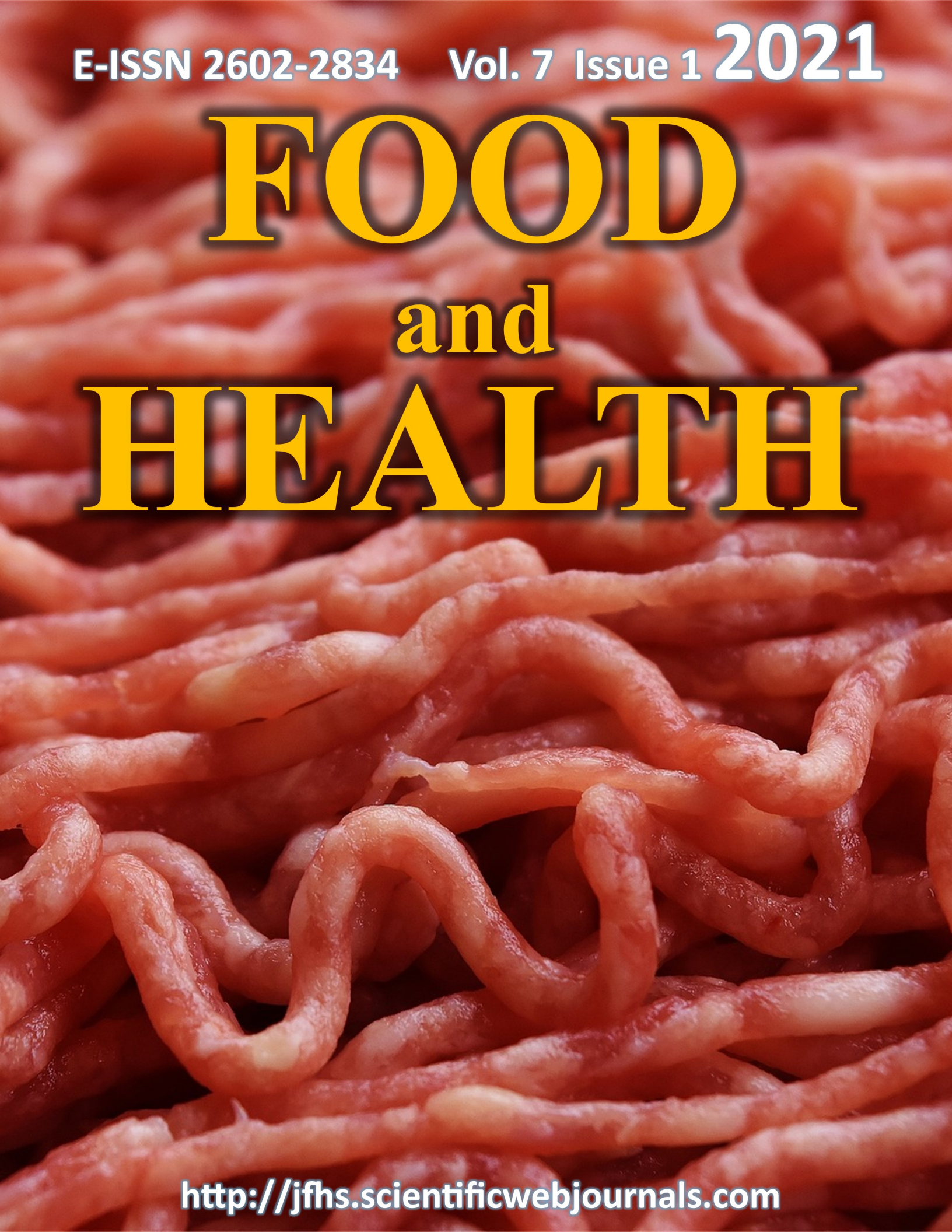
E-ISSN 2602-2834 Vol. 7 Issue 1 2021

FOOD and HEALTH



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FOOD and HEALTH

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E-ISSN 2602-2834

Publisher Özkan Özden

Adress: Abdi Bey Sok. KentPlus Sitesi No:24B D. 435 Kadıköy/İstanbul, Türkiye
E-mail: swj@scientificwebjournals.com
for submission instructions, subscription and all other information visit
http://jfhs.scientificwebjournals.com

FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Aims and Scope

FOOD and HEALTH

Abbreviation: **FOOD HEALTH**

e-ISSN: 2602-2834

Journal published in one volume of four issues per year by

http://jfhs.scientificwebjournals.com web page

"Food and Health" journal will publish peer-reviewed (double blind) articles covering all aspects of **food science and their health effect** in the form of original research articles (full papers and short communications), and review articles. Their team of experts provides editorial excellence, fast publication processes and high visibility for your paper.

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Manuscripts submitted to "**Food and Health**" journal will go through a double-blind peer-review process. Each submission will be reviewed by at least two external, independent peer reviewers who are experts in their fields in order to ensure an unbiased evaluation process. The editorial board will invite an external and independent editor to manage the evaluation processes of manuscripts submitted by editors or by the editorial board members of the journal. Our journal will be published quarterly in English or Turkish language.

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The editorial and publication processes of the journal are shaped in accordance with the guidelines of the International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal is in conformity with the Principles of

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Vol. 7 Issue 1 Page 1-74 (2021)

Content/İçerik

FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 7(1), 1-14 (2021) • https://doi.org/10.3153/FH21001 Research Article

Hastane çalışanlarının genetiği değiştirilmiş organizma (GDO), farkındalık düzeylerinin belirlenmesi: Sinop örneği

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Cite this article as:
Söyler, N., İpar, M.S., Kocatepe, D. (2021). Hastane çalışanlarının genetiği değiştirilmiş organizma (GDO), farkındalık düzeylerinin belirlenmesi: Sinop örneği. *Food and Health,* 7(1), 1-14. https://doi.org/10.3153/FH21001

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Submitted: 29.04.2020
Revision requested: 14.06.2020
Last revision received: 22.06.2020
Accepted: 13.07.2020
Published online: 13.10.2020

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Available online at
http://jfhs.scientificwebjournals.com

ÖZ

Genetiği değiştirilmiş organizmalar (GDO) veya diğer adı ile transgenik ürünler, organizmanın gen dizilimi-nin değiştirilmesi ya da organizmaya yeni bir gen aktarımı ve kendisinde bulunmayan bir özellik kazandırıl-masıyla oluşan ürünlerdir. Bu ürünlerin aşı ve ilaç üretiminde, organ naklinde ve hastalıkların tedavisinde kullanılması, bitkilerin zararlılara dayanıklılığının sağlanması, uzun raf ömrü ve gıda kalitesinin artırılması olumlu; alerjik reaksiyonlar, toksik etkiler, ekolojik çeşitliliğe zarar vermesi ise olumsuz etkilerindendir. Ger-çekleştirilen araştırmada Personelin, GDO'lar ve insan sağlığı üzerindeki etkileri hakkındaki farkındalık dü-zeylerinin ölçülmesi amaçlanmıştır. Bu kapsamda araştırmanın evrenini Sinop ilinde bulunan tüm kamu has-taneleri oluşturmakla birlikte örneklemini ise Sinop Atatürk ve Gerze Devlet Hastanesinde çalışan personel oluşturmaktadır. Araştırmanın verileri oluşturulan anket formu aracılığıyla ve yüzyüze görüşme tekniği ve tesadüfi örneklem metodu ile elde edilmiştir. Bu kapsamda toplam 312 hastane çalışanından elde edilen ve-riler, SPSS 21 paket programı ile analiz edilmiştir. Analiz sonucu araştırmaya katılanların GDO'lu gıdalara yönelik toplumsal farkındalık düzeyinin ve GDO'ların gıdalardaki varlığı ile zararlarına ilişkin farkındalık-larının yüksek olduğu belirlenmiştir. Ek olarak katılımcıların araştırma boyutlarını algılama düzeylerine göre cinsiyet ve medeni durumları bakımından farklılıklar olduğu belirlenmiştir.

Anahtar Kelimeler: Genetiği Değiştirilmiş Organizmalar, Genetiği Değiştirilmiş Gıdalar, İnsan sağlığı, Sağlık çalışanları

ABSTRACT

Determination of the genetically modified organism (GMO) awareness levels of hospital workers: example of Sinop

Genetically modified organisms (GMO), or transgenic products, are products formed by changing the gene sequence of the organism, or by transferring a new gene to the organism and giving it a feature that it does not have. The use of these products in the production of vaccines and drugs, in organ transplants and in the treatment of diseases, ensuring the resistance of plants to pests, long shelf life and increasing food quality are positive; allergic reactions, toxic effects, and damage to ecological diversity are negative effects. In the rese-arch, it was aimed to measure the awareness levels of the personnel about GMOs and their effects on human health. In this context, the universe of the research is composed of all public hospitals in Sinop province, and the sample is composed of personnel working in Sinop Atatürk and Gerze State Hospital. The data of the research were obtained through the questionnaire form created and by the face-to-face interview technique and random sampling method. In this context, data obtained from a total of 312 hospital employees were analyzed with the SPSS 21 package program. As a result of the analysis, it has been determined that the level of social awareness of the participants in GMO foods and their awareness about the presence and harm of GMOs in foods is high. In addition, it was determined that there were differences in terms of gender and marital status of participants according to their perception level of research dimensions.

Keywords: Genetically Modified Organisms, Genetically Modified Foods, Human health, Health workers

Giriş

Dünya genelinde bebeklerde ölüm oranlarının azalması, ortalama yaşam süresinin uzaması, geri kalmış ülkelerde aile planlaması kapsamındaki politikaların çeşitli nedenlerle uygulanamaması, insan sağlığını koruyucu önlemlerin alınmasına yönelik çalışmaların ileri seviyeye ulaşması gibi etkiler nedeniyle dünya nüfusu sürekli artmaktadır. 1960'lı yılların başında üç milyar kişi olan dünya nüfusu, 2020 yılında sekiz milyara ulaşmış; 2050 yılında ise bu sayının on milyara yaklaşacağı tahmin edilmektedir. Dünya üzerinde artan nüfus ile birlikte pek çok problemin yanı sıra insanların beslenme ihtiyaçlarının karşılanması da çözülmesi gereken önemli bir konu haline gelmiştir (Erbaş, 2008). Özellikle 20. yüzyılın son çeyreğinde gıda üretim endüstrisinde yer alan firmalar, artan talebi karşılamak amacıyla çeşitli arayışlar içerisine girmişler; genetiği değiştirilmiş organizmalardan gıda kaynağı olarak faydalanmaya başlamışlardır (Kıran ve Osmanağaoğlu, 2011; Özmert ve Yaman, 2011). Doğal besinlerin, sağlıklı gıdaların ve doğaya dost ürünlerin yerini, giderek çeşitli kimyasal ve biyolojik müdahaleler ile farklılaştırılmış, doymuş ve trans yağ içeriği yüksek, rafine edilmiş katkılı gıdalar ile kimyasal takviyelerle üretilen sebze, meyve ve hatta et ürünleri almaya başlamıştır. Gıda katkı maddelerinin kullanımı ile genetiği değiştirilmiş organizmalar içeren ürünlerin ortaya çıkması ile gıda üretim sektörü farklı bir ivme kazanmış (Cebirbay ve Aktaş, 2018), dünya genelinde bu tarz uygulamaların olumlu ve olumsuz özellikleri tartışılmaya açılmıştır.

Diğer taraftan büyükşehirlerdeki verimli tarım arazilerinin çeşitli nedenlerle (fazla göç, konut ve yol yapımı, kamu binaları vb.) giderek azalması da sağlıklı ve organik tarım yapılmasını engellemeye başlamıştır. Gıda israfının üst seviyelere ulaşmasının yanı sıra tüketim toplumunun gıda ihtiyaçlarına yetişebilmek için kimyasal ilaçlarla daha çok ürün veren sebze ve meyveler yetiştirilmesi, et ihtiyacını karşılayabilmek için çeşitli bileşenlerden oluşan yemlerle hayvanların beslenerek kısa sürede daha çabuk büyümesinin sağlanması gibi çeşitli uygulamalar da bir çözüm olarak görülmeye başlanmıştır.

Esasında ilk başlarda "yeşil devrim" olarak nitelendirilen çalışmalarla hem toprağın hem de tarım ürünlerinin kalitesi ve verimini arttırmaya yönelik uygulamalarda bulunulmuş, sebze ve meyvelerde fireye neden olan tarım zararlılarının etkinlikleri azaltılmaya çalışılmıştır. Daha sonra bu durum 1985-2005 yılları arasında Biyoteknoloji Devrimi olarak adlandırılan çalışmalarla giderilmeye çalışılmıştır (Yılmaz, 2014). Biyoteknoloji ilk kullanılmaya başlandığında doku kültürü, laboratuvar koşullarında seleksiyon, meristem kültürü, hücre kültürü gibi canlı organizmaların gen yapısına doğrudan müdahale etmeyen teknikler içermekteydi. Günümüzde ise gen teknolojisi ve gen transferi gibi, türlere kendi potansiyelleri dışında bazı özellikler kazandırabilen teknikleri içermektedir. Klasik biyoteknoloji, yabani türlerin evcilleştirilmesi ve ıslah edilmesi ile günümüzdeki birçok türün yetiştirilmesine olanak sağlarken; günümüz biyoteknolojisinde ise, genetiği değiştirilmiş organizmaları yetiştirmeye olanak sağlamıştır (Demir ve Pala, 2007).

Biyoteknoloji yöntemleri ile gen veya genlerin bir organizmadan başka bir organizmaya aktarılmasına gen transferi ve bu organizmalara da "genetiği değiştirilmiş organizmalar (GDO)" denilmektedir (Olhan, 2010; Kışoğlu ve Keleş, 2018). GDO, uluslararası literatürde kısaltılmış şekliyle "GM" veya "GMO" olarak geçen "Genetically Modified Organisms"in Türkçe karşılığıdır. Genleri değiştirilmiş olup hali hazırda yetiştirilen birinci nesil bitkiler çoğunlukla herbisitlere (tarımda yabani otlarla mücadele amacıyla kullanılan kimyasal ilaçlar) dirençlilik ve böcek, hastalık ve çevresel stres koşullarına dayanıklılık gibi özelliklerin kazandırıldığı bitki türleridir. Verim ve beslenme kalitesinin artırılmasının hedeflendiği ikinci nesil bitki türleri ile insan tedavisinde kullanılan çok değerli aşı ve ilaçların üretilmesi hedeflenirken; biyoyakıt potansiyeli taşıyan üçüncü nesil genetiği değiştirilmiş bitkiler üzerinde araştırma ve geliştirme çalışmalarıysa devam etmektedir (Koçer, 2009). GDO teknolojisi uygulamaları her ne kadar küresel bir strateji olmaktan çok tarım sektöründe maliyetleri düşürüp verimi artırarak kâr oranını yükseltmek amacıyla başlatılmış olsa da, bu uygulamaların uzun vadede küresel besin sorununa da çözüm getirebileceği düşünülmektedir. Bu nedenle artan dünya nüfusunun ve toplumların beslenme ihtiyaçlarının karşılanmasında pek çok gıda ve gıda üretiminde kullanılan hammaddelerin içeriğinde GDO'lu ürünler yer almaya başlamıştır. Tüm bu hususlar günümüzde başta kanser olmak üzere, immün sistem hastalıkları, kalp ve damar hastalıkları, allerjenite, antibiyotiklere karşı direnç (Atsan ve Kaya, 2008: 5), hormon hastalıkları ve obezite vb. çeşitli rahatsızlıkların da GDO'lu ürünlerin tüketiminden kaynaklandığına yönelik toplum bireylerinin tepkisini çekmeye başlamış, bu kapsamda yapılan araştırmalarda GDO'lu ürünlerin çeşitli hastalıklara neden olduğu kanıtları da tüketicilerde farkındalık oluşturmuştur. Bu kapsamda tüketicilerin GDO'lu ürünlere yaklaşımını belirlemek üzere çalışmalar literatürde yerini almıştır (Radas vd., 2008; Bakshi, 2011; Temelli ve Kurt, 2011; Özden vd., 2013; Kaya ve Akar, 2016; Cui ve Shoemaker 2017; Demiral ve Türkmenoğlu, 2018; Lefebvre vd., 2019; Sun vd., 2019; Güneş ve Yılmaz, 2019; Brosig ve Bavorova, 2019; Saka ve Sarıbaş, 2019).

Bu araştırmada ise, Sinop ilindeki sağlık çalışanlarının GDO'lu gıdalar ve insan sağlığı üzerindeki etkileri hakkındaki farkındalık düzeylerinin ölçülmesi amaçlanmıştır. Sağlık sektörünün içerisinde yer alan ancak diğer taraftan birer tüketici olan sağlık çalışanlarının GDO'lu gıdalara karşı farkındalıklarının belirlenmesi önem arz etmektedir. Ayrıca literatüre de katkı sağlayacağı düşünülen bu araştırma ile sağlık sektöründe çalışan personelin genetiği değiştirilmiş organizmalar ve insan sağlığı üzerindeki etkileri hakkındaki farkındalık düzeylerinin belirlenmesi, ülkemizde bu konuda yapılmış olan çalışmalara yeni bir boyut kazandırması yönüyle de önemlidir.

Materyal ve Metot

Araştırmanın Yöntemi ve Örneklemi

Nicel araştırma yöntemi özelliği taşıyan bu araştırmanın verileri, oluşturulan anket formu aracılığıyla ve yüzyüze görüşme tekniği ile tesadüfi örneklem metodu kullanılarak elde edilmiştir. Ankette yer alan ifadelerin anlaşılabilirliğinin belirlenmesi ve güvenirliğinin test edilmesi amacıyla 60 sağlık çalışanı ile ön test yapılmış, geri dönüşler ışığında gerekli düzeltmeler yapıldıktan sonra alan araştırmasına geçilmiştir. Araştırmanın evrenini Sinop ilinde bulunan tüm kamu hastaneleri oluşturmakla birlikte örneklemini ise Sinop Atatürk Devlet Hastanesi ve Gerze Devlet Hastanesinde çalışan sağlık personeli oluşturmaktadır. Bu kapsamda ilgili hastanelerde görev yapan toplam 312 sağlık çalışanından elde edilen veriler, SPSS 21 paket programı ile analiz edilmiştir.

Araştırmanın Etik Boyutu

Bu çalışma; Sinop Üniversitesi İnsan Araştırmaları Etik Kurulu tarafından 13.12.2018 tarih ve 2018/46 karar numarası ile onaylanmıştır. Ayrıca araştırmanın gerçekleştirildiği hastaneler için Sinop İl Sağlık Müdürlüğü'nün 30.05.2019 tarih ve 2078520-605.99 sayılı onayı ile kurumlarda anket uygulaması gerçekleştirilmiştir. Çalışmaya katılmada gönüllülük esasına uyulmuştur.

Araştırmanın Veri Toplama Aracı

Araştırma kapsamında anket sorularının hazırlanması için literatür taraması yapılmış, bu konuda yapılan araştırmalar ve ölçme araçları incelenerek uzman görüşü eşliğinde anket formu oluşturulmuştur. Anket formunun ilk bölümünde kişisel bilgilerin yer aldığı 5 adet demografik soru, ikinci bölümünde ise GDO'lu gıdalar hakkındaki farkındalığın ölçülmesi ile ilgili 17 adet önerme bulunmaktadır. Katılımcıların bu önermelere 5'li Likert ölçeği türünde "1:Kesinlikle Katılmıyorum, 2: Katılmıyorum, 3: Kararsızım, 4:Katılıyorum ve 5 Kesinlikle Katılıyorum" arasında cevap vermeleri istenmiştir.

Araştırma Verilerinin Değerlendirilmesi

Anket formları aracılığı ile elde edilen veriler SPSS 21 istatistiki programı aracılığı ile analiz edilmiştir. Verilerin yorumlanabilmesi için aritmetik ortalama, standart sapma gibi betimleyici istatistik yöntemlerinin yanı sıra faktör analizi ve boyutların katılımcılar tarafından algılanabilirlik düzeyini ölçmek için ise "bağımsız örneklem *t*-test" ve "Tek yönlü varyans analizi (ANOVA) testi" gerçekleştirilmiştir. Bu kapsamda veri setinin faktör analizine uygunluğunun değerlendirilmesi için Kaiser-Meyer-Olkin (KMO) örneklem yeterliliği ve Bartlett küresellik testi sonuçları incelenmiştir. Veri setinin faktör analizine uygun olduğunun kabul edilebilmesi için KMO değerinin 0,50'nin üzerinde bir değer alması, Bartlett Küresellik test sonucunun ise istatistiksel olarak anlamlı ($p<0,05$) olması gerekmektedir. Veri setinin KMO değeri 0,779, Bartlett Küresellik Değeri: $x2=1854,55$ $p=0.000<0,001$ bulunduğundan faktör analizine uygun olduğu görülmüştür. Yapılan faktör analizleri sonucunda anket soruları dört boyut altında toplanmıştır. Bu boyutlar; "GDO'lara Yönelik Toplumsal Bilinç Düzeyi (TBD)", "GDO'lara Yönelik Resmi Bilgilendirme, Denetim ve Farkındalık (RBDF)", "GDO'ların Gıda Sektöründeki Kullanım Zorunluluğu (GSKZ)" ve "GDO'ların Gıdalardaki Varlığına ve Zararına İlişkin Farkındalık (GVZİF)" şeklinde isimlendirilmiştir.

Bulgular ve Tartışma

Araştırmanın örneklemi Sinop Atatürk Devlet Hastanesi ve Gerze Devlet Hastanesi çalışanları olarak belirlenmiştir. Çalışmaya katılan sağlık çalışanlarının cinsiyet, yaş, meslek, medeni durum ve çocuk sahibi olma değişkenlerine yönelik sayı (s) ve yüzde (%) tablosu Tablo 1'de verilmiştir.

Katılımcıların anket formunda bulunan ve dört boyut altında toplanan her bir önermeye yönelik olarak "1:Kesinlikle Katılmıyorum, 2: Katılmıyorum, 3: Kararsızım, 4:Katılıyorum ve 5 Kesinlikle Katılıyorum" ifadeleri arasındaki değerlendirmelerine yönelik bulgular aşağıda özetlenmiştir.

GDO'lara Yönelik Toplumsal Bilinç Düzeyi Boyutuna İlişkin Değerlendirmeler

Çocukların okullarda GDO ile ilgili bilgilendirilmesi gerekliliği

Çocukların okullarda GDO ile ilgili bilgilendirilmesi gerektiğini düşünüyorum ifadesine katılımcıların %5.1'i (n=16) kesinlikle katılmıyorum, %2.6'sı (n=8) katılmıyorum, %4.2'si (n=13) kararsızım, %26.9'u (n=84) katılıyorum ve %61.2'si (n=191) tamamen katılıyorum cevabını vermişlerdir. Katılıyorum ve tamamen katılıyorum cevabını veren 275 (84+191)

kişi toplam katılımcıların %88.1'ini oluşturmaktadır. Bu verilere göre katılımcıların %88.1'i çocukların okullarda GDO ile ilgili bilgilendirilmelerini istedikleri görülmüştür.

İnsanların GDO ile ilgili bilgilenmelerini sağlayacak kamu spotu vb. olması gerekliliği

İnsanların GDO ile ilgili bilgilenmelerini sağlayacak kamu spotu vb. olması gerektiğini düşünüyorum ifadesine katılımcıların %5.4'ü (n=17) kesinlikle katılmıyorum, %3.2'si (n=10) katılmıyorum %5.4'ü (n=17) kararsızım, %28.2'si (n=88) katılıyorum ve %57.7'si (n=180) tamamen katılıyorum cevabını vermişlerdir. Katılıyorum ve tamamen katılıyorum cevabını veren 268 (88+180) kişi toplam katılımcıların %85.9'unu oluşturmaktadır. Bu verilere göre katılımcıların %85.9'u GDO ile ilgili bilgilendirilmek istedikleri görülmüştür.

GDO'lu gıdaları farkında olmadan tüketme endişesi

GDO'lu gıdaları farkında olmadan tüketmekten endişeleniyorum ifadesine ankete katılan toplam 312 kişinin %6.4'ü (n=20) kesinlikle katılmıyorum, %5.8'i (n=18) katılmıyorum %8.7'si (n=27) kararsızım, %31.7'si (n=99) katılıyorum ve %47.4'ü (n=148) tamamen katılıyorum cevabını vermişlerdir. Tamamen katılıyorum ve katılıyorum cevabını veren 247 (148+99) kişi toplam katılımcıların %78.1'ini oluştururken, bu konuda endişe taşımayan kişi sayısı 38 (18+20) dir. Elde edilen sonuçlara göre katılımcıların büyük çoğunluğunun bu konuda endişeli olduğu anlaşılmaktadır.

GDO'lu tohumların ülke tarımına zarar durumu

GDO'lu tohumların ülke tarımına zararı olduğunu düşünüyorum ifadesine katılımcıların %6,1'i (n=19) kesinlikle katılmıyorum, %9.6'sı (n=30) katılmıyorum, %14.4'ü (n=45) kararsızım, %26.3'ü (n=82) katılıyorum ve %43.6'sı (n=136) tamamen katılıyorum cevabını vermişlerdir. Katılıyorum ve tamamen katılıyorum cevabını veren 218 (82+136) kişi toplam katılımcıların %69.9'unu oluşturmaktadır. Bu verilere göre katılımcıların %69.9'u, GDO'lu tohumların ülke tarımına zararı olduğunu düşünmektedirler.

Satın alınan ürünlerde GDO işareti varlığı

Satın alacağım ürünlerde GDO'lu olduklarını belirten işaretler olsa satın almam ifadesine ankete katılanların %9.6'sı kesinlikle katılmıyorum, %6.1'i (n=19) katılmıyorum %14.1'i (n=44) kararsızım, %23.1'i (n=72) katılıyorum ve %47.1'i (n=147) tamamen katılıyorum cevabını vermişlerdir. Tamamen katılıyorum ve katılıyorum cevabını veren 246 (152+94) kişi toplam katılımcıların %78.8'ini oluştururken, üründe GDO'lu olduğunu bildiren işaret olması durumunda satın alabileceğini belirten 49 (19+30) kişi olmuştur, bu 49 kişi toplam katılımcıların %15.7'sine eşittir. Elde edilen sonuçlara göre, katılımcıların yaklaşık dörtte üçü satın alacağı ürünlerde bu işaretler olması durumunda bu tarz ürünleri almayacaklarını belirtmişlerdir.

Tablo 1. Araştırmanın çalışma grubunu oluşturan sağlık çalışanlarının değişkenlere göre dağılımı

Table 1. Distribution of the hospital workers who make up the survey group of the study by variables

Demografik Değişkenler		s	%
Cinsiyet	Kadın	204	65.4
	Erkek	108	34.6
Yaş	25 yaş altı	53	17.0
	26-30 yaş	71	22.8
	31-35 yaş	84	26.9
	36-40 yaş	46	14.7
	41 yaş üstü	58	18.6
Meslek	Doktor/Diş hekimi/Eczacı	17	5.4
	Hemşire/Ebe	97	31.1
	Sağlık memuru/ATT	40	12.8
	Tekniker	19	6.1
	Teknisyen	24	7.7
	Tıbbı Sekreter/Memur	44	14.1
	Diğer (lisans hemşirelik stajyerleri)	71	22.8
Medeni Durum	Evli	211	67.6
	Bekâr	101	32.4
Çocuk Sayısı	Evet	188	60.3
	Hayır	124	39.7

GDO'lu gıdaların insan vücudundaki alerjik etkisi

GDO'lu gıdalar insan vücudunda alerjik etki gösterebilir ifadesine ankete katılan toplam 312 kişinin %3.8'i (n=12) kesinlikle katılmıyorum, %3.8'i (n=12) katılmıyorum %23.7'si (n=74) kararsızım, %32.7'si (n=102) katılıyorum ve %35.9'u (n=112) tamamen katılıyorum cevabını vermişlerdir. Tamamen katılıyorum ve katılıyorum cevabını veren 214 (102+112) kişi toplam katılımcıların %68.8'ini oluştururken, GDO'lu gıdaların insan vücudunda alerjik etki göstermeyeceğini düşünen 24 (12+12) kişi vardır. Bu sonuçlarda en çok dikkat çeken kararsızım şeklinde fikir beyan eden 74 kişidir. Bu sayı toplam katılımcıların yaklaşık dörtte birine tekabül etmektedir. Elde edilen verilere göre katılımcıların yaklaşık %25'inin GDO'ların zararları ve faydaları hakkında çok fazla bilgiye sahip olmadıkları sonucuna varılabilir.

Hastalıkların yaygınlaşmasında GDO'ların etkisi

Günümüzde bazı hastalıkların yaygınlaşmasında GDO'ların doğrudan etkisi vardır ifadesine katılımcıların %6.1'i (n=19) kesinlikle katılmıyorum, %3.5'i (n=11) katılmıyorum %18,6'sı (n=58) kararsızım, %26.6'sı (n=83) katılıyorum ve %45.2'si (n=141) tamamen katılıyorum cevabını vermişlerdir. Katılıyorum ve tamamen katılıyorum cevabını veren 224 (83+141) kişi toplam katılımcıların %71.8'ini oluşturmaktadır. Bu verilere göre katılımcıların %71.8'inin bazı hastalıkların yaygınlaşmasında GDO'ların doğrudan etkisi olduğunu düşünmektedir.

GDO'lara Yönelik Toplumsal Bilinç Düzeyi Resmi Kuruluşlarca Yapılan Bilgilendirme, Denetimlerin Yeterliliği ve Hastaların Farkındalığı Boyutuna İlişkin Değerlendirmeler

Sağlık kurumlarında GDO ile ilgili bilgilendirme yapılması

Sağlık kurumlarında GDO ile ilgili yeterli bilgilendirme yapılmaktadır ifadesine katılımcıların %31.4'ü (n=98) kesinlikle katılmıyorum, %14.7'si (n=46) kararsızım, %33.3'ü (n=104) katılmıyorum, %11.2'si (n=35) katılıyorum ve %9.3'ü (n=29) tamamen katılıyorum cevabını vermişlerdir. Katılmıyorum ve kesinlikle katılmıyorum cevabını veren 202 (104+98) kişi toplam katılımcıların %64.7'sini oluşturmaktadır. Bu verilere göre katılımcıların %64.7'si sağlık kurumlarında GDO ile ilgili bilgilendirme yapılmadığını düşünmektedir.

Tarım ve Orman Bakanlığı'nın, ürünlerin GDO'lu olup olmadığı konusunda etkin ve yeterli denetim varlığı

Tarım ve Orman Bakanlığının, ürünlerin GDO'lu olup olmadığı konusunda etkin ve yeterli denetim yaptığını düşünüyorum ifadesine katılımcıların %28.2'si (n=88) kesinlikle katılmıyorum, %22.1'i (n=69) katılmıyorum, %30.8'i (n=96) kararsızım, %9'u (n=28) katılıyorum ve %9.9'u (n=31) tamamen katılıyorum cevabını vermişlerdir. Katılmıyorum ve kesinlikle katılmıyorum cevabını veren 157 (69+88) kişi toplam katılımcıların 50.3'ünü oluşturmaktadır. Bu verilere göre katılımcıların %50.3'ü Tarım ve Orman Bakanlığı'nca ürünlerin GDO'lu olup olmadığı ile ilgili etkin ve yeterli denetim yapılmadığını düşünmektedir.

Toplumun genetiği değiştirilmiş gıdalar hakkında bilgilendirilmesi

Toplumun genetiği değiştirilmiş gıdalar hakkında yeterince bilgilendirildiğini düşünüyorum ifadesine katılımcıların %37,8'i (n=118) kesinlikle katılmıyorum, %29,8'i (n=93) katılmıyorum %11,5'i (n=36) kararsızım, %11,2'si (n=35) katılıyorum ve %9,6'sı (n=30) tamamen katılıyorum cevabını vermişlerdir. Katılmıyorum ve kesinlikle katılmıyorum cevabını veren 211 (93+118) kişi toplam katılımcıların %67,6'sını oluşturmaktadır. Bu verilere göre katılımcıların %67,6'sı, toplumun GDO'lu gıdalar ile ilgili yeterince bilgilendirilmediklerini düşünmektedir.

Sağlık kuruluşlarında verilen tabldot yemeklerde ve kantindeki ürünlerde GDO'lu ürün kullanılması

Sağlık kuruluşlarında verilen tabldot yemeklerde ve kantindeki ürünlerde GDO'lu ürün kullanılmadığını düşünüyorum ifadesine katılımcıların %30.1'i (n=94)kesinlikle katılmıyorum, %20.2'si (n=63) katılmıyorum %33'ü (n=103) kararsızım, %8'i (n=25) katılıyorum ve %8.7'si (n=27) tamamen katılıyorum cevabını vermişlerdir. Katılmıyorum ve kesinlikle katılmıyorum cevabını veren 157 (63+94) kişi toplam katılımcıların %50.3'ünü oluşturmaktadır. Bu verilere göre katılımcıların %50.3'ü tabldot yemeklerde ve kantindeki ürünlerde GDO'lu ürün kullanıldığını düşünmektedir.

Hastaların GDO ile İlgili Farkındalıkları

Hastaların GDO ile ilgili farkındalıklarının olduğunu düşünüyorum ifadesine katılımcıların %34.6'sı (n=108) kesinlikle katılmıyorum, %30.1'i (n=94) katılmıyorum %23.4'ü (n=73) kararsızım, %7.7'si (n=24) katılıyorum ve %4.2'si (n=13) tamamen katılıyorum cevabını vermişlerdir. Katılmıyorum ve kesinlikle katılmıyorum cevabını veren 202 (94+108) kişi toplam katılımcıların %64.7'sini oluşturmaktadır. Bu verilere göre katılımcıların %64.7'si hastaların GDO ile ilgili farkındalıklarının olmadığını düşünmektedir.

GDO'lu Ürünlerin Gıda Sektöründeki Kullanım Zorunluluğu Boyutuna İlişkin Değerlendirmeler

GDO'lu gıdaların dünyadaki açlığı önlemek amacıyla kullanımı

GDO'lu gıdaların dünyadaki açlığı önlemek amacıyla kullanımı kaçınılmazdır, ifadesine katılımcıların %25.3'ü (79) kesinlikle katılmıyorum, %20.2'si (n=63) katılmıyorum %31.4'ü (n=98) kararsızım, %14,4'ü (n=45) katılıyorum ve %8.7'si (n=27) tamamen katılıyorum cevabını vermişlerdir. Katılmıyorum ve kesinlikle katılmıyorum cevabını veren 142 (63+79) kişi toplam katılımcıların %45.5'ini oluşturmaktadır. Bu verilere göre katılımcıların %45.5'i GDO'lu gıdaların dünyadaki açlığı önlemek amacıyla kullanımının kaçınılmaz olmadığını düşünmektedir.

Toprakların verimsizleşmesi ve ekolojik dengenin bozulmasıyla birlikte oluşabilecek besin yetersizliğine GDO'lu tarımın etkisi

Toprakların verimsizleşmesi ve ekolojik dengenin bozulmasıyla birlikte oluşabilecek besin yetersizliği GDO'lu tarım ile telafi edilebilir ifadesine katılımcıların %33'ü (n=103) kesinlikle katılmıyorum, %26'sı (n=81) katılmıyorum, %22.4'ü (n=70) kararsızım, %13.1'i (n=41) katılıyorum ve %5.4'ü (n=17) tamamen katılıyorum cevabını vermişlerdir. Katılmıyorum ve kesinlikle katılmıyorum cevabını veren 184 (81+103) kişi toplam katılımcıların %59'unu oluşturmaktadır. Bu verilere göre katılımcıların %59'u besin yetersizliği için GDO'lu tarımı doğru bulmamaktadır.

Fiyatının avantajlı olması sebebiyle GDO'lu ürün satın alınabilirliği

GDO'lu bir ürünün satın alınmasında fiyatının etkisini belirlemek için yöneltilen ifadede katılımcıların %38.8'i (n=121) kesinlikle katılmıyorum, %26.3'ü (n=82) katılmıyorum, %20.8'i (n=65) kararsızım, %9.6'sı (n=30) katılıyorum ve %4.5'i (n=14) tamamen katılıyorum şeklinde fikir beyan etmişlerdir. Katılmıyorum ve kesinlikle katılmıyorum cevabını veren 203 (121+82) kişi toplam katılımcıların %65.1'ini oluşturmaktadır. Elde edilen sonuçlara göre GDO'lu bir ürünün satın alınmasında avantajlı fiyatının etkisinin oldukça az olduğu, araştırmaya katılan toplam 312 kişiden sadece 44'ünün (%14.1) bu ürünleri satın alabileceği anlaşılmaktadır.

GDO'lu Gıdaların Hali Hazırda Ülkemizde Satılan Gıdalardaki Varlığı ve Zararı İle İlgili Boyutlara İlişkin Değerlendirmeler

GDO'ların insan sağlığı ve çevreye zararı

GDO'ların insan sağlığı ve çevreye zararlı olduğunu düşünüyorum ifadesine katılımcıların %10.9'u (n=34) kesinlikle katılmıyorum, %6.1'i (n=19) katılmıyorum, %4.2'si (n=13) kararsızım, %22.1'i (n=69) katılıyorum ve %56.7'si (n=177) tamamen katılıyorum cevabını vermişlerdir. Tamamen katılıyorum ve katılıyorum cevabını veren 246 (177+69) kişi toplam katılımcıların %78.8'ini oluşturmaktadır. Buna göre katılımcıların çoğunluğu GDO'ların insan sağlığı ve çevreye zararlı olduğunu düşünmektedir.

GDO'lu ürünlerin kullandığımız gıdalarda varlığı

GDO'lu ürünlerin kullandığımız gıdalarda var olduğunu düşünüyorum ifadesine katılımcıların %7.4'ü (n=23) kesinlikle katılmıyorum, %4.8'i (n=15) katılmıyorum, %9'u (n=28) kararsızım, %30.1'i (n=94) katılıyorum ve %48.7'si (n=152) tamamen katılıyorum cevabını vermişlerdir. Tamamen katılıyorum ve katılıyorum cevabını veren 246 (152+94) kişi toplam katılımcıların %78.8'ini oluşturmaktadır. Elde edilen sonuçlara göre katılımcıların büyük çoğunluğu GDO'ların insan sağlığı ve çevreye zararlı olduğunu düşünmektedir.

Katılımcıların GDO'lu Gıdalara Yönelik Farkındalıkları ve Bilgi Düzeylerine Etki Eden Boyutlara İlişkin Değerlendirmeler

Bu kısımda, araştırmanın örneklemini oluşturan katılımcıların GDO'lu Gıdalara Yönelik Farkındalıkları ve Bilgi Düzeylerini oluşturan boyutların neler olduğunu tespit etmek amaçlı analizler gerçekleştirilmiştir. Bu kapsamda veriler; faktör analizi, standart sapma ve aritmetik ortalamalardan faydalanılarak incelenmiştir.

Katılımcıların GDO'lu Gıdalara Yönelik Farkındalıkları ve Bilgi Düzeylerine Etki Eden Boyutların Tespitine İlişkin Açıklayıcı Faktör Analizi Bulguları

Katılımcıların, GDO'lu Gıdalara yönelik farkındalıkları ve bilgi düzeylerini oluşturduğu düşünülen önermeler, alan yazından yararlanılarak hazırlanmıştır. Veri setinin faktör analizine uygunluğunun değerlendirilmesi için Kaiser-Meyer-Olkin (KMO) örneklem yeterliliği ve Bartlett küresellik testi sonuçları incelenmiştir. Veri setinin faktör analizine uygun olduğunun kabul edilebilmesi için KMO değerinin 0,50'nin üzerinde bir değer alması, Bartlett Küresellik test sonucunun ise istatistiksel olarak anlamlı ($p<0,05$) olması gerekmektedir (Tablo 2).

Tablo 2. Katılımcıların GDO'lu gıdalara yönelik farkındalıkları ve bilgi düzeylerine etki eden boyutların tespitine ilişkin açıklayıcı faktör analizi sonuçları

Table 2. Exploratory factor analysis results for determining the dimensions that affect the participants' awareness of GMO foods and their level of knowledge

Genetiği Değiştirilmiş Organizmalara (GDO) Yönelik Boyutlar	**Faktör Yükleri**			
Toplumsal Bilinç Düzeyi (TBD)	**1**	**2**	**3**	**4**
Çocukların okullarda GDO ile ilgili bilgilendirilmesi gerektiğini düşünüyorum.	.859			
İnsanların GDO ile ilgili bilgilenmelerini sağlayacak kamu spotu vb. olması gerektiğini düşünüyorum.	.842			
GDO'lu gıdaları farkında olmadan tüketmekten endişeleniyorum.	.744			
GDO'lu tohumların ülke tarımına zararı olduğunu düşünüyorum.	.665			
Satın alacağım ürünlerde GDO'lu olduklarını belirten işaretler olsa satın almam.	.575			
GDO'lu gıdalar insan vücudunda alerjik etki gösterebilir.	.570			
Günümüzde bazı hastalıkların yaygınlaşmasında GDO'ların doğrudan etkisi vardır.	.441			
Resmi Bilgilendirme, Denetim ve Farkındalık (RBDF)				
Sağlık kurumlarında GDO ilgili yeterli bilgilendirme yapılmaktadır.		.809		
Tarım ve Orman Bakanlığı, ürünlerin GDO'lu olup olmadığı konusunda etkin ve yeterli denetim yaptığını düşünüyorum.		.722		
Toplumun genetiği değiştirilmiş gıdalar hakkında yeterince bilgilendirildiğini düşünüyorum.		.710		
Sağlık kuruluşlarında verilen tabldot yemekleri ve kantinde GDO'lu ürün kullanılmadığını düşünüyorum.		.645		
Hastaların GDO ile ilgili ilgili farkındalıklarının olduğunu düşünüyorum.		.636		
Gıda Sektöründeki Kullanım Zorunluluğu (GSKZ)				
GDO'lu gıdaların dünyadaki açlık önlemek amacıyla kullanımı kaçınılmazdır.			.753	
Toprakların verimsizleşmesi ve ekolojik dengenin bozulmasıyla birlikte oluşabilecek besin yetersizliği GDO'lu tarım ile telafi edilebilir.			.623	
Fiyatının avantajlı olması sebebiyle GDO'lu bir ürünü satın alabilirim.			.578	
Gıdalardaki Varlığına ve Zararına İlişkin Farkındalık (GVZİF)				
GDO'ların insan sağlığı ve çevreye zararlı olduğunu düşünüyorum.				.844
GDO'lu ürünlerin kullandığımız gıdalarda var olduğunu düşünüyorum.				.804
Özdeğerler	4.511	2.479	1.274	1.218
Faktör Varyansı Açıklama Oranı (%)	19.569	15.473	9.243	9.080
Toplam açıklanan varyans (%)	**%53.365**			
KMO Değeri	**0.779**			
Bartlett Küresellik Testi	**$x^{2=}$1854.55 p=0.000<0.001**			
Cr. Alpha=	**.669**			

Tablo 2'de katılımcıların GDO'lu gıdalara yönelik farkındalıkları ve bilgi düzeylerine etki eden boyutlara yönelik olan önermelerin değerlendirilmesi ve çalışmanın faktör analizine uygun olup olmadığının tespit edilmesi amacıyla gerçekleştirilen Kaiser-Mayer-Olkin (KMO) testi sonucunda, KMO değerinin 0.779 çıkması örneklem büyüklüğünün faktör analizi yapmak için iyi derecede yeterli olduğu, verilere ilişkin dağılımın çok değişkenli normal dağılıma uygun olduğu (*p<0.001) ve ölçeğin güvenilir olduğu (Cronbach's Alpha=.669) görülmektedir. Faktör analizi yapılarak ortaya çıkan faktörler ''Varimax'' rotasyonuna tabi tutulmuştur. Yapılan faktör analizi sonucunda 22 önermenin 4'ü (1,5,7,18 sıra numaralı) düşük faktör yük değerlerine sahip olduğundan 1 önermenin de (10) hiçbir boyut altında yer alamadığı için ölçekten çıkarılmıştır. Faktör analizinde ortaya konulan boyutların toplam varyansı açıklama oranı %53.365 olduğu ve önermelerin faktör yük değerlerinin ise 0.441 ile 0.859 arasında değişim gösterdiği görülmüştür.

Katılımcıların GDO'lu Gıdalara Yönelik Farkındalıkları ve Bilgi Düzeylerine Etki Eden Boyutları Algılama Düzeylerine İlişkin Bulgular

Gerçekleştirilen faktör analizi sonra katılımcıların ölçekte yer alan önermelere vermiş olduğu cevaplar, GDO'lara yönelik "Toplumsal Bilinç Düzeyi"; GDO'lar hakkında "Resmi Bil-

gilendirme, Denetim ve Farkındalık"; GDO'ların "Gıda Sektöründeki Kullanım Zorunluluğu" ve "Gıdalardaki Varlığına ve Zararına İlişkin Farkındalık" şeklinde 4 boyut altında toplanmıştır. Bu boyutlara ilişkin katılımcıların algı düzeyleri, aşağıdaki Tablo 3'de görüldüğü üzere aritmetik ortalama ve standart sapma değerleri ile incelenmiştir.

Araştırmanın çalışma grubunda yer alan katılımcıların, "GDO'lu Gıdalara Yönelik Farkındalıkları ve Bilgi Düzeylerine Etki Eden Boyutları Algılama Düzeylerine İlişkin Bulgular" yukarıda yer alan Tablo 3'de verilmektedir. İlgili tabloya göre katılımcıların farkındalık ve bilgi düzeyleri boyutlara göre incelendiğinde, katılımcıların en çok GDO'lara yönelik "Toplumsal Bilinç Düzeyi" boyutunda (**x̄=4.07; ss=.807)**, ve GDO'ların "Gıdalardaki Varlığına ve Zararına İlişkin Farkındalık" boyutunda (**x̄=4.07; ss=1.13)** algıya sahip olduğu; bunu GDO'ların "Gıda Sektöründeki Kullanım Zorunluluğu" boyutu (**x̄=2.35; ss=.886)** ve GDO'lar hakkında "Resmi Bilgilendirme, Denetim ve Farkındalık" boyutunun (**x̄=2.34; ss=.906)** takip ettiği görülmektedir**.** Bu noktadan hareketle, katılımcıların GDO'lu gıdalara yönelik farkındalıkları ve bilgi düzeylerini etkileyen en düşük boyutun resmi bilgilendirmeler, denetim ve farkındalık boyutunda olduğu görülmektedir.

Diğer taraftan önemli bir husus, katılımcıların her bir boyutu oluşturan önermelere verdiği cevapların değerlendirilmesidir. Bu kapsamda sırasıyla **"**GDO'lara yönelik "Toplumsal Bilinç Düzeyi" boyutunu oluşturan önermelere göre katılımcıların en yüksekten en düşük düzeye göre algıları "Çocukların okullarda GDO ile ilgili bilgilendirilmesi gerektiğini düşünüyorum (**x̄=4.07; ss=.807)"**; "İnsanların GDO ile ilgili bilgilenmelerini sağlayacak kamu spotu vb. olması gerektiğini düşünüyorum (**x̄=4.29; ss=1.080)"**; "GDO'lu gıdaları farkında olmadan tüketmekten endişeleniyorum (**x̄=4.08; ss=1.168)"; "**Günümüzde bazı hastalıkların yaygınlaşmasında GDO'ların doğrudan etkisi vardır (**x̄=4.01; ss=1.153)";** GDO'lu gıdalar insan vücudunda alerjik etki gösterebilir (**x̄=3.92; ss=1.046)";** Satın alacağım ürünlerde GDO'lu olduklarını belirten işaretler olsa satın almam (**x̄=3.91; ss=1.311)"** ve "GDO'lu tohumların ülke tarımına zararı olduğunu düşünüyorum (**x̄=3.91; ss=1.229)"** şeklinde sıralanmaktadır.

Tablo 3. Katılımcıların GDO'lu gıdalara yönelik farkındalıkları ve bilgi düzeylerine etki eden boyutları algılama düzeylerine ilişkin sonuçlar

Table 3. Results of participants' awareness of GMO foods and their perception levels of dimensions affecting their knowledge level

Önermeler	**Art. Ort. (x̄)**	**St. Sapma (ss)**
Çocukların okullarda GDO ile ilgili bilgilendirilmesi gerektiğini düşünüyorum.	4.36	1.043
İnsanların GDO ile ilgili bilgilenmelerini sağlayacak kamu spotu vb. olması gerektiğini düşünüyorum.	4.29	1.080
GDO'lu gıdaları farkında olmadan tüketmekten endişeleniyorum.	4.08	1.168
GDO'lu tohumların ülke tarımına zararı olduğunu düşünüyorum.	3.91	1.229
Satın alacağım ürünlerde GDO'lu olduklarını belirten işaretler olsa satın almam.	3.91	1.311
GDO'lu gıdalar insan vücudunda alerjik etki gösterebilir.	3.92	1.046
Günümüzde bazı hastalıkların yaygınlaşmasında GDO'ların doğrudan etkisi vardır.	4.01	1.153
Toplumsal Bilinç Düzeyi (TBD)	**4.07**	**.807**
Sağlık kurumlarında GDO ilgili yeterli bilgilendirme yapılmaktadır.	2.33	1.280
Tarım ve Orman Bakanlığı, ürünlerin GDO'lu olup olmadığı konusunda etkin ve yeterli denetim yaptığını düşünüyorum.	2.50	1.262
Toplumun genetiği değiştirilmiş gıdalar hakkında yeterince bilgilendirildiğini düşünüyorum.	2.25	1.323
Sağlık kuruluşlarında verilen tabldot yemekleri ve kantinde GDO'lu ürün kullanılmadığını düşünüyorum.	2.44	1.238
Hastaların GDO ile ilgili ilgili farkındalıklarının olduğunu düşünüyorum.	2.16	1.113
Resmi Bilgilendirme, Denetim ve Farkındalık (RBDF)	**2.34**	**.906**
GDO'lu gıdaların dünyadaki açlık önlemek amacıyla kullanımı kaçınılmazdır.	2.60	1.247
Toprakların verimsizleşmesi ve ekolojik dengenin bozulmasıyla birlikte oluşabilecek besin yetersizliği GDO'lu tarım ile telafi edilebilir.	2.32	1.213
Fiyatının avantajlı olması sebebiyle GDO'lu bir ürünü satın alabilirim.	2.14	1.169
Gıda Sektöründeki Kullanım Zorunluluğu (GSKZ)	**2.35**	**.886**
GDO'ların insan sağlığı ve çevreye zararlı olduğunu düşünüyorum.	4.07	1.353
GDO'lu ürünlerin kullandığımız gıdalarda var olduğunu düşünüyorum.	4.08	1.196
Gıdalardaki Varlığına ve Zararına İlişkin Farkındalık (GVZİF)	**4.07**	**1.113**

İkinci boyut olan GDO'ların "Gıdalardaki Varlığına ve Zararına İlişkin Farkındalık" boyutunu oluşturan önermelere göre katılımcıların en yüksekten en düşük düzeye göre algıları "GDO'lu ürünlerin kullandığımız gıdalarda var olduğunu düşünüyorum (**x̄=4.08; ss=1.196)";** ve "GDO'ların insan sağlığı ve çevreye zararlı olduğunu düşünüyorum (**x̄=4.07; ss=1.353)"** şeklinde sıralanmaktadır.

Üçüncü boyut olan "GDO'ların "Gıda Sektöründeki Kullanım Zorunluluğu" boyutunu oluşturan önermelere göre katılımcıların en yüksekten en düşük düzeye göre algıları "GDO'lu gıdaların dünyadaki açlık önlemek amacıyla kullanımı kaçınılmazdır (**x̄=2.60; ss=1.247)";** "Toprakların verimsizleşmesi ve ekolojik dengenin bozulmasıyla birlikte oluşabilecek besin yetersizliği GDO'lu tarım ile telafi edilebilir (**x̄=2.32; ss=1.213)"** ve "Fiyatının avantajlı olması sebebiyle GDO'lu bir ürünü satın alabilirim (**x̄=2.14; ss=1.169)"** şeklinde sıralanmaktadır.

Dördüncü boyut olan "GDO'lar hakkında "Resmi Bilgilendirme, Denetim ve Farkındalık" boyutunu oluşturan önermelere göre katılımcıların en yüksekten en düşük düzeye göre algıları "Tarım ve Orman Bakanlığı, ürünlerin GDO'lu olup olmadığı konusunda etkin ve yeterli denetim yaptığını düşünüyorum (**x̄=2.50; ss=1.262)";** "Sağlık kuruluşlarında verilen tabldot yemeklerde ve kantindeki ürünlerde GDO'lu ürün kullanılmadığını düşünüyorum (**x̄=2.44; ss=1.238)";** "Sağlık kurumlarında GDO ile ilgili yeterli bilgilendirme yapılmaktadır (**x̄=2.33;ss=1.280)";** "Toplumun genetiği değiştirilmiş gıdalar hakkında yeterince bilgilendirildiğini düşünüyorum (**x̄=2.25; ss=1.323)"** ve "Hastaların GDO ile ilgili farkındalıklarının olduğunu düşünüyorum (**x̄=2.16; ss=1.113)"** şeklinde sıralanmaktadır.

Katılımcıların GDO'lu Gıdalara Yönelik Farkındalıkları ve Bilgi Düzeyleri ile Demografik Özellikleri Arasındaki Farklılık Testlerine İlişkin Bulgular

Bu kısımda araştırma katılımcılarının GDO'lu Gıdalara Yönelik Farkındalıkları ve Bilgi Düzeyleri boyutları ile birtakım demografik özellikleri arasında herhangi bir fark olup olmadığına ilişkin analizler gerçekleştirilmiştir. İlgili analizlerde T-Testi ve tek yönlü varyans analizi (One-Way Anova) testi kullanılmış, sonuçlara ilişkin bulgular tablolar halinde gösterilmiş ve yorumlanmıştır.

Bu kapsamda öncelikle hipoteze ilişkin katılımcıların GDO'lu Gıdalara Yönelik Farkındalıkları ve Bilgi Düzeyleri boyutları ile cinsiyetleri arasında anlamlı bir farklılık olup olmadığına ilişkin sonuçlar aşağıdaki Tablo 4'de gösterilmiştir.

Tablo 4'de yer alan sonuçlara göre katılımcıların cinsiyetleri ile GDO'lu gıdalara yönelik "Toplumsal Bilinç Düzeyleri" arasında (sig.=0,948; p≥0,05); "Resmi Bilgilendirme, Denetim ve Farkındalıkları" arasında (sig.=0,783; p≥0,05); "Gıda Sektöründeki Kullanım Zorunluluğu" arasında (sig.=0,337; p≥0.05) anlamlılık değeri p>0,05 olduğu için farklılık bulunmamaktadır. Diğer taraftan katılımcıların cinsiyetleri ile "Gıdalardaki Varlığına ve Zararına İlişkin Farkındalık (sig.=0.030; p≤0.05) arasında anlamlılık değeri p<0.05 olduğu için farklılık bulunmaktadır. Bu veriler kadın katılımcıların (x̄=3.4393), erkek katılımcılara (x̄=3.2861) oranla GDO'ların "Gıdalardaki Varlığına ve Zararına İlişkin Farkındalıklarının daha yüksek olduğunu göstermektedir.

Tablo 4. Katılımcıların GDO'lu gıdalara yönelik farkındalıkları ve bilgi düzeyleri boyutları ile cinsiyetleri arasındaki farklılıklara ilişkin bağımsız örneklem t-Testi sonuçları

Table 4. Independent sample t-Test results regarding the differences between the participants' awareness of GMO foods and their level of knowledge and gender

BOYUTLAR	Grup	Cinsiyet	s	Ort.	St. Sapma	Levene testi			
						F	Sig.	t	p
Toplumsal Bilinç Düzeyi (TBD)	1	Kadın	204	4.0476	.83392	.004	.948	-.798	.425
	2	Erkek	108	4.1243	.75494			-.823	
Resmi Bilgilendirme, Denetim ve Farkındalık (RBDF)	1	Kadın	204	2.3735	.92384	.076	.783	.870	.385
	2	Erkek	108	2.2796	.87496			.884	
Gıda Sektöründeki Kullanım Zorunluluğu (GSKZ)	1	Kadın	204	2.3203	.85567	.924	.337	-1.061	.290
	2	Erkek	108	2.4321	.94089			-1.030	
Gıdalardaki Varlığına ve Zararına İlişkin Farkındalık (GVZİF)	1	Kadın	204	4.1201	1.07664	4.758	**.030**	.890	.374
	2	Erkek	108	4.0000	1.23425			.854	
*p<0.05									

Tablo 5. Katılımcıların GDO'lu gıdalara yönelik farkındalıkları ve bilgi düzeyleri boyutları ile medeni durumları arasındaki farklılıklara ilişkin bağımsız örneklem *t* -testi sonuçları

Table 5. Independent sample t-test results regarding the differences between the participants' awareness of GMO foods and their level of knowledge and marital status

BOYUTLAR	Grup	Medeni Durum	s	Ort.	St. Sapma	Levene testi			
						F	Sig.	t	p
Toplumsal Bilinç Düzeyi (TBD)	1	Evli	211	4.0819	.83392	.524	.470	.245	.807
	2	Bekâr	101	4.0580	.75494			.251	
Resmi Bilgilendirme, Denetim ve Farkındalık (RBDF)	1	Evli	211	2.3763	.92384	5.273	**.022**	.993	.321
	2	Bekâr	101	2.2673	.87496			1.071	
Gıda Sektöründeki Kullanım Zorunluluğu (GSKZ)	1	Evli	211	2.3207	.85567	1.312	.253	-1.103	.271
	2	Bekâr	101	2.4389	.94089			-1.134	
Gıdalardaki Varlığına ve Zararına İlişkin Farkındalık (GVZİF)	1	Evli	211	4.0474	1.07664	3.315	.070	-.701	.258
	2	Bekâr	101	4.1436	1.23425			-.749	
*p<0.05									

Tablo 5'de yer alan sonuçlara göre katılımcıların medeni durumları ile GDO'lu besinlere yönelik "Toplumsal Bilinç Düzeyleri" arasında (sig.=0.470; p≥0.05); "Gıda Sektöründeki Kullanım Zorunluluğu" arasında (sig.=0.253; p≥0.05); "Gıdalardaki Varlığına ve Zararına İlişkin Farkındalık" (sig.=0.070; p≥0.05) arasında anlamlılık değeri p>0.05 olduğu için farklılık bulunmamaktadır. Diğer taraftan katılımcıların medeni durumları ile "Resmi Bilgilendirme, Denetim ve Farkındalıkları" arasında (sig.=0.022; p<0.05) anlamlılık değeri p<0.05 olduğu için farklılık bulunmaktadır. Bu veriler evli olan katılımcıların ($\bar{x}$=2.3763), bekâr olan katılımcılara ($\bar{x}$=2.2673) oranla GDO'lara yönelik "Resmi Bilgilendirme, Denetim ve Farkındalıklarının daha yüksek olduğunu göstermektedir.

Tablo 6'da yer alan sonuçlara göre katılımcıların çocuk sahibi olma durumları ile GDO'lu gıdalara yönelik "Toplumsal Bilinç Düzeyleri" arasında (sig.=0.425; p≥0.05); "Resmi Bilgilendirme, Denetim ve Farkındalıkları" arasında sig.=0.097; p≥0.05); "Gıda Sektöründeki Kullanım Zorunluluğu" arasında (sig.=0.324; p≥0.05); "Gıdalardaki Varlığına ve Zararına İlişkin Farkındalık" (sig.=0.298; p≥0.05) boyutları arasında anlamlılık değeri p>0,05 olduğu için herhangi bir farklılık bulunmamaktadır.

Tablo 6. Katılımcıların GDO'lu gıdalara yönelik farkındalıkları ve bilgi düzeyleri boyutları ile çocuk sahibi olma durumları arasındaki farklılıklara ilişkin bağımsız örneklem *t* -testi sonuçları

Table 6. Independent sample t-test results regarding the differences between the participants' awareness of GMO foods and their level of knowledge and their status of having children

BOYUTLAR	Grup	Çocuk Durumu	s	Ortalama	Standart Sapma	Levene Testi			
						F	Sig.	t	p
Toplumsal Bilinç Düzeyi (TBD)	1	Var	188	4.1208	.83392	.637	.425	.245	.209
	2	Yok	124	4.0035	.75494			.251	
Resmi Bilgilendirme, Denetim ve Farkındalık (RBDF)	1	Var	188	2.3255	.92384	2.765	.097	.993	.711
	2	Yok	124	2.3645	.87496			1.071	
Gıda Sektöründeki Kullanım Zorunluluğu (GSKZ)	1	Var	188	2.2926	.85567	.977	.324	-1.103	.103
	2	Yok	124	2.4597	.94089			-1.134	
Gıdalardaki Varlığına ve Zararına İlişkin Farkındalık (GVZİF)	1	Var	188	4.0638	1.07664	1.086	.298	-.701	.778
	2	Yok	124	4.1008	1.23425			-.749	
*p<0.05									

Tablo 7. Katılımcıların GDO'lu gıdalara yönelik farkındalıkları ve bilgi düzeyleri boyutları ile yaşları arasındaki farklılıklara ilişkin one-way anova testi sonuçları

Table 7. One-way ANOVA test results regarding the differences between the dimensions and ages of the participants' awareness and level of knowledge about GMO foods

BOYUTLAR	Yaş	s	Ortalama	Standart Sapma	F	Sig.
Toplumsal Bilinç Düzeyi (TBD)	25 yaş ve altı	50	3.9514	.76503	1.455	.216
	26-30 yaş	70	3.9592	.90355		
	31-35 yaş	83	4.0637	.85547		
	36-40	45	4.2127	.81477		
	41 yaş ve üstü	64	4.2121	.62451		
	Toplam	312	4.0742	.80706		
Resmi Bilgilendirme, Denetim ve Farkındalık (RBDF)	25 yaş ve altı	50	2.4240	.77369	.576	.680
	26-30 yaş	70	2.2086	.76627		
	31-35 yaş	83	2.3614	.95583		
	36-40	45	2.4222	1.02089		
	41 yaş ve üstü	64	2.3375	1.00214		
	Toplam	312	2.3410	.90691		
Gıda Sektöründeki Kullanım Zorunluluğu (GSKZ)	25 yaş ve altı	50	2.5933	.90674	1.343	.254
	26-30 yaş	70	2.4000	.81175		
	31-35 yaş	83	2.2450	.87897		
	36-40	45	2.3259	.90590		
	41 yaş ve üstü	64	2.3021	.93288		
	Toplam	312	2.3590	.88619		
Gıdalardaki Varlığına ve Zararına İlişkin Farkındalık (GVZİF)	25 yaş ve altı	50	4.0700	1.13843	1.104	.355
	26-30 yaş	70	4.2214	.90744		
	31-35 yaş	83	4.0482	1.16246		
	36-40	45	4.2333	1.05852		
	41 yaş ve üstü	64	3.8594	1.34066		
	Toplam	312	4.0785	1.13314		

Tablo 7'de yer alan sonuçlara göre katılımcıların yaşları ile GDO'lu gıdalara yönelik "Toplumsal Bilinç Düzeyleri" arasında (sig.=0.216; p≥0.05); "Resmi Bilgilendirme, Denetim ve Farkındalıkları" arasında sig.=0,680; p≥0.05); "Gıda Sektöründeki Kullanım Zorunluluğu" arasında (sig.=0.254; p≥0,05); "Gıdalardaki Varlığına ve Zararına İlişkin Farkındalık" (sig.=0.355; p≥0.05) arasında anlamlılık değeri p>0.05 olduğu için farklılık bulunmamaktadır.

Genetiği değiştirilmiş organizmalar ve insan sağlığı üzerindeki etkileri hakkında farkındalık düzeylerinin ölçülmesi kapsamında Sinop ilinde bulunan kamu hastanelerinde çalışan personellere yönelik gerçekleştirilen bu araştırmanın sonucunda, katılımcıların %47.4'ünün GDO'lu gıdaları farkında olmadan tüketmekten endişelendiği; %43.6'sının GDO'lu tohumların ülke tarımına zararı olduğunu düşündüğü; %47'sinin satın alınan ürünlerde GDO işareti olması gerektiğini belirtmiş ve işaret olsa 3/4'ünün bu ürünleri satın almayacağı; 1/4'ünün GDO'lu ürünlerin alerjen etkisi konusunda kararsız oldukları; %45'inin hastalıkların yaygınlaşmasında GDO'nun etkisi olduğunu düşündükleri; %50'sine yakınının Tarım ve Orman Bakanlığı'nın GDO konusunda denetim yetersizliği olduğunu; %68'ine yakınının GDO konusunda yeterince bilgilendirilme yapılmadığını düşündükleri; %59'unun besin yetersizliği için GDO'lu tarımı doğru bulmadığı; %79'unun GDO'lu ürünlerin insan sağlığı ve çevreye zararlı olduğunu düşündükleri tespit edilmiştir.

Çalışmada Radas vd. (2008)'nin sonuçlarında görülen "tüketicilerin hangi yiyeceklerde GDO olduğunu bilmedikleri" bulgusuna benzer bir sonuç görülmüş, "GDO'lu gıdaları farkında olmadan tüketmekten endişeleniyorum" önermesine ($\bar{x}$=4.08) yüksek derecede katılmışlardır. Kaynar'ın (2009), çalışmasında ise tüketicilerin GDO'lu ürünler hakkında bilgi sahibi olmak istediği görülmekle birlikte, yine bu çalışmada da "İnsanların GDO ile ilgili bilgilenmelerini sağlayacak

kamu spotu vb. olması gerektiğini düşünüyorum" önermesine ($\bar{x}$=4.29) yüksek derecede katılmışlardır.

Özdemir ve Duran'ın (2010) ve Sönmez'in (2011) çalışmalarında tüketicilerin GDO'lardan büyük ölçüde endişelendikleri ve bu ürünlere yönelik olumsuz düşüncelere sahip oldukları bildirilmiştir. Benzer şekilde bu araştırmada katılımcıların "GDO'ların insan sağlığı ve çevreye zararlı olduğunu düşünüyorum" önermesine ($\bar{x}$=4.07), "Günümüzde bazı hastalıkların yaygınlaşmasında GDO'ların doğrudan etkisi vardır" önermesine ($\bar{x}$=4.01) yüksek derecede katılmışlardır.

Temelli ve Kurt'un (2011) çalışmasında belirttiği tüketicilerin genetiği değiştirilmiş organizmalar hakkında bilgilendirilmeleri gerektiği ifadesi çalışmamızda "Çocukların okullarda GDO ile ilgili bilgilendirilmesi gerektiğini düşünüyorum" önermesi ($\bar{x}$=4.36), ile "İnsanların GDO ile ilgili bilgilenmelerini sağlayacak kamu spotu vb. olması gerektiğini düşünüyorum" ($\bar{x}$=4.29) önermelerine yüksek derecedeki katılım ve "Hastaların GDO ile ilgili ilgili farkındalıklarının olduğunu düşünüyorum" ($\bar{x}$=2.16) önermesine çok düşük katılım olması ile desteklenmektedir.

Aydın (2012), çalışmasında katılımcıların %88'inin aldıkları ürünlerin etiketini kontrol ettiklerini, Wunderlich ve Gatto (2015), çalışmalarında GDO'lu ürünler konusunda tüketici bilincinin düşük olduğunu ayrıca Lefebvre ve ark. (2019), yaptıkları çalışmada GDO içeren ancak etiketsiz olan ürünleri satın alma olasılıklarının daha yüksek olduğunu tespit etmiştir. Tüketicilerin GDO konusundaki korkularını gidermek için şeffaflaşmanın (örneğin etiket belirtmenin) gerekli olduğunu bildirmiştir. Bu çalışmalara benzer şekilde yapılan araştırmada da katılımcıların "Satın alacağım ürünlerde GDO'lu olduklarını belirten işaret olsa satın almam" önermesine ($\bar{x}$=3.91) yüksek derecede katılmışlardır.

Cui ve Shoemaker (2018), yaptıkları çalışmada katılımcıların çok az bir kısmının GDO teknolojisinin genel prensiplerini anladığını, pek çoğunun ise "nötr" ya da "olumsuz" görüşe sahip oldukları bildirilmiştir. Benzer şekilde bu sonuç gerçekleştirilen araştırmada da katılımcıların cevaplarında yer alan, "Toplumun genetiği değiştirilmiş gıdalar hakkında yeterince bilgilendirildiğini düşünüyorum" önermesine ($\bar{x}$=2.25) ve ''Sağlık kurumlarında GDO ilgili yeterli bilgilendirme yapılmaktadır.'' önermesine ($\bar{x}$=2.33) çok düşük ölçüde katılımları ile benzerlik taşımaktadır.

Karaaslan (2017) çalışmasında, GDO'lu besinlerle beslenen canlıların görünüş veya yapısal olarak değiştiğini vurgulamış, bir başka çalışmada Güneş ve Yılmaz (2019) ise GDO'ların hastalıkların oluşması, insan ömrünün kısalması, mutasyon, erken ergenlik, besin kalitesinin azalması, biyolojik çeşitliğin azalması, doğal dengenin bozulması gibi konularda ise olumsuz fikre sahip olduklarını ifade etmişlerdir. Bu çalışmalara benzer şekilde bu araştırmada ise katılımcıların "GDO'lu gıdalar insan vücudunda alerjik etki gösterebilir" önermesine ($\bar{x}$=3.92) yüksek derecede katılmışlardır.

Çelik (2009) çalışmasında, GDO'lu bitkilerin ıslah edilmiş ya da evrimleşmiş bitki çeşitleriyle rekabet ederek onların yerini alabileceğini ve yerel tohum türlerinin yok olma tehlikesinde olduğunu belirtmiştir. Oğur vd. (2017) çalışmalarında ise katılımcıların yaklaşık %80'inin Türkiye'de GDO'lu tohumlarla üretim yapılmasını doğru bulmadığını söylemişlerdir. Benzer şekilde ilgili araştırmaların sonuçları, bu araştırmada yer alan katılımcıların "GDO'lu tohumların ülke tarımına zararlı olduğunu düşünüyorum" önermesine ($\bar{x}$=3.91) yüksek derecedeki katılımı ve "toprakların verimsizleşmesi ve ekolojik dengenin bozulmasıyla birlikte oluşabilecek besin yetersizliği GDO'lu tarım ile telafi edilebilir'' ($\bar{x}$=2.32) önermelerine yönelik düşük orandaki katılımları nedeniyle desteklenmektedir.

Yalçın (2019), çalışmasında ülkemizde GDO'lu üretim ve satış ile alakalı yapılan denetimleri yeterli ve şeffaf bulmadığını belirtmiştir; bu sonuç benzer şekilde bu çalışmada da ''Tarım ve Orman Bakanlığı, ürünlerin GDO'lu olup olmadığı konusunda etkin ve yeterli denetim yaptığını düşünüyorum'' önermesine ($\bar{x}$=2.50) katılımıların düşük oranda katılımları nedeniyle desteklenmektedir.

Hıdıroğlu vd. (2013), yaptıkları çalışmada katılımcıların %66.8'i GDO'lu ürünün fiyatı normal ürünlerden daha ucuz olsa da ürünü satın almayacağını bildirmiştir. Benzer şekilde bu çalışmada önermeler içerisinde en düşük değere sahip olan "Fiyatının avantajlı olması sebebiyle GDO'lu bir ürünü satın alabilirim" ($\bar{x}$=2.14) önermesidir.

Sonuç

Bu noktadan hareketle başta kamu kurumları olmak üzere, çeşitli sivil toplum kuruluşlarının özellikle toplum sağlığına yönelik olarak hazırlanan görsel ve işitsel yayınlarında GDO'lu gıdalar kullanılmaması, GDO'lu gıdaların insan sağlığı üzerine etkileri ve GDO'lu ile GDO'suz gıdaların nasıl fark edilebileceğine yönelik ayrıntıları vurgulaması önem arz etmektedir.

Yetkililerin GDO'lu gıdalarla ilgili gerekli yasal düzenlemeleri yaparak, bu ürünlerin denetimini etkili bir şekilde gerçekleştirmesi oldukça önemlidir. Bu bağlamda tek bir kontrol mekanizmasının yerine aşamalı ve çoklu kontrol yapılabilir. Özelikle okul ve kamu kurumu yemekhanelerinde yemek yapımında kullanılan gıdaların kontrol edilerek, GDO'lu ürün kullanımı önemli ölçüde azaltılabilir.

Ayrıca gelecek nesillerin de çeşitli bilimsel araştırmalar ile desteklendiği üzere GDO'lu gıdaların tüketiminin insan sağ-

lığı üzerine etkilerine yönelik bilinçlendirilmesi için ortaöğretim ve üniversite düzeyinde eğitim gören gençlere farkındalık ve bilgi sağlayan etkinlikler gerçekleştirilmesi için başta Milli Eğitim Bakanlığı ve Yükseköğretim Kurulu Başkanlığı işbirliği ile projeler yapılmalıdır. Bu noktada ders müfredatları içerisine de GDO'lu besinler hakkında bilgilendirici dersler eklenebilir.

Bu çalışmada elde edilen sonuçların başta ilgili alanda araştırma yapan bilim insanları olmak üzere, araştırma alanı ile ilgili (gıda, tarım ve hayvancılık, yiyecek içecek işletmeleri vb.) kamu kurum ve kuruluşları ile çeşitli sivil toplum örgütleri ve ilgili işletmelere veri sağlaması hedeflenmiştir. Ayrıca bu çalışma, konusunda bilgilenmek isteyenlere ve kamuoyunun bilinçlenmesine katkı sağlayacak, gelecekte yapılacak çalışmalara da yön gösterici olacaktır.

Etik Standart ile Uyumluluk

Çıkar çatışması: Yazarlar bu yazı için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan etmişlerdir.

Etik izin: Araştırma için Sinop Üniversitesi İnsan Araştırmaları Etik Kurulu Yönetim Kurulu'ndan 13.12.2018 tarih ve 2018/46 karar sayılı etik onay alınmış olup, araştırmanın gerçekleştirildiği hastaneler için Sinop İl Sağlık Müdürlüğü'nün 30.05.2019 tarih ve 2078520-605.99 sayılı onayı ile çalışma gerçekleştirilmiştir.

Finansal destek: -

Teşekkür: -

Açıklama: Bu çalışma 23/01/2020 tarihinde tamamlanan "Hastane Çalışanlarının Genetiği Değiştirilmiş Organizmalar (GDO) ve Sağlığımız Üzerindeki Etkileri Hakkındaki Görüşleri ve Farkındalık Düzeylerinin Belirlenmesi: Sinop Örneği" başlıklı Yüksek lisans tezi esas alınarak hazırlanmıştır.

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 7(1), 15-20 (2021) • https://doi.org/10.3153/FH21002 Research Article

Afyonkarahisar'da satışa sunulan kıymalarda *Aeromonas* spp. varlığının araştırılması

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Cite this article as:
Kara, R., Acaröz, U., Gürler, Z., Zemheri Navruz, F., Soylu, A. (2021). Afyonkarahisar' da satışa sunulan kıymalarda Aeromonas spp. varlığının araştırılması. *Food and Health,* 7(1), 15-20. https://doi.org/10.3153/FH21002

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Submitted: 15.01.2020
Revision requested: 07.06.2020
Last revision received: 26.06.2020
Accepted: 15.07.2020
Published online: 27.10.2020

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Available online at
http://jfhs.scientificwebjournals.com

ÖZ

Hayvansal ürünler arasında yaygın bir şekilde tüketilen sığır eti kıyması, hijyenik olmayan üretim ve muhafaza şartlarında bakteri gelişimine uygun olabilmektedir. *Aeromonas* spp. çevrede yaygın bulunabilmekte ve su ile ilişkilendirilmektedir. Üretimde kullanılan kontamine suyla mikroorganizmalar ürünlere geçmekte ve halk sağlığı için tehdit oluşabilmektedir. İnsanlar çoğunlukla kontamine su ve et ürünlerini tüketmek suretiyle enfekte olabilmektedir. *Aeromonas* spp. kusma, ishal ve gastroenterit gibi çeşitli rahatsızlıklara neden olmaktadır. Ayrıca, çocuklar ve yaşlılar bu bakteriye daha duyarlıdır. Yapılan bu çalışmada, Afyonkarahisar il merkezinde satışa sunulan 100 adet sığır eti kıymasında *Aeromonas* spp. varlığı klasik kültür yöntemi ile araştırılmıştır. İzole edilen bakteriler VITEK® 2 Compact ile tanımlanmıştır. Tespit edilen *A. hydrophila* şuşları PZR ile a*erA* ve *hlyA* gen varlığı yönünden araştırılmıştır. Analiz sonucunda kıyma örneklerinin %3'ünde *A. hydrophila* identifiye edilmiştir. Tanımlanan üç bakterinin de a*erA* ve *hlyA* genlerinin her ikisine de sahip olduğu tespit edilmiştir. Ayrıca identifiye edilen suşların yalnız birer tanesinin amoksisiline ve nalidiksik asite dirençli olduğu belirlenmiştir. Sonuç olarak, çiftlikten çatala tüm kırmızı et üretim aşamalarda gerekli hijyen tedbirlerin alınması, muhafaza ve ısıl işleme dikkat edilmesi ve tüketicilerin bilgilendirilmesi önerilmektedir.

Anahtar Kelimeler: *Aeromonas hydrophila*, Halk sağlığı, Kıyma

ABSTRACT

Investigation of *Aeromonas* spp. in ground beef marketed in Afyonkarahisar

Ground beef as commonly consumed among animal products may be suitable for bacterial growth under unhygienic production and storage conditions. *Aeromonas* spp. can be widely found in the environment and it is associated with water. With contaminated water used in production, microorganisms pass into products and may pose a threat to public health. People are often infected by consuming contaminated water and meat products. *Aeromonas* spp. causes various disorders such as vomiting, diarrhoea and gastroenteritis. In addition, children and older people are more susceptible to these bacteria. In this study, the presence of *Aeromonas* spp. was investigated by classical culture method in 100 ground beef samples which were sold in Afyonkarahisar city centre. The suspected *Aeromonas* spp. were confirmed with VITEK® 2 Compact. The detected *A. hydrophila* strains were further investigated by PZR in terms of a*erA* and *hlyA* genes. At the end of the analysis, *A. hydrophila* was identified in 3% of the ground beef samples. It was detected that all three bacteria have both the a*erA* and *hlyA* genes. In addition, only one of the identified strains was found to be resistant to amoxicillin and nalidixic acid. As a result, it is recommended to take necessary hygiene measures in all stages of red meat production according to farm to fork, to give attention to storage, heat treatment and inform consumers.

Keywords: *Aeromonas hydrophila*, Public health, Ground beef

Giriş

Aeromonas spp. çevrede bulunabilen ve dağılım açısından kozmopolit bir bakteridir *(*Praveen ve ark., 2016*). Aeromonas* spp, insanlarda ishalin yanı sıra özellikle bağışıklık sistemi baskılanmış bireylerde septisemi, ciddi yara enfeksiyonları, peritonit, menenjit ile göz, eklem ve kemiklerde enfeksiyonlara neden olabilmektedir (Abbott et al., 1992). *Aeromonas* türleri iki farklı gruptan oluşmaktadır. Bunlardan birincisi hareketsiz psikrofilik *A. salmonicida* ve diğer grup ise üç mezofilik hareketli olan *A. hydrophila, A. caviae ve A. sobria*'dan oluşmaktadır *(*Praveen ve ark., 2014*).*

Gıda kaynaklı bir patojen olma potansiyeline sahip olan *Aeromanas hydrophila*, ekotoksinler ve sitotoksinler dahil olmak üzere farklı virülans faktörlerini üretmektedir. Aynı zamanda psikotrof bir bakteri olan, *A. hydrophila* soğutma ve soğuk muhafaza sırasında gıdalarda gelişmekte ve toksin oluşturabilmektedir. Bu nedenle, insan tüketimi için gıdalarda *A. hydrophila* varlığının kontrol edilmesi gerektiği tavsiye edilmektedir (Daskalov, 2006).Yapılan çalışmalarda etkenin özellikle içme, kullanma ve yüzey sularından sıklıkla izole edildiği, ayrıca kanatlı eti, kırmızı et, balık, süt ve süt ürünleri gibi hayvansal gıdalarda da yüksek oranda bulunduğu bildirilmiştir (İbrahim ve Mac Rae, 1991; Gobat ve Jemni, 1993; Sachan ve Agarwal 2000; Küpüllü ve ark. 2000; İşleyici ve ark., 2006). Yapılan çalışmalarda da görüldüğü gibi insan sağlığı için son derecede önemli bir patojen bakteri olan *Aeromonas* türlerinin et ve et ürünlerinden sıklıkla izole edilmektedir. Bu nedenle yapılan bu çalışmada halk sağlığı açısından önemli tehlike arz eden *Aeromonas* spp.'nin Afyonkarahisar ilinde tüketime sunulan sığır kıymalarında varlığı ile identifiye edilen suşların antibakteriyel ilaç dirençlilikleri araştırılmıştır.

Materyal ve Metot

Materyal

Afyonkarahisar ilinden tüketime sunulan 100 adet sığır kıyma örneği, hijyenik kurallara uygun olarak 200 g'dan az olmamak üzere steril poşetlere alınarak, soğuk zincir altında laboratuvara taşınarak analize alınmıştır.

Metot

Aeromonas spp. izolasyon ve identifikasyonu

Laboratuara getirilen sığır kıyma örnekleri aynı gün içinde homojen bir şekilde 25'er g alınarak üzerine 225 mL alkali peptonlu su ilave edilmiş ve 2 dk stomacherde homojenize edildikten sonra 30 ºC'de 24 saat inkübasyona bırakılmıştır. İnkübasyon sonunda bu zenginleştirme sıvısından bir öze dolusu alınarak önceden hazırlanmış *Aeromonas* agara (Condalab Cat:1370, 5mg/l Ampicilin içeren) çizme yöntemi ile ekim yapılmıştır. Ekimi yapılan petriler 30°C de 24 saat inkübasyona bırakılmıştır. İnkübasyon sonunda şüpheli kolonilerden; gram boyama, oksidaz test, katalaz test, hareketlilik testi, Vibriostatik ajan O/129' a dirençlilik, NaCl içermeyen ve % 5 NaCl içeren Nutrient Broth'da üreme sonucu *Aeromonas* spp. olduğu tespit edilen kültürlerden tür tayini yapılmıştır (Palumbo ve ark. 1992; Anonim 2000). İdentifiye edillen *A hydrophila* suşları VITEK® 2 Compact (Biomerieux) ile gram negatif identifikasyon kartları kullanılarak tespit edilmiştir.

DNA izolasyonu ve PZR işlemi

Genetik analizler için hazırlanmış bakteri örneklerinden DNA izolasyonu ve PZR analizi yapılmıştır. İzolasyon için, DNA izolasyon kiti (Qiagen DNeasy® DNA İzolasyon Kiti, Almanya) kullanılmıştır.

Primer tasarımında NCBI web sitesinden *A. hydrophila*'ya özgü a*erA ve hlyA* genine ait tasarlanan primerler, FastPCR 6.0 (Kalendar ve ark., 2009) bilgisayar paket programından faydalanılarak kontrol edilmiştir (Tablo 1).

PCR karışımı Taq DNA Polimeraz (Ampliqon, Danimarka) kullanılarak her bir örnek için 25 µl'lik final hacimde olacak şekilde hazırlanarak, PZR cihazında analiz gerçekleştirilmiştir (T100^{TM} Thermal Cycler, Bio-Rad, ABD).Daha sona PZR ürünleri 1XTAE (Tris-Asetat-EDTA) solüsyonunda %1,5'luk agaroz jel elektroforezinde yürütüldükten sonra Safe DNA jel boyası (İnvitrogen, ABD) ile boyanarak UV altında jel görüntüleme ve analiz sistemi ile görüntülenmiştir (Vilber Lourmat, Fusion FX, Marine la Valeé, France).

Tablo 1.*A. hydrophila* a*erA* ve *hlyA*'ya ait genlerin primer dizilimleri

Table 1. Primer sequences of the gene from *A. hydrophila* a*erA* and *hlyA*

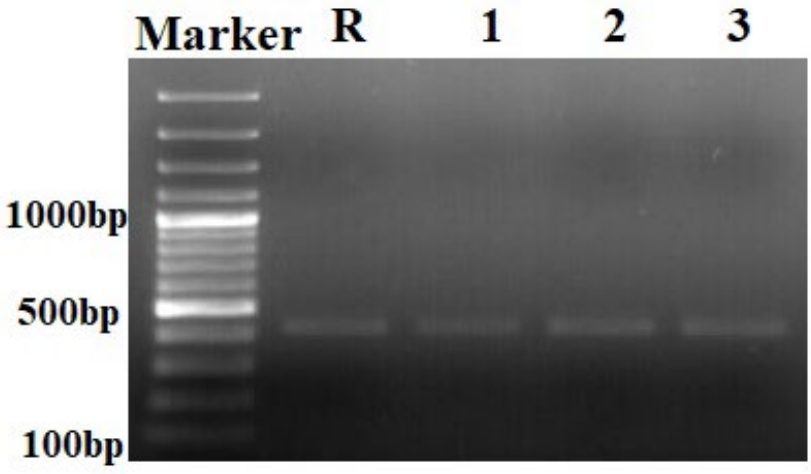
Gen		Oligonükleotid Dizisi (5'-3')	Ürün Boyutu (bç)	Gen Bankası No.
A. hydrophila (hlyA)	F	GGCCGGTGGCCCGAA-	592	JF738032.1
	R	GGCGGCGCCGGACGA-		
A. hydrophila (aerA)	F	GCCTGAGCGAGAAGGT	416	AF485772.1
	R	CAGTCCCACCCACTTC		

Antibakteriyel dirençliliğin belirlenmesi

İzole edilen bütün *Aeromonas hydrophila* (3) suşlarının sekiz adet antibiyotiğe karşı dirençlilikleri Mueller-Hinton agar kullanılarak disk diffüzyon metoduna gore araştırılmıştır. Antimikrobiyel ajan emdirilerek hazırlanmış diskler (Oxoid, Australia) kullanılmıştır. Bu amaçla Amoksisilin (25 µg), Erythromycin (5 µg) , Oksitetrasiklin (30 µg), Gentamicin (10 µg), Nalidixic Acid (30 µg), Trimetoprim Sulfametaksazol (1.25/23.75 µg), Neomisin (10 µg), Chloramphenicol (30 µg) kullanılmıştır. Sonuçlar inkübasyon sonunda tespit edilen son ölçümleri yapılarak CLSI (2014)'nin önerdiği şekilde değerlendirilmiştir.

Bulgular ve Tartışma

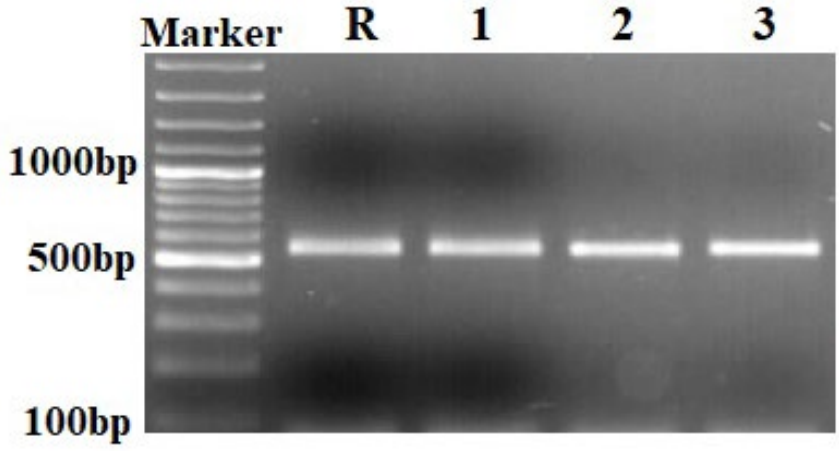
Yapılan çalışmada analize alınan 100 sığır kıyma örneğinde tespit edilen 3 *Aeromonas* türünün tamamı *A. hydrophila* olarak identifiye edilmiş ve VITEK® 2 Compact ile de tanılanmıştır. Ayrıca şuşların PZR ile a*erA* ve *hlyA* gen varlığı yönünden yapılan analizde izole ve identifiye edilen üç bakterinin de *aerA* (Resim 1) ve *hlyA* (Resim 2) genlerinin her ikisine de sahip olduğu tespit edilmiştir.



R: *A. hydrophila* (ATTC), 1-2-3 Pozitif Örnek

Resim 1. aerA Geni Pozitif *Aeromanas hydrophila*

Figure 1. *aerA* Gene Positive *Aeromonas hydrophila*



R: *A. hydrophila* (ATTC), 1-2-3 Pozitif Örnek

Resim 2. *hlyA* Geni Pozitif *Aeromanas hydrophila*

Figure 2. *hlyA* Gene Positive *Aeromonas hydrophila*

Yapılan çalışmada izole edilen *A. hydrophila* suşlarının aktimikrobiyel ilaç dirençlilik ve duyarlılıkları Tablo 2'de gösterilmiştir. Analizlere göre *A. hydrophila* suşlarının yalnız birer tanesinin Amoksisiline ve Nalidiksik asit'e dirençli olduğu tespit edilmiştir.

Kıyma örneklerinde *Aeromonas* spp. varlığına yönelik yapılan farklı çalışmalar mevcuttur. Singh, (1996) Doğu Kanada'da yaptığı çalışmada 19 sığır kıymasının 15'inde, 4 domuz kıymasının 4'ünde, 4 tavuk etinin 4'ünde, 4 hindi etinin 4'ünde ve 4 sosis örneğinin de 4 ünde olmak üzere toplam 35 örneğin 31'inden *Aeromonas spp*. saptamışlardır. Tespit edilen izolatların domuz kıymasının %97'sinden ve sığır kıymasının %87'sinden *A. hydrophila* izole etmiştir. Neyts ve ark., (2000)Belçika Flanders'te yaptıkları çalışmada 14 tane kırmızı et örneğinin 9'unda, 3 et ürününün ise 2'sinde *Aeromonas spp* elde edilmiştir. Et ve et ürünlerinden tespit edilen *Aeromonas* türlerinin %37'si *A. hydrophila,* %12'si *A. caviae* olarak identifiye etmişlerdir. Dallal ve ark., (2012) İran'da yapılan bir çalışmada rastgele perakende satış yerlerinden alınmış 158 kıyma örneğinin 27'sinden (%17) ve 92 tavuk örneğinin 53'ünden (%57.6) olmak üzere toplam 250 örneğin 80'inden (%32) *Aeromonas spp.*bulmuşlardır. *Aeromonas* türlerinin identifikasyonunda ise *A. hydrophila* %41, *A. caviae*%41, *Aeromonas sobria* %15.3, *Aeromonas jandaei* %1.8 ve *Aeromonas veronii* %0.9 oranında izole edildiği bildirilmiştir.Gowda ve ark., (2015)'nın Hindistan'da yaptıkları çalışmada, 200 adet çiğ etten (Sığır-Keçi-Domuz) 44 (%22) *Aeromonas* spp. tespit etmişlerdir. Tespit ettikleri Aeromoans türlerinin %8'i *A. hydrophila*, %9'u *A. sobria*; %6'sı *A. caviae* olarak identifiye etmişlerdir. Enany ve ark., (2013) Mısır'da yaptıkları çalışmada 50 kıyma örneğinin 8'inde (%16), 50 çiğ et örneğinin 25'inde (%50) *Aeromonas* izole etmişlerdir. Çiğ etten izole edilen 25 izolatın 12'si (%48) *A. hydrophila,* 6'sı (%24) *A.caviae*, 1'i (%16.7) *A. sobira*, 6'sı (%24) *A. schubertii* olarak; 8 kıyma örneğinden izole edilenlerin ise 3'ü (%37.5) *A.hydrophila*, 4'ü (%50) *A.caviae,* 1'i (%12.5) *A. schubertii* olarak identifiye edildiği bildirilmiştir. Benzer şekilde Mısır'da yapılan başka bir çalışmada ise Abdel-Latef, (2015) 25 kıyma örneğinin 5 tanesinde (%20) *A. hydrophila* tespit etmiştir.

Ankara'da yapılan bir başka çalışmada ise Küplülü ve ark., (2000)100 adet kıymadan 73'ünde (73%) *Aeromonas* izole etmişler; izole edilen türlerin %63'ünü *A.hydrophila, %*13.6'sını *A.sobria; %*10.9'unu *A.caviae* tespit etmişlerdir. Alişarlı ve Gökmen (2002)yaptıkları çalışmada 100'er adet sığır kıyması ve koyun kıyması örneklerinden sığır kıyma örneklerinin 55'inde (55%), *Aeromonas spp*. izole bunların 18'i *A.hydrophila*, 6 (18.75%)'sı *A. caviae* ve 8 (25%)'i *A. sobria* olarak tespit edilmiştir. Koyun kıyma örneklerinin 48'inde (48%) *Aeromonas* spp. izole edilmiş ve bunların 13 (50%)'ü *A. hydrophila*, 6 (23.07%)'sı *A. caviae* ve 7 (26.92%)'si *A.sobria* olarak identifiye edilmiştir.

Yücel ve Çitak (2003) Ankara'da yaptıkları çalışmada 59 kıymadan 40 tanesinde (67.7%) *Aeromonas spp*.'a saptamışlar ve bunların %80'ini *A.hydrophila*, %20'sini *A.sobria* olarak identifiye etmişlerdir. Arslan ve Küçüksarı, (2015) Bolu'da yaptıkları çalışmada 37 kıyma örneğinin 4 tanesinde (10,8%) *Aeromonas spp*. tespit etmişler ve izolatların tümünü *A. caviae* olarak identifiye ettiklerini bildirmişlerdir. Turgay ve Üçkardeş (2011) Kahramanmaraş'ın değişik semtlerindeki kasap ve marketlerden alınan 11 koyun ve 39 sığır kıyması örneğini *Aeromonas spp*. varlığı yönünden incelemişlerdir. Çalışmada, 11 koyun kıyma örneğinin 1'inden (%9.1), 39 sığır kıyma örneğinin 10'undan (%25.6) *Aeromonas*spp. izole etmişlerdir. Sığır kıyma örnekerinden tespir edilen Aeromonas türlerinin 6'sının (%60) *A. hydrophila,* 4'ünün (%40) *A. caviae*olduğunu; koyun kıyma örneğinden tespit edilen bir türün ise *A. hydrophila* olduğunu identifiye etmişleridir.

Yapılan bu çalışmadaidentifiye edilen *Aeromanas* spp. seviyesi genel olarak diğer araştırmacıların bulgularından düşükorana sahiptir. Aynı zamanda bizim çalışmamızda sadece *A. hydrophila* identifiye edilirken diğer çalışmalarda farklı türlerde identifiye edilmiştir. Yapılan çalışmalar arasındaki oransal farklılıklar, analize alınan örnek sayılarına bağlı olmakta birlikte ürünlerin üretim hijyenine, ham madde kalitesine, kontaminasyon durumuna, analizlerde ve onaylamalarda kullanılan metot farklılıklarına bağlı olarak şekillenmiş olabilmektedir. Ayrıca *Aeromonas* türleri denizler ve tatlı suların yanı sıra lağım, nehir, kuyu, termal sular, klorlanmış ve klorlanmamış içme suları ile kaynak sularında bulunabilmektedir (İşleyici ve Sancak, 2009). Bu nedenle kıyma örneklerinde *A. hydrophila* tespit edilemesin de işletmelerde karkas yıkama veya alet, ekipman ve çevre temizliğinde kullanılan sulardan karkas kontaminasyonu şekillenmiş olabilmektedir. Son yıllarda, tüm dünyada antibakteriyel ilaç dirençinin yaygınlaşmasına bağlı olarak enfeksiyonların tedavisinde problemler yaşanmaktadır (Onuk ve ark., 2015). Nitekim yapılan bu çalışmada *A. hydrophila* suşlarının birer tanesinin Amoksisiline ve Nalidiksik asit'e dirençli olduğu tespit edilmiştir. İdentifiye edilen suşların antibiyotik dirençliliği düzensiz antibiyotik kullanımına bağlı olabilir.

Sonuç

Sonuç olarak, analize alınan sığır kıyma örneklerinde 3 adet (%3) *Aeromonas* spp. izole edilmiştir. İzole edilen *Aeromanas* spp'nin tamamı (%3) *A. hydrophila* olarak identifiye edilmiştir. İzole edilmiş ve tanılanmış *A. hydrophila* suşlarında a*erA* ve *hlyA* genleri tespit edilmiştir. Bundan dolayı çiftlikten çatala tüm kırmızı et üretim aşamalarda gerekli hijyen

tedbirlerin alınması, kesim, parçalama, kıyma, muhafaza işlemlerine dikkat edilmesi; üretimde ve temizlikte kullanılan su kalitesine önem verilmesi, ürünlerin yeterli ısıl işlem uygulanarak tüketilmesi, antibiyotik uygulamalarının kontrollü olarak yapılması, yasal arınma sürelerine dikkat edilmesi, son olarak tüketicilerin bilgilendirilmesi ile periyodik kontrollerin yapılması önerilmektedir.

Etik Standart ile Uyumluluk

Çıkar çatışması: Yazarlar bu yazı için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan etmişlerdir.

Etik izin: Bu çalışma "Hayvan Deneyleri Etik Kurullarının Çalışma Usul ve Esaslarına Dair Yönetmelik" Madde 8 (k) gereği HADYEK iznine tabi değildir.

Finansal destek: Bu çalışma, Afyon Kocatepe Üniversitesi Bilimsel Araştırma Projeleri Koordinasyon Birimi tarafından 18.KARIYER.236 proje numarası ile desteklenmiştir.

Teşekkür: -

Açıklama: VIII. Ulusal/II. Uluslararası Veteriner Gıda Hijyeni Kongresinde (2019) özet bildiri olarak sunulmuştur.

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 7(1), 21-30 (2021) • https://doi.org/10.3153/FH21003 Research Article

Antibacterial and antioxidant activity of pulp, peel and leaves of *Feijoa sellowiana*: Effect of extraction techniques, solvents and concentration

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Cite this article as:
Karslı, B. (2021). Antibacterial and antioxidant activity of pulp, peel and leaves of *Feijoa* sellowiana: Effect of extraction technques, solvents and concentration. *Food and Health,* 7(1), 21-30. **https://doi.org/10.3153/FH21003**

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Submitted: 14.08.2020
Revision requested: 11.09.2020
Last revision received: 14.09.2020
Accepted: 14.09.2020
Published online: 07.11.2020

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Available online at
http://jfhs.scientificwebjournals.com

ABSTRACT

The present study evaluated the effect of extraction techniques (ultrasound-assisted extraction (U) and shaking water bath extraction (WB)), solvents (ethanol, acetone and distilled water) and concentration (2.5% and 5%) on total phenolic content (TPC), antibacterial and antioxidant activities of extracts obtained from feijoa leaves, peel and pulp. The antibacterial activity of feijoa extracts were tested in vitro against 6 pathogens bacteria by the disc diffusion method and the antioxidant activity was evaluated by 2,2 diphenyl-1-picrylhydrazyl radical assay. The results indicated that leaves and peel extracts exhibited stronger antibacterial activity than that of pulp. In generally, WB-A5%, WB-W5%, U-A5% and U-W 5% extracted with acetone (A) and water (W) at 5% concentration from feijoa leaves, peel and pulp showed more antibacterial activity against all tested pathogen bacteria. The leaves, peel, and pulp extracts had high antioxidant activity with 85.78-90.82%, 89.86-91.60%, and 81.49-91.31%, respectively. Peel extracts had slightly higher antioxidant activity than leaves and pulp extracts. TPC of leaves, peel, and pulp extracts were in the range of 488.99-554.00, 349.17-517, and 115.64-345.46 mg gallic acid equivalents (GAE)/100 g of extract. The overall findings suggested that different part of feijoa (especially leaves and peel) could be used as a natural antibacterial and antioxidant for functional foods.

Keywords: Antioxidant activity, Antibacterial activity, Plant extract, Total phenolic content, *Feijoa sellowiana*

Introduction

In the last decades, there has been a growing interest in the use of natural additives in foods and cosmetics worldwide due to the growing concern among consumers about potential toxicological effects of synthetic antioxidants (Chew et al., 2012). Plants are one of the most important sources of natural additives because of their antioxidant and antimicrobial agents (Basile et al., 1997). Also, plants are important part of the human diet and they have been used for thousands of years in traditional medicine and to enhance the flavor, aroma and color of foods (Neilsen and Rios, 2000). Due to these important properties of plants, scientific studies are ongoing to search for new antioxidants and antimicrobial substances from various plant sources.

The feijoa (*Feijoa sellowiana*, synonym *Acca sellowiana*) belongs to the Myrtaceae family and commonly known as guavasteen or pineapple guava, is a subtropical species whose fruits are used for human nutrition. Feijoa is originally native to South America; it is well acclimatized to some other parts of the world such as USA, Turkey, Italy, France, Australia, New Zealand and Iran (Zhu et al., 2018). The fruit is an evergreen shrub and its pulp has sweet granular, acidulous and aromatic flesh with seeds embedded in a jelly located in the central. However, the peel is green, smooth, bitter, and sour. In addition to its sweet aromatic fruits, thanks to its pleasant taste and intense color, the petals of the flowers are being eaten, usually in sweets, salads and as dish decorations (Souza et al., 2016). Feijoa fruit is used to make ice cream, smoothie, juice, yogurt, puree, jam, wine, muffin, bread spread, chocolate, candy, smoothie, and wine (Sun-Waterhouse, 2011). Feijoa is a good source of vitamin C (28 mg/100 g), low in calories and high in minerals, iodine and fibre (Basile et al., 1997). Moreover, feijoa has high content of polyphenols, especially the flavonoids. It has been reported that *F. sellowiana* has various biological activities, such as antimicrobial, antioxidant (Basile et al., 1997; Beyhan et al., 2010; Elfarnini et al., 2018), antifungal (Vuotto et al., 2000), anti-inflammatory (Rossi et al., 2007), anticancer (Bontempo et al., 2007) and immunity-stimulating (Lapcik et al., 2005) activities due to its composition rich in antioxidants flavonoids.

Food waste is a major problem facing humanity in environmental, economic and social terms. The Food and Agriculture Organization of the United Nations (FAO, 2015) reported that 1.3 billion tonnes (represent US$ 1 trillion) of food intended for human consumption are wasted. Almost half (around 45%) of the vegetables and fruits produced all over the world are wasted and thus discarding bioactive compounds which have many health benefits, such as antioxidant, anticancer, antiviral, anti-inflammatory and others (Santos et al., 2019; Viganó et al., 2015). In this respect, in order to prevent this waste parts (such as leaves and peel) of the vegetables and fruits should be evaluated.

Different extraction techniques may be used to obtain fruit waste extracts. Water, ethanol, methanol, propanol, acetone, and ethyl acetate and their combinations are commonly used as solvents for phenolic compound extraction (Aires, 2017). The conditions for extraction in the herbal food and medical industries can influence the isolation and characterization of compounds. In fact, the variations in composition and antioxidant activity are related to technical practices in various laboratories (Yakoub et al., 2018). It is well known that the determination of polyphenolic compounds is affected by their chemical nature, the extraction technique/method used, sample molecule size and stocking time, period and conditions, as well as the assay method (Poodi et al., 2018; Tanko et al., 2005).

Although many researchers have focused on determining the antimicrobial and antioxidant activity of feijoa (Basile et al., 1997; Vuotto et al., 2000; Beyhan et al., 2010; Tuncel and Yılmaz, 2015; Mosbah et al., 2018; Santos et al., 2019) little information is available about the effect of different extraction methods and conditions on total phenolic content, antimicrobial and antioxidant activities of extracts obtained from different parts of feijoa. The aim of the present study was to obtain extracts from feijoa leaves, peel and pulp by means of different extraction techniques, solvents and extract concentrations, and to compare them in terms of total phenolic content, antibacterial and antioxidant activities.

Materials and Methods

Plant Material

The fresh feijoa (*Feijoa sellowiana*) were collected from Sürmene county of Trabzon, Turkey. The mean weight and length of feijoa were 27.72 ±4.60 g and 51.73 ±5.76 mm, respectively. The plants for extraction were segregated into three parts consisting of the pulp, peel and leaves.

Extracts Preparation

For extraction purpose, the feijoa pulp, peel and leaves were dried in a drying oven (Pol-Eko-Aparatura sp. J., Poland) at 40 °C for 24 h and finely ground (18-20 mesh) using a blade mixer to produce a powder that can pass through an 18 mesh stainless steel sieve. After the drying process, total weight of pulp, peel and leaves decreased 80.11%, 78.89% and 45.79%, respectively. All the plants parts were extracted in 50 mL beakers with different extraction techniques (ultrasound-assisted extraction and shaking water bath extraction), solvents

(ethanol, acetone and distilled water) and concentration (2.5% and 5%). The shaking water bath extraction was conducted in a shaking water bath for 24 h at 40 °C. For ultrasound-assisted extraction (UAE), the mixture was dispersed by ultrasonication using a Vibra-Cell Ultrasonic Processor (Model VC505, Sonics and Materials, Inc., USA) standard probe at 20 kHz for 20 min. All extracts were filtered through Whatman filter paper grade 1 for both extraction methods. The collected filtrate of extracts obtained with ethanol and acetone solvents was placed in a rotary evaporator under reduced pressure and controlled temperature (50 °C for acetone and 70 °C for ethanol) for evaporation to dryness to remove the solvent. The final residue was re-dissolved in water using ultrasonic bath to obtain a final concentration of 50 mL. However, the samples extracted with water were not evaporated using a rotary evaporator. All extracts were then stored at -80 ºC until analysis. Detailed information on extraction conditions and relative codes for all extracts was given in Table 1.

Table 1. Extraction conditions and relative codes for feijoa leaves, peel and pulp extracts

Sample code	Extraction Techniques	Solvent	Concentration (%)
WB-E2.5%	Shaking water bath	Ethanol (80%)	2.5
WB-E5%	Shaking water bath	Ethanol (80%)	5
WB-A2.5%	Shaking water bath	Acetone (80%)	2.5
WB-A5%	Shaking water bath	Acetone (80%)	5
WB-W2.5%	Shaking water bath	Distilled Water	2.5
WB-W 5%	Shaking water bath	Distilled Water	5
U-E2.5%	Ultrasound-assisted	Ethanol (80%)	2.5
U-E5%	Ultrasound-assisted	Ethanol (80%)	5
U-A2.5%	Ultrasound-assisted	Acetone (80%)	2.5
U-A5%	Ultrasound-assisted	Acetone (80%)	5
U-W2.5%	Ultrasound-assisted	Distilled Water	2.5
U-W5%	Ultrasound-assisted	Distilled Water	5

Determination of Total Phenolic Content

Total phenolic content of feijoa leaves, peel and pulp extracts was measured using modified method of Singleton and Rossi (1965). Briefly from the stock solution of (1 mg/mL methanol) 100 µL of the extracts were made up to 3 mL with distilled water then mixed thoroughly with 250 µL of Folin–Ciocalteu reagent for 3 min, followed by the addition of 750 µL of 20% (w/v) sodium carbonate and 900 µL distilled water. The mixture was incubated at 40 °C for 30 min in a water bath and absorbance of the reaction mixtures was measured at 760 nm. Quantification was done on the basis of the standard curve of gallic acid concentration range from 100 to 800 µg/ml ($r^2 = 0.992$). Total phenolic content calculated from the calibration curve was expressed as mg of gallic acid equivalent (GAE)/g of extract.

Antioxidant Activity

(2, 2-diphenyl-1-picrylhydrazyl (DPPH))

The radical scavenging activity of the plant extracts was tested against 2,2-diphenyl-1-picryl-hydrazyl radical following the method described by Brand-Williams et al. (1995) with slight modification. 100 µL of each plant extract was mixed with 3.9 ml DPPH working solution in test tubes. Then the mixture was vortexed and the tubes incubated in dark for 60 min. The absorbance was read at 515 nm using a spectrophotometer (Shimadzu UV-1208, Japan). A blank solution containing the same amount of methanol and DPPH was prepared and measured. All the measurements were taken in triplicate and the mean values calculated. The radical scavenging activity was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{(\text{Ablank} - \text{Aextract})}{\text{Ablank}} x100$$

Microorganism

Four Gram-negative bacteria strains (*Escherichia coli* ATCC 25922, *Salmonella enterica* ATCC 13076, *Aeromonas hydrophila* ATCC 7966 and *Aeromonas sobria* ATCC 43979) and one Gram-positive strain (*Staphylococcus aureus* ATCC 25923) were used for antibacterial activity of the extracts. The pure cultures were stored in cryogenic vials with 30% (w/w) glycerol in Tryptic Soy Broth (TSB) at -80 °C.

Antimicrobial Screening by Disk Diffusion Technique

All of the bacterial strains were grown overnight on Mueller Hinton Agar (MHA) at 37 °C. The bacteria were suspended in sterile physiologic normal saline (0.9% NACI) and adjusted to the 0.5 McFarland's standard. 20 µL of the extracts were impregnated into sterile paper discs (6 mm diameter) and the paper discs were allowed to air dry under in a laminar hood for 30 min. Then discs were placed on the MHA surface previously inoculated with a sterile swab containing a suspension of each type of microorganism. Also, deionized water-loaded disc was used as negative control. Plates were incubated at 37 °C for 24 h for *E. coli*, *S. enterica* and *S. aureus* and 30 °C for 24 h for *A. hydrophila* and *A. sobria*. The inhibition zones around the disk were measured and recorded at the end of the incubation period.

Statistical Analysis

Results were expressed as the means and standard deviations. Statistical comparisons between extracts were performed with variance (ANOVA) and TUKEY test. Differences were considered significant at $P<0.05$. Statistical analyses were conducted using JMP 5.0.1 (SAS Institute, Inc., Cary, NC, USA) software. All tests were performed in triplicate.

Results and Discussion

Antibacterial Activity

The disk diffusion method was used to determine the antibacterial effect of leaves, peel and pulp extracts of feijoa against four Gram-negative (*A. sobria, A. hydrophila, E. coli, S. enterica*) and one Gram-positive *(S. aureus)* pathogen bacteria. Antibacterial activity results of extracts from the different parts of feijoa were given in Table 2.

Table 2. Antibacterial activity against pathogen bacteria of leaves, peel and pulp extracts of *Feijoa sellowiana*

Microorganism	Extracts	Inhibitions zone (mm)		
		Leaves	Peel	Pulp
A. sobria	WB-E2.5%	NI^{E}	NI^{D}	NI^{B}
	WB-E5%	7.61 ± 0.35^{Da}	NI^{Db}	NI^{Bb}
	WB-A2.5%	7.45 ± 0.03^{Da}	NI^{Db}	NI^{Bb}
	WB-A5%	9.19 ± 0.11^{Ca}	7.69 ± 0.11^{Cb}	NI^{Bc}
	WB-W2.5%	7.42 ± 0.03^{Da}	NI^{Db}	NI^{Bb}
	WB-W5%	10.26 ± 0.11^{Ba}	9.27 ± 0.37^{Ab}	6.75 ± 0.08^{Ac}
	U-E2.5%	NI^{E}	NI^{D}	NI^{B}
	U-E5%	7.94 ± 0.07^{Da}	NI^{Db}	NI^{Bb}
	U-A2.5%	7.66 ± 0.06^{Da}	NI^{Db}	NI^{Bb}
	U-A5%	9.04 ± 0.23^{Ca}	8.36 ± 0.15^{Bb}	NI^{Bc}
	U-W2.5%	7.31 ± 0.01^{Da}	NI^{Db}	NI^{Bb}
	U-W5%	12.14 ± 0.45^{Aa}	7.92 ± 0.01^{BCb}	NI^{Bc}
A. hydrophila	WB-E2.5%	7.46 ± 0.20^{Ba}	NI^{Db}	NI^{b}
	WB-E5%	8.21 ± 0.13^{Ba}	NI^{Db}	NI^{b}
	WB-A2.5%	7.89 ± 0.56^{Ba}	NI^{Db}	NI^{b}
	WB-A5%	9.82 ± 0.13^{Aa}	7.42 ± 0.06^{Cb}	NI^{c}
	WB-W2.5%	7.36 ± 0.07^{Ba}	NI^{Db}	NI^{b}
	WB-W5%	8.20 ± 0.16^{Ba}	7.84 ± 0.01^{Ba}	NI^{b}
	U-E2.5%	NI^{C}	NI^{D}	NI
	U-E5%	NI^{C}	NI^{D}	NI
	U-A2.5%	7.51 ± 0.12^{Ba}	NI^{Db}	NI^{b}
	U-A5%	9.23 ± 0.27^{Aa}	8.24 ± 0.13^{Ab}	NI^{c}
	U-W2.5%	7.92 ± 0.04^{Ba}	NI^{Db}	NI^{b}
	U-W5%	9.39 ± 0.35^{Aa}	7.88 ± 0.11^{Bb}	NI^{c}
E. coli	WB-E2.5%	NI^{E}	NI^{D}	NI
	WB-E5%	7.61 ± 0.04^{CDa}	NI^{Db}	NI^{b}
	WB-A2.5%	7.56 ± 0.04^{Da}	NI^{Db}	NI^{b}
	WB-A5%	8.83 ± 0.22^{Aa}	6.82 ± 0.04^{Cb}	NI^{c}
	WB-W2.5%	NI^{E}	NI^{D}	NI
	WB-W5%	8.09 ± 0.29^{BCa}	NI^{Db}	NI^{b}
	U-E2.5%	NI^{E}	NI^{D}	NI
	U-E5%	NI^{E}	NI^{D}	NI
	U-A2.5%	NI^{E}	NI^{D}	NI
	U-A5%	8.31 ± 0.01^{Ba}	7.81 ± 0.15^{Ab}	NI^{c}
	U-W2.5%	7.52 ± 0.14^{Da}	NI^{Db}	NI^{b}
	U-W5%	8.87 ± 0.15^{Aa}	7.51 ± 0.07^{Bb}	NI^{c}
S. enterica	WB-E2.5%	7.43 ± 0.03^{Da}	NI^{Db}	NI^{Bb}
	WB-E5%	7.79 ± 0.11^{CDa}	NI^{Db}	NI^{Bb}
	WB-A2.5%	8.26 ± 0.55^{BCDa}	NI^{Db}	NI^{Bb}
	WB-A5%	9.63 ± 0.41^{Aa}	8.42 ± 0.07^{Bb}	6.98 ± 0.23^{Ac}
	WB-W2.5%	7.47 ± 0.17^{Da}	NI^{Db}	NI^{Bb}
	WB-W5%	7.88 ± 0.42^{CDb}	9.88 ± 0.13^{Aa}	7.01 ± 0.13^{Ab}
	U-E2.5%	NI^{Ea}	NI^{Da}	NI^{Ba}
	U-E5%	NI^{Ea}	NI^{Da}	NI^{Ba}
	U-A2.5%	7.65 ± 0.09^{CDa}	7.68 ± 0.10^{Ca}	NI^{Bb}
	U-A5%	8.94 ± 0.06^{ABa}	8.31 ± 0.07^{Bb}	6.83 ± 0.01^{Ac}
	U-W2.5%	8.11 ± 0.16^{BCDa}	7.59 ± 0.04^{Ca}	NI^{Bb}
	U-W5%	8.56 ± 0.16^{BCa}	7.83 ± 0.05^{Cb}	6.96 ± 0.08^{Ac}

Table 2 continuing

S. aureus	WB-E2.5%	NI^{D}	NI^{C}	NI^{B}
	WB-E5%	7.67±0.06^{Ca}	NI^{Cb}	NI^{Bb}
	WB-A2.5%	7.54±0.17^{Ca}	NI^{Cb}	NI^{Bb}
	WB-A5%	9.52±0.04^{Ba}	7.38±0.11^{Bb}	NI^{Bc}
	WB-W2.5%	NI^{D}	NI^{C}	NI^{B}
	WB-W5%	8.29±0.22^{Ca}	7.97±0.24^{Aa}	NI^{Bb}
	U-E2.5%	NI^{Da}	NI^{Ca}	6.45±0.25^{Ab}
	U-E5%	NI^{Da}	NI^{Ca}	6.35±0.03^{Ab}
	U-A2.5%	7.97±0.11^{Ca}	7.67±0.16^{ABa}	6.46±0.06^{Ab}
	U-A5%	10.14±0.47^{ABa}	7.75±0.22^{ABb}	NI^{Bc}
	U-W2.5%	8.23±0.33^{Ca}	7.33±0.01^{Bb}	NI^{Bc}
	U-W5%	10.82±0.13^{Aa}	7.74±0.25^{ABb}	6.63±0.24^{Ac}

For each pathogen, different capital superscript letters in the same column represent significant differences (P<0.05) among the different extracts in the same part of feijoa. Different lower case superscript letters in the same line represent significant differences (P<0.05) among the same extracts in the different part of feijoa. NI: No inhibition.

The antibacterial effect against *A. sobria* of the leaves extracts was higher than those of peel and pulp extracts (P<0.05). The leaves extracts except for WB-E2.5% and U-E2.5% had inhibition zone on *A. sobria.* The WB-A5%, WB-W5%, U-A5% and U-W5% extracts from peel showed antibacterial activity against *A. sobria*, however only WB-W5% extract from pulp had an antibacterial activity against *A. sobria.* According to the results, the antibacterial activity against *A. sobria* and *A. hydrophila* of extracts prepared with water and acetone were higher than ethanolic extracts which have same concentration and extract method. The extracts from the leaves against both bacteria had greater antibacterial properties than the peel and pulp extracts (P<0.05). In particular, the WB-A5%, WB-W5%, U-A5% and U-W5% extracts possessed the greatest antibacterial activity against *A. hydrophila* (P<0.05). The pulp extracts had no antibacterial activity against *A. hydrophila.* WB-A5% and U-W5% extracts from leaves showed statistically higher value than other extracts from leaves, peel and pulp (P<0.05). As shown in Table 2, the WB-A5%, WB-W5%, U-A5% and U-W5% extracts were more effective (P<0.05) in inhibiting growth of *E. coli* than other extracts from leaves, peel and pulp. No antibacterial activity against *E. coli* was determined in pulp extracts. The maximal inhibitions were observed at 5% for *S. enterica* and the maximum inhibition zone of leaves, peel and pulp extracts was determined as 9.63 mm, 9.88 mm and 7.01 mm, respectively. On the other hand, the extracts of leaves except for U-E2.5% and U-E5% exhibited the activity on *S. enterica* compared to peel and pulp extracts (P<0.05). Extracts of pulp at 2.5% concentration did not shown any antibacterial activity against *S. enterica*. The maximum inhibition zone of feijoa extracts against *S. aureus* was found in U-W5% (10.82 mm) of leaves, WB-W5% (7.97 mm) of peel and U-W5% (6.63 mm) of pulp. Only WB-A5% and WB-W5% extracts obtained from peel by water bath method had inhibitory effect on *S. aureus* while none of the extracts from pulp by water bath method exhibited the activity on *S. aureus*. According to the results, the ultrasonic method was more effective than the water bath method in preventing the growth of *S. aureus*.

This study determined that concentration of extract is an important parameter on antibacterial activity of extracts. According to the results, the 5% concentrations of all extracts were more effective than the 2.5% to inhibit the growth of pathogenic bacteria tested in the present study (P<0.05). In general, WB-A5%, WB-W5%, U-A5% and U-W5% extracts of feijoa leaves, peel and pulp on all tested pathogen bacteria were found to be comparatively higher than other extracts (P<0.05). The highest inhibition zone of the leaves, peel and pulp extracts was determined in U-W5% (12.14 mm) against *A. sobria*, WB-A5% (9.82 mm) against *A. hydrophila*, U-W5% (8.87 mm) against *E. coli*, WB-W5% (9.88 mm) against *S. enterica* and U-W5% (10.82 mm) against *S. aureus*. The pulp extracts did not exert visible effect on growth of *A. hydrophila* and *E. coli* while WB-A5%, WB-W5%, U-A5% and U-W5% extracts from pulp showed inhibitory effect against *S. enterica*. Also, the antibacterial activity of extracts from different part of feijoa against both tested Gram-positive and Gram-negative strains was determined as leaves>peel>pulp. Similarly, Phan et al. (2019) reported that the methanolic extracts from different tissues of Australian grown feijoa have the stronger antimicrobial activity than the water extracts against *E. coli*, *S. aureus* and *C. albians* and the inhibition zones of the methanolic extracts against the three microorganisms were between 11.9-23.4 mm. Also, they determined that peel extracts had higher antibacterial activity than those of pulp and whole fruit. Vuotto et al. (2000) reported that feijoa aquatic extracts showed inhibition against all bacteria strains tested and MIC of the extracts were between 1-64 mg/L. Also, they determined that Gram-negative bacteria were more sensitive to the extracts than Gram-positive bacteria. Conversely, Basile et al. (1997) reported that the antimicrobial activity of extracts from fruit (*Feijoa sel-*

lowiana, *Actinidia chinensis*, and *Aberia caffra*) was generally more active that extracts from vegetative plant parts. The mode of antimicrobial action of feijoa extracts depends on the types of bacteria with respect to the cell wall structure. Gram-positive bacteria contain an outer peptidoglycan layer, which is an ineffective permeability barrier (Baba and Malik, 2014; Karsli et al., 2019). Also, the inhibitory activity of the plant extracts against the bacteria might be due to iron deprivation or hydrogen bonding with vital proteins needed for the growth of the bacteria (Scalbert, 1991). In addition, Safari and Ahmady-Asbchin (2019) reported that the antibacterial activity of the extract could be attributed to the high content of phenols and flavonoids. In the present study, we also determined that the extracts with higher phenolic content had higher antibacterial activity against tested pathogen bacteria. In this regard, feijoa leaves and peel extracts showed stronger antimicrobial efficacy than pulp extracts, which is well associated with our observation that feijoa leaves and peel have a higher total phenolic content than pulp.

Antioxidant Activity

The antioxidant activity of leaves, peel and pulp extracts of *F. sellowiana* was evaluated by the DPPH radical scavenging method and the results are shown in Figure 1. The feijoa fruit and leaves have high levels of antioxidants since they contain high levels of polyphenols (Beyhan et al., 2010). In the present study, the extracts of the leaves, peel and pulp had DPPH radical scavenging activity between 85.78-90.82%, 89.86-91.60% and 81.49-91.31%, respectively. The peel extracts demonstrated comparatively stronger antioxidant activity compared to the leaves and pulp extracts. Similarly, Peng et al. (2019) reported that antioxidant activity of New Zealand grown feijoa peel extracts were significantly higher than the whole fruit and flesh extracts. Amarante et al. (2017) reported that the feijoa peel extracts exhibited stronger antioxidant activity than the flesh extracts. In the present study, it was observed that antioxidant activity of the feijoa extracts increased with increasing the concentration of extracts and DPPH radical scavenging activity of 5% extracts were higher than 2.5% extracts. Turkmen et al. (2006) reported that the effect of solvent type is related to polarity of the solvents and the solubility of target compounds in them. In the present study, solvent type also had significant impact on the antioxidant capacities of feijoa leaves extracts. Indeed, the extraction with water showed the lowest scavenging activity compared to the extraction with ethanol and acetone (P<0.05). In terms of extraction efficiency, no significant differences were observed between acetone and ethanol used for extraction of different parts of feijoa except for pulp (P>0.05). Tuncel and Yılmaz (2015) reported that the acetonic (80%) feijoa extracts have higher antioxidant activity than methanolic and ethanolic extracts. In this study, significant differences were also observed among the extracts from different part of feijoa and the different extracts from same part of feijoa (P<0.05). However, no statistical difference (P<0.05) was observed between the extraction methods (ultrasonic and water bath extractions). The antioxidant activity of feijoa extracts may be linked to the presence of various bioactive compounds such as polyphenols and vitamin C (Cai et al., 2006).

Total Phenolic Content

The total phenols contents (TPC) of feijoa leaves, peel and pulp extracts are presented in Table 3. Leaves extracts had significantly (P<0.05) higher TPC than the peel and pulp extracts, while pulp extracts possessed the lowest TPC. In the present study, TPC of pulp extracts was approximately two to three times less than that of the leaves and peel. Similarly, Tuncel and Yılmaz (2015) reported that TPC of the peel was approximately two - three folds than that of the flesh. The peel of feijoa fruit contains a higher total phenolic content than flesh (Amarante et al., 2017). In this study, min and max TPC of leaves, peel and pulp extracts was between 459.44-554.00, 349.17-517.19, and 115.64-345.46 mg GAE/100 g of extract, respectively. TPC of leaves extracts showed significant difference between ultrasound-assisted extraction (USE) and shaking water bath extraction (WBE) (P<0.05). TPC value of acetonic and water extracts of leaves obtained by USE were higher than those of WBE, while TPC of ethanolic leaves extracts obtained by USE was lower than those of WBE. However, TPC value of peel and pulp extracts obtained by USE were lower than those of WBE except for WB-E2.5% and WB-A2.5% of peel and WB-A2.5% of pulp. Solvents used for total phenolic extraction also significantly (P<0.05) affected the total phenolic concentration of feijoa extracts at equal volume of solvent. Water and acetone were more effective in extracting phenolic compounds from feijoa leaves, peel and pulp than ethanol. In this respect, water can preferably be used to obtain more TPC from feijoa leaves, peel and pulp than other organic solvents. In addition, the concentration of extract was effective on the TPC of feijoa extracts and TPC of 5% extracts was generally higher than 2.5% extracts. The total phenolic concentration, antioxidant and antimicrobial activities observed in extracts of feijoa and its different parts were positively correlated, which is consistent with numerous studies. Pasquariello et al. (2015) reported that TPC of 12 feijoa cultivars fruits in Italy is between 92.88-251.02 mg GAE/100 g FW). Cecilia et al. (2016) reported that TPC of fresh fruit from 14 feijoa genotypes of Uruguay is between 197–359 mg GAE/100 g, FW. Weston (2010) has reported 59 mg of TPC in 100 g of feijoa fruit. Tuncel and Yılmaz (2015) reported that TPC of feijoa flesh

extracted with ethanol, methanol and mixture was found in the range of 767 to 1856 mg GAE/100 g dw. Beyhan et al. (2010) reported that TPC of leaf, dry fruit and fresh fruit of feijoa was 68.69, 8.69 and 17.68 µg GAE/mg, respectively. Mosbah et al. (2019) reported that TPC of feijoa leaves is 948 mg/100 g extract. The results of the present study are higher than the reported values of Pasquariello et al. (2015), Cecilia et al. (2016) and Weston (2010), however lower than the values reported by Tuncel and Yılmaz (2015), Beyhan et al. (2010) and Mosbah et al. (2019). The variation of results probably due to the plant variety, extraction techniques, type of solvent, geographical condition and the fruit size.

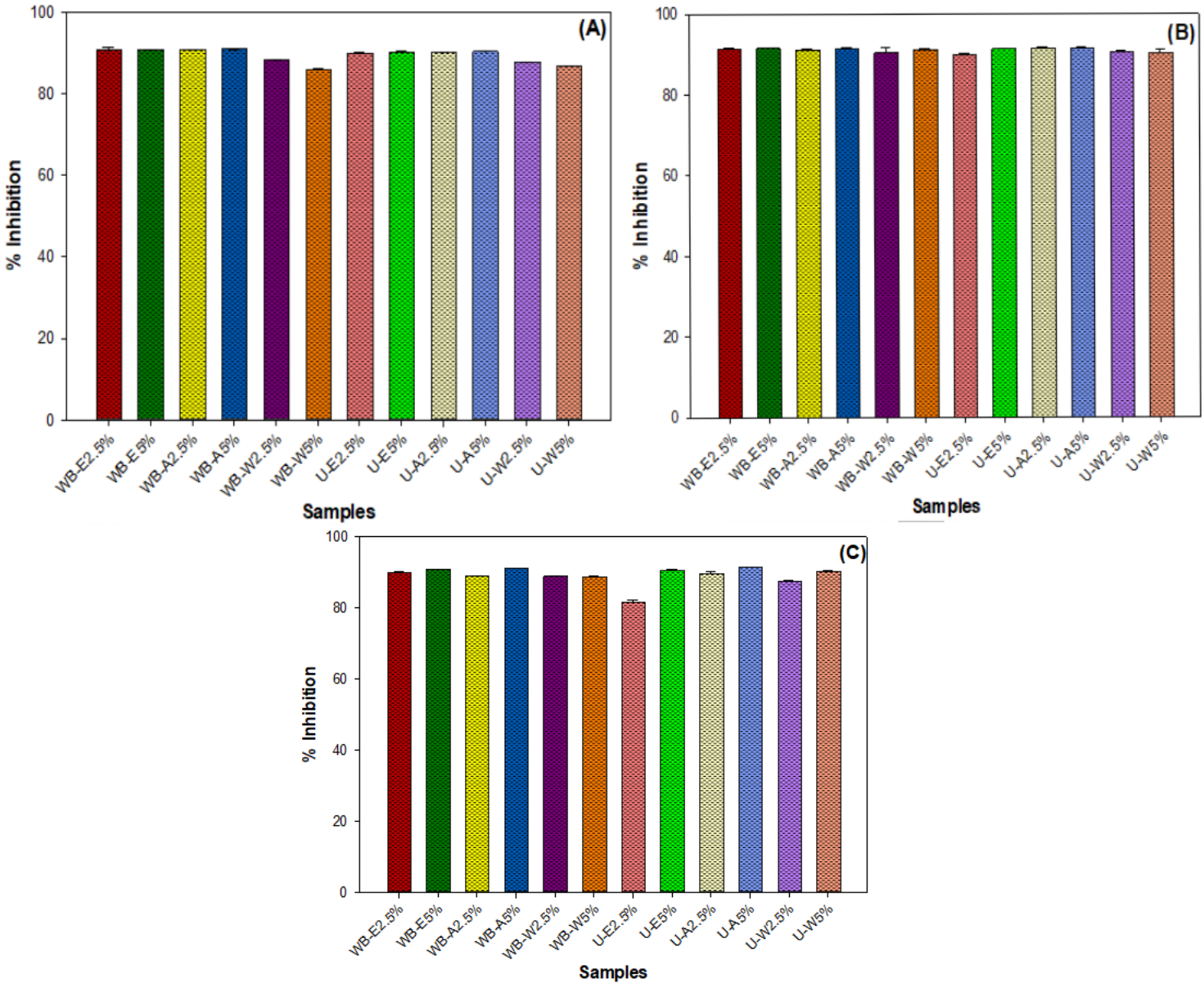

Figure 1. Antioxidant activity of feijoa leaves (A), peel (B) and pulp (C) extracted with different techniques, solvents and concentrations

Table 3. The total phenolic contents of feijoa leaves, peel and pulp extracts

Extracts	Leaves	Peel	Pulp
WB-E2.5%	$488.99\pm0.96_{F}^{a}$	$351.20\pm3.34_{E}^{b}$	$184.36\pm5.73_{DE}^{c}$
WB-E5%	$503.34\pm1.67_{DEF}^{a}$	$495.74\pm15.76_{AB}^{a}$	$276.73\pm7.88_{B}^{b}$
WB-A2.5%	$500.30\pm5.01_{EF}^{a}$	$365.72\pm11.46_{E}^{b}$	$166.13\pm13.37_{E}^{c}$
WB-A5%	$520.91\pm2.15_{BC}^{a}$	$511.62\pm9.55_{A}^{a}$	$327.22\pm10.99_{A}^{b}$
WB-W2.5%	$492.03\pm8.12_{F}^{a}$	$517.19\pm12.18_{A}^{a}$	$222.70\pm0.24_{C}^{b}$
WB-W5%	$507.23\pm5.73_{CDE}^{a}$	$514.99\pm3.34_{A}^{a}$	$345.46\pm10.99_{A}^{b}$
U-E2.5%	$460.11\pm1.19_{G}^{a}$	$411.65\pm2.87_{D}^{b}$	$115.64\pm1.67_{F}^{c}$
U-E5%	$459.44\pm1.67_{G}^{a}$	$349.17\pm8.12_{E}^{b}$	$202.94\pm5.25_{CD}^{c}$
U-A2.5%	$503.17\pm1.91_{DEF}^{a}$	$438.16\pm3.10_{CD}^{b}$	$177.61\pm5.25_{DE}^{c}$
U-A5%	$530.19\pm2.39_{B}^{a}$	$492.54\pm6.93_{AB}^{b}$	$263.90\pm5.01_{B}^{c}$
U-W2.5%	$516.18\pm1.67_{BCD}^{a}$	$468.39\pm0,88_{BC}^{b}$	$162.24\pm2.15_{E}^{c}$
U-W5%	$554.00\pm3.58_{A}^{a}$	$442.72\pm18.63_{CD}^{b}$	$271.50\pm6.21_{B}^{c}$

Different capital superscript letters in the same column represent significant differences ($P<0.05$) among the different extracts in the same part of feijoa. Different lower case superscript letters in the same line represent significant differences ($P<0.05$) among the same extracts in the different part of feijoa

Conclusions

The results of this study indicate that all part of feijoa was found to be an effective antioxidant (ranged from 81.49 to 91.31%), however the peel extracts had slightly higher antioxidant activity compared to the leaves and pulp extracts. Also, solvent type showed significant impact on the antioxidant capacities of feijoa leaves extracts. In general, the ethanolic and acetonic extracts have slightly higher DPPH radical scavenging activity compared to the water extracts. The concentration of extract was effective on antibacterial and antioxidant activity of extracts and 5% extracts had higher antibacterial and antioxidant activity than the 2.5% extracts. Water and acetone extracts were more effective than ethanol extracts in antibacterial activity and extraction of phenolic compounds. In particular, WB-A5%, WB-W5%, U-A5% and U-W5% extracts from feijoa leaves, peel and pulp had relatively higher antibacterial activity against all pathogen bacteria tested than other extracts. The leaves extracts possess the highest total phenolic content as well as antibacterial activity. Feijoa are not only interesting sources for antioxidant and antibacterial activities but also potential sources of rich phenolic compounds. Total phenolic content of leaves and peel extracts was higher two - three folds than that of the pulp. The results suggest that feijoa leaves and peel might be used as a potential source of natural antibacterial and antioxidant agent for human health and industrial purposes.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Ethics committee approval: Author declare that this study does not include any experiments with human or animal subjects.

Funding disclosure: -

Acknowledgments: -

Disclosure: -

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 7(1), 31-38 (2021) • https://doi.org/10.3153/FH21004 Research Article

In vitro risk assessment of *Padina pavonica* (Linnaeus) (Brown algae)

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Cite this article as:
Güner, A. (2021). *In vitro* risk assessment of *Padina pavonica* (Linnaeus) (Brown algae). *Food and Health,* 7(1), 31-38.
https://doi.org/10.3153/FH21004

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Submitted: 12.05.2020
Revision requested: 01.09.2020
Last revision received: 08.09.2020
Accepted: 04.10.2020
Published online: 09.11.2020

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Available online at
http://jfhs.scientificwebjournals.com

ABSTRACT

Padina pavonica (Linnaeus) Thivy 1960 is a brown algae that is antioxidant, antimicrobial, and anticancer effects and is generally used in soup, salad, and other dishes. However, no studies have been reported on safe consumption in humans to date. For this purpose, this study was conducted to determine the cytotoxic and genotoxic effects of *P. pavonica* on lymphocytes cultured from human blood. The water extract of *P. pavonica* was added into culture tubes at various concentrations (0.5-1000 µg/mL). Cytotoxic effects were determined by MTT assay. Antioxidant/oxidant status was evaluated by total antioxidant capacity (TAC) and total oxidative status (TOS) assays. Genotoxic effects were investigated by sister chromatid exchanges and micronucleus assays. Our results showed that *P. pavonica* had no genotoxic effects, even at higher concentrations. 1000 µg/mL concentration of *P. pavonica* caused an increase ($P<0.05$) TOS levels while significantly reducing cell viability. However, low concentrations (50 and 100 µg/mL) significantly increased ($P<0.05$) TAC levels. In conclusion, *P. pavonica* can be safely consumed with its non-genotoxic and antioxidant properties in a manner dose-dependent.

Keywords: Algae, Antioxidant, Genotoxic, Oxidative stress, *Padina pavonica*

Introduction

Algae is a group of photosynthetic organisms that can be found almost anywhere on the earth, consisting of multi or single-cell organisms without root, stem, and leaf differentiation. Algae has superior survival ability despite different environmental stimuli (UV, temperature, pH, heat, etc.) in their environment (Field et al., 1998; Güner et al., 2015). These features are often associated with secondary metabolites in their structure. Numerous studies reported that active metabolites of algae have antioxidant, antimicrobial, anticancer, anticoagulant, wound healing, and anti-inflammatory activities and their significant part is used in many medicines, pharmacy, agricultural, and cosmetics products (Mohamed et al., 2012; Güner et al., 2018; Güner et al., 2019; Güner et al., 2020). Algae have been also consumed as a traditional food ingredient in many countries since ancient times thanks to amino acids, vitamins, protein, terpenoids, fatty acids, minerals, sterols, and phenolic compounds in its structure. For this purpose, open and closed algae cultivation systems have been developed to meet the needs in many countries, especially in China and Japan. In particular, wakame (*Undaria* sp.), nori (*Porphyra* sp.), and Kombu (*Laminaria sp.*) that are derived from different algae family are among the most nutritious algae foods (McHugh, 2003).

Padina pavonica L. is a brown algae from the Dichtyophyceae family, is one of the common macro-algae species worldwide. Its most characteristic feature is that it has a calcareous structure and therefore it is a rich calcium carbonate deposit. Several studies revealed the antioxidant, antifungal, and antimicrobial effects of *P. pavonica* (Khaled et al., 2012; Stanojkovic et al., 2013). At the same time, *Padina* sp. is widely used in cosmetics, pharmaceutics, and medicine thanks to rich alginic acid and fucoidan ingredients. *Padina* sp. is an important food supply in coastal countries. It is especially used to add flavor to soups, salads, and fritters. Also, dried *Padina* flakes can be added are added to enrich the mineral content of many dishes such as omelet, potatoes, and salads (Pereira, 2016).

According to the literature data, over consumption of seaweeds can cause side effects such as digestive discomfort, thyroid problems, and possible exposure to heavy metals (Cherry et al., 2019). However, no information is available on the safe consumption of edible *P. pavonica*. This study was carried out to reveal whether *P. pavonica* causes cytotoxic, oxidative, and genotoxic effects on lymphocytes cultured from human blood.

Materials and Methods

P. pavonica was collected at a depth of 1-2 m, in a region of high light intensity, from the coastline of Urla, Izmir. The voucher specimen (number: 41331) was deposited in the Toxicology Laboratory of Ege University, Faculty of Science, Department of Biology. The samples were washed three times with tap water to remove salt, epiphytes, and sand attached to the surface, then carefully rinsed with fresh water, and maintained in a refrigerator at -20 °C.

Extraction

For water extraction of algae, 100 g sample was added to 500 mL distilled and boiling water using a magnetic stirrer for 15 min. Then the extracts were filtered over Whatman No. 1 paper (Güner et al., 2012).

Experimental Design

We obtained heparinized blood samples from two healthy non-smoker men, with no history of genotoxic agent exposure. Experiments were conducted with volunteer human subjects according to the Helsinki Declaration. Each blood donor was questionnaired to assess the history of exposure and signed consent forms were obtained. Approximately 4 ml of blood was collected by vein puncture from the participants on an empty stomach to minimize the potential effects of nutritional factors. Hematological and biochemical parameters were analyzed for all volunteers and no pathology was detected. Human peripheral blood lymphocyte cultures were established based on the protocol previously described by Güner et al., (2012). 3 mL of a fresh blood sample collected into an EDTA tube was transferred to a 15 ml conical centrifuge tube containing an equal amount of Histopaque-1077 (Sigma-Aldrich, St Louis, MO) and then lymphocyte cells were obtained according to the manufacturer's product protocol. Subsequently, the lymphocyte suspension (500 µL) was added to 7 ml of Chromosome Medium B (Biochrom, Leonorenstr. 2–6.D-12247, Berlin) containing 100 U/mL penicillin, 100 µg/mL streptomycin, and 0.005 µg/mL of phytohemagglutinin (Biochrom). The compounds for determining biochemical analysis and genotoxic effects were incorporated into the blood cultures following methods as mentioned below. However, mitomycin C (10^{-7} M) was used as the positive control in the cytotoxic and genotoxic assay. Hydrogen peroxide (H_2O_2) (25 µM) and ascorbic acid (10 µM) were used as the positive controls in oxidant and antioxidant analysis, respectively.

Cell Viability

MTT [3–(4, 5- dimethyl-2-thiazolyl) −2, 5-diphenyl-2H-tetrazolium bromide)] assay was set up according to a slight modification of the previous protocol (Atmaca et al., 2020). The cells were seeded at approximately 1×10^4 cells/well in a final volume of 200 µl in 96-well flat-bottom microtiter plates. After overnight incubation, cells were treated with the various concentrations (0.5, 5, 25, 50, 100, 250, 500, and 1000 µg/mL) of *P. pavonica* and incubated for 24 h at 37 °C in a 5% CO_2 incubator. At the end of incubation, 20 µL of MTT solution was added to each well and the cells were incubated for an additional 4 h. Then, the medium was removed and the formed formazan crystals were dissolved by DMSO. The amount of formazan proportional to the number of viable cells was measured by using spectrophotometer recording changes in absorbance at 570 nm (Tecan Infinite 200 PRO, Switzerland).

Total Antioxidant Capacity (TAC) and Total Oxidative Stress (TOS)

Measurements of TAC and TOS levels was carried out using commercial kits according to the manufacturer's instructions (Rel Assay Diagnostics, Gaziantep, Turkey). For these experiments, another group of cells was treated with *P. pavonica* at different concentrations (0.5-1000 µg/mL) and incubated at 37 °C in humidified 5% CO_2 for 2 hours.

Potential antioxidants in the culture medium led to the reduction of the ABTS radical (2,2'-azino-bis 3-ethyl benzothiazoline-6-sulfuric acid) in TAC analysis. Briefly, 500 µL of Reagent 1 solution was added to a quartz cuvette containing 30 µL of plasma sample and after 30 minutes, the initial absorbance was recorded at 660 nm. Then, 75 µL of Reagent 2 solution was added to the same cuvette and the absorbance was measured at 660 nm after 5 min incubation. The test was calibrated with Trolox and the obtained results were expressed in mM Trolox equivalent per liter (mmol Trolox equiv./L).

The principle of TOS assay was based on the conversion of the ferrous ion chelator complex to ferric ion by oxidants present in the medium. The TOS level was determined by mixing 500 µL of Reagent 1 with 75 µL of each plasma sample and the absorbance value of each sample was measured at 530 nm after 30 minutes. 15 µL of Reagent 2 was then added to the mixture, the absorbance was read at 530 nm again. Calibration of the assay was conducted with H_2O_2 and the results were expressed as µM H_2O_2 equivalent per liter (µmo H_2O_2 equiv./L).

Sister Chromatid Exchange (SCE) Method

5-bromo-20-deoxyuridine (Sigma, St Louis, Missouri, USA; final concentration 20 mM) was added after culture initiation to provide better visualization of SCEs (Evans and O'Riordan, 1975). Exactly 70 hours and 30 minutes after the initiation of incubations, colcemid (Sigma) was added to the cultures to obtain a final concentration of 0.5 mg/L. After hypotonic treatment (0.075 M KCl) and three repetitive cycles including fixation in methanol/acetic acid solution (3:1, v/v), centrifugation, and resuspension, the cell suspension was dropped onto chilled and grease-free microscopic slides. Then slides were air-dried, aged, and stained differently for a variety of SCE ratio according to fluorescence plus Giemsa (FPG) preparation. For each treatment, 20 well-spread second division metaphases were scored and calculated as SCEs per cell.

Micronucleus (MN) Assay

The MN test was done by adding cytochalasin B (Sigma 1; 6 mg / mL final concentration) after 44 hours of culture. After an incubation period of 72 hours, lymphocytes were fixed with ice-cold methanol: acetic acid (3:1). The cells were fixed directly on the slides using a cytospin and stained with Giemsa. The scoring criteria for micronuclei were defined by Fenech (1993). 2000 binucleated lymphocytes were screened per concentration (two cultures for each concentration) for the presence of one, two, or more micronuclei.

Statistical Analysis

Statistical analysis was performed using SPSS 18.0 (SPSS, Chicago, IL, USA). The experimental data were analyzed by one-way analysis of variance (ANOVA) and Duncan's test was performed to examine whether there were any differences between the application and control groups. The results are presented as means ± SD of at least three independent experiments and $P < 0.05$ was accepted as significant. All assays were run in triplicate.

Results and Discussion

Cell Viability

The cytotoxic effects of different concentrations of *P. pavonica* extract were evaluated by MTT assay (Figure 1). The results showed that mitomycin C, as a positive control, significantly decreased ($P<0.05$) cell viability with a fold decrease of 2.6 compared to untreated control. However, lower doses (0.5, 5, 25, 50, 100, 250, and 500 µg/mL) of *P. pavonica* did not cause ($P>0.05$) a change in cell viability while 1000 µg/mL concentration significantly inhibited ($P<0.05$) cell viability with a fold decrease of 2.6.

TAC and TOS Activity

As shown in Figures 2 and 3, ascorbic acid and H_2O_2, used as a positive control, significantly increased (P<0.05) the TAC and TOS levels with a 2.46 and 3.03-fold increase, respectively. However, only 50 (1.3-fold increase) and 100 (2-fold increase) µg/mL concentrations of *P. pavonica* led to a statistically significant increase (P<0.05) in TAC levels as compared to untreated control cells. When oxidative status after exposure treatments was investigated, the concentration of 1000 µg/mL of *P. pavonica* caused an increase (P<0.05) with a fold change of 1.4 in TOS levels.

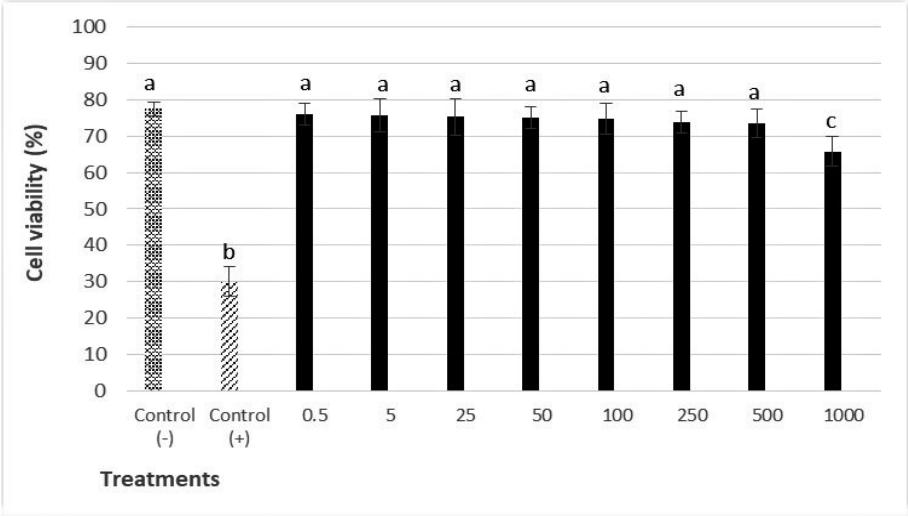

Figure 1. Effect of different concentrations of *Padina pavonica* water extract on human lymphocytes at 24 h. Values represent means ± SD of at least three experiments. Bars indicated by the different letters (a, b, c) show statistically significant differences at the P < 0.05 level. Mitomycin C (10^{-7} M) was used as a positive control.

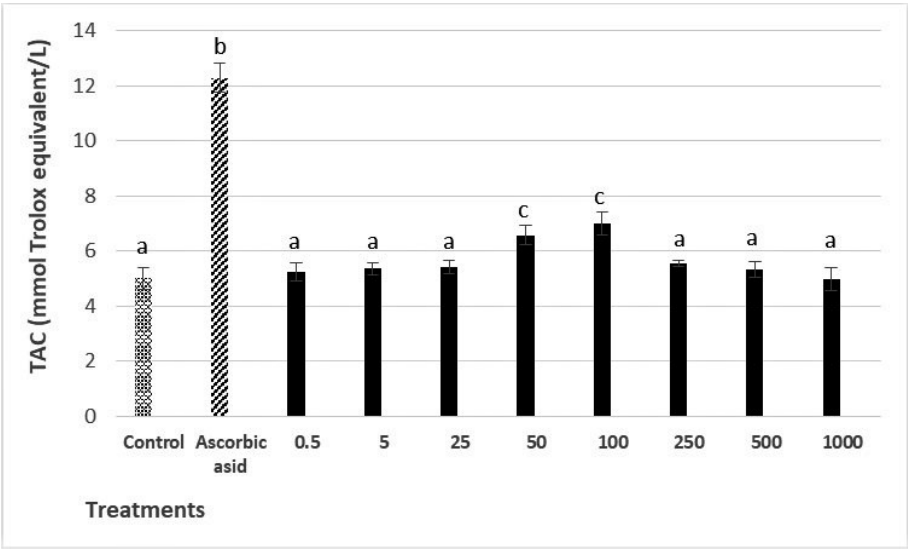

Figure 2. The TAC levels in cultured human lymphocytes exposed to various concentrations of *Padina pavonica* for 2 h. Values represent means ± SD of at least three experiments. Bars indicated by the different letters (a, b, c) show (a, b, c) statistically significant differences at the P<0.05 level. Ascorbic acid (10 mM) used a positive control.

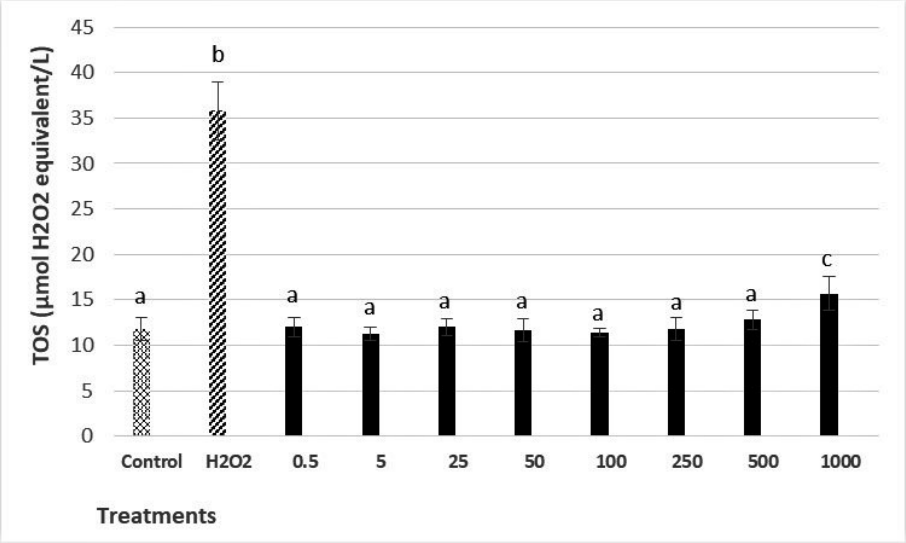

Figure 3. The TOS levels in cultured human lymphocytes exposed to various concentrations of *Padina pavonica* for 2 h. Values represent means ± SD of at least three experiments. Bars indicated by the different letters (a, b, c) show statistically significant differences at the $P < 0.05$ level. Hydrogen peroxide (H_2O_2) (25 mM) was used as a positive control.

Genotoxicity Activities

The MN and SCE frequencies on lymphocytes exposed to *P. pavonica* were depicted in Figure 4. *P. pavonica* did not induce a significant ($P>0.05$) changes in MN and SCE, even at the highest concentrations. However, mitomycin C, as a positive control, caused a significant increase ($P<0.05$) in MN and SCE ratios as compared to the untreated control.

The present study revealed for the first time cytotoxic effects of *P. pavonica* on human lymphocytes, in a dose-dependent manner. Briefly, an increase in sample dose caused a reduction in cell viability. Mashjoor et al., (2016) reported that *Padina antillarum* and *Padina boergeseni* showed cytotoxic effects in different cell lines (Vero, MCF-7, and HeLa), in a dose-dependent manner. Previous reports declared that a concentration of 50 µg/mL of *Halopteris scoparia* (brown algae) significantly inhibited viability in HEK 293 cells, in accordance with our findings (Güner et al., 2019). Another study showed that hexane, chloroform, and methanol extracts of *Sargassum swartzii* and *Cystoseira myrica* brown algae exerted cytotoxic effects in CaCo-2 and T47D while *Colpomenia sinuosa* did not cause any cytotoxicity on these cell lines (Khanavi et al., 2010). *Cystoseira compressa* extracts had no significant cytotoxic activity against Hep 3B cells in all treated concentrations (Güner et al., 2015). These different cytotoxic activities may be related to the extraction/solvent type used and the different sensitivity of the cells.

In a normal cellular process, there is a balance between antioxidant and oxidant status. When cellular damage is induced by different agents, this situation causes an increase in oxidative radical levels and consequently, many dramatic events occur for the cell. For this purpose, oxidative changes in lymphocytes after exposure to *P. pavonica* were determined by TAC and TOS tests. The major advantage of these assays is to measure all the antioxidant/oxidant capacity in the medium and not just the oxidant/antioxidant level of a compound in a culture sample (Kusano and Ferrari 2008). Lower concentrations (50 and 100 µg/mL) of *P. pavonica* led to a statistically significant increase in TAC levels as compared to untreated control cells. In other words, the algae sample at the lower dose acted as an antioxidant agent. Similarly, many studies provided the antioxidant activity of algae species. Al-Enazi et al., (2018) reported that *P. pavonica* extracts had an excellent antioxidant activity with a value of IC50 = 5.59 µg/mL, in a concentration-dependent manner. In another study comparing the biological effects of different algae samples, *P. pavonica* showed the highest antioxidant activity (Khaled et al., 2012). Previous studies have shown a highly significant correlation between antioxidant activity and polar contents such as polysaccharides, ketones, amines, phenols, aldehydes

in plants (Roopashree and Naik, 2019). The antioxidant effects of *P. pavonica* may be explained by the presence of secondary metabolites in the water extract. On the other hand, *P. pavonica* (at 500 µg/mL and below concentrations) did not cause any change in TOS levels while 1000 µg/ml treatment significantly increased TOS levels in lymphocytes as compared to control. Thus, the cytotoxic effects of *P. pavonica* could be attributed, at least in part, to oxidative stress induced by high algae contents.

When oxidative stress occurs, the evaluation of damages in DNA is one of the most important outcomes. To this end, whether the oxidative stress triggered by *P. pavonica* causes genetic damage was evaluated by the SCE and MN methods. SCE is considered to be a very simple and sensitive cytogenetic assay for evaluating the genotoxic effects of potentially mutagenic and carcinogenic agents (Das 1988). The MN assay is also a very sensitive and rapid method that can detect both clastogenic and aneugenic effects of agents (Migliore et al. 1989). Our results showed that *P. pavonica* was non-genotoxic. In other words, the algae sample did not cause any significant increases in the levels of the SCE and MN in lymphocytes as compared to control values, even at the highest concentrations. A previous study conducted by bacterial Vitotox® test and micronucleus assay reported that *Dictyopteris membranacea* (brown algae) did not cause any genotoxic effects in human C3A cells, even at the highest concentrations (Akremi et al., 2016). Similarly, another study related to the genotoxic effects of algae species declared similar results that algae species did not cause any clastogenic and DNA disrupting effects in mice bone marrow erythrocytes at the highest dose of 2000 mg/kg body (Bello et al., 2019). Sulfated polysaccharides from brown algae are one of the potential compounds used in medical applications. Previous studies have reported that fucoidan obtained from different algae species have no genotoxic effect in vivo and in vitro assay (Kim et al., 2010; Song et al., 2012).

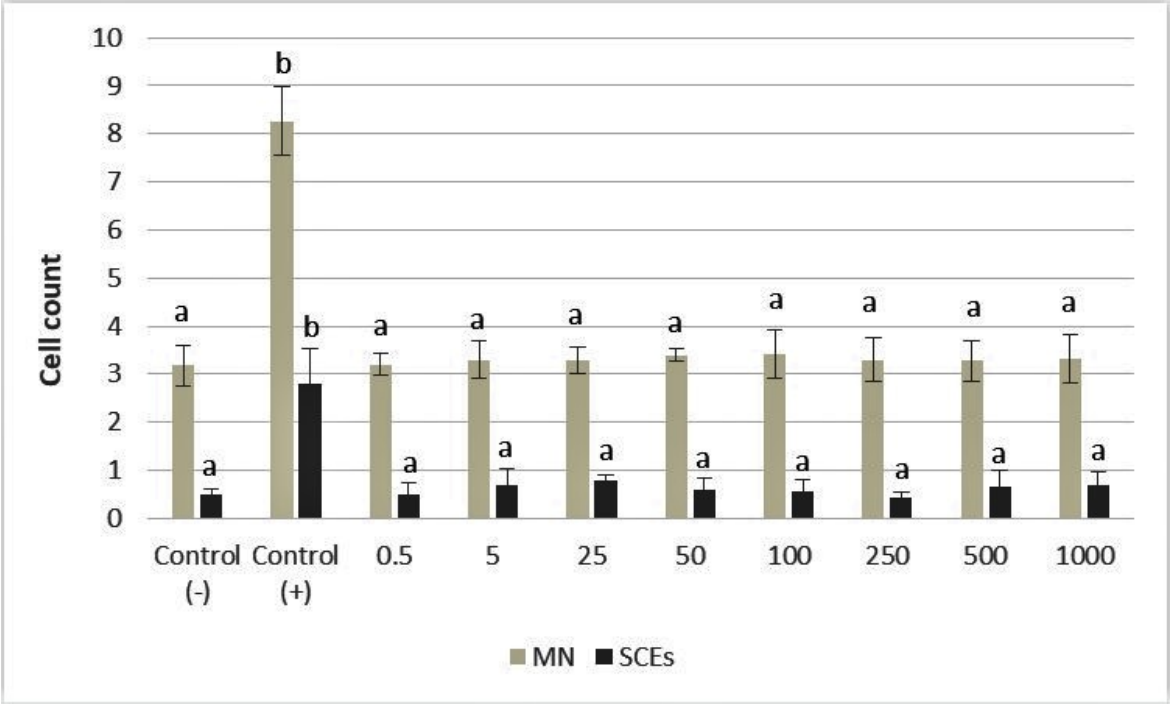

Figure 4. The frequencies of micronucleus (MN) and sister chromatid exchange (SCEs) values in human lymphocyte treated with various concentrations of *Padina pavonica* for 72 h (Positive control: Mitomycin C (10^{-7} M). Values represent means ± SD of at least three experiments. Bars indicated by the different letters (a, b, c) show statistically significant differences at the $P<0.05$ level.

Conclusions

In conclusion, the present results clearly showed that *P. pavonica* had no genotoxic effects on lymphocytes. Furthermore, this algae sample exhibited antioxidant properties dependent on the applied concentration. In this context, *P. pavonica* has the potential of being utilized as both novel bioresources and safely consumed.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Ethics committee approval: Author declare that this study does not include any experiments with human or animal subjects.

Funding disclosure: -

Acknowledgments: -

Disclosure: -

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 7(1), 39-44 (2021) • https://doi.org/10.3153/FH21005 Short Communication

Enteral beslenme ürünlerinin mezofilik aerobik bakteri ve *Cronobacter sakazakii* kontaminasyonu yönünden incelenmesi

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Cite this article as:
Omurtag Korkmaz, B.İ., Aydın, A., Kılıç, N., (2021). Enteral beslenme ürünlerinin mezofilik aerobik bakteri ve *Cronobacter skazakii* kontataminasyonu yönünden incelenmesi. *Food and Health,* 7(1), 39-44. https://doi.org/10.3153/FH21005

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Submitted: 29.06.2020
Revision requested: 24.10.2020
Last revision received: 07.11.2020
Accepted: 08.11.2020
Published online: 25.11.2020

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Available online at
http://jfhs.scientificwebjournals.com

ÖZ

Bu çalışmada Türk Gıda Kodeksi'nde özel tıbbi amaçlı diyet gıdalar altında yer alan enteral beslenme ürünlerinin Mezofilik Aerobik Bakteri ve *C.sakazakii* varlığı yönünden analizi yapılarak pH değerlerinin incelenmesi amaçlanmıştır. İstanbul ilindeki çeşitli hastane eczanelerinden 20 adet enteral beslenme ürünü temin edilerek petriye yayma plak yöntemiyle uygun besiyerlerine ekim yapılmıştır. Mikrobiyolojik analiz sonuçlarına göre örneklerin hiçbirinde MAB ve *C.sakazakii*'ye rastlanmamıştır (%100; <1 log kob/mL). Ürünlerin ortalama pH değeri 6.68 ±0.09 olarak bulunmuştur. Araştırma sonucunda incelenen enteral beslenme ürünlerinin mikrobiyolojik kontaminasyon düzeylerinin tamamının (%100) kullanıma uygun değerler içerisinde olduğu ve sağlık riski oluşturmadığı görülmüştür.

Anahtar Kelimeler: Enteral beslenme ürünleri, *Cronobacter sakazakii*, Mezofilik aerobik bakteri, pH

ABSTRACT

Investigation of enteral nutrition products for Mesophilic Aerobic Bacteria and *Cronobacter sakazakii* contamination

In this study, it is aimed to analyze for the presence of Mesophilic Aerobic Bacteria (MAB), *C.sakazakii,* and the pH values of the enteral nutrition products, which are categorized under the special medicinal dietary products in the Turkish Food Codex. 20 enteral nutrition products were obtained from various hospital pharmacies in the province of Istanbul, and the spread plate method was carried out on suitable media. According to the results of microbiological analysis, no MAB and *C.sakazakii* were found in any of the samples (100%; <1 log cfu/mL). The average pH of the products was found to be 6.68 ±0.09. As a result of the research, it has been seen that all the microbiological contamination levels (100%) of enteral nutrition products are within the values suitable for use and do not pose a health risk.

Keywords: Enteral nutrition products, *Cronobacter sakazakii*, Mesophilic aerobic bacteria, pH

Giriş

Enteral beslenme ürünleri; Türk Gıda Kodeksi'nde özel tıbbi amaçlı diyet gıdalar altında yer alan, içeriği tanımlanmış, vücudun gereksinim duyduğu besin ögelerini karşılayan, medikal kullanıma yönelik hazırlanmış, oral veya tüple beslenme yöntemleriyle uygulanabilen çoğunlukla süt temelli formulasyona sahip ürünlerdir (TGK, 2001; Önal ve Uğurcan, 2017). İçeriğinde protein kaynağı olarak süt kazeini bulundurması sebebiyle bu tip ürünlerin süt tozu ve peynir altı suyu tozu gibi düşük su aktivitesi (a_w) değerlerinde hayatta kalabilen bir bakteri olan *Cronobacter sakazakii* (*C.sakazakii*) açısından risk taşıyabileceği belirtilmektedir (Beuchat ve ark., 2009).

C.sakazakii, Enterobacteriaceae familyasında yer alan gramnegatif, spor oluşturmayan, fakültatif anaerobik özellikteki bir fırsatçı patojendir (Koluman, 2011). Bu bakteri daha önce *Enterobacter sakazakii* olarak adlandırılmış, ancak yapılan yeni sınıflandırma sonucu FAO/WHO komitesi tarafından *Enterobacter sakazakii* yerine *C.sakazakii*'nin kullanımının gerektiği belirtilmiştir (FAO/WHO, 2008). Türk Gıda Kodeksi, Mikrobiyolojik Kriterler Tebliği'nde de 2011 yılına kadar *Enterobacter sakazakii* (*E. sakazakii*) olarak ifade edilirken 2011 yılında tebliğde yapılan düzenlemeler sonucu *C. sakazakii* adıyla kullanılmaya başlanmıştır (TGK, 2011). Son zamanlarda, moleküler karakterizasyonların yaygınlaşmasıyla *Cronobacter* cinsine yeni türler de eklenmiştir. Heperkan ve ark.'nın süt tozu, peynir altı suyu tozu ve bebek formüllerinde moleküler tanımlama ile yapmış olduğu araştırmada *C.sakazakii* %6.14 oranında tespit edilmiştir (Heperkan ve ark., 2017).

Cronobacter sakazakii, bebeklerde, çocuklarda ve yetişkinlerde, özellikle yaşlı ve bağışıklığı baskılanmış bireylerde enfeksiyonlara neden olan bir patojendir. Hastalık, dünya çapında yenidoğan ve küçük bebeklerde yüksek mortalite oranına sahip olması nedeniyle önemlidir (Friedemann, 2009). Menenjit, bakteremi, sepsis ve nekrotizan enterokolit gibi şiddetli yenidoğan enfeksiyonlarında rol oynamıştır (Patrick ve ark., 2014). Yetişkinlerde, *Cronobacter* sp. pnömoni, septisemi, osteomiyelit, dalak apsesi ve yara enfeksiyonlarına neden olabilmektedir (Healy ve ark., 2010).

Özel tıbbi amaçlı kullanılan enteral beslenme ürünlerinin mikrobiyolojik açıdan steril ve tüketime uygun olması gerekir. Özellikle kritik hastalarda düşük bağışıklık sistemi, immun yanıtın yetersiz olması, en ufak bir mikrobiyolojik kontaminasyonda hastanın yaşamını tehlikeye sokabilir ve mevcut durumun kötüleşmesine sebep olabilir (Aslantaş ve Yıldız, 2008). Türk Gıda Kodeksi Mikrobiyolojik Kriterler Tebliği'nde özel tıbbi amaçlı diyet gıdalar için belirtilen şekilde kontrol edilmesi gereken mikroorganizmalar olarak *Bacillus cereus* (*B.cereus*), *Cronobacter sakazakii* (*C.sakazakii*), *Salmonella* ve *Listeria monocytogenes* (*L.monocytogenes*) yer almaktadır (TGK, 2011). Teknolojik uygulamalarla önlenmeye çalışılsa da kurutma ve ısıl işlem basamaklarına karşı dayanarak iki yıl boyunca süt tozunda hayatta kalabilen *C.sakazakii*'nin enteral beslenme ürünlerinde de izlenmesi gerekmektedir (Beuchat ve ark., 2009).

Bu sebeplerle planlanan çalışmada İstanbul ili içerisinde bulunan hastane eczanelerinden temin edilen enteral beslenme ürünleri Mezofilik Aerobik Bakteri (MAB) ve *C.sakazakii* kontaminasyonu yönünden incelenmiş, pH değerleri ölçülmüştür. Elde edilen sonuçlar Türk Gıda Kodeksi kriterleri ile karşılaştırılarak değerlendirilmiştir.

Materyal ve Metot

Örnek Toplama

Bu çalışmada Türkiye'deki farklı firmalar tarafından ithal edilmiş tüp, oral ve hem tüp hem de oral yolla kullanılabilen enteral beslenme ürünlerinin Mezofilik Aerobik Bakteri (MAB) ve *C. sakazakii* yönünden mikrobiyolojik analizi gerçekleştirilmiştir. İstanbul ili içerisinde Anadolu ve Avrupa yakalarında bulunan hastane eczanelerinden temin edilen toplam 20 adet enteral beslenme ürünü Mart-Haziran 2020 ayları arasında toplanarak analize alınmıştır.

Mikrobiyolojik Analiz

Enteral beslenme ürünlerinden, MAB ve *Cronobacter sakazakii* bulunma düzeyleri petriye ekim yapılarak incelenmiştir. MAB analizi için Plate Count Agar besiyerine 0.1 mL numune alınarak yayma plak yöntemiyle ekim gerçekleştirilmiştir. Petriler 37°C'de 48 saat aerobik olarak inkübe edilmiştir. *C.sakazakii* analizi için ise numunelerden 1 mL alınarak 9 mL Enterobacteriaceae Enrichment (EE) Broth içeren önzenginleştirme besiyeri tüplerine aktarılmıştır. Tüpler 37°C'de 24 saat aerobik olarak inkübe edilmiştir. Daha sonra kromojenik Brillance *Enterobacter sakazakii* DFI Agar'a yayma plak yöntemiyle ekilerek 37°C'de 20 sa inkubasyona bırakılmıştır. Şüpheli koloniler gram boyama ile mikroskop altında incelenmiştir. Uygulanan yöntem ve kullanılan besiyerleri referansları ile Tablo 1'de yer almaktadır.

pH ve Sıcaklık Ölçümü

Ürünlerin pH ve sıcaklık değerleri Milwaukee pH/temp marka pHmetre ile 3 tekrarlı olarak ölçülerek kaydedilmiştir.

Tablo 1. Çalışmada mikrobiyolojik analizler için kullanılan metotlar

Table 1. Methods used in the study for microbiological analysis

Araştırılan bakteri	Metot	Kaynak
MAB	Plate Count Agar (Lab M) besiyerinde 37˚C 24 sa aerobik inkubasyon.	ISO, (2003)
Cronobacter sakazakii	1 mL örnek ile 9 mL Enterobacteriaceae Enrichment Broth (Oxoid Thermo Fischer, UK) suspansiyonunu 37°C'de 24 sa. inkubasyon. Kromojenik Brillance *E.sakazakii* DFI Agar (Oxoid Thermo-Fischer, UK)'a ekim ve 37°C'de 20 sa inkubasyon. Yeşil-turkuaz renkli kolonilerden saf koloni izolasyonu için *E.sakazakii* DFI Agar'a yeniden ekim ve 37°C'de 20 sa inkubasyon. Her örnekten 3 şüpheli izolat seçilerek saflık düzeyinin belirlenmesi amacıyla mavi-yeşil renkli şüpheli kolonilerden Tryptone Soya Agar (Oxoid Thermo Fischer, UK)'a ekim.	Çetinkaya ve ark., (2013)

Veri Analizi

Excel (MS Office, 2013) kullanılarak elde edilen bulguların ortalaması alınmış, standart sapmaları ve kontaminasyon sıklıkları yüzde değerler üzerinden hesaplanmış ve One way Anova analizi ile ürünlerin pH değerleri arası olası farklılık karşılaştırılmıştır.

Bulgular ve Tartışma

Bu araştırmada 5 adet tüp ürünü, 7 adet oral ürün, 8 adet hem tüp hem de oral olarak kullanılabilen enteral beslenme ürünü incelenmiştir. İncelenen 20 üründe MAB ve *C. sakazakii* kontaminasyonlarına rastlanmamıştır (%100, <1 log kob/mL). Ürünlerin ortalama pH ve sıcaklık değerleri ise sırasıyla 6.68 ve 23.89°C olarak belirlenmiştir (Tablo 2). Ürünlerin kullanım yolu açısından pH değerleri karşılaştırıldığında istatistiki olarak herhangi bir farklılığın olmadığı görülmüştür ($p>0.05$; Tablo 3).

Tablo 2. Mikrobiyolojik analiz sonuçları (log kob/mL; koloni oluşturan birim/gram), pH ve sıcaklık ölçümü ortalama değer ve standart sapmaları

Table 2. Microbiological analysis results (log cfu/mL; colony forming unit/gram), pH and temperature mean values and standard deviations

Örnek adı	MAB	*C. sakazakii*	pH	Sıcaklık (°C)
Enteral beslenme ürünleri (n=20)	<1	<1	6.68 ±0.09	23.89 ±0.18

± Standart Sapma

Tablo 3. Kullanım şekline göre ürünlerin pH değerlerinin karşılaştırılması

Table 3. Comparison of pH value of the products according to their usage.

Kullanım şekli	n	pH ortalama	p değeri
Tüp	5	6.75 ± 0.09	0.130
Oral	7	6.61 ± 0.05	
Oral+Tüp	8	6.66 ± 0.07	

± Standart Sapma

MAB ek besin ögelerine ihtiyaç duymadan ve farklı pH değerlerinde rahatlıkla üreyebilirler (Doğan ve Tükel, 2000). MAB sayısı genel olarak bir gıdada mikroorganizmanın ne oranda bulunduğu ile ilgili bilgi vermekte olup besin güvenliği açısından önemlidir. Tokatlı (2009) yaptığı çalışmada incelediği 62 bebek mamasının 54'ünde 6 kob/g ile 2.69 x 10^3 kob/g değer aralığında MAB tespit etmiştir. Polat ve Halkman (2008), 40 adet bebek mamasını inceledikleri bir çalışmada MAB sayısını <10 – 1.95 x 10^3 kob/g aralığında bulmuştur. Aslantaş ve Yıldız (2008) yaptıkları çalışmada inceledikleri 31 enteral beslenme ürününün 1 tanesinde (%3.2) MAB sayısı yönünden üreme tespit etmişler ve oral olarak kullanılan bu üründeki kontaminasyonu 5x10^3 kob/g olarak belirlemişlerdir. Aynı çalışmada numunelerin 1'inde (%3.2) 2x10 kob/g sayıda *B. cereus* tespit ederken, ürünlerin hiçbirinde *Escherichia coli*, *Pseudoımonas aeruginosa*, *Staphylococcus aureus*, koliform bakteri, *Salmonella* türleri ve *Clostridium perfringens* tespit edilmemiştir (Aslantaş ve Yıldız, 2008). Bu araştırmada incelenen 20 adet enteral ürünün tamamında MAB bulunmamış olması ürünlerin sağlığa uygun ve güvenilir olduğunu işaret etmektedir.

Yapılan birçok araştırmada besinlere ve içeceklere *C.sakazakii*'nin bulaşabileceği belirlenmiştir. Bu bulaşma kaynaklarına örnek olarak bebek formül besinleri, mamaların hazırlanmasında kullanılan kaşık ve karıştırıcı gibi kontamine ekipman yanında süt tozu, su, pirinç, salata, peynir, çiğ kıyma, sucuk ve sebzeler verilebilir (Lehner ve Stephan, 2004). Üzüm (2006), çiğ süt örneği ile ilgili Ankara'da tüketime sunulan 100 adet besinden elde ettiği 115 izolattan 17'sini *Cronobacter* spp. (11 *C.sakazakii*, 6 *C.cloacae*) olarak tanımlamıştır. Ayrıca *C.sakazakii*'nin UHT süt fabrikasındaki üretim alanından ve sütten izole edildiği saptanmıştır (Lehner ve Stephan, 2004). Yapılan diğer araştırmalarda süt endüstrisinin dışında çikolata fabrikalarından, tahıl, baharat, patates, makarna ve türevleri üreten alanlarda da *C.sakazakii* izole edilmiştir (Kandhai ve ark., 2004; Lehner ve Stephan, 2004).

C.sakazakii çok farklı besinlerden tespit edilebilmiş olsa da Türkiye'de ve dünyada yapılan pek çok araştırma bebek beslenmesinde kullanılan mama ve formül besinler (devam sütü) ile süt tozunun kontaminasyon yönünden halen incelenmesinin gerekliliğini göstermektedir (Gültekin ve Demirel, 2006). Çakmak (2012) çalışmasında *C.sakazakii* yönünden incelediği 350 adet bebek mamasının 4'ünde (%1.14) pozitif sonuç elde etmiştir. Gurtler ve ark., bebek maması ve süt tozu örneklerinin 25 gramında *C.sakazakii*'nin varlığını incelemiş ve 170 adet süt tozu örneğinin 7'sinde, 40 adet toz bebek mamasının 1'inde tespit etmiştir (Gurtler ve ark., 2005). Hollanda'da *Cronobacter* türlerinin 4 yıl boyunca araştırıldığı bir çalışmada 182 toz bebek mamasının 1 tanesinde *Enterobacter* türleri izole edilmiştir (Kandhai ve ark., 2010).

Prematüre ve yenidoğan bebekler başta olmak üzere *C.sakazakii* menenjit, ince ve kalın bağırsak iltihabı ve birçok komplikasyona yol açmaktadır. Bu noktada enteral beslenme ürünlerini kullanan bireyler için özellikle tüple beslenenlerin, yenidoğanların ve kronik hastalığa sahip olanların bağışıklık sistemlerinin daha zayıf olduğu ve besinle bulaşan, mikrobiyolojik bir risklere karşı daha hassas oldukları klinisyenler tarafından belirtilmektedir (Aslantaş ve Yıldız, 2008). *C.sakazakii* kontaminasyonu gerçekleşmesi durumunda gastrointestinal sisteme yerleşmeyi takiben zehirlenme ve sepsis gibi birçok komplikasyon ortaya çıkabilmektedir. Enteral besinlerin içerdiği zengin makro ve mikro besin öğeleri ile a_w ve pH seviyeleri de mikrobiyal üreme için uygun ortamı pekiştirmektedir (Borges ve ark., 2011).

Özellikle tüp ürünlerinin çoğu içerdiği besin ögeleri, su aktiviteleri ve pH gibi etmenlerden dolayı mikrobiyolojik üremeye uygun bir ortam sağlamaktadır. pH'nın bakterinin optimum üreme değeri civarında olması bu noktada önemli bir etmendir. *C.sakazakii*'nin asidik pH'ya dirençli olduğu ve üreyebildiği en düşük pH değerlerinin 3.9-4.1 olduğu (Dancer ve ark., 2009), optimum olarak da 6.5 pH'ya ihtiyaç duyduğu bildirilmiştir (Pina-Pérez ve ark., 2009). Nitekim gerçekleştirilen bu araştırmada da incelenen ürünlerin ortalama pH değerlerinin 6.68±0.09 olması *C.sakazakii* kontaminasyonu olması durumunda, üremesinin de kolaylıkla gerçekleşebileceğini göstermektedir.

Enteral beslenme ürünleri özellikle kritik hastalarda, malnutrisyonlu bireylerde beslenme desteğinin oral veya gastrointestinal sistem yolu ile sağlanması için kullanılan özel tıbbi amaçlı gıdalardır (Demirkan ve ark., 2016). Başta bebekler olmak üzere immun sistemi yetersiz olan, herhangi bir kronik hastalığı olan hastalarda gerçekleşen mikrobiyolojik kontaminasyonlar ölüme yol açabilir. Bu bakımdan enteral beslenme ürünlerinin içerikleri, sterilite durumu, hazırlık aşamasında yapılan işlemler, bekletme süresi gibi etmenler önemlidir (Gavi ve ark., 2008; Gülşen-Atalay ve ark., 2019). Dolayısıyla bu ürünler için mikroorganizma kaynağının belirlenmesi, gıda kontaminasyonunu ve sonuçlarını önlemek açısından kritiktir.

Sonuç

Sonuç olarak; İstanbul ilinden toplanan 20 adet enteral beslenme ürünü arasında gerçekleştirilen bu çalışmada elde edilen mikrobiyolojik analiz sonuçları Türk Gıda Kodeksi ile karşılaştırıldığında tüm örneklerin mikrobiyolojik değerleri <1 log kob/g olarak bulunmuş olup araştırılan bakteriler yönünden risk içermediği ortaya çıkmaktadır. Protein enerji malnutrisyonu (PEM) veya kronik hastalık gibi durumlarda kritik hastalarda kullanılan enteral beslenme ürünleri, insan sağlığına etkide bulunacak bir mikroorganizmaları içermemelidir. Bu araştırma sonuçları bu yönden ürünlerin güvenli kabul edilebilir olduklarını göstermektedir. Ancak uygulama açısından bakıldığında besinlerin paketleri açılmadan önce mikrobiyolojik açıdan güvenilir olmaları yeterli değildir. Açıldıktan sonra kullanıma hazırlanırken de kontaminasyon gerçekleşebilmektedir. Hazırlama esnasında ürüne eklenen maddelerden, kullanılan araç gereçten, mevcut ortam koşullarından ve hazırlayan kişi veya hastadan kaynaklı kontaminasyonlar söz konusu olabilir. Enteral beslenme ürünleri ile ilgili sınırlı sayıda çalışma bulunmakta olup yapılan çalışmalar çoğunlukla bebek besinleri üzerinedir. Bu noktada incelenen enteral beslenme ürünlerinde sağlığı tehdit edecek herhangi bir üremenin görülmemesi bu ürünlerin üretimi sırasında uygulanan işlemlerin uygunluğunu göstermektedir. Yine de geçmiş çalışmalarda süt tozu gibi hammadde niteliğindeki ürünlerde görülen kontaminasyon değerlerine bakıldığında, benzer araştırmaların sürdürülerek ileri moleküler biyolojik tekniklerle de doğrulanmasının yerinde olacağı düşünülmektedir.

Etik Standart ile Uyumluluk

Çıkar çatışması: Yazarlar bu yazı için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan etmişlerdir.

Etik izin: Araştırma niteliği bakımından etik izin gerektirmemektedir.

Finansal destek: -

Teşekkür: Marmara Üniversitesi Sağlık Bilimleri Fakültesi, Temel Sağlık Bilimleri Bölüm Başkanı Sayın Prof. Dr. Ayşen Gargılı Keleş'e laboratuvar imkânlarını sunduğu için teşekkür ederiz.

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 7(1), 45-53 (2021) • https://doi.org/10.3153/FH21006 Review Article

Curcumin's antioxidant effects on inflammatory diseases

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Cite this article as:
Selen, H., Çomaklı, V. (2021). Curcumin's antioxidant effects on inflammatory disease. *Food and Health,* 7(1), 45-53. https://doi.org/10.3153/FH21006

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ABSTRACT

There are similar inflammatory reasons behind non-contagious chronic diseases. The prevalence of these diseases increases everyday both in our country and around the world. That's why scientists have begun looking for strong antioxidants that could help prevent and treat such inflammatory diseases. Curcumin is one of those antioxidants. Curcumin is one of the components of turmeric, which belongs to the ginger family. Many studies showed that the curcuminoids in turmeric can be used to prevent and treat cardiovascular, autoimmune and endocrine diseases, cancer as well as various inflammatory diseases. With this study, we aim to interpret these recent studies conducted with curcumin.

Keywords: Antioxidant, Curcumin, Health, Inflammatory Diseases

Submitted: 24.07.2020
Revision requested: 15.09.2020
Last revision received: 17.09.2020
Accepted: 18.09.2020
Published online: 03.12.2020

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Available online at
http://jfhs.scientificwebjournals.com

Introduction

Curcumin is a component derived from turmeric, which is used to add flavor and a yellowish color to dishes. First used by ancient Greeks, curcumin is used as a spice mostly in India and as a traditional medicine ingredient in Southeast Asia and China (Nahar et al., 2015). Curcumin, used as E100 as a food additive, can be found in mustards, cheese, canned fish, butter, pastry, and other many similar processed food (Chin et al., 2013). Curcumin is one of the main ingredients of turmeric and has anti-carcinogenic, antioxidant and anti-inflammatory effects. Thanks to these properties, curcumin is known to be effective in the protection against and treatment of many inflammatory diseases (Kumar and Sharma, 2015).

Antioxidant Mechanism of Action of Curcumin

Oxygen consumption during cell growth leads to the production of free radical oxygen intermediates as a result of a series of reactions, such as superoxide anion radicals, hydroxyl radicals, and hydrogen peroxide (H_2O_2). These radicals cause damage to biomolecules that play a critical role in maintaining life on Earth, including proteins, lipids, nucleic acids, and carbohydrates. Reactive oxygen species (ROS) lead to the development of various diseases, if they are not effectively excreted by cellular components. It is a known fact that ROS is involved in the pathophysiology of many diseases (Neeraj et al., 2008; Aksoy, 2018). Antioxidant defenses, including antioxidant enzymes or functional food ingredients, are needed to eliminate or repair these harmful effects of ROS. Functional foods, also known as antioxidant compounds, have important functions in the body such as preventing the mechanisms of free radical formation and removing the formed radicals (Ak and Gülçin, 2008). Curcumin, one of these functional foods, has been reported to be able to eliminate the harmful effects of metal ions that contribute to free radical formation by chelating them (Figure 1) and to increase antioxidant capacity by transferring electrons to the produced free radicals, or by creating a mechanism of inhibitor and activator action on the enzyme activity that plays an important role in metabolism (Ak and Gülçin, 2008; Asouri et al., 2013; Tanvir et al., 2017).

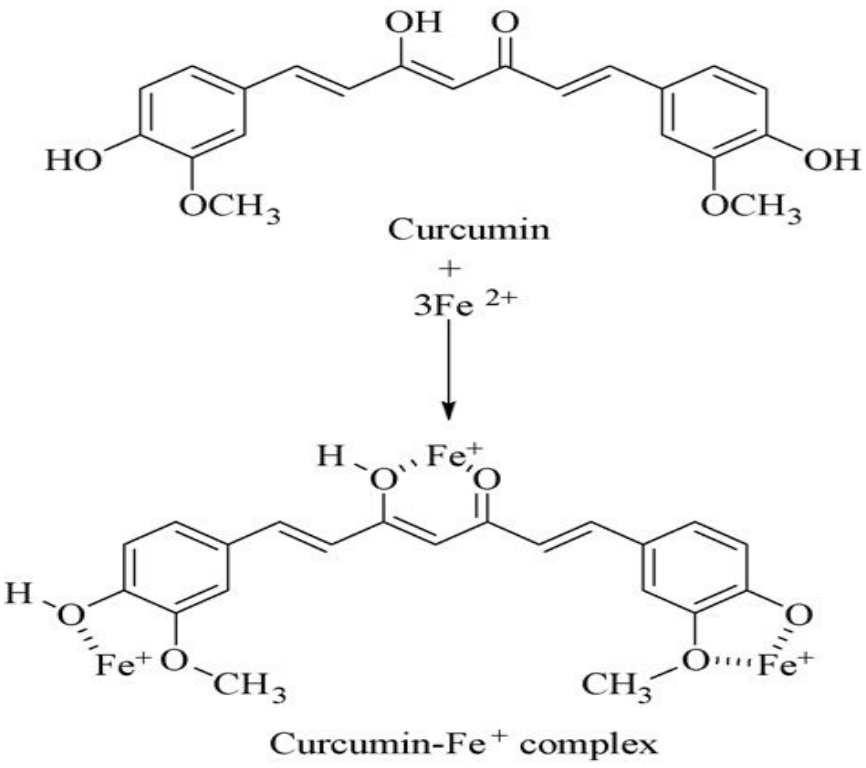

Figure 1. Proposal reaction of iron ions chelated by curcumin (Ak and Gülçin, 2008)

There are studies that support this information. For instance, in a study in which *Drosophila Melanogaster* was exposed to Al^{3+} metal ion, it has been reported that antioxidant parameters such as catalase, glutathione S-transferase and glutathione decreased and free radical precursors such as NO and H_2O_2 increased. The effect of this oxidative damage caused by Al^{3+} ion was reported to be eliminated by the curcumin molecule depending on the dose (Oyetayo et al., 2020).

Curcumin has been further shown to have very strong anti-inflammatory effects in addition to its antioxidant properties. In the literature, pro-inflammatory cytokines have been shown to form the basis for the development of non-communicable chronic diseases, including diabetes, pancreatic cell disorders, Alzheimer's disease, arthritis, cardiovascular diseases, intestinal diseases, polycystic ovary syndrome, and lipid disorders (Laveti et al., 2013). The phytochemical and polyphenol properties of curcumin inhibit the structures causing inflammation in the body, such as TNF-α, IL-1β, IL-6, MCP-1, Prostaglandin E_2, Nuclear Factor Kappa Beta (NFκB), Cyclooxygenase-2 (COX_2), and 5-Lipoxygenase (5-LOX). In contrast, curcumin has an activating or enhancing effect on cellular signal molecules such as interleukin, chemokine, cytokine, growth factors, enzymes, transcription factors, Nrf2, β-catenin, signal transduction and transcription (STAT), factors of the O class (FOXOs), and protein kinases (Figure 2).

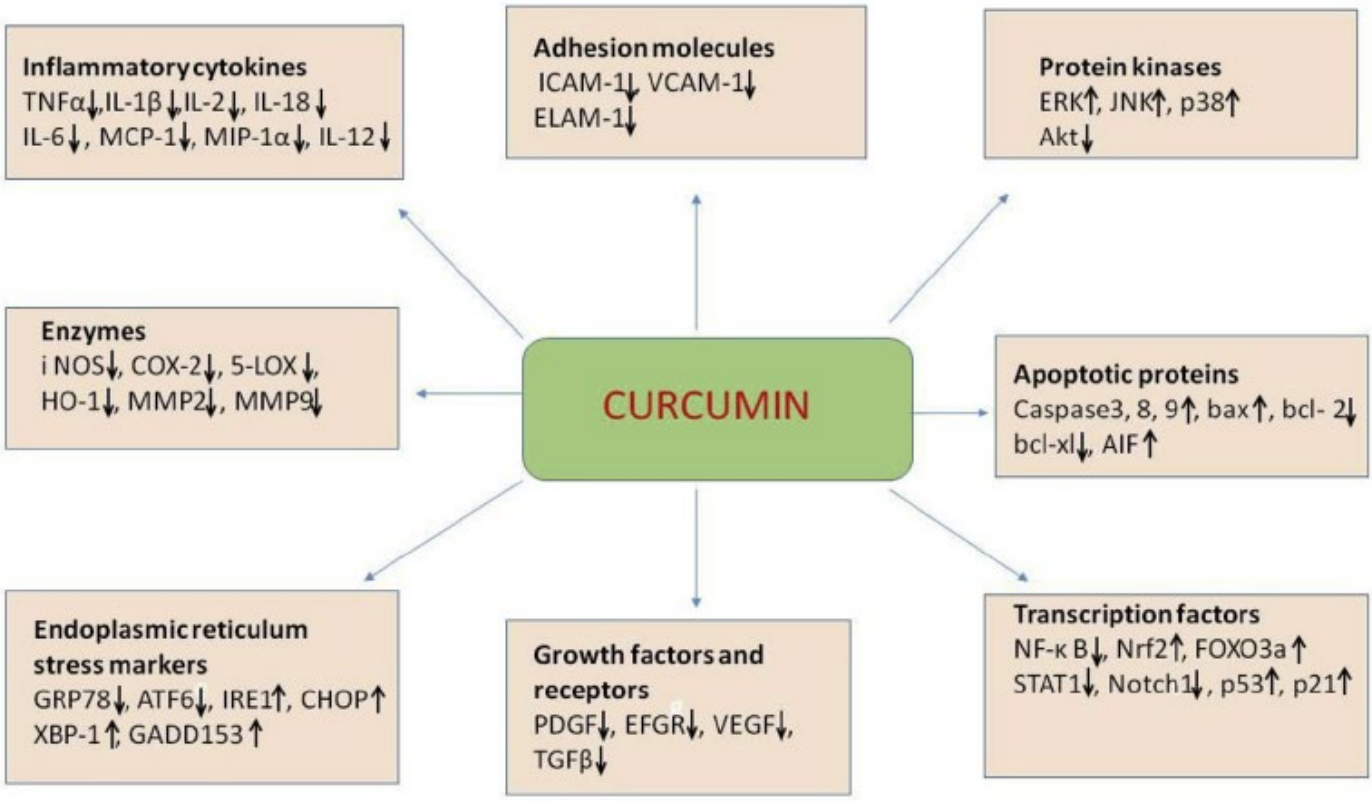

Figure 2. Molecular targets of curcumin (Ghosh et al., 2015)

Moreover, curcumin blocks the cell growth cycles in cancer cells, and reduces free radicals and inflammation, both of which can lead to cancer-causing cell mutations (Chen et al., 2010). Curcumin, a polyphenol derivative, has been reported to have positive effects on learning and verbal memory and to have a protective and therapeutic effect on health problems such as Alzheimer's and Parkinson's disease (Harish et al., 2010).

Literature Search

In a study the researchers examined the effects of curcumin on colon histology. They used azoxymethane to grow tumors in one group of rats, separated them as control and study group. Then they gave control group certain doses of curcumin along with their feed. The excrement of the rats were collected once in every four weeks for microbiological analysis and the rats were observed for 14 weeks. At the end of the study, it was seen that the rats that were given feed with the curcumin additive had higher survival rates, that the weight and length of their colons reduced and the tumor burden reduced by 0.5%. In study rats, curcumin increased the microbial richness, prevented age-related reduction in alpha diversity, increased the relative abundance of *Lactobacillales (by genus Lactobacillus)* and reduced the order of *Coriobacteriales (Actinobacteria phylum).* In other words, curcumin inhibited the genes related to inflammation, reduced or completely removed the colonic tumor burden (McFadden et al., 2015).

In another study, 20 female Wistar rats (20 months old) were separated as control and study groups. The study group rats were given curcumin extract for 12 days (300 mg/kg) in corn oil with oral gavage. After 24 hours heart tissues are taken ander anesthesia, and protein carbonyl (PC), Malondialdehyde (MDA) and Glutathione (GSH) levels were checked. GSH level of rats that were fed curcumin supplement, was found to be significantly higher compared to the study group ($p<0.05$). PC and MDA levels were found to be low, albeit not significantly ($p>0.05$). In other words, curcumin protected the heart issue of these old female rats from oxidative damage and strengthened the antioxidants defense system (Belviranlı et al., 2012).

Since prostate cancer is the most diagnosed cancer and among the biggest cause of death in the males in the USA, many studies are conducted to understand the molecular basis of the progression of this cancer. Also various efforts are underway to achieve early diagnosis and to develop new treatment strategies for the disease. Protein kinase D1 (PKD1), is a multi-functional kinase that is produced in high amounts in a normal prostate. The reduction of PKD1 expression is associated with the progression of prostate cancer (Jaggi et al., 2007). It was shown that curcumin, an active component of turmeric, activates PDK1, which in turn blocks the transcription activity of the nuclear β-katenin in prostate cancer cells and increases β-katenin signal levels. In this study, the rats with prostate cancer were divided into study and control groups and the study group were given intratumoral injection of curcumin at certain times and dosages. It was seen that compared to the control group, the tumor growth in the study group which were given curcumin additive, was inhibited 2 times more. It was explained that this effect became possible through improved membrane localization of β-katenin and the reduction of cofilin activity downstream of PDK1 (Sundram et al., 2012).

In another study, the researchers tried to understand the effect of curcumin and Kaempferol on acute pancreatitis, which was produced in rats using L-Arginine. 38 male rats were separated into 6 equal groups and the first group (control group) was administered serum physiologic (SF-NaCl) through Intraperitoneal injection (IP), the second group was administered L-arginine through IP and the third group was administered dimethyl sulfoxide (DMSO) through IP, the fourth group L-Arginine + Curcumine through IP, the fifth group L-arginine + kaempferol (flavanol) through IP and the sixth group was administered L-arginine+curcumin+kaempgerol through IP. In acute pancreatitis, although it is not meaningful, it was seen that antioxidant system indicators in treatment groups were higher clinically and the oxidative stress indicators were lower. It was also seen that cytoskeleton that was administered curcumin and kaempferol antioxidant, were preserved better compared to other groups (Turgut, 2019).

In a study was conducted to understand if the curcumin, a phytochemical compound, was effective on the preservation of remission in patients with ulcerative colitis (UC). 50 patients with active, mild and moderate UC was included in the study using the simple clinical colitis activity index (SCCAI). Patients that did not respond to non-steroidal anti-inflammatory drug (NSAID) treatment, were divided into random groups and for four weeks, curcumin tables administered to 26 patients (3 g/day) and an identical placebo was administered to 24 patients. After the study, it was seen that 14% of the curcumin administered patients clinically recovered and none of the patients in placebo group recovered. 17 patients in curcumin group and 3 patients in placebo group, achieved clinical response with 3-point reduction in their SCCAI scores. As a conclusion, in the induction of clinic and endoscopic remission, the addition of curcumin in UC treatment, was found more successful than the combination of placebo and mesalamine (Lang et al., 2015).

Another study sought to determine the effect of curcumin taken with a high-fat diet, on the antioxidant and oxidant balance in the testicles. To this end, rats were divided into four groups. First group was fed with a normal diet where the 10% of the energy came from fats, the second group with a high fat diet (HFD), where the 60% of the energy came from fats, the third group with a HFD where curcumin was added in the feed (1 g/1 kg), and the fourth group with a normal diet with a curcumin addition (1 g/1 kg). At the end of the study, reactive oxygen sorts (ROS), malondialdehyde (MDA), Glutathione (GSH), Glutathione peroxidase (GPx), Superoxide dismutase (SOD) and glutathione transferase (GST) activities were measured. HFD application increased the MDA levels and decreased the GSH levels in the testicles. Curcumin given together with HFD, decreased the MDA levels which rose due to HFD and increased the GSH levels and GST activities. However, curcumin addition to normal diet did not affect the antioxidant and oxidant indicators. In ROS, SOD and GPx values, no significant differences were observed between the groups. To conclude, it was shown that curcumin addition administered with a HFD, could preserve the antioxidant and oxidant levels in the testicles (Seyithanoğlu et al., 2020).

In a study conducted with overweight and obese female with high blood lipid profiles, the effect of the turmeric on weight loss and blood lipids was examined. Accordingly 70 females were divided into study and control groups and the control group were administered medical diet for loss weight of 0.5-1 kg per week and the study group was given 4 g of curcumin everyday in addition to the above. Biochemical parameters were measured before and after the study which lasted 8 weeks. Although the female in the study group lost more weight, these values were insignificant. Similarly, there was drop in the fasting blood glucose, total cholesterol, high density lipoprotein (HDL)-low density lipoprotein (LDL) cholesterol, triglyceride but it was insignificant. Also, the effect of curcumin administered in addition to diets prescribed by dietitians for individuals who had high blood lipid profile and who were overweight and obese, was not found statistically significant. However, considering individual differences and that the period was limited to 8 weeks, it can be suggested that similar studies can be repeated over longer periods (Atakan, 2017).

In a study, conducted to reveal if curcumin could be a treatment for Psoriasis Area and Severity Index (PASI), 63 patients with mild and moderate *Psoriasis Vulgaris* (PASI<10), and who are administered topical steroids, were randomly divided into two groups. For 12 weeks, one group was given a lecithin-based curcumin supplement (Meriva) of 2 grams next to local drug therapy, while the other group was given a placebo along with local drug therapy. Both groups saw significant drop in PASI levels but the reduction in IL-22 level was found significant in the group that was administered Meriva in addition to local steroids, compared to placebo group ($p<0.001$). It was shown that curcumin was effective as an adjacent treatment for *Psoriasis Vulgaris* and significantly reduced the IL-22 serum levels (Antiga et al., 2015).

In a study conducted with prediabetic population, the researchers wanted to determine the effect of the curcumin in delaying the development of type 2 diabetes mellitus (DM). They randomly divided 240 volunteers in two groups. For 9 months, one of the groups were administered curcumin capsules of 1500 mg, and the other group was administered equal amount of placebo. At the 3rd, 6th and 9th months, the participants were monitored to determine the number of patients that developed type 2 DM, the changes in β-cell functions (homeostasis model evaluation [HOMA]-b, C-peptit and proinsulin / insulin), insulin resistance (HOMA-IR), anti-inflammatory cytokine (adiponektin) and other parameters. After 9 months, 16.4% of the participants in placebo group were diagnosed with type 2 DM, but no patients that were treated with curcumin were diagnosed with type 2 DM. In addition, the group treated with curcumin, had higher HOMA-b, lower C-peptide and better general functioning of β cells. The group treated with curcumin, showed lower HOMA-IR levels and higher adinopektine compared to placebo group. Therefore, it was shown that curcumin administration could help prediabetic people (Chuengsamarn et al., 2012).

Another study was designed to determine if curcuminoids prevented myocardial infarction (MI) after coronary arterial bypass grafting (CABG), based on previous studies that showed curcuminoids reduced preinflammatory cytokines during cardiopulmonary bypass surgery and that it reduced the formation of cardiomiotic apoptosis after cardiac ischemic damage (Yeh et al., 2005). 121 patients that were subjected to CABG participated in the study. One group was given 4 g/day curcuminoid starting three days before the planned surgery and other group was given same amount of placebo and the administration continued until five days after the surgery. The MI prevalence in the hospital reduced to 30.0% in the placebo group and to 13.1% in curcuminoid group. Postoperative C-reactive protein, malondialdehyde and N terminal pro-B type natriuretic peptide levels were seen to be lower in curcuminoid group compared to placebo groups (Wongcharoen et al., 2012).

In a study, 65 patients with metabolic syndrome was randomly divided into study and control groups. For 12 weeks, 33 people were administered 630 mg curcumin capsules, and 32 people were administered placebo capsules three times a day. 12 weeks after curcumin consumption, there was increase in HDL-C levels, and drop in LDL-C and triglyceride levels. Curcumin consumption led to reduced cholesterol in

males and increased HDL-C in females and in both groups, it reduced T-Chol/HDL-C rates. Consumption of 1890 mg/day curcumin for 12 weeks decreased lipids but was not found significantly effective in treating weight and glucose homeostasis in metabolic syndrome patients. Daily consumption of curcumin can be an alternative option to balance the relevant parameters in metabolic syndrome patients (Yang et al., 2014). In an article where various studies were discussed, it was stated that curcumin had an anti-obesity effect (Mohamed et al., 2014).

In a study designed to determine the effect of ginger supplement on non-alcoholic fatty liver patients, study group was administered 2 g/day ginger supplement in addition to their diets which consisted of 52-55% carbohydrates, 30% fat, 15-18% protein and 20-30 g/day fiber. The groups that was administered ginger, showed significant drop in inflammatory cytokines, and parameters like liver enzymes γ-glutamyl transferase (GGT), alanine aminotransferase (ALT) (Rahimlou et al., 2016).

In a randomized study designed to determine the effect of curcumin on experimental ischemic and ischemic / reperfusion (I/R) damage in rat ovaries, 48 female wistaria rats were used. In groups that were administered curcumin, a significant reduction in the average levels of oxidant indicators of ovarian tissues and their histopathological scores, was observed (Sak et al., 2013).

Polycystic ovary syndrome (PCOS) is a very prevalent syndrome in female of reproductive age. It is often characterized by obesity, insulin resistance, hyperandrogenemia, and hirsutism (Deniz et al., 2012). In a study where curcumin supplements effect on PCOS, 72 adult female wistar rats were used. They were divided into groups of study group (healthy), PCOS group and curcumin group. After 60 days of application, ovaries were collected and analyzed for histological and Immuno-Histochemical evaluations. In curcumin group, number of corpus luteum (CL) increased, and IL-6 and C-reactive protein (CRP) inflammatory markers significantly dropped. While TNF-α expression and follicular fluid of follicles and ovary cysts in PCOS group was higher compared to control group, these expressions reduced in ovaries treated with curcumin. This study is indicative of curcumin's anti-inflammatory and antioxidant effects on PCOS (Mohammadi et al., 2017).

In a study that examined the effects of curcumin on body weight, glisemic control and serum lipids, 18-40 year old females with 60 PCOS, were divided into curcumin (n=30) and placebo (n=30) groups. The curcumin group was administered 500 mg/day curcumin for 12 weeks and the placebo group was administered same amount of placebo. Parameters were measured in the beginning of the study and after 12 weeks of application and there has been a significant improvement in their level in body mass index (BMI), serum insulin, insulin resistance, insulin sensitivity, peroxisome proliferator-activated receptor gamma (PPAR-γ), low-density lipoprotein receptor (LDLR), HDL, LDL and total cholesterol levels (Jamilian et al., 2020).

Many studies were conducted on humans and animals where it was shown that curcumin had positive effects on rheumatoid arthritis. 45 patients with rheumatoid arthritis were divided into 3 groups. The first group was administered 500 mg/day curcumin, the second was administered diclofenac sodium 50 mg/day which is a medication used for the treatment of the said disease and the third group was given a combination of the two. There was no significant difference between the groups according to the Rheumatoid Arthritis Disease Activity Score (DAS-28) and the criteria of the American College of Radiology (ACR), nevertheless, the groups that were administered curcumin, showed the best improvement. The serum CRP levels showed significant change only in the curcumin group, but no significant changes were observed in other chemical and hematologic parameters (Chandran and Goel, 2012). Similarly, in a study conducted with Wistar rats with rheumatoid arthritis, it was reported that curcumin inhibited the redness and eudema in ankles and joints of rats and also inhibited the increasing levels of pro inflammatory cytokines like IL-1β, TNF-a, MMP-1 and MMP-3 (Dai et al., 2016).

Conclusion

There are similar inflammatory reasons behind non-contagious chronic diseases. The prevalence of these diseases increases everyday both in our country and around the world. That's why scientists have begun looking for strong antioxidants that could help prevent and treat such inflammatory diseases. Curcumin is one of those antioxidants. Since no toxic effects of curcumin was determined in studies, it has been used for treatment of the aforementioned diseases for a long time.

Due to its low cost and reliability, turmeric, of which curcumin is the main ingredient, is considered promising in the prevention and treatment of diseases. Studies suggest that consumption of 1-5 g of turmeric, which equals to 150 mg curcumin, does not create any toxic effect (Sharma et al., 2005); however the joint report of World Health Organization and United Nations Food and Agriculture Organization states that the side effects of curcumin should be studied and the maximum daily dosage must be 1 mg/kg (WHO, 2000).

We believe that in the face of changing life conditions, unhealthy diets and the life style brought about by sedentary life, having turmeric in our daily diet will prove preventive against diseases.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Ethics committee approval: The authors declare that this study does not require ethical permission.

Funding disclosure: -

Acknowledgments: -

Disclosure: -

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 7(1), 54-63 (2021) • https://doi.org/10.3153/FH21007 Review Article

A potential antiviral and food-derived healthy ingredient: Resveratrol

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Cite this article as:
Arslaner, A., Türkoğlu, Z. (2021). A potential antiviral and food-derived healty ingredient: Resveratrol. *Food and Health,* 7(1), 54-63.
https://doi.org/10.3153/FH21007

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Submitted: 27.05.2020
Revision requested: 30.09.2020
Last revision received: 05.10.2020
Accepted: 05.10.2020
Published online: 09.12.2020

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Available online at
http://jfhs.scientificwebjournals.com

ABSTRACT

Polyphenols are the secondary metabolites of plants and has an important role in human nutrition as the leading antioxidants. According to the carbon number-based classification of polyphenols, resveratrol is a natural polyphenol in the stilbene group with antioxidant and anticarcinogenic effects. Its beneficial effects on cardiovascular diseases have also been reported. It was first identified in 1940 and has recently gained importance especially in medicine and pharmacy. Researchers have carried out various studies on resveratrol and its time-honored use in traditional eastern medicine has been reported. Resveratrol is a food ingredient that has the potential to be used in the treatment of various diseases, but also has antiviral effects. The study focuses on resveratrol's physical and chemical properties, effects on health, antiviral effects and use in foods as a functional component.

Keywords: Resveratrol, Food, Health, Nutrition, Anti-viral

Introduction

When facing a stress factor, plants synthesize a type of secondary metabolite with low molecular weight called phytoalexin as a defense mechanism. Trans-resveratrol is a polyphenolic phytoalexin from the stilbene group (Fremont, 1999). Polyphenols are secondary metabolites of plants and the most common antioxidants in human nutrition (Bravo,1998; Emirdağ, 2014). Resveratrol is in the stilbene group according to the carbon number-based classification of polyphenols (; Athar et al., 2007). It is a natural polyphenol with reported antimicrobial, antioxidant and anticarcinogenic effects in addition to its beneficial effect on cardiovascular diseases; it joins the structure of various plants, is formed by the bonding of two aromatic rings with the methylene bond, contains three hydroxyl groups and can be found both in cis and trans configurations (Athar et al., 2007; Tokuşoğlu et al., 2005). It was first identified in 1940 in the roots of white hellebore and then, was found in the roots of *Polygonum cuspidatum*, also known as Kojo-kon in Japan. It was identified in the leaf epidermis and pericarp of grape berries in 1976 (Shishodia and Aggarwal, 2006). The study focuses on resveratrol's physical and chemical properties, effects on health, antiviral effects and use in foods as a functional component.

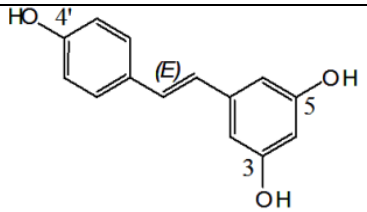
The Physical and Chemical Properties of Resveratrol

Many beneficial components such as antioxidants and phenolic substances in the structure of foods have biological regulatory roles, protective and nutritional properties in our body. During the metabolism of nutrients in our body, free radicals and other reactive oxygen species (ROS), also called toxins, are released. Free radicals are chemical structures containing one or more unpaired electrons in their outer orbitals. These structures lead to oxidation in our body, resulting in diseases and aging. Antioxidants neutralize free radicals by maintaining their own electrons in order to reduce the formed ROS and maintain their stability (Lobo et al., 2010). Antioxidants are secondary metabolites that can be produced spontaneously in the body or can be taken from the outside, usually through plants or synthetic drugs. Antioxidants help prevent or treat diseases as well as increase body resistance by reducing the effects of free radicals or altering their structure. Antioxidant activity, which is a measure of how much of the free radicals can be inactivated by antioxidants, can therefore be defined as free radical capture capacity (Kumarasamy et al., 2007).

Antioxidants are used to increase body resistance and protect human health, as well as to extend the shelf life of nutrients. Synthetic antioxidants are mainly used to extend the shelf life of nutrients in industrial processes. Today, BHA and BHT are used as synthetic antioxidants in many countries. Daily intake is reported by both Joint FAO/WHO Expert Committee on Food Additives (JECFA) and Commission of the European Communities as 0.5 mg/kg for BHA (EFSA, 2011) and JECFA allocated an ADI of 0-0.3 mg/kg bw/day for BHT (EFSA, 2012). Meanwhile, it has also been reported that the amount of consumption of antioxidants may be higher than specified and may cause liver and carcinogenic effects (Fremont, 1999). Synthetic forms of antioxidants are not preferred due to their toxic potential and interest in phenolic compounds with natural antioxidant properties increases day by day.

Polyphenols are the secondary metabolites of plants and has an important role in human nutrition as the leading antioxidants. According to the carbon number-based classification of polyphenols, resveratrol formed by connecting two aromatic rings with methylene bond; containing 3 hydroxy groups, available in cis and trans configurations is a natural polyphenol in the stilbene group. Resveratrol, which is found in the structure of many plant species, has been reported to have antimicrobial, antioxidant, anticarcinogenic effects and cardiovascular diseases decreasing effect (Tokuşoğlu et al., 2005; Lobo et al., 2010). Table 1 shows the chemical and physical properties of resveratrol.

Table 1. Physical and chemical properties of resveratrol (Haneke, 2002)

Molecular Formula	$C_{14}H_{12}O_3$
Structural Formula	
Systematic Name	5-[(E)-2-(4-hidroxyphenyl)-ethenyl] benzene-1,3-diol
Other Names	Trans-resveratrol Trans-3,5,4'-trihydrozystilbene 3,4',5-stilbenetriol (E)-5- (p-hydroxy styryl) resorcinol 3,5,4'-trihydroxy-cis-stilbene 3,5,4'-trihydroxy-trans-stilbene
Molecular Weight	228.25 g/mol
Boiling Point	253 -255℃
Physical Structure	White – Solid
Solubility	Easily dissolves in water, methanol and acetone.

Resveratrol is available in cis and trans isomers or glycolyzed form. It is mostly in glycolyzed (3-O-D-glucoside) form in plants. Glycolysis protects resveratrol from oxidative degradation (Athar et al., 2007). Glycolyzed resveratrol is very stable and water-soluble, easily and highly absorbed from the gastrointestinal tract. After absorption, it is metabolized in the liver to trans-resveratrol-3-O-glucuronite and trans resveratrol- 3-O-sulphate (Signorelli and Ghidoni, 2005). In vivo studies have shown that in healthy people, resveratrol is metabolized to the 3 and 4'-O-sulfate and 3-O-glucuronide conjugates less than 2 hours after consumption (Sing et al., 2015).

Resveratrol is formed by the combination of 3 molecules of CoA and 1 molecule of 4-cuamoryl CoA. Here, it is important that the enzyme needed for the synthesis of resveratrol is not normally active and activated when the plant is faced with a stress factor (Soleas et al., 1997). Figure 1 shows the biosynthesis of resveratrol.

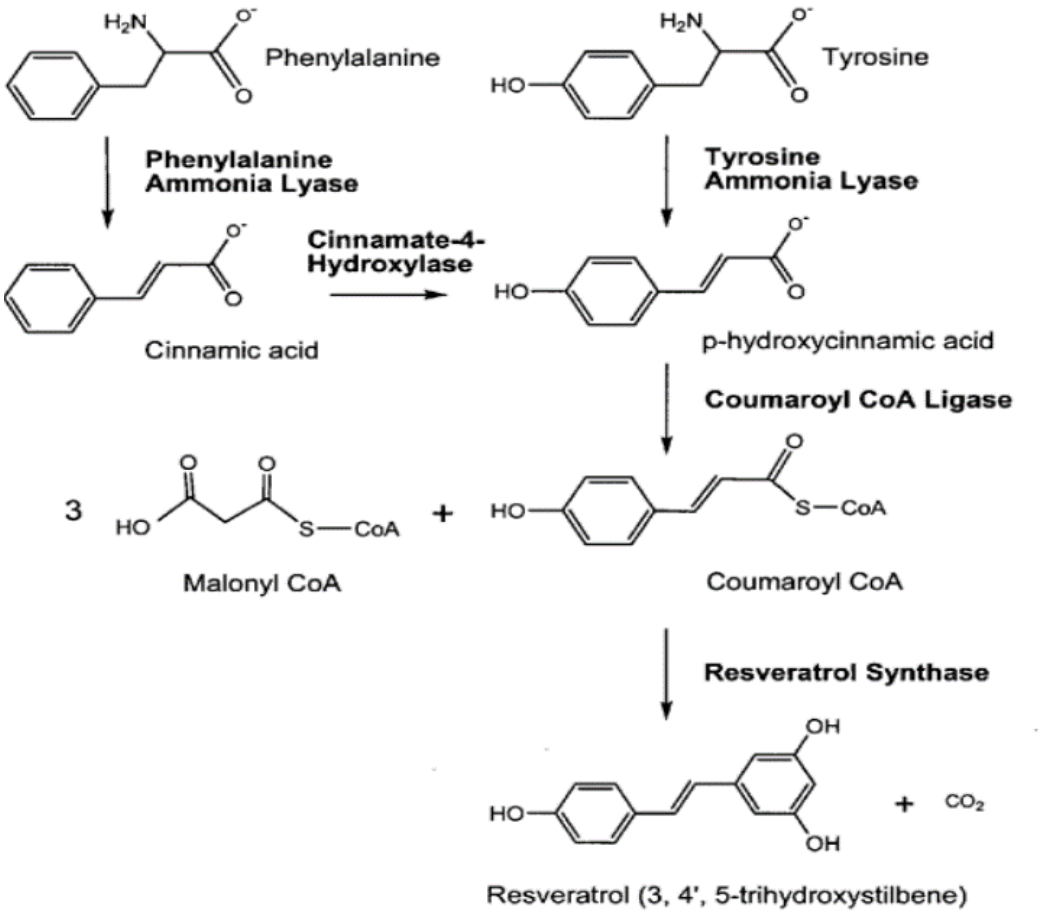


Figure 1. Resveratrol biosynthesis from phenylalanine (Becker et al. 2003, Huang et al. 2007)

The Sources of Resveratrol

Resveratrol is a secondary metabolite synthesized as a defense compound against various external factors and found most abundantly in the product obtained by the drying of the roots of *Polygonum cuspidatum* (Konjo-Kon or Itadori tea), a plant traditionally used in Japan and China (Nonomura et al., 1963; Savouret and Quesne, 2002). Vastano et al. (2000) reported that *Polygonum cuspidatum* consist 2960-3770 ppm resveratrol. Chen et al. (2013) mentioned that the root contains a much higher level of resveratrol than the stem and leaf, and it is accumulated in its highest level in October. Prince Edward Island, Canada-grown knotweed contains similar levels of resveratrol and polydatin compared to Chinese samples collected in the month of October.

In studies on resveratrol and its derivative stilbene compounds, mainly grape and grape products (Concenco et al., 2019). Mnari et al. (2016) reported that resveratrol contents of Tunisian raisins varieties in range of 0.02-0.12 mg/g dry weight. Lyons et al (2003) indicated that wild blueberry and bilberries might serve as another dietary source of resveratrol. Shrikanta et al. (2015) stated that some of the less consumed fruits such as mulberry, jamun and jackfruit are rich in resveratrol and can be processed by processing into functional drinks. Values of resveratrol in European plum (*Prunus domestica*) cultivars detected average 1.0 µg/1g (Sebasti`a et al., 2012).

In addition, resveratrol has been identified in black chocolate and cocoa liquor and hops used in beer making (Athar et al., 2007). Salvador et al. (2018) observed the highest contents of trans-resveratrol in alkalized cocoa powder and natural cocoa powder (13.53 µg/kg and 11.40 µg/kg) respectively.

Some studies have shown that resveratrol glucoside was the major form available in most vegetable and the content of resveratrol forms in different varieties and regions were different (Peng et al., 2005), also the content varying with seasonal, geographical, and environmental factors (Kurita et al., 2013). Some of specific vegetable foods such as celery (originated in West China, 783.29 µg/100g), red radish (North China, 194.4 µg/100g), *Coprinus comatus* (Midland China, 573.74 µg/100g) have been reported to be important sources of resveratrol (Peng et al., 2005). Sebastia et al. (2017) announced that trans-resveratrol contents varied from 20 µg/100g (tomato and strawberry) to 300 µg/100g in dates (*Phoenix dactylifera* L.).

Some of the wild and ornamental plant species, edible mushrooms have also been reported to be an important source of resveratrol. Average trans-resveratrol amounts have been determined 1.07 µg/g and 0.7960 µg/g in *Solanum americanum* ripe fruit pulp and peel, respectively (Vagula et al., 2016). Akyüz et al. (2012) detected resveratrol in some edible mushroom samples in the range of 0.25-0.75 µg/g.

Alkan (2007) pointed out that grape skins, which are rich in resveratrol amount, which are separated as waste during the production of molasses, can be evaluated in this direction. Silva et al. (2014) studied guava and Surinam cherry byproduct and concluded that the content of resveratrol in the byproducts 25.67 mg/100g and 112.51 mg/100g dry basis, respectively. The researchers underlined the nutraceutical potential and future application in the food industry of the agrifood byproducts (Silva et al., 2014; Annunziata et al., 2018).

The Effects of Resveratrol on Health

Free radicals are chemical structures that contain one or more unpaired electrons in their outer orbitals. Although the reactive chemical compounds are formed in organisms due to oxidation and reduction reactions, they can also be formed due to external factors such as radiation, UV lights, air pollution, and combustion products of fossil fuels, some insecticides, pesticides, viruses, infections and drugs. In the case where they are not eliminated in an organism, free radicals cause pathological disorders and aging. Antioxidants reduces the effects of free radicals in the body and foods and/or alter their structures so that the oxidation reactions are significantly inhibited or delayed (Shahidi and Ambigaipalan, 2015).

Various studies have emphasized the cardiovascular protective, antithrombotic, antioxidant, anti-inflammatory, blood sugar reducing and anticancer activities of resveratrol. It naturally has two different isomeric forms and its trans form has been reported to be more important and stable and, thus, more widely used in studies. Trans-resveratrol has different biological properties and has been reported to have stronger antioxidant and cardioprotective effects (Gliemann et al., 2016). Preventing the cancer development in organisms may be possible with sufficient levels of optimized analogs of the resveratrol molecule and increased stability and bioavailability in organisms (Kiskova et al., 2020).

Recent studies have shown that resveratrol had protective effects against neurodegenerative diseases such as Alzheimer's disease and obesity in addition to its effectiveness in the treatment of osteoporosis in postmenopausal women with low breast cancer risk (Kuršvietienė et al., 2016). Resveratrol inhibited lipid peroxidase in linoleic acid emulsion at a higher rate (89.1%) when compared with synthetic antioxidants at the same concentration (Gülçin, 2010). Its antiaging effect and enhancing effect on insulin sensitivity, the enzymatic activity of the sirtuin gene (SIRT 1-7) and mitochondria number

were associated with its antioxidant activity (Kındır and Güvenç, 2010).

The current literature suggests that resveratrol prevents cancer through various mechanisms including anti-proliferative, anti-inflammatory and anti-angiogenesis mechanisms by initiating apoptosis (Singh et al., 2015). Apoptosis induction is the key mechanism in the inhibition of the formation of many tumors. Resveratrol induces p53-mediated apoptosis in cancer cells, including prostate cancer, colon cancer and breast cancer (Yu et al., 2012).

Resveratrol may inhibit enzymatic activity in another mechanism through which resveratrol is believed to inhibit the development of cancer cells. Cyclooxygenase and decarboxylase are included in the cancer-causing enzyme groups. Epidemiological studies have shown that long-term cyclooxygenase inhibition reduced the development of various cancer types and cancer caused by the enzyme was prevented with the deletion of the cyclooxygenase-II (COX2)-coding gene (Yu et al., 2012). Its effect on tumor formation stems from its inhibitory effect on the antimutagens and free radicals in animal models and antioxidant properties. The inhibition of COX-1 reduces tumor progression and resveratrol have been reported to substantially inhibit the COX-1 enzyme (Savouret and Quesne, 2002).

Although French people consume fat and cholesterol-rich foods, they are 40% less likely to develop cardiovascular diseases than the rest of Europe. This is associated with the consumption of resveratrol in red wine and referred to as the "French Paradox" (Kopp, 1998; Yu et al., 2012). Plaque formation in veins leads to thrombosis and consequently embolism. The main cause of cardiovascular diseases is embolism. Atherosclerosis is a result of the impairment of the reactions between the normal cell elements of the artery-related wall and blood. Atherosclerosis can be prevented by consuming anti-atherogenic foods. As a polyphenol, resveratrol has a good protective effect on different parts of atherosclerosis (Das and Da, 2007). The protective mechanism of resveratrol against cardiovascular diseases was suggested to operate by blocking the thrombocyte aggregation and reducing cholesterol through its anti-inflammatory effects (Keskin et al., 2009).

Alzheimer's disease (AD) is not only a growing health issue but also a social and economic load. AD occurs due to the accumulation of β- amyloid plaques on the nerve cells in brain and its cause is still not known. Its severity increases over years and currently, there is not a cure for AD (Granzatto and Zatta, 2014). Of the affected individuals, 15% are in the 65-74 age group and 44% are in the 75-84 age group. Aging and genetic disposition are classically considered the unavoidable risk factors for AD. On the other hand, environmental factors, insulin resistance, obesity and metabolic syndromes are avoidable risk factors for AD. Factors such as regular physical activity, Mediterranean diet (fruits, vegetables, hazelnut, beans, olive oil, etc.), calorie restriction and maintaining the ideal body weight (or intermittent fasting), reducing smoking, controlling diseases such as diabetes, hypertension, and lifelong learning can delay or prevent aging-related cognitive decline (Sawda et al., 2017). Preclinical studies support the potential role of resveratrol in the treatment and prevention of neurodegenerative diseases such as Huntington's disease, Parkinson disease and AD (Sun et al., 2010). Resveratrol can protect against neurodegenerative diseases by eliminating reactive oxygen species (ROS), hydrogen peroxide and free radicals, NO, Aβ and other intra- and extracellular toxins through the SIRT1 activation mechanism (Graff et al., 2013).

Resveratrol is a food ingredient that has the potential to be used in the treatment of various diseases, but also displays antimicrobial activity against a surprisingly wide range of bacterial, viral and fungal species (Vestergaard and Ingmer, 2019). In 1976, one of the first studies showing the antiviral activity of polyphenols was carried out and it was found that especially the grape juice phenolic components separated by a membrane filtration showed a preventive activity against the poliovirus (Annunziata et al., 2018).

It has been stated that in combating many drugs-resistant viruses (HSV-1, HSV-2 etc.) natural food-derived matrices such as grape pulp rich in resveratrol and other polyphenols, which have no side-effect, can be evaluated (Annunziata et al., 2018).

Antiviral mechanisms and effects of resveratrol have been reported to be widely investigated in such as influenza virus, herpes simplex virus, respiratory syncytial virus, human immunodeficiency virus (HIV), hepatitis C virus and multiple sclerosis (MS). Most of these studies have announced that the progression of the disease and recession of the viral infections after administration of resveratrol (except to MS and hepatitis C). Antiviral mechanisms of resveratrol arise through inhibition of viral protein synthesis, inhibition of various transcription and signaling pathways, and inhibition of viral associated gene expressions (Annunziata et al., 2018).

Mohd et al. (2019) suggested that resveratrol exhibited direct virucidal activity against Zika virus (ZIKV) and possessed anti-ZIKV replication properties, highlighting the need for further exploration of resveratrol as a potential antiviral molecule against ZIKV infection.

Eighty-Six percent of the genetic sequence of SARS-CoV is the same as SARS-CoV-2, the virus responsible for the global pandemic caused by infectious disease COVID-19 (Chan et al., 2020). The angiotensin converting enzyme-2 (ACE2) cellular receptor is responsible for the pathogenesis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), thus affecting the entrance and clearance of the virus (Zhu et al., 2020). Overall, ACE2 activity is protective against SARS-CoV pathogenesis. Recent research suggests that the nutrients in our daily diet may affect the expression and function of the ACE2 gene, and resveratrol has the potential to contribute to ACE2 activity. Therefore, adding resveratrol to the diet can help reduce the harmful effects of high-fat diets on ACE2 gene expression. It has also been suggested to be used in regulating diet strategies in order to reduce disease severity in COVID-19 pandemic (Horne and Vohl, 2020).

The bioavailability of resveratrol after ingestion, the potential of dietary or direct intake in humans and animals, in the treatment of viral infections should be explored in more detail.

The Use of Resveratrol as a Functional Component in Foods

In order to increase product functionality, studies in which resveratrol is added to the food matrix are not common. Resveratrol is found small quantities in the diet; any protective effect of this molecule is unlikely at normal nutritional intakes (Manach et al., 2004). Therefore, a detailed investigation of the effects of resveratrol on humans and determination of how the resveratrol concentration especially in foods and beverages that are biologically related to resveratrol can be increased are needed (Pastor et al., 2019). It has been reported that encapsulation of resveratrol, which has a higher solubility in food-grade oils, can be used to improve its water-dispersibility, chemical stability, and bioavailability, thus it can be incorporate resveratrol into aqueous based products that have lower fat and calorie contents, such as some beverages, yogurts, sauces, dressings, and desserts. (Davidov-Pardo and McClements, 2014).

Acar (2011) investigated the effects of the interaction between resveratrol and milk proteins on the Maillard reaction and found that around percentage 80 of resveratrol cross-linked with milk proteins, but the concentration of resveratrol did not have a significant effect on the Maillard reaction.

Emirdağ (2014) investigated the effects of the interaction between resveratrol and milk proteins on the textural properties and water-holding capacity of yogurt, which are among important parameters in yogurt production. To determine the interaction of resveratrol with milk proteins and how the interaction occurs, resveratrol was analyzed with RP-HPLC and the results showed that around 85% of resveratrol interacted with milk proteins.

In a pilot study investigating the addition of resveratrol instead of sulphur dioxide, an antioxidant, during wine production, Pastor et al. (2015) reported that the addition of two different concentrations of resveratrol (150 mg/L and 300 mg/L) did not have any negative effect on the organoleptic and sensory properties of the products. Researchers have also suggested that the negative effects of sulphur dioxide on health can be prevented with the addition of resveratrol.

In their study on the production of a functional yogurt that is suitable for the consumption of all age groups, Türkoğlu (2019) added 25mg/100g, 50mg/100g, 75mg/100g and 100mg/g resveratrol to the set-type traditional yogurts produced with *Lactobacillus bulgaricus* + *Streptococcus thermophilus* and probiotic yogurts containing *Lb. bulgaricus* + *S. thermophilus*: *Lb. acidophilus* (1:1). The results of the study revealed that, considering all parameters in terms of the physicochemical and microbiological properties and sensory scores at the end of day 28, 50mg/100g resveratrol-added traditional and probiotic yogurts were new functional products suitable for consumption.

Yu et al. (2020), investigated potential of resveratrol in mitigating advanced glycation end-products formed in baked milk and baked yogurt and the results of the research provided a promising strategy for inhibiting the AGEs formed in baked milk and baked yogurt with the addition of a proper concentration of resveratrol.

In the future, the number of studies investigating the enrichment of products frequently consumed in the daily diet with the addition of resveratrol may increase.

Conclusions

As a natural polyphenol, resveratrol has various biochemical and physiological effects such as anti-inflammatory, antioxidative, antiproliferative and chemopreventive effects. Recent studies have shown its positive effects especially on cardiovascular diseases, cancer, type 2 diabetes and neurological disorders. In addition, studies reported that resveratrol has shown a high antiviral potential in both human and animal viral infections. The bioavailability of resveratrol after ingestion, the potential of dietary or direct intake in humans and animals, in the treatment of viral infections should be explored in more detail. It appears that more than the amount naturally found in food is needed to provide the expected benefits from resveratrol. Moreover, variety, region, seasonal, geographical, and environmental conditions have affected resveratrol contents of food. For this reason, it is often not

possible to consume adequate resveratrol in the diet. Agricultural food waste and byproducts should be investigated in terms of resveratrol potential. As our understanding of the positive effects of resveratrol on health grows, the antioxidant will continue to be the subject of many scientific studies.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Ethics committee approval: The authors declare that this study does not require ethical permission.

Funding disclosure: -

Acknowledgments: -

Disclosure: A part of this research was presented as oral and published form at the 3rd International Conference Advanced Engineering Technologies (ICADET'19), 19-21 September 2019, Bayburt, Turkey.

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 7(1), 64-74 (2021) • https://doi.org/10.3153/FH21008 *Review Article*

Pectin: Properties and utilization in meat products

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Cite this article as:
Sharefiabadi, E., Serdaroğlu, M. (2021). Pectin: Properties and utilization im meat products. *Food and Health,* 7(1), 64-74.
https://doi.org/10.3153/FH21008

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Submitted: 18.09.2020
Revision requested: 16.10.2020
Last revision received: 21.10.2020
Accepted: 22.10.2020
Published online: 11.12.2021

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Available online at
http://jfhs.scientificwebjournals.com

ABSTRACT

In recent years, there is increased awareness of conscious consumers about the fact that foods they eat are related directly to their health. In meat industry research and development, studies have accelerated to formulate healthier meat products formulations using plant sources as additive which are also expected to improve the functional properties of the product. Pectin is a water soluble fiber with a structural complexity that occurs naturally in the cell walls of fruits and vegetables, contributes to reducing the risk of cancer, and has some health benefits. Gelation is the most unique property of pectin; it forms a gel in the presence of Ca^{2+} ions or sugar and acid. Pectin presents good water and fat binding property. Therefore, it can be used as a gelling agent, film/coating, and emulsifier and in low-calorie meat products as fat and /or sugar substitution (dietary fiber), as a natural component contributes to phosphate substitution and medical delivery systems in meat products. In this paper, it was aimed to discuss the physico-chemical properties, health implications of pectin and its potential applications in meat products.

Keywords: Pectin, Meat products, Gelling agent, Restructured meat, Low-fat products

Introduction

Pectin is a type of structural fiber found in the primary cell wall and intracellular layer of plant cells mainly in fruits, such as apples, oranges, lemons, and so on (Mudgil, 2017).

Pectin contains a polysaccharide backbone with an a-(1-->4)-linked D-galacturonic acid. The acid groups along the chain are largely esterified with methoxy groups in the natural product. There can also be acetyl groups present on the free hydroxy groups. As shown in Figure 1, esterification is the reaction between carboxylic acid and alcohol or compounds containing the hydroxyl group (usually methanol) to form ester and water (Parkinson, 2014).

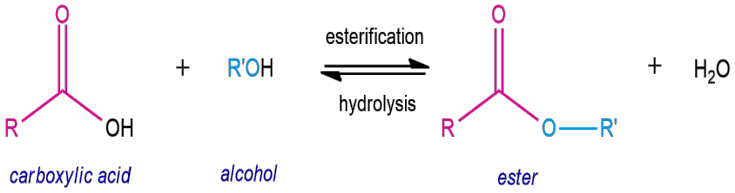

Figure 1. Esterification mechanism of pectin (Parkinson, 2014).

Degree of methyl esterification ranges between 0-100% and on the basis of esterification, there are two different types of peçtin; high methoxy (HMP) and low methoxy pectin (LMP) (Ramirez-Suarez et al., 2017).

Table 1 shows pectin content of various plants. Apple pomace and citrus fruit peels are commercially acceptable sources of pectin (Thakur et al., 1997). However, it has been extracted from different plants such as sunflower head, mango peel, soybean hull, sugar beet pulp, chickpea husk, etc. (Fajardo et al.,2016). Pectin from apple forms a more elastic-viscous gel however, citrus pectin displays a more elastic-brittle gel (Masuelli, 2019).

Utilization of pectin has been allowed in all countries, FAO/WHO committee recommended acceptable daily intake of pectin has no limitation and it is as a safe additive except where specified by good manufacturing practice. Pectin is used as a thickening agent, gelling agent, texturizer, emulsifier, stabilizer, and fat or sugar replacer in food industry, the major application of pectin is based on its gelling properties (Thakur et al., 1997). The source and the method of extraction have an influence on the structure and properties of pectin such as viscosity and gelling ability and thus their application in food industry (Gawkowska et al., 2018).

In the present review, it was aimed to investigate the functional properties of pectin and its usages in meat product formulations.

Table 2. Pectin content of various plants

Plants	Pectin	Reference
Sugar beet and sunflower head	10-20%	Yancheva et al., 2016
Cocoa husks	about 9%	Yancheva et al., 2016
Beet and potato pulp and Soy hull	26-28%	Yancheva et al., 2016
Apple pomace	10-17%	Sharma et al., 2014; Fajardo et al., 2016
Citrus peels	20-30%	Panchami & Gunasekaran, 2017; Fajardo et al., 2016
Plants of *Lupinus* genus	1.5%-7%	Valdés et al., 2015
Burdock from the *Arctium* genus	Higher than 21%	Valdés et al., 2015
Peach	up to 10%	Valdés et al., 2015
Orange peel/ pomace and seed	30-50 %	Begum et al., 2017
Pineapple	54%	Begum et al., 2017

Pectin Extraction Methods

According to the previous studies, some parameters mainly pH, time and temperature influence pectin extraction (Yapo et al., 2007; Fathi et al., 2012). Pectin can be extracted by various methods from the cell-wall material in laboratory-scale such as cold/hot water or buffer solutions, cold diluted sodium hydroxide, cold/hot solutions of chelating agents and hot diluted acids (Levigne et al., 2002). Figure 2 shows an optimized method of pectin extraction. Pectin can have physical harm in structure when extracted fully by acidic extraction and acid extraction of pectin has no environmental safety. Therefore, the combination of acid extraction and other methods is used. In recent years, searching for alternative methods that have fewer disadvantages is continued such as using microbial enzymes or enzyme complexes and ultrasound-assisted, ohmic- assisted, microwave-assisted, etc. extractions (Ptichkina et al., 2008; Gavahian et al., 2019). As an example, sonication of pectin leads to increased antioxidant capacity, 200 W and 400 W sonicated pectin have higher oxygen radical absorbance capacity and FRAP values than native pectin. Therefore, ultrasound offers an effective and green process for pectin transformation and creation of antioxidant potent pectin products (Ogutu and Mu, 2017).

Characteristics of Pectin

Solubility and Dispersibility

There are two types of pectin depending on the solubility: water-soluble and water-insoluble pectin. This property is determined by the number and distribution of methoxy groups and the degree of polymerization. It means that a decrease in molecular weight and an increase the (degree of esterification) DE cause to increase solubility increases. Other parameters that have an impact on solubility are pH, temperature, and the nature and concentration of the solute present (Axelos and Thibault, 1991).

Dispersibility means the ease of solubilization of pectin, which is more important than solubility. When dry powdered of pectin added to water, attends to hydrate rapidly and forming clumps (Kachare et al., 2020). Formation of the clump can be prevented by using water-soluble carrier material with dry powdered pectin or improving the dispersibility of pectin by mixing (5/10 parts by weight) fine-powdered sugar or D-glucose (the common dispersing agents) and pectin (Axelos and Thibault, 1991).

Gelation

Gelation is the most unique property of peçtin, pectin forms gels in the presence of Ca^{2+} ions or sugar and acid also traps the liquid by forming a three-dimensional network due to the merging or cross-linking of long polymer chains (Narasimman and Sethuraman, 2016). Two different pectin types form gels under completely different conditions. The ester group is less hydrophilic than the carboxyl group; therefore, HMP makes gel at a higher temperature than the LMP. Low methoxy pectin forms gel in pH (2-6) and presence of divalent ions such as calcium, high methoxy pectin forms a gel in the presence of sugar (>50%) and acid (pH: 3.1-3.6) (Narasimman and Sethuraman, 2016).

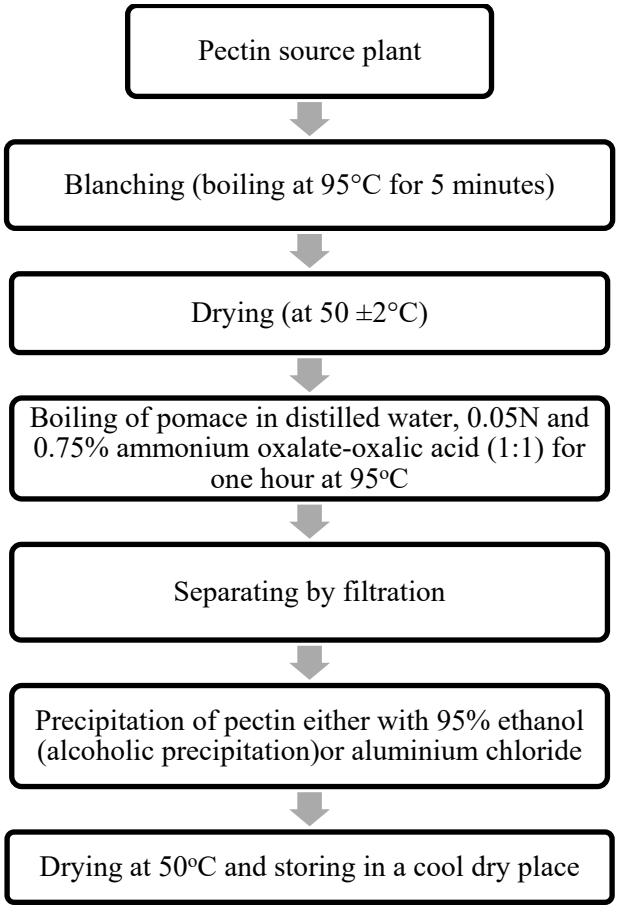

Figure 3. An optimized method for pectin extraction (Sharma et al., 2014)

Table 3. The yield of pectin extraction methods (Vales et al., 2015)

Sources	Methods	Yield
Sugar beet pulp	Citric acid, 99°C	23.95
Watermelon seed	HCl, 85°C	19.75
Tomato peel	Oxalic acid and ammonium oxalate, 90°C (two steps 24 and 12 h)	32.0
Mango peel	Sulfuric acid in the water, 90 °C	>70
Sunflower head	Sodium citrate, 85 °C	16.90

Pectin as Emulsifier

Emulsions assist encapsulation of the bioactive compounds. Depending on the disperse phase, there are two types of liquid emulsion: oil droplet in water (O/W) and water droplet in oil (W/O). Meat emulsion is an example of O/W emulsion, margarine and butter, by contrast are examples of W/O emulsion (Fajardo et al., 2016). A multiple (double) emulsion system defined as 'emulsion in emulsion', in which oil-in-water (O/W) and water-in-oil (W/O) are together. For example in W1/O/W2 double emulsion, W1 and W2 are internal and external water phases respectively, there are two different interface layers W1-O (internal water droplets are surrounded by oil) and O-W2 (oil droplets are surrounded by water (Öztürk et al., 2016).

Emulsifiers are used to mix two liquids that are normally immiscible for manufacturing desirable products. Emulsifiers divided into small-molecular surfactants (lecithin, etc.) and macro-molecular emulsifiers (proteins and plant-based polymers such as soy polysaccharide, pectin, etc.). Good emulsifiers should have low molecular weight, rapidly reduce the interfacial tension and soluble in the external phase. Pectin has been reported to exhibit surface-active property in oil-water interface and thereby stabilizing the oil droplets in emulsion systems (Funami et al., 2007)

O/W emulsion is stabilized by steric and electrostatic interaction of pectin. Pectin improves the stability of emulsion, with the addition of pectin viscosity of the emulsion increased, therefore movement of oil droplets are limited. Molecular weight effects emulsifying capacity of pectin. Small emulsion droplets are efficiently stabilized by low molecular weight pectin. Researches also indicated that pectin increases colloidal binding and coagulation of soluble proteins. The high molecular weight of pectin is an important factor that prevents protein coagulation. Many factors may influence the interactions, such as DE of pectin, pH and processing conditions. Pectins can stabilize the protein in acidic media through conjugation or complexation. The rate of structure development in pectin gels depends on temperature, pectin concentration and hydration of pectin. (Axelos and Thibault, 1991; Yapo et al., 2007; Fajardo et al., 2016)

Pectin-protein molecules form a network that surrounded oil droplets in emulsion based food products. Nowadays, pectin is used in emulsified low-fat meat products, dairy products, etc. (Masuelli, 2019).

Usage of Pectin in Meat Products

Depending on the functional properties such as gelling ability, water binding ability and acting as an emulsifier pectin is one of the natural ingredients for healthy meat products formulations. Pectin is widely used in meat products to form a gel and/or as a thickener. This property is related to the size, shape, chain length and total negative charge in galacturonic acid structure of pectin molecule.

The interactions between pectin and meat proteins showed in Figure 3, 4 and 5. Actin interacts with pectin through eighteen amino acids; there are eight H-bonds that play an important role in the correct positioning of pectin into the actin surface (Figure 3). Myosin interacts with pectin through thirteen amino acid and twelve H-bonds play an important role in the correct positioning of pectin into the myosin surface (Figure 4). Collagen interacts with pectin through thirteen amino acid and a total of six H-bonds play an important role in the correct positioning of pectin into the collagen surface (Figure 5).

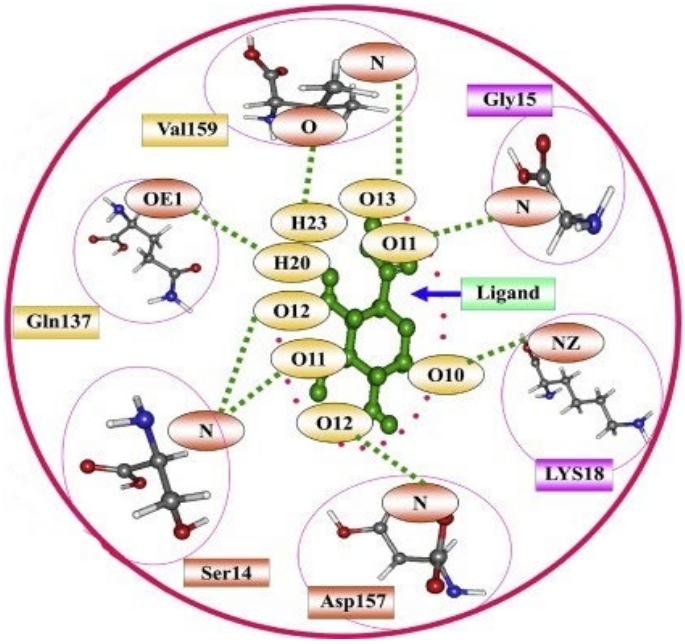

Figure 3. Complex structure pectin + actin (Ahmad et al., 2020)

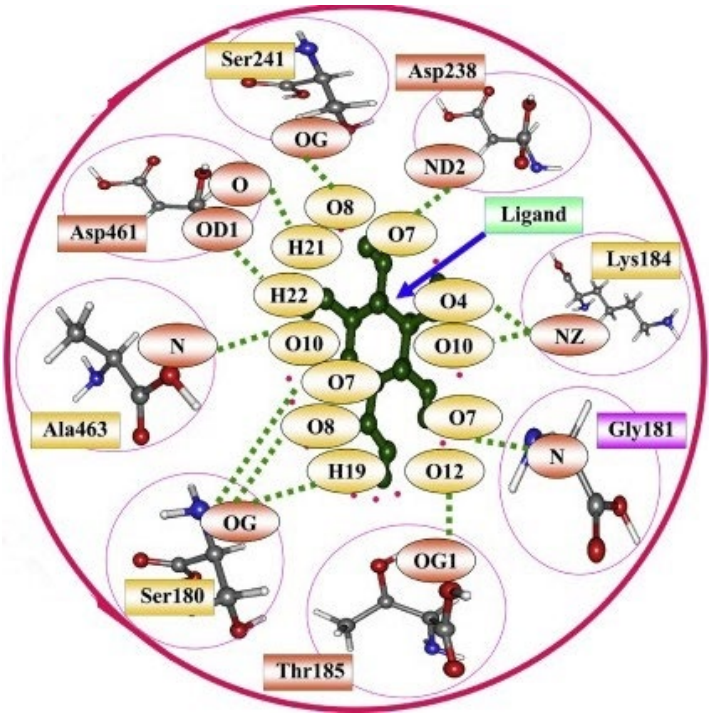

Figure 4. Complex structure pectin + myosin (Ahmad et al., 2020)

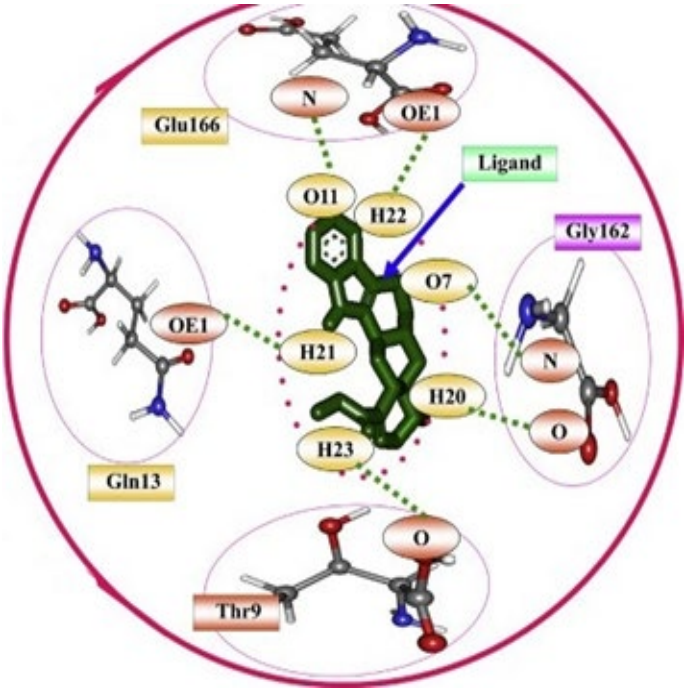

Figure 5. Complex structure pectin + collagen (Ahmad et al., 2020)

Myofibrillar proteins represent the functional part of meat proteins, they are extracted from the muscle tissue, followed by the accumulation around oil droplets and/or participation in the formation of a three-dimensional network gel during meat processing, ultimately contributing to improved emulsion stability, water-holding capacity (WHC), and texture of meat products. The examination of zeta potential and hydrodynamic size has demonstrated that histidine inhibits the aggregation of myosin and increases the solubility of myosin. Recently, it was highlighted that both lysine and arginine improve the water holding capacity and texture. The myosin molecule has an asymmetric structure with two globular heads and a rod-like tail. It associates with insoluble fibers through electrostatic tail-to-tail interaction under physiological conditions. Consequently, pectin has a good interaction with meat proteins based on the H-bond formation and free binding energy (Ahmad et al., 2020).

Film/Coating

Films or coatings are defined as a layer on the food surface or placed between food components (Korkmaz, 2018). Their function is to extend the shelf life of the products and provide a barrier against various hazards. Films should provide mechanical properties and restrict the migration of gas in food wrapping and/or coating. Edible films can represent physical protection, reduction of moisture and lipid transition, limit absorption of oxygen, improve handling features and can be contacted directly with food. Some natural components such as carbohydrates and proteins are used for the manufacturing of films, which have nutritional value, biodegradability, and environmental compatibility (Maftoonazad et al., 2007). Pectin and its derivatives are used in many biodegradable packaging materials that serve as moisture, oil, and aroma barrier, reduce respiration rate and oxidation of food (Ciolacu et al., 2014). Pectin-based edible films and coatings, either alone or enriched with antimicrobial and antioxidant substances, were investigated in meat and meat products (Tural et al., 2017).

Edible films are developed to be used as carrier for additive with specific functions such as anti-browning, antimicrobial agents, texture enhancers, nutrients, probiotics, and flavors (Falguera et al., 2011). As an additional barrier against pathogenic and spoilage microorganisms, antimicrobials are incorporated into edible films to inhibit food surface contamination. Ravishankar et al., (2012) are investigated the incorporation of carvacrol and cinnamaldehyde as antimicrobials into films against *Listeria monocytogenes* on contaminated ham and bologna. The effectivity of pectin films on ham was more than bologna (Ravishankar et al., 2012). Borges et al., (2016) evaluated the effect of free nisin and nisin-loaded pec-

tin nanoparticles on the growth rate of *Listeria innocua* in fermented pork meat model at different temperatures and fermentation conditions during 96 hours. Using both nisin and nisin-loaded pectin nanoparticles had significant inhibition effect on *Listeria innocua* (Borges et al., 2016).

Casings prepared with pectin or combination of gelatin/sodium alginate have been used for sausage production. Sensory analysis of sausages indicated that pectin casings were more preferred to gelatin/sodium alginate casings for sausages (Liu et al., 2007). Kang et al., (2007) studied the physicochemical, microbiological and sensory quality of irradiated cooked pork patties coated with pectin containing green tea leaf extract, found that lipid oxidation and microbial growth decreased in coated samples.

Pectin as Fat Substitution

Fat has an important role in meat products, including stabilizing meat emulsions, increasing cooking yield and water holding capacity, and improving texture. Fat also has an impact on binding, rheological and structural properties of meat products. However, high-fat diets are related to obesity, high blood pressure, cardiovascular and coronary heart diseases (Choi et al., 2016). Three different techniques can be applied to reduce the fat content in meat product formulations; changing the chemical composition of the carcass, using meat with low-fat content (manipulating animal raw materials or selecting lean meat) and using fat substitutes. Different fat substitutes have been used to ensure the functionality of fat. Substitutes derived from carbohydrates are generally hydrophilic because they have large number of hydrogen bonds with water, which forms an emulsion system on the targeted food tissue (Schmiele et al., 2015; Tufeanu and Tita, 2016).

Using pectin can be considered a feasible way to replace or reduce animal fat in meat products. Méndez-Zamora et al. (2015) studied the effect of substitution of animal fat with different formulations of pectin and inulin on chemical composition, textural, and sensory properties of emulsion type sausages and they recorded combined using of 15% pectin and 15% inulin could be used as animal fat replacer.

Effects of carrageenan and/or pectin gel (20%) were evaluated in low-fat beef frankfurters, frankfurters formulated with either carrageenan or carrageenan/ pectin gel had acceptable sensory scores (Candogan and Kolsarıcı, 2003). In another study, different fat replacers were investigated in low-fat frankfurters. According to the results, the emulsion stability of the batter was affected in different way due to the addition of different hydrocolloids. Samples including 1% pectin concentration had the highest cooking yield and water holding capacity (WHC) similar to control. TBA values decreased by addition of 0.5% pectin. Sensory analysis showed that one of the closest samples to the control were 0.5% pectin by the consumers (Jafarzadeh Yadegari, 2015). Han and Bertram (2017) evaluated using pectin in fat-reduced pork meat model system; pectin added samples showed similar properties with the normal fat controls.

Replacing pork back fat with 35% olive oil resulted positive scores in sensory characteristics when 0.45% of pectin was added (Pappa et al., 2000).

The substitution of 5% mango peel pectin to fat content in Chinese sausage enhanced color and conserve the physical qualities as well as sensory attribute

Plant sources containing pectin were also used in meat industry. Apple pomace was used in different meat products such as chicken sausages (Yadav et al., 2016), buffalo meat sausage (Younis and Ahmad, 2015) and reduced-fat chicken sausages (Choi et al., 2016). All these studies showed an increment in cooking yield, WHC, emulsion stability. Çoksever (2009) investigated the effects of bitter orange albedo at different concentrations on the quality of naturally fermented Turkish style sausages and results were similar to pectin uses studies. Decreasing in weight loss and increasing in cooking yield were observed by the addition of 1% soy hull pectin in both fresh and frozen/thawed beef patties while TBARS value, statistically not affected and sensory scores were similar to control (Kim et al. 2016).

Pectin Assisted Phosphate-Free Meat Products

Phosphates are multi-functional and low-cost compounds. Phosphates enhance product yield by increasing water holding capacity, flavor, and texture as well as having antioxidant functions as a metal chelating agent. However, researches show that high phosphate intake has several health risks. Therefore, there is a trend for reducing the amount of use in formulations or replacing them with natural components that meet their effects (Tabak et al., 2019). In phosphate free meat products, natural calcium powders are widely used as alkaline ingredients to increase the pH value of products. Since low methoxy pectins form gels through calcium ions, low methoxy pectin is a promising additive for phosphate free meat product formulations. Besides this due to its water-binding property pectin can assist or enhance water-binding properties of phosphate reduced and phosphate free meat products (Ko et al., 2014; Cho et al. 2017; Cho et al. 2018).

Tabak et al., (2019) showed combined using of eggshell powder with pectin or carrageenan enhanced the technological and sensory qualities of phosphate free chicken patties.

Using Pectin in Restructured Meat Products

Pectin specially amidated low methoxy pectin (ALMP) have certain functionality in gel products. ALMP is an anionic carbohydrate that its anionic groups interact with the cationic groups of protein and hydrogen bonds consequently, increase the functional and mechanical properties of protein systems. Therefore, its addition can affect the gelling process, depending on concentration and raw matter (Ramirez-Suarez et al., 2017). Gel matrix is formed by binding small pieces of meat as a result of solubilizing myofibrillar proteins in restructured meat products. Addition of pectin can improve one or more properties of solid food (patties) such as cohesion, firmness, juiciness, freeze/thaw stability or texture resistance to shrinking during cooking (Deo et al., 2019). Urestia et al., (2003) used different concentrations of ALMP for producing restructured fish to improve the quality properties of surimi. Results observed that 5% of ALMP decreased expressible water content in restructured products. 1% concentration of ALMP recommended to improved texture and gel strength in restructured fish (Urestia et al., 2003). Ramirez-Suarez et al., (2017) evaluated the effects of different concentration ALMP pectin on quality properties of jumbo squid (*Dosidicus gigas*) muscle gels due to the low functionality of muscle proteins and limited use. Texture was improved by adding ALMP. The highest WHC was shown in samples contain 3% ALMP compared to control (Ramirez-Suarez et al., 2017). As shown in Table 3 besides other properties, pectin has potential as a preservative, carrier of materials, and provides good rheological properties.

Health Benefits of Pectin

Pectin presents cholesterol-lowering, serum glucose-lowering and anti-cancer activities due to the specific structural domains, carrying bioactive properties. Pectin as soluble fiber decreases blood serum total cholesterol and low-density lipoprotein cholesterol, without having any effect on high-density lipoprotein cholesterol. Pectin has a good potential in food delivery, pectic-oligosaccharides obtained in a refined form from apple pomace presented prebiotic effects, which contribute to a healthy environment (Naqash et al., 2017). Pectin reduces blood sugar when consumed with food and studies have shown that pectin reduces the risk of some cancer types. For example, studies showed that extraction of pectin from citrus fruits prevents the formation of spontaneous prostate cancer cells in the body. These studies have also demonstrated the relationship between pectin and decreasing the risk of prostate cancer (Çoksever, 2009).

Table 3. Review of recent researches

Products	Applications	Results	References
Beef, chicken filet, shrimps and whiting (fish)	Edible film containing pectin for freshness	High sensitivity to gaseous amines and good standard degradation markers	Dudnyk et al., 2018
Raw-fermented sausage	Incorporation of 3% pectin	Similar rheological properties as the full-fat control	Zeeb et al., 2018
Emulsion-type sausage	Incorporation of 1.88% pectin	Higher in softening value	Zeeb et al., 2018
Meat batter	15% inulin and 15% pectin as a fat replacer	No effects on physical properties	Silva-Vazquez et al., 2018
Raw beef meat	Edible pectin-fish gelatin films	Improving oxygen barrier and delaying the lipid oxidation	Bermúdez-Oria et al., 2019
Meat sausage	LM pectin(4%)-encapsulated-fat	Preventing fat digestion and absorption/ improving texture	Santiaguín-Padilla et al.,2019
Hamburger patty (semi-finished products)	Alginate-pectin with whey protein concentrate	Inhibiting lipid oxidation, improving tenderness and better quality	Barybina et al., 2019
Fresh pork loin	Nanoemulsion loaded pectin edible coating	The best preservation and stability in 15 days 4°C	Xiong et al.,2020

Conclusion

Pectin is a water-soluble fiber and used in food industry as emulsifier, stabilizer, gelling, and thickening agent. Pectin presents in the cell walls of most plants, apple pomace and citrus peel are the two major sources of commercial pectin. Pectin extraction is a multiple-stage process in which hydrolysis and extraction of the pectin macromolecules from plant tissue and their solubilization takes place under the influence of different factors. Pectin has been used successfully for many years in food industry as a thickening, gelling agent and a stabilizer. Consumers have preferred products with natural additives while having the sensory properties of traditional food. Pectin is one of the promising natural additives for low fat meat products and also can be used in phosphate free formulations and emulsion type products due to the gelling and water binding ability. Pectin edible films incorporated with natural antimicrobials have potential application in preservation as active packaging materials for meat products.

Compliance with Ethical Standard

Conflict of interests: The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

Ethics committee approval: The authors declare that this study does not require ethical permission.

Funding disclosure: -

Acknowledgments: -

Disclosure: -

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Limitations, drawbacks, and the shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

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FOOD and HEALTH
E-ISSN 2602-2834

References

Reference System is APA 6th Edition

In-text Citation with APA

The APA style calls for three kinds of information to be included in in-text citations. The **author's last name** and the work's **date of publication** must always appear, and these items must match exactly the corresponding entry in the references list. The third kind of information, the page number, appears only in a citation to a direct quotation.

....(Crockatt, 1995).

Direct quote from the text

"The potentially contradictory nature of Moscow's priorities surfaced first in its policies towards East Germany and Yugoslavia," (Crockatt, 1995, p. 1).

Major Citations for a Reference List in Table 2.

Note: All second and third lines in the APA Bibliography should be indented.

REVISIONS

When submitting a revised version of a paper, the author must submit a detailed "Response to the reviewers" that states point by point how each issue raised by the reviewers has been covered and where it can be found (each reviewer's comment, followed by the author's reply and line numbers where the changes have been made) as well as an annotated copy of the main document. Revised manuscripts must be submitted within 30 days from the date of the decision letter. If the revised version of the manuscript is not submitted within the allocated time, the revision option may be cancelled. If the submitting author(s) believe that additional time is required, they should request this extension before the initial 30-day period is over.

Accepted manuscripts are copy-edited for grammar, punctuation, and format. Once the publication process of a manuscript is completed, it is published online on the journal's webpage as an ahead-of-print publication before it is included in its scheduled issue. A PDF proof of the accepted manuscript is sent to the corresponding author and their publication approval is requested within 2 days of their receipt of the proof.

Table 2.

Material Type	Reference List/Bibliography
A book in print	**Baxter, C. (1997).** *Race equality in health care and education.* Philadelphia: Ballière Tindall, p. 110-115, ISBN 4546465465
A book chapter, print version	**Haybron, D.M. (2008).** Philosophy and the science of subjective well-being. In M. Eid & R. J. Larsen (Eds.), *The science of subjective well-being* (p. 17-43). New York, NY: Guilford Press. ISBN 4546469999
An eBook	**Millbower, L. (2003).** *Show biz training: Fun and effective business training techniques from the worlds of stage, screen, and song*. p. 92-90. Retrieved from http://www.amacombooks.org/ (accessed 10.10.2015).
An article in a print journal	**Carter, S., Dunbar-Odom, D. (2009).** The converging literacies center: An integrated model for writing programs. *Kairos: A Journal of Rhetoric, Technology, and Pedagogy,* 14(1), 38-48.
Preview article in a journal with DOI	**Gaudio, J.L., Snowdon, C.T. (2008).** Spatial cues more salient than color cues in cotton-top tamarins (*Saguinus oedipus*) reversal learning. *Journal of Comparative Psychology,* https://doi.org/10.1037/0735-7036.122.4.441
Websites - professional or personal sites	***The World Famous Hot Dog Site.* (1999, July 7).** Retrieved January 5, 2008, from http://www.xroads.com/~tcs/hotdog/hotdog.html (accessed 10.10.2015).
Websites - online government publications	**U.S. Department of Justice. (2006, September 10).** Trends in violent victimization by age, 1973-2005. Retrieved from http://www.ojp.usdoj.gov/bjs/glance/vage.htm (accessed 10.10.2015).
Photograph (from book, magazine or webpage)	**Close, C. (2002).** *Ronald.* [photograph]. Museum of Modern Art, New York, NY. Retrieved from http://www.moma.org/collection/object.php?object_id=108890 (accessed 10.10.2015).
Artwork - from library database	**Clark, L. (c.a. 1960's).** *Man with Baby.* [photograph]. George Eastman House, Rochester, NY. Retrieved from ARTstor.
Artwork - from website	**Close, C. (2002).** *Ronald.* [photograph]. Museum of Modern Art, New York. Retrieved from http://www.moma.org/collection/browse_results.php?object_id=108890 (accessed 10.10.2015).