant reduction in their blood glucose levels following the administration of the walnut male flowers extract. The antidiabetic activity of walnuts has been extensively investigated in both *in vitro* and *in vivo* studies, encompassing various parts of the walnut plant, including its leaves, fruit, and bark [31-34]. For instance, walnut leaves were extensively utilized in the studies, constituting around 79% of the plant material employed. These leaves were commonly prepared in powdered form or extracted through decoction, oil extraction, microwave, or Soxhlet extraction. In addition, it was also reported that the fruits (14.28%) and bark (7%) exhibited notable anti-diabetic effects when subjected to maceration or powdering [25]. In a clinical study conducted by Hosseini et al. [35] the use of walnut in patients with type 2 diabetes was shown to be both effective and safe. Administration of a daily dose of 200 mg over a period of 3 months resulted in reduced fasting blood glucose, HbA1c, cholesterol, and triglyceride levels. These positive effects can be attributed to the abundant presence of phenolic acids and flavonoids in the plant. However, further investigations are required to elucidate the precise mechanism underlying the antidiabetic activity of walnut male flowers. Additionally, *in vivo* analyses should be conducted to assess the potential toxicity associated with their use. Subsequently, clinical studies can be undertaken to comprehensively explore the therapeutic potential of walnut male flowers in the management of diabetes.

CONCLUSION

In conclusion, this study addresses a knowledge gap regarding the inhibitory activity of *Juglans regia* (walnut) male flowers on α-amylase and α-glucosidase enzymes. Results suggest that walnut male flowers could be utilized in diabetes management due to their abundant polyphenol content. The study demonstrated that the high phenolic content in walnut male flowers might results in greater inhibitory activity against α-glucosidase enzymes compared to the chemical drug acarbose. However, acarbose exhibited stronger inhibitory activity against α-amylase. Additionally, the walnut male flower extract exhibited antioxidant activity. Considering the global cultivation and accessibility of walnut species, combined with the notable health-enhancing attributes of its extract, these flowers could possess substantial potential as a valuable source of natural antioxidants and antidiabetic agents in various biotechnology products. However, further research is required, including *in vivo* analysis and clinical studies, to explore the potential of this valuable raw material. Ongoing scientific exploration holds the potential for the advancement of efficient therapeutic approaches through the utilization of isolated individual active

compounds or combinations derived from the walnut male flowers.

REFERENCES

[1] Sun, H., Saeedi, P., Karuranga, S., Pinkepank, M., Ogurtsova, K., Duncan, B.B., Stein, C., Basit, A., Chan, J.C., Mbanya, J.C., Pavkov, M.E., Ramachandaran, A., Wild, S.H., James, S., Herman, W.H., Zhang, P., Bommer, C., Kuo, S., Boyko E.J., Magliano, D. (2022). IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Research and Clinical Practice*, 183, 109119.

[2] Bhatti, J.S., Sehrawat, A., Mishra, J., Sidhu, I., Navik, U., Khullar , N., Kumar, S., Bhatti G.K., Reddy, P.H. (2022). Oxidative stress in the pathophysiology of type 2 diabetes and related complications: Current therapeutics strategies and future perspectives. *Free Radical Biology and Medicine*, 184, 114-134.

[3] WHO (2016). World Health Organization, 2016. [Online]. Available: https://www.who.int/publications/i/item/9789241565257.

[4] Kadiroğlu, P., Ekici, H. (2018). Yeşil ceviz kabuklarının biyoaktif özelliklerinin FT-IR spektroskopi yöntemiyle tahmin edilmesi. *Akademik Gıda*, 16(1), 20-26.

[5] Zhang, W.E., Wang, C.L., Shi, B.B., Pan, X.J. (2017). Effect of storage temperature and time on the nutritional quality of walnut male inflorescences. *Journal of Food and Drug Analysis*, 25(2), 374–384.

[6] FAOSTAT, (2023). Food and agriculture organization of the United Nations. 1 1 2021. [Online]. Available: https://www.fao.org/faostat/en/#data/QC. [Accessed 01 03 2023].

[7] TÜİK, (2021). Bitkisel Üretim İstatistikleri. 2021. [Online]. Available: https://biruni.tuik.gov.tr/medas/?kn=92&locale=tr.

[8] Pop, C., Suharoschi, R., Pop, O.L. (2021). Dietary fiber and prebiotic compounds in fruits and vegetables food waste. *Sustainability*, 13(13), 7219.

[9] Chrzanowski, G., Leszczynski, B., Czerniewicz, P., Sytykiewicz, H., Matok H., Krzyzanowski, R. (2011). Phenolic acids of walnut (*Juglans regia* L.). *Herba Polonica*, 57(2), 22-27.

[10] Sharma, M. Sharma, M., Sharma, M. (2022). A comprehensive review on ethnobotanical, medicinal and nutritional potential of walnut (*Juglans regia* L.). *Proceedings of the Indian National Science Academy*, 88(4), 601–616.

[11] Hosseini, S.E., Karimzadeh, K. (2013). Effects of a hydroalcoholic extract of walnut male flowers on diabetic rats. *Zahedan Journal of Research in Medical Sciences*, 5(11), 55-58.

[12] Tanoeyadi, S., Tsunoda, T., Takuya, I., Benjamin, P., Mahmud, T. (2023). Acarbose may function as a competitive exclusion agent for the producing bacteria. *ACS Chem. Biol.*, 18(2), 367–376.

[13] Li, Y., Guo, C., Yang, J., Wei, J., Xu, J., Cheng, S. (2006). Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food Chemistry*, 96(2), 254-260.

[14] Dorman, H.J.D., Peltoketo, A., Hiltunen, R., Tikkanen, M.J. (2003). Characterisation of the antioxidant properties of de-odourised aqueous extracts from selected *Lamiaceae* herbs. *Food Chemistry*, 83(2), 255–262.

[15] Nyambe-Silavwe, H., Villa-Rodriguez, J.A., Ifie, I., Holmes, M., Aydin, E., Jensen J.M., Williamson, G. (2015). Inhibition of human α-amylase by dietary polyphenols. *Journal of Functional Foods*,19, 723–732.

[16] Gao, H., Huang, Y.N., Xu, P.Y., Kawabata, J. (2007). Inhibitory effect on α-glucosidase by the fruits of *Terminalia chebula* Retz. *Food Chemistry*, 105(2), 628–634.

[17] Wang, C., Zhang W., Pan, X. (2014). Nutritional quality of the walnut male inflorescences at four flowering stages. *Journal of Food Nutrition Research*, 2(8), 457–464.

[18] Yu, C., Li, S., Zhang, X., Ma, A., Cao, Z., Qi, G., Guo S., Tian, Y. (2022). Purification and ultra-high-performance liquid chromatography tandem mass spectrometry analysis of phenolics extracted from male walnut flowers. *International Journal of Food Properties*, 25(1), 792–1803.

[19] Żurek, N., Pawłowska, A., Pycia, K., Grabek-Lejko D., Kapusta, I.T. (2022). Phenolic profile and antioxidant, antibacterial, and antiproliferative activity of *Juglans regia* L. male flowers. *Molecules*, 27(9), 2762.

[20] Pereira, J.A., Oliveira, I., Sousa, A., Valentão, P., Andrade, P.B., Ferreira, I.C. F.R. Ferreres, F., Bento, A. Seabra, R., Estevinho, L. (2007). Walnut (*Juglans regia* L.) leaves: Phenolic compounds, antibacterial activity and antioxidant potential of different cultivars. *Food and Chemical Toxicology*, 45(11), 2287–2295.

[21] Pycia, K., Kapusta, I., Jaworska, G. (2019). Impact of the degree of maturity of walnuts (*Juglans regia* L.) and their variety on the antioxidant potential and the content of tocopherols and polyphenols. *Molecules*, 24(16), 2936.

[22] Zhang, Y.G., Kan, H., Chen, S.X., Thakur, K., Wang, S., Zhang, J G., Shang, Y.F., Wei, Z.F. (2020). Comparison of phenolic compounds extracted from *Diaphragma juglandis* fructus, walnut pellicle, and flowers of *Juglans regia* using methanol, ultrasonic wave, and enzyme assisted-extraction. *Food Chemistry*, 321, 126672.

[23] Muzaffer, U., Paul, V.I. (2018). Phytochemical analysis, in vitro antioxidant and antimicrobial activities of male flower of *Juglans regia* L. *International Journal of Food Properties*, 21(1), 345–356.

[24] Nabavi, S.F., Ebrahimzadeh, M.A., Nabavi, S.M., Mahmoudi M., Rad, S.K. (2011). Biological activities of *Juglans regia* flowers. *Revista Brasileira de Farmacognosia*, 21, 465–470.

[25] Bourais, S., Elmarrkechy, D., Taha, Y., Mourabit, A., Bouyahya, M., El Yadini, O., Machich, S., El Hajjaji, H., El Boury, N., Dakka, Iba, N. (2022). Review on medicinal uses, nutritional value, and antimicrobial,

antioxidant, anti-inflammatory, antidiabetic, and anticancer potential related to bioactive compounds of *J. regia*. *Food Reviews International,* p. 1–51.

[26] Asgary, S.. Parkhideh, S.. Solhpour, A. Madani, H. Mahzouni P., Rahimi, P. (2008). Effect of ethanolic extract of *Juglans regia* L. on blood sugar in diabetes-induced rats. *Journal of Medicinal Food*, 11(3), 533–538.

[27] Ćorković, A., Pichler, I., Buljeta, J., Šimunović, J., Kopjar, M. (2021). Carboxymethylcellulose hydrogels: Effect of its different amount on preservation of tart cherry anthocyanins and polyphenols. *Current Plant Biology*, 28, 100222.

[28] De Souza, V.B., Thomazini, M., Echalar Barrientos, M.A., Nalin, C.M., Ferro-Furtado, R., Genovese M.I., Favaro-Trindade, C.S. (2018). Functional properties and encapsulation of a proanthocyanidin-rich cinnamon extract (*Cinnamomum zeylanicum*) by complex coacervation using gelatin and different polysaccharides. *Food Hydrocolloids*, 77, 297–306.

[29] Forino, M., Stiuso, P., Lama, S., Ciminiello, P., Tenore, G.C., Novellino, E., Taglialatela-Scafati, O. (2016). Bioassay-guided identification of the antihyperglycaemic constituents of walnut (*Juglans regia*) leaves. *Journal of Functional Foods*, 26, 731–738.

[30] Hosseini, S., Jamshidi, L., Mehrzadi, S., Mohammad, K., Najmizadeh, A.R., Alimoradi H., Huseini, H.F. (2014). Effects of *Juglans regia* L. leaf extract on hyperglycemia and lipid profiles in type two diabetic patients: A randomized double-blind, placebo-controlled clinical trial. *Journal of Ethnopharmacology*, 152(3), 451–456.

[31] Krishnan, V., Verma, P., Saha, S., Singh, B., Vinutha, T., Kumar, R.R., Kulshreshta, A., Singh, S.P., Sathyavathi, T.A., Sachdev, A., Praveen, S. (2022). Polyphenol-enriched extract from pearl millet (*Pennisetum glaucum*) inhibits key enzymes involved in post prandial hyper glycemia (α-amylase, α-glucosidase) and regulates hepatic glucose uptake. *Biocatalysis and Agricultural Biotechnology*, 43, 1-13.

[32] Lavelli, V., Sri Harsha, P.S., Pagliarini, E. (2017). Degradation kinetics of encapsulated grape skin phenolics and micronized grape skins in various water activity environments and criteria to develop wide-ranging and tailor-made food applications. *Innovative Food Science & Emerging Technologies*, 39, 156–164.

[33] Liu, W,. Zhang, J., Zhang, Q., Shan, Y. (2018). Effects of postharvest chilling and heating treatments on the sensory quality and antioxidant system of daylily flowers. *Horticulture, Environment, and Biotechnology*, 59(5), 671–685.

[34] Mollica, G., Zengin, M., Locatelli, A., Stefanucci, G., Macedonio, G., Bellagamba, O., Onaolapo, A., Onaolapo, F., Azeez, A., Ayileka, Novellino, E. (2017). An assessment of the nutraceutical potential of *Juglans regia* L. leaf powder in diabetic rats. *Food and Chemical Toxicology*, 107, 554–564.

[35] Rahimzadeh, M., Jahanshahi, S., Moein, S., Moein, M.R. (2014). Evaluation of alpha-amylase inhibition by *Urtica dioica* and *Juglans regia* extracts. *Iranian Journal of Basic Medical Sciences*, 17(6), 465.

SİDAS MEDYA
Akademik Gıda®
ISSN Online: 2148-015X
https://dergipark.org.tr/tr/pub/akademik-gida

Akademik Gıda 21(2) (2023) 119-131, DOI: 10.24323/akademik-gida.1350935

Research Paper / Araştırma Makalesi

Comparison of Physicochemical, Microbiological, and Sensorial Characteristics of Fermented Probiotic Drinks Produced from Corn and Cow Milks

Emine Mine Çomak Göçer[1], Firuze Ergin Zeren[2], Ahmet Küçükçetin[2]

[1]Department of Nutrition and Dietetics, Faculty of Health Sciences, Akdeniz University, Antalya, Turkey
[2]Department of Food Engineering, Faculty of Engineering, Akdeniz University, Antalya, Turkey

Received (Geliş Tarihi): 22.05.2023, Accepted (Kabul Tarihi): 22.07.2023
Corresponding author (Yazışmalardan Sorumlu Yazar): kucukcetin@akdeniz.edu.tr (A. Küçükçetin)
+90 242 310 6569 +90 242 310 6306

ABSTRACT

This study was focused on preparing corn milk by boiling corns, and producing fermented probiotic drinks by adding inulin and sugar into this milk as well as producing a probiotic drink from cow's milk. Milks were fermented by using a yogurt starter culture and *Lactobacillus acidophilus* LA-5. Fermented probiotic drinks were stored at 4°C for 30 days, and the physicochemical, microbiological, and sensorial characteristics of the drinks were compared during storage. The probiotic drink made from cow's milk exhibited the highest protein (2.25%), titratable acidity (0.46%), L* color (84.41) values and general sensory liking score (4.09) while having the lowest pH (4.46), syneresis (4.50 mL/50 mL) and apparent viscosity (0.09 Pa.s) values. The titratable acidity, syneresis, and apparent viscosity values of drinks increased during storage as the counts of *S. thermophilus, L. delbrueckii* subsp. *bulgaricus*, and *L. acidophilus* decreased. The power-law model showed that the probiotic drinks exhibited a pseudoplastic flow behavior. Notably, the apparent viscosity value of probiotic drinks produced from corn milk was higher than that of the other samples ($p<0.05$). Additionally, the probiotic drink produced from corn milk had the lowest average counts of *S. thermophilus, L. delbrueckii* subsp. *bulgaricus*, and *L. acidophilus*. During storage, the highest decrease (%) in the counts of *L. acidophilus* was determined in probiotic drinks produced from corn milk (8.54%), followed by corn milk and sugar (5.50%), corn milk and inulin (5.46%), and cow's milk (4.30%).

Keywords: Corn milk, Cow's milk, Probiotic drink, *Lactobacillus acidophilus,* Physicochemical characteristics

Mısır ve İnek Sütlerinden Üretilen Fermente Probiyotik İçeceklerin Fizikokimyasal, Mikrobiyolojik ve Duyusal Özelliklerinin Karşılaştırması

ÖZ

Bu çalışmada inek sütünden probiyotik içecek üretiminin yanı sıra haşlanmış mısırdan mısır sütü elde edilmiş ve mısır sütüne inülin ve şeker ilave edilerek fermente probiyotik içecek üretimi gerçekleştirilmiştir. Sütler, yoğurt starter kültürü ve *Lactobacillus acidophilus* LA-5 kullanılarak fermente edilmiştir. Fermente probiyotik içecek örnekleri 4°C'de 30 gün süreyle depolanmış ve fizikokimyasal, mikrobiyolojik ve duyusal özellikleri açısından karşılaştırma yapılmıştır. İnek sütünden yapılan probiyotik içeceğin en yüksek protein (%2.25), titrasyon asitliği (%0.46) ve L* renk (84.41) değerleri ile genel beğeni puanına (4.09) ve en düşük pH (4.46), serum ayrılması (4.50 mL/50 mL), görünür viskozite (0.09 Pa.s) değerlerine sahip olduğu belirlenmiştir Depolama süresi boyunca örneklerin titrasyon asitliği, serum ayrılması ve görünür viskozite değerleri artarken, *S. thermophilus, L. delbrueckii* subsp. *bulgaricus* ve *L. acidophilus* sayılarında azalma olmuştur. Power Law modeline göre, probiyotik içecek örnekleri psödoplastik akış davranışı göstermiştir. Özellikle mısır sütünden yapılan probiyotik içeceğin görünür viskozite değeri diğer örneklere göre daha yüksek bulunmuştur ($p<0.05$). Ayrıca, mısır sütünden yapılan probiyotik içeceğin en düşük ortalama *S. thermophilus,*

L. delbrueckii subsp. *bulgaricus* ve *L. acidophilus* sayılarına sahip olduğu belirlenmiştir. Depolama süresince *L. acidophilus* sayısında en fazla düşüş (%) mısır sütünden yapılan probiyotik içecekte (%8.54), bunu takiben mısır sütü ve şeker (%5.50), mısır sütü ve inülin (%5.46) ve inek (%4.30) sütünden yapılan içeceklerde tespit edilmiştir.

Anahtar Kelimeler: Mısır sütü, İnek sütü, Probiyotik içecek, *Lactobacillus acidophilus*, Fizikokimyasal özellikler

INTRODUCTION

The nutritional value of food and beverages plays a crucial role in meeting consumers' dietary needs and reducing the risk of chronic diseases. In supermarkets worldwide, there is a strong recommendation for chemical-additive-free beverages made from various fruits and vegetables with minimal processing [1]. In developing countries, where milk and its products are expensive and there is a preference for avoiding cow's milk due to vegetarianism or allergies, significant efforts are being directed toward producing yogurt from a variety of food sources [2]. Non-dairy alternatives, particularly cereal-based products, have gained global attention in response to the growing trends of lactose intolerance, vegetarianism, veganism, and low-fat diets [3]. Consequently, the demand for vegetable milk, which serves as a substitute for animal milk and dairy products not suitable for a vegan diet, has been steadily increasing [4]. In line with the rising popularity of vegetarianism, there is also a growing appreciation for the benefits of probiotics [5].

The term "probiotics" is defined as live microorganisms that, when consumed in adequate amounts, provide health benefits to the host [6]. Numerous studies have investigated the dietary and therapeutic properties of *Lactobacillus acidophilus*, which is considered reliable probiotic bacteria used in food production. Fermented milk products produced with *L. acidophilus* undergo a pre-fermentation process, resulting in increased nutritional value and improved digestibility compared to regular milk [7]. While the dairy industry has traditionally played a major role in developing probiotic products, other sectors, such as the nut, cereal, and vegetable milk industries, have also become involved. Vegetable milks, in particular, are noteworthy because they offer nutritional and health benefits, along with the inclusion of prebiotic compounds such as inulin, making them suitable for producing synbiotic products (combining probiotics and prebiotics). Furthermore, prebiotics like inulin not only provides health benefits but also offer technological advantages to fermented products by increasing viscosity and promoting the survival of probiotics during processing and storage [8]. In recent years, functional soft drinks like probiotic-enriched soy milk, germinated rice beverage, and corn milk have gained popularity among health-conscious consumers [9].

Corn, scientifically known as *Zea mays L.*, is a widely cultivated tropical crop that serves as a common staple food. It primarily consists of starch, with low protein content and an inadequate amino acid profile [10]. The isolated corn fiber derived from corn kernels has been identified as a prebiotic suitable for promoting the growth of both *Bifidobacterium* and *L. acidophilus*. With its cultivation spanning over 160 countries, corn plays a significant role in the diets of millions of people [11]. This versatile crop is utilized in the production of various food items, including noodles, porridge, bread, tortillas, and corn drinks. Corn milk, a soft beverage, offers potential health benefits due to its inclusion of several phytonutrients, such as dietary fiber, vitamins, antioxidants, and minerals [9]. However, fermented probiotic corn-based yogurt-like products, particularly corn drinks, remain limited in the market [11].

The objective of this study was to produce probiotic drinks using corn milk and cow's milk and to compare their physicochemical, microbiological, and sensorial properties over a 30-day storage period at 4°C. Inulin and sugar (sucrose) were utilized as additional ingredients in the production of probiotic drinks using corn milk. The study also assessed the acceptability of the samples as preferable carriers for *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *L. acidophilus* by calculating the reduction (%) of these microorganisms at the end of the storage period.

MATERIALS and METHODS

Materials

Corn kernels (Superfresh, Kerevitaş Food Industry and Trade Inc., Bursa, Türkiye) were obtained from a local market, while raw cow's milk was bought from Akdeniz University Faculty of Agriculture's Cattle Farm. The yogurt starter culture (CHR M790) and *L. acidophilus* LA-5 were acquired from Chr. Hansen A/S (Horsholm, Denmark). The preparation of corn milk and fermented probiotic drink samples took place at the Department of Food Engineering, Akdeniz University.

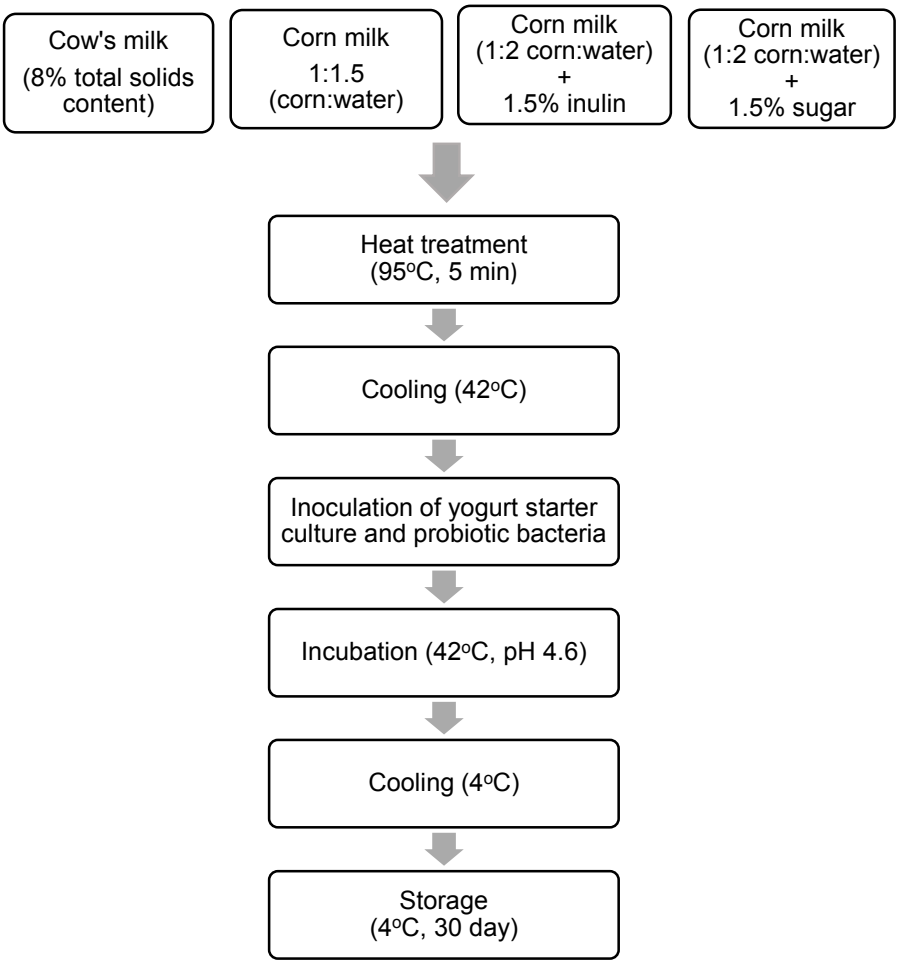
Production of Corn Milk

Distilled water was boiled, and corn kernels were added to the boiling water. The mixture was boiled for 10 minutes. After filtering the boiled water, distilled water was added to the boiled corn at corn/water ratios of 1/1.5 (w/w) and 1/2 (w/w). The mixture was then blended for 5 minutes using a Kenwood Thermoresist Glass Blender AT338. The resulting slurry was passed through a 1 mm^2 sieve, and the filtrate was collected in a clean container, yielding corn milk.

Production of Fermented Probiotic Drink from Corn and Cow's Milk

The total solids content of cow milk was adjusted to 8% for use in the production of the probiotic drink. The corn milk, prepared with a corn/water ratio of 1/1.5 (w/w), was

used to produce 100% corn milk without the addition of sugar or inulin. For the corn milk prepared with a corn/water ratio of 1/2 (w/w), 1.5% inulin or 1.5% sugar was added. The cow milk and corn milk, with adjusted total solids content of 8%, underwent heat treatment at 95°C for 5 min and then cooled to 42°C. The cooled milk was inoculated with a yogurt starter culture (0.05 g/L) containing *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus*, as well *as L. acidophilus* LA-5 (0.05 g/L). The mixture was incubated at 42°C until the pH reached 4.6. The resulting probiotic drink samples were filled into 200 mL plastic cups with lids and stored at 4°C for 30 days (Schema 1).



Schema 1. Schematic diagram of the production steps of fermented probiotic drinks

Physicochemical Analyses

The protein, total solids, and ash contents of the milk and probiotic drink samples were analyzed using the AOAC standard methods [12] on the first day of storage. The titratable acidity of the milk and probiotic drink samples was determined by titration using 0.1 N NaOH (Merck KGaA, Darmstadt, Germany) following the AOAC standard method [12]. The titratable acidity (TA) value was expressed as a percentage of lactic acid. The pH of the probiotic drink samples was measured at 25°C using a calibrated pH meter (Orion 2 Star, Thermo Scientific, Singapore). To assess the color parameters of the probiotic drink samples, a color measuring device (Minolta Colorimeter CR-400, Konica Minolta, Japan) was employed. The CIE L*, a*, b*, and ΔE color parameters were obtained directly from color measuring device. Before measuring the samples, the device was calibrated using a white calibration plate (L=95.14, a=-0.13, b=2.71, and ΔE=0.57). To analyze syneresis, probiotic drink samples were placed in a volumetric cylinder. The total volume of separated whey was measured during storage and expressed as milliliters of whey per 50 mL of probiotic drink [13].

Rheological Analyses

Rheological measurements of probiotic drink samples prepared from cow milk and corn milk were conducted on the 1st, 15th, and 30th days of storage at 4°C. The rheological parameters of the probiotic drink samples were determined using a Brookfield R/S plus stress-controlled rheometer (Brookfield Engineering, Middleboro, MA, USA) equipped with a concentric cylinder double gap (DG3) measurement system. The measurements were carried out at 10°C in a water bath (Brookfield TC-502). For the measurement, 19 mL of the sample was deposited into the rheometer gap after gently mixing it with ten up-and-down spoon motions in the cup. After allowing 2 min for temperature

equilibrium, the measurements were recorded. The samples were subjected to shear by linearly increasing the shear rate from 0.1 to 300 s^{-1} for 5 minutes, followed by reducing it to 0.1 s^{-1} for another 5 minutes. The rheological parameters of the samples were determined using the power-law model and Rheo3000 software (Rheotec Messtechnik GmbH, Berlin, Germany). The apparent viscosity values were calculated at a shear rate of 50 s^{-1} [14].

Microbiological Analyses

Microbiological analyses were performed to determine the counts of *L. acidophilus* LA-5 in the probiotic drink samples. MRS agar with bromocresol green and clindamycin (MRSBC agar) was used for the analysis, while the plates were incubated anaerobically at 37°C for 72 hours. The counts of *L. delbrueckii* subsp. *bulgaricus* were determined using MRS agar, while the plates were incubated at 45°C for 72 hours. The counts of *S. thermophilus* were conducted on M17 agar (Merck KGaA, Darmstadt, Germany) containing 1% (w/v) lactose, followed by incubation at 45°C for 24 hours [15].

The percentage reduction in the counts of *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, and *L. acidophilus* in the samples stored for 1, 15, and 30 days was calculated using the equation 1 provided by Ergin et al. [16].

$$R = \left[\left[1 - \frac{logN\ (cfu)}{logNo\ (cfu)}\right]\right] \times 100 \qquad \text{(cfu: colony forming unit)} \qquad (1)$$

where N_0 represents the microbial count in the samples stored for 1 day, while N represents the microbial count in the samples stored for 15 and 30 days.

Sensory Analyses

A group of 40 individuals, consisting of academic staff and graduate students from the Department of Food Engineering and Department of Nutrition and Dietetics at Akdeniz University, participated in the sensory evaluation of the samples. The panelists, ranging in age from 22 to 40, had no known medical conditions that could impact their sense of smell and taste. The sensory evaluation took place on the 1st, 15th, and 30th days of storage and the hedonic scale, which ranges from 1 (very bad) to 5 (very good) was used to evaluate the sensory quality (color and appearance, texture and consistency, taste and smell, and general liking) of samples, The evaluations were conducted at room temperature, approximately 2 hours before or after meals. Transparent plastic cups containing 25 mL of probiotic drink samples at 8°C were presented to the panelists for evaluation. The samples were coded with three-digit numbers and evaluated in a randomized order. Each panelist conducted the evaluation individually in a well-lit environment and was instructed to drink water between samples [17].

Statistical Analysis

The statistical analysis was performed with two replications conducted simultaneously. The average values obtained from the analyses were subjected to variance analysis using the SAS statistical program (SAS System 9.0, SAS Institute Inc., Cary, NC, USA). To determine the significance of the sources of variation, Duncan Multiple Comparison Test was used to assess the levels of effects.

RESULTS and DISCUSSION

Physicochemical Properties

In Table 1, the average contents of total solids, ash, and protein are presented for milk and probiotic drink samples on the 1st day of storage. Additionally, the pH and titratable acidity values of milk samples on the 1st day are included. Among the milk samples with an adjusted total solids content of 8%, cow's milk exhibited the highest levels of ash content, protein content, and pH value ($p<0.05$). The probiotic drink made from cow's milk also had the highest average ash and protein contents ($p<0.05$). Figure 1 illustrates the pH and titratable acidity values of the fermented probiotic drink samples throughout the storage period.

Table 1. Composition of milk and probiotic drink samples

Type of sample		Total solids (%)	Ash (%)	Protein (%)	pH	Titratable acidity (%)
Milk	Corn	7.99±0.16 a	0.20±0.02 c	1.62±0.03 b	6.29±0.03 b	0.11±0.01 a
	Cow	7.92±0.01 a	0.31±0.01 a	2.20±0.01 a	6.41±0.03 a	0.12±0.00 a
	Corn + Inulin	7.79±0.23 a	0.23±0.01 b	1.37±0.02 c	6.23±0.02 b	0.09±0.01 b
	Corn + Sugar	8.05±0.05 a	0.21±0.02 bc	1.39±0.01 c	6.29±0.03 b	0.09±0.01 b
Probiotic drink	Corn	8.05±0.01 a	0.20±0.02 b	1.76±0.11 b		
	Cow	8.04±0.11 a	0.34±0.06 a	2.25±0.08 a		
	Corn + Inulin	8.08±0.08 a	0.21±0.01 b	1.39±0.01 c		
	Corn + Sugar	8.07±0.08 a	0.18±0.04 b	1.41±0.01 c		

Values are expressed as mean ± standard deviation. Values with different letters (a-c) show the difference in the same column ($p<0.05$).

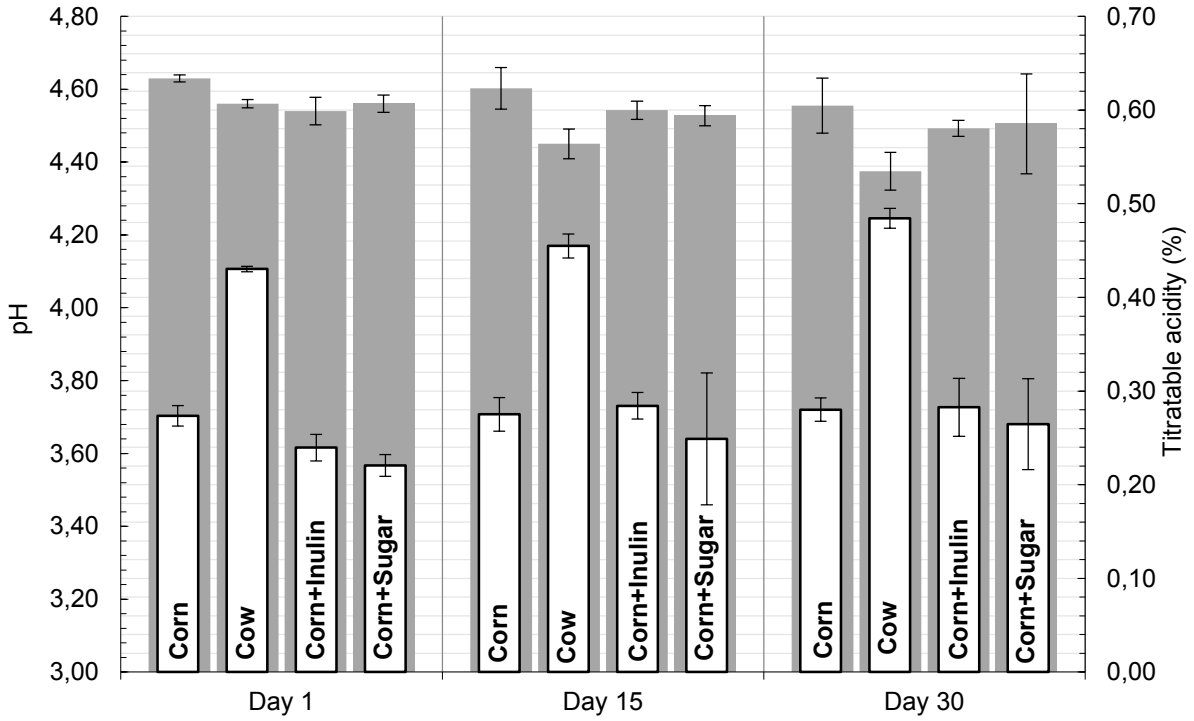


Figure 1. pH (■) and titratable acidity (□) values of fermented probiotic drink samples during storage

The impact of sample variety and storage time on the titration acidity values of probiotic drink samples stored at 4°C for 30 days was found to be statistically significant, with a significance level of $p<0.001$ for the sample variety and $p<0.01$ for the storage time (Table 2). Among the probiotic drink samples, the one made from cow's milk exhibited the highest titration acidity value, while the drink made from corn milk and sugar had the lowest titration acidity value ($p<0.05$). Additionally, the probiotic drink made from corn milk had the highest pH value, whereas the drink made from cow's milk had the lowest pH value. This difference between the corn milk and cow's milk samples could be attributed to the lower buffering capacity of cow's milk. It should be noted that *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* can metabolize lactose and fructose, converting them into lactic acid through the Embeden-Meyerhof-Parnas (EMP) pathway. However, *L. delbrueckii* subsp. *bulgaricus* lacks invertase, which prevents it from converting sucrose into lactic acid through the EMP pathway [18]. This could explain the lower titration acidity value observed in the sample containing sucrose. The increase in titratable acidity and decrease in pH during storage is likely attributed to the production of lactic acid from lactose by lactic acid bacteria [15].

Table 2. Effect of the sample type and storage time on titratable acidity and pH values of fermented probiotic drinks

	Titratable acidity (%)	pH
Type of sample	***	***
Corn	0.28±0.01 b	4.60±0.06 a
Cow	0.46±0.02 a	4.46±0.09 c
Corn + Inulin	0.27±0.03 b	4.52±0.03 b
Corn + Sugar	0.24±0.05 c	4.53±0.08 b
Storage time (days)	**	***
1	0.29±0.09 b	4.57±0.04 a
15	0.32±0.02 a	4.53±0.07 b
30	0.33±0.02 a	4.48±0.10 c

Values are expressed as mean ± standard deviation. Values with different letters (a-c) show the difference in the same column $p<0.05$, *$p<0.05$, **$p<0.01$, ***$p<0.001$

The color of food products plays a crucial role in determining their quality and consumer acceptance [1]. The lightness (L*) scale ranges from 0 (black) to 100 (white). The coordinate a* represents reddish colors with positive values and greenish colors with negative values, while the coordinate b* represents yellowish colors with positive values and bluish colors with negative values [17, 19]. Figure 2 displays the L*, a*, b*, and ΔE values of fermented probiotic drink samples throughout the storage period.

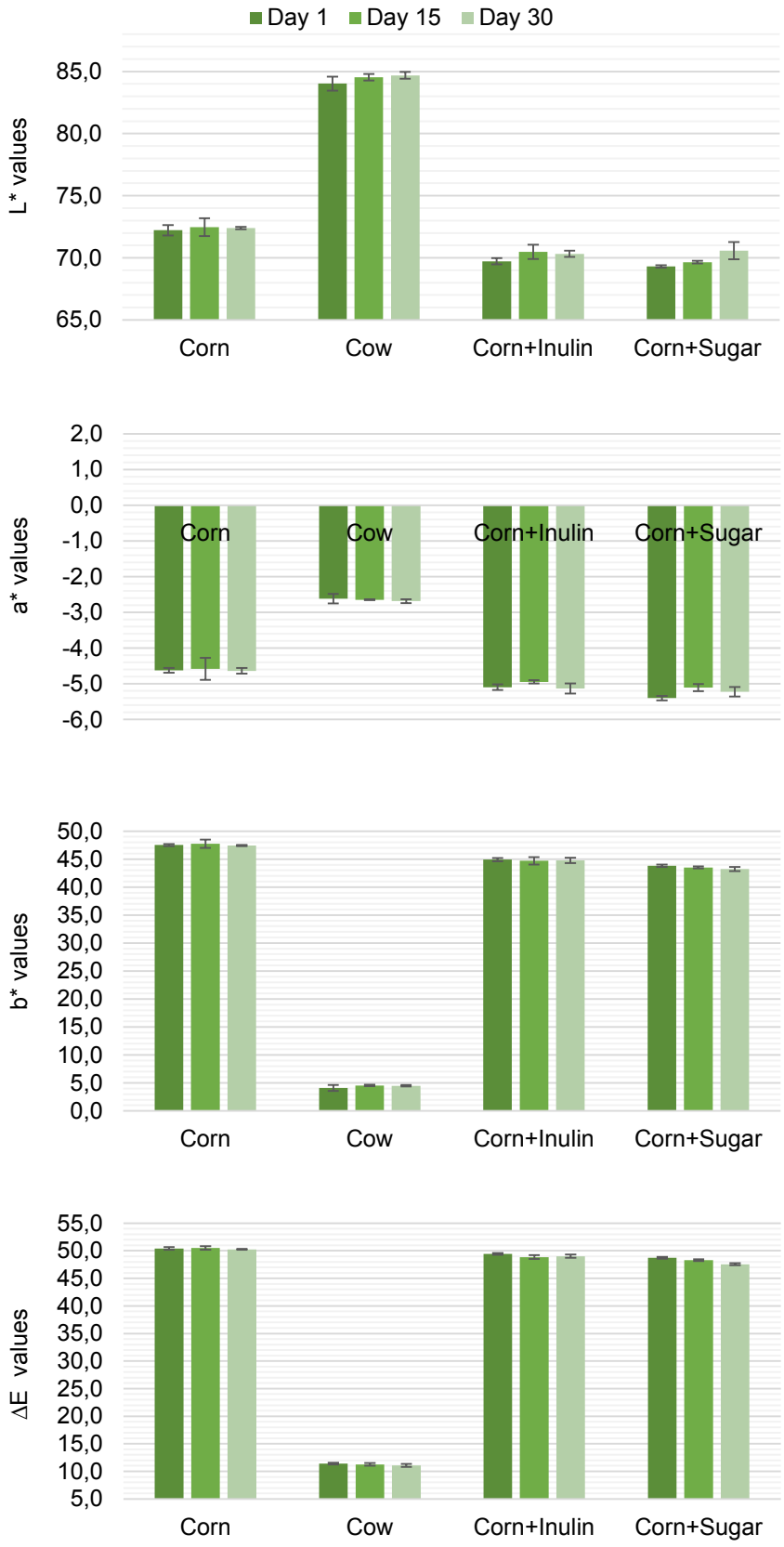


Figure 2. The L*, a*, b* and ΔE values of fermented probiotic drink samples during storage

Probiotic drink samples made from cow's milk exhibited the highest L* and a* values while showing the lowest b* and ΔE values ($p<0.05$). On the other hand, probiotic drink samples made from corn milk had the highest b* and ΔE values. Notably, when considering the b* value, it was observed that the yellowness of the probiotic drink

produced from corn milk exceeded that of the other samples. Among the samples, the probiotic drink made from corn milk and sugar displayed the lowest a* value (Table 3). The primary factor influencing the yellow color of the probiotic drink samples made from corn milk was the presence of corn, which is the main ingredient containing carotenoids such as lutein and zeaxanthin [20, 21]. The difference observed between the samples produced from corn milk is likely attributed to the amount and type of pigments present in the milk composition. Additionally, a* statistically significant increase in the mean L* and ΔE values of probiotic drink samples during storage was observed ($p<0.05$).

Table 3. Effect of sample type and storage time on color parameters of fermented probiotic drink samples

	L*	a*	b*	ΔE
Type of sample	***	***	***	***
Corn	72.36±0.45 b	-4.62±0.17 b	47.56±0.44 a	50.40±0.25 a
Cow	84.41±0.46 a	-2.65±0.08 a	4.38±0.36 d	11.25±0.25 d
Corn + Inulin	70.17±0.49 c	-5.06±0.12 c	44.79±0.47 b	49.10±0.36 b
Corn + Sugar	69.85±0.67 c	-5.24±0.16 d	43.51±0.35 c	48.20±0.53 c
Storage time (days)	***	*		***
1	73.82±1.55 b	-4.44±1.12 b	35.09±0.31 a	40.01±0.19 a
15	74.28±6.22 a	-4.32±1.03 a	35.13±0.44 a	39.73±0.27 b
30	74.49±6.15 a	-4.42±1.06 b	34.97±0.27 a	39.48±0.20 c

Values are expressed as mean ± standard deviation. Values with different letters (a-d) show the difference in the same column ($p<0.05$), *$p<0.05$, **$p<0.01$, ***$p<0.001$

Figure 3 illustrates the apparent viscosity and syneresis values during the storage period. In this study, the average apparent viscosity values of probiotic drink samples ranged from 0.08 to 0.55 Pa.s on the 1st day of storage, 0.09 to 0.59 Pa.s on the 15th day of storage, and 0.1 to 0.64 Pa.s on the 30th day of storage, respectively. Additionally, Figure 4 showcases the consistency coefficient and flow behavior index values of the probiotic drink samples throughout the storage period. The consistency coefficient values of probiotic drink samples ranged from 0.99 to 9.68 Pa.sn on the 1st day of storage, 1.08 to 11.60 Pa.s on the 15th day of storage, and 1.36 to 13.51 Pa.s on the 30th day of storage. For the analysis of flow behavior, various models including Newtonian flow, Power-law, Bingham, and Herschel-Bulkley were employed in this study. The Power-law model provided a high correlation coefficient (R^2) ranging from 0.94 to 0.98, accurately describing the rheological properties of the samples. The flow behavior index (n) values, which indicate the tendency of fluids to behave in Newtonian flow, were less than 1 for the probiotic drink samples produced in our study, ranging from 0.13 to 0.34 on the 1st day, 0.11 to 0.33 on the 15th day, and 0.09 to 0.32 on the 30th day of storage, suggesting non-Newtonian pseudoplastic flow behavior.

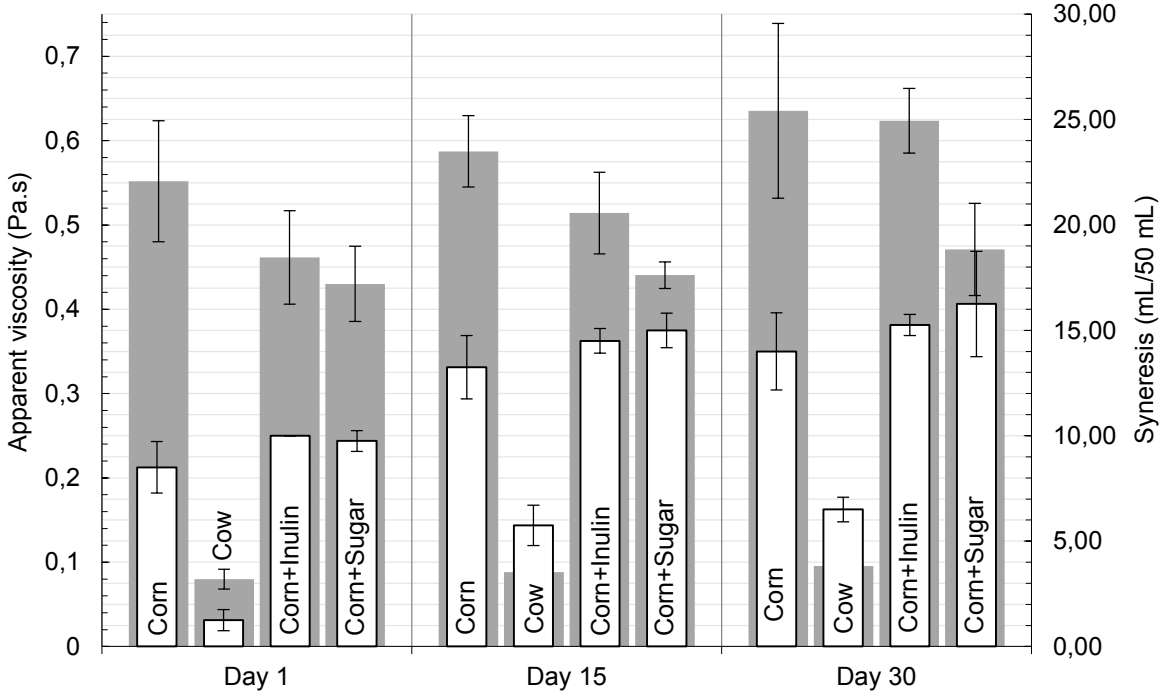


Figure 3. Apparent viscosity (■) and syneresis (□) values of the probiotic drink samples obtained during the storage period.

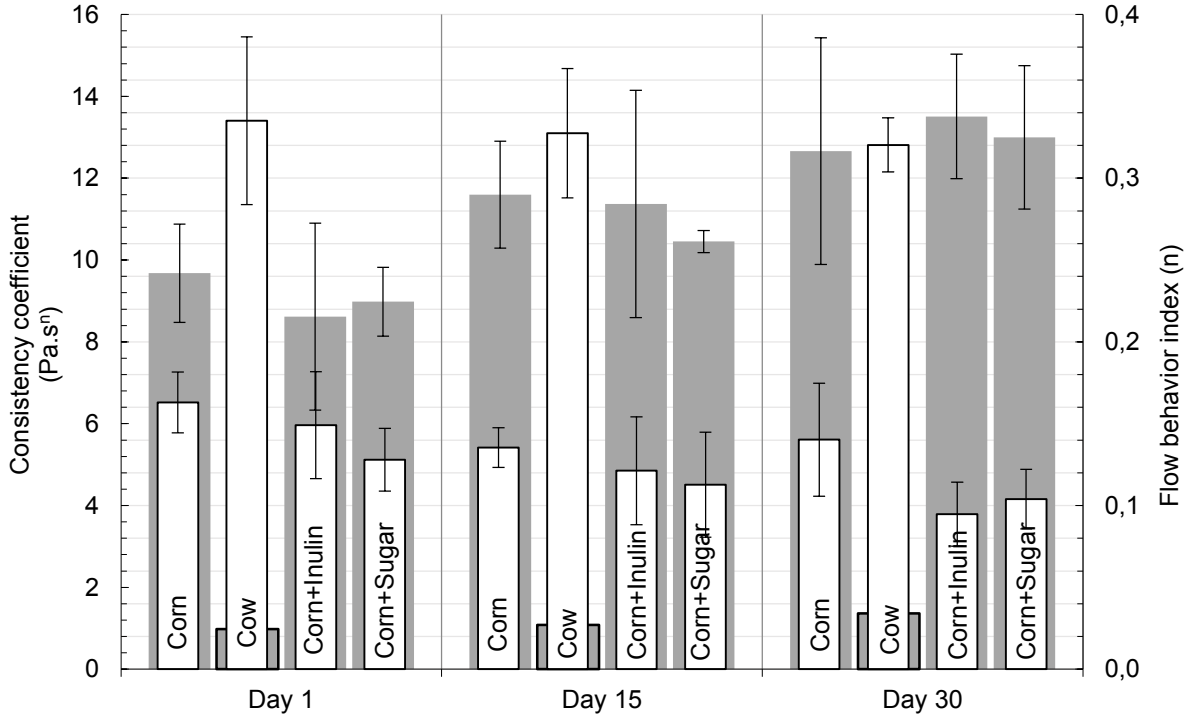


Figure 4. Consistency coefficient (■) and flow behavior index (□) values of the probiotic drink samples during the storage period.

Syneresis, apparent viscosity, consistency coefficient, and flow behavior index values of probiotic drink samples were affected by sample type and storage time at the $p<0.001$. Table 4 presents the effect of sample type and storage time on the syneresis, apparent viscosity, consistency coefficient, and flow behavior index values of fermented probiotic drink samples. It was observed that the average apparent viscosity values of probiotic drink samples made from corn milk were higher compared to the other samples, while the probiotic drink made from cow's milk exhibited the lowest syneresis, apparent viscosity, and consistency coefficient values. Furthermore, the probiotic drink sample made from cow's milk had the highest flow behavior index value ($p<0.05$).

Table 4. Effect of the sample type and storage time on the syneresis, apparent viscosity, consistency coefficient, and flow behavior index values of fermented probiotic drink samples

	Syneresis (mL/50mL)	Apparent viscosity (Pa.s)	Consistency coefficient (Pa.s^n)	Flow behavior index (n)
Type of sample	***	***	***	***
Corn	11.92±1.51 b	0.59±0.07 a	11.31±0.62 a	0.15±0.01 b
Cow	4.50±0.68 c	0.09±0.01 d	1.14±0.30 b	0.33±0.03 a
Corn + Inulin	13.25±0.36 a	0.53±0.05 b	11.17±0.84 a	0.12±0.04 c
Corn + Sugar	13.67±1.27 a	0.45±0.04 c	10.81±0.58 a	0.12±0.02 c
Storage time (days)	***	***	***	*
1	7.38±0.56 c	0.38±0.10 b	7.07±0.96 c	0.20±0.09 a
15	12.13±0.97 b	0.41±0.04 b	8.63±0.18 b	0.18±0.10 ab
30	13.00±1.35 a	0.46±0.05 a	10.13±0.37 a	0.17±0.02 b

Values are expressed as mean ± standard deviation. Values with different letters (a-d) show the difference in the same column ($p<0.05$), *$p<0.05$, **$p<0.01$, ***$p<0.001$

Syneresis, a significant quality defect, occurs when the protein network breaks down during storage, leading to the loss of the gel structure's ability to retain the serum phase [22]. The protein structure membrane surrounding fat globules enhances emulsion stability by reducing interfacial energy and surface tension. Increasing acidity during fermentation disrupts the protein structure membrane, promoting fat globule contact and flocculation. Consequently, emulsions with low protein content may experience increased phase separation [23]. As indicated in Table 1, probiotic drink samples produced from corn milk had lower protein content, which affected the syneresis values. Additionally, bacteria surviving in the probiotic drink hydrolyze proteins during storage, leading to the release of trapped serum within the protein structure [24]. In our study, an increase in syneresis values during storage was observed ($p<0.05$). Various factors, including structural differences, amino acid composition, surface polarity, or hydrophobicity of proteins in milk

composition, can influence syneresis [25]. Based on our results, it was inferred that the differences between the samples may be attributed to variations in titration acidity values and protein structures between cow's milk protein and corn milk derived from corn.

The particle diameter of fat globules, casein micelles, and whey proteins in cow's milk ranges between 0.1-10 µm, 20-400 nm, and 3-10 nm, respectively [26]. However, corn milk is obtained by passing corn slurry through a 1 mm^2 sieve. Consequently, the fermented probiotic drink produced from corn milk displayed higher apparent viscosity and consistency coefficient values compared to the drink made from cow's milk. Kucukcetin et al. [27] demonstrated that apparent viscosity and consistency coefficient values of ayran increased while flow behavior index values decreased with increasing particle size. Ergin [28] established a correlation between apparent viscosity values, consistency coefficient values, and particle size of probiotic yogurt samples, suggesting that protein content can also influence apparent viscosity values. Wang et al. [11] similarly found that the viscosity of a product increased with higher soy protein content in a probiotic corn-based yogurt-like product. In our study, the apparent viscosity values of fermented probiotic drinks produced solely from corn milk were higher than those produced from corn milk with inulin and sugar. This might be attributed to the higher protein content of the fermented probiotic drink made solely from corn milk (Table 1). The addition of inulin to corn milk resulted in higher apparent viscosity compared to the addition of sugar in the samples, potentially due to the water-holding and thickening properties of inulin [29]. The apparent viscosity and consistency coefficient values of the samples increased during storage. It is known that protein-protein interactions and slow protein rearrangements in fermented products continue during cold storage, which is associated with a decrease in pH [30].

Microbiological Properties

Figure 5 depicts the logarithmic values of *S. thermophilus, L. delbrueckii* subsp. *bulgaricus*, and *L. acidophilus* counts in probiotic drink samples during storage, along with the temporal variations in these values.

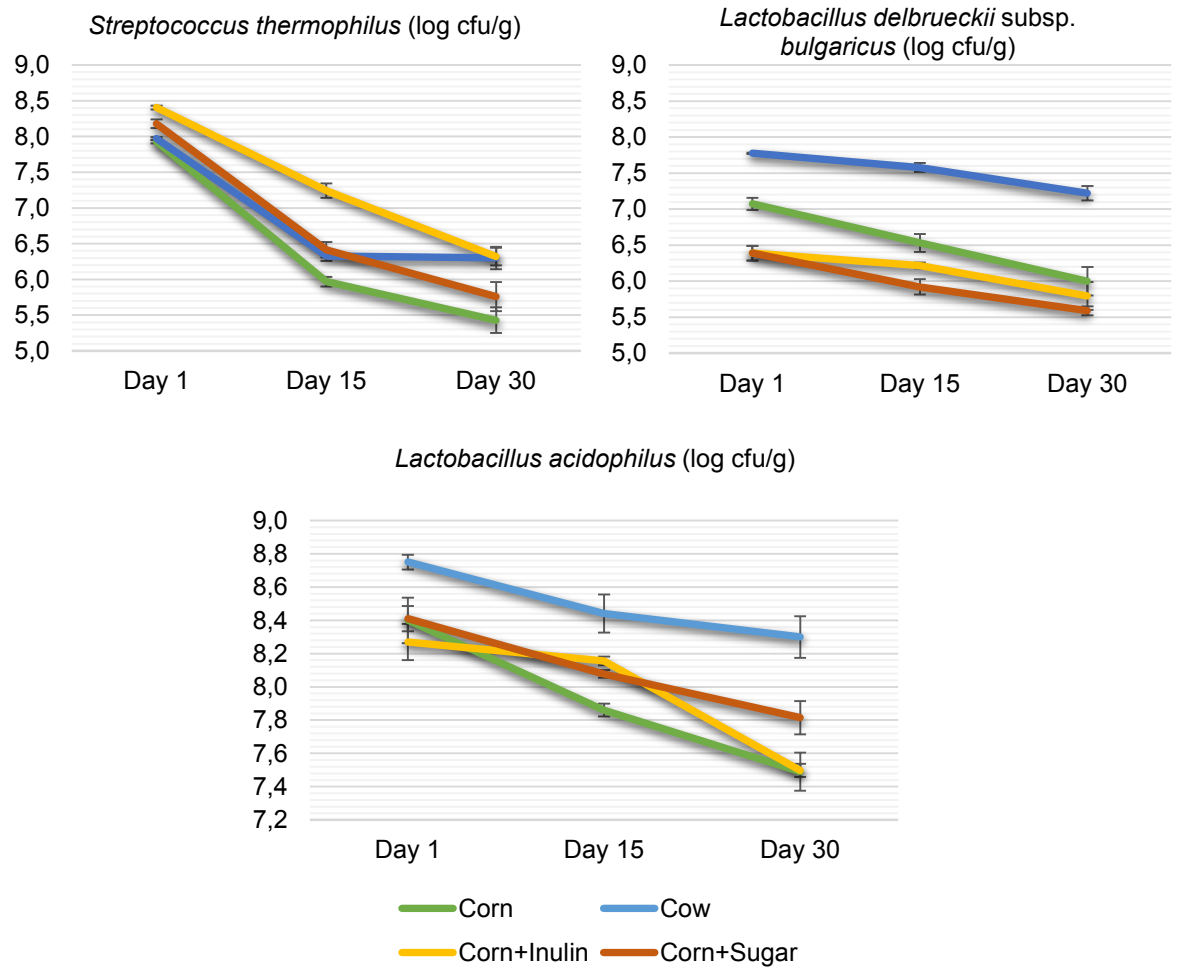


Figure 5. Survival of (a) *Streptococcus thermophilus*, (b) *Lactobacillus delbrueckii* subsp. *bulgaricus,* (c) *L. acidophilus* in probiotic drink samples on 1st, 15th and 30th day of storage.

The counts of *S. thermophilus, L. delbrueckii* subsp. *bulgaricus*, and *L. acidophilus* in probiotic drink samples were found to be influenced by both the sample type and storage time at a significant level of p<0.001. The probiotic drink made from cow's milk exhibited the highest average counts for *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, and *L. acidophilus*, while the probiotic drink made from solely corn milk had the lowest counts for these bacteria (p<0.05) (Table 5). Comparing the *L. acidophilus* counts among the samples made from corn milk, the probiotic drink sample made from corn milk and inulin had the highest count. This can be attributed to the fact that inulin acts as a prebiotic substance. In our study, although the counts of *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, and *L. acidophilus* showed statistically significant differences among the samples, the viability of *L. delbrueckii* subsp. *bulgaricus* and *L. acidophilus* during storage did not differ by more than 1 log cfu/g.

Table 5. Effect of the sample type and storage time on the microbiological properties of fermented probiotic drink samples

	Streptococcus thermophilus (log cfu/g)	*Lactobacillus delbrueckii* subsp. *bulgaricus* (log cfu/g)	*Lactobacillus acidophilus* (log cfu/g)
Type of sample	***	***	***
Corn	6.44±0.32 c	5.97±0.36 d	7.92±0.43 c
Cow	7.32±0.26 a	7.52±0.29 a	8.50±0.22 a
Corn + Inulin	6.87±0.24 b	6.54±0.48 b	8.10±0.27 b
Corn + Sugar	6.78±0.31 b	6.13±0.27 c	7.97±0.36 c
Storage time (days)	***	***	***
1	8.12±0.20 a	6.91±0.60 a	8.46±0.21 a
15	6.49±0.12 b	6.56±0.65 b	8.14±0.22 b
30	5.95±0.42 c	6.15±0.68 c	7.77±0.38 c

Values are expressed as mean ± standard deviation. Values with different letters (a-d) show the difference in the same column (p<0.05), *p<0.05, **p<0.01, ***p<0.001

The survival of probiotic microorganisms is crucial for them to exhibit their beneficial properties during food processing, storage, and gastrointestinal transit. To ensure the desired effects of probiotic bacteria, these specific microorganisms must remain viable, active, and abundant in the product until the expiration date, with cell counts ranging from 10^6 to 10^9 cfu/g [31]. The dosage reaching the target site in the body plays a significant role in the manifestation of characteristic effects of probiotic bacteria [32]. In our study, the counts of *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, and *L. acidophilus* in probiotic drink samples decreased during storage (p<0.05) (Table 5). The effects of sample type and storage time on the percentage reduction of microorganism counts in the samples are presented in Table 6. The percentage reduction of microorganism counts increased during storage (p<0.05). The probiotic drink sample made from corn milk exhibited the highest decrease in the counts of *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, and *L. acidophilus*. The type of milk used can influence the fermentation of milk products, as microorganisms can be stimulated or inhibited by compounds present in milk [33]. Antibacterial and polyphenolic compounds, such as phenolic acids and flavonoids, found in corn milk may hinder the growth of *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, and *L. acidophilus* in probiotic drink during storage. When comparing the reduction rates (%) of *L. acidophilus*, no significant difference (p<0.05) was observed between the reduction rates in the probiotic drink samples made from cow's milk, corn milk and inulin, and corn milk and sugar (Table 6).

Table 6. Effect of the sample type and storage time on the reduction of microorganism counts (%)

	Reduction of *S. thermophilus* counts (%)	Reduction of *L. delbrueckii* subsp. *bulgaricus* counts (%)	Reduction of *L. acidophilus* counts (%)
Type of sample	**	***	***
Corn	28.10±1.39 a	11.41±1.62 a	8.54±1.42 a
Cow	19.34±2.13 c	4.82±1.23 b	4.30±0.61 b
Corn + Inulin	20.82±0.55 c	6.11±1.32 b	5.46±1.50 b
Corn + Sugar	25.55±1.62 b	9.91±1.25 a	5.50±0.97 b
Storage time (days)	***	***	***
15	20.19±4.20 b	5.10±0.68 b	3.84±0.53 b
30	26.72±4.61 a	11.02±1.03 a	8.06±0.86 a

Values are expressed as mean ± standard deviation. Values with different letters (a-d) show the difference in the same column (p<0.05), *p<0.05, **p<0.01, ***p<0.001

Sensorial Properties

Figure 5 shows the color & appearance, texture & consistency, taste & smell, and general liking scores of probiotic drink samples during storage.

Table 7 presents the sensory evaluation results of probiotic drink samples, focusing on taste & smell, as well as general liking. The probiotic drink made from corn milk and sugar received the lowest scores in taste & smell and general liking (Table 7). Over the course of 30 days of storage, there was a significant decrease in sensory scores ($p<0.05$). The lower scores in texture and consistency of the fermented products could be attributed to serum separation, as reported by Kizzie-Hayford et al. [23]. In our study, the probiotic drink sample made from cow's milk showed the lowest serum separation value (Table 4) and received the highest scores in texture & consistency. Regarding color & appearance, the probiotic drink sample made from cow's milk, which had the highest L* (brightness) parameter, received the highest score. When considering the general liking and taste and smell scores, it was observed that the probiotic drink sample made from cow's milk was preferred more than the samples made from corn milk.

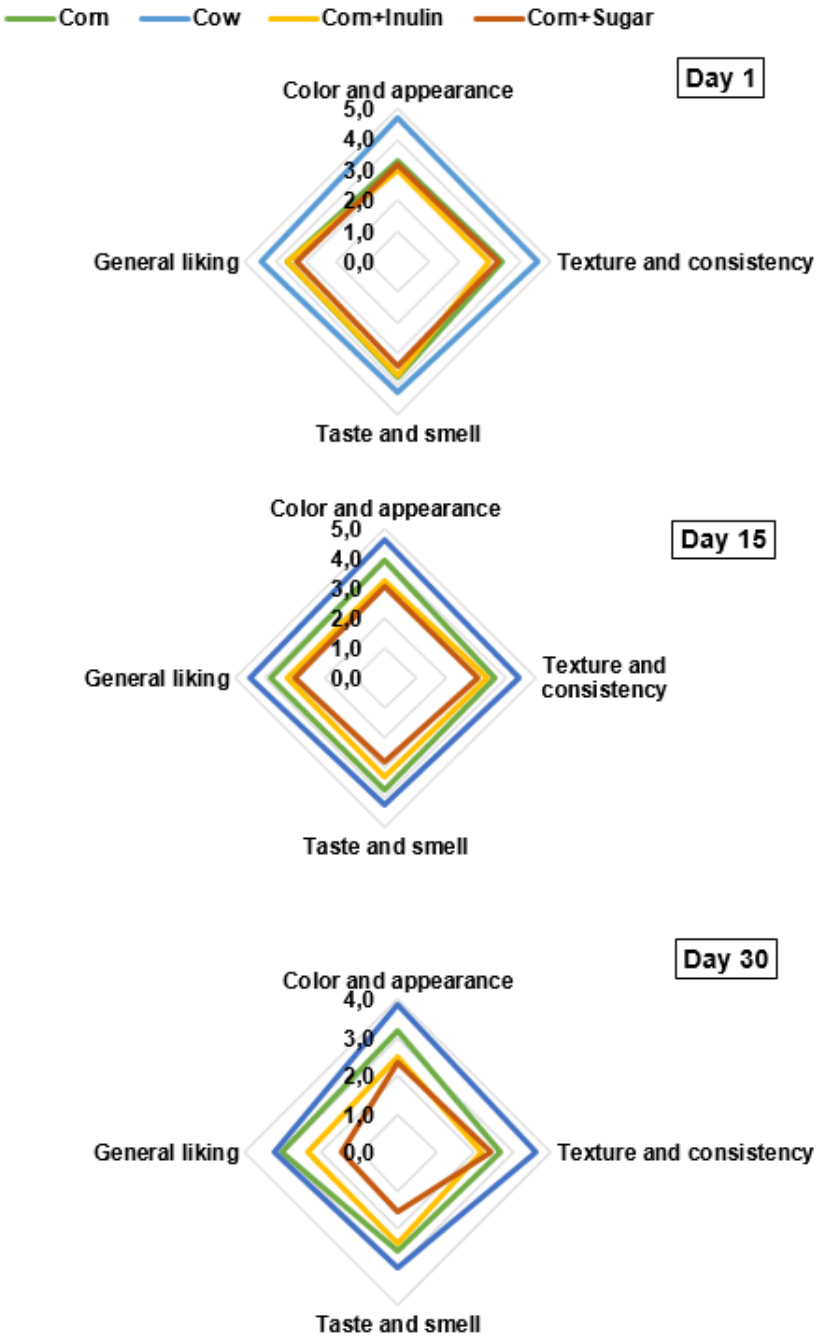


Figure 6. Color & appearance, texture & consistency, taste & smell, and general liking scores of fermented probiotic drink samples during storage (full score = 5; 1 point: very bad, 2 points: bad, 3 points: fair, 4 points: good, 5 points: very good)

Table 7. Effect of the sample type and storage time on the sensory qualities of fermented probiotic drink samples

	Color and appearance	Texture and consistency	Taste and smell	General liking
Type of sample	***	***	**	***
Corn	3.48±0.37 b	3.23±0.46 b	3.38±0.26 ab	3.50±0.20 b
Cow	4.40±0.42 a	4.21±0.48 a	3.84±0.27 a	4.09±0.27 a
Corn + Inulin	2.92±0.41 c	2.86±0.61 b	3.15±0.31 b	3.08±0.26 bc
Corn + Sugar	2.88±0.50 c	2.94±0.53 b	2.61±0.38 c	2.61±0.13 c
Storage time (days)	***	***	***	***
1	3.55±0.77 a	3.56±0.68 a	3.82±0.15 a	3.77±0.18 a
15	3.72±0.68 a	3.63±0.61 a	3.53±0.22 a	3.64±0.22 a
30	2.99±0.65 b	2.74±0.62 b	2.38±0.23 b	2.55±0.30 b

Values are expressed as mean ± standard deviation. Values with different letters (a-d) show the difference in the same column ($p<0.05$), *$p<0.05$, **$p<0.01$, ***$p<0.001$

CONCLUSION

In this study, it was observed that probiotic drink samples made from corn milk exhibited certain distinct characteristics compared to the probiotic drink made from cow's milk. Specifically, probiotic drink samples made from corn milk had higher pH values, a* values, syneresis, apparent viscosity, and consistency coefficient values. The color of the probiotic drinks made from corn milk was noticeably more yellow in comparison to the probiotic drink made from cow's milk. Regarding the microbiological properties, probiotic drink samples produced from cow's milk demonstrated high probiotic viability (>10^8 cfu/g) at the end of the storage period, while the viability of *L. acidophilus* (>10^7 cfu/g) was maintained in all samples (Figure 5). However, the counts of *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, and *L. acidophilus* decreased during storage, with the lowest reduction (%) observed in the probiotic drink made from cow's milk. Among the different probiotic drink samples, the probiotic drink made from cow's milk was found to be the most preferred in terms of sensory attributes. On the other hand, fermented probiotic drinks produced from corn milk with inulin can be recommended to vegans, with relatively high apparent viscosity values and probiotic bacteria count.

REFERENCES

[1] Shiekh, K.A., Luanglaor, T., Hanprerakriengkrai, N., Jafari, S., Kijpatanasilp, I., Asadatorn, N., Worobo, R.W., Bekhit, A.E., Assatarakul, K. (2023). Antioxidants and quality changes of thermally processed purple corn (*Zea mays* L.) milk fortified with low sucrose content during cold storage. *Foods,* 12, 277.

[2] Donald, I., Eucharia, C.N. (2018). Nutritional and sensory evaluation of African breadfruit-corn yoghurt. *African Journal of Food Science*, 12(4), 73-79.

[3] Menezes, A.G.T., Ramos, C.L., Dias, D.R., Schwan, R.F. (2018). Combination of probiotic yeast and lactic acid bacteria as starter culture to produce maize-based beverages. *Food Research International*, 111, 187-197.

[4] Çomak Göçer, E.M., Koptagel, E. (2023). Production of milks and kefir beverages from nuts and certain physicochemical analysis. *Food Chemistry*, 402, 134252.

[5] Muncey, L., Hekmat, S. (2021). Development of probiotic almond beverage using *Lacticaseibacillus rhamnosus* GR-1 fortified with short-chain and long-chain inulin fibre. *Fermentation*, 7, 90.

[6] FAO/WHO. (2001). Report on Joint FAO/WHO Expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Available at: www.ftp.fao.org/es/esn/food/probio_report_en.pdf (accessed 11 May 2023).

[7] Çomak Göçer, E.M., Ergin, F., Aşcı Arslan, A., Küçükçetin, A. (2016). Farklı inkübasyon sıcaklığı ile inkübasyon sonlandırma pH'sının probiyotik yoğurdun fizikokimyasal ve mikrobiyolojik özellikleri üzerine etkisi. *Akademik Gıda* 14(4), 341-350.

[8] Bernat, N., Cháfer, M., Chiralt, A., González-Martínez, C. (2015). Development of a non-dairy probiotic fermented product based on almond milk and inulin. *Food Science and Technology International*. 21(6), 440-53.

[9] Sangkam, J., Apichartsrangkoon, A., Baipong, S., Sriwattana, S., Tiampakdee, A., Sintuya, P. (2019). Pre-blanching corn and pressurization effects on the physicochemical and microbiological qualities of corn milk, *Food Bioscience*, 31, 100446.

[10] Ajala, L., Ologunde, M., Adetuyi, F.O. (2013). Physicochemical and sensory qualities of spiced soy-corn milk. *African Journal of Biotechnology*, 12(17), 2262-2265.

[11] Wang, C., Zheng, H., Liu, T., Wang, D., Guo, M. (2017). Physiochemical properties and probiotic survivability of symbiotic corn-based yogurt-like product. *Journal of Food Science*, 82(9), 2142-2150.

[12] AOAC. (2000). Official methods of analysis of AOAC International (17th ed.). Gaithersburg, MD, USA: Association of Official Analytical Communities.

[13] Yalçın, S., Ergin, F., Küçükçetin, A. (2021). Effects of homogenization and heat treatment of milk with different fat content on physical properties of ayran. *Annals of the Brazilian Academy of Sciences*, 93(3), e20200517.

[14] Çomak Göçer, E.M., Koptagel, E. (2023). Production and evaluation of microbiological &

rheological characteristics of kefir beverages made from nuts. *Food Bioscience* 52, 102367.

[15] Çomak Göçer, E.M., Ergin, F., Özen Küçükçetin, İ., Küçükçetin, A. (2021). In vitro gastrointestinal resistance of *Lactobacillus acidophilus* in some dairy products. *Brazilian Journal of Microbiology*, 52(4), 2319-2334

[16] Ergin, F., Atamer, Z., Çomak Göçer E.M., Demir, M., Hinrichs, J., Kucukcetin, A. (2021). Optimization of *Salmonella* bacteriophage microencapsulation in alginate-caseinate formulation using vibrational nozzle technique. *Food Hydrocolloids*, 113, 106456.

[17] Çomak Göçer, E.M., Koptagel, E. (2023). Farklı yağlı tohumlardan elde edilen bitkisel sütlerden üretilen kefirlerin bazı fiziksel ve duyusal özellikleri. *The Journal of Food*, 48(1), 227-241.

[18] Estedez, A.M., Majia, J., Figuerola, F. and Escobar, B. (2008). Effect of solid content and sugar combination on the quality of soymilk yoghurt, *Journal of Food Processing and Preservation,* 34: 87-97.

[19] Paredes, J.L., Escudero-Gilete, M.L., Vicario, I.M. (2022). A new functional kefir fermented beverage obtained from fruit and vegetable juice: Development and characterization. *LWT*, 154, 112728,

[20] Aini, N., Sumarmono, J., Sustriawan, B., Prihananto, V., Priscillia, E. (2020). The quality of corn milk-based cheese analogue made with virgin coconut oil as a fat substitute and with various emulsifiers. IOP Conference Series: Earth and Environmental Science, 443, 012039.

[21] Scott, C.E., Eldridge, A.L. (2005) Comparison of carotenoid content in fresh, frozen and canned corn. *Journal of Food Composition and Analysis*, 18, 6,551-559.

[22] Rasika, D.M.D., Vidanarachchi, J.K., Rocha, R.S., Balthazar, C.F., Cruz, A.G., Sant'Ana, A.S., Ranadheera, C.S. (2021). Plant-based milk substitutes as emerging probiotic carriers. *Current Opinion in Food Science*, 38: 8-20.

[23] Kizzie-Hayford, N., Jaros, D., Zahn, S., Rohm, H. (2016). Effects of protein enrichment on the microbiological, physicochemical and sensory properties of fermented tiger nut milk. *LWT*, 74, 319-324.

[24] Yeniçeri, Ş.A, Çomak Göçer, E.M., Küçükçetin, A. (2021). Probiyotik bakteri içeren ayranın fizikokimyasal ve mikrobiyolojik özellikleri. *Akademik Gıda*, 19(4), 414-423.

[25] Ozturkoglu-Budak, S., Akal, C., Yetisemiyen, A. (2016). Effect of dried nut fortification on functional, physicochemical, textural, and microbiological properties of yogurt. *Journal of Dairy Science*, 99(11), 8511-8523.

[26] Walstra, P., Walstra, P., Wouters, J. T., Geurts, T. J. (2005). Dairy science and technology. (2nd ed.), CRC press, Boca Raton.

[27] Küçükçetin, A., Comak, E.M, Aşcı, A., Demir, M., Şık, B. (2012). Effect of casein to whey protein ratio of skim milk on the physical properties of a yoghurt drink, Ayran. *Milclwissenschaft,* 67(3), 233–248.

[28] Ergin, F. (2022). Süt tozu, peyniraltı suyu izolatı veya inülin ile kurumaddesi arttırılan ve kalsiyum klorür eklenen sütlerden üretilen probiyotik yoğurtların bazı fizikokimyasal, mikrobiyolojik ve duyusal özellikleri. *Akademik Gıda*, *20*(2), 145-160.

[29] Chetachukwu, A. S., Thongraung, C., Yupanqui, C. T. (2018). Effect of short-chain inulin on the rheological and sensory characteristics of reduced fat set coconut milk yoghurt. *Journal of Texture Studies*, *49*(4), 434-447.

[30] Güler-Akın, M., Ferliarslan, I., Serdar Akın, M. (2016) Apricot probiotic drinking yoghurt supplied with inulin and oat fiber. *Advances in Microbiology*, 6, 999-1009.

[31] Casarotti, S.N., Penna, A.L.B. (2015). Acidification profile, probiotic in vitro gastrointestinal tolerance and viability in fermented milk with fruit flours. *International Dairy Journal*, 41, 1-6.

[32] Ascı Arslan, A., Çomak Göçer, E.M., Demir, M., Atamer, Z., Hinrichs, J., Küçükçetin, A. (2016). Viability of *Lactobacillus acidophilus* ATCC 4356 incorporated into ice cream using three different methods. *Dairy Science and Technology*, 96,477-478.

[33] Atalar, İ. (2019). Functional kefir production from high pressure homogenized hazelnut milk. *LWT - Food Science and Technology*, 107, 256-263.

SİDAS MEDYA
Akademik Gıda®
ISSN Online: 2148-015X
https://dergipark.org.tr/tr/pub/akademik-gida

Akademik Gıda 21(2) (2023) 132-140, DOI: 10.24323/akademik-gida.1350944

Research Paper / Araştırma Makalesi

Aflatoxin M1 Levels in Milk Samples Produced in the Northern Part of Cyprus

Cengiz Bereket[1,2], Gozde Girgin[2], Gonul Sahin[1]

[*1]Eastern Mediterranean University, Faculty of Pharmacy, İbn-i Sina Street, 99628, Famagusta, North Cyprus, via Mersin 10 Türkiye
[2]Hacettepe University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, 06100, Sıhhiye, Ankara, Türkiye

Received (Geliş Tarihi): 27.08.2022, Accepted (Kabul Tarihi): 07.06.2023
Corresponding author (Yazışmalardan Sorumlu Yazar): cengiz.bereket@emu.edu.tr (C. Bereket)
+90 392 630 24 01 +90 392 630 2819

ABSTRACT

Aflatoxin M_1 (AFM_1) is the hydroxylated metabolite of aflatoxin B_1 (AFB_1), which is formed in the liver by cytochrome P450 enzymes and can be secreted into the urine, feces, and milk of mammals. AFM_1 is a carcinogenic, cytotoxic, teratogenic, mutagenic and genotoxic agent that poses a significant health risk to both humans and animals. This study was conducted to determine the presence of AFM_1 in both raw and ultra-high temperature (UHT) cow's milk samples produced in the northern part of Cyprus, and to determine whether it poses a risk to public health. In this survey, a total of 20 UHT cow's milk samples from 2 different milk brands produced in the northern part of Cyprus, and 22 raw cow's milk samples collected from the different dairies were analyzed for the presence of AFM_1 by high performance liquid chromatography (HPLC) with fluorescence detector after immunoaffinity cleanup. AFM_1 could not be detected in any of the analyzed raw and UHT cow milk samples. The LOD and LOQ values of the HPLC-FLD method were 1.038 µg/kg and 3.145 µg/kg, respectively. The mean recovery and repeatability values of the method were 95.6% and 4.9%, respectively. Although the presence of AFM_1 in milk samples produced in the northern part of Cyprus poses no major risk to public health, more milk samples and animal feed must be monitored on a regular basis to decrease potential consumer exposure.

Keywords: Mycotoxin, Aflatoxin M_1, Milk, High performance liquid chromatography (HPLC)

Kıbrıs'ın Kuzeyinde Üretilen Süt Örneklerinde Aflatoksin M_1 Düzeyleri

ÖZ

Aflatoksin M_1 (AFM_1), sitokrom P450 enzimleri tarafından karaciğerde oluşan ve memelilerin idrar, dışkı ve sütüne salgılanabilen, aflatoksin B_1 (AFB_1)'in hidroksillenmiş metabolitidir. AFM_1 karsinojenik, sitotoksik, teratojenik, mutajenik ve genotoksik bir ajan olduğundan hem insanlar hem de hayvanlar üzerinde önemli sağlık riskleri teşkil etmektedir. Bu çalışma, Kıbrıs'ın kuzeyinde üretilen çiğ ve ultra yüksek ısı (UHT) inek sütü örneklerinde AFM_1 varlığını tespit etmek ve halk sağlığı açısından risk oluşturup oluşturmadığını belirlemek amacıyla yapılmıştır. Çalışmada, Kıbrıs'ın kuzeyinde üretilen 2 süt markasından toplam 20 UHT inek sütü ve farklı mandıralardan toplanmış 22 adet çiğ inek sütü örneği, immünoaffinite kolon temizlemesinden sonra floresans dedektörlü yüksek performanslı sıvı kromatografisi (HPLC) ile AFM_1 varlığı yönünden analiz edilmiştir. Analiz edilen çiğ ve UHT inek sütü örneklerinin hiçbirinde AFM_1 tespit edilebilecek düzeyde bulunamamıştır. HPLC-FLD yönteminin LOD ve LOQ değerleri sırasıyla 1.038 µg/kg ve 3.145 µg/kg idi. Yöntemin ortalama geri kazanım ve tekrarlanabilirlik değerleri sırasıyla, %95.6 ve %4.9 olarak bulunmuştur. Kıbrıs'ın kuzeyinde üretilen süt örneklerindeki AFM_1 içeriği, halk sağlığı açısından önemli bir risk teşkil etmese de daha fazla sayıda süt örneğinin ve hayvan yeminin sürekli takibi, olası tüketici maruziyetini azaltmak için gereklidir.

Anahtar Kelimeler: Mikotoksin, Aflatoksin M_1, Süt, Yüksek performanslı sıvı kromatografisi (HPLC)

INTRODUCTION

Aflatoxins are secondary metabolites produced by diverse species of the fungal genus *Aspergillus* and are the most investigated group of mycotoxins [1, 2]. *A. parasiticus* and *A. flavus* are the most prevalent strains that produce aflatoxins under particular conditions (relative humidity above 70%, 24-35°C, 2.5-6.5 pH). *A. nomius*, *A. pseudotamarii*, *A. bombycis*, *A. ochraceoroseus*, and *A. australis* strains, on the other hand, are only seldom capable of producing them. Aflatoxins occur naturally in a wide variety of food commodities such as cereals (corn, sorghum, wheat, rice), oilseeds (soybean, peanut, sunflower, cottonseeds), spices (cayenne pepper, black pepper, coriander, turmeric, ginger), nuts, dried fruits, milk, and meat [3–6]. Aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2), aflatoxin M_1 (AFM_1) and aflatoxin M_2 (AFM_2) are the most prominent ones among the more than 20 types of aflatoxin molecules identified [7]. While acute exposure to high doses of aflatoxins in mammals usually results in hepatotoxicity, nephrotoxicity, and in some cases death; chronic exposure causes a variety of toxic effects, including immunosuppression, teratogenicity, carcinogenicity, mutagenicity, cytotoxicity, reproductive and estrogenic disorders [8, 9].

AFB_1 is the most well-known human hepatocarcinogen and the most potent hepatotoxin among the aflatoxins [10]. According to research, AFB_1 is involved in 4.6-28.2% of hepatocellular carcinoma cases, which is the third leading cause of cancer death worldwide [6]. The International Agency for Research on Cancer (IARC) concluded that there is sufficient evidence for the carcinogenicity of AFB_1 in humans and has classified this mycotoxin as a “Group 1” carcinogen [9, 11].

AFM_1, the hydroxylated metabolite of AFB_1, is formed in the liver by microsomal enzymes and is excreted into urine, feces, and milk in mammals [12, 13]. AFM_1 has approximately 10% carcinogenicity and 10% mutagenicity of AFB_1, according to *in vivo* and *in vitro* studies, respectively [14]. Hence, AFM_1 has been identified as a potential risk to human health and has been classified as a “Group 1” carcinogen by the International Agency for Research on Cancer [3]. Humans are exposed to AFM_1 mostly through the consumption of contaminated milk and dairy products or through AFB_1 metabolism in the liver. AFM_1 may be present in milk and dairy products, since it is the major excretion product in the milk of lactating animals fed with AFB_1-contaminated diets. According to the data from several research, the carry-over rate of AFB_1 as AFM_1 into the milk of dairy cows varies between 0.3% and 6.2%. The extent of carry-over of AFB_1 from feed to milk in dairy cows is influenced by various factors including season, animal feeding regimen, milking mode, rate of ingestion, rate of digestion, hepatic biotransformation capacity, lactation stage, and actual milk production [14–16].

Since the presence of AFM_1 and its by-products in milk and dairy products causes global concern, many countries have established the maximum acceptable limits for AFM_1 in milk and dairy products, and legislative laws have been drafted accordingly. For example, the European Union (EU) has set the maximum acceptable AFM_1 limit for adults in raw milk, milk for manufacturing of milk-based products and heat-treated milk at 0.05 µg/kg (*ppb*). However, the more restrictive maximum acceptable limit for AFM_1 was set at 0.025 µg/kg for infant formulae and follow-on formulae, including infant milk and follow-on milk [17]. In accordance with European Union regulations, Turkey and the northern part of Cyprus have set the maximum AFM_1 level in milk and dairy products for adults and infants at 0.05 µg/kg and 0.025 µg/kg, respectively [18].

Due to their high stability, no significant reduction in the amount of AFM_1 in milk and milk-based products has been reported with pasteurization, ultra-high temperature, sterilization, cooking, ionizing radiation, addition of enzymes, and other conventional food processing methods [19, 20]. Therefore, the most effective strategy to prevent the occurrence of AFM_1 in the food chain is to inhibit mold growth in agricultural products and the subsequent production of AFB_1 in livestock feed [21].

The presence of AFM_1 in milk and dairy products is one of the most public health issues. The presence of this toxin in milk, even at low levels, poses a risk for consumers of large quantities of milk such as children and adults, especially in long-term exposure [22]. Although milk and dairy products are the primary sources of nutrition for infants, children, and adults, there is no literature data on the determination of AFM_1 levels in milk samples consumed in the northern part of Cyprus. To date, only a study on the determination of AFM_1 levels in Cypriot traditional cheese (Hellim), has been reported by Öztürk et al. [23].

This study aimed to measure the AFM_1 levels both in ultra-high temperature (UHT) cow's milk samples produced in the northern part of Cyprus and raw cow’s milk samples collected from several local dairy farms, and evaluate them in terms of current limits.

MATERIALS and METHODS

In this study, a total of 20 UHT cow's milk samples, 10 from each of the two dairy companies produced in the northern part of Cyprus, and 22 raw cow's milk samples obtained from different dairy farms were analyzed for the presence and concentration of AFM_1. On September 2020, 20 UHT cow's milk samples, all of which were manufactured on different dates, were collected from various markets in their original packaging (200 mL or 1 L) and transported to the Eastern Mediterranean University, Faculty of Pharmacy Laboratory within a cold chain. These samples were stored in the refrigerator at +4°C and analyzed as soon as possible. In September, 22 raw cow’s milk samples from 22 distinct cows were collected from dairy farms in three different villages in the Famagusta region by using 50 mL falcon tubes. Raw cow milk samples were transported to the Eastern Mediterranean University Faculty of Pharmacy

Laboratory within a cold chain and kept in the refrigerator at -20°C until analysis.

AFM_1 levels in milk samples were detected by high performance liquid chromatography (HPLC) coupled with a fluorescence detector after immunoaffinity column (containing monoclonal AFM_1 antibodies immobilized to a solid support) purification. For sample preparation and immunoaffinity column (IAC) clean-up, the manufacturer's (R-Biopharm Rhone LTD.) protocols were followed [24].

Preparation of UHT Cow's Milk Samples

Prior to the analysis,100 mL of the milk samples were warmed to 37°C in an ultrasonic bath, then centrifuged at 4,000 rpm for 20 minutes. After centrifugation, the remaining fat on the surface was separated and discarded. In order to completely remove the fat layer, milk samples were filtered through whatman No:4 filter paper. 50 mL of the filtrate were passed through the immunoaffinity column (RP70N Easi-Extract Aflatoxin) at a flow rate of 2 mL per minute. Following the passage of the filtrate through the column, the column was washed with 20 mL of phosphate buffered saline (PBS) at a flow rate of approximately 5 mL per minute. Afterwards, the air was passed through the column to remove residual liquid. 1.25 mL of methanol: acetonitrile (40:60, v/v) solution was passed through the column at a flow rate of 1 drop per second to elute the AFM_1 from the column and collected in an amber glass vial. Backflushing was applied and repeated 2-3 times during this process. Following the elution, 1.25 mL of water was passed through the column and collected in the same vial to give a 2.5 mL total volume. The eluate collected in the amber vial was mixed by vortex and injected into the HPLC system in a volume of 100 µL.

Preparation of Raw Cow's Milk Samples

Minor changes were made in the preparation of raw cow's milk samples compared to UHT cow's milk samples. According to these changes, 100 mL of the milk samples were centrifuged at 4,000 rpm for 20 minutes. After centrifugation, the remaining fat on the surface was separated and discarded. To increase the filtration rate and prevent clogging of the immunoaffinity column, milk samples were warmed to 37°C in an ultrasonic bath. After warming, milk samples were filtered through whatman No:4 filter paper to remove the fat layer completely. 50 mL of the filtrate were passed through the immunoaffinity column (RP70N Easi-Extract Aflatoxin) at a flow rate of 2 mL per minute. Following the passage of the filtrate through the column, the column was washed with 20 mL of phosphate buffered saline (PBS) at a flow rate of approximately 5 mL per minute. Afterwards, the air was passed through the column to remove residual liquid. 1.25 mL of methanol: acetonitrile (40:60, v/v) solution was passed through the column at a flow rate of 1 drop per second to elute the AFM_1 from the column and collected in an amber glass vial. Backflushing was applied and repeated 2-3 times during this process. Following the elution, 1.25 mL of water was passed through the column and collected in the same vial to give a 2.5 mL total volume. After mixing the eluate by vortex, it was filtered through an RC 0.45 µm membrane filter into a new amber vial. Finally, 100 µL of the eluate collected in the amber vial was injected into the HPLC system.

High Performance Liquid Chromatography (HPLC) Analysis

Agilent Technologies 1260 Infinity HPLC system (SEM Lab Inc., Turkey) equipped with an autosampler and fluorescence detector (Agilent FLD cell 8 µL, Germany) was used for AFM_1 analysis. The excitation and emission wavelengths of the fluorescence detector were set to 365 and 435 nm, respectively. An Inertsil ODS-3V 4.6x150 mm column was used and the column temperature was set to 25°C. The isocratic mobile phase (acetonitrile/water, 25:75, v/v) with a flow rate of 1 mL/min was used. The retention time of AFM_1 was determined by injecting 100 µL of AFM_1 reference standard into the HPLC, which was prepared for analysis by passing the mobile phase for a certain period of time. Quantification was performed based on the calibration curve constructed using AFM_1 reference standard solutions for a total of six concentration points (2, 5, 10, 20, 50 and 100 µg/L).

Method Validation

The linearity, limit of detection (LOD), limit of quantification (LOQ), repeatability, and recovery of the method were validated with an in-house model according to the decision of the European Union Commission 657/2002 [25].
Linearity was evaluated by injection of AFM_1 reference standard solutions at 6 concentration points (three replicates for each). LOD and LOQ values were calculated using Equation 1 and Equation 2, respectively:

$$LOD = 3.3 * S_a / b \quad (1)$$
$$LOQ = 10 * S_a / b \quad (2)$$

Where s_a is the standard deviation of the intercept; and b is the slope of the regression line obtained from the calibration curve [26].

Blank samples (milk samples which did not exhibit AFM_1 presence) spiked at three fortification levels (12.5, 25 and 37.5 µg/kg corresponding to 2.5, 5 and 7.5 µg/kg in eluate) were used to calculate repeatability and recovery. Analyzes were repeated 6 times for each concentration level.

Statistical Analysis

Data were analyzed by one-way ANOVA. The confidence level was established at 95% in all tests and probabilities less than 5% ($P<0.05$) were evaluated as significant. All statistical analyzes were made with Microsoft Excel statistical program.

RESULTS and DISCUSSION

Validation of HPLC Analysis

The HPLC method fulfilled the conditions outlined in Commission Regulation (EC) No. 401/2006 [27]. The calibration curve was constructed using the peak areas obtained from AFM_1 standard solutions at different concentrations. The calibration curve was found to be linear between 2 and 100 µg/L, with linear equation y=0.1463x+0.0652. The coefficient of determination (R^2) of the calibration curve was calculated as 0.9998 (Figure 1). The mean recovery and repeatability of the method were 95.6% and 4.9%, respectively as reported in Table 1. The chromatograms of the UHT cow's milk sample with and without the AFM_1 standard solution added, and the chromatogram of the AFM_1 standard solution at 5 µg/L concentration used for evaluating recovery are presented in Figures 2, 3 and 4, respectively.LOD and LOQ values were found to be 1.038 and 3.145 µg/kg, respectively.

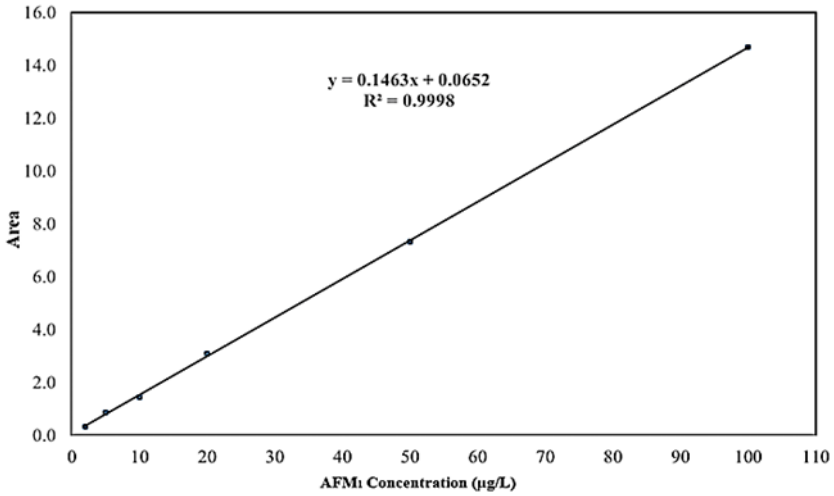


Figure 1. Calibration curve of AFM_1 standard solutions.

Table 1. Repeatability and recovery data.

Parameters	AFM_1 Spike Doses		
	12.5 µg/kg	25 µg/kg	32.5 µg/kg
Repeatability (coefficient of variation, % CV)	4.6	3.6	6.4
Recovery (% ± standard deviation, SD)	96.6±4.5	91.8±3.3	98.6±6.3

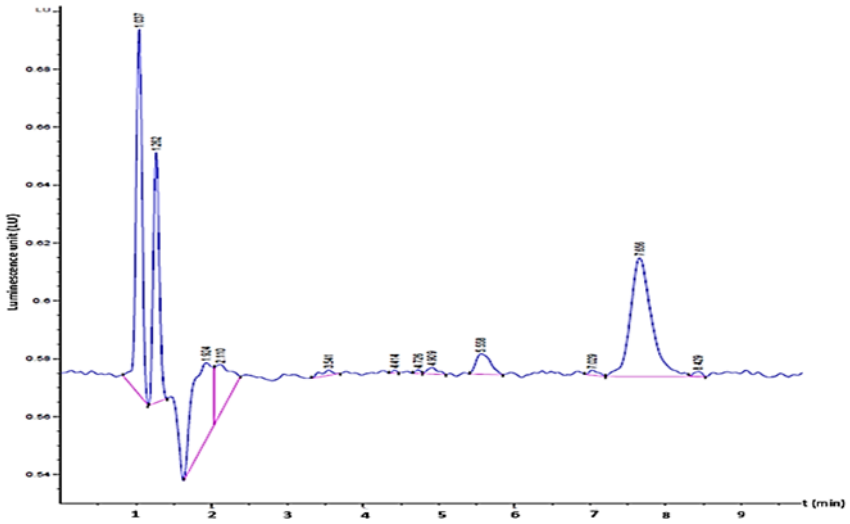


Figure 2. Chromatogram of UHT cow's milk sample containing 5 µg/kg AFM_1 standard solution.

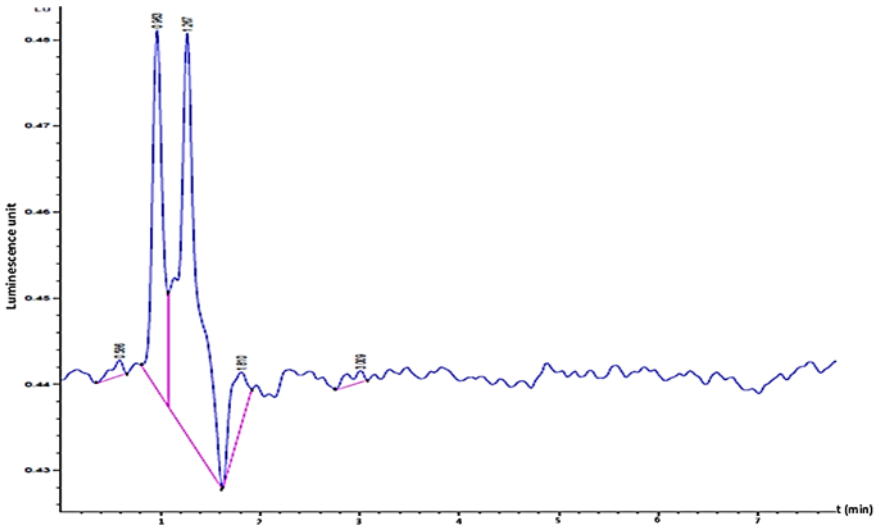


Figure 3. Chromatogram of UHT cow's milk sample without the addition of AFM_1 standard solution.

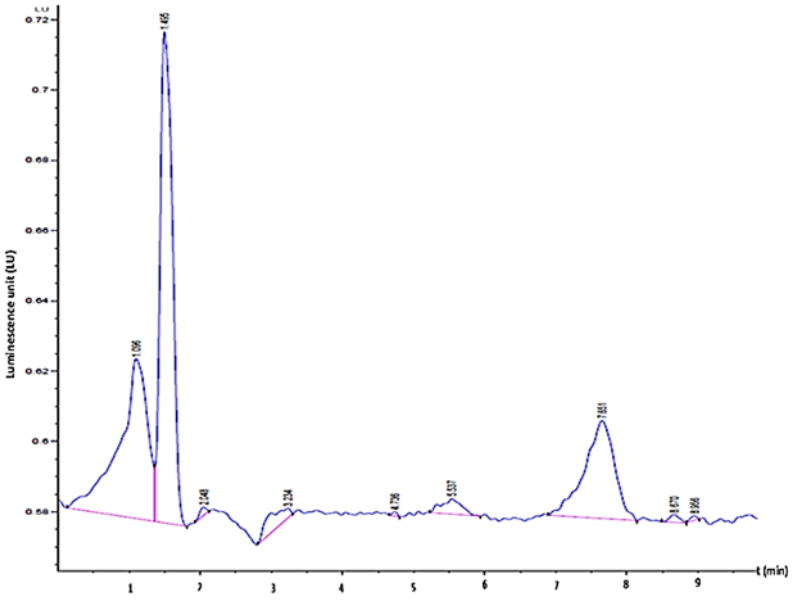


Figure 4. Chromatogram of AFM_1 standard solution at 5 µg/L concentration.

Sample Analysis

20 µg/L of AFM_1 standard solution was injected into HPLC and the retention time of AFM_1 was found to be 7.637 minutes. It was aimed to measure the AFM_1 levels in milk samples by comparing the chromatograms obtained from milk samples with the chromatograms obtained from AFM_1 standard solutions. AFM_1 was not detected in any of the 22 raw cow's milk and 20 UHT cow's milk samples analyzed. In the analyzed UHT cow's milk and raw cow's milk samples, some examples of the chromatograms of AFM_1 levels that could not be detected are shown in Figures 5 and 6, respectively.

In this study, the presence of AFM_1 was determined in ultra-high temperature (UHT) cow's milk samples manufactured in the northern part of Cyprus and raw cow's milk samples collected from several local dairy farms. AFM_1 was not detectable in any of the 22 raw cow's milk and 20 UHT cow's milk samples analyzed. According to the Meteorology Department in the northern part of Cyprus, the average temperature and the standardized precipitation index (SPI) in September 2020 (when the milk samples were collected) was 28°C, and between 0.50 and - 0.50 ('near normal', which is between slightly humid and slightly arid), respectively [42, 43]. It is well known that the optimum water activity, temperature and relative humidity required for the aflatoxin formation are 0.99, 33°C and >70%, respectively [6]. Therefore, the lack of ideal conditions for aflatoxin formation could explain the undetectable AFM_1 levels in milk samples.

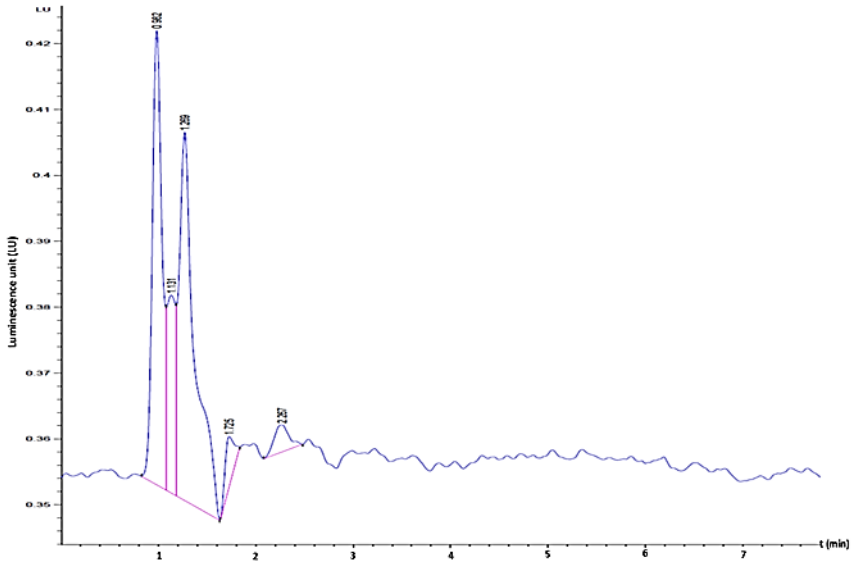


Figure 5. Chromatogram of UHT cow's milk sample which AFM_1 level could not be detected.

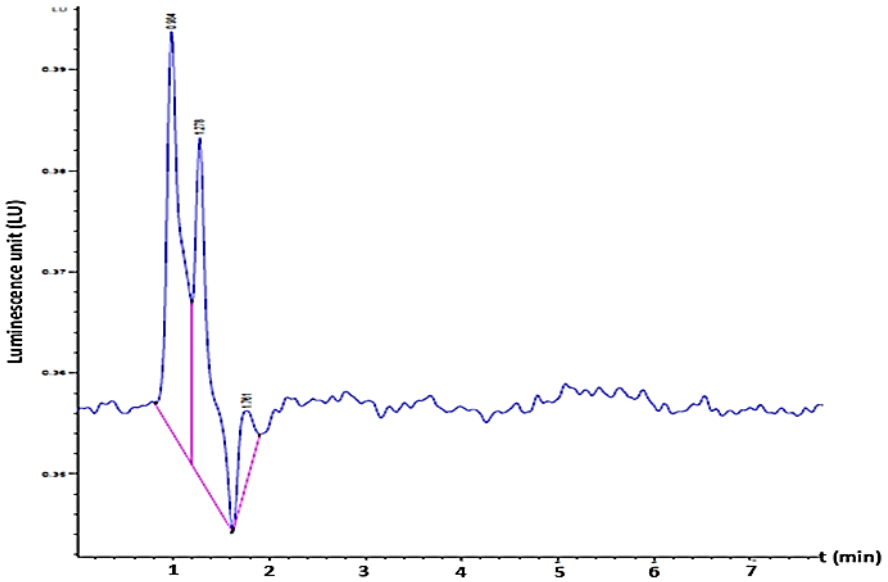


Figure 6. Chromatogram of raw cow's milk sample which AFM_1 level could not be detected.

In the study conducted by Kutlubay and Sökmen at Giresun University, AFM_1 was not detected in any of the 30 cow milk samples analyzed [28]. Bellio et al. [29] reported that only eight (0.5%) of a total of 1668 cow milk samples collected and evaluated in Italy, did not meet the EU regulation limit for the presence of AFM_1. Piva et al. [30] examined 276 milk samples for the presence of AFM_1 and reported that only 7 had AFM_1 levels of more than 50 ng/L. In any of the 100 pasteurized milk samples produced in Iran, AFM_1 levels did not exceed 50 ng/L, which is the maximum limit for milk, according to Behfar et al. [31]. In a study conducted by Cammilleri and his colleagues, AFM_1 levels in 170 cow's milk samples collected in Southern Italy were found to be below the European Commission regulation 1881/2006 limit [26], [27]. In a study of AFM_1 formation in 100 raw milk samples in South Korea, none of the samples exceeded the Korean legal limit of 0.5 µg/kg for AFM_1 [32]. The fact that the AFM_1 levels in the milk samples analyzed in the above-mentioned studies did not exceed the legal limits, or only exceeded the legal limits in a few samples, is consistent with our findings. The level of AFM_1 in milk increases in proportion to the amount of AFB_1 in the feeds [33]. In comparison to the winter months, when animals are fed more concentrated feed, AFM_1 contamination is lower in the spring and summer, when fresh grass and roughage are more abundant and pasture feeding is the common practice [34]. Therefore, various factors such as geographical, territorial and seasonal differences, feeding regimes of animals, lactation period from which milk samples were taken, and different analysis methods used are among the reasons for the different findings regarding the prevelance of AFM_1 in milk [35].

The HPLC-FLD method's LOD and LOQ values were found to be 1.038 and 3.145 µg/kg, respectively, which were much higher than the values determined by other

researchers [29, 36–40]. On the other hand, the linearity, repeatability and recovery values of the HPLC method were compatible with the criteria specified in the commission regulation [41], proving the reliability and validity of the method.

CONCLUSION

The AFM_1 levels in any of the 22 raw cow's milk and 20 UHT cow's milk samples analyzed in this study were not detectable, and the AFM_1 level in the milk samples did not exceed the Turkish Food Codex (TGK) and European Union (EU) limit values [18, 44].

It is a pleasing situation for both dairy consumers and producers that the AFM_1 levels detected in the analyzed milk samples did not exceed the regulatory limits. The findings of the study can be explained by drying the animal feeds properly after harvest, storing them in appropriate conditions, and avoiding exposure to high temperatures and humidity. Although the AFM_1 content in milk samples produced in the northern part of Cyprus poses no major risk to public health, the need for larger research with more milk samples is becoming apparent. Moreover, an ongoing surveillance program is required to monitor the risk of aflatoxin contamination throughout the animal feed supply chain. Apart from all these considerations, the likelihood of total aflatoxin or mycotoxin exposure based on consumer dietary habits, also poses a concern. When all factors are considered, it has been concluded that good agricultural practices, good preservation practices, good hygiene practices, and application of hazard analysis and critical control points (HACCP) based food safety systems will be beneficial in preventing aflatoxin exposure in the food chain.

ACKNOWLEDGEMENTS

We thank Prof. Dr. Muberra Kosar for her support and assistance in the usage of the HPLC-system.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

FUNDING

The authors received no financial support for the research, authorship, and/or publication of this article.

DATA AVAILABILITY STATEMENT

Raw data were generated at Eastern Mediterranean University. Derived data supporting the findings of this study are available from the corresponding author, Bereket, C. on request.

REFERENCES

[1] Hussein, H., Brasel, J. (2001). Toxicity, metabolism, and impact of mycotoxins on humans and animals. *Toxicology*, 167(2), 101-134.

[2] Mehenktaş, C. (2019). Süt ve süt ürünlerinde aflatoksin M1. *Akademik Gıda*, 17, 439-443.

[3] International Agency for Research on Cancer. (2002). International agency for research on cancer ıarc monographs on the evaluation of carcinogenic risks to humans. *Iarc Monographs on the Evaluation of Carcinogenic Risks to Humansarc Monographs on the Evaluation of Carcinogenic Risks to Humans*, 82, 1-556.

[4] Sweeney, M., Dobson, A. (1998). Mycotoxin production by aspergillus, fusarium and penicillium species. *International Journal of Food Microbiology*, 43(3), 141-158.

[5] Yentür, G., Er, B. (2012). The evaluation of the aflatoxin presence in foods. *Turk Hijyen ve Deneysel Biyoloji Dergisi*, 69(1), 41-52.

[6] Neme, K., Mohammed, A. (2017). Mycotoxin occurrence in grains and the role of postharvest management as a mitigation strategies - A review. *Food Control*, 78, 412-425.

[7] Ismail, A., Gonçalves, B.L., de Neeff, D.V., Ponzilacqua, B., Coppa, C.F.S.C., Hintzsche, H., Sajid, M., Cruz, A.G., Corassin, C.H., Oliveira, C.A.F. (2018). Aflatoxin in foodstuffs: Occurrence and recent advances in decontamination. *Food Research International*, 113, 74-85.

[8] Luo, Y., Liu, X., Li, J. (2018). Updating techniques on controlling mycotoxins - A review. *Food Control*, 89, 123-132.

[9] Benkerroum, N. (2020). Chronic and acute toxicities of aflatoxins: Mechanisms of action. *International Journal of Environmental Research and Public Health*, 17(2), 1-28.

[10] Soni, K.B., Lahiri, M., Chackradeo, P., Bhide, S.V., Kuttan, R. (1997). Protective effect of food additives on aflatoxin-induced mutagenicity and hepatocarcinogenicity. *Cancer Letters*, 115(2), 129-133.

[11] Rawal, S., Kim, J.E., Coulombe, R. (2010). Aflatoxin B1 in poultry: Toxicology, metabolism and prevention. *Research in Veterinary Science*, 89(3), 325-331.

[12] Bognanno, M., Fauci, L.L., Ritieni, A., Tafuri, A., De Lorenzo, A., Micari, P., Renzo, L. Di, Ciappellano, S., Sarullo, V., Galvano, F. (2006). Survey of the occurrence of aflatoxin m1 in ovine milk by HPLC and its confirmation by MS. *Molecular Nutrition and Food Research*, 50(3), 300-305.

[13] Santini, A., Ritieni, A. (2013). Aflatoxins: Risk, exposure and remediation. *Aflatoxins - Recent Advances and Future Prospects*. INTECH Open Access Publisher,343p.

[14] Iqbal, S., Jinap, S., Pirouz, A., Faizal, A. (2015). Aflatoxin m1 in milk and dairy products, occurrence and recent challenges: A review. *Trends in Food Science & Technology*, 46(1), 110-119.

[15] Duarte, S.C., Almeida, A.M., Teixeira, A.S., Pereira, A.L., Falcão, A.C., Pena, A., Lino, C.M. (2013). Aflatoxin m1 in marketed milk in Portugal: Assessment of human and animal exposure. *Food Control*, 30(2), 411-417.

[16] Giovati, L., Magliani, W., Ciociola, T., Santinoli, C.,

Conti, S., Polonelli, L. (2015). AFM1 in milk: Physical, biological, and prophylactic methods to mitigate contamination. *Toxins*, 7(10), 4330-4349.

[17] The Commission of the European Communities. (2010). Commission Regulation (EU) No 165/2010 of 26 February 2010 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards aflatoxins. *Official Journal of the European Union*, 50(2009), 8-12.

[18] Türk Gıda Kodeksi. (2011). Türk gıda kodeksi bulaşanlar yönetmeliği mikotoksinler (Ek1). *Resmi Gazete Tarih: 29*, *12.*

[19] Santini, A., Raiola, A., Ferrantelli, V., Giangrosso, G., Macaluso, A., Bognanno, M., Galvano, F., Ritieni, A. (2013). Aflatoxin m1 in raw, UHT milk and dairy products in Sicily (Italy). *Food Additives and Contaminants:* Part B, 6(3), 181-186.

[20] Matabaro, E., Ishimwe, N., Uwimbabazi, E., Lee, B. (2017). Current immunoassay methods for the rapid detection of aflatoxin in milk and dairy products. *Comprehensive Reviews in Food Science and Food Safety*, 16(5), 808-820.

[21] Choudhary, A.K., Kumari, P. (2010). Management of mycotoxin contamination in preharvest and post harvest crops: Present status and future prospects. *Journal of Phytology*, 2(7), 37-52.

[22] Rodríguez Velasco, M., Calonge Delso, M., Ordónez Escudero, D. (2003). ELISA and HPLC determination of the occurrence of aflatoxin m1 in raw cow's milk. *Food Additives and Contaminants*, 20(3), 276-280.

[23] Öztürk, B., Çelik, F., Çelik, Y., Kabaran, S., Ziver, T. (2014). To determine the occurence of aflatoxin M1 (AFM1) in samples of cyprus traditional cheese (halloumi): A cross-sectional study. *Kafkas Universitesi Veteriner Fakultesi Dergisi*, 20(5), 773-778.

[24] R-Biopharm Rhone. (2017). *Rp70n easi extract aflatoxin milk and milk powder V11-2017-09*.

[25] European Commission (EC). (2002). Commission decision of 12 August 2002 implementing Council Directive 96/23/EC concerning the performance of analytical methods and the interpretation of results. *Official Journal of the European Communities*, 221, 8-36.

[26] Cammilleri, G., Graci, S., Collura, R., Buscemi, M., Vella, A., Macaluso, A., Giaccone, V., Giangrosso, G., Cicero, Antonello., Maria Lo Dico, G., Pulvirenti, A., Cicero, N., Ferrantelli, V. (2019). Aflatoxin m1 in cow, sheep, and donkey milk produced in Sicily, Southern Italy. *Mycotoxin Research*, 35(1), 47-53.

[27] European Commission (EC). (2006). Commission Regulation (EC) No 118/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Union,* (364), 5-24.

[28] Kutlubay, Z., Sökmen, B. (2016). Investigation of aflatoxin M1 in milks in the Giresun region. *Giresun Üniversitesi Fen Bilimleri Enstitüsü,* 1-67.

[29] Bellio, A., Bianchi, D.M., Gramaglia, M., Loria, A., Nucera, D., Gallina, S., Gili, M., Decastelli, L. (2016). Aflatoxin M1 in cow's milk: Method validation for milk sampled in northern Italy. *Toxins (Basel)*, 8(3), 57.

[30] Piva, G., Pietri, A., Galazzi, L., Curto, O. (1988). Aflatoxin M1 occurrence in dairy products marketed in Italy. *Food Additives & Contaminants*, 5(2), 133-139.

[31] Behfar, A., Khorasgani, Z.N., Alemzadeh, Z., Goudarzi, M., Ebrahimi, R., Tarhani, N. (2012). Determination of aflatoxin M1 levels in produced pasteurized milk in Ahvaz city by using HPLC. *Jundishapur Journal of Natural Pharmaceutical Products,* 7(2), 80-84.

[32] Kim, H.J., Lee, J.E., Kwak, B.M., Ahn, J.H., Jeong, S.H. (2010). Occurrence of aflatoxin M1 in raw milk from South Korea winter seasons using an immunoaffinity column and high performance liquid chromatography. *Journal of Food Safety,* 30(4), 804-813.

[33] Çiğdem, A., Arif, A. (2004). Ankara'da işlenen sütlerde aflatoksin M1 varlığının ve düzeylerinin HPLC ile araştırılması. *Ankara Üniversitesi Veteriner Fakültesi Dergisi*, 51(3), 175-179.

[34] İşleyici, Ö., Morul, F., Sancak, Y.C. (2012). Van'da tüketime sunulan UHT sterilize inek sütlerinde aflatoksin M1 düzeyinin araştırılması. *Yüzüncü Yıl Üniversitesi Veteriner Fakültesi Dergisi*, 23(2), 65-69.

[35] Tunail, N. (2000). Mikrobiyal enfeksiyonlar ve intoksikasyonlar -Gıda mikrobiyolojisi ve uygulamaları. *Ankara Üniversitesi Ziraat Fakültesi Gıda Mühendisliği Bölümü Yayını*, 82-88.

[36] Muscarella, M., Lo, S., Palermo, C., Centonze, D. (2007). Validation according to European Commission Decision 2002 / 657 / EC of a confirmatory method for aflatoxin m1 in milk based on immunoaffinity columns and high performance liquid chromatography with fluorescence detection. *Analytica Chimica Acta*, 594, 257-264.

[37] Kabak, B., Ozbey, F. (2012). Aflatoxin m1 in UHT milk consumed in Turkey and first assessment of its bioaccessibility using an in vitro digestion model. *Food Control*, 28(2), 338-344.

[38] de Oliveira, P.C., de Fátima Ferreira Soares, N., de Oliveira, T.V., Baffa Júnior, J.C., da Silva, W.A. (2013). Aflatoxin M1 occurrence in ultra high temperature (UHT) treated fluid milk from Minas Gerais/Brazil. *Food Control*, 30(1), 90-92.

[39] Iha, M.H., Barbosa, C.B., Okada, I.A., Trucksess, M.W. (2013). Aflatoxin M1 in milk and distribution and stability of aflatoxin M1 during production and storage of yoghurt and cheese. *Food Control*, 1(29), 1–6.

[40] Iqbal, S.Z., Asi, M.R., Selamat, J. (2014). Aflatoxin M1 in milk from urban and rural farmhouses of Punjab , Pakistan. *Food Additives & Contaminants: Part B*, 7(1), 17-20.

[41] Emea, Chmp, Ewp. (2011). Guideline on bioanalytical method validation. *European Medicines Agency Science Medicines Health*, 1-23.

[42] *Kuraklık Analizi - K.K.T.C. Meteoroloji Dairesi*. (2020). Retrieved November 20, 2020, from http://kktcmeteor.org/verianaliz/kuraklik-analizi

[43] *Sıcaklık Analizi - K.K.T.C. Meteoroloji Dairesi*.

(2020). Retrieved November 20, 2020, from http://kktcmeteor.org/verianaliz/Analyze-sic

[44] Alimentarius, C. (2001). Comments submitted on the draft maximum level for aflatoxin M1 in milk. *Codex Committee on Food Additives and Contaminants 33rd Session, Hague, The Netherlands Commission Regulation (EC),* 25, 1-6.

SİDAS MEDYA
Akademik Gıda®
ISSN Online: 2148-015X
https://dergipark.org.tr/tr/pub/akademik-gida

Akademik Gıda 21(2) (2023) 141-150, DOI: 10.24323/akademik-gida.1350948

Research Paper / Araştırma Makalesi

Kinetics of Antioxidant Activity and Color Degradation in Tomatoes during Hot Air Drying

Adeviye Rana Gokmen[1], Engin Demiray[1] ✉, Yahya Tulek[1], Yusuf Yilmaz[2]

[1]Pamukkale University, Faculty of Engineering, Department of Food Engineering, Denizli, Turkey
[2]Burdur Mehmet Akif Ersoy University, Faculty of Engineering and Architecture, Department of Food Engineering, Istiklal Campus, 15200, Burdur, Turkey

Received (Geliş Tarihi): 17.05.2022, Accepted (Kabul Tarihi): 07.06.2023
✉ *Corresponding author (Yazışmalardan Sorumlu Yazar): edemiray@pau.edu.tr (E. Demiray)*
+90 258 296 3090 +90 258 296 3262

ABSTRACT

The antioxidant activity (AA) and color degradation were monitored in tomato quarters (*Rio Grande*) during hot air drying in a cabinet drier at five temperatures (60, 70, 80, 90 and 100°C) at an airflow rate of 0.2 m/s and 20% relative humidity. AA values of fresh tomatoes determined by total phenolic content (TPC), FRAP and DPPH assays were 85.3 mg GAE, 26.2 µmol TE and 31.3 µmol TE/100g dm, respectively. Increasing drying temperature resulted in a reduction in Hunter Lab and a/b color values of tomatoes as well as their AA values. During hot air drying, the degradation of AA and color values of tomatoes followed a first-order reaction. Activation energy values for AA degradation determined by TPC, FRAP and DPPH assays were 24.36, 22.91 and 23.67 kJ/mol, respectively. High correlations were found among the TPC, DPPH and FRAP values and lycopene and β-carotene contents of tomatoes during hot air drying. Degradation kinetic data revealed that color values and tomatoes AA are susceptible to drying temperature.

Keywords: Tomato, Hot air drying, Lycopene, β-Carotene, Antioxidant activity

Sıcak Hava ile Kurutma Sırasında Domateslerde Antioksidan Aktivite ve Renk Bozulmasının Kinetiği

ÖZ

Antioksidan aktivite (AA) ve renk bozulması, 0.2 m s^{-1} hava akış hızında, %20 bağıl nemde ve beş sıcaklıkta (60, 70, 80, 90 ve 100°C) bir kabin tipi kurutucuda çeyrek dilimler şeklinde kesilmiş domateslerde sıcak havayla kurutma sırasında incelenmiştir. Toplam fenolik madde miktarı (TPC), FRAP ve DPPH deneyleri ile belirlenen taze domateslerin AA değerleri sırasıyla 85.3 mg GAE, 26.2 µmol TE ve 31.3 µmol TE/100g yaş madde olarak hesaplanmıştır. Artan kurutma sıcaklığı ile domateslerin Hunter Lab renk değerlerinde, a/b oranında ve AA değerlerinde azalma meydana gelmiştir. Sıcak hava ile kurutma sırasında, domateslerin AA ve renk değerlerinin bozunması birinci dereceden reaksiyon modeline uyumlu olduğu belirlenmiştir. TPC, FRAP ve DPPH analizleri ile belirlenen AA bozunması için aktivasyon enerjisi değerleri sırasıyla 24.36, 22.91 ve 23.67 kJ/mol olarak hesaplanmıştır. Sıcak hava ile kurutma sırasında domateslerin TPC, DPPH ve FRAP değerleri ile likopen ve β-karoten içerikleri arasında yüksek korelasyonlar bulunmuştur. Bozunma kinetik verileri, renk değerlerinin ve domates AA'nın kuruma sıcaklığına duyarlı olduğunu ortaya çıkarmıştır.

Anahtar Kelimeler: Domates, Sıcak hava ile kurutma, Likopen, β-Karoten, Antioksidan aktivite

INTRODUCTION

Tomato production (about 165 million tons/year) is the eighth agricultural product worldwide among the commodities with the greatest value, and the leading tomato growing countries include China, the United States, India, Turkey, and Egypt [1]. Tomatoes (*Lycopersicum esculentum*) contain a number of health functional constituents such as red-colored carotenoid lycopene and other flavonoids, phenolic acids (especially chlorogenic acids) and ascorbic acid in addition to basic nutritional compounds [2, 3]. High levels of antioxidants present in tomatoes and tomato products help prevent oxidative damage that is hazardous for humans [4]. Major carotenoids present in tomato fruits include lycopene, responsible for the red color in tomatoes, and β-carotene (7% of the total carotenoid content) [5]. β-Carotene has a provitamin A activity, and lycopene acts as an antioxidant, anticarcinogenic and antimutagenic agent [6]. Lycopene concentration increases with the maturity of the tomato berries, causing the development of red color [7].

Drying provides one of the oldest and most effective means of preserving foods from spoilage. Once dried, many foods can be stored successfully for years without refrigeration, if appropriately packaged [8]. Due to its simplicity, hot air drying is frequently used to dry foods [9]. Drying kinetics of foods is generally used to describe the combined macroscopic and microscopic mechanisms of heat and mass transfer during drying, and it is influenced by several factors such as drying conditions, type of dryers and characteristics of materials to be dried. Since on-line measurement of temperature and moisture is difficult and time-consuming for drying, the drying kinetics models are essential for equipment design, process optimization and product quality improvement [10].

Tomatoes are mostly dried at high temperatures in the presence of oxygen, and dried tomato products (e.g. tomato halves, slices, quarters and powders) show the highest sensitivity to oxidative damage [11]. Drying conditions such as high temperature, long duration of exposure and the presence of oxygen may increase the degradation of total phenolics, flavonoids [12] and lycopene [13] during drying, and reduce antioxidant activity of tomatoes. Degradation of major carotenoids in tomatoes by thermal processing and non-enzymatic Maillard reaction during drying are mostly responsible for color changes of tomatoes [14]. Drying tomato quarters of three cultivars, commercially grown in New Zealand, at 42°C in a forced-air drier for 48h, Kerkhofs et al. [15] reported a decrease in total phenolic content between 8 and 33% while extractable lycopene content of tomatoes increased considerably. The authors suggested that bound lycopene in the tissue could be released at lower drying temperatures but lycopene degrades at high temperatures [14, 16, 17]. Drying process may reduce total phenolic, flavonoid and ascorbic acid contents and antioxidant activity of tomatoes [18].

Most of the studies in the literature are focused on the individual and/or combined effect of drying conditions such as pre-treatment, temperature and drying method on antioxidant activity and color values of tomatoes. These parameters are usually determined at the beginning and the end of a drying process. Drying temperature and time are two major factors influencing the degradation kinetics of tomato constituents. No study is available on the degradation kinetics of antioxidant activity and color of tomatoes during drying. Therefore, this present study was conducted to determine the degradation kinetics of antioxidant activity and color in tomato quarters during hot air drying at temperatures varying from 60 to 100°C.

MATERIALS and METHODS

Materials

Fresh tomatoes of *Rio Grande* variety with a diameter about 7 cm were obtained from a local farmer in a town of Acipayam, Denizli (Tukey) in mid-August and onwards. Healthy tomatoes, homogeneous for intense red-color and blemish- and bruise-free, were visually selected. Tomatoes in polyethylene packages were kept refrigerated until drying. The initial moisture content of samples (94.5%) was determined by the AOAC method [19]. All chemicals were of analytical grade unless stated. Solvents used in antioxidant assays were of HPLC grade. Gallic acid, 2,2 -diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tripyridyl-s-triazine (TPTZ) were purchased from Fluka (Switzerland) while iron(III) chloride hexahydrate (FeCl3.6H2O), sodium carbonate were from Riedel-de Haen (Germany). Folin-Ciocalteu reagent was purchased from Merck (Germany). Trolox® (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and (+) catechin were obtained from Sigma (St. Louis, MO, USA).

Methods

Drying Procedure

Tomato quarters were dried in a cabinet drier (70×55×100 cm, W×D×H) manufactured by the Yücebaş Makine Ltd. Inc. (İzmir, Turkey). The cabinet had four removable stainless steel gauze trays (40×60 cm). For each drying experiment at 60, 70, 80, 90 and 100°C, 5 kg of tomatoes in uniform ripeness, color and size were used. The temperature and relative humidity of the dryer were stabilized for an hour. At all temperatures, airflow rate and relative humidity were 0.2 m/s and 20%, respectively. Relative humidity in the drying chamber was measured by a relative humidity sensor (accuracy ±2%) (Elimko, E-RHT-10, Istanbul, Turkey). The airflow rate in the drying chamber was measured with a Tri-Sense hot wire probe anemometer (accuracy ±2%) (Tri-Sense, 37000-90, Cole-Parmer Instrument Co., Illinois, USA). Air flowed vertical to the drying surfaces of samples. And hot air used in the drying process was circulated in the cabinet. Drying air used was automatically exhausted when the relative humidity was over 20%. Tomatoes were cut into quarters longitudinally, and approximately 200-250 g of quarters

was placed on each tray as a single layer with a thickness of 2.2±0.2 cm. For each temperature, one kilogram of tomatoes was used to monitor the time-dependent weight loss. The rest was either used to determine the initial dry matter (dm) content of tomato quarters or wrapped in aluminum foil in polyethylene packages that are kept at -20°C for further analyses. Tomato quarters were dried until their water content reached approximately 15 g/100 g (wet basis). Three independent measurements were taken for each experiment.

Degradation Kinetics

The relationship between the reaction rate and temperature was determined by the Eq. 1, the Arrhenius equation [20].

$$k = k_0 e^{-E_a/RT} \quad (1)$$

where k is the reaction rate constant (h^{-1}), k_0 is the pre-exponential constant (h^{-1}), Ea is the activation energy (kJ/mol), R is the universal gas constant (kJ/mol.K) and T is the absolute temperature (K).

Temperature coefficient (Q_{10}) is the criterion indicating the effect of raising the temperature by 10°C on the rate of reaction, and it was calculated by the Eq. 2 [21].

$$Q_{10} = (k_2/k_1)^{10/(T_2-T_1)} \quad (2)$$

where k_1 and k_2 are reaction rate constants at temperatures T_1 and T_2, respectively (h^{-1}).

The half time ($t_{1/2}$) is the time required for the antioxidant activity or color values of dried tomatoes to decay down to 50% of its initial concentration, and it was calculated by the Eq. 3 [17].

$$t_{1/2} = -\ln(0.5) \times k^{-1} \quad (3)$$

where k is the reaction rate constant.

The order of degradation reactions was calculated by the Eq. 4. By replacing AA with respective color values (CV), this equation was also used to determine the reaction order for color degradation during drying.

$$\ln AA = \ln AA_0 - k.t \quad (4)$$

where AA is the antioxidant activity (μmol TE or mg GAE /g dm) at time *t*, AA_0 is the initial antioxidant activity of lipophilic extracts, and *k* is the reaction rate constant.

Color Measurements

The Hunterlab MiniScan XE colorimeter (Hunter Associates Laboratory, USA) was used to monitor the changes in Hunter color values of tomatoes during drying. Color readings were taken at three different points of tomatoes for better representation of average color values, and expressed in Hunter Color Scale (Lab). The red–yellow ratio (a/b) was reported to indicate the redness of tomatoes [22].

Extraction of Lipophilic Constituents

For the extraction of lipophilic constituents from tomatoes, the method suggested by Lin and Chen [23] was used with some modifications. Fresh or dried tomato samples were mixed with ethanol-hexane solution (4:3, v/v) containing 1% BHT (w/v) at a ratio of 1:10 (w/v). The mixture was homogenized by a homogenizer (Miccra D-8, ART Prozess- & Labortechnik GmbH & Co. KG, Müllheim, Germany), and then transferred into a polypropylene centrifuge tube. After centrifuging (Universal 30RF, Hettich Zentrifugen, Tuttlingen, Germany) at 11,000*g* at 5°C for 15 min, supernatants were transferred into amber bottles by using glass Pasteur pipettes. The use of extract in ethanol-hexane mixture created cloudiness in the working solutions of antioxidant activity assays. Therefore, 0.5 mL of the extract was fully evaporated with nitrogen flash, and then the residue was redissolved in 0.2 mL of the appropriate working solutions in the antioxidant activity assays. The samples were vortexed briefly and sonicated for 5 min to dissolve the residue completely.

Antioxidant Activity Assays

Total phenol contents (TPC) of tomato extracts were determined by the Folin-Ciocalteu method [24]. Gallic acid was used as a standard. A UV-Vis spectrophotometer with 8 cells (T80 Model, PG Instruments, England) was used to determine the total phenol contents of extracts in terms of gallic acid equivalents (GAE). The FRAP and DPPH assay procedures described by Thaipong et al. [25] were used to determine antioxidant activities of tomato extracts. For FRAP assay, absorbance of ferrous tripyridyltriazine complex was measured at 593 nm with a spectrophotometer. For DPPH assay, the absorbance readings of extracts were taken at 515 nm wavelength. The linear standard curves used in both FRAP and DPPH assays were between 10 and 50 μM Trolox®. Antioxidant activity of tomato extracts in FRAP and DPPH assays were expressed in μmol TE/g dry matter.

Statistical Analysis

Drying experiments were performed in triplicates and the measurements were performed in duplicates. Data were analyzed using the Statistical Analysis System software [26]. PROC CORR was used to determine Pearson's correlation coefficients (R) among the parameters studied. Lycopene and β-carotene contents of processed tomatoes reported in a study by Demiray et al. [17] were used to determine correlation coefficients among total phenolic content, antioxidant activity, and lycopene and β-carotene contents of tomatoes during drying.

RESULTS and DISCUSSION

Degradation Kinetics of Total Phenolic Content

Drying is used to preserve foods, and food components like phenolics may degrade during this process. With drying, the time taken to reduce the moisture content of tomatoes from the initial value 95.2±0.2% (w.b.) to a final value 10±0.2% (w.b.) was 20, 14, 12, 10 and 8 h at 60, 70, 80, 90 and 100°C, respectively. In this study, degradation in TPC during drying of tomatoes followed a first-order reaction. Plot of natural logarithm of TPC against time for each temperature is shown in Fig. 1A. Equations used to explain the time-dependency of antioxidant activity are indicated in Table 1, and coefficients of determinations (R^2) were higher than 0.95, confirming that the reaction of antioxidant activity degradation is a first order.

Table 1. Linear equations for the antioxidant activity and color degradation of tomatoes during hot air drying at five different temperatures (y, natural logarithm of either antioxidant activity or color values; x, the drying time in h; R^2, the coefficients of determination, are shown in parenthesis).

Parameter	Temperature (°C)				
	60	70	80	90	100
TPC	y = -0.1689x + 7.4360 (0.9984)	y = -0.2331x + 7.3234 (0.9974)	y = -0.3262x + 7.4570 (0.9973)	y = -0.3556x + 7.4048 (0.9994)	y = -0.4418x + 7.3270 (0.9989)
FRAP	y = -0.1863x + 6.1276 (0.9972)	y = -0.2359x + 6.0987 (0.9910)	y = -0.2844x + 6.0636 (0.9955)	y = -0.3426x + 6.1275 (0.9922)	y = -0.4696x + 6.2292 (0.9943)
DPPH	y = -0.1632x + 5.6361 (0.9963)	y = -0.2549x + 5.7112 (0.9977)	y = -0.2900x + 5.6914 (0.9971)	y = -0.3480x + 5.7279 (0.9966)	y = -0.4368x + 5.7070 (0.9918)
Hunter color L	y = -0.0067x + 3.4092 (0.9929)	y = -0.0137x + 3.2830 (0.9893)	y = -0.0186x + 3.3542 (0.9981)	y = -0.0287x + 3.3462 (0.9972)	y = -0.0432x + 3.3355 (0.9951)
Hunter color a	y = -0.0111x + 3.2509 (0.8980)	y = -0.0230x + 3.2901 (0.9943)	y = -0.0322x + 3.2898 (0.9740)	y = -0.0502x + 3.3247 (0.9699)	y = -0.0699x + 3.2522 (0.9898)
Hunter color b	y = -0.0037x + 2.5814 (0.9822)	y = -0.0071x + 2.5755 (0.9233)	y = -0.0118x + 2.5560 (0.9851)	y = 0.0123x + 2.5623 (0.9919)	y = -0.0126x + 2.5576 (0.8992)
a/b	y = -0.0074x + 0.6694 (0.8118)	y = -0.0107x + 0.7275 (0.9591)	y = -0.0205x + 0.7336 (0.9386)	y = -0.0431x + 0.7489 (0.9667)	y = -0.0467x + 0.7209 (0.7612)

Calculated rate constants (k) and other kinetic parameters of antioxidant activity loss in tomatoes during various drying conditions are given in Table 2. Reaction rate constants for the loss of TPC in tomato quarters were in the range of 0.17-0.44 h^{-1} and significantly affected by drying temperature. Temperature dependence of reaction rate constants followed the Arrhenius relationship.

Results of this present study were in good agreement with the data reported in the literature. Indeed, a first order kinetic model was suggested for the thermal degradation of lycopene in tomatoes paste [21] and in model systems [27]. Activation energy for TPC was 24.36 kJ/mol. The effect of increasing temperature from 60 to 70ºC was similar to temperature increase from 70 to 80°C, which is reflected by similar Q_{10} values for the total phenolic contents of tomatoes (Table 2). Half-life times for TPC degradation in Table 2 support that at elevated drying temperatures TPC loss in tomatoes becomes faster. Results indicated that the drying temperature of 70°C is more suitable to minimize the degradation of TPC in tomato quarters during hot air drying even though the drying takes place longer. Studying eight different dried tomato (*Lycopersicum esculentum*) samples (preserved in oil) marketed in Brazil, de Abreu et al. [28] reported that total phenolic contents of hydrophilic extracts of processed tomatoes ranged from about 338 to 836 mg GAE/100 g dm. In a recent study by Aktürk Gümüşay et al. [29], TPC of fresh tomatoes reduced from 792 mg to 314, 346, 356 and 654 mg GAE/100 g dm for sun-dried, oven-dried, vacuum oven-dried and freeze-dried tomatoes, respectively. They reported that oxidative enzymes like polyphenoloxidase and peroxidase could be activated during drying and lead to a loss in TPC values of tomatoes. Studying the degradation kinetics of TPC, antioxidant capacity and vitamin C content of mandarin slices during drying (oven and vacuum) at 55, 65 and 75°C, Akdas and Baslar [30] reported that degradation kinetics for TPC were of a first-order model and activation energy values for the TPC degradation of oven and vacuum dried mandarin slices were about 53 and 55 kJ/mol, respectively.

Tomatoes contain a number of flavonoids and phenolic acids that can contribute to a healthy diet, and besides flavonoids, stilbenoids and other phenolics, tomato is the most important source of lycopene, a red-colored carotenoid associated with several health benefits [2]. Flavonoids are regarded as potentially useful compounds, with implications for inflammation, cardiovascular diseases and cancer [31]. Chlorogenic acids and related compounds are the main phenolic compounds besides flavonoids in tomatoes, which may also be responsible for an astringent taste [32]. Food processing conditions may result in a reduction in total phenolic contents and antioxidant activity of tomatoes [33]. In a study by Toor and Savage [18], total phenolic content of three tomato cultivars (Excell, Tradiro, and Flavourine) reduced from 404 to 300 mg GAE/100 g dm at the end of a drying process at 42°C for 18h in a forced-air drier.

Degradation Kinetics of Ferric Reducing Antioxidant Power

The degradation rate of FRAP values in tomato quarters increased with temperature (Table 2). In this present study, when tested in the FRAP assay, the antioxidant

activity of tomatoes dried at 60ºC changed from 305.24 to 58.02 mg/100 g dm at the end of drying. But at 100ºC, it dropped to 12.96 mg. The kinetics of degradation of antioxidant activity with the FRAP assay followed a first-order reaction like TPC. The reaction constants of AA in dried tomatoes were determined by plotting the natural logarithm of AA (µmol TE / g dm) against time for each temperature (Fig. 1B). Depending on drying temperature, AA degradation rate increased. Half life time was calculated as 3.72 h at 60ºC, which dropped to 1.48 h at 100ºC. Activation energy was 22.91 kJ/mol. When temperature increased from 90 to 100ºC, the degradation rate of AA in tomatoes was affected more than other temperatures ranges (i.e. Q_{10} value was the highest for the temperature increase from 90 to 100°C). Studying the antioxidant capacity of several tomato varieties, Martinez-Valverde et al. [34] reported that the antioxidant activity of tomato extracts is mostly dependent on the tomato variety and the assay method used. The authors stated that lycopene and ferulic and caffeic acids are distinctive compounds that are highly related to the antioxidant capacity of tomatoes.

Table 2. Reaction rate constants and other kinetic parameters[a] for antioxidant activity loss in tomatoes during drying at five different temperatures

Antioxidant Activity Method	Temperature (ºC)	Q_{10} Value	k (h^{-1})	$t_{1/2}$ (h)	E_a (kJ/mol)
TPC	60		0.1689	4.10	24.36
	70	1.38	0.2331	2.97	
	80	1.40	0.3262	2.12	
	90	1.09	0.3556	1.95	
	100	1.24	0.4418	1.57	
FRAP	60		0.1863	3.72	22.91
	70	1.27	0.2359	2.94	
	80	1.21	0.2844	2.44	
	90	1.20	0.3426	2.02	
	100	1.37	0.4696	1.48	
DPPH	60		0.1632	4.25	23.67
	70	1.56	0.2549	2.72	
	80	1.14	0.2900	2.39	
	90	1.20	0.3480	1.99	
	100	1.26	0.4368	1.59	

[a]: Q_{10}, k, $t_{1/2}$ and E_a: temperature coefficient, reaction rate constant, reaction half life time and activation energy, respectively.

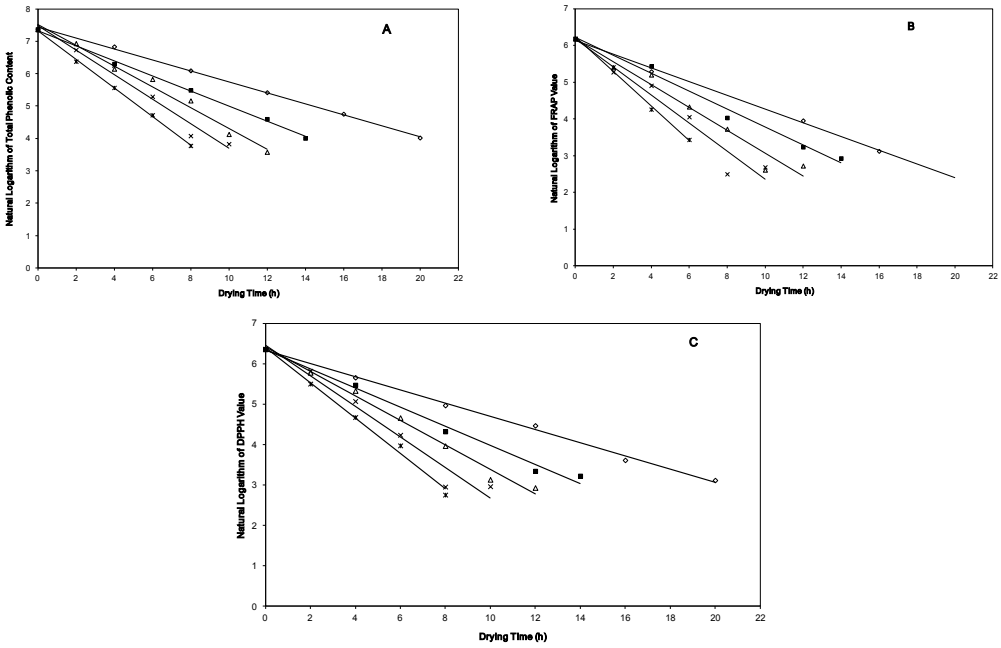


Figure 1. Degradation kinetics of antioxidant activity (A, TPC; B, FRAP and C, DPPH values) in tomatoes dried at 60°C (♦), 70°C (□), 80°C (△), 90°C (×) and 100°C (∗). Lines indicate linear regression for each drying temperature, and each point reflects the average of triplicates

Degradation Kinetics of DPPH

Higher DPPH radical scavenging activity of hydrophilic extracts of tomato and tomato products than hydrophobic extracts were previously reported by several authors [28, 35, 36] although conflicting results were also present in the literature [37]. Using the ABTS assay, Toor and Savage [18] reported that drying tomatoes at 42°C for 18h in a forced air drying reduced the total antioxidant activity of fresh tomatoes from 2.73 to 1.78 mmol TE/100 g dm. Similar reductions in the total antioxidant activity of different cultivars were also reported by Kerkhofs et al. [15]. In this present study, DPPH radical scavenging activity of dried tomatoes was determined in hydrophobic extracts. The degradation kinetics of DPPH values followed a first-order reaction like TPC and FRAP. Reaction constants for DPPH values in dried tomatoes were determined by plotting the natural logarithm of DPPH values (µmol TE/g dm) versus time for each temperature (Fig. 1C). The plots were mostly linear (R^2 = 0.904–0.980), confirming that the reaction of DPPH value degradation is towards a first order. Kinetic data at various drying conditions are shown in Table 2. Reaction rate constants for antioxidant activity loss in terms of DPPH values were in the range of 0.1632-0.4368 h^{-1} and significantly influenced by drying temperature. As drying temperature increased, the degradation of DPPH values also increased. For example, half life time ($t_{1/2}$) of DPPH values in tomatoes during drying was 4.25 h at 60ºC; however, it decreased to 1.59 h at 100ºC. Results indicated that DPPH values are highly influenced by drying temperature, and results were similar to total phenolic contents and FRAP values of tomatoes during drying. Akdas and Baslar [30] dried mandarin slices in oven or vacuum drying conditions at 55, 65 and 75°C, and determined the degradation kinetics of DPPH radical scavenging activity of slices. They suggested a first-order reaction for antioxidant activity degradation and reported that activation energy values for the degradation of antioxidant activity in oven and vacuum dried mandarin slices were about 40 and 42 kJ mol^{-1}, respectively. In this present study, the degradation of DPPH values in tomato quarters during hot air drying was a first-order reaction, and activation energy was 23.67 kJ mol^{-1}.

Degradation Kinetics of Color Values

Degradation of color values followed a first-order reaction ($ln\ CV = lnCV_0 - k.t$) where CV is the color value (Hunter L, a, b or a/b value) at time t, CV_0 is the respective initial color value of processed tomatoes, and k is the reaction rate constant). Plots of natural logarithm of color values against time for each temperature are shown in Fig. 2A-D while the equations explaining the time-dependency of color values of processed tomatoes are indicated in Table 1. Coefficients of determinations (R^2) were in the range of 0.90 to 0.99, which is a good indicator for a first order reaction. The calculated rate constants (k) and other kinetic parameters of color values in tomatoes during various drying conditions are given in Table 3. The reaction rate constants for the color values of tomato quarters during drying were in the range of 0.01-0.07 h^{-1} and significantly affected by drying temperature. Temperature dependence of the reaction rate constants followed the Arrhenius relationship.

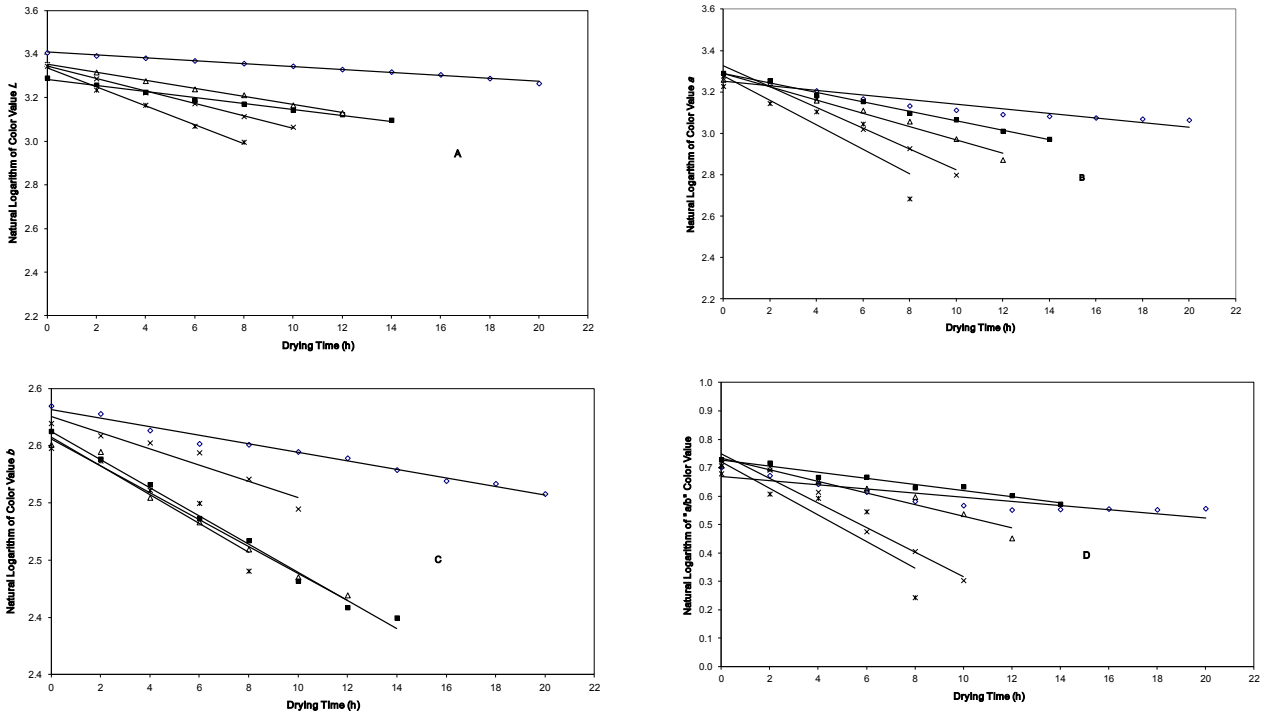


Figure 2. Degradation kinetics of color values (A, Hunter L; B, Hunter a; C, Hunter b and D, a/b values) in tomatoes dried at 60°C (♦), 70°C (□), 80°C (△), 90°C (×) and 100°C (∗). Lines indicate linear regression for each drying temperature, and each point reflects the average of triplicates.

Changing drying temperature from 60 to 70°C resulted in the highest Q_{10} value of Hunter L, a and b color parameters in processed tomatoes in comparison to other temperature changes. On the other hand, the highest Q_{10} value (2.102) was determined in a/b values of processed tomatoes when temperature was increased from 80 to 90°C. Color variation in tomatoes is mostly related to a/b value and a low a/b value represents an orange to brown color due to the breakdown of lycopene and the formation of the Maillard reaction products by the intensive heat treatment [38, 39]. Half-life time for a/b value was calculated as 51 min at 60ºC, which dropped to 9 min at 100ºC. The activation energy was 52.58 kJ/mol.

Drying method itself has a significant influence on color values of tomatoes [40]. In a study by Izli and Isik [41], tomato halves were dried at microwave, convective, and microwave-convective driers to determine changes in color and microstructure of tomatoes. Authors reported that both drying temperature and drier type influenced tomato color, and reduction in drying temperature resulted in a better retention of color. Similarly, studying the effect of drier type (microwave, vacuum-microwave and hot air) on drying kinetics, antioxidant activity and color changes of tomato quarters, Orikasa et al. [42] reported that the use of a vacuum-microwave drier increased the retention of antioxidant activity in tomato quarters while leading to a lighter color than other two methods. Kerkhofs et al. [15] drying tomato quarters of three cultivars (Aranka, Encore and Flavourine) in a forced-air drier at 42°C for 18 h to a final moisture content of 23% resulted in a reduction of $a*/b*$ ratio by 25%. Under similar drying conditions, Toor and Savage [15] reported that drying reduced CIE L* values while increasing a*/b* ratios of tomatoes (Excell, Tradiro and Flavourine). Demiray and Tulek [43] drying red pepper slices in a vacuum dryer at three different temperatures (45, 55 and 65°C) and two absolute pressures (21.5 kPa and 48.0 kPa). Authors reported that the color values (Hunter L, a and b) decreased, while ΔE (The color difference) increased during drying. Mathematical modeling of color degradation kinetics indicated that both the zero-order and first-order kinetic model were found to describe the Hunter L, a and b values. However, ΔE followed zero-order kinetic model.

Table 3. Reaction rate constants and other kinetic parameters[a] for color values in tomatoes during drying at five different temperatures

Hunter Color Value	Temperature (ºC)	Q_{10} Value	k (h^{-1})	$t_{1/2}$ (h)	E_a (kJ/mol)
L	60		0.0067	1.72	46.29
	70	2.044	0.0137	0.84	
	80	1.357	0.0186	0.62	
	90	1.543	0.0287	0.40	
	100	1.505	0.0432	0.27	
a	60		0.0111	1.04	46.28
	70	2.019	0.0230	0.50	
	80	1.332	0.0322	0.36	
	90	1.522	0.0502	0.23	
	100	1.392	0.0699	0.17	
b	60		0.0037	3.12	31.49
	70	1.920	0.0071	1.63	
	80	1.662	0.0118	0.98	
	90	1.042	0.0123	0.94	
	100	1.024	0.0126	0.92	
a/b	60		0.0074	0.85	52.58
	70	1.445	0.0107	0.56	
	80	1.915	0.0205	0.31	
	90	2.102	0.0431	0.16	
	100	1.083	0.0467	0.15	

[a]: Q_{10}, k, $t_{1/2}$, k_0 and E_a: temperature coefficient, reaction rate constant, reaction half life time, preexponential constant and activation energy, respectively.

Correlation among Color Values, Antioxidant Activity, Lycopene and β-Carotene Contents

In this present study, significant correlation coefficients ($p<0.001$) were found among the parameters studied (Table 4). Total phenolic content, DPPH and FRAP values and lycopene and β-carotene contents of tomatoes during hot air drying were highly correlated with each other (i.e. Pearson correlation coefficients > 0.90). Arias et al. [44] reported a good correlation between color of hydroponic tomatoes and their lycopene content, and they proposed an equation explaining the relation between the lycopene content and the ratio of a*/b* color values during maturity. Studying the changes in carotenoids, phenolic

compounds and vitamin C contents of red and yellow tomatoes during technical processing and lyophilization, George et al. [45] reported lower a*/b* ratios and β-carotene contents in yellow tomatoes than in red tomatoes. The authors stated that the parameter b* was higher in yellow tomato than in red tomato, and they concluded that the b* parameter was not a good indicator of the β-carotene content. Insignificant correlation between total phenolic content and CIE Lab color values of 167 tomato samples of five different cultivars was reported by Hernandez et al. [46] However, the authors reported that correlation between lycopene content and color value a*, which represents red color, was significant. Ilahy et al. [47] reported that antioxidant activity (ABTS and FRAP values) of hydrophilic extracts of high-lycopene tomato cultivars was significantly correlated with total phenolic contents of extracts while antioxidant activity of lipophilic extracts was highly and significantly correlated with total carotenoid and lycopene contents of tomato extracts. Kim et al. [48] reported the highest correlation (r= 0.893) between the total phenolic content and reducing power of hydrothermal extracts of watermelons and the lowest correlation between ABTS values and reducing power (r= 0.605). The authors also reported high correlation between the TPC and DPPH values of hydrothermal extracts of watermelons. In this present study, all parameters studied were correlated with each other, and the main reason for this could be the fact that the correlations were determined during hot air drying of tomatoes. Drying process had a significant effect on the parameters including color values, total phenolic contents and antioxidant activity of tomatoes.

Table 4. Pearson correlation coefficients among color values, lycopene and β-carotene contents, TPC, DPPH and FRAP values of air-dried tomatoes (n=26). Lower values in parentheses (p values) indicate that parameters are highly correlated with each other. Coefficients higher than 0.90 are shown in bold.

Parameters	L	a	b	a/b	TPC	DPPH	FRAP	Lycopene	β-Carotene
L	1.000	0.837 (<0.0001)	0.727 (<0.0001)	0.721 (<0.0001)	0.798 (<0.0001)	0.780 (<0.0001)	0.751 (<0.0001)	0.819 (<0.0001)	0.698 (<0.0001)
a		1.000	0.736 (<0.0001)	**0.936** (<0.0001)	0.878 (<0.0001)	0.875 (<0.0001)	0.846 (<0.0001)	**0.919** (<0.0001)	0.890 (<0.0001)
b			1.000	0.452 (0.0200)	0.717 (<0.0001)	0.711 (<0.0001)	0.681 (<0.0001)	0.764 (<0.0001)	0.750 (<0.0001)
a/b				1.000	0.774 (<0.0001)	0.773 (<0.0001)	0.751 (<0.0001)	0.824 (<0.0001)	0.793 (<0.0001)
TPC					1.000	**0.994** (<0.0001)	**0.982** (<0.0001)	**0.949** (<0.0001)	**0.917** (<0.0001)
DPPH						1.000	**0.992** (<0.0001)	**0.946** (<0.0001)	**0.924** (<0.0001)
FRAP							1.000	**0.927** (<0.0001)	0.896 (<0.0001)
Lycopene								1.000	**0.954** (<0.0001)
β-Carotene									1.000

CONCLUSION

This study indicated that degradation kinetics of antioxidant activity and color values in tomato quarters during hot air drying followed a first-order reaction. Reaction rate constants for these constituents of tomatoes were highly dependent on the drying temperature, and activation energy values for antioxidant activity determined by three TPC, FRAP and DPPH assays were 24.36, 22.91 and 23.67 kJ/mol, respectively. Prolonged drying time increased the degradation rate of antioxidant activity of tomatoes during hot air drying. In lipophilic extracts, significant correlations were found among the TPC, DPPH and FRAP values and lycopene and β-carotene contents of tomatoes during hot air drying. Main reason for high correlations could be the fact that hot air drying has a significant influence on parameters studied including color values and antioxidant activity of tomatoes. Kinetic data revealed that color values and antioxidant activity of tomatoes are susceptible to drying temperature. Under the conditions studied, tomatoes should be dried at temperatures lower than 70°C in order to obtain better retention of antioxidant activity and color in final products. Results of this present study could be useful to optimize drying conditions for tomatoes with superior total phenolic content and antioxidant activity as well as desired color values.

ACKNOWLEDGEMENT

This research was partially supported by the Pamukkale University Research Fund (2008MHF004).

REFERENCES

[1] FAO, http://faostat3.fao.org/browse/Q/QC/E. Accessed 15 October 2016.

[2] Slimestad, R., Verheul, M. (2009). Review of flavonoids and other phenolics from fruits of different tomato (*Lycopersicon esculentum* mill.) cultivars. *Journal of the Science of Food and Agriculture*, 89, 1255-1270.

[3] Veillet, S., Busch, J., Savage, G. (2009). Acceptability and antioxidant properties of a semi-dried and smoked tomato product. *Journal of Food, Agriculture and Environment* 7(2), 70-75.

[4] Halliwell, B. (2000). Why and how should we measure oxidative DNA damage in nutritional studies? How far have we come? *The American Journal of Clinical Nutrition* 72(5), 1082-1087.
[5] Gould, W. A. (1983). Tomato production, processing, and quality evaluation. The AVI Publishing Company Inc., Westport, USA.
[6] Pfander, H. (1992). Carotenoids, In: Methods in Enzymology, ed. By Packer L., Elsevier Science Publishing, San Diego, United States, pp. 3-13.
[7] Ellis, R. J. (1979). The Plastids: Their Chemistry, Structure, Growth and Inheritance. *Trends in Biochemical Sciences*, 4 p. N305.
[8] Durance, T.D., Wang, J.H. (2002). Energy consumption, density, and rehydration rate of vacuum microwave and hot-air convection dehydrated tomatoes. *Journal of Food Science*, 67(6), 2212-2216.
[9] Bazyma, L.A., Guskov, V.P., Basteev, A.V., Lyashenko, A.M., Lyakhno, V., Kutovoy, V.A. (2006). The investigation of low temperature vacuum drying processes of agricultural materials. *Journal of Food Engineering*, 74(3), 410-415.
[10] Giri, S. K., Prasad, S. (2007). Drying kinetics and rehydration characteristics of microwave-vacuum and convective hot-air dried mushrooms. *Journal of Food Engineering*, 78(2), 512-521.
[11] Giovanelli, G., Zanoni, B., Lavelli, V., Nani, R. (2002). Water sorption, drying and antioxidant properties of dried tomato products. *Journal of Food Engineering*, 52(2), 135-141.
[12] Chang, C.H., Liu, Y.C. (2007). Study on lycopene and antioxidant contents variations in tomatoes under air-drying process. *Journal of Food Science*, 72(9), 532-540.
[13] Preedy, V.R. (2012). Vitamin A and carotenoids: Chemistry, analysis, function and effects", Royal Society of Chemistry Publishing, Cambridge, United Kingdom.
[14] Zanoni, B., Peri, C., Nani, R., Lavelli, V. (1998). Oxidative heat damage of tomato halves as affected by drying. *Food Research International*, 31(5), 395-401.
[15] Kerkhofs, N.S., Lister, C.E., Savage, G.P. (2005). Change in colour and antioxidant content of tomato cultivars following forced-air drying. *Plant Foods for Human Nutrition*, 60(3), 117-121.
[16] Shi, J., Le Maguer, M., Kakuda, Y., Liptay, A., Niekamp, F. (1999). Lycopene degradation and isomerization in tomato dehydration. *Food Research International*, 32(1), 15-21.
[17] Demiray, E., Tulek, Y., Yilmaz, Y. (2013). Degradation kinetics of lycopene, β-carotene and ascorbic acid in tomatoes during hot air drying. *LWT-Food Science and Technology*, 50(1), 172-176.
[18] Toor, R.K., Savage, G.P. (2006). Effect of semi-drying on the antioxidant components of tomatoes. *Food Chemistry*, 94(1), 90-97.
[19] AOAC, (1990). Official Methods of Analysis, 15th Ed. Washington, DC: Association of Official Analytical Chemists.
[20] Goula, A.M., Adamopoulos, K.G., Chatzitakis, P.C., Nikas, V.A. (2006). Prediction of lycopene degradation during a drying process of tomato pulp. *Journal of Food Engineering*, 74(1), 37-46.
[21] Sharma, S.K., Le Maguer, M. (1996). Kinetics of lycopene degradation in tomato pulp solids under different processing and storage conditions. *Food Research International*, 29(3-4), 309-315.
[22] Min, S., Jin, Z.T., Zhang, Q.H. (2003). Commercial scale pulsed electric field processing of tomato juice. *Journal of Agricultural and Food Chemistry*, 51(11), 3338-3344.
[23] Lin, C.H., Chen, B.H. (2005). Stability of carotenoids in tomato juice during processing. *European Food Research and Technology*, 221(3), 274-280.
[24] Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent" ed. By Packer L., Elsevier Science Publishing, San Diego, United States, pp. 152-178.
[25] Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., Byrne, D. H. (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis*, 19(6-7), 669-675.
[26] SAS Institute, (2002). SAS User's Guide: Statistics, Version 9.00. Cary, NC: SAS Institute.
[27] Lee, M.T., Chen, B.H. (2001). Separation of lycopene and its *cis* isomers by liquid chromatography. *Chromatographia*, 54(9), 613-617.
[28] de Abreu, W.C., Barcelos, M.D.F.P., de Barros Vilas Boas, E.V., da Silva, E.P. (2014). Total antioxidant activity of dried tomatoes marketed in Brazil. *International Journal of Food properties*, 17(3), 639-649.
[29] Gümüşay, Ö.A., Borazan, A.A., Ercal, N., Demirkol, O. (2015). Drying effects on the antioxidant properties of tomatoes and ginger. *Food Chemistry*, 173, 156-162.
[30] Akdaş, S., Başlar, M. (2015). Dehydration and degradation kinetics of bioactive compounds for mandarin slices under vacuum and oven drying conditions. *Journal of Food Processing and Preservation*, 39(6), 1098-1107.
[31] Fossen, T., Andersen, O.M. (2006). Chemistry, Biochemistry and Applications: Spectroscopic Techniques Applied to Flavonoids In: Flavonoids eds. Anderson, O.M., Markham, K.R., Taylor & Francis, New York, USA, pp. 37-142.
[32] De Bruyn, J. W., Garretsen, F., Kooistra, E. (1971). Variation in taste and chemical composition of the tomato (*Lycopersicon esculentum* Mill.). *Euphytica*, 20(2), 214-227.
[33] Sahlin, E., Savage, G.P., Lister, C.E. (2004). Investigation of the antioxidant properties of tomatoes after processing. *Journal of Food composition and Analysis*, 17(5), 635-647.
[34] Martínez-Valverde, I., Periago, M.J., Provan, G., Chesson, A. (2002). Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (*Lycopersicum esculentum*).

Journal of the Science of Food and Agriculture, 82(3), 323-330.

[35] Larrosa, M., Espín, J.C., Tomás-Barberán, F.A. (2003). Antioxidant capacity of tomato juice functionalised with enzymatically synthesised hydroxytyrosol. *Journal of the Science of Food and Agriculture*, 83(7), 658-666.

[36] Powell, Z.D.L.C. (2001). Antioxidant capacity of lycopene-containing foods. *International Journal of Food Sciences and Nutrition*, 52(2), 143-149.

[37] Ishida, B.K., Chapman, M.H. (2004). A comparison of carotenoid content and total antioxidant activity in catsup from several commercial sources in the United States. *Journal of Agricultural and Food Chemistry*, 52(26), 8017-8020.

[38] Le Maguer, M., Shi, J. (2000). Lycopene in tomatoes: chemical and physical properties affected by food processing. *Critical Reviews in Food Science and Nutrition*, 40(1), 1-42.

[39] Krebbers, B., Matser, A.M., Hoogerwerf, S.W., Moezelaar, R., Tomassen, M.M., van den Berg, R.W. (2003). Combined high-pressure and thermal treatments for processing of tomato puree: evaluation of microbial inactivation and quality parameters. *Innovative Food Science & Emerging Technologies*, 4(4), 377-385.

[40] Askari, G. R., Emam-Djomeh, Z., Tahmasbi, M. (2009). Effect of various drying methods on texture and color of tomato halves. *Journal of Texture Studies*, 40(4), 371-389.

[41] Izli, N., Isik, E. (2015). Color and microstructure properties of tomatoes dried by microwave, convective, and microwave-convective methods. *International Journal of Food Properties*, 18(2), 241-249.

[42] Orikasa, T., Koide, S., Sugawara, H., Watanabe, T., Okada, M., Matsushima, U., Tagawa, A. (2014). Drying kinetics and quality of tomato fruits dehydrated by a vacuum-microwave method. In: XXIX International Horticultural Congress on Horticulture: Sustaining Lives, Livelihoods and Landscapes (IHC2014), Brisbane, Australia, pp. 375-380.

[43] Demiray, E., Tulek, Y. (2020). Color and ascorbic acid degradation kinetics of red pepper (*Capsicum annuum* L.) slices during vacuum drying. *Akademik Gıda*, 18(1), 19-26.

[44] Arias, R., Lee, T.C., Logendra, L., Janes, H. (2000). Correlation of lycopene measured by HPLC with the L*, a*, b* color readings of a hydroponic tomato and the relationship of maturity with color and lycopene content. *Journal of Agricultural and Food Chemistry*, 48(5), 1697-1702.

[45] Georgé, S., Tourniaire, F., Gautier, H., Goupy, P., Rock, E., Caris-Veyrat, C. (2011). Changes in the contents of carotenoids, phenolic compounds and vitamin C during technical processing and lyophilisation of red and yellow tomatoes. *Food Chemistry*, 124(4), 1603-1611.

[46] Hernández, M., Rodríguez, E., Díaz, C. (2007). Free hydroxycinnamic acids, lycopene, and color parameters in tomato cultivars. *Journal of Agricultural and Food Chemistry*, 55(21), 8604-8615.

[47] Ilahy, R., Hdider, C., Lenucci, M.S., Tlili, I., Dalessandro, G. (2011). Antioxidant activity and bioactive compound changes during fruit ripening of high-lycopene tomato cultivars. *Journal of Food Composition and Analysis*, 24(4-5), 588-595.

[48] Kim, S.J., Matsushita, Y., Fukushima, K., Aoki, D., Yagami, S., Yuk, H.G., Lee, S.C. (2014). Antioxidant activity of a hydrothermal extract from watermelons. *LWT-Food Science and Technology*, 59(1), 361-368.

SİDAS MEDYA
Akademik Gıda®
ISSN Online: 2148-015X
https://dergipark.org.tr/tr/pub/akademik-gida

Akademik Gıda 21(2) (2023) 151-157, DOI: 10.24323/akademik-gida.1350967

Research Paper / Araştırma Makalesi

Gluten Status in Gluten-Free Pastry and Bakery Products Produced in Istanbul, Turkey

Yeliz Miral Kaya[1], Ayşen Çoban Dinçsoy[2]

[1]Institute of Graduate Programs, Istanbul Gedik University, Kartal, Istanbul, 34876, Turkey
[2] Department of Gastronomy and Culinary Arts, Istanbul Gedik University, Kartal, Istanbul, 34876, Turkey

Received (Geliş Tarihi): 01.12.2021, Accepted (Kabul Tarihi): 15.07.2023
Corresponding author (Yazışmalardan Sorumlu Yazar): coban.aysen@gmail.com (A. Çoban Dinçsoy)
444 5 438 +90 216 452 8717

ABSTRACT

Gluten is a complex protein that forms the basis of bakery products, including pastry products, with its elasticity. Gluten proteins are constituted by gliadin and glutenin. Gliadin, which is in the water-insoluble protein group, is very difficult to digest. Many immune disorders influence a growing number of people in relation to the consumption of wheat flour-based foods. The aim of this study is to determine the status of gluten-free products with the legal limits of the gluten-free products sold in pastry shops and bakeries in Istanbul, Turkey. Ninety samples in total including gluten-free bread, cakes, cookies, snacks, and cereals were collected from various regions of Istanbul in November 2020. The samples obtained were examined by ELISA (Enzyme-Linked Immunosorbent Assay) for the presence of gluten. As a result, the presence of gluten was found to be lower than 5 ppm in 61 samples (67.7%), between 5-20 ppm in 8 samples (8.8%), and 20 ppm and above in 21 samples (23%). Within the scope of Turkish Food Codex Regulation on Food Labeling and Consumer Information Number 29960, some foods were inappropriate products which offered to consumption as gluten-free in patisseries and bakeries. It could be thought that risks might be reduced to the maximum extent with hygiene and sanitation training in food businesses, analysis in terms of gluten in raw materials and final products, control of contamination from raw materials, personnel and environment during the production phase, and gluten analysis at critical points.

Keywords: Gluten, Contamination, Gluten intolerance, ELISA

İstanbul İlinde Pastane ve Fırınlarda Üretilen Glutensiz Ürünlerde Gluten Varlığı

ÖZ

Gluten, esneklik özelliği ile pastacılık ürünleri dahil olmak üzere unlu mamullerin temelini oluşturan kompleks bir proteindir. Gluten proteinleri gliadin ve glutenin tarafından oluşturulur. Suda çözünmeyen protein grubunda yer alan gliadinin sindirimi oldukça zordur. Birçok bağışıklık bozukluğu, buğday unu bazlı gıdaları tüketen insanları artan oranda etkilemektedir. Bu çalışmanın amacı, İstanbul ilinde pastane ve fırınlarda glutensiz olarak tüketime sunulan ürünlerin yasal limite (20 ppm) uygunluğunun araştırılmasıdır. 2020 yılının Kasım ayında, İstanbul'un farklı bölgelerinde glutensiz ekmek, kek, kurabiye, atıştırmalık ve tahıl gevrekleri olmak üzere 90 adet örnek toplanmıştır. Gluten varlığı analizi için örnekler Enzim Bağlı İmmünosorbent Testi (ELISA, Enzyme-Linked Immunosorbent Assay) ile incelenmiştir. Sonuç olarak, incelenen örneklerin gluten varlığı 61 (%67.7) adedinde 5 ppm'den düşük, 8 (%8.8) adedinde 5-20 ppm arasında ve 21 (%23) adedinde ise 20 ppm ve üzerinde tespit edilmiştir. Pastane ve fırınlarda glutensiz olarak tüketime sunulan ürünlerin 29960 sayılı Türk Gıda Kodeksi Gıda Etiketleme ve Tüketicileri Bilgilendirme Yönetmeliği kapsamına uygun olmadığı belirlenmiştir. Gıda işletmelerinde hijyen ve sanitasyon eğitiminin son derece önemli olduğu düşünülmektedir. Ek olarak, hammadde ve son üründe gluten yönünden

analizlerin yapılması, üretim aşamasında hammadde, personel ve ortam kaynaklı kontaminasyonunun kontrolü ve kritik noktalarda gluten analizi ile risklerin azami ölçüde azalacağı düşünülmektedir.

Anahtar Kelimeler: Gluten, Kontaminasyon, Gluten intolerans, ELISA

INTRODUCTION

The word gluten means glue derived from the Latin word “glue” [27]. Gluten substance is a storage protein in wheat consisting of “gluten and gliadin” fractions. Based on dry matter, gluten contains 75-86% protein while gliadins protein makes up about 30% of all proteins [35, 36]. Gluten is generally separated into two classes: soluble fraction in alcohol called gliadins (monomer unit) which contribute to the cohesiveness and extensibility of the gluten, and insoluble-glutenin (polymer unit, soluble in dilute acids and bases) which play a role in the maintenance of the elasticity and strength of the gluten. Both proteins constitute 80–85% of gluten proteins and define viscoelastic properties of dough [35]. In reference to Codex Alimentarius, ‘Gluten is a protein fraction of wheat, rye, barley, or their crossed varieties and derivatives thereof, being insoluble in water and NaCl 0.5 mol/L’ [4].

The structure and organization of glutenin and gliadin has difference, despite their similar utilitarian relationship [36]. It is stated that the effects of gluten proteins on dough and bread are mostly combined [38]. Gluten contributes to the appearance and crumb structure of many bakery products. When gluten is removed from the bread formulation, low volume, friability, color, and other quality defects occur in the bread [12].

The characteristics of the products covered by the Turkish Food Codex Regulation on Gluten-Free Foods are defined in two sections. The first part, in foodstuffs defined as gluten reduced, the gluten substance in the dry part is 200 mg/kg; in the second part, in food products defined as gluten-free, the gluten substance should not exceed 20 mg/kg in the dry part. Instead of gluten-free foods used as an alternative to basic foods such as flour and bread, they should contain the same number of vitamins and minerals as the food products they consume [33].

Gliadins include intra-molecular disulfide bonds, the breaking of which leads to opening of the protein molecule and are responsible for binding property of gluten [3, 18]. Glutenin is multi-chained and appeared to be mainly polymerized by disulfide bonds [7]. They are mainly responsible for the viscoelasticity properties of dough and gliadins provide to dough extensibleness [19, 35]. The relevant ratio between proteins is responsible for important rheological properties as viscosity, extensibility, and elasticity [14, 17]. The functional properties of gluten proteins depend on their physical and chemical properties that affect their behavior in food systems [6].

Grain and cereal products containing gluten protein, as the main source of energy and nutrients in nutrition can cause discomfort in many people. The source of toxic effects of gluten protein is prolamins which are known as “gliadin” in wheat, “secalin” in rye, “hordein” in barley and “avenin” in oats [21]. Gluten-related disorders are known to affect about 10% of the general population. The only known treatment today is avoiding consumption of gluten during the lifelong. Therefore, accurate and reliable information about the presence of gluten in foods is of great importance [10]. However, the foods made from wheat flour can negatively impact human health, inducing adverse reactions in genetically vulnerable individuals [37].

Gluten-related diseases (GRD) are also called reactions that occur by consuming gluten protein. Dermatitis herpetiformis (DH), gluten ataxia, wheat allergy, non-celiac gluten sensitivity (NCGS), celiac disease (CD) are gluten-related diseases [30, 31]. Celiac disease (CD) is commonly known as GRD in which genetic and environmental factors in addition to gluten intolerance are the primary causes of natal and adaptative immune responses [1, 24]. CD is typical by damaged and shortened small intestine mucosa, partial, or total intestinal villi atrophy and nutrient malabsorption [16]. The prevalence of CD globally is estimated about 2% in the general population and 0.3–2.9% in children [23, 29].

Additionally, the gluten-free diet is practiced by many people and is gaining vital importance and popularity. Regarding this issue, it is known that following a search for "gluten-free diet" on the internet, 4.2 million results were displayed, and result in an informational search for “gluten-free diet and weight loss”, over 5 million results [13, 15].

On the other hand, cross contamination can occur in packaged food products, as well as in institutions that produce large capacity food. It is due to the untrained and carelessness of kitchen personnel at catering, hotel, restaurant, and cafe where food is produced. Notably, by the reason of not to paying attention to the use of counter and equipment can spread gluten protein through direct or cross contamination. Thus, it can occur in such gluten-free food products [8]. Within the scope of Turkish Food Codex Regulation on Food Labeling and Consumer Information No 29960, some foods are inappropriate products which offered to consumption as gluten-free in patisseries and bakeries.

In this study, it was aimed to investigate the suitability of gluten presence according to legal limits with the R5 ELISA RIDASCREEN Gliadin method in pastry and bakery products sold as gluten-free in Istanbul, Turkey.

MATERIALS and METHODS

In this study, 90 gluten-free products that were produced and sold on site were directly collected to able to analyze all samples for three various days as materials in different 8 cities of Istanbul in November 2020. The sampling was randomly selected from the products produced by the boutique patisseries located in the regions where gluten-free products notably are manufactured, which is frequently preferred in terms of gastronomy in Istanbul. Since the products are not packaged products, they were collected under the hygiene conditions in line with the options from their own products.

Table 1. Type and distribution of the samples

Region	Number of Samples	Collection Date	Type of Samples
Plant 1	2 (%2,2)	08.11.2020	Cake, Cookie
Plant 2	13 (%14,4)	08.11.2020 16.11.2020	Bread, Cookie, Snack
Plant 3	2 (%2,2)	15.11.2020	Cracker
Plant 4	1 (%1,1)	15.11.2020	Cracker
Plant 5	19 (%21,1)	08.11.2020	Bread, Cake, Cookie, Cracker, Snack
Plant 6	28 (%31,1)	15.11.2020	Cake, Cookie, Cracker, Snack
Plant 7	3 (%3,3)	08.11.2020	Bread, Cookie, Snack
Plant 8	22 (%24,4)	08.11.2020	Cake, Cookie, Snack
Total		90 (%100)	

*Plant number indicates each different collection point.

Table 2. Type and distribution of the samples

	Bread	Cake	Cookie	Snack	Crispy
	Bread (n:5)	Cake (n:20) Brownie (n:6) Muffin (n:5) Cakepop (n:4)	Cookie (n:32)	Bar (n:4) Truf (n:6) Wafer (n:1)	Cracker (n:5) Grissini (n:2)
Total	5	35	32	11	7

Following the sampling, samples were analyzed in the Laboratory of the Department of Food Hygiene and Technology at the Istanbul University - Cerrahpasa Faculty of the Veterinary Medicine under the cold chain considering the contamination risks in the thermo-box.

The Sandwich ELISA immunological method, which uses the R5 antibody, which is accepted to be the most sensitive, was chosen for examination of gluten analysis methods performed in recent years internationally. Gluten-free foods were analyzed with the R5 Sandwich ELISA, R5 ELISA Ridascreen Gliadin method, which is given as the ELISA R5 Mendez Method gluten determination method in the gluten detection method section of the Codex Alimentarius (39). The samples were analyzed by R7001 Ridascreen Gliadin (R-Biopharm AG, Darmstadt, Germany) test kit and R7006 Cocktail solution (R-Biopharm AG, Darmstadt, Germany) and 96% EtOH (Merck) according to supplier's protocol, respectively.

To prepare samples, 40% EtOH was applied to remove possible gliadin contamination of the equipment during analysis of the laboratory's existing work areas and instrument/equipment in the work area. It is the pre and past version of the analysis. Subsequently, if there are packaged products, it needs to be cleaned with 40% EtOH, just in case. Additionally, analysis grade gloves were used. Even when giving numbers to the tubes, contamination was considered.

Finally, result of test was calculated by RIDA SOFT Win Z9999 (Version 1.79, R-Biopharm, Darmstadt, Germany) software.

RESULTS and DISCUSSION

In this study, the presence of gluten was investigated in a total of 90 food samples, including bread, cake, cookie, snacks and crisp, produced and sold in patisseries and bakeries manufacturing in different regions of Istanbul. Information on the region taken, the number of samples, dates of purchase and sample types of the samples taken for analysis is given (Table 1).

It has been determined that the products in the bread category contain 60% gluten between 20-80 ppm and 40% gluten above 80 ppm. Although there is no contamination between 20-80 ppm of the products in the cake category, the presence of gluten contamination at a rate of 5.7% above 80 ppm was detected. It was determined that the products in the cookie category had 18.75% contamination between 20-80 ppm and 15.6% contamination above 80 ppm. Gluten contamination was detected at a rate of 28.5% between 20-80 ppm in the friable category and 14.2% above 80 ppm (Table 3).

In summary, in line with the results of the study, it was determined that 21 (23.31%) of bakery and pastry food products offered for gluten-free consumption contain 20 ppm gluten, which is the legal limit value specified in the Codex Alimentarius [4]. It was determined that 8 (8.8%)

products were between 5-20 ppm, while the other 61 (67.7%) products were below 5 ppm (Tablo 3).

As a result of the examination in terms of legal limit value in terms of main ingredients, 100% of the product containing corn flour, 60% of the product containing buckwheat, 40% of the product containing almond flour, 5% of the product containing hazelnut flour, The legal limit was determined above 20 ppm for 75% of the product, 75% of the product containing chickpea flour, and 100% of the other flour-mixed products. When the main raw materials of gluten-free products containing gluten above the limit value are examined, buckwheat flour at 60%, flour mixture at 100%, chickpea flour at 75%, corn flour at 100%, almond flour at 40%, hazelnut flour at 5%. It was determined that 75% of einkorn flour was used (Table 4).

Table 3. Gluten amounts of samples according to the legal limit

Product Type	Total Product Number	Product Number Over Legal Limit	<5 ppm	5-20 ppm	20-80 ppm	>80 ppm
Bread	5	5	-	-	3	2
Cake	35	2	29	4	-	2
Cookie	32	11	18	3	6	5
Snack	11	-	10	1	-	-
Crispy	7	3	4	-	2	1
Total	90	21 (23.3%)	61 (67.7%)	8 (8.8%)	11 (12.2%)	10 (11.11%)

Table 4. Type and rate of the main component of gluten-free products over the legal limit

Main component	Product number	≥20 ppm	Positive rate (%)
Corn flour	1	1	%100
Buckwheat	5	3	%60
Almond flour	10	4	%40
Hazelnut floır	20	1	%5
Walnut flour	11	-	0
Coconut flour	7	-	0
Einkorn flour	4	3	%75
Chickpea flour	4	3	%75
Mix flour	6	6	%100
Other	22	-	0
Total	90	21	(23.3%)

When the studies conducted were examined, it was found that they were generally correlated with our study. Within the scope of studies conducted in Turkey, gluten contamination was found that 17.5% of 200 gluten-free labeled foods had gluten above the legal limit of 20 ppm. When the mentioned contamination sources are examined, it was determined that it is sourced from food raw materials with 68% buckwheat content, 23% grain mixtures, 6% corn and 3% rice origin [2].

In Finland, 59 natural gluten-free and 24 wheat starch-based products were collected in the market in different years. As a result of the study, in 13 (22%) of 59 natural gluten-free products, it was determined that 11 (45.8%) of 24 wheat-starch-based gluten-free products contain gluten above 20 ppm [5]. According to the study conducted in Brazil, 180 gluten-free food products obtained from 60 different food services were analyzed by ELISA; stated that 2.8% of the samples and at least 6.7% of the food services were contaminated with gluten above the legal limit of 20 ppm [9].

Bustamente et al. (2017) evaluated the gluten content of grain-based gluten-free foods from 1998 to 2016 in their study [43]. The products are divided into 8 categories as flour, breakfast cereal, bakery products, pasta, bread, dough, snacks, and yeasts. 3141 grain-based gluten-free products sold in Spain between 1998-2016 were selected, and the products were divided into 2 subgroups as those with gluten-free logo and without gluten content on the label. Analysis was carried out using 2 different ELISA kits between 1998 and 2016. The Transia Plate Gluten kit, which was extracted with 40% EtOH between 1998 and 2001, and using the ω-gliadin antibody, was used for analysis. Between 2001-2016, Ridascreen R 7001 gliadin test kit and INGEZIM gluten quick kit using R5 antibody were used for analysis. Gluten was detected in 371 of 3141 products. Yeast category was determined as 22.2%, breakfast cereal category with 21.5%, as the categories with the highest contamination. According to another study conducted in Brazil; As a result of the analysis of 130 samples from gluten-free bakery products by ELISA, the gluten contamination rate was determined to be 21.5% [8]. According to a study conducted in India; Gluten contamination was found above the legal limit in 36.7% of the products made using gluten-free flour in 160 food samples offered for sale in the supermarkets. In addition, it was stated that 35.9% of naturally gluten-free flour samples and 85% of oat flour were contaminated with gluten [28]. As a result of the ELISA analysis of 275 food samples with gluten-free labels and 186 food samples without gluten-free labels supplied from supermarkets in the United States of America; It was found that 1.1% and 19.4%, respectively, were contaminated with gluten [32]. In another study, samples were taken from 22 gluten-free grains, seeds and flour containing no gluten-free label was analyzed with the Ridascreen Gliadin sandwich R5 enzyme-linked immunosorbent test. As a result, it was determined that

13 of the 22 samples (59%) were below 5 ppm, in 9 (41%) it ranged between 2.9 - 8.5 ppm, and 7 (32%) of them contained gluten above 20 ppm [34]. Approximately 88% (n:133) of oat samples were found to be contaminated above 20 ppm, after confirming that oat-containing samples obtained from commercial outlets in Canada were heavily contaminated with gluten [20]. As a like our study, Verma et al. (40) analyzed gluten contamination by Sandwich ELISA sensitive to gliadin and prolamins in wheat coated with R5 antibody in 32 gluten-free products in Italy. It was found that 6 (19.4%) products that do not contain gluten due to the nature of the raw materials and 1 (3%) product with the gluten-free logo contain more than 20 ppm of gluten. The contaminations were found in buckwheat, oat, and lentil-based products. Gluten amounts range from 30 ppm to 53 ppm. It has been emphasized that these amounts will cause damage to the intestinal mucosa of celiacs. Moreover, gluten contamination was investigated in simultaneously purchased gluten-free food products to examine the awareness of gluten-related ailments by food preparation and service personnel in Ireland. It has been determined that 2.7% of 260 gluten-free food samples offered for consumption in restaurants contain between 21-100 ppm, 7.7% above 100 ppm and 10% in total contains gluten [26].

Gluten contamination was detected above 20 ppm limit value of 0.5% from 205 gluten-free bread, pasta, pastry, biscuits, pizza, breakfast cereals and foods covered with breadcrumbs collected in four countries, including Italy, Spain, Norway, and Germany [11]. Furthermore, 78 gluten-free labeled samples from the United States were analyzed using the gliadin competitive enzyme-linked immunosorbent assay. While 48 (61.5%) samples were lower than 10 ppm for gluten; 16 samples (20.5%) were found to contain 20 ppm and above gluten ranging from 20.3 to 60.3 ppm. It was stated that gluten content in 5 of 8 breakfast cereal samples was above 20 ppm [22]. To verify whether the gluten content of several commercial food products sold in Brazil comply with the labeling, Méndez ELISA R5 sandwich method analyzed 437 samples; it has been stated that it is 70% gluten-free, contains 26% gluten and is not labeled for gluten at 4% [25].

Another study was conducted on 84 food samples including 52 labeled gluten-free foods (L-GFF) and 32 naturally gluten-free foods (N-GFF), regarding six various categories (cake, cookies and cakes, baker`s yeast, dried vegetables, dried fruits, and cereals). To determine their gluten content, samples were analyzed using a sandwich enzyme immunosorbent assay (R5 ELISA Ridascreen® gliadin Mendez), considering 20 mg/kg (ppm) as the contamination limit. As a result of the test, the contamination rate was 23.8% (L-GFF: 21.9%, N-GFF: 25%). The cake, cookies, and baker's yeast products did not result in any contamination. The contamination rate was 5.3% in dried vegetables, 25% in dried fruits, and 42.1% in cereals. However, all oat samples were the most contaminated food. L-GFF locally manufactured were more often contaminated than those imported (28.6 vs. 16.7%) [41].

Verma et al. (42) investigated the gluten variety with the Ridascreen R-7001 Gliadin Sandwich ELISA kit in products with gluten production and gluten-free logo in the markets in Italy, as a continuation of the previous study of them (41). Between April and October 2016, the most preferred 200 product purchases from the markets in Ancona, Italy, are the two-component sections with the gluten-free logo and gluten-free products by nature. 93 products with the gluten-free logo and 107 gluten-free products were collected. According to analysis, more than 20 ppm of gluten was found in 1% of the guidelines with the gluten-free logo, in 8% of the gluten-free guidelines, and in 9% of a total of 200 manufacturers. It has been stated that gluten contamination is found in gluten-free products in Italy.

Table 5. Comparison of gluten contamination results for some countries with Turkey

	Collin et al. [5]	Lee et al. [22]	Mattioni et al. [25]	Sharma et al. [32]	Verma et al. [40]	Verma et al. [42]	Bustamente et al. [43]	Guennouni et al. [41]	Our study
Country	Finland	USA	Brazil	USA	Italy	Italy	Spain	Morocco	Turkey
Number of labeled gluten-free	83	78	437	275	32	93	3141	84	90
Number of gluten-free over the limit	28.9%	20.2%	30%	1.1%	3%	1%	12%	42.1%	23.3%

CONCLUSION

Individuals with gluten sensitivity prefer foods such as bread, crackers, biscuits, and cereals as the basic carbohydrate source that should be taken daily in a healthy diet. To produce healthy and reliable gluten free products, cross contamination prevention and control plans should be implemented from production to consumption. The gluten-free product production line should be separated. Food personnel should be educated. Additionally, packaging should be in appropriate features and during storage, packaging, and transportation. Direct/indirect contact with gluten-containing products should be prevented during harvest, preparation and serving.

REFERENCES

[1] Asri, N., Rostami-Nejad, M., Barzegar, M., Nikzamir, A., Rezaei-Tavirani, M., Razzaghi, M.,

Reza Zali, M. (2020). Suppressive mechanisms induced by Tregs in celiac disease. *Iranian Biomedical Journal,* 24(3), 140-147.

[2] Atasoy, G., Gökhisar, Ö.K., Turhan, M. (2019). Gluten contamination in manufactured gluten-free foods in Turkey. *Food Additives & Contaminants: Part A*, 37(3), 363-373.

[3] Barak, S., Mudgil, D., Khatkar, B.S. (2014). Biochemical and functional properties of gliadins: A review. *Critical Reviews in Food Science and Nutrition*, 357-368.

[4] Codex Alimentarius, (2008). Codex Standard for foods for special dietary use for persons intolerant to gluten, CODEX STAN 118-1979 (revised 2008, amendment 2015).

[5] Collin, P., Thorell, L., Kaukinen, K., Maki, M. (2004). The safe threshold for gluten contamination in gluten-free products. Can trace amounts be accepted in the treatment of coeliac disease? *Alimentary Pharmacology and Therapeutics,* 19(12), 1277-1283.

[6] Day, L., Augustin, M.A., Batey, I.L. (2006). Wheat-gluten uses and industry needs. *Trends in Food Science & Technology*, 17(2), 82-90.

[7] Ewart, J.A.D. (1979). Glutenin structure. *Journal of Science of Food and Agriculture*, 30, 482-492.

[8] Farage, P., de Medeiros Nobrega, Y.K., Pratesi, R., Gandolfi, L., Assuncao, P., Zandonadi, R.P. (2017). Gluten contamination in gluten-free bakery products: a risk for coeliac disease patients. *Public Health Nutrition,* 20(3), 413-416.

[9] Farage, P., Puppin Zandonadi, R., Cortez Ginani, V., Gandolfi, L., Yoshio Nakano, E., Pratesi, R. (2018). Gluten-free diet: From development to assessment of a checklist designed for the prevention of gluten cross-contamination in food services. *Nutrients,* 10(9), 1274.

[10] Falcomer, A.L., Santos Araújo, L., Farage, P., Santos Monteiro, J., Yoshio Nakano, E., Puppin Zandonadi, R. (2020). Gluten contamination in food services and industry: A systematic review. *Critical Reviews in Food Science and Nutrition*, 60(3), 479-493.

[11] Gibert, A., Kruizinga, A.G., Neuhold, S., Houben, G.F., Canela, M.A., Fasano, A., Catassi, C. (2013). Might gluten traces in wheat substitutes pose a risk in patients with celiac disease? A population-based probabilistic approach to risk estimation [corrected] [Published erratum appears.]. *American Journal of Clinical Nutrition*, 97(1), 109-116.

[12] Gallagher, E., Gormley, T.R., Arendt, E.K. (2004). Recent advances in the formulation of gluten free cereal-based products. *Trends in Food Science and Technology*, 15, 143-152.

[13] Golley, S., Corsini, N., Topping, D., Morell, M., Mohr, P. (2014). Motivations for avoiding wheat consumption in Australia: results from a population survey. *Public Health Nutrition*, 18(3), 490-499.

[14] Gomez, A., Ferrero, C., Calvelo, A., Anon, M.C., Puppo, M.C. (2011). Effect of mixing time on structural and rheological properties of wheat flour dough for breadmaking. *International Journal of Food Properties*, 14, 583-598.

[15] Gaesser, G.A., Angadi, S.S. (2012). Gluten-free diet: Imprudent dietary advice for the general population? *Journal of the Academy of Nutrition and Dietetics on Science Direct*, 112(9), 1330-1333.

[16] Kagnoff, M.F. (2007). Celiac disease: pathogenesis of a model immunogenetic disease. *The Journal of Clinical Investigation,* 117(1), 41-49.

[17] Khatkar, B.S., Bell, A.E., Schofield, J.D. (1995). The dynamic rheological properties of glutens and gluten subfractions from wheats of good and poor bread-making quality. *Journal of Cereal Science*, 22, 29-44.

[18] Khatkar, B.S., Fido, R.J., Tatham, A.S., Schofield, J.D. (2002). Functional properties of wheat gliadins: 2. Effects on dynamic rheological properties of wheat gluten. *Journal of Cereal Science*, 35, 307–313.

[19] Khatkar, B.S., Fido, R.J., Tatham, A.S., Schofield, J.D. (2002). Functional properties of wheat gliadins: 1. Effects on mixing characteristics and bread making quality. *Journal of Cereal Science*, 35, 299–306.

[20] Koerner, T.B., Cleroux, C., Poirier, C., Cantin, I., Alimkulov, A., Elamparo, H. (2011). Gluten Contamination in the Canadian commercial oat supply. *Food Additives & Contaminants: Part A,* 28(6), 705-710.

[21] Lee, A., Newman, J.M. (2003). Coeliac diet: Its impact on quality of life. *Journal of the American Dietetic Association*, 103(11), 1533-1535.

[22] Lee, H.J., Anderson, Z., Ryu, D. (2014). Gluten contamination in foods labeled as 'gluten free' in the United States. *Journal of Food Protection*, 77(10), 1830-1833.

[23] Ludvigsson, J., Green, P. (2011). Clinical management of celiac disease. *Journal of Internal Medicine,* 269, 560-571.

[24] Malekzadeh, R., Sachdev, A., Ali, A.F. (2005). Coeliac disease in developing countries: Middle East, India and North Africa. *Best Practice & Research Clinical Gastroenterology,* 19(3), 351-358.

[25] Mattioni, B., Scheuer, P.M., Antunes, A.L., Paulino, N., De Francisco, A. (2016). Compliance with Gluten-Free labelling regulation in the Brazilian food industry. *Cereal Chemistry*, 93(5), 518-522.

[26] McIntosh, J., Flanagan, A., Madden, N., Mulcahy, M., Dargan, L., Walker, M., Burns, D.T. (2011). Awareness of coeliac disease and the gluten status of 'Gluten-Free' food obtained on request in catering outlets in Ireland. *International Journal of Food Science & Technology,* 46(8), 1569-1574.

[27] O'Neill, J. (2011). Gluten–free foods: Trends, Challenges and Solutions. *Cereal Foods*, 55, 220-223.

[28] Raju, N.R., Joshi, A.K., Vahini, R., Deepika, T., Bhaskarachari, K., Devindra, S. (2020). Gluten contamination in labelled and naturally gluten-free grain products in southern India. *Food Additives & Contaminants Part A Chemistry Analysis Control Exposure & Risk Assessment*, 37(4), 531-538.

[29] Rostami-Nejad, M., Taraghikhah, N., Ciacci, C., Pourhoseingholi, M.A., Barzegar, F., Rezaei-

Tavirani, M., Aldulaimi, D., Reza Zali, M. (2020). Anxiety symptoms in adult celiac patients and the effect of a gluten-free diet: an Iranian Nationwide study. *Inflammatory Intestinal Diseases,* 5(1), 42-48.

[30] Sapone, A., Bai, J.C., Ciacci, C., Dolinsek, J., Green, P.H., Hadjivassiliou, M., Kaukinen, K., Rostami, K., Sander, S.D., Schumann, M., Ullrich, R., Villalta, D., Volta, U., Catassi, C., Fasano, A. (2012). Spectrum of gluten-related disorders: consensus on new nomenclature and classification. *BMC Medicine*, 10(1), 1-12.

[31] Sharma, N., Bhatia, S., Chunduri, V., Kaur, S., Sharma, S., Kapoor, P., Kumari, A., Garg, M. (2020). Pathogenesis of celiac disease and other gluten related disorders in wheat and strategies for mitigating them. *Frontiers in Nutrition*, 7.

[32] Sharma, G.M., Pereira, M., Williams, K.M. (2015). Gluten detection in foods available in the United States–A market survey. *Food Chemistry,* 169(15), 120-126.

[33] TGKY, (2012). Turkish Food Codex, Communiqué on Foods Suitable for Individuals with Gluten Intolerance No: 2012/4, R.G. Number: 28163.

[34] Thompson, T., Lee, A.R., Grace, T. (2010). Gluten contamination of grains, seeds, and flours in the United States: A pilot study. *Journal of the American Dietetic Association*, 110(6), 937-940.

[35] Weiser, H. (2007). Chemistry of glutenin proteins. *Food Microbiology*, 24, 115-119.

[36] Mendez Xiomara, P., Una Antonio, J., Vega-Fernandez, S., Santos Angeles, M. (2022). The Ability of the yeast *Wickerhamomyces anomalus* to hydrolyze immunogenic wheat gliadin proteins. Foods, 11, (4105).

[37] Taraghikhah, N., Ashtari, S., Asri, N., Shahbazkhani, B., Al-Dulaimi, D., Rostami-Nejad, M., Rezaei-Tavirani, M., Razzaghi, M.R., Zali, M.R. (2020). An updated overview of spectrum of gluten-related disorders: Clinical and diagnostic aspects. *BMC Gastroenterology*, 20 (258).

[38] Dizlek, H. (2013). Gluten kompleksinin hamur ve ekmek nitelikleri üzerindeki etkileri. *Akademik Gida*, 11(1) (2013) 102-106.

[39] Codex Alimentarius (2008). Standard For Foods for Special Dietary Use For Persons Intolerant To Gluten, *Codex Standard 118-1979*, (revised 2008, amendment 2015).

[40] Verma, A.K., Gatti, S., Galeazzi, T., Monachesi, C, Padella, L, Giada Del, B. (2016). Detection of gluten content in the naturally gluten free and 'gluten free' labelled commercially available food products in Italy. *Digestive and Liver Disease*, 48, 279.

[41] Guennouni, M., Elmoumou, L., Admou, B., Hazime, R., Elkhoudri, N., Hakmaoui, A., Bourrahouat, A., Hilali, A. (2022). Detection of gluten content in both naturally and labelled gluten-free products available in Morocco. *Journal of Consumer Protection and Food Safety*, 17, 137-144.

[42] Verma, A.K., Gatti, S., Galeazzi, T., Monachesi, C, Padella, L, Giada Del, B., (2017). Gluten contamination in naturally or labelled gluten free products marketed in Italy. *Nutrients*, 9, 115.

[43] Bustamante, M.A., Fernandez-Gil, M.P., Churruca, I., Miranda, J., Lasa, A., Navarro, V. (2017). Evolution of gluten content in cereal-based gluten-free products: An overview from 1998 to 2016. *Nutrients,* 9, 21.

SİDAS MEDYA
Akademik Gıda®
ISSN Online: 2148-015X
https://dergipark.org.tr/tr/pub/akademik-gida

Akademik Gıda 21(2) (2023) 158-166, DOI: 10.24323/akademik-gida.1350972

Araştırma Makalesi / Research Paper

Trabzon Ekmeği Ekşi Hamurlarının Bazı Fizikokimyasal ve Mikrobiyolojik Özellikleri

Merve Yurttaş[1], Nevzat Şahin[2], Ahmet Hilmi Çon[3]

[1]Amasya Üniversitesi, Sağlık Bilimleri Fakültesi, Beslenme ve Diyetetik Bölümü, Amasya
[2]Ondokuz Mayıs Üniversitesi, Fen Edebiyat Fakültesi, Moleküler Biyoloji ve Genetik Bölümü, Samsun
[3]Ondokuz Mayıs Üniversitesi, Mühendislik Fakültesi, Gıda Mühendisliği Bölümü, Samsun

Geliş Tarihi (Received): 21.04.2022, Kabul Tarihi (Accepted): 04.07.2023
Yazışmalardan Sorumlu Yazar (Corresponding author): ahmeth.con@omu.edu.tr (A.H. Çon)
0 362 312 1919 0 362 457 6035

ÖZ

Ekşi hamur ekmeği ülkemizde laktik asit bakterileri ve mayaların görev aldığı fermantasyon yöntemiyle üretilen geleneksel bir ekmek çeşididir. Fermantasyon boyunca ekşi hamur ekmeğinin teknolojik özellikleri gelişmekte, karakteristik tat ve aroması oluşmaktadır. Mineral biyoyararlanımı artan, raf ömrü uzayan ve glisemik indeksi düşen ekşi hamur ekmeği tüketici tarafından arzu edilen ekmek özelliğini taşımaktadır. Sonuçta, ekşi hamur ekmeğinin üretimi her geçen gün yaygınlaşmaktadır. Bu çalışmada; yaz ve kış döneminde Orta ve Doğu Karadeniz bölgelerinden elde edilen 54 adet ekşi hamur örneğinin bazı fizikokimyasal ve mikrobiyolojik özellikleri araştırılmıştır. Örneklerin pH değerinin 3.30-5.43, asitlik derecesi, toplam asitlik, kuru madde ve kül içeriklerinin sırasıyla 2.50-20.50, %0.23-1.79, %49.05-65.91 ve %0.34-0.95 arasında değiştiği belirlenmiştir. Ekşi hamur örneklerinin laktik asit (LAB), toplam aerobik mezofilik bakteri (TAMB) ve maya sayısı sırasıyla 3.65-8.97 log KOB/g, 4.19-7.20 log KOB/g, 4.17-7.52 log KOB/g olarak belirlenmiştir. Örneklerin asitlik ve pH düzeylerinin yöresel, mevsimsel ve fırınlar arasında önemli farklılıklara sahip olduğu tespit edilmiştir. Mikroorganizma sayısı, kuru madde ve kül miktarları analiz edilen örneklerin bu değerler bakımından fırınlar arasında önemli farklılıklara sahip olduğu saptanmıştır. Bu sonuçlar, Orta ve Doğu Karadeniz bölgelerinde ekşi hamur ekmeği üretiminde standardizasyonun olmadığını göstermektedir.

Anahtar Kelimeler: Ekşi hamur, Ekmek, Mikrobiyolojik özellikler, Teknolojik özellikler

Some Physicochemical and Microbiological Properties of Trabzon Bread Sourdoughs

ABSTRACT

Sourdough bread is a traditional bread form produced in Türkiye using the fermentation method of lactic acid bacteria and molds. Through fermentation, the technological characteristics of sourdough bread are developed, resulting in the emergence of its characteristic taste and aroma. This process also inreases mineral bioavailability, extends shelf life and decreases glisemic index, making it highly desirable for consumers. As a result, the production of sourdough bread has become increasingly widespread. This study examines the physicochemical and microbiological characteristics of 54 samples of sourdough obtained from the Middle and Eastern Black Sea regions during summer and winter periods. It was determined that the pH values of the samples ranged from 3.30 to 5.43, and while the degree of acidity, total acidity, dry matter and ash contents ranges from 2.50 to 20.50, 0.23 to 1.79%, 49.05 to 65.91%, and 0.34 to 0.95%, respectively. The number of lactic acid bacteria (LAB), total aerobic mesophilic bacteria and molds of the sourdough samples were found to be in the range from 3.65 to 8.97 log CFU/g, 4.19 TO 7.20 log CFU/g and 4.17 to 7.52 log CFU/g, respectively. It was determined that the samples' acidity and pH levels had significant differences between regions, seasons and different bakeries. The number of microorganisms, dry matter and ash quantities of samples showed significant differences among bakeries in terms of these values. These results

indicated a lack of standardization in the production of sourdough bread in the regions of the Middle and East Black Sea regions.

Keywords: Sourdough, Bread, Microbiological properties, Technological properties

GİRİŞ

Ekmek, ülkemizde en çok tüketilen tahıl ürünlerinden birisidir [1]. Ekmeğin enerji verici, ucuz, vitamin ve mineral içeriğinin zengin olması onu temel besin kaynağımız haline getirmektedir [2]. Asırlar boyunca varlığı bilinen [3] ekşi hamur ekmeği; un tipi, uygulanan teknoloji, fermantasyon işlemindeki farklılıklar nedeniyle çeşitlilik göstermektedir [4]. Son yıllarda katkısız, besin değeri yüksek ve uzun raf ömrüne sahip ürünlere olan talep artışı, spontane fermantasyondan dolayı aromatik tat ve uzun raf ömrüne sahip olan ekşi hamur ekmeğine olan talebi de artırmıştır [5-8].

Ekşi hamur, un ve su karışımının laktik asit bakterileri ve mayaların fermantasyonu sonucu elde edilen bir hamur çeşididir [9-12]. Ekmeğe karakteristik özelliğini veren mikrobiyotasındaki LAB ve mayalar ile bu mikroorganizmalar arasındaki interaksiyonlardır [13]. Fermantasyonla üretilen organik asitler, enzimler, eksopolisakkaritler gibi çeşitli metabolitler ekmeğe özgü tat ve aroma kazandırmakta, ekmeğin hacim ve tekstürünü iyileştirmekte ve raf ömrünü uzatmaktadır [14-16]. Buğdayda fosforun depo halinde bulunma formu olması dolayısıyla ekmekte de bulunan; Ca, Mg, Cu, Fe, Zn, Mn gibi önemli mineralleri bağlayarak vücut tarafından emilimini engelleyen fitik asit, fermantasyonla parçalanmakta ve ekmeğin besin değeri artmaktadır [17]. Ayrıca fermantasyon, organik asitler ve diğer metabolitlerin oluşumuyla birlikte nişasta emilimini azaltarak ekmeğin glisemik indeksini de düşürmektedir [18]. Yapılan çalışmalar ekşi hamur yöntemiyle üretilen ürünlerin glüteni parçaladığını ve bu sebeple çölyak hastaları tarafından da tüketilebileceğini belirtmektedir [19-21]. Glisemik indeksi düşük, besin değeri yüksek, katkısız, uzun raf ömrüne sahip olması bu ekmeğin tercih edilirliğini artırmaktadır. Bu çalışmada, Karadeniz bölgesi taranarak ekşi hamur ekmeği üreten fırınlardan alınan ekşihamur örneklerinin bazı fizikokimyasal ve mikrobiyolojik özellikleri ile mevsimsel değişimlerinin belirlenerek standardizasyon durumlarının aydınlatılması hedeflenmiştir.

MATERYAL ve METOT

Materyal

Kış ve yaz dönemi ekşi hamur örnekleri Ocak-Şubat ve Temmuz-Ağustos ayları içerisinde Trabzon, Giresun, Ordu ve Samsun illerinde ekşihamur ekmeği (Trabzon ekmeği) üretimi yapan toplam 27 farklı fırından alınmış ve 5^{o}C'de 8 saat içerisinde laboratuara taşınarak bekletilmeksizin analiz edilmiştir.

Metot

Mikrobiyolojik Analizler

Mikrobiyolojik sayım için 10 g ekşi hamur örneği 90 ml peptonlu fizyolojik su ile homojenize edilmiş ve 10^{-7}'ye kadar dilusyonları hazırlanmıştır. LAB sayımı %0.01 siklohekzimid ilaveli MRS-5 agarda (MRS-5C) 30^{o}C sıcaklıkta %5 karbondioksit (CO_2) içeren ortamda 48 saat; TAMB sayımı Plate Count Agar (PCA) (Merck 1.05463) ortamında 30°C'da 48 saat; maya sayımı da Dichloran Rose Bengal Chloramphenicol (DRBC) Agar ortamında (Merck 1.00466) 28-30^{o}C sıcaklıkta 2 gün inkübasyon sonunda tespit edilmiştir [22-24].

Fizikokimyasal Analizler

Örneklerin pH değeri, katı pH metre elektrotu (Hanna HI4221) kullanılarak her ekşi hamur örneği için 3 farklı bölgeye batırılarak saptanmış ve ortalamaları alınmıştır. Örneklerin toplam asitlik ve asitlik derecesi analizleri için Gül [25], kurumadde ve kül analizleri için ise Anonim [26] tarafından verilen yöntem kullanılmıştır [25, 26].

İstatistiksel Analiz

Fizikokimyasal ve mikrobiyolojik analizler yaz ve kış dönemi ile iller (Samsun, Trabzon, Ordu, Giresun) olmak üzere 2x4 faktöriyel düzende deneme planına göre 2 tekerrürlü olarak yürütülmüştür. Elde edilen veriler için SPSS 22 paket programı kullanılmış, örnekler arasındaki farkı karşılaştırmak amacıyla Duncan testi uygulanmıştır ($P<0.05$).

BULGULAR ve TARTIŞMA

Karadeniz bölgesinde ekşi hamur ekmeği (Trabzon ekmeği) üretimi yapan 27 farklı fırından kış ve yaz döneminde alınan toplam 54 ekşi hamur örneğinin bazı özellikleri belirlenmiş ve elde edilen sonuçlar Tablo 1'de verilmiştir.

Karadeniz Bölgesi ekşi hamur örneklerinin kış döneminde 4.08-5.43 pH, yaz döneminde de 3.30-4.48 pH arasında değişen pH sonuçları arasındaki farklılık ekşi hamur mayası kullanım oranı, fermantasyon süresi ve ekşi hamur florasındaki LAB türlerinin muhtemel farklılığına bağlanmıştır. Yaz döneminde kış dönemine göre belirgin şekilde daha düşük belirlenen pH değeri ise fırınlarda iklimlendirme sistemleri olmaması nedeni ile mevsimsel sıcaklık farklılığına bağlanabilir.

Tablo 1. Karadeniz bölgesi ekşi hamurlarının bazı fizikokimyasal özellikleri
Table 1. Some physicochemical properties of Black Sea region sourdoughs

İl	İlçe	Kış Dönemi					Yaz Dönemi				
		pH	Asit Derecesi	Toplam Asit (%)	Kuru Madde (%)	Kül (%)	pH	Asit Derecesi	Toplam Asit (%)	Kuru Madde (%)	Kül (%)
Samsun	Ondokuzmayıs	4.47	7.25	0.64	54.87	0.85	4.09	8.75	0.74	56.5	0.68
	Ondokuzmayıs	4.95	5.00	0.45	52.83	0.83	3.76	8.50	0.79	55.1	0.62
	Bafra	4.60	6.25	0.50	53.30	0.95	4.48	4.50	0.41	54.9	0.59
	Merkez	4.75	4.00	0.45	52.51	0.64	4.15	5.50	0.56	55.7	0.55
	Tekkeköy	5.33	2.50	0.26	52.98	0.79	3.82	7.25	0.76	59.1	0.50
	Çarşamba	4.36	3.00	0.81	65.91	0.66	3.73	11.25	0.98	59.1	0.62
	Terme	5.43	2.50	0.23	57.38	0.61	3.30	20.50	1.79	61.0	0.65
	Ortalama	**4.84**	**4.36**	**0.48**	**55.69**	**0.76**	**3.90**	**9.46**	**0.86**	**57.3**	**0.60**
Giresun	Espiye	5.21	4.00	0.25	49.59	0.63	4.29	4.00	0.38	60.58	0.54
	Görele	4.57	7.00	0.33	52.98	0.48	3.81	5.75	0.54	51.23	0.49
	Çavuşlu	4.18	7.75	0.54	53.32	0.63	3.82	9.25	0.93	52.64	0.53
	Çavuşlu	4.44	6.25	0.71	51.95	0.65	4.23	5.75	0.67	54.33	0.62
	Piraziz	4.76	6.00	0.61	53.94	0.54	3.54	7.50	0.71	61.60	0.48
	Bulancak	4.44	6.25	0.66	52.93	0.57	4.24	6.00	0.37	55.31	0.55
	Merkez	4.46	6.25	0.66	58.08	0.65	3.74	4.50	0.52	62.17	0.50
	Ortalama	**4.58**	**6.21**	**0.54**	**53.26**	**0.59**	**3.95**	**6.11**	**0.59**	**56.84**	**0.53**
Trabzon	Beşikdüzü	4.33	5.50	0.40	58.66	0.67	3.40	7.75	0.85	56.20	0.61
	Vakfıkebir	4.32	7.00	0.40	52.83	0.60	4.07	5.00	0.62	54.40	0.59
	Vakfıkebir	4.36	5.75	0.45	53.36	0.56	3.76	3.25	0.83	57.13	0.34
	Vakfıkebir	4.14	7.75	0.53	56.67	0.55	3.55	6.75	0.85	58.17	0.54
	Akçaabat	4.61	8.00	0.49	50.27	0.88	3.39	10.00	1.04	49.05	0.64
	Akçaabat	4.47	4.50	0.35	54.12	0.66	3.95	4.75	0.61	52.94	0.59
	Merkez	4.84	5.00	0.23	52.68	0.54	3.63	5.00	0.76	51.88	0.61
	Merkez	5.06	3.50	0.26	54.00	0.57	4.05	4.75	0.57	52.74	0.56
	Ortalama	**4.52**	**5.88**	**0.39**	**54.07**	**0.63**	**3.72**	**5.91**	**0.77**	**54.06**	**0.56**
Ordu	Ünye	4.68	6.25	0.51	52.31	0.57	3.37	9.75	0.75	54.78	0.54
	Fatsa	4.08	8.25	0.88	54.18	0.63	3.86	6.50	0.63	55.06	0.48
	Bolaman	4.48	5.50	0.59	59.11	0.45	3.41	9.25	0.91	57.99	0.57
	Merkez	4.28	7.75	0.66	56.95	0.59	4.33	4.00	0.40	54.26	0.56
	Gülyalı	4.21	7.50	0.76	52.51	0.69	4.12	3.00	0.33	59.16	0.53
	Ortalama	**4.35**	**7.05**	**0.68**	**55.01**	**0.59**	**3.82**	**6.50**	**0.60**	**56.25**	**0.54**
Genel Ortalama		**4.58**	**5.83**	**0.51**	**54.47**	**0.64**	**3.85**	**6.99**	**0.71**	**56.0**	**0.56**

Kotancılar ve ark. [27] tarafından, Trabzon ekmeğinin ekşi hamur katkısı arttıkça ve fermantasyon süresi uzadıkça ekmeklerin pH değerlerinin önemli derecede azaldığı, süreye bağlı olarak 4.85-5.34 pH arasında değişim gösterdiği saptanmıştır. Ekşi hamurların pH değerinin Yağmur [28] tarafından 3.77-5.44 pH; Manini ve ark. [29] tarafından 4.1-5.6 pH; Rizzello ve ark. [30] tarafından 4.26-4.47 pH; Plessas ve ark. [31] tarafından 4.3-4.7 pH; Galli ve ark. [32] tarafından 4.03-4.04 pH; Gerçekaslan ve ark. [33] tarafından 4.06-4.50 pH arasında değiştiği bildirilmiştir.

Bu veriler çalışma sonuçlarından özellikle kış döneminde elde edilen sonuçlar ile uyumludur. Bununla birlikte pH sonuçlarını yaz dönemi çalışma sonuçlarına benzer şekilde daha düşük olarak bildiren literatürler de bulunmaktadır. Örneğin ekşi hamurun pH değerinin, Gül ve ark. [34] 3.65-5.55 pH; Spicher ve ark. [34] 3.8-4.4 pH; Şimşek [35] 3.50-3.94 pH; Çetin-Babaoğlu ve ark. [37] 3.88-6.26 pH; Meroth ve ark. [38] 3.4-3.9 pH; Pepe ve ark. [39] 3.5-4.3 pH; Kömen [40] 3.84-3.52 pH; Akgün [41] 3.86-4.16 pH; Minervini ve ark. [42] 3.7-4.28 pH; Paramithiotis ve ark. [43] 3.35-6.5 pH; Vrancken ve ark. [44] 3.3-3.7 pH; Lhomme ve ark. [9] 3.23-4.01 pH; Dertli ve ark. [45] 3.37-3.95 pH; Yıldız ve ark. [46] 3.69-4.15 pH; Örü ve Hendek Ertop [47] 3.27-4.89 pH; Liu ve ark. [48] 3.89-4.55 pH; Fraberger ve ark. [11] 3.69-4.36 pH arasında değiştiğini ve Franco ve ark. [49] 4.0 pH olduğunu belirlemiştir [9, 11, 27, 28, 29, 30, 31, 32, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49]

Ekşi hamur örneklerinin kış döneminde asitlik derecesi değeri 2.50-8.25 arasında değişmiş ve ortalama 5.83 olarak; toplam asitlik değeri de %0.23-0.88 arasında değişmiş ve ortalama %0.51 olarak belirlenmiştir. Yaz döneminde ise asitlik derecesi 3.00-20.50 arasında değişmiş ve ortalama 6.99 olarak; toplam asitlik değeri de %0.33-1.79 arasında değişmiş ve ortalama %0.71 olarak belirlenmiştir. Ekşi hamurların asitlik derecesi değeri, Meroth ve ark. [38] tarafından 4.4-20.5; Şimşek [35] tarafından 7.6-19.3; Yağmur [28] tarafından 4.03-14.63; Gül ve ark. [34] tarafından 4.2-14.0 Manini ve ark. [29] tarafından 4.4 ve 10.2; Lhomme ve ark. [9] tarafından 8.3-22.1; Rizzello ve ark. [30] tarafından 8.9-11.5; Plessas ve ark. [31] tarafından 7.1-11; Menezes ve ark. [50] tarafından 1.40-12.63 arasında değiştiği; Kömen [40] tarafından da ekşihamurların 24 saatlik fermantasyon sonunda 7.17-12.63, 48 saatlik fermantasyon sonunda 13.49-17.34 arasında bulunduğu bildirilmiştir. Literatürde verilen aralık değerlerin bir kısmı yüksek olmakla birlikte çalışmada belirlenen asitlik derecelerinin büyük kısmı verilen aralıklar içerisindedir. Çetin-Babaoğlu ve ark. [37] tarafından bildirilen toplam asitlik değeri de (%0.24-0.85 arasında) kış mevsim sonuçları ile tam bir uyum içerisindedir [9, 28, 29, 30, 31, 34, 36, 38, 40, 50].

Ekşi hamur örneklerinin kuru madde oranları kış döneminde %49.59-65.91 arasında değişmiş ve ortalama %54.47; kül oranları da %0.45-0.95 arasında değişmiş ve ortalama %0,64 olarak belirlenmiştir. Yaz döneminde de kuru madde oranları %49.05-62.17 arasında değişmiş ve ortalama %56.05 olarak; kül oranları da %0.34-0.68 arasında değişmiş ve ortalama % 0.56 olarak belirlenmiştir. Ekşi hamurların kuru madde içerikleri Gül ve ark. [34] tarafından %38.3-61.3 (ort. %53.02), Şimşek [35] tarafından %43.0-58.6 (ort. %50.4) ve Yağmur [28] tarafından %52.48-60.11 arasında belirlenmiştir. Literatür verileri çalışma sonuçları ile uyumludur. Kül miktarının ise ekşi hamur ekmeklerinde Rizzello ve ark. [30] tarafından %0.9-1.0, Çağlıyan [51] tarafından %0.92-1.43 arasında olduğu;

Ogunsakin ve ark. [52] tarafından da *Pediococcus pentosaceus* içeren sorgum ekşi hamur ekmeğinde %1.5 olarak belirlendiği bildirilmiştir. Bu değerler elde ettiğimiz verilerden yüksektir. Bu sonuç ekmek yapımında kullanılan materyalin farklılığı ile birlikte bu sonuçların ekmekte elde edilmesine bağlanmıştır [34, 36, 28, 30, 51, 52].

Karadeniz Bölgesi ekşi hamurlarının kış ve yaz dönemindeki bileşim farklılıkları il bazında değerlendirilmiş ve sonuçlar Tablo 2'de verilmiştir. İllere göre yapılan değerlendirmede pH değerleri açısından kış dönemi ekşi hamurları içerisinde Samsun örneklerinin en yüksek, Ordu örneklerinin en düşük pH değerine sahip olduğu ve istatistiki olarak da birbirlerinden farklı bulundukları belirlenmiştir (P<0.05). Yaz dönemi örnekleri arasında ise iller arasında istatistiki olarak önemli bir fark bulunmamıştır (P>0.05). Asitlik derecesi ise kış döneminde en yüksek Ordu örneklerinde belirlenmiş ve bunu sırası ile Giresun ve Trabzon örnekleri takip etmiştir. En düşük asitlik derecesi değeri ise Samsun örneklerinde saptanmıştır. Asitlik derecesinde Samsun örneklerinin Giresun ve Ordu örneklerden farklı bulunduğu (P<0.05) saptanmıştır. Yaz döneminde ise en yüksek asitlik derecesi Samsun örneklerinde belirlenmiş bunu sırası ile Ordu, Giresun ve Trabzon örnekleri takip etmiştir. Fakat aralarındaki fark istatistiki olarak önemsiz bulunmuştur (P>0.05).

Tablo 2. Ekşi hamurun bazı fizikokimyasal özelliklerinin illere göre değişimi*
Table 2. Variation of some physicochemical properties of sourdough according to provinces

Sezon	İl	N	pH Değeri	Asitlik Derecesi	Toplam Asitlik (%)	Kuru Madde (%)	Kül (%)
Kış Dönemi	Samsun	7	4.84±0.41^{a}	4.36±1.88^{b}	0.48±0.20^{b}	55.68±4.82^{a}	0.76±0.13^{a}
	Giresun	7	4.58±0.33^{ab}	6.21±1.15^{a}	0.54±0.18^{ab}	53.26±2.55^{a}	0.59±0.06^{b}
	Trabzon	8	4.52±0.30^{ab}	5.88±1.59^{ab}	0.39±0.11^{b}	54.07±2.57^{a}	0.63±0.11^{b}
	Ordu	5	4.35±0.24^{b}	7.05±1.14^{a}	0.68±0.14^{a}	55.01±2.95^{a}	0.59±0.09^{b}
	Ortalama	27	4.59±0.36	5.79±1.71	0.50±0.18	54.45±3.30	0.65±0.12
Yaz Dönemi	Samsun	7	3.90±0.38^{a}	9.46±5.35^{a}	0.86±0.45^{a}	57.39±2.38^{a}	0.60±0.06^{a}
	Giresun	7	3.95±0.30^{a}	6.11±1.78^{a}	0.59±0.20^{a}	56.84±4.52^{a}	0.53±0.05^{a}
	Trabzon	8	3.73±0.28^{a}	5.91±2.15^{a}	0.77±0.16^{a}	54.06±3.02^{a}	0.56±0.09^{a}
	Ordu	5	3.82±0.42^{a}	6.50±3.03^{a}	0.60±0.24^{a}	56.25±2.18^{a}	0.54±0.04^{a}
	Ortalama	27	3.85±0.33	6.99±3.50	0.71±0.29	56.05±3.33	0.56±0.07

*: İstatistiki değerlendirme kış ve yaz dönemi örneklerinde kendi içerisinde yapılmıştır. Sütunlarda farklı harfle işaretlenen örnekler arasındaki fark önemlidir.
**: Statistical evaluation was made within the winter and summer period samples. The difference between examples marked with different letters in the columns is significant*

Örneklerin toplam asitlik değeri de kış döneminde en yüksek Ordu örneklerinde belirlenmiş ve bunu sırası ile Giresun, Samsun ve Trabzon örnekleri takip etmiştir. Trabzon ve Samsun ekşi hamurlarının toplam asitlik değerinin istatistiki olarak da Ordu örneklerinden farklı bulunduğu (P<0.05) belirlenmiştir. Yaz döneminde ise toplam asitlik değeri en yüksek Samsun İli örneklerinde belirlenmiş ve bunu sırası ile Trabzon, Ordu ve Giresun örnekleri takip etmiş ancak örnekler arasındaki farklılık istatistiki olarak önemsiz bulunmuştur (P>0.05). Kış döneminde en düşük pH değerine sahip Ordu örneklerinde en yüksek asitlik değeri ve % asitliğin belirlenmesi; en yüksek pH değerine sahip Samsun örneğinde de en düşük asitlik derecesi ve Trabzon ile birlikte en düşük % asitlik değerlerinin belirlenmesi anlamlı ve birbirini destekler bulunmuştur.

Ekşi hamur örneklerinin kuru madde ve kül değerleri hem kış hem de yaz döneminde en yüksek olarak Samsun örnekleri arasında belirlenmiş olmakla birlikte, kuru madde değerleri açısından her iki dönemde de, kül açısından ise yaz döneminde iller arasındaki farklılıklar istatistiki olarak önemsiz bulunmuştur (P>0.05).

Kış ve yaz dönemleri birbiri ile karşılaştırıldığında da; ekşi hamur örneklerinin pH ve toplam asitlik değerlerinin birbirlerinden önemli derecede farklı olduğu (P<0.05), asitlik derecesi, kuru madde ve kül değerlerinin ise farksız (P>0.05) olduğu belirlenmiştir.

Çalışmada analiz edilen toplam 54 ekşi hamur örneklerinin mikrobiyolojik sayım sonuçları Tablo 3'de verilmiştir. Karadeniz Bölgesi'nden toplanan ekşi hamur örneklerinin LAB sayısı kış dönemi 6.14-8.97 log KOB/g arasında ve ortalama 7.59 log KOB/g; yaz döneminde de 3.65-8.59 log KOB/g arasında ve ortalama 7.54 log KOB/g olarak belirlenmiştir. Yaz ve kış dönemleri için birbirine çok yakın bulunan LAB ortalama değerlerinin, farklı fırınlara ait ekşi hamur örnekleri açısından karşılaştırıldığında farklılıklara sahip olduğu saptanmıştır. Bu farklılığın ekşi hamur mayası kullanım oranı ile ekşi hamurların fermantasyon süresinin farklılığından kaynaklanabileceği düşünülmektedir. Örneklerin pH, asitlik derecesi ve toplam asitlik değerleri arasındaki önemli farklılıklar da bu yaklaşımımızı desteklemektedir.

Tablo 3. Karadeniz bölgesi ekşi hamurlarının mikrobiyolojik özellikleri (log KOB/g)*
Table 3. Microbiological properties of Black Sea region sourdoughs (log CFU/g)

İl	İlçe	Mikroorganizma Sayısı					
		Kış Dönemi			Yaz Dönemi		
		LAB	TAMB	Maya	LAB	TAMB	Maya
Samsun	Ondokuzmayıs	8.97	6.53	6.52	6.27	6.27	6.48
	Ondokuzmayıs	7.29	6.65	6.64	7.32	6.27	6.29
	Bafra	7.50	6.83	6.78	6.18	6.45	6.32
	Merkez	7.43	6.77	6.56	7.77	6.75	6.72
	Tekkeköy	6.54	6.71	6.53	6.58	6.50	6.31
	Çarşamba	7.59	5.47	5.46	8.43	6.40	6.23
	Terme	7.57	7.19	7.02	7.00	4.19	4.17
	Ortalama	7.56	6.59	6.50	7.72	6.41	6.36
Giresun	Espiye	6.14	7.20	6.53	5.42	5.82	6.75
	Görele	7.74	6.91	6.73	8.59	7.03	6.86
	Çavuşlu	7.48	6.70	6.62	7.50	7.00	7.52
	Çavuşlu	7.35	6.59	6.79	3.65	6.64	6.44
	Piraziz	8.11	6.82	6.85	4.31	4.64	6.80
	Bulancak	7.84	6.60	6.66	5.31	6.10	6.55
	Merkez	8.26	6.90	6.74	5.01	4.59	5.79
	Ortalama	7.56	6.82	6.70	7.78	6.59	6.93
Trabzon	Beşikdüzü	7.72	6.71	6.76	6.41	6.59	6.47
	Vakfıkebir	6.96	6.78	6.75	4.96	6.64	6.55
	Vakfıkebir	7.35	6.66	6.51	6.62	6.20	6.18
	Vakfıkebir	7.76	6.65	6.55	7.49	6.17	6.23
	Akçaabat	7.13	6.49	6.42	6.40	6.32	6.38
	Akçaabat	7.71	6.59	6.67	7.70	6.70	6.72
	Merkez	7.56	6.71	6.68	5.21	6.40	6.20
	Merkez	7.54	7.05	6.85	7.72	6.67	6.41
	Ortalama	7.47	6.71	6.65	7.25	6.50	6.43
Ordu	Ünye	8.08	7.07	7.08	5.71	6.56	6.26
	Fatsa	8.49	6.62	6.63	4.34	5.71	6.50
	Bolaman	7.5	6.59	6.60	5.02	4.86	6.00
	Merkez	7.06	6.77	6.77	4.10	5.83	6.68
	Gülyalı	8.17	7.15	6.92	5.49	6.37	6.68
	Ortalama	7.86	6.84	6.80	5.28	6.16	6.49
Genel	Ortalama	7.59	6.73	6.65	7.54	6.46	6.62

*: LAB: Laktik asit bakterisi, TAMB: Toplam aerobik mezofilik bakteri
**: LAB: Lactic acid bacteria, TAMB: Total aerobic mesophilic bacteria*

Salovaara [53] tarafından tam olarak fermente olmuş ekşi hamurların 10^9 KOB/g laktobasil içerdiği; Lönner ve ark. [54] tarafından da İsveç çavdar unundan hazırlanan ekşi hamurda LAB sayısının hemen hemen toplam canlı sayısına eşit olduğu ($3.4x10^8$-$1.96x10^9$ KOB/g) bildirilmiştir. Ekşi hamur LAB sayısı; Meroth ve ark. [38] tarafından 7.83-9.50 log KOB/g; Paramithiotis ve ark. [55] tarafından $1.7x10^9$-$3.2x10^9$ KOB/g; Akgün [41] tarafından 8.04-8.80 log KOB/g; Rosenquist ve Hansen [56] tarafından 8.43-9.14 log KOB/g; Minervini ve ark. [42] tarafından 8.75-9.13 log KOB/g; Yağmur [28] tarafından 6.71-9.16 log KOB/g; Rizzello ve ark. [30] tarafından 8.7-9.0 log KOB/g; Rizzello ve ark. [57] tarafından 9.3-9.7 log KOB/g; Örü ve Hendek Ertop [47] tarafından $5x10^9$-$12x10^9$ KOB/g arasında belirlenmiştir. Bu veriler çalışma sonuçlarından biraz yüksektir. Bununla birlikte çalışma sonuçlarına benzer şekilde ekşi hamur LAB sayısı Özcangaz [58] tarafından $3.3x10^7$-$5.0x10^8$ KOB/g; Şimşek [35] tarafından $2.0x10^6$-$7.9x10^8$ KOB/g (ort. $7.8x10^7$ KOB/g); Pepe ve ark. [39] tarafından 6-8.7 log KOB/g; Gül ve ark. [34] tarafından 5.28-9.66 log KOB/g (ort. 7.34 log KOB/g); Dertli ve ark. [45] tarafından 8.35-8.96 log KOB/g; Franco ve ark. [49] tarafından 8,35-8,40 log KOB/g; Fraberger ve ark. [11] tarafından 5,00-9,59 log KOB/g ve Çetin-Babaoğlu ve ark. [37] tarafından da 6,77-9,15 log KOB/g arasında olarak belirlenmiştir [53, 54, 38, 55, 41, 56, 42, 28, 30, 57, 47, 58, 36, 39, 34, 45, 49, 11, 37]

TAMB sayısı Karadeniz Bölgesi ekşi hamur örneklerinde kış döneminde 5.47-7.20 log KOB/g arasında (ort. 6.73 log KOB/g); yaz döneminde de 4.19-7.03 log KOB/g arasında (ort. 6.46 log KOB/g) belirlenmiştir. Yaz döneminde örnekler arasındaki farklılıkların kış dönemine göre biraz daha fazla olduğu ve ortalama değerin biraz düşük bulunduğu belirlenmiştir. Yazın daha düşük ortalama sayıya rastlanmamasının fermantasyon süresinin kısa tutulmasından kaynaklanabileceği düşünülmektedir. Ekşi hamur örneklerinin TAMB sayısı; Dığrak ve Özçelik [59] tarafından $4.7x10^7$-$7.6x10^8$ KOB/g; Şimşek [36] tarafından $9.0x10^5$-$6.5x10^7$ KOB/g (ort. $1.9x10^7$ KOB/g);

Gül ve ark. [34] tarafından 5.97-9.57 log/KOB/g (ort. 7,94 log KOB/g) ve Yağmur [28] tarafından 5.51-8.08 log KOB/g arasında belirlenmiştir. Ekşi hamur TAMB sayılarında elde edilen farklı sonuçlar farklı hammadde kullanımı ve farklı fermantasyon süresi uygulanmasına bağlanmıştır [59, 36, 34, 28].

Ekşi hamur örneklerinin maya sayısı da kış döneminde 5.46-7.08 log KOB/g (ort. 6.65 log KOB/g); yaz döneminde 4.17-7.52 log KOB/g arasında (ort. 6.62 log KOB/g) değişmiştir. Yaz döneminde örnekler arasında daha büyük farklılıklar olmakla birlikte, ortalama değerler birbirine çok yakındır. Ekşi hamur örneklerinin maya sayısı, Gobbetti [60] tarafından 10^8 KOB/g; Dığrak ve Özçelik [59] tarafından $1.5x10^6$-$2.2x10^8$ KOB/g; Rosenquist ve Hansen [56] tarafından 6.00-8.04 log KOB/g; Gül ve ark. [34] tarafından 6.33-9.96 log KOB/g (ort. 8.94 log KOB/g); Fraberger ve ark. [11] tarafından da 5,00-7,90 log KOB/g arasında belirlenmiştir. Bu çalışmalarda elde edilen üst sınır maya sayısı, çalışma sonuçlarından oldukça yüksektir. Bu değişkenlik bazı yörelerde üretimde normal ekmek mayası da kullanılmasına bağlanabilir. Bununla birlikte, çalışma sonuçlarına daha yakın olarak ekşi hamurların maya sayısı Şimşek [35] tarafından $1.7x10^6$-$4.9x10^7$ KOB/g (ort. $1.4x10^7$ KOB/g); Minervini ve ark. [42] tarafından 7.30 log KOB/g; Yağmur [28] tarafından 5.27-7.32 log KOB/g; Dertli ve ark [45] tarafından 6.71-6.96 log KOB/g ve Özcangaz [58] tarafından $2.8x10^7$-$5.2x10^7$ KOB/g arasında belirlenmiştir [60, 59, 55, 34, 11, 36, 42, 28, 45, 58]

Karadeniz Bölgesi'nden temin edilen ekşi hamurların kış ve yaz dönemindeki mikrobiyolojik özelliklerinin il bazındaki farklılıklarına ait değerlendirme yapıldığında (Tablo 4); kış dönemi ekşi hamurları içerisinde LAB sayısı açısından iller arasında fark bulunmamıştır ($P>0.05$). Yaz döneminde ise en yüksek LAB sayısı Samsun örneklerinde belirlenmiş ve en az sayıya sahip Ordu örneklerinden istatistiki olarak farklı bulunmuştur ($P<0.05$). Örneklerin TAMB ve maya sayılarının da hem kış, hem de yaz döneminde iller arasında istatistiki olarak fark içermediği ($P>0.05$) belirlenmiştir.

Tablo 4. Kış ve yaz dönemi ekşi hamurlarının mikrobiyolojik özelliklerinin illere göre değişimi* (log KOB/g)
Table 4. Variation of microbiological properties winter and summer sourdoughs according to province (log CFU/g)

Sezon	İl	N	LAB	TAMB	Maya
Kış Dönemi	Samsun	7	7.56±0.72^a	6.59±0.54^a	6.50±0.49^a
	Giresun	7	7.56±0.70^a	6.82±0.21^a	6.70±0.11^a
	Trabzon	8	7.47±0.29^a	6.71±0.16^a	6.65±0.14^a
	Ordu	5	7.86±0.57^a	6.84±0.26^a	6.80±0.20^a
	Ortalama		7.59±0.57	6.73±0.32	6.65±0.29
Yaz Dönemi	Samsun	7	7.08±0.82^a	6.12±0.87^a	6.07±0.86^a
	Giresun	7	5.68±1.75^{ab}	5.97±1.03^a	6.67±0.52^a
	Trabzon	8	6.56±1.07^a	6.46±0.22^a	6.39±0.19^a
	Ordu	5	4.93±0.70^b	5.87±0.67^a	6.42±0.29^a
	Ortalama		6.17±1.37	6.14±0.74	6.39±0.55

*: İstatistiki değerlendirme kış ve yaz dönemi örneklerinde kendi içerisinde yapılmıştır. Sütunlarda farklı harfle işaretlenen örnekler arasındaki fark önemlidir.
**: Statistical evaluation was made within the winter and summer period samples. The difference between examples marked with different letters in the columns is significant.*

SONUÇ

Geleneksel ürünlerin yaygınlaşması, endüstriyel boyutlarda üretilmesi ve tüketici taleplerine cevap verebilmesi için standart üretim yöntemi ve ürün özelliklerine sahip olması büyük önem taşımaktadır. Önemli geleneksel ürünlerimizden ekşi hamur ekmeğinde standardizasyonun sağlanabilmesi için öncelikle ekşi hamur üretimi basamağında asitlik düzeyinde standardizasyonun sağlanması gerekmektedir. Ekşi hamur ekmeklerinde toplam asitliğin yüksekliği; fermantasyon süresinin uzunluğu ile sıcaklığının yüksekliği, daha güçlü aroma ve daha ekşi bir tat oluşumuna işaret etmektedir. Yüksek toplam asitlik sonucu düşen pH değeri ile hem ekmek tat ve aromasını etkileyen enzimler aktive olmakta, hem de lezzet ve aroma oluşumunda etkili olan maillard reaksiyonlarında artış meydana [61] gelmektedir. Yine, hem ulaşılan pH değeri, hem de asit üretiminden sorumlu laktik asit bakterileri tarafından üretilen antimikrobiyal metabolitler ekmeğin raf ömrünü de uzatmaktadır. Ayrıca uzun hamur fermantasyon süresi ile artan çeşitli biyoaktif bileşikler [62] ekmeğin besin değerini ve sağlık üzerindeki yararlı etkilerini de artırmaktadır. Yapılan çalışmada, tüketici beğenisi ve besin değeri üzerine etkileri vurgulanan asitlik ve pH düzeylerinin ekşi hamurlarda hem yöresel, hem mevsimsel, hem de fırınlar arasında önemli farklılıklara sahip olduğu (asitlik derecesi 2.50-20.50, toplam asitlik % 0.23-1.793 ve pH 30-5.43 arasında) saptanmıştır. Bu farklılıkların başta gelen nedeni olarak; uygulanan fermantasyon süresi ile fırınlarda iklimlendirme sistemlerinin olmamasından kaynaklanan sıcaklık farklılıkları ve florada bulunan LAB türleri ile aşılama materyali katma miktarı farklılığı gösterilebilir. Ekşi hamur örnekleri arasındaki bu farklılıklar fırınlarda standart bir üretimin olmadığını göstermektedir. Ekşi hamur örneklerinin kuru madde ve kül değerleri de

fırınlar arasında farklılıklara sahip olmakla birlikte (kuru madde %49.05-65.91 kül %0.34-0.95 arasında), kuru madde içeriğinde hem kış hem de yaz döneminde; kül içeriğinde ise yaz döneminde iller arasındaki farklılıklar istatistiki olarak önemsiz (P>0.05) bulunmaktadır. Bu durum olumlu bulunmakla birlikte, fırınlar arası farklılıklara neden olan hammadde ve üretimdeki uygulama farklılıklarının azaltılarak standardizasyonun sağlanması uygun olacaktır.

Ekşi hamurların teknolojik özelliklerinin oluşmasında/ gelişmesinde ve fırının üretim koşulları ile hijyenik kalitesi hakkında genel bir bilgi veren mikrobiyolojik içerik değerlendirildiğinde: kış ve yaz sezonlarında birbirine çok yakın ortalama değerlere sahip olduğu (sırasıyla LAB sayısı 7.59 ve 7.54 log KOB/g; TAMB sayısı 6.73 ve 6.46 log KOB/g; maya sayısı da 6.65 ve 6.62 log KOB/g) saptanmıştır. Bununla birlikte fırınlar arasındaki farklılıklar her iki sezonda da önemli bulunmuştur (LAB sayısı 3.65-8.97 log KOB/g; TAMB sayısı 4.19-7.20 log KOB/g; maya sayısı 4.17-7.52 log KOB/g arasında). Bu farklılıkların aşılama materyali katma miktarı, ekşi hamurların fermantasyon süresi ve sıcaklıklarının farklılıkları ve hammadde kalitesindeki farklılıklardan kaynaklanabileceği düşünülmektedir.

Bu çalışma sonucu ekşi hamur örnekleri arasında saptanan farklılıklar; standart üretimin eksikliğini göstermiş, fırınlarda teknolojik ekipman ve sistem eksikliklerinin giderilip, üretim teknolojisi ve hijyen bilgilerinin tamamlanarak uygulamaya aktarılmasının gerekliliğini ortaya koymuştur. Standart üretimin sağlanması hem üreticilerin karlılığının artmasını sağlayacak, hem de tüketicilerin kaliteli, besleyici ve güvenli gıdaya ulaşmasını kolaylaştıracaktır.

KAYNAKLAR

[1] Gültekin, F., Akın, S., Elgün, A. (2019). Ekmek hakkında güncel bir değerlendirme: sağlık etkileri, gıda katkı maddeleri ve helallik sorunu. *Journal of Halal Life Style*, 1(1), 1-17.

[2] Kotancılar, G., Çelik, İ., Ertugay, Z. (1995). Ekmeğin besin değeri ve beslenmedeki önemi. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi*, 26(3), 431-441.

[3] Ventimiglia, G., Alfonzo, A., Galluzzo, P., Corona, O., Francesca, N., Caracappa, S., Moschetti, G., Settanni, L. (2015). Codominance of *Lactobacillus plantarum* and obligate heterofermantative lactic acid bacteria during sourdough fermantation. *Food Microbiology*, 51, 57-68.

[4] De Vuyst, L., Vancanneyt, M. (2007). Biodiversity and identification of sourdough lactic acid bacteria. *Food Microbiology*, 24, 120-127.

[5] Hendek Ertop, M., Hayta, M. (2016). Ekşi hamur fermantasyonunun ekmeğin biyoaktif bileşenleri ve biyoyararlanımı üzerindeki etkileri. *Gıda*, 41(2), 115-122.

[6] Kerrebroeck, S.V., Bastos, F.C.C., Harth, H., De Vuyst, L. (2016). A low pH does not determine the community dynamics of spontaneously developed backslopped liquid wheat sourdoughs but does influence their metabolite kinetics. *International Journal of Food Microbiology*, 239, 54-64

[7] Di Monaco, R., Torrieri, E., Pepe, O., Masi, P., Cavella, S. (2015). Effect of Sourdough with exopolysaccharide (eps)-producing lactic acid bacteria (LAB) on sensory quality of bread during shelf life. *Food and Bioprocess Technology*, 8, 691-701.

[8] Erdoğmuş, S.F., Bostancı, B. (2020). Kefir örneklerinden laktik asit bakterilerinin izolasyonu, identifikasyonu ve antimikrobiyal etkilerinin değerlendirilmesi. *Gıda*, 45(1), 72-800.

[9] Lhomme, N., Lattanzi, A., Dousset, X. Minervini, F., De Angelis, M., Lacaze, G., Onno, B., Gobbetti, M. (2015). Lactic acid bacterium and yeast microbiotas of sixteen French traditional sourdoughs. *International Journal of Food Microbiology*, 215, 161-170.

[10] Bakırcı, F., Köse, E. (2017). Ekşi hamurlardan laktik asit bakterileri ve mayaların izolasyonu ve tanımlanması. *Akademik Gıda*, 15(2), 149-154.

[11] Fraberger, V., Unger, C., Kummer, C., Domig, K.J. (2020). Insights to microbial diversity of traditional Austrian sourdough. *Food Science and Technology*, 127.

[12] Arora, K., Ameur, H., Polo, A., Di Cagno, R., Rizzello, G.C., Gobbetti, M. (2021). Thirty years of knowledge on sourdough fermentation: A systematic Review. *Trends in Food Science & Technology*, 108, 71-83.

[13] Hendek Ertop, M. (2017). Farklı fermantasyon ve kurutma yöntemleriyle üretilmiş toz ekşi hamurun bazı mikrobiyolojik nitelikleri ve ekmekteki küf gelişimi üzerine etkileri. *Gıda* 42(5), 609-619.

[14] Blandino, A., Al-Aseeria, M.A., Pandiella, S.S., Cantero, D., Webb, C. (2003). Cereal-based fermented foods and baverages. *Food Research International*, 36, 527-543.

[15] Galle, S., Arendt, E.K. (2014). Exopolysaccharides from sourdough lactic acid bacteria. *Critical Reviews in Food Science and Nutrition*, 54(7), 891-901.

[16] Torrieri, E., Pepe, O.Ventorino, V., Masi, P., Cavella, S. (2014). Effect of sourdough at different concentrations on quality and shelf life of bread. *Food Science and Technology*, 56, 508-516.

[17] Yıldırım, R.M., Arıcı M. (2019). Effect of fermentation temperature on the degradation of phytic acid in whole-wheat sourdough bread. *Food Science and Technology*, 112.

[18] Gobbetti, M., De Angelis, M., Di Cagno, R.Calasso, M., Archetti, G., Rizzello, C.G. (2019). Novel insights on the functional/nutritional features of the sourdough fermentation. *International Journal of Food Microbiology*, 302, 103-113.

[19] Gobbetti, M., Rizzello, C.G., Di Cagno, R., De Angelis, M. (2007). Sourdough lactobacilli and celiac disease. *Food Microbiology*, 24, 187-196.

[20] Üstü, Y. (2018). Enteropatiler düşündüğümüzden daha sık olabilir mi? *Ankara Medical Journal*, 18(4), 704-705.

[21] Lynch, K.M., Coffey, A., Arendt, E.K. (2018). Exopolysaccharide producing lactic acid bacteria: their techno-functional role and potential application in glüten-free bread products. *Food*

Research International, 110, 52-61.

[22] Çon, A.H. (1995). Sucuktan bakteriosin-benzeri antimikrobiyal metabolit üreten laktik asit bakterilerinin izolasyonu ve identifikasyonu ve çeşitli gıda zararlısı ve/veya gıda kaynaklı patojen bakterilere karşı antagonistik aktivite araştırması. Doktora tezi, Atatürk Üniversitesi Fen Bilimleri Enstitüsü Gıda Mühendisliği Anabilim Dalı, 84 sayfa, Erzurum.

[23] Anonim, (2005). Merck Gıda Mikrobiyolojisi Uygulamaları. Başak Matbaacılık Ltd.Şti., Ankara.

[24] Scheirlinck, I., Meulen R.V., Schoor, A.V., Huys, G., Vandamme, P., De Vuyst L., Vancanneyt, M. (2007). *Lactobacillus crustorum* sp. nov., isolated from two traditonal Belgian wheat sourdoughs. *International Journal of Systematic and Evolutionary Microbiology*, 57, 1461-1467.

[25] Gül, L.B. (2013). Tarhanadan izole edilen bakteriyosin üreticisi laktik asit bakterilerinin endüstriyel özellikleri ve *S. cerevisiae* ile etkileşimi. Yüksek Lisans Tezi, Ondokuz Mayıs Üniversitesi Fen Bilimleri Enstitüsü Gıda Mühendisliği Anabilim Dalı, 165 sayfa, Samsun.

[26] Anonim, (1990). American Association of Cereal Chemists. Approved Methods of the AAC, 8th edition, The Association: St. Paul, MN.

[27] Kotancılar, H.G., Karaoğlu, M.M., Gerçekaslan, K.E. ve Uysal, P. (2006.) Ekşi hamur katkısının beyaz tava ekmeğinin bayatlaması üzerine etkisi. *Atatürk Üniversitesi Ziraat Fakültesi Dergisi*, 37(1), 103-110.

[28] Yağmur, G. (2013). Ekşihamur fermantasyonunda etkili olan laktik asit bakterilerinin ve mayaların belirlenmesi ve bunlardan elde edilen sıvı ekşihamurun ekmek kalitesi üzerine etkisinin araştırılması. Doktor Tezi, Çukurova Üniversitesi Fen Bilimleri Enstitüsü Gıda Mühendisliği Anabilim Dalı, 178 sayfa, Adana.

[29] Manini, F., Casiraghi, M.C., Poutanen, K., Barsca, M., Erba, D., Plumed-Ferrer, C. (2016). Characterization of lactic acid bacteria isolated from wheat bran sourdough. *Food Science and Technology*, 66, 275-283.

[30] Rizzello, C.G., Cavoski, I., Turk, J., Ercolini, D., Nionelli, N., Pontonio, E., De Angelis, M., De Flippis, F., Gobbetti, M., Di Cagno, R. (2015). Organic cultivation of *Triticum turgidum* subsp. *durum* is reflected in the flour-sourdough fermentation bread axis. *Applied and Environmental Microbiology*, 81(9), 3192-3204.

[31] Plessas, S., Alexopoulos, A., Mantzourani, I., Koutinas, A., Voidaoru, C., Stavropoulou, E., Bezirtzoglou, E. (2009). Application of novel starter cultures for sourdough bread production. *Anaerobe*, 17, 486-489.

[32] Galli, V., Venturi, M., Pini, N., Guerrini, S., Granchi, L., Vincenzini, M. (2019). Liquid and firm sourdough fermentation: microbial robustness and interactions during consecutive backsloppings. *Food Science and Technology*, 105, 9-15.

[33] Gerçekaslan, K.E., Kotancılar, H.G., Kaban, G., Karaoğlu, M.M. (2012). Vakfıkebir Ekmek Hamurundan Laktik Asit Bakterilerinin İzolasyonu ve Tanısı. Akademik Gıda, 10(3), 47-50.

[34] Gül, H. Özçelik, S., Sağdıç, O., Certel, M. (2005). Sourdough bread production with lactobacilli and *S. cerevisiae* isolated from sourdoughs. *Process Biochemistry*, 40(2), 691-697.

[35] Spicher, G., Rabe, E., Sommer, R., Stephan, H. (1981). The Microflora of Sourdough XIV., Communication on the Behavior of Homofermentative Sourdough Bacteria and Yeasts in Mixed Culture. *Zeitchrift Für Lebensmittel-Unturshung und Forschung*, 173(4), 29, 291-296.

[36] Şimşek, Ö. (2003). Uşak ve yöresi ekşihamurlarından izole edilen antimkrobiyal aktiviteye sahip laktik asit bakterilerinin tanımlanması ve bazı metabolik özelliklerinin belirlenmesi. Yüksek Lisans Tezi, Pamukkale Üniversitesi Fen Bilimleri Enstitüsü Gıda Mühendisliği Anabilim Dalı, 90 sayfa, Denizli.

[37] Çetin-Babaoğlu, H., Arslan-Tontul, S., Akın, N. (2021). Yer elması tozu ilavesinin ekşi hamur fermantasyonuna etkisi. *Gıda*, 46(2), 367-375.

[38] Meroth, C.B., Water, J., Hertel, C., Brandt, M.J., Hammes, V.P. (2003). Monitoring the bacterial population dynamics in sourdough fermentation processes by using PCR-Denaturing gradient gel electrophoresis. *Applied and Environmetal Microbiology*, 69(1), 475-482.

[39] Pepe, O., Blaiotta, G., Anastasio, M., Moschetti, G., Ercolini, D. and Villani, F. (2004). Technological and moleculer diversity of Lactobacillus plantarum strains isolated from naturally fermented sourdoughs. *Systematic and Applied Microbiology*, 27, 443-453.

[40] Kömen, G. (2010). Structural changes of gliadins during sourdough fermentation as a promising approach to gluten-free diet. Master's Thesis, İzmir Institute of Technology Graduate School of Engineering and Sciences Food Engineering Department, 106, İzmir.

[41] Akgün, F.B. (2007). Ekşi hamur tozu eldesi ve ekmek üretiminde kullanılabilme olanakları. Yüksek Lisans Tezi, Pamukkale Üniversitesi Fen Bilimleri Enstitüsü Gıda Mühendisliği Anabilim Dalı, 68 sayfa, Denizli.

[42] Minervini, F., Di Cagno, R., Lattanzi, A., De Angelis, M., Antonielli, L., Cardinali, G., Cappelle, S., Gobbetti, M. (2011). The lactic acid bacteria and yeast microbiota of nineteen sourdoughs used fort the manufacture of traditional/typical İtalian breads: interactions between ingredients and microbial species diversity. *Applied and Environmental Microbiology*, 78(4), 1251-1264.

[43] Paramithiotis, S., Sofou, A., Tsakalidou, E., Kalantzopoulos, G. (2007). Flour carbohydrate catabolism and metabolite production by sourdough lactic acid bacteria. *World Journal Microbial Technology*, 23, 1417-1423.

[44] Vrancken, G., Rimaux, T., Weckx, S., Leroy, F., De Vuyst, L. (2011). Influence of temperature and backslopping time on the microbiota of a Type I propagated laboratory wheat sourdough fermentation. *Applied and Environmental Microbiology*, 77(8), 2716-2726.

[45] Dertli, E., Mercan, E., Arıcı, M., Yılmaz, M.T., Sağdıç, O. (2016). Characterisation of lactic acid

bacteria from Turkish sourdough and determination of their exopolysaccharide (EPS) production characteristics. *Food Science and Technology*, 71, 116-124.

[46] Yıldız, B., Çakıcı, A., Uslu, D.Y., Uslu, H. (2021). Ekmek üretiminde ekşi maya üzerine taze meyvelerin kullanımının etkisi. *Niğde Ömer Halisdemir Üniversitesi Mühendislik Bilimleri Dergisi*, 10(1), 150-159.

[47] Örü, F. ve Hendek Ertop, M. (2021). Siyez ve ekmeklik buğday kepeğinin ekşi hamur üretiminde kullanım olanağının değerlendirilmesi. *Gıda*, 46(2), 396-407.

[48] Liu, T., Li, Y., Yang, Y., Yi, H., Zhang, L., He, G. (2020). The influence of different lactic acid bacteria on sourdough flavor and a deep insight into sourdough fermentation through RNA sequencing. *Food Chemistry*, 307.

[49] Franco, W., Péréz Díaz, I.M., Connelly, L., Diaz, J.T. (2020). Isolation of exopolysaccharide-producing yeast and lactic acid bacteria from Quinoa sourdough fermentation. *Foods*, 9, 337.

[50] Menezes, L.A.A., Molognoni, L., De Sa Ploencio, L.A., Costa, F.B.M., Daguer, H., De Dea Lindner, J. (2019). Use of sourdough fermentation to reducing FODMAPs in breads. *European Food Research and Technology*, 245, 1183-1195.

[51] Çağlıyan, B.İ. (2008). İzmir piyasasında satılan bazı ekmek çeşitlerinin nitelikleri ve yapım teknikleri. Doktora tezi, Ege Üniversitesi Fen Bilimleri Enstitüsü Gıda Mühendisliği Anabilim Dalı, 117 sayfa, İzmir

[52] , O.A., Banwo, K., Ogunremi, O.R., Sanni, A.I. (2015). Microbiological and physicochemical properties of sourdough bread from sorghum flour. *International Food Research International*, 22(6), 2610-2618.

[53] Salovaara, H. (1993). Lactic Acid Bacteria in Cereal Based Products, In Lactic Acid Bacteria, New York.

[54] Lönner, C., Welander, T., Malin, N., Dostalek, M. (1986). The microflora in a sour dough started spontaneously on typical Swedish rye meal. *Food Microbiology*, 3(1), 3-12.

[55] Paramithiotis, S., Gioulatos, S., Tsakalidou, E., Kalantzopoulos, G. (2006). Interactions between *Saccharomyces cerevisiae* and lactic acid bacteria in sourdough. *Process Biochemistry*, 41(12), 2429-2433.

[56] Rosenquist H., Hansen Å. (2000). The microbial stability of two bakery sourdoughs made from conventionally and organically grown rye. *Food Microbiology*, 17, 241-250.

[57] Rizzello, C.G., Lorussa, A., Montemurro, M., Gobbetti, M. (2016). Use of sourdough made with quinoa (*Chenopodium quinoa*) flour and autochthonous selected lactic acid bacteria for enhancing the nutritional, textural and sensory features of white bread. *Food Microbiology*, 56, 1-13.

[58] Özcangaz, Ç. (2000). Türk hamur ekmeğindeki laktik suşların karakterizasyonu ve maya ile olan etkileşimleri. Yüksek Lisans Tezi, Orta Doğu Teknik Üniversitesi Fen Bilimleri Enstitüsü Gıda Mühendisliği Anabilim Dalı, 101 sayfa, Ankara

[59] Dığrak, M., Özçelik, S. (1991). Elazığ ve yöresinde kullanılan ekşi mayanın bileşimi, morfolojik, fizyolojik ve biyokimyasal özellikleri. *Gıda*, 16(5), 325-331.

[60] Gobbetti, M. (1998). The sourdough micrflora interactions of lactic acid bacteria and yeasts. *Trends in Food Science & Technology*, 9, 267-274.

[61] Semić, A., Oručević, S., Bauman, I., Muminović, Š., Spaho, N., Klepo, B. (2009). Effects of Increasing Sourness Of Bread Dough On Bread Quality. *5th International Congress FLOUR-BREAD '09; 7th Croatian Congress of Cereal Technologists*, UDC 664.66 : 664.642, 416-424.

[62] Koistinen, V.M., Mattila, O., Katina, K., Poutanen, K., Aura A-N., Hanhineva, K. (2018). Metabolic profiling of sourdough fermented wheat and rye bread. *Scientific Reports*, 8, Article number: 5684, 1-11.

SİDAS MEDYA
Akademik Gıda®
ISSN Online: 2148-015X
https://dergipark.org.tr/tr/pub/akademik-gida

Akademik Gıda 21(2) (2023) 167-173, DOI: 10.24323/akademik-gida.1351008

Araştırma Makalesi / Research Paper

Chlorella vulgaris Türü Mikroalglerde B Vitamini İçeriklerinin Uzun Süreli Pişirme Koşulunda Değişimi

Berat Zeki Haznedaroğlu

Boğaziçi Üniversitesi, Çevre Bilimleri Enstitüsü, İstanbul

Geliş Tarihi (Received): 09.01.2023, Kabul Tarihi (Accepted): 19.06.2023
Yazışmalardan Sorumlu Yazar (Corresponding author): berat.haznedaroglu@boun.edu.tr (B.Z. Haznedaroğlu)
0 212 359 7974 0 212 257 5033

ÖZ

Bu çalışmada besleyici öğeler açısından zengin, farklı fonksiyonel gıdalarda kullanımı giderek yaygınlaşan *Chlorella vulgaris* türü mikroalglerde bulunan B vitamini içeriklerinin 125°C sıcaklıkta ve 35 dakikalık pişirme koşulları altında değişimi incelenmiştir. Ultra yüksek performanslı sıvı kromatografisi-yüksek çözünürlüklü kütle spektrometresi (UHPLC-HR/MS) kullanılarak gerçekleştirilen ölçümlerde 35 dakikalık pişirme süresi sonrası B_1 (tiamin), B_2 (riboflavin), B_3 (niasin), ve B_6 (piridoksin) vitaminlerinin pişirme işlemine maruz bırakılmayan kontrol grubuna kıyasla istatistiki olarak anlamlı şekilde ($p<0.05$) arttığı belirlenmiştir. B_7 (biyotin) ve B_{12} (metilkobalamin) miktarların ise 35 dakikalık pişirme işlemi sonrası kontrol grubuna göre bir miktar arttığı, ancak aradaki farkın istatistiki olarak anlamlı olmadığı ($p>0.05$) gözlenmiştir. Otuz beş dakikalık uzun ısıl işlemlerinin, kalın bir hücre çeperine sahip *Chlorella vulgaris* mikroalg türünde daha fazla B vitamini açığa çıkmasına yardımcı olabileceği; böylelikle ısıl işlemlere karşı hassas olan ve pişirme sonrası bozunduğu bilinen B vitaminlerinin, *Chlorella vulgaris* türü mikroalglerde pişirme sırasında korunarak fonksiyonel gıda ürünlerinde kullanılabileceği değerlendirilmiştir.

Anahtar Kelimeler: Mikroalg, *Chlorella*, Fonksiyonel gıda, B vitaminleri

Changes in Vitamin B Complex of *Chlorella vulgaris* during Long Term Baking Conditions

ABSTRACT

In this study, *Chlorella vulgaris* microalgae, commonly used in functional foods due to its rich nutritious compounds, have been subjected to 35-min cooking durations at 125°C to determine changes in its vitamin B content. Using Ultra-Performance Liquid Chromatography–High-Resolution Mass Spectrometry (UHPLC-HR/MS), long-term 35-min cooking caused significant increases ($p<0.05$) in vitamins B_1 (thiamine), B_2 (riboflavin), B_3 (niacin) and B_6 (pyridoxine) compared to raw (non-baked) samples. Vitamins B_7 (biotin) and B_{12} (methylcobalamin) were both higher in 35-min-baked samples although these changes were statistically insignificant ($p>0.05$). These observations were attributed to the fact that long-term heat treatment during cooking might help breakage of thicker cell walls present in *Chlorella vulgaris* leading to higher vitamin B concentrations compared to raw samples. As such, it was concluded that cooking processes might help preserve vitamin B-rich content of *Chlorella vulgaris* and contribute to their use in functional food products.

Keywords: Microalgae, *Chlorella*, Functional food, Vitamin B

GİRİŞ

Farklı alg türleri Aztek ve Maya gibi kadim topluluklara kadar varan dönemlerden beri gıda tüketiminde kullanılmakta olup, besleyici karakterlerine yönelik çalışmaların artması ile birlikte son dönemlerde fonksiyonel gıda üretiminde öne çıkmaktadır [1-4]. Algler, alternatif protein [5, 6], gıda boyaları [7], aljin ve aljinat türevleri [8, 9], karagenan [10], karotenoidler [11], omega-3 ve diğer doymamış yağ asitleri [12] gibi çeşitli gıda bileşenlerinin üretiminde yaygınca kullanılmaktadır. Küresel iklim krizi sebebiyle etkileri ve sıklıkları giderek artan kuraklık, sel, yangın gibi aşırı iklim olayları ve afetler, tarım ve hayvancılık faaliyetlerini olumsuz etkilerken, artan dünya nüfusu karşısında güvenli ve sağlıklı besin kaynaklarına erişimin giderek zorlaşmasından dolayı, alglerin gıda ürünlerinde kullanımının önümüzdeki dönemlere artması beklenmektedir [13-15].

Makroalglere göre boyutları çok daha küçük olan *Chlorella vulgaris* türü yeşil mikroalgler ise "Spirulina" genel ismi ile bilinen *Arthrospira* mavi-yeşil alg türleri gibi tam biyokütle (İng. whole biomass) olarak doğrudan veya farklı gıda ürünlerine kuru ve/veya sıvı formda eklenerek kullanılabilmektedir [16-18]. Amerikan Gıda ve İlaç Kurumu (US FDA) ile Avrupa Gıda Güvenliği Kurumu (EFSA) tarafından "Genellikle Güvenilir Kabul Edilen (İng. Generally Recognized as Safe - GRAS) statüsünde bulunması sebebiyle gıda ürünlerinde kullanımı ve tüketimi hızla yaygınlaşmaktadır [19]. *Chlorella vulgaris* türü mikroalgler, beta-karoten [20], lutein [21], zeakzantin gibi karotenoidler ile çeşitli proteinler [22, 23], A, E, B vitamin kompleksleri [24], esansiyel amino asitler [25], polisakkaritler [16], omega-3 vb. çoklu doymamış yağ asitleri ve çeşitli mineraller içermektedir [26, 27]. Psödo-kobalaminden farklı olarak doğal biyolojik formu olan metil-kobalamin içerdiğinden dolayı *Chlorella vulgaris,* önemli bitkisel B_{12} vitamin kaynakları içinde gösterilmektedir [28, 29].

İçeriklerindeki yüksek besi öğelerine rağmen, gıda pişirme proseslerinin mikroalglerde yarattığı etkilerin araştırıldığı çalışmalar literatürde oldukça sınırlıdır. Örnek olarak, antioksidan özelliği oldukça yüksek, doğal mavi gıda boyası (Lina Blue®) olarak yaygınca kullanılan ve farklı alg türlerinden elde edilebilen fikosiyanin pigmentinin 70°C ve üstü sıcaklıklarda denatüre olduğu bilinmektedir [30, 31]. Benzer şekilde C vitaminin 70°C ve üstü sıcaklıklarda bozulduğu bildirilirken [32]; B_1 (tiyamin), B_2 (riboflavin), B_6 (piridoksin) ve B_9 (folik asit) vitaminlerinin ısıya karşı duyarlı oldukları raporlanmıştır [33, 34].

Gerçekleştirilen bu çalışmada ise, B vitaminleri içeriği açısından zengin *Chlorella vulgaris* türü mikroalglerin, 125°C sıcaklıkta uzun süreli (35 dakika) pişirme koşullarına maruz bırakıldıklarında B vitamini konsantrasyonlarının nasıl bir değişim gösterdikleri sorusu araştırılmış, analitik ölçümler ultra yüksek performanslı sıvı kromatografisi-yüksek çözünürlüklü kütle spektrometresi (UHPLC-HR/MS) kullanılarak gerçekleştirilmiştir.

MATERYAL ve METOT

Materyal

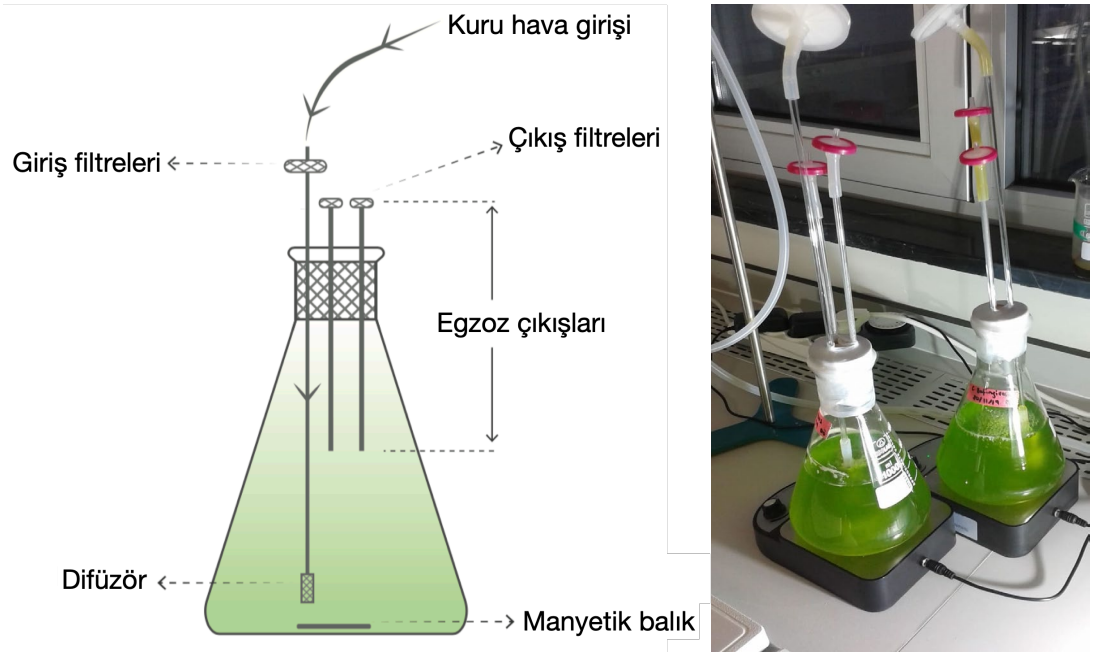
Bu çalışmada, Chlorophyceae sınıfına ait *Chlorella vulgaris* yeşil mikroalg türünün, Culture Collection of Algae and Protozoa (İskoçya, Birleşik Krallık) koleksiyonundan temin edilen CCAP 211/11B suşu kullanılmıştır. Besi yeri olarak bu türün kültürlenmesinde yaygın olarak tercih edilen Modified Bold's 3N (MB3N) kullanılmıştır [35]. Besi yeri hazırlanmasında kullanılan tüm kimyasallar ve analitik standartlar, Merck KGaA (Darmstadt, Almanya) firmasından temin edilmiştir.

Mikroalg Kültürlenmesi

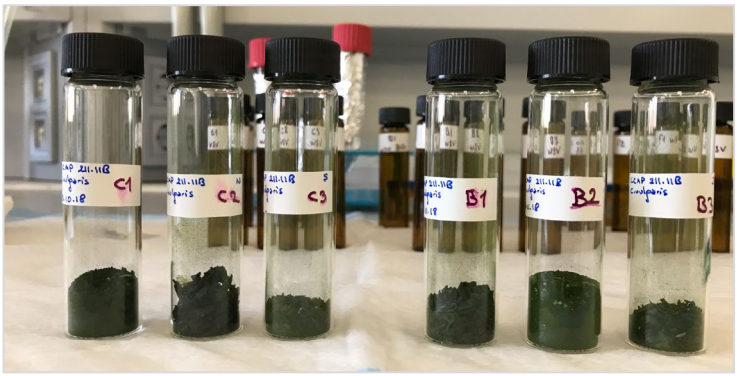
Mikroalg kültürleri 2-L hacimli Erlen şişeler kullanılarak tasarlanan fotobiyoreaktörlerde (Şekil 1), 110 µmol foton/m^2/s fotosentetik foton akı yoğunluğuna sahip beyaz LED ışık altında, 14 saat aydınlık, 10 saat karanlık çevrimine maruz bırakılarak, 25±2°C sıcaklıkta büyütülmüştür. Reaktörler 0.2 µm filtreden geçirilen ve 400 mL/dk akış oranlı kuru hava ile beslenmiş ve reaktör pH seviyesi 8±0.5 olarak sabit tutulmuştur. Kesikli (batch) besleme modunda çalıştırılan fotobiyoreaktörlerde, mikroalg biyokütle büyüme değerleri günlük olarak spektrofotometrik (680 nm dalga boyunda) ve standart ışık mikroskobu altında hemositometrik hücre sayım teknikleri kullanılarak ölçülmüştür. Sekiz günlük büyüme sonrası orta-katsal faza gelen mikroalg kültürleri santrifüj yardımıyla (3140xg, 10 dk) nem oranları %18-20 seviyesinde olacak şekilde hasatlanmıştır. Kuru hücre ağırlıkları, alikotlanan birim hacimli mikroyosun biyokütlesinin daha önceden darası alınmış 0.44 µm gözenek çaplı membran filtrelerden geçirilerek, 105°C fırınlarda 4 saat kurutulma işlemini takiben 2 saat desikatörde nem oranları %4-6 seviyesinde tutularak hassas terazi yardımıyla belirlenmiştir.

Pişirme Koşulları

Mikroalg kültürleri 35 dakikalık uzun süreli pişirme koşulu olarak 125°C sıcaklıkta tutulan fırınlarda (ON-105, Daihan, G. Kore), Pyrex® cam petri kapları (Merck, Almanya) kullanılarak pişirilmiştir [36]. Karşılaştırma yapılan kontrol grubu ise herhangi bir pişirmeye maruz bırakılmamıştır (Şekil 2). Mikroalg örneklerinin pişirme öncesi ve sonrası nem tayinleri yakın kızılaltı (NIR) analizörü (SpectraStar XT, Unity Scientific, Brookfield, ABD) kullanılarak yapılmıştır.



Şekil 1. Çalışmada kullanılan mikroalg türlerinin kültürlendiği fotobiyoreaktör sistemi
Figure 1. Fotobioreactor system used to cultivate microalgae species for the stud



Şekil 2. Çalışmada kullanılan mikroalg örnekleri (C1, C2 ve C3 pişirme işlemine tabi tutulan biyolojik tekrar örneklerini; B1, B2 ve B3 pişirme işlemine tabi tutulmayan gruba ait biyolojik tekrar örneklerini ifade etmektedir)
Figure 2. Microalgae samples used for the study (C1, C2 and C3 represent biological replicates of the baked group; B1, B2, and B3 represent biological replicates of the no-bake group)

Homojenizasyon ve Vitamin Ekstraksiyon İşlemleri

Kontrol grubu ve 35 dakikalık pişirme işlemine tabi tutulan mikroalg kültürlerinden alındıan nem oranı %4-6 olan 250 mg'lık örnekler, 1 mL 4:5 kloroform:metanol (v/v) solvent karışımı içerisinde 0.1 mm ve 0.5 mm çaplarında cam bilyeler kullanılarak homojenize edilmiştir. Homojenizasyon işlemleri, bilyeli öğütme sistemi (Precellys, Bertin, Fransa) yardımıyla 2500 rpm'de 1 dakikalık öğütme ve 2 dakikalık buz üstünde soğutma adımlarının 8 kez tekrar edilmesi ile gerçekleştirilmiştir. Homojenizasyon sonrası örnekler, amber renkli koyu vialler kullanılarak 4 mL asetonitril ve 80 µL formik asit içeren ekstraksiyon solüsyonuna alınarak 1 dk boyunca vorteks yardımıyla karıştırılmıştır. Karanlık bir odada 10 dk boyunca roller tüp karıştırıcı kullanılarak tekrar karıştırılan örneklerin santrifüjleme işlemi sonrası (3140xg, 15 dk) üst fazları cam Pastör pipetler yardımıyla cam viallere alınmıştır. Son adımda, 0.22 µm gözenek açıklığına sahip filtreler kullanılarak elde edilen örneklerden toplam 900 µL'lik kısım, 100 µL iç standart (B_{12c}-siyanokobalamin) ile karıştırılarak analitik ölçümlere hazır hale getirilmiştir.

Analitik İşlemler ve Vitamin Analizleri

Örneklerin kromatografik ayırma işlemi Ultimate 3000 UHPLC sistemi (Thermo Fisher Scientific, Waltham, MA, USA) kullanılarak gerçekleştirilmiştir. Ayırma kolonu olarak 15 cm Thermo Scientific kolon (Accucore RP-MS, 150X2.1mm, 2.6um) kullanılmış olup kolon fırın sıcaklığı 50°C olarak ayarlanmıştır. Her ikisi de 0.1% formik asit içeren su (A) ve metanol (B) çözücülerinden oluşan ikili

mobil faz sistemi, Tablo 1'de verilen gradyan elüsyon programı uygulanarak kromatografik ayırma işlemi gerçekleştirilmiştir.

Tablo 1. Kromatografik ayırma işlemi sırasında kullanılan gradyan elüsyon programı
Table 1. Gradient elution program for the chromatographic separation of samples

No	Zaman	Akış (mL/dakika)	%A	%B
1	-2.000	Dengelenme		
2	0.000	Başlangıç		
3	0.000	0.400	98	2
4	5.500	0.400	50	50
5	8.000	0.400	20	80
6	8.100	0.400	2	98
7	8.100	0.600	2	98
8	11.000	0.600	2	98
9	11.100	0.600	98	2
10	14.400	0.600	98	2
11	14.500	0.400	98	2
12	15.000	Bitiş		

Vitaminlerin kromatografik ayırma işlemini takiben kantitatif analizleri için Ultimate 3000 UHPLC sistemiyle birlikte çalışan Q Exactive™ Orbitrap kütle spektrometresi (Thermo Fisher Scientific, Waltham, MA, ABD), pozitif modda ısıtılmış elektrosprey iyonizasyon (HESI) kaynağı kullanılarak, yüksek çözünürlükte veri toplama işlemi gerçekleştirilmiştir. HESI parametreleri sprey voltajı, 3 kV; gaz akış hızları; sprey gazı akış hızı, 50 AU; kurutma gazı akış hızı, 15 AU; süpürme gazı akış hızı, 1 AU; kapiler sıcaklığı, 380°C; kurutma gazı sıcaklığı, 350°C olacak şekilde ayarlanmıştır.

Örnekler paralel reaksiyon izleme (PRM) modunda Tablo 2'de verilen inklüzyon listesi kullanılarak kantitatif analiz gerçekleştirilmiştir. Analiz parametreleri 35000 çözünürlük; normalize çarpışma enerjisi 35, AGC hedefi 10000, Maximum IT 100 ms ve analiz süresi 15 dk. olarak ayarlanmıştır.

Tablo 2. Kütle spektrometri paralel reaksiyon izleme inklüzyon listesi
Table 2. Mass spectrometry parallel reaction monitoring inclusion list

Molekül İsmi	Molekül Formülü [M]	Kütle [m/z]	Polarite	Başlangıç (dk.)	Bitiş (dk.)
Niasin	$C_6H_5NO_2$	124.03930	Pozitif	0.07	1.80
Piridoksin	$C_8H_{11}NO_3$	170.08117	Pozitif	0.60	1.25
D-pantotenik asit)	$C_9H_{17}NO_5$	220.11795	Pozitif	3.00	4.80
Biyotin	$C_{10}H_{16}N_2O_3S$	245.09544	Pozitif	5.55	6.00
Tiamin	$C_{12}H_{17}N_4OS$	265.11176	Pozitif	0.50	0.95
Riboflavin	$C_{17}H_{20}N_4O_6$	377.14556	Pozitif	5.75	6.20
Folik asit	$C_{19}H_{19}N_7O_6$	442.14696	Pozitif	4.95	5.40
Metilkobalamin	$C_{63}H_{91}CoN_{13}O_{14}P$	673.79121	Pozitif	4.35	5.60
Siyanokobalamin	$C_{63}H_{88}CoN_{14}O_{14}P$	678.29098	Pozitif	5.41	5.85

İstatistiksel Analizler

Çalışma kapsamındaki tüm deney setleri üçerli bağımsız biyolojik tekrarlar şeklinde gerçekleştirilmiş olup, sonuçlar ortalama ± standart sapma olarak raporlanmıştır. İstatistiksel değerlendirmeler SPSS Statistics (v25, IBM, Chicago, IL, ABD) programı kullanılarak iki uçlu bağımsız örneklem t-testi ile %95'lik güven aralığında belirlenmiştir.

BULGULAR ve TARTIŞMA

Besinlerde vitamin içeriklerinin sıcaklık temelli gıda hazırlama (pastörizasyon, vb.) ve/veya pişirme işlemleri sırasında gösterdiği değişikliklerin araştırılması, gıda sektöründe önem taşıyan konulardandır. Lee ve ark. [37] tarafından gerçekleştirilen çalışmada kaynatma, buğulama, mikrodalga gibi farklı pişirme işlemlere maruz bırakılan sebzelerde bulunan vitaminlerden ağırlıklı olarak Vitamin C ve yağda çözünen vitaminlerin (A, D) korunduğu, bazılarının ise kaybolduğu (K vitamini gibi) raporlanmıştır. Rickman ve ark. [38] ise özellikle konserveleme öncesi ısıl işleme bırakılan meyve ve sebzelerde B ve C vitaminlerinin azaldığını belirlemiştir. Kaynatma işleminin süt içerisinde bulunan B_{12} vitamin içeriğini oldukça düşürdüğü [39]; benzer şekilde kavurma işleminin fıstık ürünlerinde B_1 vitamini içeriğini azalttığı raporlanmıştır [40].

B vitamini içerikleri açısından oldukça zengin *C. vulgaris* türü mikroalglerin ekmek [41], makarna [42], kurabiye [36], vb. gıda ürünlerinde kullanımı yaygınlaşmaktadır. Bu çerçevede, farklı gıda ürünlerin pişirme sürelerini kapsayacak şekilde 125°C sıcaklıkta 35 dk'lık uzun pişirme süresinin B vitamini içeriklerine olan etkisinin araştırıldığı bu çalışmada, *C. vulgaris* türü mikroalglerde B_9 (folik asit) dışında tespit edilen tüm diğer B vitamin içeriklerinin, pişirme işlemine tabi tutulamayan kontrol grubuna göre korunduğu ve pişirme işleminin daha fazla B vitamini çıkmasına yardımcı olabileceği belirlenmiştir (Tablo 3).

Tablo 3. Otuzbeş dakikalık pişirme işlemine maruz bırakılan mikroalg örneklerindeki B vitamini içeriklerinin kontrol grubuna göre değişimi

Table 3. Changes in Vitamin B contents of 35-min baked microalgae samples compared to no-bake control group

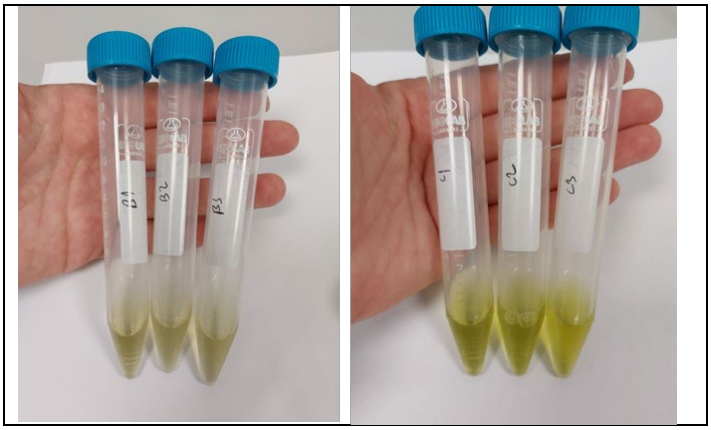
Vitamin türü	Kontrol grubu (µg/100g kuru ağırlık)	35 dk. Pişirme (µg/100g kuru ağırlık)
B_1 (Tiamin)	579.04±19.34	680.66±62.15*
B_2 (Riboflavin)	1.82±0.45	63.20±17.27*
B_3 (Niasin)	4814.64±390.06	7973.80±2022.08*
B_5 (Pantotenik asit)	127.65±12.96	127.57±0.41
B_6 (Piridoksin)	2.08±0.19	7.05±0.07*
B_7 (Biyotin)	8.47±0.33	9.78±1.02
B_9 (Folik asit)	ND	ND
B_{12m} (Metilkobalamin)	243.39±25.91	264.98±18.24

*İstatistiksel olarak kontrol grubundan farklı ($p<0.05$) (n=3).

UHPLC-HR/MS kullanılarak gerçekleştirilen analizlerde B_1 (tiamin), B_2 (riboflavin), B_3 (niasin), ve B_6 (piridoksin) vitaminlerinin 35 dk pişirme sürelerine maruz bırakılan örneklerde kontrol grubuna kıyasla istatistiki olarak anlamlı şekilde ($p<0.05$) daha fazla bulunduğu belirlenmiştir (Çizelge 3). Vitamin B_7 (biyotin) ve B_{12m} (metilkobalamin) konsantrasyonları incelendiğinde ise 35 dk pişirme işlemine tabi tutulan örneklerin kontrol grubuna göre bir miktar fazla konsantrasyonda oldukları ancak bu farkın istatistiki olarak anlamlı olmadığı ($p>0.05$) gözlenmiştir. Vitamin B_5 (pantotenik asit) içeriklerinde ise 35 dk pişirme sonrasında kontrol grubuna göre değişim gözlenmemiştir (Tablo 3).

Yakın zamanda yayınlanan bir derleme çalışmada, mikrodalga kullanılarak pişirilen makroalglerde vitamin ve diğer metabolit içerikleri daha fazla korunurken, kaynatma yöntemi tercih edilerek hazırlanan makroalglerde ise daha fazla metabolit kaybı olduğu değerlendirilmiştir [44]. Literatürde genel olarak B vitaminlerinin ısıl işlemlere tabi tutulduklarında bozuldukları belirtilmekle birlikte, özellikle B_1 (tiamin), B_5 (pantotenik asit), B_6 (piridoksin), B_9 (folik asit) ve B_{12m} (metilkobalamin) vitaminlerinin diğer vitaminlere kıyasla ısıya karşı daha fazla hassas oldukları raporlanmaktadır [33, 43, 45]. Bu çalışma kapsamında ise bu yaygın görüşün tersine 125°C sıcaklıkta 15 dk ve 35 dk'lık sürelerde pişirilen *C. vulgaris* türü mikroalg örneklerinde B vitaminlerinin kontrol grubuna göre daha yüksek konsantrasyonlarda olduğu belirlenmiştir (Tablo 3). Bu sonuçlar, bitkisel hücre yapısına benzer kalın hücre çeperlerine sahip *C. vulgaris* türü mikroalglerin [46], pişirme işlemi sırasında sıcaklığa maruz bırakılmasıyla birlikte daha fazla B vitamini elde edilmesine sebep olabileceği değerlendirilmiştir. Bu görüşün dayanağı olarak, B vitaminlerinin ekstraksiyonu sonrasında ve analitik ölçümlerden hemen önce alınan görüntülerde 35 dk pişirme işlemine maruz bırakılan örneklerin başlangıç biyokütle miktarları aynı olmasına rağmen daha koyu lizatların elde edildiği gözlenmiştir (Şekil 3).

Şekil 3'den görüldüğü üzere pişirme işleminin mikroalg hücre duvarının parçalanmasına yardımcı olabileceği, böylelikle ısıl işleme tabi tutulmayan kontrol grubundan daha fazla B vitamini kompleksinin elde edilebileceği değerlendirilmiştir. Literatürde ısıl işleme tabi tutulmayan ham ve pişirilen örnekler ile yapılan çalışmalar karşılaştırıldığında, ızgara yapılan Afrika Kedi Balığı'nın pişirilmeyen balıklara göre daha fazla B_2 (riboflavin) ve B_3 (niasin) vitamini içerdiği gözlenmiştir [47]. Benzer ve kapsamlı bir başka çalışmada ise balık ve balık ürünlerinin buğulama, ızgara ve tava gibi farklı pişirme koşullarında B_2 ve B_3 vitaminlerin, pişirilmemiş ürünlere göre daha fazla olduğu kaydedilmiştir [48]. Bu durum, literatürdeki yaygın örneklere kıyasla bazı gıda ürünlerinin pişirme yöntemi ile ısıl işleme tabi tutulduklarında B vitamini içeriklerinin daha fazla açığa çıkabileceği ihtimalini doğurmakla birlikte, bu alanda daha kapsamlı ve kontrollü çalışmalara ihtiyaç duyulmaktadır.



Şekil 3. Mikroalg örneklerinin ekstraksiyon işlemi sonrası görüntüleri [Kontrol grubu (sol), 35 dk. pişirme (sağ)]

Table 3. Images of microalgae samples following extraction process [No-bake control group (left), 35-min baked group (right)]

SONUÇ

Özetle, bu çalışmada B vitaminleri içeriği açısından zengin olan ve son dönemlerde farklı fonksiyonel gıdalarda kullanımı yaygınlaşan *Chlorella vulgaris* türü mikroalglerin, karıştırılarak kullanıldığı ürünler itibariyle ekmek, kurabiye, vb. ürünlerin pişirme yöntemi ile ısıl işlemlere maruz bırakıldığında bazı B vitaminlerinin pişirilmeyen örneklere göre daha fazla açığa çıktığı, diğerlerinin ise maruz kaldıkları ısıl işleme rağmen korunduğu belirlenmiştir. Isıl işlemlere karşı hassas olduğu bilinen vitaminlerin mikroalg hücre çeperi içinde korunduğu, ısıl işlemin pişirme sonucu gıdada korunan B vitamini içeriklerine olumlu katkıda bulunabileceği raporlanmıştır.

TEŞEKKÜR

Bu çalışma Avrupa Birliği ve Türkiye Cumhuriyeti tarafından ortak finanse edilen, Sanayi ve Teknoloji Bakanlığı, AB ve Dış İlişkiler Genel Müdürlüğü, AB Mali Programları Daire Başkanlığı tarafından yürütülen Rekabetçi Sektörler Programı EuropeAid/140111/ IH/SUP/TR numaralı "Biyoekonomi Odaklı Kalkınma için Entegre Biyorafineri Konsepti" Proje kapsamında gerçekleştirilmiştir. Analitik ölçümlere katkıda bulunan Boğaziçi Üniversitesi İstanbul Mikroyosun Biyoteknolojileri Ar-Ge Birimi ekibinden Dr. Engin Bayram'a ve Duygu Özçelik'e; fotobiyoreaktör çizimi için Derya Gelgör'e teşekkür ederiz.

KAYNAKLAR

[1] Aaronson, S. (1986). A role for algae as human food in antiquity. *Food and Foodways,* 1(3), 311-315.

[2] Adjali, A., Clarot, I., Chen, Z., Marchioni, E., Boudier, A. (2021). Physicochemical degradation of phycocyanin and means to improve its stability: A short review. *Journal of Pharmaceutical Analysis*, 12(3), 406-414.

[3] Akyıl, S., İlter, I., Mehmet, K., Kaymak-Ertekin, F. (2016). Alglerden elde edilen yüksek değerlikli bileşiklerin biyoaktif/biyolojik uygulama alanları. *Akademik Gıda,* 14(4), 418-423.

[4] Alçay, A.Ü., Sağlam, A., Yalçın, S., Bostan, K. (2018). Possible protein sources for the future. *Akademik Gıda,* 16(2), 197-204.

[5] Ambati, R.R., Gogisetty, D., Aswathanarayana, R.G., Ravi, S., Bikkina, P.N., Bo, L., Yuepeng, S. (2019). Industrial potential of carotenoid pigments from microalgae: Current trends and future prospects. *Critical Reviews in Food Science and Nutrition,* 59(12), 1880-1902.

[6] Berry Ottaway, P. (2010). Stability of vitamins during food processing and storage. In Chemical Deterioration and Physical Instability of Food and Beverages, Edited by L.H. Skibsted, J. Risbo, M.L. Andersen, Woodhead Publishing, 80 High Street, Cambridge, CB22 3HJ United Kingdom. 539p.

[7] De Ruiter, G.A., Rudolph, B. (1997). Carrageenan biotechnology. *Trends in Food Science & Technology,* 8(12), 389-395.

[8] Dias, M.G., Sanchez, M., Bartolo, H., Oliveira, L. (2003). Vitamin content of fish and fish products consumed in Portugal. *Electronic Journal of Environmental, Agricultural and Food Chemistry,* 2(4), 510-515.

[9] Ersoy, B., Özeren, A. (2009). The effect of cooking methods on mineral and vitamin contents of African catfish. *Food chemistry,* 115(2), 419-422.

[10] Ferreira, A.S., Ferreira, S.S., Correia, A., Vilanova, M., Silva, T.H., Coimbra, M.A., Nunes, C. (2020). Reserve, structural and extracellular polysaccharides of *Chlorella vulgaris*: A holistic approach. *Algal Research,* 45, 101757.

[11] Fradique, M., Batista, A.P., Nunes, M.C., Gouveia, L., Bandarra, N.M., Raymundo, A. (2010). Incorporation of *Chlorella vulgaris* and *Spirulina maxima* biomass in pasta products. Part 1: Preparation and evaluation. *Journal of the Science of Food and Agriculture,* 90(10), 1656-1664.

[12] Fuliaş, A., Vlase, G., Vlase, T., Oneţiu, D., Doca, N., Ledeţi, I. (2014). Thermal degradation of B-group vitamins: B1, B2 and B6. *Journal of Thermal Analysis and Calorimetry,* 118(2), 1033-1038.

[13] Ghafari, M., Rashidi, B., Haznedaroglu, B.Z. (2018). Effects of macro and micronutrients on neutral lipid accumulation in oleaginous microalgae. *Biofuels,* 9(2), 147-156.

[14] Godde, C., Mason-D'Croz, D., Mayberry, D., Thornton, P.K., Herrero, M. (2021). Impacts of climate change on the livestock food supply chain; A review of the evidence. *Global Food Security,* 28, 100488.

[15] Gouveia, L., Batista, A.P., Miranda, A., Empis, J., Raymundo, A. (2007). *Chlorella vulgaris* biomass used as colouring source in traditional butter cookies. *Innovative Food Science & Emerging Technologies,* 8(3), 433-436.

[16] Graça, C., Fradinho, P., Sousa, I., Raymundo, A. (2018). Impact of *Chlorella vulgaris* on the rheology of wheat flour dough and bread texture. *LWT,* 89, 466-474.

[17] Helliwell, K.E., Lawrence, A.D., Holzer, A., Kudahl, U.J., Sasso, S., Kräutler, B., Smith, A.G. (2016). Cyanobacteria and eukaryotic algae use different chemical variants of vitamin B12. *Current Biology,* 26(8), 999-1008.

[18] Hildebrand, G., Poojary, M.M., O'Donnell, C., Lund, M.N., Garcia-Vaquero, M., Tiwari, B.K. (2020). Ultrasound-assisted processing of *Chlorella vulgaris* for enhanced protein extraction. *Journal of Applied Phycology,* 32(3), 1709-1718.

[19] İlter, I., Akyıl, S., Mehmet, K., Kaymak-Ertekin, F. (2016). Alglerden elde edilen stabilize edici maddeler. *Akademik Gıda,* 14(3), 315-321.

[20] Jayappriyan, K., Baskar, B., Vijayakumar, M., Brabakaran, A., Rajkumar, R., Elumalai, S. (2021). Food and nutraceutical applications of algae. In Algae for Food, Edited by K.R. Jayappriyan, B. Baskar, M. Vijayakumar, A. Brabakaran, R. Rajkumar, S. Elumalai, CRC Press, 5 Howick Place, London SW1P 1WG, England, 83p.

[21] Ji, X.J., Ren, L.J., Huang, H. (2015). Omega-3 biotechnology: a green and sustainable process for

omega-3 fatty acids production. *Frontiers in Bioengineering and Biotechnology,* 3, 158.
[22] Koyande, A.K., Chew, K.W., Manickam, S., Chang, J.S., Show, P.L. (2021). Emerging algal nanotechnology for high-value compounds: a direction to future food production. *Trends in Food Science & Technology,* 116, 290-302.
[23] Kulkarni, S., Nikolov, Z. (2018). Process for selective extraction of pigments and functional proteins from *Chlorella vulgaris*. *Algal Research,* 35, 185-193.
[24] Lee, S., Choi, Y., Jeong, H.S., Lee, J., Sung, J. (2018). Effect of different cooking methods on the content of vitamins and true retention in selected vegetables. *Food Science and Biotechnology,* 27(2), 333-342.
[25] Liu, S., Gifuni, I., Mear, H., Frappart, M., Couallier, E. (2021). Recovery of soluble proteins from *Chlorella vulgaris* by bead-milling and microfiltration: Impact of the concentration and the physicochemical conditions during the cell disruption on the whole process. *Process Biochemistry,* 108, 34-47.
[26] Misiou, O., Koutsoumanis, K. (2021). Climate change and its implications for food safety and spoilage. *Trends in Food Science & Technology*, 126, 142-152.
[27] Munawaroh, H., Darojatun, K., Gumilar, G., Aisyah, S., Wulandari, A. (2018). Characterization of phycocyanin from *Spirulina fusiformis* and its thermal stability. *4th International Seminar of Mathematics, Science and Computer Science Education,* October 14, 2017, Bandung, Indonesia, Book of Proceedings, 012205p.
[28] Onwezen, M.C., Bouwman, E.P., Reinders, M.J., Dagevos, H. (2021). A systematic review on consumer acceptance of alternative proteins: Pulses, algae, insects, plant-based meat alternatives, and cultured meat. *Appetite,* 159, 105058.
[29] Palabıyık, İ. (2017). Liyofilize bazı mikroalg türlerinin sakız bileşiminde doğal renklendirici olarak kullanımı. *Gıda,* 42(6), 676-681.
[30] Panahi, Y., Darvishi, B., Jowzi, N., Beiraghdar, F., Sahebkar, A. (2016). *Chlorella vulgaris*: a multifunctional dietary supplement with diverse medicinal properties. *Current Pharmaceutical Design,* 22(2), 164-173.
[31] Pires, J.C. (2017). COP21: the algae opportunity? *Renewable and Sustainable Energy Reviews,* 79, 867-877.
[32] Qazi, W.M., Ballance, S., Kousoulaki, K., Uhlen, A.K., Kleinegris, D.M., Skjånes, K., Rieder, A. (2021). Protein enrichment of wheat bread with microalgae: *Microchloropsis gaditana*, *Tetraselmis chui* and *Chlorella vulgaris*. *Foods,* 10(12), 3078.
[33] Rani, K., Sandal, N., Sahoo, P. (2018). A comprehensive review on chlorella-its composition, health benefits, market and regulatory scenario. *The Pharma Innovation Journal,* 7(7), 584-589.
[34] Rickman, J.C., Barrett, D.M., Bruhn, C.M. (2007). Nutritional comparison of fresh, frozen and canned fruits and vegetables. Part 1. Vitamins C and B and phenolic compounds. *Journal of the Science of Food and Agriculture,* 87(6), 930-944.
[35] Rojas, A.M., Gerschenson, L.N. (2001). Ascorbic acid destruction in aqueous model systems: An additional discussion. *Journal of the Science of Food and Agriculture,* 81(15), 1433-1439.
[36] Ryley, J., Kajda, P. (1994). Vitamins in thermal processing. *Food chemistry,* 49(2), 119-129.
[37] Safi, C., Zebib, B., Merah, O., Pontalier, P.Y., Vaca-Garcia, C. (2014). Morphology, composition, production, processing and applications of *Chlorella vulgaris*: A review. *Renewable and Sustainable Energy Reviews,* 35, 265-278.
[38] Sayeda, M., Ali, G.H., El-Baz, F.K. (2015). Potential production of omega fatty acids from microalgae. *International Journal of Pharmaceutical Sciences Review and Research,* 34(2), 210-215.
[39] Seyfabadi, J., Ramezanpour, Z., Amini Khoeyi, Z. (2011). Protein, fatty acid, and pigment content of *Chlorella vulgaris* under different light regimes. *Journal of Applied Phycology,* 23(4), 721-726.
[40] Stuetz, W., Schlörmann, W., Glei, M. (2017). B-vitamins, carotenoids and α-/γ-tocopherol in raw and roasted nuts. *Food Chemistry,* 221, 222-227.
[41] Syed, S., Arasu, A., Ponnuswamy, I. (2015). The uses of *Chlorella vulgaris* as antimicrobial agent and as a diet: The presence of bio-active compounds which caters the vitamins, minerals in general. *International Journal of Bio-Science and Bio-Technology,* 7(1), 185-190.
[42] Tuğçe, Ö., Bayram, B. Spirulina mikroalginin besinsel özellikleri ve sağlık üzerine potansiyel etkileri. *Akademik Gıda,* 20(3), 296-304.
[43] Usov, A.I. (1999). Alginic acids and alginates: Analytical methods used for their estimation and characterisation of composition and primary structure. *Russian Chemical Reviews,* 68(11), 957-966.
[44] Ho, K., Redan, B.W. (2022). Impact of thermal processing on the nutrients, phytochemicals, and metal contaminants in edible algae, *Critical Reviews in Food Science and Nutrition*, 62(2), 508-526.
[45] Watanabe, F. (2007). Vitamin B12 sources and bioavailability. *Experimental Biology and Medicine,* 232(10), 1266-1274.
[46] Watanabe, F., Yabuta, Y., Bito, T., Teng, F. (2014). Vitamin B12-containing plant food sources for vegetarians. *Nutrients,* 6(5), 1861-1873.
[47] Weber, S., Grande, P.M., Blank, L.M., Klose, H. (2022). Insights into cell wall disintegration of *Chlorella vulgaris*. *Plos One,* 17(1), e0262500.
[48] West, V.A. (2015). Stability of selected B vitamins in thermally-treated pinto beans, Department of Nutrition, Dietetics and Food Science, Brigham Young University Yüksek Lisans Tezi, Provo, Utah, Amerika Birleşik Devletleri, 105 s.

SİDAS MEDYA
Akademik Gıda®
ISSN Online: 2148-015X
https://dergipark.org.tr/tr/pub/akademik-gida

Akademik Gıda 21(2) (2023) 174-186, DOI: 10.24323/akademik-gida.1351175

Research Paper / Araştırma Makalesi

Physicochemical and Phytochemical Properties of Different Extracts of Sumac Plant (*Rhus coriaria* L.) Grown in Tunceli, Türkiye

Esra Yuksel[1], Olcay Kaplan Ince[2]

[1]Munzur University, Institute of Graduate Studies, Department of Food Engineering, 62000, Tunceli, Türkiye
[2]Munzur University, Faculty of Fine Arts, Design and Architecture, Department of Gastronomy and Culinary Arts, 62000, Tunceli, Türkiye

Received (Geliş Tarihi): 23.06.2022, Accepted (Kabul Tarihi): 16.05.2023
Corresponding author (Yazışmalardan Sorumlu Yazar): olcaykaplan@munzur.edu.tr (O. Kaplan Ince)
+90 428 213 17 94 +90 428 213 18 61

ABSTRACT

In this study, sumac plant (*Rhus coriaria* L.) naturally grown in Tunceli (Türkiye) was collected from 5 different provinces and investigated for their phytochemical properties. In sumac samples, pH, color, ash amount, total phenolic content (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging capacity, metal chelating capacity, copper (II) ion reducing antioxidant capacity (CUPRAC), reducing power, mineral matter content, organic acids as tartaric acid, malic acid and citric acid, phenolic compounds as gallic acid, vanillic acid, caffeic acid, routine, resveratrol, (-)- epicatechin, and (+)- catechin hydrate contents were determined. The highest TPC was found in the acidified methanol extract (AME) and the DPPH free radical scavenging capacity was found in the acidified acetonitrile extract (AAE). It was determined that all extracts of sumac sample 5 (S5) collected from Pertek district, metal chelating capacity was higher than the other samples but lower than the metal chelating capacity of ethylene diamine tetra acetic acid (EDTA). CUPRAC was detected at the highest concentration of 118.0±3.0 mg caffeic acid equivalent kg^{-1} (mg CAE kg^{-1}) in the AME, at the lowest 10.2±0.6 mg CAE kg^{-1} in the AAE for S5 sample. It was found that the reducing powers of all samples were found to be lower than the reducing power of synthetic antioxidants (butylhydroxytoluene (BHT), α tocopherol, and vitamin C), and the samples were rich in mineral substances, the predominant organic acid was malic acid, and phenolic compound was gallic acid.

Keywords: Antioxidant capacity, Phenolic substance, Mineral, Organic acid, Sumac

Tunceli'de Yetişen Sumak Bitkisinin (*Rhus coriaria* L.) Farklı Ekstraktlarının Fizikokimyasal ve Fitokimyasal Özellikleri

ÖZ

Bu çalışmada, Tunceli'de doğal olarak yetişen sumak bitkisinin (*Rhus coriaria* L.) 5 farklı bölgeden toplanarak fitokimyasal bileşenleri araştırılmıştır. Sumak örneklerinde pH, renk, kül miktarı, toplam fenolik madde (TFM), 2,2-difenil-1-pikrilhidrazil (DPPH) serbest radikal temizleme kapasitesi, metal şelatlama kapasitesi, bakır (II) iyonu indirgeme antioksidan kapasitesi (CUPRAC), indirgeme kuvveti, mineral madde içeriği, tartarik asit, malik asit ve sitrik asit gibi organik asitler, gallik asit, vanilik asit, kafeik asit, rutin, resveratrol, (-)- epikateşin ve (+)- kateşin hidrat gibi fenolik bileşikler belirlenmiştir. En yüksek TFM miktarı asitlendirilmiş metanol ekstraktında (AME) ve DPPH serbest radikal süpürme kapasitesi asitlendirilmiş asetonitril ekstraktında (AAE) bulundu. Pertek bölgesinden toplanan sumak örneğinden (S5) elde edilen tüm ekstraktların metal şelatlama kapasitesi diğer numunelere göre daha yüksek, ancak etilen diamin tetra asetik asit (EDTA) metal şelatlama kapasitesinden ise daha düşük olduğu bulunmuştur. CUPRAC değerinin S5 örneğinin AME'nda en yüksek, 118,0±3,0 mg kafeik asit eşdeğeri kg^{-1} (mg KAE kg^{-1}), AAE'ında ise en

düşük, 10,2±0,6 mg KAE kg^{-1} olduğu tespit edilmiştir. Tüm örneklerin indirgeme kuvvetinin sentetik antioksidanların (bütil hidroksitoluen (BHT), α tokoferol, ve vitamin C), indirgeme kuvvetinden daha düşük olduğu ve örneklerin mineral maddelerce zengin olduğu, baskın organik asidin malik asit ve fenolik bileşiğin gallik asit olduğu belirlenmiştir.

Anahtar Kelimeler: Antioksidan kapasite, Fenolik madde, Mineral, Organik asit, Sumak

INTRODUCTION

Sumac (*Rhus coriaria* L.), which belongs to *Anacardiaceae* family, is a small tree that reaches a height of 4 m and with imparipinnate leaves [1, 2]. It is shrub with reddish-brown colored fruit and with one seed fruit which is 4–6 mm slightly fleshy, lenticular drupes, surrounded by short glandular hairs. It is usually wild grown in the Mediterranean region extending from the Canary Island to Iran and Afghanistan besides southeast of Anatolia-Türkiye [1–4]. Sumac is traditionally used as a spice and flavoring agent in several Mediterranean and Middle Eastern countries including Iran, Lebanon, Jordan, and Syria as well as Türkiye, and has a widespread preference in medicine and nutritional applications [3]. For enhancing taste of vegetable dishes, it has been preferable as a condiment both in Türkiye and Iran [4]. Because of antibacterial, hypolipidemic, antiinflammatory, antifungal, hypoglycemic and antioxidant activities. It has used in many area including cosmetic, dying agent, nutrition especially in pharmacy and other industries [1, 3, 5]. Sumac is a rare plant that is frequently preferred in traditional medicine as medicinal herb, due to these superior and versatile properties [6].

As reported in various studies about sumac composition, over 200 components such as phenolic compounds, flavonoids, organic acids, terpenoids, anthocyanins and their derivates compounds have been isolated from the sumac plant [3]. On the other hand, as can be understood from the studies conducted to evaluate its potential antibacterial, antifungal and antioxidant effects, it has also been proven that the sumac in question has analgesic, antilipidemic and hypoglycemic effects [5]. Because of the various phytochemical components which contains such as bioactive compounds and phenolic compounds, sumac has been frequently used as a treatment agent for various diseases including diabetes, stomach pain, smallpox, hypertension, dysentery, diarrhea, paralysis, hematemesis, ophthalmia, diuresis, atherosclerosis, measles, and liver disease, tooth and gum diseases. Moreover, it has been preferred for cancer treatment in traditional medicine [1, 3, 7].

Sumac, a natural antibacterial agent, is also used as a natural preservative in food products, as well as an ingredient in beverages and sauces, and as a natural acidifier in recipes. Based on previous investigations, it has been clearly reported that sumac addition to foods or water can have a beneficial effect on both human and animal health [1]. Recently, the hypoglycemic effect and chemopreventive effect of sumac, which is frequently consumed as herbal medicine due to its anti-fibrogenic, antimicrobial and anti-inflammatory activities, has also attracted the attention of many researchers [2]. In addition, it has been reported that the alcohol extract of sumac is genoprotective and hydroalcoholic extracts inhibit skin proinflammatory mediators significantly, and it is emphasized that this activity is due to the tannins it contains [2].

Owing to the advantages of using the sumac plant in different areas, there are various studies in the literature on different parts such as stick, leaf and fruit. Abu-Reidah et al.[8] extracted the fruit epicarps' using methanol and investigated phytochemical composition by using high performance liquid chromatography-mass spectrometry method (HPLC-MS). Al-Boushi et al. [9] determined phenolic compounds by using HPLC in leaves and sticks. Gallic acid, 4- hydroxyl benzoic acid, synergic acid, and vanillic acid concentrations were found higher in leaves than in sticks, only caffeic acid concentration was higher in sticks than in leaves. Ereifej et al. [10] extracted sumac fruit by various solvents at various temperatures and they reported that total phenolic content was significantly affected by solvent type and temperature.

The main goal of the present study was carried out a detailed investigation about sumac grown naturally in Tunceli, Türkiye. Sumac samples were collected from five different provinces in Tunceli. pH, color, ash amount, total phenolic content (TPC), DPPH free radical scavenging capacity, metal chelating capacity, CUPRAC, reducing power, mineral matter content, organic acids as tartaric acid, malic acid and citric acid, phenolic compounds as gallic acid, vanillic acid, caffeic acid, routine, resveratrol, (-)- epicatechin and (+)- catechin hydrate contents were determined and evaluated. In this context, various sumac extracts including water, acidified methanol were prepared and investigated. This study is the most comprehensive study on Tunceli (Türkiye) sumac.

MATERIALS and METHODS

Materials

Sumac plant samples were collected from different districts of Tunceli (Türkiye) province. Collected places are given in Figure 1. The collected sumac samples were cleaned and dried at room temperature, then separated from the seeds. The cleaned and dried samples were ground with a grinder. All analyses were performed by using ground samples and triplicate.

(a) (b)

Figure 1. (a) Image of sumac, (b) Sampling points of sumac samples (S1: Mazgirt, S2: Tunceli-Center, S3: Çemişgezek, S4: Pülümür, S5: Pertek)

Methods

pH

Five grams of ground sumac was taken and 10 mL of ultra-pure water was added. After vortexing 5 min, pH was measured with digital pH meter (Thermo Scientific, Orion3Star, MA, USA) [11].

Color Analysis

Hunter (L, a, b) color values were measured by colorimeter (Chroma Meter, CR-400, Konica Minolta Sensing, Inc., Osaka, Japan) at room temperature in the appropriate light [11].

Ash Content Amount

For the determination of ash, 3 g of ground sumac sample was weighed and placed in a beaker. The temperature of the ash oven (Nuve Furnace, MF110, Ankara, Turkey) was gradually increased and kept at 450°C until white ash was obtained. Samples were cooled in desiccator and then weighed [12].

Preparation of Samples for Antioxidant Capacity Tests

For antioxidant tests sumac samples were extracted using 3 different solvents, as acidified methanol, acidified acetonitrile and acidified water. 3 g of sumac sample was taken and 20 mL of acidified solvent was added. After shaking for 2 h using the orbital shaker (Orbital Shaker, JSOS-500, JS Research Inc. Gonju, Korea), then centrifuged 5 min at 3000 rpm. The obtained supernatant was passed through a 0.45 µm injection filter and stored in a refrigerator until to be used for antioxidant tests [13].

Total Phenolic Content

The total phenolic content (TPC) in the extracted samples was determined by some modifications to the method developed by Singleton and Rossi [14] using the Folin-Ciocalteu reagent. 0.1 mL of the gallic acid standards at different concentrations was added and then 5 mL of pure water and 0.5 mL of Folin-Ciocalteu solution were added and vortexed. The solution was incubated in the dark at room temperature for 3 min. After addition of 1.5 mL of 2% Na_2CO_3 vortexed and again incubated for 2 h at room temperature in the dark. The absorbances were determined by using ultraviolet visible (UV-Vis) spectrophotometer (Shimadzu, UV-1601, Kyoto, Japan) at 760 nm. The blank sample was done at same way by using ultra pure water instead of sumac extract. Gallic acid was used as standard. Using gallic acid standard graph, the TPC of the samples was calculated. The TPC was then expressed in terms of mg gallic acid equivalent kg^{-1} dry weight (mg GAE kg^{-1} dw).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Capacity

Blois [15] method was applied by making some modifications for determination DPPH free radical scavenging capacity. 0.1 mL was taken from the sumac samples extract and the final volume was completed to 3 mL with methanol. After addition of 1 mL of DPPH solution mixture was vortexed and incubated at room temperature in the dark for 30 min. Then, absorbance of mixture was measured by UV-Vis spectrophotometer at 517 nm. The same procedure was carried out using methanol instead of sumac extract for the control solution. The inhibition values were calculated according to the following formula (Eq. 1).

DPPH Free Radical Scavenging Capacity (%) = [(Absorbance of control - Absorbance of sample) / Absorbance of control] × 100 (1)

Metal Chelating Capacity

For the determination of chelating capacity of iron (II) ions, Dinis et al. [16] method was carried out by applying some changes. 1 mL of sumac extracts at different concentrations was taken. 3.7 mL of ultra pure water and 100 µL of 2 mM $FeCl_2$ were added and vortexed. Mixture was incubated at room temperature in the dark for 30 min. Then added 200 µL 5 mM of ferrozine and vortexed for 10 min at the same conditions. Absorbance measurements were done at 562 nm by UV-Vis spectrophotometer. Ethylene diamine tetra acetic acid (EDTA) was used as standard. The same procedure was

carried out using ultra pure water instead of sumac extract for the control solution.

Copper (II) Ion Reducing Antioxidant Capacity (CUPRAC)

For the determination of CUPRAC Apak et al. [17] method was used. 1 mL of 1.0×10^{-2} M $CuCl_2$, 7.5×10^{-3} M neocuproine and 1 M NH_4Ac (pH 7) was taken. 0.5 mL sumac extract and 0.5 mL of ultra pure water were added to this solution. The solution was vortexed and then incubated 30 min in the dark. Absorbance was determined at 450 nm. Caffeic acid at different concentrations was used as standard and all procedures were repeated for standards. The same procedure was carried out using pure water instead of the sample as the control solution. The CUPRAC values of the samples were expressed in mg caffeic acid equivalent kg^{-1} dry weight (mg CAE kg^{-1} dw).

Reducing Power

Reducing power was determined according to Oyaizu [18] method by making some changes. This method is based on the reduction of Fe^{3+} to Fe^{2+} by the antioxidant substances. 1 mL of sumac extracts at different concentrations was taken. 2.5 mL of phosphate buffer and 2.5 mL of 1% $K_3Fe(CN)_6$ were added, vortexed and incubated at 50°C for 20 min. After addition of 2.5 mL of 10% trichloroacetic acid, it was centrifuged. 2.5 mL of supernatant was taken. 2.5 mL of ultra pure water and 0.5 mL of 0.1% $FeCl_3$ solution were added and vortexed. The absorbance was determined at 700 nm. Ultra pure water was used instead of the sumac extract for the control solution and the same procedure was carried out. The increase in absorbance was evaluated as the reducing power capacity of the sumac extract.

Mineral Matter Content

For the analysis of mineral content, 0.3 g of sumac sample was taken and 5 mL of concentrated nitric acid was added on it. Then it was solubilized by applying the steps given in Table 1 in the microwave oven (Berghof Speedwave 2, Berghof, Eningen, Germany) [19].

Table 1. Microwave oven digestion steps

Step	1	2	3
Temperature (°C)	145	190	100
Power (%)	75	90	40
Time (min)	5	10	10

Samples solubilized in microwave oven were centrifuged and clear solutions were obtained. The obtained clear solutions were analyzed by flame atomic absorption spectrometer (FAAS) (PerkinElmer AAnalyst 800, Shelton, CT, USA) according to operation conditions in Table 2.

Table 2. Operation conditions of FAAS

Element	Acetylene flow rate (L min^{-1})	Air flow rate (L min^{-1})	Wave length (nm)	Slit range (nm)
Ca	2.0	17.0	422.7	0.7
Cu	2.0	17.0	324.8	0.7
Fe	2.0	17.0	248.3	0.2
K	2.0	17.0	766.5	0.7
Mg	2.0	17.0	285.2	0.7
Mn	2.0	17.0	279.5	0.2
Na	2.0	17.0	589.0	0.2
Zn	2.0	17.0	213.9	0.7

Organic Acid Composition

The amount of organic acid was determined by making some changes on the method developed by Bevilacqua and Califano [20]. For determination of organic acids (citric acid, malic acid, and tartaric acid), 3 g of sumac sample was taken and 20 mL 0.004 M H_2SO_4 was added. After shaking for 2 h in an orbital shaker, centrifuged 15 minutes at 3000 rpm. Obtained supernatant was passed through 0.45 µm injection filter and then SEP-PAK C18 cartridge. 1 mL of solution was taken and analyzed by high performance liquid chromatography (HPLC) (Schimadzu, Prominence LC-20A) method. HPLC apparatus was equipped with diode array detector (DAD) (SPD-M20A), column oven (CTO-10ASVP), pump (LC-20AT), and degasser (DGU-20A5). H_2SO_4 (0.004 M) was used as mobile phase and flow rate was 1 mL min^{-1}. A 5 µM 4.6×250 mm (Inertsil ODS-3) column was used and temperature was set to 30°C. Sample volume was 20 µL.

Phenolic Compounds Composition

For the determination of phenolic compounds (gallic acid, vanillic acid, caffeic acid, routine, resveratrol, (-)- epicatechin and (+)- catechin hydrate), 3 g of sumac sample was taken and 20 mL of acidified water was added. The mixture was shaked for 2 h in an orbital shaker and then centrifuged 15 minutes at 3000 rpm. The supernatant was passed through a 0.45 µm injection filter. UniverSil HS C18 (5µm, 250×4.6 mm) column was used for HPLC analysis. Methanol and 2% acetic acid were used as mobile phase in gradient elution [13].

RESULTS and DISCUSSION

pH

The measured pH values of the sumac samples are given in Table 3. The highest pH was determined in S2 (3.28±0.23) sample and the lowest pH was determined in S3 (2.78±0.15) sample. Fereidoonfara et al. [21], analyzed Iranian sumac samples and samples pH values were varied in the range from 2.66 to 3.90, and Caliskan and Dirim [22] found sumac extracts pH in the range from 3.13 to 3.23.

Table 3. pH, ash amount and color (L, a, and b) values of sumac samples

Sample	pH	Ash amount (%)	L	a	b
S1	2.99±0.14	4.0±0.4	35.6±1.2	7.6±0.4	20.7±0.2
S2	3.28±0.23	5.0±0.3	37.6±1.2	7.6±0.2	19.1±0.4
S3	2.78±0.15	5.3±0.5	23.1±0.8	13.5±0.2	12.8±0.2
S4	2.83±0.25	4.3±0.4	43.0±0.7	9.5±0.8	19.0±0.8
S5	2.94±0.26	4.6±0.4	34.6±2.4	9.7±0.8	17.3±0.4

Ash

The ash contents (%) of the sumac samples are given in Table 3. Among the sumac samples, the highest ash content was found in sample S3 and the lowest in sample S1. It was observed that the amount of ash varied between 4.0±0.4% and 5.3±0.5%. Ash amount of Syrian sumac was determined as 2.66±0.33% and Chinese sumac as 5.37±0.14% [23]. The ash content of the sumac extracts obtained by spray drying at different temperatures was investigated in the range of 1.15 and 3.37% on a wet basis [22].

Color

Color values of sumac samples are given in Table 3. The L value is between 0 and 100, and the darker value increases as it approaches 0, while the lighter value increases as it approaches 100. The sample S4 has the highest L value, that is, the brightest. The sample with the lowest L value is the sample S3 and has a darker color than the others. a value takes + and - value and "+" denotes redness, "-" denotes greenery. Since a was positive in the results, the highest degree of redness was found in the sample S3, and at least in the samples S1 and S2. b value takes the value of "+" and "-" and denotes "+" yellowness, while "-" denotes blueness. Again, since the b value results were positive, the highest degree of yellowness was found in the sample S1 and the least in the sample S3. Caliskan and Dirim [24] detected the L* value of sumac berries and sumac extract 36.77±0.89 and 32.17± .13, respectively. a* value of sumac berries and sumac extract 8.00±1.29 and 2.25±0.08, respectively. b* value of sumac berries and sumac extract 5.56±0.55 and 0.93±0.03, respectively.

Total Phenolic Content

The TPC in the acidified methanol extract (AME), acidified acetonitrile extract (AAE), and acidified water extract (AWE) of the sumac plant was determined by Folin Ciocalteu reagent. Linear regression equation and correlation coefficient were calculated as $y = 0.0007x+0.0598$ and $R^2 = 0.09973$, respectively. The TPC of samples of various extracts are given in Table 4.

Table 4. TPC and CUPRAC values of sumac samples

	TPC (mg GAE kg^{-1})			CUPRAC (mg CAE kg^{-1})		
Sample	AME	AAE	AWE	AME	AAE	AWE
S1	1100±44	494±40	672±50	90.3±2.5	69.1±2.5	100.0±5.7
S2	1388±33	350±5	754±47	96.0±0.3	26.0±0.3	52.3±1.1
S3	811±48	269±14	331±33	54.5±2.3	17.0±0.8	20.0±0.2
S4	797±5	231±10	631±64	53.3±1.0	34.0±0.9	41.2±0.3
S5	1929±63	526±67	904±39	118.0±3.0	10.2±0.6	70.0±1.3

As seen in Table 4, when the extracts of the same sumac in different solvents were compared, the highest TPC was determined in AME. This is followed by AWE and AAE, respectively. TPC of AME of different sumac samples were S5>S2>S1>S3>S4, respectively. TPC of different sumac samples from the highest to the lowest S5>S1>S2>S3>S4 for AAE and S5>S2>S1>S4>S3 for AWE. When the TPC of all samples in different solvents were compared, it was detected that the sample S5 was highest in terms of TPC.

Romeo et al. [25], extracted sumac fruit and leaf with water and different proportions of ethanol-water mixtures. The sumac leaf TPC was found higher than fruit. TPC of sumac fruit in different solvents were found to be in the range of 2.80±0.01-9.47±0.01 g GAE kg^{-1}, and in range of 15.22±0.13-29.38±0.24 g GAE kg^{-1} in the leaf. In a study, the TPC was determined by extracting Syrian and Chinese sumac samples under different experimental conditions such as ethanol concentration, extraction time, particle size and ratio of solvent amount to sumac amount. Optimum experimental conditions were found to be the same for Syrian and Chinese sumac samples except extraction time. Under optimum conditions, the TPC was determined as 159.32 mg GAE g^{-1} for Syrian sumac and 150.68 mg GAE g^{-1} for Chinese sumac [16]. Bashash et al. [27], extracted sumac samples with different solvents and compared TPC. TPC was found highest in extract of methanol in brown sumac fruit, in extract of ethanol-methanol mixture in brown sumac powder and in ethanol extract in red sumac powder samples. In all samples water extract TPC were the lowest. Bursal and Köksal [28], calculated water and ethanol extracts of sumac samples' TPC as GAE (per 1 mg extract). The TPC of ethanol and water extract were found as 15 µg GAE and 63 µg GAE, respectively. Zannou et al. [29] used acidic deep eutechtic solvents and conventional solvents to extract sumac samples by using homogenate and ultrasound assisted extraction methods. TPC was found highest, 124.96 ± 3.43 mg GAE g^{-1}, by ultrasound assisted extraction method.

DPPH Free Radical Scavenging Capacity

The inhibition (%) graphs of the DPPH free radical scavenging capacity of the sumac samples and different extracts are given in Figure 2. It was found that the DPPH free radical scavenging capacity of the samples extracted with acidified acetonitrile was the highest in all samples. The DPPH free radical scavenging capacity of samples extracted with acidified water was the lowest in samples S1 and S5, while the DPPH free radical scavenging capacity of samples extracted with acidified methanol was found to be the lowest in samples S2, S3, and S4.

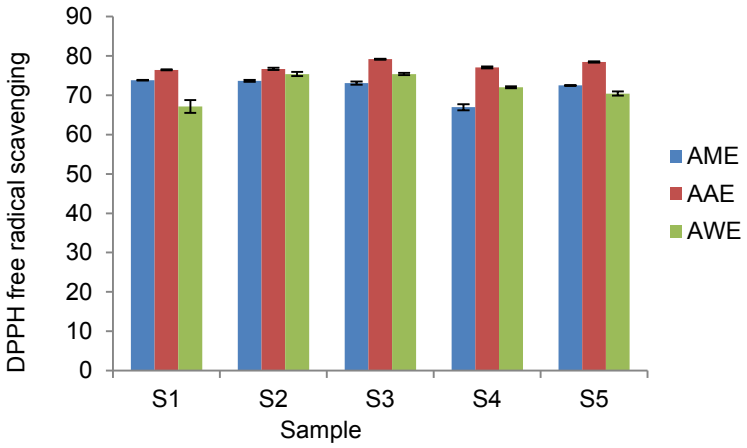


Figure 2. DPPH radical scavenging capacity of AME, AAE, AWE of sumac samples

DPPH free radical scavenging capacities of AME were 74.0±0.1% for S1, 73.7±0.3% for S2, 73.1±0.4% for S3, 67.0±0.8% for S4, and 72.5±0.2% for S5, in AAE, DPPH free radical scavenging capacities were 76.5±0.1% for S1, 76.7±0.3% for S2, 79.2±0.1% for S3, 77.1±0.2% for S4, and 78.5±0.1% for S5 and in AWE, DPPH free radical scavenging capacities were 67.2±1.6% for S1, 75.4±0.5% for S2, 75.4±0.3% for S3, 72.0±0.2% for S4, and 70.4±0.5% for S5, respectively.

Kossah et al. [30], reported 0.01 mg L^{-1}-1 mg L^{-1} of Syrian sumac extracts' DPPH radical scavenging capacity in the range of 34.53%±0.25 -95.42%±0.01. In a study, the leaves and fruits of the sumac plant were extracted with ethanol, and then pure extracts were obtained by removing the ethanol with a rotary evaporator. The obtained pure extracts were used for analysis. The DPPH radical scavenging capacity of sumac leaf was found in the range of 59%-100% and the fruit DPPH radical scavenging capacity was in the range of 39%-92% [31].

Metal Chelating Capacity

Metal ions can cause lipid peroxidation to form free radicals and lipid peroxides. Therefore, metal chelating capacity shows antiradical and antioxidant properties [28]. The metal chelating capacity of the sumac plant in AME, AAE and AWE was determined. EDTA was used for the standard graphic (Figure 3) and the metal chelating capacity of the extracts was compared with the metal chelating capacity of EDTA (Figure 4).

Metal chelating capacity of EDTA was found higher than AME (Figure 4(a)), AAE (Figure 4(b)) and AWE (Figure 4(c)). When different extracts of sumac samples compared among themselves S5 has the highest metal chelating capacity. Sample S5's in AME metal chelating capacity value was close to EDTA's metal chelating capacity. At the same time, the metal chelating capacities of other extracts were found to be much lower than that of EDTA, and the metal chelating capacities of the samples in these extracts were found to be close to each other.

Işnel [31] collected sumac samples Diyarbakır-Silvan. Metal chelating capacity was investigated in sumac fruit and leaves. As a result of the analysis metal chelating capacity was determined as %38-%100 in fruits and as %12-%60 in leaves.

Copper (II) Ion Reducing Antioxidant Capacity (CUPRAC)

The antioxidant capacity is calculated by using the ability of the Cu (II)-neocuproin complex formed by neocuproin to be reduced to Cu (I)-neocuproin chelate. Caffeic acid was used as standard for determination of copper (II) ion reducing antioxidant capacity. Caffeic acid calibration graph linear regression equation and correlation coefficient were obtained as $y = 0.0406x + 0.1352$ and $R^2 = 0.998$, respectively. Using this standard graph, CUPRAC amounts of the samples were calculated as caffeic acid equivalent (mg CAE kg^{-1}). CUPRAC values of AME, AAE and AWE of sumac plant are given in Table 4.

As seen in Table 4, when the extracts of the same sumac in different solvents were compared, the highest CUPRAC value in the sample S1 was seen in AWE, while the highest CUPRAC value in other samples was found in AME. AAE of all examples has the lowest CUPRAC value. AME of different sumac have the highest CUPRAC values, respectively, S5>S2>S1>S3>S4. AAE of different sumac has the highest CUPRAC values, respectively S1>S4>S2>S3>S5. AWE of sumac plants' CUPRAC values decreased respectively as S1>S5>S2>S4>S3.

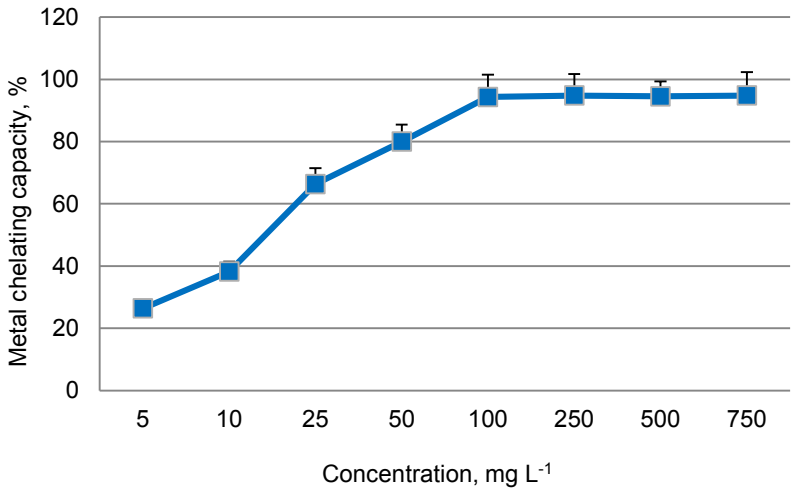
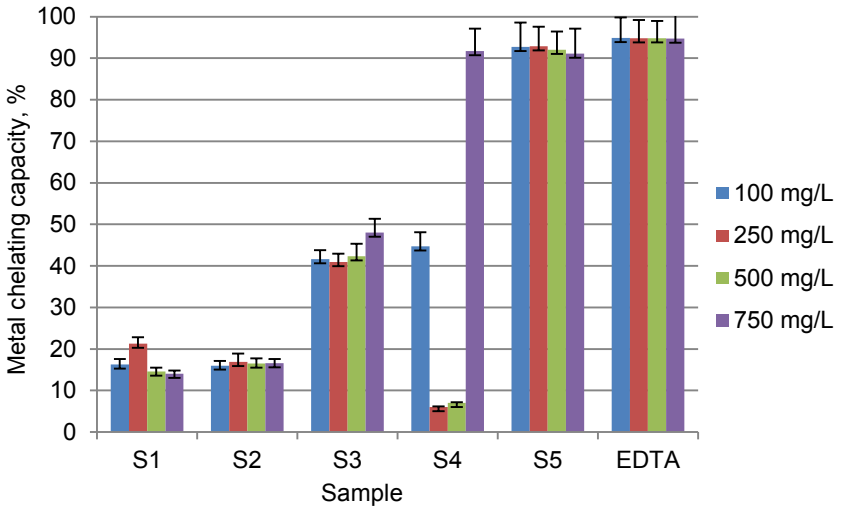
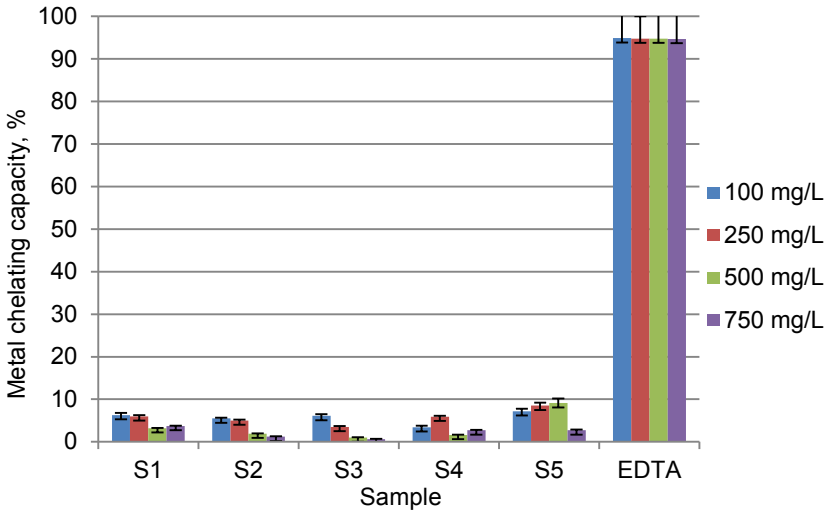


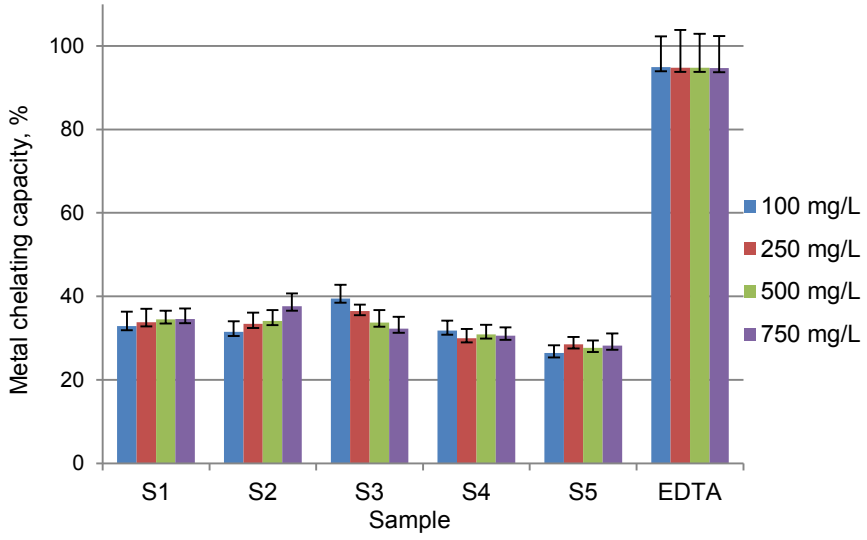
Figure 3. Calibration graph of EDTA



(a)



(b)



(c)

Figure 4. Metal chelating capacity of (a) EDTA and AME of sumac samples, (b) EDTA and AAE of sumac samples, (c) EDTA and AWE of sumac samples

Bursal and Köksal [28] stated in their study on sumac that the CUPRAC value of water extract is higher than ethanol extract. No other source has been found in the literature for the determination of CUPRAC in sumac. In the study in which the sumac plant grown in Şırnak was extracted with methanol for 24 h, the CUPRAC analysis value was reported as 3.33±0.17 mmol trolox equivalent antioxidant capacity (TEAC) g^{-1} [32].

Reducing Power

All of the sumac extracts used in the study were compared among themselves and synthetic antioxidants as butylhydroxytoluene (BHT), α tocopherol and vitamin C standards. When compared the reducing power of the sumac samples AME and synthetic antioxidants was changed as vitamin C>BHT>α tocopherol>S5>S1>S2>S4>S3, respectively. Sumac samples AAE and synthetic antioxidants was changed as vitamin C>BHT>α tocopherol>S5>S1>S2>S4>S3, and sumac samples AWE and synthetic antioxidants was changed as vitamin C>BHT>α tocopherol>S5>S2>S4>S1>S3, respectively. As a result, sample S5 had higher reducing power than others while sample S3 had the lowest reducing power in all of the solvents.

Al-Muwaly et al. [33] investigated Iraqi sumac seeds and extracted 24 h with distilled water, methanol and ethanol. The obtained extracts were dried in a lyophilizer, then they were dissolved with distilled water and used for analysis. The reducing power of the extracts prepared at different concentrations and ascorbic acid that used as a standard were determined. It was determined that the reducing force increased as the extract concentration increased. Statistically, the highest reducing force was determined as 2.103±0.397 in 117.64 ppm methanol extract. In another study reducing power of synthetic antioxidants, water extract and ethanol extract was compared and the reducing respectively decreased as BHA>trolox>BHT>α tocopherol>water extract>ethanol extract [28].

Mineral Concentration

Standard solutions of Ca, Fe, K, Mg, Mn, Na and Zn minerals were used in order to determine the mineral content of the sumac plant. Calibration values and mineral contents of sumac samples are given in Table 5. In terms of Fe content, S4 and S1 have the highest and lowest values, respectively. It was observed that the sample richest in Ca was S4 and the sample containing the least Ca was S1. In terms of K, S3 has the lowest value, while S5 has the highest value. In terms of Na content, S3 has the highest value and S5 is the lowest. S2 has the most Zn, while S3 is the sumac with the least Zn amount. The richest sample in Mn was S4 and the sample containing the least Mn was S3. The highest Mg amount was determined in S5 and the lowest in S3.

Özcan and Hacıseferoğulları [34] carried out mineral analysis of sumac samples collected from Mersin by using ICP-AES and element concentrations was found 144.53±3.76 mg kg^{-1} for Fe, 10.93±0.84 mg kg^{-1} for Zn, 855.95±17.63 mg kg^{-1} for Mg, 3661.57±25.71 mg kg^{-1} for Ca, 7963.35±47.85 mg kg^{-1} for K, 114.06±3.65 mg kg^{-1} for Na, and 10.49±1.32 mg kg^{-1} for Mn. Fe, Zn and Ca concentrations in sumac samples are lower than present study, and Mn concentrations are similar to present study. Ünver [11] reported that sumac samples collected from different provinces are rich in Al, Ca, K, Fe and Mg. Fe was in the range of 69-611 mg kg^{-1}, Zn was in the range of 3.70-6.92 mg kg^{-1}, Mg was in the range of 342.9-700.1 mg kg^{-1}, Ca was in the range of 1000.4-3577.5 mg kg^{-1}, K was in the range of 8094-17361 mg kg^{-1}, and Na was in the range of 730.9-1249.3 mg kg^{-1}. Kossah et al. [23], analyzed Syrian and Chinese sumacs and the mineral concentrations were found 174.15±0.18 mg kg^{-1}

and 180.00±0.67 mg kg^{-1} for Fe, 55.74±0.38 mg kg^{-1} and 17.20±0.38 mg kg-1 for Zn, 3155.53±0.41 mg kg^{-1} and 3098.00±0.52 mg kg^{-1} for Ca, 605.74±0.51 mg kg^{-1} and 871.00±0.42 mg kg^{-1} for Mg, 101.04±0.15 mg kg^{-1} and 183.00±0.26 for Na mg kg^{-1}, 7441.25±0.07 mg kg^{-1} and 5576.00±0.68 mg kg^{-1} for K, 10.57±0.39 mg kg^{-1} and 11.60±0.35 mg kg^{-1} for Mn, respectively.

Organic Acid

Citric acid, malic acid and tartaric acid were determined in sumac samples and organic acid standards were used for calibration curves. Calibration values for organic acids are given in Table 6. Figure 5 represents organic acids chromatogram including tartaric acid, malic acid and citric acid.

In the samples S1, S2, S3, S4 and S5, citric acid, malic acid and tartaric acid were determined (Table 7). The predominant organic acid in sumac samples is malic acid. Kossah et al. [23], determined that the sumac fruit grown in Syria contains more organic acid than the sumac fruit grown in China. The predominant organic acid in both sumac is malic acid. It has also been detected in small amounts of tartaric acid and citric acid in sumac in Syria and China. It has been revealed that the Syrian sumac fruit is more acidic than the Chinese sumac fruit. The concentration of malic acid among organic acids in sumac was 1568.04 mg kg^{-1}, citric acid 56.93 mg kg^{-1} and tartaric acid 2.15 mg kg^{-1}. In another study sumac samples that were different genotypes collected from Kahramanmaras province were analyzed and mean concentrations of citric acid and malic acid were calculated as 70.82 mg kg^{-1} and 1822.82 mg kg^{-1} [4].

Phenolic Compounds

Calibration values for phenolic compounds are given in Table 8. Since the amount of gallic acid has the highest value among the phenolic compounds in sumac. It was determined that gallic acid was the highest amount in the sample S1 (Figure 6) and the least in sample S3. + (-) catechin hydrate S2 is the most in the sample, while at least S1 is in the sample. - (-) epicatechin is in the sample numbered S4 at most and in the sample number S5 at least. Vanilic acid is mostly in the S1 sample of sumac and could not be determined in S4. While resveratrol could not be determined in S2 and S4, it showed the highest value in S5. Rutin and caffeic acid could not be determined in S4, but the highest values were found in the sample numbered S5.

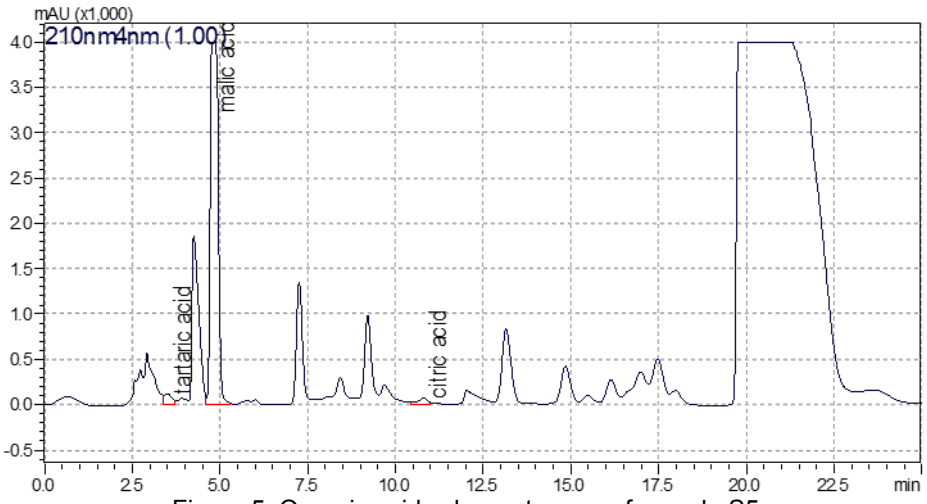


Figure 5. Organic acids chromatogram of sample S5

Table 5. Calibration values and mineral contents (mg kg^{-1}) of sumac samples

	S1	S2	S3	S4	S5	Linear regression equation and correlation coefficient
Ca	59153±1955	69011±4869	67747±2151	178174±10255	132360±12670	y = 0.0211x + 0.0004 R² = 0.9978
Fe	6117±724	10131±928	9236±199	16281±904	14776±635	y = 0.0202x - 0.002 R² = 0.9951
K	9490±594	7262±527	4259±183	4841±463	10297±329	y = 1.0324x - 0.0485 R² = 0.9999
Mg	1662±54	1676±79	532±30	1149±28	2022±191	y = 0.4537x + 0.009 R² = 0.9988
Mn	10.0±0.2	13.5±0.6	9.0±0.8	22.0±0.5	16.0±0.4	y = 1.0139x - 0.0205 R² = 1
Na	115±10	68±3	223±11	38±4	16±2	y = 0.9839x + 0.0245 R² = 0.9968
Zn	52±2	100±3	31±2	34±2	48±3	y = 0.2783x + 0.004 R² = 0.9997

Table 6. Calibration values of organic acids

Organic acid	Wavelength (nm)	Retention time (min)	LOD (mg L^{-1})	LOQ (mg L^{-1})	Linear regression equation and correlation coefficient
Citric	210	10.6	0.6061	2.0202	y = 1345.8x – 4335.5 R^2 = 0.9951
Malic	210	4.9	0.4320	1.4398	y = 1927.4x - 37877 R^2 = 0.9985
Tartaric	210	3.5	0.1735	0.5782	y = 2075.2x + 4713.6 R^2 = 0.9997

Table 7. Organic acid contents of sumac samples (mg kg^{-1})

Sample	Citric acid	Malic acid	Tartaric acid
S1	5559±490	156767±8200	1040±55
S2	2356±122	154885±6570	20360±358
S3	6916±433	207704±8875	2273±82
S4	1268±58	169515±5480	1993±124
S5	7463±215	219521±7758	6160±221

Table 8. Calibration values for phenolic compounds

Phenolic compound	Wavelength (nm)	Retention time (min)	LOD (mg L^{-1})	LOQ (mg L^{-1})	Linear regression equation and correlation coefficient
Gallic acid	280	10.14	0.0589	0.1963	y = 25982x – 6164.6 R^2 = 0.9989
+(-)Catechin hydrate	280	22.92	0.1149	0.3829	y = 7022.6x – 1253.3 R^2 = 0.9998
-(-) Epicatechin	280	34.83	0.1442	0.4806	y = 6741.4x - 4572,6 R^2 = 0.9982
Vanilic acid	280	29.94	0.0693	0.2310	y = 17509x – 3030.1 R^2 = 0.9998
Resveratrol	320	51.54	0.0128	0.0427	y = 75487x – 2297.2 R^2 = 0.9996
Rutin	360	50.82	0.0834	0.2781	y = 15755x + 3820.9 R^2 = 0.9988
Caffeic acid	320	31.77	0.0241	0.0805	y = 57030x – 6926.9 R^2 = 0.9997

The amounts of some phenolic compounds in the sumac samples are given in Table 9. In another study, gallic acid concentration (5.97±0.02 mg g^{-1}) of staghorn sumac fruit was found lower than present study [35]. Tohma et al.[7] used water and ethanol to determine phenolic compound in sumac samples. Gallic acid concentration was evaluated as 86.77 mg kg^{-1} and 19.31 mg kg^{-1}, in ethanolic and water extract, respectively. In ethanolic extract epicatechin and rutin did not detected but in water extract epicatechin was 21.2 mg kg^{-1} and rutin was 0.49 mg kg^{-1}. In another study gallic acid was detected as 67.56 mg g^{-1} in sumac samples collected in Çanakkale and detected 19.01 mg g^{-1} in sumacs collected in Siirt [11].

Table 9. Amounts of some phenolic compounds in sumac samples (mg kg^{-1})

Sample	Gallic acid	+(-)Catechin hydrate	-(-) Epicatechin	Vanilic acid	Resveratrol	Rutin	Caffeic acid
S1	15199±760	88±7	143±11	175±19	12±1	143±17	27±2
S2	14696±1175	228±23	131±8	33±2	ND*	86±8	34±3
S3	6093±305	129±8	163±8	15±1	59±3	50±3	5.0±0.5
S4	8629±690	119±7	599±54	ND	ND	ND	ND
S5	11045±773	142±14	93±6	88±10	237±19	183±13	52±5

*:ND: Not Detected

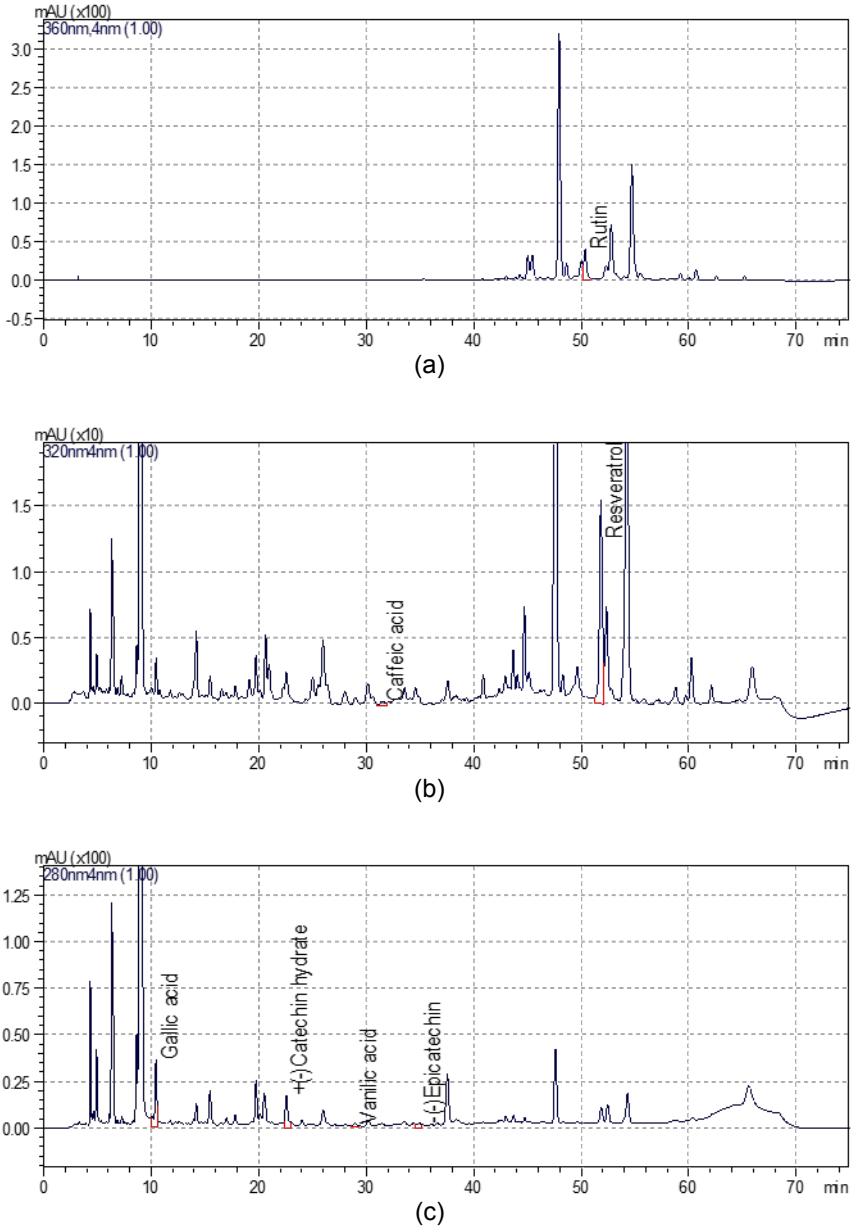


Figure 6. HPLC chromatogram of sample S1 at (a) 280 nm, (b) 320 nm, (c) 360 nm

CONCLUSION

In the present study, sumac samples were extracted using three different solvents and obtained results were presented:

- The pH values of sumac plants were found to be close to each other. It was determined that the pH value of S3 (2.78±0.15) was lower than the other samples, and S2 had the highest pH value (3.28±0.23).
- The lightest color has S4, the darkest color was seen in S3 sample and the others were found to be similar.
- The highest redness value was observed in S3 sample, the lowest value in S1 and S2 samples.
- The highest yellowness value is in S1 sample and the lowest in S3 sample. S2, S4, S5 showed similar results.
- Mineral content of sumac samples were found as for Ca (59153±1955 mg L^{-1}-178174±10255 mg L^{-1}), for (6117±724 mg L^{-1}- 16281±904 mg L^{-1}) Fe, for K (4259±183 mg L^{-1}-10297±329 mg L^{-1}), for Mg (532±30 mg L^{-1}-2022±191 mg L^{-1}), for Mn (9.0±0.8 mg L^{-1}-22.0±0.5 mg L^{-1}), for Na (16±2 mg L^{-1}-223±11 mg L^{-1}) and for Zn (31±2 mg L^{-1}-100±3 mg L^{-1}). Obtained results revealed that S5 was found to have the highest concentration in terms of K, but the lowest concentration in terms of Na. Ca, Fe and Mn concentrations were higher in S4 sample. It was determined that Na concentration was higher than the others in S3, while Zn, Mg, K and Mn were lower. Generally, the mineral content of sumac samples is high. As a result, the most abundant mineral in sumac in Tunceli province is Ca.
- Sumac samples DPPH radical scavenging capacities were found as AAE (76.5±0.1%-79.2±0.1%)>AWE

(67.2±1.6%-75.4±0.5%)>AME (67.0±0.8%-74.0±0.1%).

- Metal chelating capacities of extracted sumacs were decreased as EDTA>AWE>AME>AAE.
- While the highest CUPRAC value was obtained in the AME (118.0±3 mg CAE kg^{-1}), the lowest value was found in the AAE (10.2±3 mg CAE kg^{-1}).

The sumac plant analyzed in the study has its own color and taste and is therefore used as a spice. It was found that it is rich in minerals, organic acids and phenolic compounds that are of great importance for health, and its antioxidant capacity is high. It is also important that sumac grow naturally. Since the interest in natural nutrition has increased due to the increasing diseases in recent years, the consumption of naturally grown plants such as sumac should be encouraged. This plant, which is beneficial for human health in many ways, can be consumed by processing different products other than spices.

FUNDING

This study was supported by The Scientific Research Projects Coordination Unit of Munzur University. Project Number: YLMUB016-24

DATA AVAILABILITY STATEMENT

The data that support this study are available in the article and accompanying online supplementary material.

REFERENCES

[1] Dziki, D., Cacak-Pietrzak, G., Hassoon, W.H., Gawlik-Dziki, U., Sułek, A., Różyło, R., Sugier, D. (2021). The fruits of sumac (*Rhus coriaria* L.) as a functional additive and salt replacement to wheat bread. *LWT-Food Science and Technology*, 136, 110346.

[2] Grassia, M., Sarghini, F., Bruno, M., Cinquanta, L., Scognamiglio, M., Pacifico, S., Fiorentino, A., Geraci, A., Schicchi, R., Corona, O. (2021). Chemical composition and microencapsulation suitability of sumac (*Rhus coriaria* L.) fruit extract. *European Food Research and Technology*, 247, 1133–1148.

[3] Alsamri, H., Athamneh, K., Pintus, G., Eid, A.H., Iratni, R. (2021). Pharmacological and antioxidant activities of *Rhus coriaria* L. (sumac). *Antioxidants,* 10, 73.

[4] Ozcan, A., Susluoglu, Z., Nogay, G., Ergun, M., Sutyemez, M. (2021). Phytochemical characterization of some sumac (*Rhus coriaria* L.) genotypes from southern part of Türkiye. *Food Chemistry*, 358, 129779.

[5] Sakhr, K., El Khatib, S. (2020). Physiochemical properties and medicinal, nutritional and industrial applications of Lebanese sumac (Syrian Sumac - *Rhus coriaria*): A review. *Heliyon,* 6, e03207.

[6] Reidel, R.V.B., Cioni, P.L., Majo, L., Pistelli, L. (2017). Evolution of volatile emission in *Rhus coriaria* organs during different stages of growth and evaluation of the essential oil composition. *Chemistry and Biodiversity,* 14, e1700270.

[7] Tohma, H., Altay, A., Köksal, E., Gören, A.C., Gülçin, I. (2019). Measurement of anticancer, antidiabetic and anticholinergic properties of sumac (*Rhus coriaria*): Analysis of its phenolic compounds by LC–MS/MS. *Journal of Food Measurement and Characterization,* 13, 1607–1619.

[8] Abu-Reidah, I.M., Ali-Shtayeh, M.S., Jamous, R.M., Arráez-Román, D., Segura-Carretero, A. (2015) HPLC-DAD-ESI-MS/MS screening of bioactive components from *Rhus coriaria* L. (sumac) fruits. *Food Chemistry,* 166, 179-191.

[9] Al-Boushi, M.A., Haj Hamdo, H., Herbali, J. (2014). Extraction and study of the phenolic compounds in the leaves and sticks of the Syrian sumac plant (*Rhus coriaria* L.). *International Journal of ChemTech Research*, 6, 2414-2420.

[10] Ereifej, K.I., Feng, H., Rababah, T.M., Tashtoush, S.H.,Aludatt, M., Gammoh, S., Al-Rabadi, G. (2016). Effect of extractant and temperature on phenolic compounds and antioxidant activity of selected spices. *Food and Nutrition Sciences*, 7, 362-370.

[11] Ünver, A., 2006. Sumak (*Rhus coriaria* L.) Meyvelerinden Oleorezin Üretimi Üzerine Araştırma. Doktora Tezi, Selçuk Üniversitesi Fen Bilimleri Enstitüsü, Konya.

[12] AOAC, Association of Official Analytical Chemists. (1997). Official Methods of Analysis (16th ed.) Association of Official Analytical Chemists Washington DC.

[13] Cikcikoglu Yildirim, N., Türkoğlu, S., Kaplan Ince, O., Ince, M. (2013). Evaluation of antioxidant properties elemental and phenolic contents composition of wild nettle *Urtica dioica* L. from Tunceli Türkiye. *Cellular and Molecular Biology*, 59, 1882–1888.

[14] Singleton, V.L., Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16, 144–158.

[15] Blois, M.S. (1958). Antioxidant determinations by the use of stable free radical. *Nature,* 181, 1199–1200.

[16] Dinis, T.C.P., Madeira, V.M.C., Almeid, L.M. (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Archives of Biochemistry and Biophysics*, 315, 161–169.

[17] Apak, R., Güçlü, K., Özyürek, M., Karademir, S.E. (2004). Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. *Journal of Agricultural and Food Chemistry*, 52, 7970–7981.

[18] Oyaizu, M. (1988). Antioxidative activities of browning products of glucosamine fractionated by organic solvent and thin-layer chromatography. *Japanese Society for Food Science and Technology*, 35, 771–775.

[19] Unal, I., Kaplan Ince, O. (2017). Characterization of antioxidant activity, vitamins, and elemental composition of ciris (*Asphodelus aestivus* L.) from Tunceli, Türkiye. *Instrumentation Science & Technology*, 45(5), 469-478.

[20] Bevilacqua, A.E., Califano, A. (1989). Determination of organic acids in dairy products by high

performance liquid chromatography. *Journal of Food Science*, 54, 1076–1079.

[21] Fereidoonfar, H., Salehi-Arjmand, H., Khadivi, A., Akramian, M., Safdari, L. (2019). Chemical variation and antioxidant capacity of sumac (*Rhus coriaria* L.). *Industrial Crops and Products*, 139, 111518.

[22] Caliskan, G., Dirim, S.N. (2013). The effects of the different drying conditions and the amounts of maltodextrin addition during spray drying of sumac extract. *Food and Bioproducts Processing*, 91(4), 539-548.

[23] Kossah, R., Nsabimana, C., Zhao, J., Chen, H., Tian, F., Zhang, H., Chen, W. (2009). Comparative study on the chemical composition of Syrian sumac (*Rhus coriaria* L.) and Chinese sumac (*Rhus typhina* L.) fruits. *Pakistan Journal of Nutrition,* 8, 1570–1574.

[24] Caliskan, G., Dirim, S.N. (2016). The effect of different drying processes and the amounts of maltodextrin addition on the powder properties of sumac extract powders. *Powder Technology*, 287, 308-314.

[25] Romeo, F.V., Ballistreri, G., Fabroni, S., Pangallo, S., Li Destri Nicosia, M.G., Schena, L., Rapisarda, P. (2015). Chemical characterization of different sumac and pomegranate extracts effective against *Botrytis cinerea* rots. *Molecules*, 20, 11941–11958.

[26] Kossah, R., Nsabimana, C., Zhang, H., Chen, W. (2010). Optimization of extraction of polyphenols from Syrian sumac (*Rhus coriaria* L.) and Chinese sumac (*Rhus typhina* L.) fruits. *Research Journal of Phytochemistry*, 4, 146–153.

[27] Bashash, M., Bolandi, M., Zamindar, N. (2012). Phenolic content of selected sumac fruits from Iran, extracted with different solvents. *Journal of Chemical Health Risks*, 2, 17–20.

[28] Bursal, E., Köksal, E. (2011). Evaluation of reducing power and radical scavenging activities of water and ethanol extracts from sumac (*Rhus coriaria* L.). *Food Research International,* 44, 2217–2221.

[29] Zannou, O., Pashazadeh, H., Galanakis, C., Alamri, A., Koca, I. (2022). Carboxylic acid-based deep eutectic solvents combined with ınnovative extraction techniques for greener extraction of phenolic compounds from sumac (*Rhus coriaria* L.). *Journal of Applied Research on Medicinal and Aromatic Plants*, 30, 100380.

[30] Kossah, R., Nsabimana, C., Zhang, H., Chen, W. (2013). Evaluation of antimicrobial and antioxidant activities of Syrian sumac fruit extract. *Journal of Natural Products Research Paper*, 6, 96–102.

[31] İşnel, İ. (2022) Diyarbakır Silvan (Yuva Dağı) Bölgesindeki Bazı Bitkilerin Antioksidan ve Antimikrobiyal Aktiviteleri. Yüksek Lisans Tezi, Nevşehir Hacı Bektaş Veli Üniversitesi Fen Bilimleri Enstitüsü, Nevşehir.

[32] Yarar, R. (2017). Şırnak Bölgesi'ndeki Halk Arasında Tıbbi Amaçlo Kullanılan Bazı Bitkilerin Antioksidan ve Biyolojik Aktivitelerinin Belirlenmesi. Yüksek Lisans Tezi, Artvin Çotuh Üniversitesi Fen Bilimleri Enstitüsü, Artvin.

[33] Al-Muwaly, K.Y., Al-Flayeh, K.A., Ali, A.A. (2013). Antioxidant and free radical scavenging effects of Iraqi sumac (*Rhus coriaria* L). *Baghdad Science Journal*, 10(3), 921-933.

[34] Özcan, M., Hacıseferoğulları, H. (2004). A condiment (sumac fruits): some physico-cheminal properties. *Bulgarian Journal of Plant Physiology*, 30(3-4), 74-84.

[35] Wu, T., McCallum, J.L., Wang, S., Liu, R., Zhu, H., Tsao, R. (2013). Evaluation of antioxidant activities and chemical characterisation of staghorn sumac fruit (*Rhus hirta* L.). *Food Chemistry*, 138, 1333–1340.

SİDAS MEDYA
Akademik Gıda®
ISSN Online: 2148-015X
https://dergipark.org.tr/tr/pub/akademik-gida

Akademik Gıda 21(2) (2023) 187-197, DOI: 10.24323/akademik-gida.1351186

Derleme Makale / Review Paper

Sürdürülebilir Gıda, Gıda Takviyesi ve Gıda Katkı Maddesi Üretiminde Alglerin Önemi

Muazzez Kumkapu, Neşe Şahin Yeşilçubuk

İstanbul Teknik Üniversitesi, Kimya Metalurji Fakültesi, Gıda Mühendisliği Bölümü, 34467 Sarıyer, İstanbul

Geliş Tarihi (Received): 01.12.2021, Kabul Tarihi (Accepted): 09.07.2023
Yazışmalardan Sorumlu Yazar (Corresponding author): kumkapu15@itu.edu.tr (M. Kumkapu)
0 212 285 7341 0 212 285 2925

ÖZ

Dünya nüfusunun hızla artması, çevresel bozulma, gıda kaynakları için rekabet ve tarımın uluslararası ekonomiye entegrasyonu gibi nedenler gıda sürdürülebilirliği için tehdit oluşturmaktadır. Günümüzde potansiyel yeni gıda kaynağı arayışı ön plandadır. Bu noktada algler öne çıkmaktadır. Algler içeriğinde protein, çoklu doymamış yağ asitleri, polisakkarit, pigment, sterol, vitamin ve mineraller gibi önemli biyoaktif bileşenler bulundurmaktadır. Bu değerli biyoaktif bileşenlere ek olarak alglerin doğal ve sürdürülebilir gıda kaynağı olarak görülmesinin nedenleri arasında alglerin bölünerek çoğalıp hızla biyokütle oluşturmaları ve uygun koşullarda açık sistemler kullanılarak düşük maliyetlerle yetiştirilebilmesi gibi faktörlerde bulunmaktadır. Alglerin gıda olarak tüketimi özellikle Uzak Doğu'da geleneksel bir uygulama olmasına rağmen, günümüzde alglerin gıda endüstrisinde ticarileşme potansiyeli, alglerin gıda katkısı, gıda takviyesi veya gıda bileşeni olarak kullanılmalarıyla artmaktadır. Tüm bu bilgiler doğrultusunda alglerin gıda endüstrisindeki öneminin yakın gelecekte artması beklenmektedir. Bu çalışmada biyoaktif bileşenler için potansiyel alg kaynakları, elde edilen ürünler ve günümüzdeki ticari üretimi konusunda bilgiler verilmiştir.

Anahtar Kelimeler: Sürdürülebilirlik, Alg, Gıda, Gıda takviyesi

Importance of Algae in Production of Sustainable Food, Food Supplements and Food Additives

ABSTRACT

Rapid increase in the world population, environmental degradation, competition for food resources, and the integration of agriculture into the global economy pose threats to food sustainability. Currently, there is a prominent search for potential new food sources, and in this context, algae have emerged as a notable candidate. Algae contain significant bioactive compounds such as proteins, polyunsaturated fatty acids, polysaccharides, pigments, sterols, vitamins, and minerals. In addition to these valuable bioactive compounds, algae are perceived as a natural and sustainable food source due to their ability to rapidly multiply and generate biomass under favorable conditions, as well as their ability to be cultivated at low costs using open systems. While algae consumption as a food has traditionally been prevalent in the Far East, the commercial potential of algae in the food industry has recently increased with their utilization as food additives, dietary supplements, and food ingredients. Based on all these insights, it is expected that the importance of algae in the food industry will continue to grow in the near future. This review provides information on potential algae sources for bioactive compounds, derived products, and current commercial production, emphasizing their significance.

Keywords: Sustainability, Algae, Food, Food supplement

GİRİŞ

Sürdürülebilirlik, sürekliliği olan herhangi bir sistemin işlerini kesintisiz, bozulmadan, aşırı kullanımla tüketmeden ya da sistemin yaşama bağlı olan ana kaynaklara aşırı yüklenmeden sürdürülebilmesi olarak tanımlanmaktadır [1]. Sosyal, ekonomik ve çevresel olmak üzere üç boyutlu bir sürdürebilirlik tanımı evrensel olarak yaygınlaşmıştır [2]. Sürdürülebilir gıda ve sürdürülebilir gıda tüketimi gibi konular son yıllarda oldukça önem verilen konular haline gelmiştir. Sıkı bağları olan tarım ve gıda endüstrilerini içeren 'yeşil tedarik zinciri' çalışmaları hız kazanmaktadır [3]. Devlet kurumları ve sivil toplum örgütlerinin yanı sıra uluslararası şirketlerin politikaları arasına sürdürülebilir gıda, sürdürülebilir gıda tüketimi ve sürdürülebilir ambalajlama gibi başlıklar yer almaktadır. Ayrıca, şirketler bu politikaları sosyal sorumluluk projeleri ile desteklemektedirler. Brklacich ve ark. [4]' e göre, gıda sürdürülebilirliğini tehdit eden dört etken çevresel bozulma, kaynaklar için rekabet, artan gıda talepleri ve tarımın uluslararası ekonomiye entegrasyonu olarak sıralanmaktadır. 2050 yılına kadar Dünya nüfusunun dokuz milyarı bulması beklenmekte olup, zamanda küresel gıda üretiminin yaklaşık %60 oranında artması gerekmektedir [5]. Gıda ve Tarım Örgütü (FAO), Uluslararası Tarımsal Kalkınma Fonu (IFAD) ve Dünya Gıda Programı (WFP) tarafından yayınlanan 'Dünyadaki Gıda Güvencesizliğinin Durumu' Raporu' na göre, son üç yılda açlık çeken insan sayısında hafif bir artış yaşanmasına rağmen, bugün Dünyada 820 milyondan fazla insan hâlen açlık çekmektedir. Açlık ve yetersiz beslenmenin yaklaşık yüzde 20' lik bir oranla en yaygın görüldüğü kıta Afrika'dır [6]. Hem nüfus artışına bağlı gıda talebinin artması hem de mevcut durumda dahi yetersiz beslenmenin yoğun yaşanmasından dolayı mevcut kaynakların verimliliğinin arttırılması ve yeni gıda kaynak arayışı hızla artmaktadır. Yabani yenilebilir bitkiler, yenilebilir çiçekler, yenilebilir böcekler, tohumlar, saksı balı/poleni ve deniz canlıları alternatif gıda kaynakları kapsamında incelenmektedir. [7, 8]. Alternatif protein ve et arayışı günümüzde ticari karşılık bulmaktadır. Sentetik et, bitkisel proteinler, böcek kaynaklı proteinler ve farklı bakteri, alg, küf ve mayaları içeren mikrobiyal proteinler alternatif protein kaynağı kapsamında araştırma konusu olmaktadır [9, 10]. Global pazarda bitkisel proteinlerden hazırlanmış hamburgerler, böcek tozu kullanılarak hazırlanmış atıştırmalıklar, farklı mikrobiyal kaynaklardan ekstrakte edilmiş protein tozları ve farklı et çeşitlerini simüle eden sentetik etler gibi çok çeşitli ürünler bulunmaktadır. Tüm bu bilgiler ışığında, algler sürdürülebilir ve alternatif gıda, protein ve diğer değerli biyoaktif bileşenler bakımından önemli bir araştırma konusu olarak gündeme gelmiştir. Alglerin sürdürülebilir kaynak olarak öne çıkmasının ana nedenleri, değerli bileşime sahip olmaları, hali hazırda ekosistemde doğal koşullarda bulunabilmeleri ve açık sistemlerde düşük yatırım maliyetiyle üretilebilmeleridir. Algler bölünerek çoğalıp hızla biyokütle oluşturmaları ve uygun koşullarda açık sistemler kullanılarak düşük maliyetlerle üretim yapılabilmesi nedeniyle ticari olarak da alg yetiştiriciliğine ilgi artmaktadır [11, 12]. Alglerin gıda endüstrisinde popülerliğinin diğer nedenleri ise yüksek protein içeriği sebebiyle vegan beslenmeye uygun olması ve tüketicilerin doğal gıda/katkı maddesine olan ilgisidir.

Bu derlemenin amacı, alglerin sürdürülebilir gıda endüstrisindeki önemine vurgu yaparak biyoaktif bileşenler için potansiyel alg kaynakları, elde edilen ürünler ve ticari üretimleri gibi güncel konulara odaklanmaktır.

ALGLER HAKKINDA GENEL BİLGİLER

Algler, insan yaşamı ve dünya için bilinenin ötesinde önemli bir role sahiptir. Algler dünya yüzeyinin %7' ini oluşturan okyanuslar ve denizler için birincil üreticiler olarak belirtilmekte olup, insanlar ve diğer kara hayvanlarının solunumu için mevcut net küresel oksijenin yaklaşık yüzde 30 ila 50' sini üretmektedir [13]. Algler aerobik fotosentetik organizmalardır. Protista aleminin bitki benzeri 'plant like' olarak adlandırılan üyesidir. Algler genellikle sucul habitatlarda yaşayan, selüloz çeperi bulunan, ototrof, basit yapılı ve fotosentez yapabilen ökaryotik canlılar olarak tanımlanır [14]. Bu genel tanımın aksine prokaryot ve fotosentez yapamayan şubeleri de vardır. Tek hücreli ya da çok hücreli olarak bulunabilirler. Algler, yaklaşık olarak %70' i deniz ve göllerde bulunmasına rağmen, yeryüzünün her alanında yaygın olarak bulunabilirler [15]. Her alg türü farklı bir özelliğe sahip olabilmekte ve bu özellikleri doğrultusunda gereksinim duyduğu sıcaklık, ışık, karbon ihtiyacı gibi koşullar değişiklik göstermektedir [12]. Algler morfolojik açıdan tek hücreli ve ipliksi formlardan, karışık yapıda olan türlere kadar farklı biçimlerde gözlenebilmektedir. Algler ökaryotik ve prokaryotik olarak iki gruba ayrılırlar. Prokaryotik algler mikroalg olarak adlandırılır ve bakteriler alemine dahildir. Ökaryotik algler ise makroalg olarak adlandırılır. Makroalgler genel olarak yosun olarak bilinmektedir. Mavi-yeşil algler (*Cyanophyta*) mikroalgler olarak bilinirler. Makroalgler ise kamçı taşımalarına veya pigmentasyonlarına göre beş şubeye ayrılabilir. Bu şubeler kahverengi algler (*Phaeophyta*), kırmızı algler (*Rhodophyta*), yeşil algler (*Chlorophyta*), diyatomeler ve kamçılı algler (*Flagelleta*)'dir [15].

ALGLERİN GIDA OLARAK TÜKETİMİ

Algler, Uzak Doğu' da geleneksel olarak farklı formlarda tüketilmektedir. Uzakdoğu mutfağında taze ya da kurutulmuş olarak birçok farklı çeşitte alg kaynaklı yemeği bulunmaktadır. Makroalgler, deniz ve göllerden toplanarak taze salata olarak veya çorba, yemek, sos, baharat ve çay gibi şekillerde kurutulması ve pişirilmesi suretiyle tüketilmektedir [16]. En yaygın olarak tüketilen makroalgler arasında kırmızı alglerden *Porphyra* (nori, kim, laver), *Asparagopsis taxiformis* (limu), *Gracilaria*, *Chondrus crispus* (İrlanda yosunu) ve *Palmaria palmata* (dulse), *Laminaria* (kombu), *Undaria* (wakame), *Macrocystis* ve yeşil alglerden *Caulerpa* spp., *Codium* spp. ve *Ulva* spp.' dır [17]. Mikroalglerin gıda olarak kullanılması fikri İkinci Dünya Savaşı'ndan sonra ortaya atılmıştır [18]. Çünkü bu dönemde dünya genelinde açlık, fakirlik ve kaynak sıkıntısı yaşanmaktaydı. Bu sebeple, araştırmacılar sürdürülebilir gıda kaynağı arayışına girmişlerdir. En iyi alternatif olarak algler

görülmüş ve bu dönemde pilot ölçekli olarak laboratuvarlarda çalışılmaya başlanmıştır [18]. Yapılan çalışmalar sonucunda, çok sayıda mikroalg türünün protein, karbonhidrat, lipit ve diğer biyoaktif bileşikler açısından zengin olduğu bildirilmiştir. Örneğin, yüksek protein içeriği nedeniyle *Spirulina* spp., *Chlorella* spp., *Dunaliella* spp. ve *Aphanizomenon* spp. önem kazanmıştır [19].

Alglerin gıda olarak kullanımının yanında gıda katkı maddesi olarak gıda endüstrisinde önemli bir yeri vardır. Alglerden elde edilen polisakkaritler, reçel, içecek ve fırıncılık gibi endüstrilerde kıvam arttırıcı ve jelleştirici olarak kullanılmaktadır. Alglerden kıvam arttırıcı ve jelleştirici üretimi için pazar büyüklüğü yaklaşık 1 milyar dolardır [20]. Alglerin katkı maddesi olarak kullanımı, doğal renklendirici ve pigment ekstraksiyonu yoluyla gerçekleştirilir. Son yıllarda alglerin gıda sektöründe doğal renklendirici olarak alternatif kullanımı da yaygınlaşmaktadır. Alglerden elde edilen başlıca pigmentler klorofil, fikosiyanin, fikoeritrin, astaksantin, kantaksantin, β-karoten, lutein ve fukoksantindir [21].

ALGLERDEN ÜRETİLEN BİYOAKTİF BİLEŞENLER

Alglerin sürdürülebilir bir kaynak olmasındaki en temel neden, bünyesinde barındırdığı değerli bileşenlerdir. Bu yüksek değerli bileşenler protein, çoklu doymamış yağ asitleri, polisakkarit, pigment, sterol, vitamin ve minerallerdir [19]. Özellikle makroalglerin gıda olarak tüketilmesinin yanı sıra, alglerin gıda takviyesi ve gıda katkı maddesi olarak kullanılması içerdiği biyoaktif bileşenler sayesinde olmuştur. Bu kısımda, bahsedilen biyoaktif bileşenler potansiyel alg kaynaklarıyla birlikte incelenecektir.

Protein

Proteinler beslenmede çok önemli bir yer tutmaktadır. Vücutta yaşamsal fonksiyonlar için gerekli olan temel polimerdir. Alglerin türü ve üreme koşullarına bağlı olarak içerdikleri maddelerin yaklaşık %16-70 oranını protein oluşturmaktadır [22]. Toplam kuru maddenin %50' sinden fazlasını protein oluşturan türler arasında *Spirulina* spp., *Chlorella* spp., *Dunaliella* spp., *Anabeana* spp., *Euglena* spp., *Aphanizomenon* spp., ve *Scenedesmus spp.* yer almaktadır [19, 23]. Yüksek protein içeriği nedeniyle özellikle *Spirulina* spp. ve *Chlorella* spp. cinsleri ön plandadır.

Spirulina spp., çok hücreli ve filamentli bir mavi-yeşil alg türüdür ve içerdiği yüksek oranda protein ile bilinmektedir. *Spirulina* spp. genel olarak olarak kuru bazda ağırlıkça %40-70 arasında protein içerir [23]. *Spirulina* spp.' ya Dünya Sağlık Örgütü (WHO) tarafından 'süper gıda' etiketi verilmiş ve hatta besin özellikleri nedeniyle Ulusal Havacılık ve Uzay İdaresi (NASA) tarafından uzaya gönderilmiştir [24]. Becker' a göre [23], *Spirulina* spp. cinsi içerisinde protein kaynağı olarak öne çıkan iki tür *Spirulina platensis* ve *Spirulina maxima*' dır. Toplam kuru maddede protein içeriği *S. platensis* için %46-63 iken, *S. maxima* için %60-71' dir [23].

Chlorella spp., küçük küresel hücreli bir yeşil alg (*Chlorophyta*)'dir [25]. *Chlorella* spp. içerdiği yüksek protein ve elzem amino asit bileşimi sayesinde son zamanlarda besin takviyesi olarak popülerliği artan bir alg cinsidir [26]. Algal protein üretiminde *Chlorella* cinsi içinde öne çıkan iki tür *C. vulgaris* ve *C. pyrenoidosa*' dır ve toplam kuru maddede protein oranları *C. vulgaris* için %51-58 iken, *C. pyrenoidosa* için %57' dir [23]. Farklı alg kaynakları için toplam kuru maddedeki genel kompozisyonları Tablo 1' de verilmiştir.

Tablo 1. Kuru maddede (%) alglerin genel yapısal kompozisyonu [23]
Table 1. General structural composition of algae in dry matter (%) [23]

Alg	Protein	Karbonhidrat	Yağ
Spirulina platensis	46-63	8-14	4-9
Spirulina maxima	60-71	13-16	6-7
Chlorella vulgaris	51-58	12-17	14-22
Chlorella pyrenoidosa	57	26	2
Dunaliella salina	57	32	6
Scenedesmus obliquus	50-56	10-17	12-14
Anabaena cylindrica	43-56	25-30	4-7

Spirulina ve *Chlorella* cinsleri yüksek protein içerikleri nedeniyle son yıllarda çok popüler hale gelmiştir. Algler, yüksek protein içeriğinin yanında elzem amino asitleri içermekte olup algal proteinlerin standart bitki proteinlerinden daha üstün olduğu ifade edilmektedir [27]. Algler gıda takviyesi olarak kullanılmakta olup, toz, pul ve tablet formları satılmaktadır. Açık sistemlerde en çok yetiştirilen alg türleri *Spirulina* spp. ve *Chlorella* spp. olduğu bildirilmektedir [28]. Algal protein üretimi açısından endüstrileşmenin yüksek olduğu hatta *Spirulina* üretimi açısından yıllık 200 ton kurutulmuş biyokütle üretimine sahip Çin de bir şirket ve *Chlorella* açısından yıllık 400 ton kurutulmuş biyokütle üretimi ile Tayvan da şirket global markette zirveye sahip oldukları bilinmektedir [29]. Son yıllarda algal protein ve ürün satışı yapan firmaların sayısı artmıştır.

Son yıllarda özellikle ticari verimi arttırmak amacıyla alglerin protein içeriğini yükseltecek üretim stratejileri gündemdedir. Bu stratejiler arasında spesifik her alg türü için optimum sıcaklık, ışık uygulamasının süre ve değeri, karıştırma ve ortamın besin içeriği uygulamaları vardır [30]. Güncel konulardan diğeri ise alglerden protein ekstraksiyonunun ardından proteaz enzimi uygulanmasıyla birlikte biyoaktif peptitlerin elde edilmesi yaklaşımıdır. Bu sayede algal kaynakların fonksiyonelliği arttırılmaktadır [31].

Çoklu Doymamış Yağ Asitleri

Algler gerekli koşullar sağlandığında toplam kuru maddesinin yaklaşık %50' sine kadar yağ depolayabilmektedir [28]. Algal lipitler, enerji üretimi için kullanılan 14-20 karbon içeren yağ asitleri ve sağlıklı besin takviyeleri olarak kullanılan 20' den fazla karbon içeren çoklu doymamış yağ asitleri olarak ikiye ayrılabilmektedir [32]. Çoklu doymamış yağ asitlerinin kalp dolaşım hastalıkları, aterosklerozis, koroner kalp hastalıkları ve kanda yüksek lipit içeriği gibi sorunlar üzerinde olumlu etkileri bulunmaktadır ve bu nedenle diyetle alınmaları çok önemlidir [33]. Son yıllarda yapılan çalışmalarda ise alglerden elde edilen çoklu doymamış yağ asitlerinin sahip olduğu bağışıklık biyoaktivitesi sayesinde COVID-19 tedavisinde kullanılabilecek potansiyel bir kaynak olduğu gösterilmiştir [34]. Çoklu doymamış yağ asitleri özellikle elzem yağ asitleri olan omega-3 ve omega-6 içermesi sebebiyle çok değerlidir. Elzem yağ asitleri insan vücudunda üretilmeyen ve beslenme yoluyla alınması gereken yağ asitleridir. Omega-6 yağ asitlerinden elzem olan linoleik asit (LA) metabolik reaksiyonlar sonucunda sırasıyla gamma linolenik asit (GLA) ve araşidonik asit (AA)'e dönüşmektedir [35]. Omega-3 yağ asitlerinden elzem olan α-linolenik asit (ALA) ise metabolik reaksiyonlar sonucunda sırayla stearidonik asit (SDA), eikosapentaenoik asit (EPA) ve dokosahekzaenoik asit (DHA)'e dönüşmektedir [28]. Bazı algal yağlar, EPA, DHA ve GLA ve araşidonik asit (AA) gibi önemli yağ asitlerini içermektedir [36]. Tüm bu sebeplerden dolayı, alglerin çoklu doymamış yağ asiti üretimi konusunda popülerliği artmış ve mikroalgler endüstriyel EPA ve DHA üretimi için en önemli doğal kaynak olarak kabul edilmektedir [37].

Eleren ve Öner' e göre [28], yüksek yağ içeriğine sahip mikroalg cinsleri şöyle sıralanabilir; *Chlorella* spp., *Spirulina* spp., *Dunaliella* spp., *Phaeodactylum* spp, *Scenedesmus* spp., ve *Chlamydomonas* spp.. *Chrysophyceae, Xanthophycea* ve *Eustigmatophyceae* sınıfı bazı alglere ait lipitler yüksek oranda EPA içermektedir [33]. *Schizochytrium* spp., *Cryptocodinium cohnii*, *Amphidinium* spp., *Prorocentrum triestinum* DHA sentezleyebilirken, *Chlorella minutissima, Nannochloropsis* spp., *Porphyridum cruentum, Phaeodactylum tricornutum* ve *Isochrysis galbana* EPA sentezleyebilmektedir [38, 39, 40]. *Chrysophyceae, Gyrodinium* ve *Crypthecodinium* türleri hem EPA hem de DHA kaynağıdır [41]. *Arthrospira* spp. ve *Porphyridium cruentum* ise sırasıyla GLA ve AA kaynağı olarak rapor edilmiştir [42]. *Spirulina platensis'* nin içerdiği yağın yaklaşık %20' si GLA' dır [33]. Diatomlarda temel ÇDYA' nin EPA olduğu bilinmektedir [43]. Bazı algal türler toplam kuru maddede yağ içeriği bilgisi Tablo 1' de verilmiştir. Tablo 2' de ise farklı alglerin içerdiği ÇDYA içeriği toplam yağ asitinin %' si şeklinde özetlenmektedir.

Tablo 2. Farklı alg kaynaklarının ÇDYA içeriği [44]
Table 2. PUFA content of different algal sources [44]

Alg Türü	ÇDYA Çeşidi	ÇDYA İçeriği (Toplam yağ asitinin yüzdesi)
Phaeodactylum tricornutum	EPA	9-57
Crypthecodinium cohnii	DHA	36-44
Nannochloropsis spp.	EPA	9-27
Porphyridium spp.	AA	22-35
	EPA	3-27
Schizochytrium spp.	DHA	33-37
Isochrysis galbana	EPA	12-27
	DHA	5-14

Algler içerdiği ÇDYA ile özellikle omega-3 ve omega-6 içeriği ile ilgi görmektedir. Elzem yağ asidi eksikliği konusunda gıda takviyesi olarak önerilmektedir. Balık yağları çoklu doymamış yağ asitlerinin ana kaynağı olarak bilinmesine rağmen balıklar çoklu doymamış yağ asitlerini üretemezler, dışarıdan mikroalg tüketerek bünyelerine katarlar [37]. Balık yağı yüksek oranda omega-3 içermesine rağmen, gıda takviyesi olarak arzu edilmeyen koku ve tada sahip olması nedeniyle kullanımı kısıtlanmaktadır [36]. Bu noktada, algal kaynakların gıda takviyesi olarak tavsiye edilmesi artmıştır. Sadece gıda takviyesi olarak değil, mikroalglere dayalı yeni tasarlanmış ürünlerin geliştirilmesinde omega-3 ve omega-6 kaynağı olarak algler öne çıkmaktadır. Örneğin omega-3 kaynağı olan algal makarna ve pesto formülasyonu geliştirilmiştir [45]. Mikroalglerin üretim hacminin %75' inden fazlası sağlıklı gıda pazarında beslenmeyi arttırmak amacıyla kullanılmaktadır. Omega-3 ve omega-6' nın yeri çok kritiktir ve yükselen bir pazarı temsil etmektedir [39]. DSM' in ekstrakte ettiği algal DHA, ABD' deki tüm bebek mamalarının %99' unda bulunduğu bilinmektedir [46]. ÇDYA kaynağı olarak endüstriyel olarak kullanılan ilk algler *Crypthecodinium cohnii*. *Schizochytrium* spp. ve diatomlar olmuştur [47, 48]. Alglerden çoklu doymamış yağ asitleri (ÇDYA) üretimi endüstriyel olarak gerçekleştirilmekte ve önemli bir pazar değeri taşımaktadır. 2019 yılında küresel omega-3 yağ asitleri pazarı büyüklüğü 2.3 milyar USD olarak değerlendirilmiş ve 2019-2027 döneminde yıllık bileşik büyüme oranı (CAGR) %7.44 olarak tahmin edilmektedir [49]. Mikroalglerden elde edilen yağlar, EPA/DHA pazar hacminin %3' ünü ve değerinin %18' ini oluşturmaktadır ve pazarda ki bu artış eğilimi algal kaynaklı ÇDYA üzerinde de etkili olması beklenmektedir [50]. Ticari EPA/DHA üreticilerinin başında DSM/Evonik şirketine bağlı çalışan Martek Biosciences firması gelmektedir [50, 51]. Martek firması, *Crypthecodinium cohnii* ve *Schizochytrium* spp. mikroalglerini kullanarak algal DHA yağlarının satışını yapmaktadır [51]. Pazarda algal omega-3 ve omega-6 üretimi yapan şirketler DSM/Evonik, Lonza, Cellana, Algae Biosciences, BASF

ve NPC, SB oils, Source Omega, Qualitas Health ve Fraunhofer olarak sıralanabilir [50]. DSM eski adıyla Martek şirketi bu alanda öncüdür. DSM, besleyici lipitler kapsamında alglerden elde edilen %48 oranında omega-6 içeren life's ARA, DHA kaynağı olarak life's DHA, EPA ve DHA karşımını içeren life's OMEGA ürünlerinin satışını yapmaktadır [52]. FDA, 2001 yılında Martek firmasının, DSM şirketine dahil olmadan, ürettiği algal yağ katkı maddelerini 'grass' (generally recognised as safe/ genel olarak güvenilir zararsız kabul edilen) statüsünde kabul etmiştir [53].

Polisakkarit

Polisakkaritler çok sayıda monosakkarit birimlerinden oluşan karbonhidratlardır. Algal polisakkaritler, gıda sektöründe stabilizatör, emülgatör ve kıvam arttırıcı olarak büyük öneme sahip olup, aynı amaçlarla ilaç sektöründe de kullanılmaktadır [36]. Çeşitli alg türlerindeki toplam polisakkarit konsantrasyonları kuru ağırlığın %4-76 arasında değişmektedir [54]. Algal polisakkaritler hücre duvarı polisakkaritleri, depo polisakkaritleri ve yapısal polisakkaritler olarak sınıflandırılabilir [54, 55]. Alglerden elde edilen ve ekonomik açıdan önemli olan polisakkaritler arasında karragenan, aljinat ve agar yer almaktadır [20]. Karragenan yapısına bağlı sülfat grubunun sayısı ve konumuna göre endüstriyel olarak kappa, iyota ve lambda olmak üzere 3 gruba ayrılmaktadır [56]. Bu sınıflandırma hangi amaç ve fonksiyon için kullanılacağını etkilemekte olup, kappa karragenan sert ve dayanıklı jel formunda, iyota karragenan elastik ve su tutucu jel yapıda, lambda karragenan ise jel özelliği göstermeyen durumlarda kullanılması önerilmektedir [54, 57]. Aljinat, pek çok gıda ürününde kıvam arttırıcı, emülgatör ve stabilize edici ajan olarak kullanılmaktadır. Agar, jöleli tatlılar, marshmallow, toffees gibi şekerleme ürünlerinde ve fırınlanmış ürünlerinde dolgu malzemelerinde olduğu gibi birçok alanda jelleştirici olarak kullanılmaktadır [58].

Kırmızı algler, karragenan ve agar kaynağı olarak bilinirken kahverengi algler aljinat kaynağı olarak bilinmektedir [59, 60]. *Chondrus* spp., *Eucheuma* spp., *Kappaphycus* spp., *Gigartina* spp., *Hypnea* spp., *Iridaea* spp. kütlece karragenan içeren alg cinsleridir [54]. *Gelidiella* spp., *Gelidiopsis* spp., *Gelidiopsis* spp., *Gelidium* spp., *Gracilaria* spp. ve *Pterocladia* spp. ise agar üretimi için kullanılan cinslerdir [54]. Bakterilerden de aljinat üretilebilmesine rağmen günümüzde ticari olarak tüm aljinat üretimi alglerden yapılmaktadır ve bu alg türleri şöyle sıralanabilir; *Laminaria hiperborea*, *Laminaria digitata*, *Laminaria japónica*, *Macrocystis pyrifera*, *Ascophyllum nodosum*, *Eclonia maxima*, ve *Sargassum* spp. [11, 61].

Tablo 3. Farklı alg cins/türlerinin polisakkarit içerikleri [62]
Table 3. Polysaccharide contents of different algal genus/species [62]

Algal Kaynak	Polisakkarit Tipi	Polisakkarit İçeriği (% toplam kuru maddede)
Laminaria japonica	Aljinat	%30-40
Gracilaria spp.	Agar	%40-50
Eucheuma spp.	Karragenan	%40-50
Ascophyllum nodosum	Aljinat	%30-40

Alglerden üretilen polisakkaritler günümüzde ticarileşmiş gıda katkı maddesi haline gelmiştir. Alglerden elde edilen polisakkaritlerin kıvam arttırıcı ve jelleştirici olarak reçel, içecek, fırıncılık ürünleri gibi endüstrilerde kullanıldığı bilinmektedir. 2010 yılında, bu üç polisakkaritin yıllık yaklaşık 90 ton ticari üretimi olup 1.016 milyar dolar pazar değerine sahiptir ve büyük oranda gıda endüstrisinde kullanılmaktadır [20].

Alglerden elde edilen polisakkaritler sadece gıda katkı maddesi olarak kullanılmamaktadır. Güncel kaynaklar alglerden elde edilen polisakkaritlerin farmasötik, kozmetik ve beslenme takviyeleri gibi geniş uygulama alanlarına sahip olduğunu teyit etmektedir [63]. Alglerden elde edilen aljinat, agar, karragenan ve ulvan gibi polisakkaritlerin, 3D gıda yazıcılarında potansiyel bir kaynak olarak kullanılabileceği belirtilmektedir [64]. Sürdürülebilir gıda ambalaj çalışmalarında da incelenen kaynaklardandır [65].

Pigment

Gıdaların satın alınması ve duyusal analizlerinde en önemli özelliklerden biri gıdanın rengidir. Çünkü renk fiziksel olarak en dikkat çekici ve ilk izlenimi oluşturan etmendir. Bu nedenlerle gıda sektöründe renklendiricilerin yeri büyüktür. Doğal ve sentetik olmak üzere iki tip pigment vardır [66]. Sentetik pigmentler kimyasal maddelerin türevi olup, özellikle gıdalarda kullanımı ile ilgili tartışmalar olmaktadır [21]. Hatta, sentetik renklendiricilerin alerjen, tahriş edici ve karsinojen etkiye sahip olması gibi sağlık üzerine negatif etkilerinin olduğu bilinmektedir [67]. Sentetik pigmentlerin bu olumsuz özelliklerinden dolayı doğal pigmentlere talep artmaktadır. Bunun yanında, doğal pigmentler antioksidan ve antitümoral aktiviteleri sayesinde sağlık açısından pozitif etki sağlamaktadır [68]. Yapılan araştırmalar sayesinde alglerin pigment üretimi için uygun bir kaynak olarak görülmesinin üzerine literatür ve ticari anlamda alglere olan ilgi bu konuda artmıştır [69].

Klorofil, karotenoid ve fikobilinler doğal pigmentlerin altında değerlendirilir [21]. Fikosiyanin, fikoeritrin, astaksantin, kantaksantin, β-karoten, lutein ve fukoksantin alglerden doğal olarak elde edilen pigmentler olarak bilinmektedir [17, 70]. En iyi β-karotenoid üreticisi *Dunaliella* spp. olarak bilinmektedir [71, 72]. β-karoten dünyada gıda renklendiricisi olarak margarin, peynir, meyve suları, süt ürünleri gibi ürünlerde sarı-turuncu renklerini vermek için en çok kullanılan pigmenttir [71]. β-karotenin gıdalarda

renklendirici olması dışında A vitamini öncüsü ve yüksek antioksidan özelliğine sahip olması nedeniyle sağlık açısından da gıdaların fonksiyonel özelliklerini geliştirmektedir [73]. Astaksantin üretimi yapan alg *Haematococus* spp. olarak bilinmektedir [71, 72]. Astaksantin pembe renk veren bir pigment olmasının yanında daha çok yüksek antioksidan aktivitesi ile öne çıkmaktadır [74]. Astaksantin, su ürünleri yetiştiriciliğinde ana bir pazar olarak öne çıkar çünkü somon ve alabalık gibi türlerde pigmentasyon kaynağı olarak kullanılır [75]. Fikosiyanin ve fikoeritrin üretimi özellikle *Porphyridium* spp. tarafından üretilmesinin yanında *Spirulina* spp., *Synechococcus* spp., *Phormidium* spp., ve *Nostoc* spp.' den yapılmaktadır [21, 72]. Sakız, jöleler, şekerler ve süt ürünlerinde mavi renk kaynağı olarak fikosiyanın kullanılmaktadır [76]. *Chlorella* spp. ve *Scenedesmus* spp. cinslerinden lutein pigmenti üretilmektedir [72]. Lutein gıda renklendiricisi, antioksidan ve antienflamatuvar aktivitesi yanı sıra göz sağlığına faydalı pigment olarak bilinmektedir [77]. Hazır çorba, alkollü ve alkolsüz içecek, bisküvi, sos, kek ve şekerleme endüstrilerinde sıklıkla kullanılan bir pigmenttir [21]. *Undaria* spp. *ve Sargassum* spp. cinslerinden fukoksantin pigmenti üretilmektedir [21]. Fukoksantin pigmenti renklendirici özelliğinine ek olarak, antiobesite, antitümör, antidiyabet, antioksidan, antiinflamatuar ve kardiyovasküler koruyucu gibi etkilerinin bulunduğu tespit edilmiştir [78]. Tablo 4'te farklı alglerin methanol çözgeni ile pigment ekstraksiyonu sonuçları toplam klorofil ve karotenoid cinsinden µg/g alg olarak ifade edilmiştir.

Tablo 4. Farklı alg kaynaklarının µg/g alg olarak pigment içeriği [79]
Table 4. Pigment content of different algal sources in µg/g algae [79]

Alg	Toplam Klorofil (µg/g alg)	Toplam Karotenoid (µg/g alg)
Himanthalia elongata	66.0	2.3
Undaria pinnatifida	352.2	54.6
Laminaria ochroleuca	168.9	27.0
Porphyra spp.	509.7	70.8
Spirulina spp.	10253.0	1263.9

Literatür bilgilerinin yanı sıra günümüzde ticari olarak da alglerden pigment üretimi yapılmaktadır. Algal pigment pazar değerinin 2025 yılına kadar 452.4 milyon USD' e ulaşması beklenmektedir [80]. Ticari üretime örnek olarak, *Sprirulina platensis*'ten klorofil a üretimi, *Undaria pinnatifida*'dan fukoksantin üretimi, *Haematococcus* spp.'ten astaksantin üretimi, *Dunaliella spp.*'den β-karoten üretimi verilebilir [81, 82, 83, 84].

Pigmentlerin hem renk verme hem de sağlık etkilerinin gözlenebilmesi için öncelikle algal kaynaktan ekstrakte edilmelidir. Geleneksel yöntemlerin yanında, SC-CO_2 (Süperkritik CO_2 ekstraksiyonu), MAE (Mikrodalga destekli ekstraksiyon), UAE (Ultrason destekli ekstraksiyon) ve EAE (Enzim destekli ekstraksiyon) gibi inovatif ekstraksiyon metotları çalışılmaktadır [85]. Yapılan araştırmalar sonucunda, pigmentlerin gıda renklendiricisi olmanın yanı sıra antioksidan, antimutajen, antiobesite, antitümör, antidiyabet ve antiinflamatuar etkilere sahip oldukları görülmüş, bu nedenle gıdaların fonksiyonel ve pozitif sağlık etkilerini artırdığı tespit edilmiştir. Örneklerden de açıkça anlaşıldığı üzere, alglerin ve alglerden elde edilen pigmentlerin doğal ve sürdürülebilir gıda renklendiricisi olarak önemi giderek artmaktadır.

Sterol

Sterol, çoğunlukla ökaryotik hücrenin zarlarında bulunan yapısal lipittir [86]. Kolesterol hayvan dokularındaki ana sterol iken, stigmasterol ve ergosterol sırasıyla bitkiler ve mantarlardaki ana sterol bileşenidir [86]. Algler, çeşitli sterolleri içermektedirler ve algal steroller kolesterol, fukosterol, izofukosterol, klionosterol ve stigmasterol olarak sıralanabilir [11]. Yeşil algler izofukokolesterol, kolesterol ve sitosterol; kahverengi algler fukosterol, kolesterol ve brassikasterol; kırmızı algler ise desmosterol, kolesterol, sitosterol ve fukosterol içermektedir [87]. Dinoflagellatların nispeten küçük bir grubu olan dinotomlar son yıllarda sterol kaynağı olarak araştırma konusu olmuştur [88]. Fitosterollerin antioksidan, antikanser, antidiyabetik, antihipertansif, antienflamatuar, hiperkolesterolemik, antifungal ve antibakteriyel gibi çeşitli pozitif biyolojik aktiviteleri olduğu literatürde belirtilmektedir [89]. Alglerden elde edilen fitosteroller arasında özellikle fukosterolün incelendiği ve yapılan çalışmalar sonucunda antioksidan, antiinflamatuar, kolesterol düşürücü özelliklerinin yanı sıra antidiyabetik, antiobezite, antiAlzheimer, antiaging, antikanser ve hepatoprotektif etkilerinin olduğu görülmüştür [90]. Sterollerin sağlık üzerindeki olumlu etkilerinden yararlanabilmek için emülsiyon, lipozom, oleojel veya nanolipozom gibi taşıma sistemleri aracılığıyla vücuda alınmalarının önemli olduğu belirtilmektedir [91]. Luo ve ark. [92]' in çalışmasında, fukosterol kaynağı olarak *Turbinaria conoides*, *Himanthalia elongate*, *Undaria pinnatifida*, *Phorphyra* spp., *Chondus crispus*, *Cystoseira* spp. ve *Ulva* spp.; sitosterol kaynağı olarak *Porphyridium cruentum, Bigelowiella natans, Gymno chlorastellata* ve *Lotharella amoeboformis*; stigmasterol kaynağı olarak ise *Porphyridium cruentum* gösterilmiştir. Farklı türleri içeren çalışmalarda, sterol içeriğinin kuru bazda %6.5'e kadar yükseldiği ve bu değerin yaz döneminde en yüksek seviyeye ulaştığı gözlenmiştir [93].

Zoosterol yani hayvansal sterol olan kolesterol insanlar tarafından sentezlenebilirken, fitosteroller sentezlenememekte ve dışarıdan alınması gerekmektedir [11, 94]. Eksiklik durumunda gıda takviyesi olarak alınması gerekmektedir. Ticari fitosterol üretimi için ana kaynak yüksek gelişmiş ve doğal olarak bulunan bitkilerdir [95]. Fitosteroller için küresel pazarın 2022 yılında 935 milyon dolara ulaşması beklenmektedir [96]. Hem sterollerin sağlık üzerine pozitif etkilerinin bilinmesi hem de alglerin sterol kaynağı olarak

görülmesi nedeniyle bu konuda yapılan çalışmalar artmaktadır. Kanola, soya ve ayçiçeği gibi ticari fitosterol kaynaklarıyla kıyaslandığında *Porphyridium* spp., *Isochrysis* spp., *Pavlova lutheri* ve *Thalassiosira pseudonana* gibi alglerin mg/g yağ cinsinden daha yüksek fitosterol içeriğine sahip oldukları belirlenmiştir [96]. Alglerin sterol üretiminde sürdürülebilir ve önemli bir kaynak olarak kabul edildiği ifade edilmektedir.

Vitamin ve Mineraller

Vitaminler ve mineraller, vücudumuzun normal olarak gelişmesi ve çalışması için ihtiyaç duyduğu temel maddelerdendir. Vitamin A, D, E, K, C, tiamin, riboflavin, niasin, piridoksin, folik asit, biyotin, pantotenik asit ve B_{12} olmak üzere 13 tane elzem vitamin türü vardır ve hepsinin sağlık üzerine spesifik pozitif özellikleri bulunmaktadır [97]. Makromineraller, sodyum, klor, potasyum, kalsiyum, fosfor, magnezyum ve kükürt gibi elementleri içerirken, iz mineralleri demir, çinko, iyot, bakır, selenyum, flor, mangan, krom ve molibden gibi elementlerden oluşmaktadır [98].

Algler, A, B_1, B_2, B_6, B_{12}, C ve E vitaminleri açısından ve potasyum, demir, magnezyum, kalsiyum ve iyot gibi mineraller açısından oldukça zengindir [15]. *Spirulina* spp., *Chlorella* spp. ve *Dunaliella* spp. cinsleri dünya üzerinde en çok yetiştiriciliği yapılan cinslerdir ve bu üç cins yüksek A vitamini içeriğine sahiptir [99, 100]. İlter ve ark. [11]' nın çalışmasında C ve E vitamini kaynağı olan türler *Dunaliella tertiolecta*, *Nannochloropsis oculata*, *Spirulina platensis*, *Tetraselmis suecica* ve *Euglena gracilis* olarak belirtilmiştir. Askorbik asit bakımından zengin olan ve büyük miktarlarda C vitamini biriken deniz diyatomu *Skeletonema marinoi.* yeni kaynaklar arasına girmektedir [101]. Yeşil, kahverengi ve kırmızı alglerde yüksek miktarda potasyum, kalsiyum ve sodyum içeriği bulunmaktadır [102]. 25 farklı yeşil mikroalg incelendiğinde, *Bracteacoccus minor* ve *Chlorococcum humicola* türlerinin demir, çinko ve kobalt içeriğinin yüksek olduğu görülmüştür [103]. Ticari olarak en çok yetiştiriciliği yapılan *Spirulina* ve *Chlorella* cinslerinin vitamin içerikleri mg ya da µg/100 g kuru maddede olarak Tablo 5'te verilmiştir. Görüldüğü üzere, *Spirulina* spp. E ve tiamin vitamini kaynağı olarak daha zengin iken, *Chlorella* spp. A, C ve niasin vitamini bakımından daha zengin bir içeriğe sahiptir.

Tablo 5. *Spirulina* ve *Chlorella* cinslerinin 100 g kuru maddede vitamin içeriği [104]
Table 5. Vitamin content of Spirulina and Chlorella genus in 100 g dry matter [104]

Vitamin Türü	*Chlorella* spp.	*Spirulina* spp.
A vitamini	30.77 mg	0.34 mg
C vitamini	10.4 mg	10.1 mg
B_1 vitamini (Tiamin)	1.7 mg	2.4 mg
B_2 vitamini (Riboflavin)	4.3 mg	3.7 mg
B_3 vitamini (Niasin)	23.8 mg	12.8 mg
B_5 vitamini (Pantotenik asit)	1.1 mg	-
B_6 vitamini (Piridoksin)	1.4 mg	0.4 mg
B_9 vitamini (Folik asit)	94 µg	94 µg
E vitamini	1.5 mg	5.0 mg
K vitamini	-	25.5 µg

Alglerden ticari olarak vitamin ve mineral üretildiği konusunda bilgi bulunamamıştır. Algal vitamin ve mineraller gıda takviyesi olarak incelenmektedir. Literatür bilgileri doğrultusunda zengin vitamin ve mineral içeren spesifik alg cinsleri bulunmaktadır. Bu nedenle, doğal ve sürdürülebilir vitamin ve mineral kaynağı olarak algler potansiyele sahiptir.

SONUÇ

Bu çalışmada, gıda sürdürülebilirliğinin önemi ve bu noktada alglerin yeri incelenmiştir. Alglerin sürdürülebilir bir kaynak olmasının en önemli nedeni içerdiği protein, çoklu doymamış yağ asidi, polisakkarit, pigment, sterol, vitamin ve mineraller gibi biyoaktif bileşenlerdir. Günümüzde algal protein, ÇDYA, sterol, vitamin ve mineraller gıda takviyesi olarak, polisakkarit ve pigmentler ise gıda katkı maddesi olarak kullanılmaktadır. *Spirulina* spp., *Chlorella* spp., *Dunaliella* spp., *Crypthecodinium* spp., *Schizochytrium* spp., *Laminaria* spp., *Gracilaria* spp. ve *Undaria* spp. cinsleri ticari olarak üretimi yapılan alg türlerine örnek olarak verilebilir. Algler son dönemde hem akademik çalışmalarda hem de endüstriyel uygulamalarda popülerliğini arttırmaktadır. Bunun yanı sıra yeni ve sürdürülebilir gıda kaynağı olması ve vegan beslenmeye uygun olması algleri en güncel araştırma konuları arasında tutmaya yetmektedir. Alglere olan gereksinimin artmasıyla bu alanda endüstriyelleşmenin giderek artacağı öngörüsünü yapmak doğru olacaktır.

KAYNAKLAR

[1] Glavič, P., Lukman, R. (2007). Review of sustainability terms and their definitions. *Journal of Cleaner Production,* 15(18), 1875-1885.

[2] Purvis, B., Mao, Y., Robinson, D. (2019). Three pillars of sustainability: in search of conceptual origins. *Sustainability Science,* 14(3), 681-695.

[3] Rueda, X., Garrett, R.D., Lambin, E.F. (2017). Corporate investments in supply chain sustainability: Selecting instruments in the agri-

food industry. *Journal of Cleaner Production,* 142*,* 2480-2492.

[4] Brklacich, M., Bryant, C.R., Smit, B. (1991). Review and appraisal of concept of sustainable food production systems. *Environmental Management,* 15(1), 1-14.

[5] Usmani, M.A., Toppo, K., Nayaka, S., Suseela, M.R., Sheikh, S. (2015). Role of algae in sustainable food, health and nutritional security: An overview. *Uttar Pradesh State Biodiversity Board,* 2015, 83-88.

[6] Food and Agriculture Organization. (2019). State of Food Insecurity in the World 2019 Report, http://www.fao.org/3/ca5249tr/ca5249tr.pdf, Haziran 2020.

[7] Mariutti, L. R. B., Rebelo, K. S., Bisconsin-Junior, A., de Morais, J. S., Magnani, M., Maldonade, I. R., & Cazarin, C.B.B. (2021). The use of alternative food sources to improve health and guarantee access and food intake. *Food Research International,* 149, 110709.

[8] Tan, K., Zhang, H., Li, S., Ma, H., Zheng, H. (2022). Lipid nutritional quality of marine and freshwater bivalves and their aquaculture potential. *Critical Reviews in Food Science and Nutrition*, 62(25), 6990-7014.

[9] Grossmann, L., Weiss, J. (2021). Alternative protein sources as technofunctional food ingredients. *Annual Review of Food Science and Technology*, 12, 93-117.

[10] Thavamani, A., Sferra, T. J., Sankararaman, S. (2020). Meet the meat alternatives: The value of alternative protein sources. *Current Nutrition Reports*, 9, 346-355.

[11] İlter, I., Akyıl, S., Koç, M., Kaymak-Ertekin, F. (2016). Alglerden elde edilen stabilize edici maddeler. *Akademik Gıda*, 14(3), 315-321.

[12] Barsanti, L., Gualtieri, P. (2014). Algae: Anatomy, Biochemistry, and Biotechnology. Taylor & Francis, Boca Raton.

[13] Lewin, R., Andersen, R. (2019). Algea. In: Encyclopædia Britannica.

[14] Baweja, P., Sahoo, D. (2015). Classification of Algae. In: The Algae World Cellular Origin. In Life in Extreme Habitats and Astrobiology, Edited by D. Sahoo, J. Seckbach, Springer, Dordrecht, pp. 31–55.

[15] Cebe, A.S. (2010). Alglerin genel özellikleri, kullanım alanları ve eczacılıktaki önemi. *Ankara Üniversitesi Eczacılık Fakültesi Dergisi,* 39(3), 237–264.

[16] Oğur, S. (2016). Kurutulmuş alglerin besin değeri ve gıda olarak kullanımı. *Su Ürünleri Dergisi*, 33(1), 67-79.

[17] Borowitzka, M.A. (1998). Algae As Food. In Microbiology of Fermented Foods, Edited by B.J.B. Wood, Springer, Boston.

[18] Edwards, M. (2010). Algae History and Politics, Cambridge University Press, Cambridge. pp. 205.

[19] Koyande, A.K., Chew, K.W., Rambabu, K., Tao, Y., Chu, D.T., Show, P.L. (2019). Microalgae: A potential alternative to health supplementation for humans. *Food Science and Human Wellness,* 8(1), 16–24.

[20] Bixler, H.J., Porse, H. (2011). A decade of change in the seaweed hydrocolloids industry. *Journal of Applied Phycology,* 23, 321-335.

[21] İlter, I., Akyıl, S., Koç, M., Kaymak-Ertekin, F. (2017). Alglerden elde edilen ve gıdalarda doğal renklendirici olarak kullanılan pigmentler ve fonksiyonel özellikleri. *Türk Tarım-Gıda Bilim ve Teknoloji Dergisi,* 5(12), 1508-1515.

[22] Katırcıoğlu, H., Aksöz, N. (2003). Tek hücre proteini. *Orlab On-Line Mikrobiyoloji Dergisi,* 1(8), 34-49.

[23] Becker, E.W. (2007). Micro-algae as a source of protein. *Biotechnology Advances,* 25(2), 207-210.

[24] Khan, Z., Bhadouria, P., Bisen, P.S. (2005). Nutritional and therapeutic potential of Spirulina. *Current Pharmaceutical Biotechnology,* 6(5), 373-379.

[25] Masojídek, J., Torzillo, G. (2014). Mass cultivation of freshwater microalgae. *Reference Module in Earth Systems and Environmental Sciences*, 2014, 1-13.

[26] Bleakley, S., Hayes, M. (2017). Algal proteins: extraction, application, and challenges concerning production. *Foods,* 6(5), 33.

[27] Habib, M.A.B. (2008). Review on culture, production and use of Spirulina as food for humans and feeds for domestic animals and fish. Food and agriculture organization of the united nations.

[28] Eleren, S.Ç., Öner, B. (2019). Sürdürülebilir ve çevre dostu biyoyakıt hammaddesi: mikroalgler. *Pamukkale Üniversitesi Mühendislik Bilimleri Dergisi,* 25(3), 304-319.

[29] Spolaore, P., Joannis-Cassan, C., Duran, E., Isambert, A. (2006). Commercial applications of microalgae. *Journal of Bioscience and Bioengineering,* 101(2), 87-96.

[30] Geada, P., Moreira, C., Silva, M., Nunes, R., Madureira, L., Rocha, C.M., Teixeira, J.A. (2021). Algal proteins: Production strategies and nutritional and functional properties. *Bioresource Technology*, 332, 125125.

[31] O'Connor, J., Garcia-Vaquero, M., Meaney, S., Tiwari, B.K. (2022). Bioactive peptides from algae: Traditional and novel generation strategies, structure-function relationships, and bioinformatics as predictive tools for bioactivity. *Marine Drugs*, 20(5), 317.

[32] Yen, H.W., Hu, I.C., Chen, C.Y., Ho, S.H., Lee, D.J., Chang, J.S. (2013). Microalgae-based biorefinery–from biofuels to natural products. *Bioresource Technology,* 135, 166-174.

[33] Darcan, S., Sarıgül, N. (2015). Mikroorganizmalardan tek hücre yağları üretimi. *Türk Mikrobiyoloji Cemiyeti Dergisi,* 45(2), 55-67.

[34] Ziyaei, K., Ataie, Z., Mokhtari, M., Adrah, K., Daneshmehr, M.A. (2022). An insight to the therapeutic potential of algae-derived sulfated polysaccharides and polyunsaturated fatty acids: Focusing on the COVID-19. *International Journal of Biological Macromolecules*, 209, 244-257.

[35] Kent, L. (2009). Çoklu Doymamış Yağ Asitleri Eldesi: Tepki Yüzey Metodolojisi İle

Optimizasyonu. Doktora tezi, İTÜ Fen Bilimleri Enstitüsü, İstanbul, pp. 10-50.

[36] Akyıl, S., İlter, I., Koç, M., Kaymak-Ertekin, F. (2016). Alglerden elde edilen yüksek değerlikli bileşiklerin biyoaktif/biyolojik uygulama alanları. *Akademik Gıda,* 14(4), 418-423.

[37] Mishra G. (2015) Polyunsaturated Fatty Acids from Algae. In The Algae World, Edited by D. Sahoo, J. Seckbach, Springer, Dordrecht, pp. 57-75.

[38] Medina, A.R., Grima, E.M., Gime´nez, A.G., Gonza´lez, M.J.I. (1997). Downstream processing of algal polyunsaturated fatty acids. *Biotechnology Advances,* 16(3), 517-580.

[39] Kyle, D.J. (2001). The large-scale production and use of a single-cell oil highly enriched in docosahexaenoic acid. *ACS Symposium Series Omega-3 Fatty Acids,* 2, 92-107.

[40] Bellou, S., Aggelis, G. (2013). Biochemical activitiesin Chlorella sp. and Nannochloropsissalina during lipid and sugar synthesis in a lab-scale open pondsimulating reactor. *Journal of Biotechnology,* 164(2), 318-329.

[41] Makri, A., Bellou, S., Birkou, M., Papatrehas, K., Dolapsakis, N.P., Bokas, D., Papanikolaou, S., Aggelis, G. (2011). Lipid synthesized by micro-algaegrown in laboratory and industrial-scale bioreactors. *Engineering in Life Sciences,* 11(1), 52-58.

[42] Cohen, Z., Heimer, Y.M. (1992). Production of Polyunsaturated Fatty Acids (EPA, ARA and GLA) by The Microalgae Porphyridium and Spirulina. In Industrial Applications of Single Cell Oils, Edited by D.J. Kyle, C. Ratledge. CRC Press, New York, pp. 243-273.

[43] Volkman, J.K. (2003). Sterols in microorganisms. *Applied Microbiology and Biotechnology,* 60(5), 495-506.

[44] Robertson, R., Guihéneuf, F., Schmid, M., Stengel, D.B., Fitzgerald, G., Ross, P., Stanton, C. (2013). Algae-derived polyunsaturated fatty acids: implications for human health. *Polyunsaturated Fatty Acids: Sources, Antioxidant Properties and Health Benefits*, 2013, 45-99.

[45] Pina-Pérez, M.C., Brück, W., Brück, T., Beyrer, M. (2020). Microalgae as Healthy İngredients for Functional Foods. In The Role of Alternative and İnnovative Food İngredients and Products ın Consumer Wellness, Edited by C.M. Galanakis, Academic Press, New York, pp. 103-137.

[46] Rahman, K.M. (2020). Food and High Value Products from Microalgae: Market Opportunities and Challenges. In Microalgae Biotechnology for Food, Health and High Value Products, Springer, Singapore, pp. 3-27.

[47] Harwood, J.L. (2019). Algae: critical sources of very long-chain polyunsaturated fatty acids. *Biomolecules,* 9(11), 708.

[48] Chen, W., Li, T., Du, S., Chen, H., Wang, Q. (2023). Microalgal polyunsaturated fatty acids: Hotspots and production techniques. *Frontiers in Bioengineering and Biotechnology*, 11, 1146881.

[49] K. Ahuja, K. Mamtani. (2022). EPA/DHA (omega 3) ingredients market and Share Report 2026. https://www.gminsights.com/industry-analysis/EPA-DHA-omega-3-ingredients-market

[50] Van der Voort, M.P., Spruijt, J., Potters, J.I., Elissen, H.J.H. (2017). Socio-Economic Assessment of Algae-Based PUFA Production. The Value Chain from Microalgae to PUFA Chain, Project no: 613303.

[51] Ansorena, D., Astiasarán, I. (2013). Development Of Nutraceuticals Containing Marine Algae Oils. In Functional Ingredients from Algae for Foods and Nutraceuticals, Edited by H.D. González, Woodhead Publishing, Philadelphia, pp. 634-657.

[52] DSM. (2021). Nutritional Lipids. https://www.dsm.com/markets/humannutrition/en/products/ nutritional-lipids.html, Mart 2021.

[53] Harris, R.P. (2006). Omega 3 fatty acids. Novinka Books, New York, pp. 17.

[54] Kraan, S. (2012). Algal Polysaccharides, Novel Applications and Outlook. In Carbohydrates-Comprehensive Studies on Glycobiology and Glycotechnology, Edited by C. Chang, InTech, Rijeka, pp. 65-80.

[55] Mišurcováa, L., Orsavováb, J., Ambrožováa, J.V. (2014). Algal Polysaccharides and Health. In Polysaccharides: Bioactivity And Biotechnology, Edited by K.G. Ramawat, J. Mérillon, Springer, Cham, pp. 95.

[56] Campo, V.L., Kawano, D.F., da Silva Jr, D.B., Carvalho, I. (2009). Carrageenans: biological properties, chemical modifications and structural analysis–A review. *Carbohydrate polymers,* 77(2), 167-180.

[57] Ak, İ. (2015). Sucul ortamın ekonomik bitkileri; makro algler. *Dünya Gıda Dergisi*, 12, 88-97.

[58] Pegg, A.M. (2012). The Application of Natural Hydrocolloids to Foods and Beverages. In Natural Food Additives, İngredients and Flavourings, Woodhead Publishing, Cambridge, pp. 175-196.

[59] McHugh, D.J. (2003). A guide to the seaweed industry. *FAO Fish Technology,* 441, 1-105.

[60] Draget, K.I., Smidsrød, O., Skjåk-Bræk, G. (2005). Alginates from algae. *Biopolymers Online: Biology, Chemistry, Biotechnology, Applications,* 6, 22-45.

[61] Draget, K.I. (2009). Alginates. In Handbook of Hydrocolloids, Edited by G.O. Phillips, P.A. Williams, CRC press, Boca Raton, pp. 53-64.

[62] Gotteland, M., Riveros, K., Gasaly, N., Carcamo, C., Magne, F., Liabeuf, G., Beattie, A., Rosenfeld, S. (2020). The pros and cons of using algal polysaccharides as prebiotics. *Frontiers in Nutrition*, 7, 163-169.

[63] Patel, A.K., Vadrale, A.P., Singhania, R.R., Michaud, P., Pandey, A., Chen, S.J., Dong, C.D. (2022). Algal polysaccharides: current status and future prospects. *Phytochemistry Reviews,* 1-30.

[64] Mandal, S., Nagi, G.K., Corcoran, A.A., Agrawal, R., Dubey, M., Hunt, R.W. (2022). Algal polysaccharides for 3D printing: A review. *Carbohydrate Polymers*, 120267.

[65] Thiviya, P., Gamage, A., Liyanapathiranage, A., Makehelwala, M., Dassanayake, R. S.,

Manamperi, A., Madhujith, T. (2022). Algal polysaccharides: Structure, preparation and applications in food packaging. *Food Chemistry*, 134903.

[66] Erdal, P., Ökmen, G. (2013). Gıdalarda kullanılan mikrobiyal kaynaklı pigmentler. *Türk Bilimsel Derlemeler Dergisi,* 6(2), 56-68.

[67] Alam, T., Najam, L., Al-Harrasi, A. (2018). Extraction of natural pigments from marine algae. *Journal of Agricultural and Marine Sciences,* 23(1), 81-91.

[68] Beutner, S., Bloedorn, B., Frixel, S., Hernández-Blanco, I., Hoffmann, T., Martin, H.D., Schülke, I. (2001). Quantitative effect of antioxidant properties of natural colorants and phytochemicals: carotenoids, flavonoids, phenols and indigoids. *Journal of the Science of Food and Agriculture,* 81(6), 559-568.

[69] Dufossé, L., Galaup, P., Yaron, A., Arad, S.M., Blanc, P., Murthy, K.N.C., Ravishankar, G.A. (2005). Microorganisms and microalgae as a source of pigment for food use: a scientific oddity or an industrial reality?. *Trends in Food Science & Technology,* 16(9), 389-406.

[70] Prasanna, R., Sood, A., Suresh, A., Nayak, S., Kaushik, B. (2007). Alg pigmentlerinin biyoloji ve endüstrideki potansiyelleri ve uygulamaları. *Acta Botanica Hungarica,* 49(2),131-156.

[71] Çelikel, N., Kınık, Ö., Gönç, S., Kavas, G. (2006). Mikroalglerin gıdalarda renk verici madde (pigment) kaynağı olarak kullanımı. *Türkiye 9. Gıda Kongresi,* Mayıs 24-26, 2006, Bolu, Türkiye, Bildiri Kitabı, pp. 447-450.

[72] Dring, M.J. (1998). The Biology of Marine Plants. Cambridge University Press, Cambridge, pp. 43-76.

[73] da Costa Cardoso, L.A., Kanno, K.Y.F., Karp, S.G. (2017). Microbial production of carotenoids A review. *African Journal of Biotechnology,* 16(4), 139-146.

[74] Hu, I.C. (2019). Production of Potential Coproducts from Microalgae. In Biofuels from Algae. Elsevier, New York, pp. 345-358.

[75] Lorenz, R.T., Cysewski, G.R. (2000). Commercial potential for Haematococcus microalgae as a natural source of astaxanthin. *Trends in Biotechnology,* 18(4), 160-167.

[76] Santiago-Santos, M.C., Ponce-Noyola, T., Olvera-Ramírez, R., Ortega-López, J., Cañizares-Villanueva, R.O. (2004). Extraction and purification of phycocyanin from Calothrix sp.. *Process Biochemistry,* 39(12), 2047-2052.

[77] Buscemi, S., Corleo, D., Di Pace, F., Petroni, M. L., Satriano, A., Marchesini, G. (2018). The effect of lutein on eye and extra-eye health. *Nutrient,* 10(9), 13-21.

[78] Zhang, H., Tang, Y., Zhang, Y., Zhang, S., Qu, J., Wang, X., Liu, Z. (2015). Fucoxanthin: A promising medicinal and nutritional ingredient. *Evidence-Based Complementary and Alternative Medicine*, 2015, 1-10.

[79] Osório, C., Machado, S., Peixoto, J., Bessada, S., Pimentel, F.B.C, Alves, R., Oliveira, M.B.P.P. (2020). Pigments content (Chlorophylls, fucoxanthin and phycobiliproteins) of different commercial dried algae. *Separations*, 7(2), 33.

[80] Patel, A.K., Albarico, F.P.J.B., Perumal, P.K., Vadrale, A.P., Nian, C.T., Chau, H.T.B., ... Singhania, R.R. (2022). Algae as an emerging source of bioactive pigments. *Bioresource Technology*, 351, 126910.

[81] Scheer, H. (2013). Chlorophylls and Carotenoids. In Encyclopedia of Biological Chemistry, Edited by W.J. Lennarz, M.D. Lane, Academic Press, Oxford.

[82] Pereira, R., Yarish, C. (2009). Mass Production of Marine Macroalgae. In Encyclopedia Of Ecology and Environmental Management, Edited by P.P. Calow, John Wiley & Sons, New York, pp. 2236-2247.

[83] Fung, A., Hamid, N., Lu, J. (2013). Fucoxanthin content and antioxidant properties of Undaria pinnatifida. *Food Chemistry,* 136(2), 1055-1062.

[84] Ambati, R.R., Gogisetty, D., Aswathanarayana, R.G., Ravi, S., Bikkina, P.N., Bo, L., Yuepeng, S. (2019). Industrial potential of carotenoid pigments from microalgae: Current trends and future prospects. *Critical Reviews İn Food Science And Nutrition,* 59(12), 1880-1902.

[85] Cikoš, A.M., Šubarić, D., Roje, M., Babić, J., Jerković, I., Jokić, S. (2022). Recent advances on macroalgal pigments and their biological activities (2016–2021). *Algal Research*, 65, 102748.

[86] Hazra, S., Ghosh, S., Hazra, B. (2017). Phytochemicals with Antileishmanial Activity: Prospective Drug Targets. In Studies in Natural Products Chemistry, Edited by A. Rahman, Elsevier, Amsterdam, pp. 303-336.

[87] Guedes, A.C., Amaro, H.M., Sousa-Pinto, I., Malcata, F.X. (2019). Algal spent biomass—A pool of applications. In Biofuels from algae, Edited by A. Pandey, Elsevier, Amsterdam, pp. 397-433.

[88] Leblond, J.D., Vandergrift, S.L. (2022). Sterols of the 'dinotom'Durinskia baltica (Dinophyceae) are of dinoflagellate origin. *Phycological Research*, 70(1), 35-41.

[89] Kim, S.K., Van Ta, Q. (2011). Potential Beneficial Effects of Marine Algal Sterols on Human Health. In Advances in Food and Nutrition Research, Edited by S.L. Taylor, Academic Press, Burlington, pp. 191-198.

[90] Hannan, M.A., Sohag, A.A.M., Dash, R., Haque, M.N., Mohibbullah, M., Oktaviani, D.F., Moon, I.S. (2020). Phytosterols of marine algae: Insights into the potential health benefits and molecular pharmacology. *Phytomedicine,* 69, 153-201.

[91] Zhang, R., Han, Y., McClements, D. J., Xu, D., Chen, S. (2022). Production, characterization, delivery, and cholesterol-lowering mechanism of phytosterols: a review. *Journal of agricultural and food chemistry*, 70(8), 2483-2494.

[92] Luo, X., Su, P., Zhang, W. (2015). Advances in microalgae-derived phytosterols for functional food and pharmaceutical applications. *Marine Drugs,* 13(7), 4231-4254.

[93] Klein, B., Davis, R. (2023). Algal Biomass Production via Open Pond Algae Farm

Cultivation: 2022 State of Technology and Future Research (No. NREL/TP-5100-85661). National Renewable Energy Laboratory (NREL), Golden, CO (United States).

[94] Lopes, G., Sousa, C., Valentao, P., Andrade, P.B. (2013). Sterols in Algae and Health. In Bioactive Compounds from Marine Foods, Edited by B. Hernandez-Ledesma, M. Herrero, John Wiley, Chichester, pp.173-191.

[95] Fernandes, P., Cabral, J.M.S. (2007). Phytosterols: applications and recovery methods. *Bioresource Technology,* 98(12), 2335-2350.

[96] Randhir, A., Laird, D.W., Maker, G., Trengove, R., Moheimani, N.R. (2020). Microalgae: a potential sustainable commercial source of sterols. *Algal Research*, 46, 101772.

[97] Combs, G.F., McClung, J.P. (2017). The Vitamins: Fundamental Aspects İn Nutrition and Health. Academic Press, Amsterdam.

[98] Mason, J.B. (2007). Vitamins, trace minerals, and other micronutrients. *Cecil Textbook of Medicine* 23, 1626-1639.

[99] Tang, G., Suter, P.M. (2011). Vitamin A, nutrition, and health values of algae: Spirulina, Chlorella, and Dunaliella. *Journal of Pharmacy and Nutrition Sciences,* 1(2), 111-118.

[100] Priyadarshani, I., Rath, B. (2012). Commercial and industrial applications of micro algae–A review. *Journal of Algal Biomass Utilization,* 3(4), 89-100.

[101] Uma, V. S., Usmani, Z., Sharma, M., Diwan, D., Sharma, M., Guo, M., .Gupta, V. K. (2022). Valorisation of algal biomass to value-added metabolites: Emerging trends and opportunities. *Phytochemistry Reviews*, 1-26.

[102] Wells, M.L., Potin, P., Craigie, J.S., Raven, J.A., Merchant, S.S., Helliwell, K.E., Smith, A.G., Camire, M.E, Brawley, S.H. (2017). Algae as nutritional and functional food sources: revisiting our understanding. *Journal of Applied Phycology,* 29(2), 949-982.

[103] Santhakumaran, P., Ayyappan, S.M., Ray, J. G. (2020). Nutraceutical applications of twenty-five species of rapid-growing green-microalgae as indicated by their antibacterial, antioxidant and mineral content. *Algal Research*, 47, 101878.

[104] Andrade, L.M., Andrade, C., Dias, M., Nascimento, C., Mendes, M. (2018). Chlorella and Spirulina microalgae as sources of functional foods. *Nutraceuticals, and Food Supplements*, 6(1), 45-58.

SİDAS MEDYA
Akademik Gıda®
ISSN Online: 2148-015X
https://dergipark.org.tr/tr/pub/akademik-gida

Akademik Gıda 21(2) (2023) 198-201, DOI: 10.24323/akademik-gida.1351195

Opinion / Görüş

Food Safety and Law Enforcement in Türkiye

Enver Kaşlı[1,]

Ankara Police Department, 06560 Yenimahalle, Ankara

Received (Geliş Tarihi): 17.01.2023, Accepted (Kabul Tarihi): 07.06.2023
Corresponding author (Yazışmalardan Sorumlu Yazar): kaslienver@gmail.com (E. Kaşlı)
+90 312 303 6524 +90 312 384 0772

ABSTRACT

Food is an indispensable basic need for human beings. Food safety has become one of the elements of public order, as technological developments bring many new threats to food safety in contrast to the advantages it provides. Safe food does not pose a danger to human health, is ready for consumption, and complies with the legislation. Food law enforcement is an activity that requires technical knowledge and expertise and is among the private law enforcement agencies. To ensure food safety in Türkiye, the Ministry of Agriculture and Forestry carries out food law enforcement activities. In this study, food safety and food law enforcement organization, powers, and sanctions of food law enforcement are examined by Turkish legislation.

Keywords: Food safety, Food law enforcement, Hygiene, Food legislation

Türkiye'de Gıda Güvenliği ve Gıda Kolluğu

ÖZ

Gıda, insan için vazgeçilmez temel bir ihtiyaçtır. Teknolojideki gelişmeler sağladığı avantajların yanı sıra gıda güvenliği açısından da birçok yeni tehdidi ortaya çıkardığından dolayı gıdanın güvenliği, kamu düzeninin unsurları arasına girmiştir. Güvenli gıda; insan sağlığına tehlike oluşturmayan, tüketime hazır ve mevzuata uygun gıdadır. Gıda kolluğu, teknik bilgi ve uzmanlık isteyen bir faaliyet olup özel kolluk teşkilatları arasında yer almaktadır. Türkiye'de gıda güvenliğini sağlamak amacıyla Tarım ve Orman Bakanlığı, gıda kolluğu faaliyetlerini yürütmektedir. Bu çalışmada Türk mevzuatına uygun olarak gıda güvenliği ve gıda kolluğu teşkilatı, gıda kolluğunun yetkileri ve yaptırımlar incelenmektedir.

Anahtar Kelimeler: Gıda güvenliği, Gıda kolluğu, Hijyen, Gıda mevzuatı

INTRODUCTION

Today, law enforcement activities are not limited to just policing. To protect public order, there are many law enforcement agencies besides the police. Food law enforcement is a private law enforcement activity. The purpose of food law enforcement is to ensure the safety of food that people need to feed. Food is an indispensable basic need for human beings. Technological advancements have also deeply affected the food industry and created new food-related safety concerns. Since public order means people live in safety and peace, ensuring food safety has become an element of public order.

To ensure food safety in Türkiye, the Ministry of Agriculture and Forestry carries out food law enforcement activities. These law enforcement activities are regulated in the Veterinary Services, Phytosanitary, Food and Feed Law No. 5996 (shortly Food Law), which came into force in 2010. The Food Law was enacted to harmonize with the European Union legislation [1, 2]. In this study, firstly, food safety will be examined according to Turkish legislation, then the food law enforcement

organization will be explained, then the powers and sanctions of the food law enforcement will be analyzed.

DETERMINATION of FOOD as SAFE

Food law enforcement is an activity that requires technical knowledge and expertise. Food law enforcement needs technical and special knowledge, mainly food engineering science. Therefore, the basic concepts of food law enforcement are specific to its field of activity. The most basic concept of food law enforcement is food. People have to feed to live, food is used to feed. Food is used to express processed, partially processed, or unprocessed product that is eaten, drunk or expected to be eaten or drunk by humans [3] (article 3). The basis of food safety is the risk of food harming human health. Concerns about food safety are not a new phenomenon. It is claimed that 1.8 million people die each year because of diarrheal diseases caused by unsafe food and water [4]. Safe food does not pose a physical, chemical, or biological hazard to human health is ready for consumption, and complies with the legislation [3] (article 21).

Certain criteria are taken into account to determine safe food. In determining whether the food is safe; production stages, label information and health warning information, and daily normal usage conditions by people are taken into consideration [5]. In determining whether the food is harmful to human health; in addition to the possible immediate, short, or long-term effects on the health of the consumer, the effects on future generations, possible cumulative toxic effects, and the special health sensitivities of certain consumer groups are also taken into consideration [3] (article 21).

Safe food complies with the Turkish food codex [6]. In the food codex, there are regulations regarding minimum technical and hygiene criteria regarding food, additives, sampling, packaging, labeling, transportation, storage principles, and analysis methods. The Ministry's National Food Codex Commission prepares the food codex. The Ministry can cooperate with relevant institutions and organizations while preparing the food codex [3] (article 23). The production and placing on the market of food and food contact materials contrary to the food codex is prohibited [3] (article 21). Safe food must comply with the hygiene conditions in the food codex. Hygiene is any measure required to be taken at all stages of the food chain to ensure that food is suitable for human consumption [3] (article 3).

Adulteration and counterfeiting violate food safety. Acts such as counterfeiting and adulteration are experienced especially during periods of increased demand in the food market [8]. Adulteration is the removal of all or some of the elements and nutritional values that give food its basic feature or the change of its amount in violation of the legislation. Another type of adulteration is the addition of another substance that does not have the same value as if it were the same substance instead of that substance. On the other hand, counterfeit is the presentation of food as having features that are not found in its structure in terms of shape, composition, and qualities, or as if it is the same as another product [3] (article 3). Counterfeiting and adulteration of food are prohibited. The imitated and adulterated product cannot be processed or placed on the market [3] (article 24). Food law enforcement has a great responsibility to ensure that people are fed with safe food.

ORGANIZATION of FOOD LAW ENFORCEMENT

Law enforcement protects public order and intervenes in events disrupting public order. Peace and order in social life is public order. The scope of public order is expanding and enriching. While law enforcement generally meant the police in the past, today many activities such as urbanization, protection of the environment, combating climate change, and disaster preparedness are carried out as law enforcement activities. Food must be safe for health to maintain public order. Throughout history, states have taken measures to ensure that people are fed from safe sources [2]. Food law enforcement is the whole of inspection, investigation, control, monitoring, and enforcement activities to ensure food safety to protect human health [11].

Private law enforcement is only authorized and in charge of a certain type of activity. Since food safety requires technical knowledge and expertise, food law enforcement is a special law enforcement activity. The Ministry of Agriculture and Forestry is responsible for ensuring food safety in Türkiye. Since the food production stages are interconnected, the authorities related to food safety are gathered in a single institution. The Ministry consists of central and provincial units. Its central organization is in Ankara, the capital of Türkiye. The provincial organization is located in 81 provinces of the country. The ministry is the contact point of the international Codex Alimentarius Commission [3] (article 23). Ministry as food law enforcement is responsible for preparing the food codex [3] (article 23), determining the hygiene conditions [3] (article 29), making the regulations regarding new foods [3] (article 21), taking the necessary measures in case of risk [3] (article 25), and carrying out official control activities [3] (article 31). Consumers can assist food law enforcement by calling. Consumers have been able to make their food safety-related notices and complaints to the Call Center 174 Food Line since 2009.

Businesses engaged in food-related activities must obtain permission from the Ministry [3] (article 30). Food businesses are also responsible for ensuring food safety. Food businesses must comply with the food law. Food businesses are obliged to collect their products and inform the Ministry of the situation if they consider that their products are not reliable or safe. The food business operator must also inform the consumer about the reason for the collection. Food businesses; keep the records related to food activities up to date and submit them to the Ministry during official controls. Depending on the type of business, food businesses must employ at least one staff member who has received a bachelor's degree in the subject [3] (article 22). In the event of a serious risk to human health, food businesses and other

interested parties must comply with the Ministry's measures [3] (article 25).

WARRANTS of FOOD LAW ENFORCEMENT

Ensuring food safety is a long and complex process. Food safety is the observance and control of the rules at every stage of production to ensure the production of foods that are harmless to human health from the first producer to the last seller.

Inspections made to ensure food safety is called official controls. The control officer carries out official controls. The control officer may be Ministry personnel or authorized persons. The control officer has the authority to enter any place at any time for control and to request samples from the food business operators during the control without paying any price. Control officer has to take decisions impartially, objectively, and independently, away from any kind of influence and conflict of interest [3](article 31). Analyzes of the samples related to the official controls are carried out in the laboratories of the Ministry or other laboratories approved by the Ministry [3] (article 33). Those concerned have the right to object to the Ministry about the results of official control and inspection within seven days from the date of notification [3] (article 31).

Food law enforcement has to intervene when it detects an incident that endangers or violates food safety. Food law enforcement's interventions are based on the powers granted by the Food Law. Food law enforcement has the discretion to decide which powers to apply according to the circumstances of the case. In food law enforcement, intervening in an event that endangers food safety is preventive power, and intervening in an event that violates food safety is judicial power.

- Restriction and Prohibition of Foodstuff: The Ministry may restrict or prohibit the use of certain substances and products as food or in food production, or may bind their use to certain principles, taking into account human health [3](article 21).
- Determination of Import and Export Conditions: The Ministry is authorized to determine the import and export conditions related to food [3](article 34). However, in the presence of bilateral or multilateral international agreements, the provisions of the agreements shall apply to imports and exports.
- Granting Approval: The Ministry is authorized to approve before being placed on the market. The product owner has to submit the information and documents requested by the Ministry for approval. The production, importation, and placing of these products on the market without approval are prohibited [3](article 22).
- Restriction and Prohibition of Import, Export, and Supply to the Market: The Ministry may restrict or prohibit the placing on the market, transportation, entry, and exit of the food, which, as a result of official control, is determined not to meet the requirements of the legislation and to pose a danger to human health [3] (article 32). Even if the food complies with the conditions determined by the Ministry, if there is sufficient doubt that the food is unsafe, the Ministry may restrict the supply of the food to the market or have the food brought to the market recalled [8] (article 21).
- Suspension of Sale and Withdrawal from the Market: The Ministry may suspend the sale of food, which, as a result of official control, is determined not to meet the requirements of the legislation and to pose a danger to human health, and may request that they be collected from the market by the owner or operator [3] (article 32).
- Making up for the Deficiency: when a deficiency that can be corrected in terms of legislation is detected, if the food does not pose any danger to human health, the products may be allowed to be placed on the market, provided that they are brought into compliance with the legislation [3] (article 32).
- Evaluation for Other Purposes: The Ministry may permit the evaluation of the food, which, as a result of official control, does not meet the requirements of the legislation and poses a danger to human health, under the control of the Ministry and other relevant institutions [3] (article 32).
- Disposal: The Ministry may destroy the food that is determined as a result of official control that does not meet the requirements of the legislation and poses a danger to human health [3] (article 32).
- Reporting a crime: When the Ministry detects a crime related to food safety, it is obliged to report the situation to the prosecutor's office.

SANCTIONS and JUDICIAL REMEDY

Sanction is the legal reaction against the violation of the rule of law. To ensure food safety, judicial and administrative sanctions are regulated for those who violate the legislative rules. Judicial sanctions are decided by the criminal courts as a result of the criminal investigation. The most important responsibility of food law enforcement for requiring criminal sanctions is to report a crime and submit the evidence to the prosecutor's office. Provincial directors and control officers serving as food law enforcement have the authority to impose administrative sanctions on incidents they encounter during inspections [3] (article 42).

The acts that require judicial sanctions are as follows:

- Acts of producing, importing, and placing on the market food that will endanger people's life and health are punished with imprisonment from one year to five years and a judicial fine. These foods are collected from the market at the expense of the responsible person, and their property is transferred to the public and destroyed [3] (article 40).

The acts that require administrative sanctions are as follows:

- Administrative fines are imposed on those who violate the regulations set by the Ministry regarding new foods [3] (article 40).
- Administrative fines shall be imposed on those who produce food and substances and materials that come into contact with food, and those who place them on the market, in violation of the food codex [3] (article 40).
- Administrative fines are imposed on those who violate the restrictions or prohibitions determined by the Ministry [3] (article 40).
- Administrative fines are imposed on those who place food on the market without approval [3] (article 40).
- Administrative fines are imposed on those who do not comply with the measures taken by the Ministry in case of risk [3] (article 40).
- An administrative fine is imposed on those who do not employ trained personnel [3] (article 40).
- Administrative fines are imposed on those who imitate and adulterate. The ownership of food is passed to the public [3] (article 40).
- An administrative fine is imposed on those who do not comply with the hygiene rules [3] (article 41).

Disposal procedures are carried out under the supervision of the Ministry, with all costs borne by the owner. Administrative fines are paid within thirty days [3] (article 40). Related persons may seek rights under criminal procedure law against acts that require judicial sanction. Those concerned have the right to file a lawsuit against the acts requiring administrative sanction before the administrative courts.

CONCLUSION

For the health of society, people must be fed with adequate and safe food. The measures to be taken by food law enforcement have an important place to prevent the threats to food safety from turning into harm. The law regulating the duties and powers of food law enforcement in Türkiye entered into force in 2010. The Ministry of Agriculture and Forestry, which carries out food law enforcement activities, has been given many important tasks such as preparing the food codex, determining the hygiene conditions, making the regulations regarding new foods, taking the necessary measures in case of risk, and carrying out official control activities. To fulfill this duty, food law enforcement has been given the authority to make regulations for foodstuffs, to allow food businesses, and to take measures for market activities. Food law enforcement can apply administrative sanctions to ensure food safety and to apply to the courts. As a result, food law enforcement has the necessary preventive and judicial powers at the level of law to ensure food safety.

DISCLAIMER

Opinions expressed within the content reflect only the author's views. The contents of the published opinion do not necessarily represent the views of the government agency of Police Department in Türkiye.

REFERENCES

[1] Koç, G., Uzmay, A. (2015). Gıda güvencesi ve gıda güvenliği: Kavramsal çerçeve, gelişmeler ve Türkiye. *Tarım Ekonomisi Dergisi*, 21(1), 39-48.
[2] Gökçe, R., Ergezer, H. (2016). Gıda mevzuatımız; nereden, nereye?. *Akademik Gıda*, 14(2), 225-229.
[3] Veterinary Services, Phytosanitary, Food and Feed Law No. 5996. Republic of Türkiye Official Gazette, 13/06/2010, 27610.
[4] World Health Organization. (2006). Five Keys to Safer Food Manual. France.
[5] Erkmen, O. (2010). Gıda kaynaklı tehlikeler ve güvenli gıda üretimi. *Çocuk Sağlığı ve Hastalıkları Dergisi*, 53(3), 220-235.
[6] Putri, S. (2018). Challenge to enforce food safety law and regulation in Indonesia. *IOP Conference Series: Earth and Environmental Science*, 175, 1-6.
[7] Assan, N. (2019). The Challenges of Food Law Enforcement: Perceptions of Environmental Health Practitioners in the Northwest of England. Unpblished Doctorate Thesis, Salford University.

SİDAS MEDYA
Akademik Gıda® / Academic Food Journal
ISSN Online: 2148-015X
http://dergipark.gov.tr/akademik-gida

Akademik Gıda Dergisi Yazım Kuralları

Akademik Gıda dergisi gıda bilimi ve teknolojisi alanında hazırlanmış özgün araştırma ve derleme makalelerin yayınlandığı **hakemli** bir dergidir. Araştırma notu, mini derleme, görüş ve editöre mektup gibi yazılar da yayın için değerlendirilir. Dergi 3 ayda bir basılmakta olup 4 sayıda bir cilt tamamlanır. Dergide Türkçe ve İngilizce makaleler yayınlanır.

Akademik Gıda dergisinde yayınlanması istenen çalışmalar derginin www.academicfoodjournal.com web sayfasında bulunan elektronik makale gönderim sistemi üzerinden gönderilmelidir. E-posta ile gönderilen makaleler değerlendirilmeyecektir. Elektronik makale gönderim sistemi ile ilgili sorularınız için ogursoy@yahoo.com e-posta adresinden editörle irtibata geçebilirsiniz.

- Gönderilecek çalışmanın dergide hangi tür makale olarak (Araştırma Makalesi, Derleme Makale, Araştırma Notu, Mini Derleme, Görüş ve Editöre Mektup) yayınlanması istendiği yazar(lar) tarafından mutlaka belirtilmelidir.
- Yazar(lar) tarafından çalışmayı değerlendirilebileceği düşünülen ve yazar(lar)la çıkar çatışması/çakışması olmayan en az 3 potansiyel hakem iletişim bilgileri de (yazışma adresi, e-posta ve telefon numarası) verilerek önerilmelidir. Önerilecek hakemler yazarın kendi kurumu dışından olmalıdır.
- Gönderilecek çalışmalar yazım ve imla hataları içermemelidir. İngilizceden Türkçeye tercüme edilen teknik terimler "Gıda Mühendisliği Teknik Terimler Rehberi"nde [Gıda Mühendisleri Odası, Kitaplar Serisi No: 17, Filiz Matbaacılık, Ankara, 232s, ISBN: 978-9944-89-407-4] tavsiye edilen şekliyle kullanılmalıdır.
- Gönderilen çalışmaların daha önce hiç bir yerde yayınlanmadığı yazar(lar) tarafından garanti edilmelidir.
- Yayın Kurulu yayına kabul edilmiş çalışmalarda gerekli değişiklikleri yapmaya yetkilidir.

Makalelerin Değerlendirilmesi

Yayımlanmak üzere Akademik Gıda dergisine gönderilen çalışmalar öncelikle Editörlerin ön incelemesinden geçmektedir. İlk incelemeyi geçen çalışmalar, değerlendirilmek üzere en az iki bağımsız hakeme gönderilmektedir. Çalışmaların değerlendirilmesinde hakemlerin makale yazar(lar)ını, makale yazar(lar)ının hakemleri görmediği çift-kör (double-blind) değerlendirme sistemi kullanılmaktadır. Editörler (i) dergi kapsamı dışında olan, (ii) teknik açıdan yetersiz, (iii) kendi içerisinde bütünlük ve tutarlılık arz etmeyen sonuçlar içeren veya (iv) kötü yazılmış çalışmaları doğrudan reddetme hakkına sahiptir.

Yayın Ücreti

Sidas Medya Limited Şirketi'nin 5 Ocak 2023 tarihli kararı uyarınca, 15 Ocak 2023 tarihinden sonra Akademik Gıda dergisine gönderilen Türkçe makaleler için "kabul/red şartına bağlı olmaksızın" yazar/yazarlar tarafından katkı payı olarak 300 TL (KDV Dahil) ödenmesi uygun görülmüştür. İngilizce olarak dergiye gönderilen makalelerden herhangi bir ücret talep edilmemektedir.

Etik Beyanı

Dergi yayın politikası, makalelerin değerlendirilmesi ve etik hususlar ile ilgili detaylı bilgilere Etik Beyanı kısmından ulaşılabilir.

Çalışmaların Hazırlanması

1. Çalışmalar A4 boyutunda hazırlanmalı, üstten 2.45 cm, alttan 2.45 cm, sağ ve soldan 1.75 cm boşluk bırakılmalı ve tek kolon olarak hazırlanmalıdır. Metin çift satır aralıklı yazılmalı, paragraflar arasında tek satır boşluk bırakılmalıdır. Metinde bütün satırlar (sürekli) numaralandırılmalıdır.

2. Çalışma başlığı 14 punto Arial, koyu, küçük harflerle ve ortalanmış olarak yazılmalıdır. Başlıktan sonra bir satır boşluk bırakılmalı (11 punto); yazar isimleri (yalnızca ilk harfler büyük) 10 punto Arial ve ortalanmış olarak verilmelidir. Yazarların adresleri, telefon ve faks bilgileri ile yazışmalardan sorumlu yazarın e-posta adresi hemen alt satırda 9 punto Arial, ilk harfler büyük olacak şekilde ve ortalanmış olarak yazılmalıdır. Yazarların çalıştıkları kuruluşlar (ve/veya adresler) farklı ise her bir yazar isminin sonuna rakamlarla üst indis konulmalıdır.

3. Metin içindeki kısımların başlıkları (ÖZ, ABSTRACT, GİRİŞ vb.) 10 punto Arial ve koyu olarak büyük harflerle yazılmalı, başlıktan sonra bir satır boşluk bırakılarak metine geçilmelidir. Alt başlıklarda ilk harfler büyük, 10 punto Arial ve koyu yazı karakteri kullanılmalıdır. ÖZ'ün altına bir satır boşluk bırakıldıktan sonra en fazla 5 adet Anahtar Kelime konmalıdır. Anahtar Kelimelerden sonra bir satır boşluk bırakılarak İngilizce başlık ve altına ABSTRACT ve Keywords yazılmalıdır. Bir satır boşluk bırakılarak ana metine geçilmelidir.

4. Ana metin 9.5 punto Arial olarak hazırlanmalıdır.

5. Çalışma başlıca şu kısımlardan oluşmalıdır: Başlık, Yazar İsimleri, Adresleri, İletişim Bilgileri, Yazışmalardan Sorumlu Yazarın E-posta adresi, Öz, Abstract, Ana Metin (Giriş, Materyal ve Metot, Bulgular ve Tartışma, Sonuç), Teşekkür (gerekiyorsa), Kısaltmalar (gerekiyorsa), Kaynaklar.

6. Öz ve Abstract 250 kelimeyi geçmemeli, çalışmanın amacını, metodunu ve önemli sonuçlarını içermelidir. Öz tek paragraf olarak yazılmalı ve öz içinde kaynaklara atıf yapılmamalıdır.

7. Çalışma içerisinde geçen mikroorganizma isimleri ile Latince ifade ve isimler italik olarak yazılmalı ve kısaltmalarda uluslararası yazım kuralları göz önünde bulundurulmalıdır.

8. Tablo başlıkları tablonun üstüne, şekil başlıkları ise şeklin altına yazılmalı ve numaralandırılmalıdır. Kullanılan tablo ve şekillere metin içinde mutlaka atıf yapılmalıdır. Metin içinde geçen veriler tablo ve şekillerin tekrarı olmamalıdır. Tablo ve şekillerin başlıkları içerikleriyle uyumlu ve anlaşılabilir olmalıdır. Şekiller ve resimlerin yüksek çözünürlükte olmasına dikkat edilmelidir. Resimler (ve gerekiyorsa Şekiller) *.jpg formatında metin içerisinde yer almalıdır.

9. Metin içerisinde atıflar köşeli parantez içerisinde rakamlarla yapılmalı [1] ve Kaynaklar bölümünde bu numara sırasıyla detayları yazılmalıdır. Kaynakların numaralandırılması MS Word Numaralandırma Kitaplığı kullanılarak yapılmalıdır.

10. Kullanılan matematiksel denklemler numaralandırılmalı ve metin içerisinde bu denklemlere atıf yapılmalıdır.

11. Kaynaklar kısmı APA yazım stili kullanılarak hazırlanmalıdır. Kaynakların yazımında aşağıdaki örnek yazım biçimleri kullanılmalı ve makalelerin yayınlandığı dergi isimleri kısaltma kullanılmadan ve italik olarak yazılmalıdır. Web adreslerine atıf yapılacağında (mümkün olduğunca Resmi web sayfalarına atıf yapılmalıdır) mutlaka ilgili web adresine erişim tarihi verilmelidir.

Makale

[1] Bozkurt, H., İçier, F. (2009). İnegöl köfte üretiminde ohmik pişirmenin uygulanabilirliğinin incelenmesi. *Akademik Gıda,* 9(1), 6-12.

Kitap

[2] Kılıç, S. (2001). Süt Endüstrisinde Laktik Asit Bakterileri. Ege Üniversitesi Ziraat Fakültesi Yayınları, Ege Üniversitesi Matbaası, Bornova, İzmir.

Kitap Bölümü

[3] Gibson, G.R., Saavedra, J.M., MacFarlane, S., MacFarlane, G.T. (1997). Probiotics and Intestinal Infections. In Probiotics 2: Applications and Practical Aspects, Edited by R. Fuller, Chapman & Hall, 2-6 Boundary Row, London SE1 8HN, England, 212p.

Kongre-Sempozyum Bildirisi

[4] Gürsoy, O., Akdemir, O., Hepbaşlı, A., Kınık, Ö. (2004). Recent situation of energy consumption in Turkey dairy industry. *International Dairy Symposium: Recent Developments in Dairy Science and Technology*, May 24-28, 2004, Isparta, Turkey, Book of Proceedings, 10-16p.

12. Hakem görüşleri doğrultusunda düzeltilmek üzere yazar(lar)a gönderilen çalışmaların gerekli düzeltmeleri yapılarak yayın ofisine ulaştırılması gereklidir. Editörler tarafından belirtilen süre zarfında gönderilmeyen çalışmalar "ilk defa gönderilmiş çalışma" olarak değerlendirilecektir.

13. Yukarıdaki kurallara uygun olarak hazırlanmamış çalışmalar değerlendirmeye alınmaz.

SİDAS MEDYA
Akademik Gıda® / Academic Food Journal
ISSN Online: 2148-015X
http://dergipark.gov.tr/akademik-gida

Guidelines to Authors

Akademik Gıda® (Academic Food Journal) is a peer reviewed journal where original research and review articles are published in the field of food science and technology. Research notes, mini-reviews, opinions and letters to the editor are also considered for publication. The journal is published trimonthly and each volume is composed of 4 issues per year. Journal articles are published either in Turkish or English. Manuscripts in either good American or British English usage are accepted, but not a mixture of these.

Manuscripts for the Akademik Gıda® (Academic Food Journal) must be sent via the electronic article submission system, which can be located in the official website of the journal, www.academicfoodjournal.com. Manuscripts sent by e-mail are not considered for evaluation. For questions related to the electronic article submission system, contact the editor via e-mail at ogursoy@yahoo.com.

- Authors must specify the type of the manuscript (research articles, review articles, research briefs, mini-review articles, comments and letters to the editor).
- Authors should provide at least 3 potential referees and their contact information (mailing address, e-mail address and phone number).
- Manuscripts to be submitted should be free from any spelling or grammatical error.
- Authors must guarantee that the submitted manuscript is not published anywhere previously and will not be submitted to anywhere before the editorial board makes a final decision on the manuscript.
- The editorial board is authorized to make necessary changes in manuscripts accepted for publication.

Peer review policy

Manuscripts pass through initial screening in the editorial office followed by internal review by Editors. After the first evaluation, manuscripts are double-blind-reviewed by a peer review system involving at least two independent reviewers to ensure high quality of manuscripts accepted for publication. The Editors have the right to decline formal review of a manuscript if it is (i) on a topic outside the scope of the Journal, (ii) lacking technical merit, (iii) fragmentary and providing marginally incremental results or (iv) poorly written.

Publication fee

There is a 300 TL submission fee for Turkish manuscripts submitted after January 15, 2023. This fee may not be waived. No fee is charged for articles submitted to the journal in English.

Ethics Statement

Detailed information about journal publication policy, evaluation of manuscripts and ethical issues can be found in the Ethics Statement section.

Preparation of a manuscript

1. Manuscripts should be prepared in A4 size, and the text must be prepared in a single column format. The text must be double-spaced, and a single space should be left between paragraphs. All lines and pages must be continuously numbered.

2. The title must be 14pt Arial, bold, small letters and centered. A blank line should be left after the title, and the names of authors should be given in 10pt Arial and centered. In addition to each author's contact address, the phone and fax numbers and e-mail address of the corresponding author should be provided. If the institutions of the authors are different, superscript numbers should be used to indicate their addresses.

3. The headings (e.g. Abstract, Introduction, Materials and Methods etc.) must be 10pt Arial, and should be typed in bold capital letters. Each heading should appear on its own separate line. A blank line should be left after each heading. A list of keywords, a maximum of 5, should be provided below the abstract section of the manuscript.

4. The main text should be prepared in 9.5pt Arial.

5. Typical articles mainly consist of the following divisions: Title, Author Names, Addresses, Contact Information, Corresponding author's e-mail address, Abstract, Main text (Introduction, Materials and Methods, Results and Discussion, Conclusions), Acknowledgements (if necessary), Abbreviations (if necessary) and References.

6. The abstract should not exceed 250 words, and the main purpose and method and the most significant result and conclusion should be presented in the abstract. The abstract should be prepared as a single paragraph, and should not include any citation.

7. Latin names in the text should be in italics, and names and abbreviations should follow international rules. If abbreviations that are not standard are unavoidable, they must be defined at their first mention in the text. Consistency of abbreviations throughout the article must be ensured. Internationally accepted rules and conventions must be followed, and the international

system of units (SI) must be used. If other units are mentioned, their equivalents in SI must be provided.

8. Table headings should be on the top of each table and figure captions below each figure. Each table or figure must be numbered consecutively in accordance with their appearance in the text. All figures and tables should be cited in the text. The data presented in the tables and figures should not be repeated in the text. Table headings and figure captions should be self-explanatory. Figures and pictures must be provided in high resolution, and pictures (and, if necessary figures) should be included in the text as *. jpg format.

9. References in the text should be cited in numbers in square brackets [1] and details of the citations must be provided in the Literature or References section with their respective numbers.

10. Mathematical equations should be numbered and cited in the text.

11. References should be given according to the APA manual of style. The following formats should be used for the details of cited references, and the journal names must be typed in italics. References to the Web addresses (if necessary, the official web pages should be preferred) must include full web address and the date of access.

Article

[1] Güzeler, N., Kaçar, A., Say, D. (2011). Effect of milk powder, maltodextrin and polydextrose use on physical and sensory properties of low calorie ice cream during storage. *Akademik Gıda,* 9(2), 6-12.

Book

[2] Kilic, S. (2001). Lactic Acid Bacteria in Dairy Industry. Ege University Faculty of Agriculture Publications, Ege University Press, Bornova, Izmir, Turkey.

Book Chapter

[3] Gibson, G.R., Saavedra, J.M., MacFarlane, S., MacFarlane, G.T. (1997). Probiotics and Intestinal Infections. In Probiotics 2: Applications and Practical Aspects, Edited by R. Fuller, Chapman & Hall, 2-6 Boundary Row, London, England, 212p.

Proceedings of the Congress-Symposium

[4] Gursoy, O., Akdemir, O., Hepbasli, A., Kinik, O. (2004). Recent situation of energy consumption in dairy industry in Turkey. *International Dairy Symposium: Recent Developments in Dairy Science and Technology*, May 24-28, 2004, Isparta, Turkey, Book of Proceedings, 10-16p.

12. A list of the corrections requested by the referees must be provided by the authors, and it must be sent to the editorial office.

13. Studies that are not prepared in accordance with the rules above will not be considered for evaluation.

SİDAS MEDYA
Akademik Gıda®
ISSN Online: 2148-015X
https://dergipark.org.tr/tr/pub/akademik-gida

Etik Beyanı

Akademik GIDA®, gıda bilimi ve teknolojisi alanında orijinal araştırma ve derleme makalelerinin yayınlandığı hakemli bir dergidir. Dergi üç ayda bir Sidas Medya Ltd. Şti. (Çankaya, İzmir, Türkiye) tarafından yayınlanmaktadır. Derginin genel bilimsel kalitesini iyileştirmek için yayıncı tarafından aşağıdaki yönergeler belirlenmiştir.

Yayın Politikası

Akademik Gıda dergisine gönderilen tüm makaleler Dergi Editörleri için Davranış Kuralları ve En İyi Uygulama Kılavuzları ve Dergi Yayıncıları için Davranış Kurallarında (Code of Conduct and Best Practice Guidelines for Journal Editors and Code of Conduct for Journal Publishers) belirtilen Genel Kılavuzlara uygun olarak değerlendirilmektedir. Bilimsel yazılar dergiye gönderilmeden önce derginin Yazım Kurallarının okunmasını önemle tavsiye ederiz. Yazarlar aynı zamanda Avrupa Bilim Editörleri Birliği'nin (EASE) (European Association of Science Editors) İngilizce olarak basılacak makaleler için "Bilimsel Makalelerin Yazarları ve Çevirmenleri İçin Rehber"e uymalıdır. Yazarlar, insan veya hayvan verilerini içeren araştırmaları için Uluslararası Tıp Dergisi Editörleri Komitesinin (International Committee of Medical Journal Editors) önerilerini takip etmelidir.

Makalelerin Değerlendirilmesi

Dergiye gönderilen tüm makaleler, bilimsel içeriklerinin özgünlüğü ve kalitesi ölçütlerine göre değerlendirilir.

- Dergiye gönderilen tüm yazılar, ilk olarak yayın ofisindeki (teknik ve genel kalite değerlendirilmesi açısından) eleme işleminden geçer ve ardından teknik ve bilimsel editörler tarafından değerlendirilir.
- İlk değerlendirmeden sonra, editörler (i) dergi kapsamı dışında kalan bir konu hakkında hazırlanmış makaleleri (ii) teknik olarak eksik/yetersiz makaleleri, (iii) kısmi ve marjinal artan sonuçları içeren makaleleri veya (iv) kötü yazılmış makaleleri reddetme hakkına sahiptir.
- İlk inceleme sonucunda makalenin ileri değerlendirme için uygun olduğuna karar verilirse, dergide yayımlanmak üzere kaliteli makalelerin seçimini yapmak amacıyla, makaleler çift-körlü (hakemin ve yazar/yazarların birbirlerini görmedikleri) değerlendirme sistemi ile en az iki bağımsız hakemden oluşan bir değerlendirme sürecinde bilimsel incelemeye alınır.
- Hakemler tarafından talep edilirse, makalenin hakem görüşleri doğrultusunda yazarlar tarafından revize edilmiş versiyonu orijinal hakemler tarafından tekrar değerlendirilir. Değerlendirmelerin ardından editörler hakem önerileri doğrultusunda makale hakkındaki nihai kararlarını verirler. Gerekirse editörler, hakemlerin istedikleri tüm şartların yerine getirilmesi için yazarlardan ilave revizyon isteyebilir.
- Kabul edilen makalelerin son versiyonu, yayın öncesi taslağın (galley proof) hazırlanması için teknik editörlere gönderilir. Yazarlardan, makalelerinin dizgisi hazırlanmış taslaklarını son kontrol için yayın öncesinde incelemeleri istenir.
- Tüm makaleler, nihai formlarında DOI numarası almış ve çevrimiçi olarak pdf dosyaları halinde yayımlanır. İlgili veritabanlarında bu şekilde indekslenir.

Yayın Ücreti

Akademik Gıda dergisinde makalelerin yayınlanması için herhangi bir yayın ücreti talep edilmemektedir.

Gizlilik

Editörler, Akademik Gıda'ya gönderilen tüm makaleleri tam bir gizlilikle ele alır. Editörler, hakemler haricinde, COPE tavsiyelerine uyulmadığı takdirde, üçüncü şahıslara makale ile ilgili hiçbir bilgi vermezler. Yayınlanmak üzere dergiye gönderilen makaleler hakemler için de gizlidir ve bilimsel değerlendirme için aldıkları makalelerin herhangi bir bölümünü üçüncü şahıslarla paylaşmalarına veya dağıtmalarına izin verilmez. Suiistimal şüphesi olduğunda, hakemlerin derhal gizli bir şekilde yayın ofisine başvurmaları önerilir. Hakemler ayrıca, Dergi Editörleri İçin Davranış Kuralları ve En İyi Uygulama Kuralları ile Dergi Yayıncıları için Davranış Kuralları'nı (Code of Conduct and Best Practice Guidelines for Journal Editors and Code of Conduct for Journal Publishers) takip ederek editöre gizli yorumlarında belirli bir eylem önerebilirler.

Akademik Gıda, çift-kör bir hakem inceleme süreci yürütür, yani çalışmanın eleştirel değerlendirmesini sağlamak için hakemlerin isimleri gizlidir. Hakemlerden, raporlarında adlarını veya irtibat bilgilerini açıklamamaları istenir. Hakem raporları yazarlara gönderilemeden önce bu açıdan kontrol edilir.

Yazarlık

Bir yazar, bir araştırmanın fikrine veya tasarımına, verilerin elde edilmesine, verilerin analizine veya yorumlanmasına büyük ölçüde katkıda bulunan, makalenin hazırlanmasında, yazılmasında veya gözden geçirilmesinde entelektüel içeriğe eleştirel katkı yapan bireydir. Katkıda bulunanlar diğer kişiler makalenin Teşekkür bölümünde belirtilmelidir ve çalışmanın yazarı olarak kabul edilemez. Tüm yazarların doğru ve tam isimleri ile ORCID kimlikleri dergiye gönderilen

makalenin başlık sayfasında yer almalıdır. Yazarların isimlerinin yanında çalıştıkları kurumlar ve yazışmalardan sorumlu yazarın geçerli bir adresi verilmelidir. Yazışmalardan sorumlu yazarın telefon ve faks numaraları ile e-posta adresi makalenin ilk sayfasında belirtilmelidir. Tüm yazarlar, gönderilen makalenin daha önce herhangi bir yerde yayınlanmadığını ve makale hakkında Akademik Gıda dergisi nihai bir karar vermeden önce makaleyi başka bir dergiye göndermeyeceklerini garanti etmelidir.

Destekleyen/Finans Sağlayan Kuruluşlar

Araştırmanın tüm finans kaynaklarına ilişkin detaylar, Teşekkür bölümünde belirtilmelidir. Yazarlar, resmi finansman kurum/larının tam isimlerini ve proje/hibe numaralarını belirtmelidir.

Yazarlarda Değişiklik

Makalenin Akademik Gıda'ya sunulmasından sonra yazar isimlerinde değişiklik ancak revizyon sırasında gerekli olan ek çalışmalar durumunda olabilir. Makalenin yayına kabul edilmesinden sonra herhangi bir değişikliğe izin verilmez. Yazarlıktaki değişiklik, hakem görüşlerine verilen cevaplar sırasında yazışmalarda belirtilmeli ve tüm yazarlar tarafından kabul edilmelidir. Yazışmalardan sorumlu yazar, yazarların sırası da dahil olmak üzere makalenin revize edilmiş versiyonundaki değişikliklerden sorumludur.

Çalışma Verilerinde Düzeltme

Yayınlanan verilerin doğruluğundan tüm yazarlar sorumlu olmalıdır. Verilerin düzeltilmesi için, yazışmalardan sorumlu yazardan yayın öncesi taslağı (galley proof) incelemesi ve makalenin yayınlanmasından 4 gün önce dikkatlice düzeltmesi istenir.

Makalenin Geri Çekilmesi

Bir makalenin geri çekilmesi, gönderim veya yayın hatalarını düzeltmek için kullanılır. Yazarlar makaleyi geri çekebilir ve bu durumda Yayın Etiği Komitesi (COPE) Geri Çekme Kurallarına [(COPE) retraction guidelines] uymalıdır. Tekrarlanan veya benzerlik oranı yüksek bir yayın, verilerin hileli kullanımı, intihal veya etik dışı araştırma yapılması durumunda, makale editör tarafından geri çekilecek ve geri çekilen makale linklerine bağlantı korunacak ancak elektronik veri tabanına (makale sayfasına) bir geri çekme bildirimi eklenecektir.

Etik Hususlar

Çıkar çatışması:

- Yazar/lar başvuru sırasında herhangi bir çıkar çatışması varsa beyan etmelidir. Yazar/ların başvuru sırasında bilimsel değerlendirme için en az üç potansiyel hakem önermeleri istenir. Önerilen hakemler çalışma arkadaşları, ortak çalıştıkları kişiler veya çalıştıkları kurumların üyeleri olamazlar.
- Hakemler makaleyi değerlendirmelerini önleyen herhangi bir çıkar çatışması olması durumunda Editörleri bilgilendirmesi ve bu konuda COPE kurallarına uyması tavsiye edilmektedir.
- Editörler Kurulu üyeleri veya kurul üyelerinin ortak çalıştıkları kişiler tarafından dergiye gönderilen makaleler için, değerlendirme sırasındaki önyargıları en aza indirgemek amacıyla, değerlendirme süreci ilgili kurul üyelerini dışarıda tutacak şekilde değiştirilerek uygulanır.
- Düzeltmeler (revizyonlar) sırasında, editörler Dergi Editörleri İçin Davranış Kuralları ile En İyi Uygulama Kılavuzu ve Dergi Yayıncıları İçin Davranış Kurallarını (Code of Conduct and Best Practice Guidelines for Journal Editors and Code of Conduct for Journal Publishers) takip ederler.

İnsan denekleri, hayvan veya bitki içeren araştırmalar

- Araştırmanın insan denekleri veya hayvanları içermesi durumunda, yazarların Uluslararası Tıp Dergisi Editörleri Komitesinin (the International Committee of Medical Journal Editors) yönergelerini izlemeleri önerilir.
- İnsan denekleri içeren çalışmalarda, deneklerin çalışmaya katılmak için imzaladıkları onamlar yazarlar tarafından sağlanmalıdır. 18 yaşın altındaki deneklerin çalışmaya katılmaları için ebeveyn veya velileri tarafından izin verilmelidir.
- Test edilen tüm denekler için, makalenin, ilgili kurallara ve/veya uygun izinlere veya lisanslara uyumunu gösteren belgelerin sunulması gerekir.
- Hayvanlar üzerinde yapılacak her türlü araştırma kurumsal, ulusal veya uluslararası kurallara uygun olmalı ve etik kurul tarafından onaylanmalıdır.
- Bitki materyallerinin toplanması dahil, bitkiler üzerinde yapılan deneysel araştırmalar, kurumsal, ulusal veya uluslararası kurallara uygun olmalıdır.
- Saha çalışmaları yerel mevzuata uygun olarak yapılmalı ve uygun izinleri ve/veya lisansları belirten bir açıklama makalede yer almalıdır.

Yayın suistimali

- Akademik Gıda dergisi, Dergi Editörleri İçin Davranış Kuralları ile En İyi Uygulama Kılavuzları ve Dergi Yayıncıları İçin Davranış Kurallarını (Code of Conduct and Best Practice Guidelines for Journal Editors and Code of Conduct for Journal Publishers) takip eder.
- Makalenin aynı anda birden fazla dergiye gönderilmesi, intihal, yayınlanmış makalenin yeniden yayınlanması, etik kuralların ihlali vb. şüpheli bir suiistimal durumunda, araştırmacılar, hakemler veya okuyucular Yayın Ofisi (ogursoy@yahoo.com) ile iletişime geçmeye teşvik edilir.
- Makaledeki benzerlik oranı tek bir kaynaktan %10'dan fazla olmamak üzere en fazla %25 ile sınırlandırılmıştır. Bu koşula uymayan makaleler reddedilir. Bu şartların ihlal edilmesi durumunda, COPE (COPE recommendations) tavsiyeleri izlenecek ve ilgili tüm taraflara bildirilecektir.

SİDAS MEDYA
Akademik Gıda®
ISSN Online: 2148-015X
https://dergipark.org.tr/tr/pub/akademik-gida

Ethics and Publication Malpractice Statement

Akademik GIDA® is a peer-reviewed journal where original research and review articles are published quarterly by Sidas Media Agency Advertisement Consultation Ltd. (Cankaya, Izmir, Turkey) in the field of food science and technology. In order to improve the overall scientific quality of the journal, following guidelines have been established by the publisher.

Editorial Policy

General Guidelines stated in the Code of Conduct and Best Practice Guidelines for Journal Editors and Code of Conduct for Journal Publishers are followed by all papers submitted to Academic GIDA. Prior to submission, authors are highly recommended to read the Journal's Instructions to Authors. Authors should also follow the European Association of Science Editors (EASE) Guidelines for Authors and Translators of Scientific Articles to be Published in English. For any research involving human or animal data, the recommendations of the International Committee of Medical Journal Editors should be followed by the authors of the manuscripts.

Peer Review

All contributions are evaluated according to the criteria of originality and quality of their scientific content.

- All manuscripts pass through an initial screening process (technical and overall quality evaluation) in the editorial office followed by an internal review by the technical and scientific editors.
- After the first evaluation, editors have the right to decline formal review of a manuscript if it is (i) on a topic outside the scope of the Journal, (ii) lacking technical merit, (iii) fragmentary and providing marginally incremental results or (iv) poorly written.
- If the manuscript is considered suitable for further evaluation, manuscripts are double-blind-reviewed by a peer review system involving at least two independent reviewers to ensure high quality of manuscripts accepted for publication.
- If requested, the revised version is evaluated by the reviewers, and editors make a decision about final acceptance based on their suggestions. If necessary, further revision can be asked for to fulfil all the requirements of the reviewers.
- The final version is then sent to the technical editor in order to produce a galley proof, and the authors receive this proof for final check before publishing.
- All manuscripts are posted online as pdf files in their final form, indexed in databases with the assigned DOI numbers.

Publication Fee

Akademik GIDA welcomes article submissions and does not charge any publication fee.

Confidentiality

Editors handle all papers submitted to Akademik GIDA in strict confidence. With the exception of reviewers, they do not disclose any information regarding submissions to third parties, unless in case of a suspected misconduct, where COPE recommendations are followed. Submissions are also confidential for reviewers and they are not allowed to share or distribute any part of the manuscripts which they receive for evaluation to third parties. For a case of suspected misconduct, reviewers are encouraged to contact the editorial office immediately in a confidential manner. Reviewers can also recommend a particular course of action in their confidential comments to the editor, following Code of Conduct and Best Practice Guidelines for Journal Editors and Code of Conduct for Journal Publishers.

Akademik GIDA conducts a double-blind peer review process, i.e. the names of the reviewers are confidential to ensure the critical evaluation of the work. Reviewers are asked not to disclose their names or contact details in their comments for authors.

Authorship

An author is an individual who substantially contributed to the idea or design of a research, acquisition of data, analysis or interpretation of data, was involved in drafting, writing or revising the manuscript critically for important intellectual content. Other contributors should be mentioned in the Acknowledgements section of the manuscript and cannot be considered as authors of the study. Correct and full names of all authors and their ORCID IDs should be on the title page of the manuscript. Names of authors must be supplemented with their affiliations and a valid address of the corresponding author. The phone and fax numbers and e-mail address of the corresponding author should be stated in the first page of the manuscript. All authors must guarantee that the submitted manuscript is not published anywhere previously and will not be submitted to anywhere before the editorial board makes a final decision on the manuscript.

Funding Sources

Details for all funding sources of the research should be stated in the Acknowledgements. Authors should provide

the full official funding agency name(s) and grant number(s).

Alteration in Authorship

Alteration in authorship after the submission of the manuscript to Akademik GIDA can be justified only by the additional work required during the revision. Any change is not allowed after the acceptance of the manuscript for publication. Alteration in authorship should be indicated in the responses to reviewers, and should be accepted by all authors. The corresponding author is primarily responsible for any alteration in the revised version of the manuscript, including the order of authors.

Correction of Data

All authors should be responsible for the accuracy of the published data. For the correction of data, the corresponding author receives the galley proof of the paper and is asked to correct it carefully within 4 days before publication.

Retraction of an Article

A retraction of an article is used to correct errors in submission or publication. Authors can retract the paper and should follow the Committee on Publication Ethics (COPE) retraction guidelines. In case of a duplicate or overlapping publication, fraudulent use of data, plagiarism or unethical research, the paper will be retracted by the editor, and a retraction notice will be included into the electronic database while all links to the retracted article will be maintained.

Ethical Considerations

Conflict of interest:

- Authors should declare any conflict of interest in their submission form. Authors are requested to suggest at least three potential reviewers before submission, and these reviewers cannot be their colleagues, collaborators or members of their institutions.
- Reviewers should notify the editors on any conflict of interest which prevents them from reviewing the paper, and they are recommended to follow the COPE guidelines.
- For the manuscripts submitted by the members of the Editorial Board or their collaborators, peer reviewing is modified to exclude them from the entire evaluation process in order to minimize any bias during the evaluation.
- During revision, the editors follow the Code of Conduct and Best Practice Guidelines for Journal Editors and Code of Conduct for Journal Publishers.

Research involving human subjects, animals or plants:

- If the research involves humans or animals, the authors are recommended to follow the guidelines of the International Committee of Medical Journal Editors.
- In studies involving human subjects, their informed consent to participate in the study should be supplied by the authors. For subjects under the age of 18, their parents or guardians should give the permission for their participation in the study. For all tested subjects, the manuscript must accompany with a statement detailing compliance with relevant guidelines and/or appropriate permissions or licenses.
- Any research on animals must comply with institutional, national or international guidelines and, where possible, should be approved by an ethics committee.
- Any experimental research on plants, including collection of plant materials, must comply with institutional, national, or international guidelines.
- Field studies should be conducted in compliance with local legislation, and a statement specifying the appropriate permissions and/or licences should be included in the manuscript.

Publication misconduct:

- The Journal follows the Code of Conduct and Best Practice Guidelines for Journal Editors and Code of Conduct for Journal Publishers.
- In a case of a suspected misconduct such as redundant or duplicate submission, plagiarism, text recycling, violation of ethical norms, etc., researchers, reviewers or readers are encouraged to contact the Editorial Office (ogursoy@yahoo.com).
- The overlapping in the manuscript is highly restricted to the maximum of 25% with no more than 10% from a single source; otherwise, the manuscript will be rejected. If these terms are violated, COPE recommendations will be followed and all parties involved will be notified.

Copyright

numbers, and the link to the published version on the Journal's website should be provided. Copyright notices and disclaimers must not be deleted.

Fevzipaşa Blv. Çelik İş Merkezi No:162 K:3 D:302 Çankaya / İZMİR
Tel: +90 232 441 60 01 Fax: +90 232 441 61 06 E-mail: sidasmedya@gmail.com

SİDAS MEDYA