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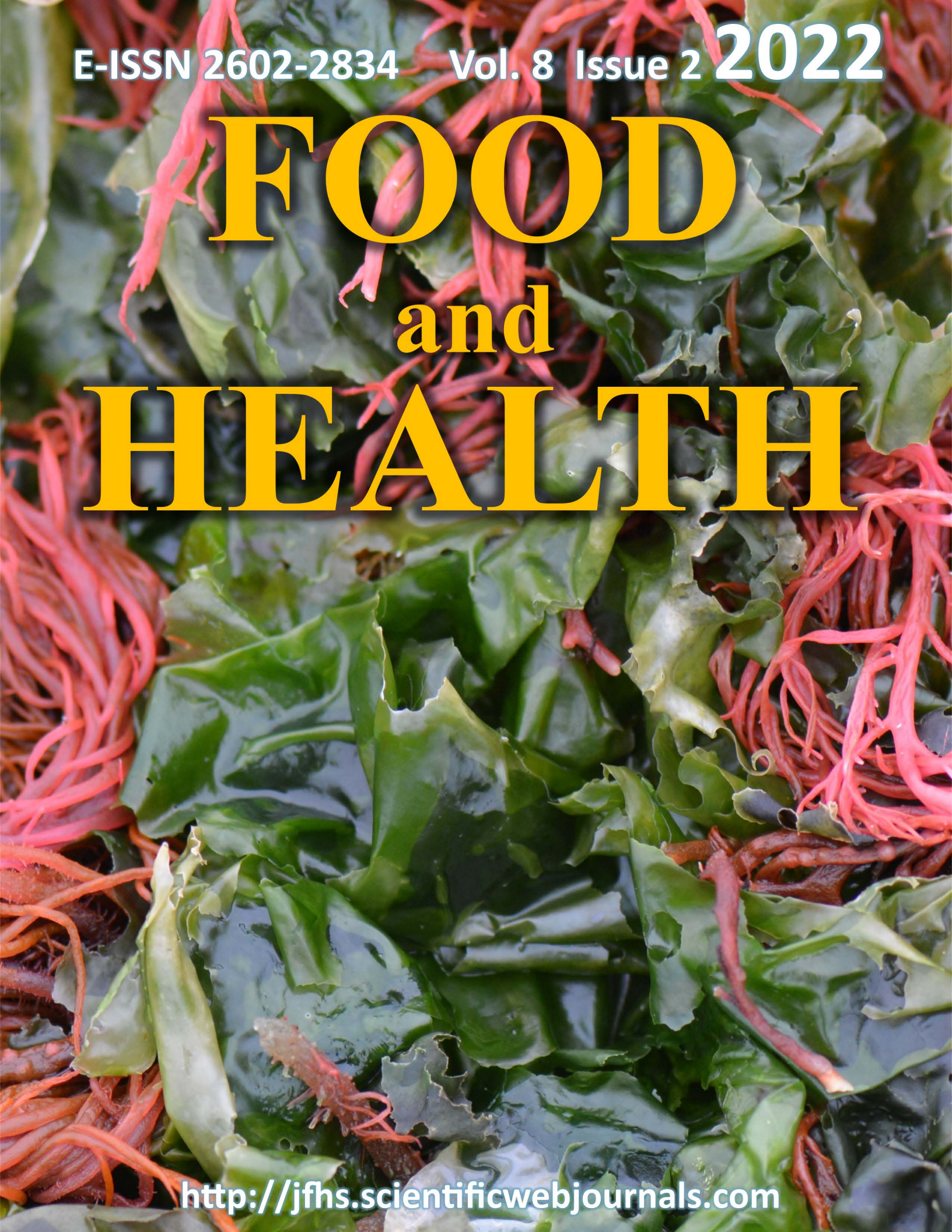

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"Food and Health" journal will publish peer-reviewed (double-blind) articles covering all aspects of **food science and their health effect** in the form of original research articles (full papers and short communications), and review articles. Their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

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Gıda ve Yem için Hızlı Alarm Sistemi'nde yer alan çevresel kirletici bildirimleri

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ÖZ

Gıda ve Yem için Hızlı Alarm Sistemi; Avrupa Komisyonu tarafından oluşturulmuş, gıda zincirinde halk sağlığına yönelik riskler tespit edildiğinde hızlı şekilde bilgi akışını ve tepki verilmesini sağlayan bir veri tabanıdır. Bu çalışma kapsamında, 2000-2020 yılları arasında Hızlı Alarm Sistemi veri tabanında yer alan gıdalarda bulunan çevresel kirletici kaynaklı bildirimler ile ilgili ayrıntılı bir rapor hazırlanmıştır. Sistemden elde edilen tüm veriler Microsoft Office Excel 2010 kullanılarak değerlendirilmiştir. Yirmi bir yıl içerisinde gerçekleştirilmiş 774 bildirim incelendiğinde, bildirimlerin %32'sinin "balık ve balık ürünleri", %29'unun "sıvı ve katı yağlar" ve %11'inin "diyetetik ürünler ve gıda takviyeleri" hakkında oluşturulduğu belirlenmiştir. Temel problemin (bildirimlerin %12'si) "polisiklik aromatik hidrokarbonlar" olduğu saptanmıştır. Tüm bildirimler içinde %33'ünün "ciddi risk" derecesine sahip olduğu bulunmuştur. En fazla bildirim (81 bildirim) 2001 yılında bulunmaktadır. Ek olarak, bildirimlerin %61'i "alarm", %13'ü "bilgi", %11'i "dikkat gerektiren bilgi", %11'i "sınır iadesi" ve %4'ü "takip gerektiren bilgi" bildirimleri türündedir. Bildirimlerin büyük bir kısmı (415 bildirim) piyasadaki resmi kontroller sonucunda oluşturulmuştur. Almanya, 178 bildirim ile en yüksek bildirimde bulunan ülkedir. Bildirimler sonucunda genellikle "ürünlerin piyasadan toplatılması" ve "üretici firmanın ürünleri piyasadan geri çekmesi" yaptırımları uygulanmıştır.

Anahtar Kelimeler: Çevresel kirleticiler, Gıda güvenliği, Hızlı Alarm Sistemi, Rapid Alert System for Food and Feed (RASFF), RASFF bildirimleri

ABSTRACT

Environmental pollutants notifications in Rapid Alert System for Food and Feed

Rapid Alarm System for Food and Feed (RASFF); is a database created by the European Commission that provides rapid information flow and response when risks to public health are identified in the food chain. Within the scope of this study, a detailed report was prepared on the notifications of environmental pollutants in foods in the Rapid Alarm System database between 2000 and 2020. All data obtained from the system were evaluated using Microsoft Office Excel 2010. When 774 notifications made in 21 years were examined, it was determined that 32% of the notifications were about "fish and fish products", 29% about "oils and fats" and 11% about "dietetic products and food supplements". The main problem (12% of the notifications) was determined to be "polycyclic aromatic hydrocarbons". Among all notifications, 33% of them were found to have a "serious risk" degree. The highest number of notifications (81 notifications) was detected in 2001. Additionally, 61% of the notifications were in the "alert", 13% "information", 11% "information for attention", 11% "border rejection" and 4% "information follow-up" notifications. Most of the notifications (415 notifications) were created as a result of official controls in the market. Germany was the country with the highest notifications, with 178 notifications. As a result of the notifications, sanctions were generally imposed on "withdrawal of products from the market" and "withdrawal of products from the market by the manufacturer". Considering the decrease in PAH notifications from food-related environmental pollutants over the years, it can be thought that the Rapid Alert System was effective in the decrease in the number of notifications.

Keywords: Environmental pollutants, Food safety, Rapid Alert System for Food and Feed (RASFF), RASFF notifications

Giriş

Gıda güvenliği; gıdalarda oluşabilecek biyolojik, kimyasal ve fiziksel tüm zararları ortadan kaldırmak amacıyla alınan önlemlerin tümü anlamına gelmektedir (Tarım ve Köyişleri Bakanlığı, 2008). Birleşmiş Milletler Gıda ve Tarım Örgütü (FAO)'ne göre ise gıda güvenliği insanların sağlıklı bir yaşam için güvenli ve yeterli gıdaya ulaşabilmeleridir (Karabal, 2019). Gıda ve Yem için Hızlı Alarm Sistemi (RASFF); Avrupa Komisyonu tarafından geliştirilmiş, Avrupa Birliği (AB) üye ülkeleri için gıda güvenliği ile ilgili bilgileri toplamak amacıyla merkezi bir veri tabanı olarak hizmet veren izleme ve bildirim aracıdır (Dada ve ark., 2021). Bu çevrimiçi veri tabanı "tarih, ülke, tip (gıda, yem), ürün (kategori), risk durumu, referans numarası, bildirim konusu" gibi kriterler kullanılarak gıda güvenliği ile ilgili bilgilere ulaşılmasına imkan tanır (RASFF, 2021). İnsan ve hayvan sağlığı açısından riskli bir durum ortaya çıktığı takdirde üyeler tarafından bildirimler oluşturularak, komisyona haber verilir (Çınar ve ark., 2017). Veri tabanı üzerinde 24 saat kesintisiz bilgi akışı mevcuttur (Dada ve ark., 2021). Sistem gıda güvenliğinin yanı sıra gıda sahtekarlığı, ikameler ve doğrudan gıda tehlikesi olarak kabul edilmese dahi ekonomik önem taşıyan tağşişleri izlemek için de kullanılabilir (Dada ve ark., 2021).

Hızlı Alarm Sistemi'nin kuruluşu Avrupa Konseyi'nin (EC) 178/2002 sayılı kararı olan "Genel Gıda Yasası" ile resmileştirilmiştir (Çınar ve ark., 2017; RASFF, 2021). Bu sistemin yasal temeli Avrupa Konseyi Yönetmeliği'nin 50. maddesinde belirtilmektedir. Oluşturulmuş bu bilgi ağı içerisinde AB'nin 28 üye ülkesi, Avrupa Ekonomik Alanı (AEA)'nın üç ülkesi Norveç, İzlanda ve Lihtenştayn, ek olarak İsviçre, Avrupa Komisyonu-Sağlık ve Gıda Güvenliği Genel Müdürlüğü ve Avrupa Gıda Güvenliği Otoritesi (EFSA) tarafından temsil edilen Avrupa Komisyonu (EC), Avrupa Serbest Ticaret Birliği (EFTA) Gözetim Kurumu (ESA) yer almaktadır (Caldeira ve ark., 2021). Sistemde yer alan bildirimler; alarm, bilgi, sınır iadesi ve haber bildirimleri olarak dört grup altında incelenmektedir. Alarm bildirimleri, ciddi sağlık riski taşıyan bir gıda veya yem ürünü piyasaya sunulduğunda, hızlı önlem alınması gerektiği durumlarda gerçekleştirilir. Bilgi bildirimleri, piyasaya sürülen ürünler ile ilgili bir risk tespit edildiğinde ancak, henüz ürünün pazarlarına ulaşmadığı ya da artık pazarlarında bulunmayan ülkelerde, hızlı önlem gerektirmeyen durumlarda oluşturulur. Sınır iadesi, sağlık riski tespit edildiğinde AB'nin dış sınırlarından reddedilen ürünler için gerçekleştirilirken, haber bildirimleri ise hakkında alarm, bilgi ve sınır iadesi bildirimleri oluşturulmamış ürünler ile ilgili yetkililerin dikkatini çeken bir durum ortaya çıktığı takdirde üyeleri bilgilendirmek amacıyla oluşturulmaktadır (RASFF, 2021).

Son yıllarda Hızlı Alarm Sistemi'nden; gıdalarda yabancı madde kontaminasyonlarının incelenmesinde, deniz ürünlerini etkileyen uygunsuzlukların değerlendirilmesinde, gıda kirleticileri (gıda ile temas eden maddeler, farmakolojik olarak aktif maddeler ve diğer gıda kirleticileri) hakkında yer alan bildirimlerden faydalanılarak kirletici madde maruziyetinin daha hızlı tespitinde, *Listeria monocytogenes* ile kontamine olmuş gıda ürünlerine ilişkin bildirimler analiz edilerek tüketicinin gıda kaynaklı listeriosis hastalığından korunmasında, gıda alerjenleri ile ilgili bildirimler esas alınarak 1169/2011 sayılı AB tüzüğü ile bildirimler arasındaki ilişkinin belirlenmesinde ve mikotoksinler ile ilgili bildirimlerin analizinde faydalanılmıştır (Djekic ve ark., 2017; Amico ve ark., 2018; Fürst ve ark., 2019; Lüth ve ark., 2019; Pádua ve ark., 2019; Pigłowski, 2019). Ayrıca Alshannaq ve Yu (2021) tarafından yapılan çalışmada, 2010-2019 dönemi için başta aflatoksin olmak üzere mikotoksinlerle kirlenmiş gıda ve yem ürünlerine ilişkin bildirimler incelenmiştir. Caldeira ve ark. (2021) tarafından yapılan çalışmada, 1979-2019 yılları arasında balıklarda gözlenen bir parazit türü ile ilgili gerçekleştirilmiş olan bildirimler değerlendirilmiştir. Son olarak Dada ve ark. (2021) tarafından yapılan çalışmada ise, 2000-2020 yılları arasında Asya ve Pasifik bölgesinden gelen gıdaların mikrobiyolojik güvenliğini test etmek amacıyla Hızlı Alarm Sistemi'nde yer alan bildirimlerden faydalanılmıştır.

Ülkemiz kaynaklı bildirimlerin incelendiği çalışmalar da literatürde mevcuttur. Çebi ve Olhan (2017) tarafından yapılan çalışmada, 2011-2015 yılları arasında ülkemiz kaynaklı gıdalar için oluşturulmuş bildirimler incelenmiş ve en problemli ürün kategorilerinin meyve ve sebzeler ile sert kabuklu yemişler olduğu belirtilmiştir. Bu çalışmada mikotoksin ve pestisit kaynaklı bildirimler en tehlikeli bildirim konularını oluşturmuştur. Çınar ve ark. (2017) tarafından yapılan çalışmada, 2009-2016 yılları arasında Hızlı Alarm Sistemi'nde yer alan ülkemiz kaynaklı bildirimler değerlendirilmiş ve ihraç edilen ürünlerdeki temel problemin aflatoksin olduğu bulgulanmıştır. Bu çalışmada da problemli gıda kategorileri meyve, sebze, kuru yemiş ve tohumlar olarak belirlenmiştir. Deniz Şirinyıldız ve Yorulmaz (2019) tarafından yapılan çalışmada, 2004-2019 yılları arasında ülkemiz için önemli bir ihraç ürünü olan kuru incir ile ilgili bildirimler değerlendirilmiş ve en temel problem olarak ürünlerde aflatoksin varlığı tespit edilmiştir. Çalışmada en fazla bildirimde bulunan iki ülkenin Fransa ve Almanya olduğu, bildirimlerin büyük bir kısmının sınır kontrolleri sırasında oluşturulduğu ve ciddi risk derecesine sahip olduğu rapor edilmiştir. Kürekci ve Şahin (2019) tarafından yapılan çalışmada, 1992-2018 yılları arasında kanatlı eti ve ürünleri ile ilgili gerçekleştirilen bildirimler gıda güvenliği açısından incelenmiştir. Kanatlı eti ve ürünleri ile

ilgili esas sorunun patojen mikroorganizmalar veya toksinleri olduğu saptanmıştır. Sağlam ve Masatcıoğlu (2020) tarafından yapılan çalışmada ise, 2009–2018 yılları arasında sistemin üye ülkeleri ve ülkemiz kaynaklı gıdalarda yapılmış olan bildirimler incelenmiş ve en problemli ürün kategorisi olarak önceki çalışmalarla benzer şekilde meyve ve sebzeler (%20) bulgulanmıştır. En fazla bildirim yapılan konu yine mikotoksinler (özellikle aflatoksinler-%89) olmuştur.

Çevre kirliliği insan sağlığını etkileyen en ciddi problemlerden biridir. Çevre kirliliği, dünyanın fiziksel ve biyolojik bileşenlerinin normal çevresel süreçleri olumsuz yönde etkileyecek şekilde zararlı kimyasallarla kirlenmesi olarak tanımlanmıştır. Bu kimyasal maddeler, olması gereken seviyelerin üzerinde biriktiklerinde veya zehirli olduklarında "çevresel kirletici" olarak kabul edilirler ve hava, toprak ile suyun kirlenmesine neden olurlar (Gupta, 2007; Suzuki ve ark., 2020). Polisiklik aromatik hidrokarbon (PAH)'lar, heterosiklik aminler ve poliklorlu aromatik bileşikler (dioksinler, dibenzofuranlar ve bifeniller) çevresel kirleticilere örnek verilebilir (Hoffman ve ark., 1991; Suzuki ve ark., 2020). Vücutta bu kirleticilerin konsantrasyonlarının belirli eşiği aşması durumunda insan sağlığı olumsuz yönde etkilenebilir (Suzuki ve ark., 2020). Postolache ve ark. (2020) tarafından yapılan çalışmada, 2000-2020 yılları arasında süt ve süt ürünleri için Hızlı Alarm Sistemi'nde yer alan bildirimler analiz edildiğinde çevresel kirleticiler (öncelikle dioksin ve dioksin benzeri bileşikler) süt ürünleri için en önemli kimyasal kirleticilerden biri olarak belirtilmiştir. Süt ve süt ürünleri hakkında fazla sayıda bildirimde bulunan ülkeler Fransa, Almanya ve İtalya olurken; bildirimler genellikle şirketlerin kendi kontrolleri ve piyasadaki resmi kontroller sırasında oluşturulmuştur. Bildirimler sonucunda geri çağırma, geri çekme ve imha olmak üzere farklı yaptırımlar uygulanmıştır.

Çevresel kirleticiler içerisinde bahsedilen PAH'lar, organik malzemelerin (örneğin kömür, yağ, petrol ve odun) eksik yanması sırasında açığa çıkan ve her yerde bulunabilen çevresel kirleticilerdendir. PAH'ların başlıca kaynakları arasında konut ısıtması, asfalt, kok, alüminyum üretimi, petrol rafinerilerindeki faaliyetler, motorlu araçların egzoz gazları vb. yer almaktadır. PAH'ların özellikle atmosferde taşınmaları çok kolaydır ve birçoğu toksik, mutajenik ve/veya karsinojenik özelliklere sahiptir. PAH'lar yağda yüksek oranda çözünebildiklerinden, insan vücuduna alınması halinde gastrointestinal sistemde kolayca emilerek, sağlık açısından büyük risk oluşturmaktadır (Abdel-Shafy ve Mansour, 2016).

Bu çalışmanın amacı, 2000-2020 yılları arasında Gıda ve Yem için Hızlı Alarm Sistemi veri tabanında yer alan bildirimlerden yola çıkılarak çevresel kirleticiler ile ilgili ayrıntılı bir rapor hazırlamaktır.

Materyal ve Metot

Çalışma kapsamında materyal olarak 1 Ocak 2000 ile 31 Aralık 2020 tarihleri arasında Hızlı Alarm Sistemi'ne yapılan gıda kaynaklı "Çevresel Kirletici" bildirimlerine ait veriler kullanılmıştır. Sistemin resmi sitesinde tür (gıda), tarih (01/01/2000-31/12/2020) ve tehlike (çevresel kirletici) bilgileri girilerek, bildirimde bulunulan ürünler ile ilgili "ürün kategorisi", "bildirim konusu", "risk derecesi", "bildirim yılı", "bildirim türü", "bildirim kaynağı", "bildirimde bulunan ülke", "uygulanan yaptırım" ve "ürünün dağıtım bilgileri" hakkındaki veriler kategorilere ayrılarak incelenmiştir. Elde edilen veriler Microsoft Office Excel 2010 (Microsoft Corp., Redmond, ABD) kullanılarak işlenmiştir ve ilgili grafikler ile tablolar oluşturulmuştur.

Bulgular ve Tartışma

Yapılan incelemeler sonucunda 2000 ile 2020 yılları arasında Hızlı Alarm Sistemi'nde çevresel kirleticiler hakkında gıda kaynaklı toplam 774 bildirim tespit edilmiştir.

Ürün Kategorisine Göre Bildirimler

Farklı ürün gruplarına ait bildirim sayı ve oranları Tablo 1'de verildiği gibidir. Tüm bildirimler ürün kategorilerine göre incelendiğinde, en fazla bildirimin %32 ile "balık ve balık ürünleri" hakkında olduğu gözlenmiştir. Bu ürün kategorisini %29 dağılım oranı ile "katı ve sıvı yağlar" takip etmiştir. Ayrıca "diyetetik gıdalar, gıda takviyeleri ve güçlendirilmiş gıdalar", "et ve et ürünleri (kümes hayvanları dışında kalan)", "meyve ve sebzeler", "yumurta ve yumurta ürünleri", "otlar ve baharatlar", "kakao ve kakao karışımları, kahve, çay", "tahıllar ve unlu mamuller", "alkolsüz içeçekler", "kabuklular ve kabuklu ürünleri", "kanatlı eti ve ürünleri", "çorbalar, et suları, soslar ve çeşniler", "süt ve süt ürünleri", "yumuşakçalar ve kabuklular hariç vahşi avlanmış balık ürünleri", "gıda katkı maddeleri ve tatlandırıcılar" "şekerlemeler", "yumuşakçalar ve ürünleri", "çerezler ve tohumlar", "hazır yemekler ve atıştırmalıklar", "içme suyu", "şaraplar", "diğer gıda ürünleri, karışımlar", "doğal maden suyu" ve "gıda ile temas eden maddeler" hakkında da bildirimlerde bulunulmuştur. Hızlı Alarm Sistemi hakkında yapılan farklı çalışmalar incelendiğinde özellikle meyve, sebze ve kuru yemişler problemli ürün kategorileri olarak karşımıza çıkmıştır (Çebi ve Olhan, 2017; Çınar ve ark., 2017). Mevcut çalışma kapsamında bu kategoriler ile ilgili oluşturulmuş olan bildirimlerin oranı düşüktür.

Konularına Göre Bildirimler

Ürün konularına ait bildirim sayı ve oranları Tablo 2'de verildiği gibidir. Tablo incelendiğinde en çok bildirime konu olan çevresel kirleticinin polisiklik aromatik hidrokarbonlar

olduğu görülmektedir. PAH'ları sırasıyla dioksinler, mineral yağlar ve dioksin benzeri olmayan poliklorobifeniller takip etmiştir. Polisiklik aromatik hidrokarbonların, füme tavuklarda ve balıklarda, dioksin ve dioksin benzeri bileşiklerin özellikle süt ve süt ürünlerinde ciddi birer çevresel kirletici olduğunu belirten çalışmalar da literatürde yer almaktadır (Beia ve ark., 2020; Postolache ve ark., 2020).

Bu çalışma kapsamında gazyağı, bromat, dizel yakıt, toluen, benzen, perklorat, petrol hidrokarbonları, izinsiz kullanılan gıda katkı maddeleri ve diğer yağlı maddeler ile kontaminasyon gibi konuların da gıda kaynaklı çevresel kirletici bildirimlerinin gerçekleştirilmesine sebep olduğu bulgulanmıştır. En büyük problem olarak karşımıza çıkan PAH'lar hakkında gerçekleştirilen bildirimlerin her geçen yıl azaldığı gözlenmiştir. PAH bildirimlerinin yıllara göre değişimi Şekil 1'de verildiği gibidir. En fazla PAH bildirimi 2000 yılında 80 bildirim olarak saptanmıştır. En az bildirim ise 1 bildirim sayısı ile 2020 yılında gerçekleştirilmiştir.

Risk Derecelerine Göre Bildirimler

Tüm bildirimler gıda güvenliği açısından değerlendirildiğinde ciddi risk derecesine sahip, kararsız kalınmış ve ciddi olmayan risk derecesine sahip bildirimler olmak üzere 3 gruba ayrılmıştır. Risk derecesine göre bildirim sayı ve oranları Tablo 3'te verildiği gibidir. Tespit edilen 774 bildirimin %65'inin risk derecesini belirleme konusunda kararsız kalınmıştır. Gerçekleştirilmiş olan bildirimlerin %33'ünün ciddi risk derecesine sahip olduğu saptanmıştır. Yalnızca %2'lik bir kısım ciddi risk derecesine sahip olmayan bildirimler olarak adlandırılmıştır.

Yıllara Göre Bildirimler

Bildirim sayılarının yıllara göre değişimi Şekil 2'de verildiği gibidir. En az bildirim sayısı 2000 yılında 2 bildirim olarak belirlenirken, en fazla bildirim sayısı 80 bildirim ile 2001 yılında tespit edilmiştir. İlerleyen yıllar ile birlikte bildirim sayılarında kararlı bir değişim gözlenmemektedir.

Türlerine Göre Bildirimler

Hızlı Alarm Sistemi'nde yer alan gıda kaynaklı çevresel kirleticiler ile ilgili oluşturulmuş bildirimler; alarm bildirimleri, bilgi bildirimleri, dikkat gerektiren bilgi bildirimleri, takip gerektiren bilgi bildirimleri ve sınır reddi olarak 5 grupta yer almıştır. Türlerine göre bildirim sayı ve oranları Tablo 4'te verildiği gibidir. En fazla bildirim, alarm türünde bulunmaktadır. Sistemde 2011 yılına kadar yalnızca alarm, bilgi ve sınır reddi bildirimleri yer alırken, 2011 yılı sonrası bilgi bildirimleri detaylandırılmıştır ve bahsedilen üç bildirim türüne dikkat gerektiren bilgi bildirimleri ve takip gerektiren bilgi bildirimleri de ilave edilmiştir. En fazla bildirimin oluşturulduğu 2001 yılında gerçekleştirilmiş olan 81 bildirimin ise 80'inin alarm türünde, yalnızca 1'inin bilgi bildirimi türünde olduğu bulgulanmıştır.

Kaynağına Göre Bildirimler

Kaynaklarına göre bildirimlerin 2000-2020 yılları arasındaki dağılımı Tablo 5'te verildiği gibidir. Tüm bildirimlerin %54'ü, piyasadaki resmi kontroller sırasında problemli ürünlerin belirlenmesi sonucu oluşturulmuş bildirimlerdir. Hızlı Alarm Sistemi'nde yer alan 126 bildirim için ise kaynak belirtilmemiştir. Bildirimlerin 110 adedi sınır kontrolleri sonucunda ürünlerin geri alınması üzerine; diğerleri üretici firmaların kendi kontrolleri sırasında, sınır kontrolünde ürünlerin geçişine izin verilmesi üzerine, tüketici şikayeti sonrası, Hızlı Alarm Sistemi bildirimleri üzerine yapılan kontroller sonucunda, sınır kontrolü esnasında gümrükte yapılan sevkiyatta, üye olmayan ülkelerde gerçekleştirilen resmi kontroller sırasında ve medyanın takibi üzerine oluşturulmuştur. Şirketlerin kendi kontrollerinin ve piyasadaki resmi kontrollerin problemli ürünleri bulmada etkili olduğu farklı çalışmalarda da tespit edilmiştir (Postolache ve ark., 2020).

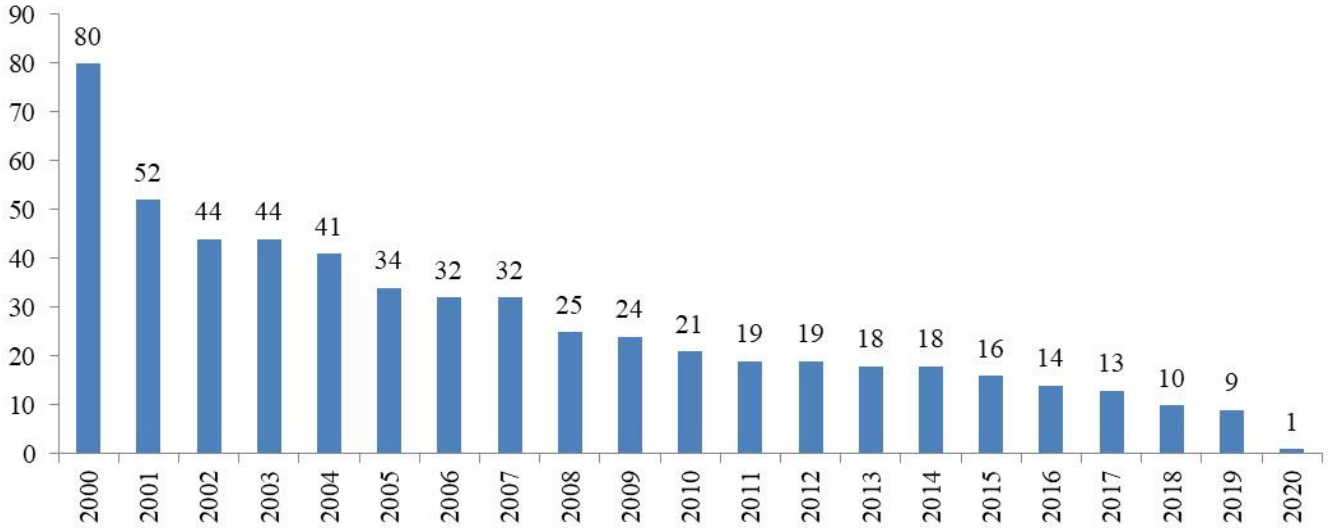
Ülkelere Göre Bildirimler

Gıda ve Yem için Hızlı Alarm Sistemi'ne bildirimde bulunan ülkelerin bildirim sayıları ve oranları Tablo 6'da verildiği gibidir. Almanya en fazla bildirimde bulunan ülke olmuştur. Ardından sırasıyla Birleşik Krallık, Hollanda, Slovakya, Belçika, İtalya, Polonya, Avustralya, Fransa, Finlandiya, Çekya, Litvanya, İrlanda, Norveç, İsveç, Estonya, Macaristan, Letonya, Yunanistan, Slovenya, Danimarka, Portekiz, İspanya, Lüksemburg, Romanya, İsviçre, Komisyon Hizmetleri, İzlanda ve Kıbrıs gelmektedir. Farklı çalışmalar incelendiğinde de Almanya, Fransa ve İtalya yüksek sayıda bildirimde bulunan ülkeler olarak öne çıkmaktadır (Deniz Şirinyıldız ve Yorulmaz, 2019; Postolache ve ark., 2020).

Uygulanan Yaptırımlara Göre Bildirimler

Hızlı Alarm Sistemi'nde yer alan tüm bildirimler sonucunda uygulanan yaptırımlar Tablo 7'de verildiği gibidir. Tablo incelendiğinde ağırlıklı olarak ürünlerin piyasadan çekildiği, geri çağırıldığı, imha edildiği görülmektedir. Ayrıca, kimi ürünlerle ilgili uygulanan yaptırımlar hakkında bilgi verilmemiş ve kimi ürünler de tüketiciden geri çağırılmıştır. Ek olarak, gerçekleştirilen diğer bildirimlerin ardından yeni ürün talep edilmesi, resmi olarak alıkoyma, para iadesi, alıcıların bilgilendirilmesi, ürünlere el konulması (haciz), yetkililerin bilgilendirilmesi, göndericiye ürünün geri iadesi ve stokta ürün tutmama gibi yaptırımlar da uygulanmıştır. Geri kalan bildirimler için farklı yaptırım türleri (ürünlerin bağlanması, daha ayrıntılı kontroller, ticaret yasağı konulması, firmanın ithalat yetkisini kaldırma, kamu uyarısı, basın bildirisi, satış yapan kişilerce alıkonma, göndericiyi konu hakkında bilgilendirme,

ürünü marketlerden kaldırma, ürünün varış noktasını değiştirme, imha işleminin tekrarlanması, fiziksel ya da kimyasal bir işlemin uygulanması, ürünlerin geri çağırılması, ürünlerin gıda ya da yem dışında farklı amaçlar için kullanılması) uygulanırken, 17 bildirim sonucunda hiçbir yaptırıma ihtiyaç duyulmamıştır.



Şekil 1. Polisiklik aromatik hidrokarbon bildirim sayılarının yıllar içindeki değişimi

Figure 1. Changes in the number of polycyclic aromatic hydrocarbon notifications over the years

Şekil 2. Bildirim sayılarının yıllar içindeki değişimi

Figure 2. Changes in the number of notifications over the years

Dağıtım Bilgilerine Göre Bildirimler

Hızlı Alarm Sistemi'nde çevresel kirleticilere ilişkin bildirim oluşturulmuş ürünlerin dağıtım bilgileri Tablo 8'de verildiği gibidir. Hakkında bildirimde bulunulan ürünlerin önemli bir kısmı ile ilgili dağıtım bilgisi yer almamaktadır. Oluşturulan 151 bildirim ile ilgili ürünler piyasaya dağıtılmış olan ürünlerdir, 145 bildirim ile ilgili ürünler ise diğer üye ülkelerde de dağıtılmıştır. Ayrıca dağıtımı yalnızca bildirimde bulunan ülkeler ile sınırlı olan, dağıtımı gerçekleştirilmeyen, dağıtımı piyasaya arz edilmeyen, artık piyasada olmayan, bildirimde bulunan ülkeden dağıtımı söz konusu olmayan, henüz dağıtım bilgisi girilmeyen, diğer üye ülkelere dağıtımı yapılmayan, stoğu bulunmayan, üye olmayan ülkelere dağıtım yapılmış, kullanım süresi bitmiş, çoktan tüketimi gerçekleştirilmiş, gümrük mühürleri altında varış noktasına seyahat etmesine izin verilmiş, son tüketim tarihi geçmiş ve çevrimiçi işlem gören ürünler için de Hızlı Alarm Sistemi'nde bildirimler mevcuttur.

Tablo 1. Ürün kategorilerine göre bildirim sayı ve oranları

Table 1. Number and rates of notifications by product categories

Ürün kategorisi	Bildirim sayısı (adet)	Bildirim oranı (%)
Balık ve balık ürünleri	251	32,43
Katı ve sıvı yağlar	222	28,68
Diyetetik gıdalar, gıda takviyeleri, güçlendirilmiş gıdalar	88	11,37
Et ve et ürünleri (kümes hayvanları dışında)	37	4,78
Meyve ve sebzeler	27	3,49
Yumurta ve yumurta ürünleri	22	2,84
Otlar ve baharatlar	20	2,58
Kakao ve kakao karışımları, kahve ve çay	19	2,45
Tahıllar ve unlu mamuller	16	2,07
Alkolsüz içecekler	12	1,55
Kabuklular ve kabuklu ürünleri	10	1,29
Kanatlı eti ve ürünleri	8	1,03
Çorbalar, et suları, soslar ve çeşniler	7	0,90
Süt ve süt ürünleri	6	0,78
Yumuşakçalar ve kabuklular hariç vahşi avlanmış balık ürünleri	5	0,65
Gıda katkı maddeleri ve tatlandırıcılar	5	0,65
Şekerlemeler	4	0,52
Yumuşakçalar ve ürünleri	3	0,39
Çerezler ve tohumlar	3	0,39
Hazır yemekler ve atıştırmalıklar	2	0,26
İçme suyu	2	0,26
Şaraplar	2	0,26
Diğer gıda ürünleri, karışımlar	1	0,13
Doğal maden suyu	1	0,13
Gıda ile temas eden maddeler	1	0,13

Tablo 2. Konularına göre bildirim sayı ve oranları

Table 2. Number and rates of notifications by subject

Bildirim konusu	Bildirim sayısı (adet)	Bildirim oranı (%)
Polisiklik aromatik hidrokarbonlar	566	73,13
Dioksinler	106	13,70
Mineral yağlar	30	3,88
Dioksin benzeri olmayan poliklorobifeniller	15	1,94
Diğerleri	57	7,36

Tablo 3. Risk derecelerine göre bildirim sayı ve oranları

Table 3. Number and rates of notifications according to risk decision

Risk derecesi	Bildirim sayısı (adet)	Bildirim oranı (%)
Kararsız kalınmış	504	65,12
Ciddi risk derecesi	253	32,69
Ciddi risk derecesine sahip olmayan	17	2,20

Tablo 4. Türlerine göre bildirim sayı ve oranları

Table 4. Number and rates of notifications by notification type

Bildirim türü	Bildirim sayısı (adet)	Bildirim oranı (%)
Alarm bildirimleri	473	61,11
Bilgi bildirimleri	97	12,53
Dikkat gerektiren bilgi bildirimleri	89	11,50
Sınır reddi	82	10,59
Takip gerektiren bilgi bildirimleri	33	4,26

Tablo 5. Kaynağına göre bildirim sayı ve oranları

Table 5. Number and rates of notifications by notification basis

Kaynağına göre bildirimler	Bildirim sayısı (adet)	Bildirim oranı (%)
Piyasadaki resmi kontroller sırasında	415	53,62
Kaynak belirtilmemiş	126	16,28
Sınır kontrolleri sonucunda ürünlerin geri alınması üzerine	110	14,21
Üretici firmaların kendi kontrolleri sırasında	58	7,49
Sınır kontrolünde ürünlerin geçişine izin verilmesi üzerine	51	6,59
Tüketici şikayeti sonrası	8	1,03
Bildirimler sonucu kontrollerde	2	0,26
Sınır kontrolü esnasında gümrükte yapılan sevkiyatta	2	0,26
Üye olmayan ülkelerde gerçekleştirilen resmi kontrollerde	1	0,13
Medyanın takibi üzerine	1	0,13

Tablo 6. Ülkelere göre bildirim sayı ve oranları

Table 6. Number and rates of notifications by country

Ülke adı	Bildirim sayısı (adet)	Bildirim oranı (%)
Almanya	178	23,00
Diğer	148	19,12
Birleşik Krallık	64	8,27
Hollanda	63	8,14
Slovakya	45	5,81
Belçika	44	5,68
İtalya	41	5,30
Polonya	41	5,30
Avustralya	34	4,39
Fransa	33	4,26
Finlandiya	31	4,01
Çekya	27	3,49
Litvanya	25	3,23

Tablo 7. Uygulanan yaptırımlara göre bildirim sayı ve oranları

Table 7. Number and rates of notifications according to the action taken

Ülke adı	Bildirim sayısı (adet)	Bildirim oranı (%)
Ürünün piyasadan çekilmesi	170	21,96
Ürünlerin geri çekilmesi	128	16,54
Diğer yaptırım türleri	66	8,53
Ürünlerin imha edilmesi	59	7,62
Yaptırım hakkında bilgi verilmemiş	55	7,11
Ürünlerin tüketiciden geri çağırılması	53	6,85
Yeni ürün talep etme	49	6,33
Resmi olarak alıkoyma	36	4,65
Para iadesi	33	4,26
Alıcıları bilgilendirme	32	4,13
El konulması, haciz	31	4,01
Yetkilileri bilgilendirme	23	2,97
Hiçbir yaptırım uygulanmamış	17	2,20
Göndericiye iade etme	11	1,42
Stokta ürün tutmama	11	1,42

Tablo 8. Dağıtım bilgilerine göre bildirim sayı ve oranları

Table 8. Number and rates of notifications according to distribution status

Ülke adı	Bildirim sayısı (adet)	Bildirim oranı (%)
Dağıtım bilgisi yok	159	20,54
Piyasaya dağıtılmış	151	19,51
Diğer üye ülkelere dağıtılmış	145	18,73
Dağıtımı yalnızca bildirimde bulunan ülkeler ile sınırlı	99	12,79
Henüz dağıtımı gerçekleştirilmemiş	77	9,95
Henüz piyasaya arz edilmemiş	38	4,91
Artık piyasada olmayan ürünler	30	3,88
Bildirimde bulunan ülkeden dağıtılmamış	22	2,84
Henüz dağıtım bilgisi girilmemiş	20	2,58
Diğer üye ülkelere dağıtımı yapılmamış	6	0,78
Stoğu yok	6	0,78
Üye olmayan ülkelere dağıtım yapılmış	5	0,65
Kullanım süresi bitmiş	5	0,65
Tüketilmiş	4	0,52
Gümrük onayı ile seyahatine izin verilmiş	3	0,39
Son tüketim tarihi geçmiş	3	0,39
Çevrimiçi işlem gören ürün	1	0,13

Sonuç

Bu çalışma ile Hızlı Alarm Sistemi'nde 2000-2020 yılları arasında yer alan gıdalarda çevresel kirleticiler kaynaklı bildirimler hakkında ayrıntılı bilgi verilmiştir. En problemli ürün kategorisi balık ve balık ürünleri iken, en temel problem olarak PAH'lar öne çıkmıştır. PAH'ların yıllara göre bildirim sayısında düşüş olduğu dikkat çekmektedir. Bildirimlerin büyük bir kısmının ciddi risk derecesine sahip olması, daha güçlü tedbirlerin alınması gerekliliğini göz önüne sermektedir.

Hızlı Alarm Sistemi'nde yer alan bildirimlerin ayrıntılı olarak incelenmesi, gıda endüstrisinde gıda güvenliğinin sağlanması ve sürdürülebilirliği için büyük önem taşımaktadır. Ayrıca gıda ihracatı ve ithalatı sırasında karşılaşılan temel sorunların tespiti ve alınması gereken tedbirler açısından da son derece önemlidir. Avrupa Birliği özellikle ithal ettiği ürünlerin güvenliği konusunda oldukça titiz davranmaktadır. Bu veri tabanı takibi ile risk yönetimi geliştirilip uluslararası gıda güvenliği standartlarında üretim sağlanarak, AB pazarında rekabet gücü arttırılabilir. Bu sistem tüketicinin korunmasında, dolayısıyla halk sağlığında etkili olma potansiyeline sahiptir. Mevcut çalışmanın sonuçlarının, endüstriyi ve tüketiciyi bilinçlendirmek adına faydalı olacağı düşünülmektedir.

Etik Standart ile Uyumluluk

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

Etik izin: -

Finansal destek: -

Teşekkür: Derya DENİZ ŞİRİNYILDIZ 100/2000 YÖK Doktora Bursu programı kapsamında desteklenmiştir.

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Effect of black carrot (*Daucus carota* L.) pomace in cake and cookie formulations as a functional ingredient on sensory analysis

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ABSTRACT

In this study, it was aimed to determine the properties of the black carrot pomace and its use in pastries. For this purpose, black carrot pomace was dried at 150ºC by using drum drier. Dietary fiber (64.93 %), total sugar (1.16 %), fat (1.60 %), protein (8.82 %), anthocyanins (0.21 mg/g) and total phenolic substances (2.30 mg/g) of the black carrot pomace were determined. The functional properties of the carrot pomace such as water retention capacity (1.53 g/g), swelling (1.59 mL/g), alcohol insoluble residue (737.5 g/kg) and emulsion capacity (30.64 mL/g) were calculated. Dried pomace was used at three different levels in modified cookie and cake formulations. Ranking test was applied for the sensory evaluation of each derived formulations. The results of the sensory analysis showed that the use of black carrot pomace in cookie formulations was more suitable and more preferred.

Keywords: *Daucus carota*, Anthocyanin, Total phenolic substances, Functional food

Introduction

The fruit and vegetable processing industry produces significant volume of by-products, which could cause problems in their disposal. Despite considerable improvements in processing techniques including the use of depolymerizing enzymes, mash heating, and decanter technology, approximately one third of the raw material remains as pomace. Food industry wastes might be recycled using new washing, drying and fermentation techniques. Usually, these products are used in animal feeding. However, the high amount of dietary fiber content could permit the use of these products in developing new natural ingredients for the food industry. The conditions that are used in pomace processing industry could be effective on the chemical and physical characteristics and this might be important for the utilization purpose of the pomace (Constenla et al., 2002; Pagan et al., 2001; Schieber et al., 2001). The composition of the dietary fibers of various plant-originated pomaces was discussed in many literatures. The health benefits of dietary fiber have led to increased consumption of fiber-rich products and a search for new sources of fiber as food ingredients. In order to take advantage of the dietary and functional properties of fiber, some high dietary fiber formulated foods are currently being developed (Figuerola et al., 2005).

Dietary fibers are not only desirable for their nutritional properties, but also for their functional and technological properties and because of those they can also be used to upgrade agricultural products and by-products for use as food ingredients (Schieber et al., 2001; Figuerola et al., 2005). Thus, the functional properties, such as particle size, water retention, swelling, alcohol insoluble residue, emulsion capacity, emulsion stability, water and oil absorbtion, dietary fiber etc., which have been more useful for understanding the physiological effects of dietary fiber should be studied, than the chemical composition alone (Figuerola et al., 2005; Robertson et al., 2000).

Black carrot (*Daucus carota L.*) is cultivated in the southern regions of Turkey. The extracts of the roots are used in a traditional drink "Şalgam" also, it is known as a rich anthocyanin source (Keskin et al., 2021). The anthocyanin pigment composition of black carrot (*Daucus carota L.*) was detected as cy-3-rut-glu-gal acylated with one cinnamic acid (Akhtar et al., 2017; Giusti et al., 2003). Nawirska (2005) reported that carrot pomace contains mainly cellulose (% 51.6) and comparatively high amounts of lignin (32.2 %) also pectin (% 3.88) and hemicellulose (12.3 %). Chau et al. (2004) has revealed that carrot pomace was rich in insoluble fiber-rich fraction 56.3 g/100 g of pomace, which was mainly composed of pectic polysaccharides, hemicellulose, and cellulose. This insoluble fiber-rich fraction was found to have desirable physicochemical properties and in vitro hypoglycemic properties as compared with cellulose. Various attempts were made at utilizing carrot pomace in food such as milk, bread, cake, dressing and pickles, and for the production of functional drinks (Stoll et al., 2003; Pandey et al., 2021).

In this study, it was aimed to investigate the proximate composition and the functional properties of the black carrot pomace to be used as a potential fiber rich source in the enrichment of cereal products and to produce dietary cakes and cookies. For this purpose, total dietary fiber, water retention capacity, swelling, emulsion capacity, total phenolic content and anthocyanin content of black carrot pomace were determined; hence this pomace was used in three different cake and cookie formulations.

Materials and Methods

The black carrot pomace used in the study was supplied in 5 kg plastic bottles from a local fruit and vegetable processing facility, Etap Tarım ve Gıda Ürünleri Ambalaj San. Ve Tic. A.Ş. Mersin, Turkey. The supplied black carrot pomace was dried in drum drier (VDS 430) at Ege University Vocational Training School Pilot Plant with drum speed: 6 rpm, vapor pressure: 75 psi, drying temperature: 150 ºC. The dried pomace was blended in Warring blender. The dried pomace, which has the particle size varying between 425-850 µm, was used in different cake and cookie formulations.

Production of Cakes and Cookies:

As a result of the preliminary research on the chemical and the functional properties of carrot pomace, three cookie and three cake formulations were derived in which dried carrot pomace to be used as an ingredient. The three formulations modified to involve 3%, 5% and 7% dried pomace, respectively (Table 1 and Table 2).

Chemical Analysis and Functional Properties of the Black Carrot Pomace

Moisture content of the dried pomace was determined in vacuum oven at 80ºC and 600-800 mbar. The ash content was estimated according to AOAC-1995 method 942.05, total

sugar was determined by Lane Eynon Method (AOAC-1990 method 923.09). Fat content was determined by Soxhelet Method (Food and Agriculture Organization, 1986). Protein content (AOAC-1990 method 920.53) and dietary fiber content of the dried pomace was calculated (AOAC-1994 method 991.43).

Total phenolic content was measured by using the Folin–Ciocalteu method (Vinson et al., 1995) and the results were expressed as mg gallic acid equivalents (GAE) per gram of sample (mg GAE/g). Total anthocyanins were determined by using a pH differential method (Fuleki and Francis, 1968) and the results were expressed as miligram of cyanidin-3-glucoside equivalents per gram of sample.

Water retention capacity (WRC) 1 g of pomace was hydrated in 30 mL distilled water in a centrifuge tube at room temperature. After equilibration (18 h), the samples were centrifuged (3,000*g*; 20 min), the supernatant was decanted and the sample was transferred into a weighed sinter to drain. Sample fresh weight was recorded prior to drying. WRC was calculated as the amount of water retained by the pellet (g/g dry weight) after transferring to the sinter and the WRC was used to measure the water retained by the insoluble matrix (Robertson et al., 2000).

Swelling the sample (~0,1g) was hydrated in 10 mL of distilled water, in a calibrated cylinder (1.5 cm diameter) at room temperature. After equilibration (18 h), the bed volume was recorded and expressed as volume/g original substrate dry weight (Robertson et al., 2000).

Alcohol insoluble residues (AIR) AIR were prepared to characterize the relative contribution of polymeric material from each test substrate. Samples (~1 g dry weight) were extracted (x3) in boiling ethanol (30 mL; 5 min; 80-85% v/v). Alcohol-insoluble material from each extraction step was recovered by extraction step was recovered from filtration. Ethanolic supernatants were discarded and the ethanol insoluble material was dried by solvent exchange through absolute ethanol (x2) and acetone (x2) to yield a dry powder (Robertson et al., 2000).

Emulsion capacity one gram of each sample was mixed with 34 mL 1% NaCl solution in a Waring micro blender for 3 second. While continuing blending, 30 mL vegetable oil was added at a rate of 10 mL / min. Blending was continued for an additional 30 sec. Each sample was transferred to a 50 mL graduated centrifuge tube, kept in water bath at 80 ºC for 1 min, and then centrifuged at 3000 g for 40 min. The volume of oil separated from each sample after centrifugation was read directly from the tube. Emulsion capacity was expressed as the amount of oil emulsified and held per gram of sample (Okezie and Bello, 1988).

Water and oil absorbtion one gram of sample was dispersed in 50 mL of distilled water for water absorbtion and in 50 mL vegetable oil for oil absorbtion. Then the samples were centrifuged at 5000g for 30 mins. The volume of water and oil was measured to calculate the amount of water/oil absorbed by the sample. The results were reported as mL water/oil absorbed per g of sample.

Sensory Analysis

Sensory evaluation was conducted in an air conditioned sensory test laboratory equipped with individual booths. Cakes and cookies were served at room temperature to involve 3 cakes and 3 cookies on white plastic plates coded by using randomly selected 3 digit numbers. Panelists' sensitivity to major tastes and odors were tested according to ISO-3972:2011. Ranking test (Altuğ and Elmacı, 2005) was applied to evaluate the preference of trained panelists for appearance, texture, flavor and overall impression of cake and cookie formulations. 7 panelists attended the evaluation of the cookies while 10 panelists attended the evaluation of the cakes. The ranking test forms used for the evaluation of cake and cookie samples are given in Figure 1 and 2.

Name:..... Product:Cake Date:.....

Evaluate and rank the cake samples by assigning sample code of the most like/preferred sample to "1" and the least liked/preferred sample to "3" for appearance, texture, flavor and overall impression.

	Appearance		Texture		Flavor	O. Impression	
Most preferred	1	Tender	1	Most preferred	1	Most preferred	1
	2		2		2		2
Least preferred	3	Tough	3	Least preferred	3	Least preferred	3

Figure 1. Ranking test form for the cake samples

Name:..... Product: Cookie Date:.....

Evaluate and rank the cookie samples by assigning sample code of the most like/preferred sample to "1" and the least liked/preferred sample to "3" for appearance, texture, flavor and overall impression.

	Appearance		Texture		Flavor		O. Impression
Most preferred	1	Tough	1	Most preferred	1	Most preferred	1
	2		2		2		2
Least preferred	3	Tender	3	Least preferred	3	Least preferred	3

Figure 2. Ranking test form for the cookie samples

Results and Discussion

The results of the chemical analyses applied to the black carrot pomace after drying were shown in Table 3. Since the aim was to produce dietary cake and cookies, it can be concluded that the low levels of lipid and sugar content is an advantage. Total phenolic content of the dried pomace was detected to be 2.30 mg/g dw and the anthocyanin content was calculated as 0.21 mg/g dw. Kamiloğlu et al. (2017) used black carrot pomace for enhancing the nutritional value of cake in addition they investigated the digestive stability of polyphenols from black carrot pomace and traced the changes in antioxidant capacity by using a standardized static *in vitro* digestion model. They reported a dose-dependent increase in anthocyanins (72–267 µg/g dw), phenolic acids (49–148 µg/g dw), total phenolics (54–202 mg GAE/100 g dw) and total antioxidant capacity (21–129 to 153–478 mg TE/100 g dw).

In our study the dietary fiber content of the sample was calculated as (64.93 g/100g dw). According to the study performed by Figuerola et al. (2005) the dietary fiber contents were found as: grapefruit (44.2-62.6 %), lemon pomace (60.1-68.3 %), orange pomace (64.3-78,2 %) and apple pomace (60.2-89.8 %) and also Nawirska et al. (2005) calculated the dietary fiber contents of apple pomace as 98.74 % and carrot pomace as 54.2 %. In addition, Chau et al. (2004) reported the dietary fiber of carrot pomace within the ranges 50.1-67.4 %. It can be deducted that black carrot pomace can be used in dietary cake formulations due to its high dietary fiber content.

The dried black carrot pomace used in this study has the water retention capacity of 1.53 g/g and the swelling was determined to be 1.59. Figuerola et al. (2005) detected the water retention capacity of grapefruit (2.09-2.26 g/g), lemon pomace (1.74-1.85 g/g), orange pomace (1.65 g/g) and apple pomace (1.62-1.87 g/g) and the swelling values of grapefruit (6.69-8.02), lemon pomace (7.32-9.19), orange pomace (6.11) and apple pomace (6.29-8.27) (Figuerola et al.. 2005). As seen in the above figures the water retention and swelling values of the black carrot pomace was found to be lower than the fruit pomaces analyzed in other studies. This difference is assumed to originate from the difference between the dietary fiber composition of vegetables and the fruits.

Oil adsorption capacity depends on surface properties, overall charge density, thickness, and hydrophobic nature of the fiber particle. In this study the oil absorbtion of the black carrot pomace was calculated as 2.77 mL/g. Likewise the oil absorbtion capacities of grapefruit (1.20-1.52 g/g), lemon pomace (1.30-1.48 g/g), orange pomace (1.81 g/g) and apple pomace (0.60-1.45 g/g) (Figuerola et al.. 2005). The oil absorbtion value of the black carrot pomace, which was found to be higher than the fruit pomaces in the literature, must be taken into consideration in the fat containing food formulations.

Alcohol insoluble residue was prepared to characterize the relative contribution of polymeric material from the pomace. In this study, it was calculated as 737.5 g/kg. In comparison with the AIR values in Robertson et al. (2000) of pea hull (953.2 g/kg), apple pulp (690.5 g/kg) and citrus pulp (484.8 g/kg), the AIR value of the black carrot pomace is considerably high.

The results of the sensory analysis (Table 5) applied to the cakes showed that the appearance of the 3% carrot pomace containing cakes are superior than the 5 and 7% carrot pomace containing cakes ($p<0.05$). The texture of the 3% carrot pomace containing cakes were ranked to be preferred more than the 5 % and 7% carrot pomace containing cakes ($p<0.05$). There was no significant difference among the 3, 5 and 7% carrot containing cakes in flavor ($p<0.05$). Also no

significant difference was detected between the 3 and 5% carrot containing cakes in overall impression and 7% carrot pomace containing cakes were found to be inferior ($p<0.05$).

Table 1. The ingredients of the cake formulations used in sensory analysis

	Amount (%)		
Ingredients	**Formulation No.1**	**Formulation No.2**	**Formulation No.3**
Margarine	9	9	9
Egg	20	20	19
Sugar	14	14	13
Flour	21	20	20
Baking Powder	0.8	0.8	0.8
B.Carrot Pomace	3	5	7

Table 2. The ingredients of the cookie formulations used in sensory analysis

	Amount (%)		
Ingredients (g)	**Formulation No.1**	**Formulation No.2**	**Formulation No.3**
Margarine	9	9	9
Egg	20	20	19
Sugar	14	14	13
Flour	21	20	20
Baking Powder	0.8	0.8	0.8
B.Carrot Pomace	3	5	7
Milk	32	31	30.5

Table 3. Proximate composition of dried black carrot pomace

Property	**Result**
Moisture	6.06 ±0.12 g/100g
Ash	5.65 ±0.08 g/100g dw
Lipid	1.60 ±0.21 g/100g dw
Protein	8.82 ±0.28 g/100g dw
Total sugar	1.16 ±0.03 g/100g dw
Dietary fiber	64.93 ±2.92 g/100g dw
Total Phenolic Compound	2.30 ±0.36 mg/g dw
Anthocyanin	0.21 ±0.03 mg/g dw

Table 4. Functional properties of dried black carrot pomace

Property	Result
Water Retention Capacity	1.53 ±0.52 g/g
Swelling	1.59 ±0.20 mL/g
Alcohol Insoluble Residue	737.5 ±10 g/kg
Emulsion Capacity	30.64 ±0.40 mL/g
Water Absorbtion	7.46 ±0.88 mL/g
Oil Absorbtion	2.77 ±0.31 mL/g

Table 5. The ranking test results of the cakes and cookies

	Cakes			Cookies		
	3%	5%	7%	3%	5%	7%
Appearance	11	22	27	7	14	21
Texture	15	24	21	10	11	21
Flavor	18	19	23	9	14	19
Overall Impression	16	17	27	8	13	21

Similarly the sensory analysis applied to the cookies revealed that the appearance of the 3 % carrot pomace containing cookies were superior ($p<0.05$), also the 7% pomace containing cookies were found to be inferior. No significant difference existed between the texture characteristics of the 3 and 5% carrot pomace containing cookies and these were more preferred to the 7 % carrot pomace containing cookies which were evaluated as inferior ($p<0.05$). The flavor of the 3% carrot pomace containing cookies were found to be superior and 7 % carrot pomace containing cookies found to be inferior among the cookies ($p<0.05$). The general impression of the 3% carrot pomace containing cookies were superior whereas the 7 % was found to be inferior ($p<0.05$).

Baltacıoğlu et al. (2019) used fermented black carrot powder waste (FBCW) to biscuits in various amounts (10, 20 and 30 %). They reported that 30 % FBCW added samples were found to be superior in terms of crust color and softness; control group were chosen as the best in terms of inner biscuit color, pore homogeneity and smell; 10 % FBCW added samples were found to be superior in terms of taste and crispiness and finally 20% FBCW added samples were detected to be superior in terms of greasy feeling in the mouth by the panelists. The authors also reported that 10 % FBCW added samples were superior in terms of general impression.

Carson et al. (1994) used dried, powdered unrefined pomace of three apple cultivars as an ingredient in pie filling and oatmeal cookies. Two pie filling or three oatmeal cookies were evaluated by the panelists according to their pomace amounts. Both food products were rated as moderately liked and it was reported that pomace amount did not affect color, cookie size or sensory scores

Canett Romero et al. (2004) made cookies containing four different levels of deseeded grape pomace (0, 5, 7.5 and 10 %). In result, cookies were well accepted by panelists showing no significant differences among the four levels of deseeded grape pomace addition. The addition of deseeded grape pomace imparted a darker color to the cookies indicated by the lower L color value. The researchers reported that deseeded grape pomace is a potential ingredient to some food products.

In many literature studies excessive amounts of pomace were disliked by the panelists since it disrupts some sensory properties i.e. texture, color, size, hardness/softness, mouth feeling, greasy feeling in mouth. Likewise, in our study the 3% carrot pomace containing cakes and cookies were detected superior to 5 and 7 % containing samples in most of the sensory quality attributes.

Conclusion

In this study it was determined that the black carrot pomace is a valuable by-product. The final produced black carrot pomace added cakes and cookies has better functional properties compared to the control groups. The panelists mostly preferred 3% carrot pomace containing carrots and cookies in sensory quality attributes texture, flavor, appearance and overall impression. In the literature lots of studies on the dietary fiber content of apple, peach, grape and citrus pomaces

can be accessible, but limited research was carried out on carrot and other vegetable pomaces. As a result due to the compositional and functional properties of the black carrot pomace, which is rich in dietary fiber content, have a potential to be used as an ingredient in dietary products.

Compliance with Ethical Standard

Conflict of interests: The author declares that for this article they have no actual, potential or perceived conflict of interests.

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

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Comparison of total phenolic contents and antioxidant activities of propolis in different solvents

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ABSTRACT

This study aims to determine which solvent is the best for the solubility of the propolis by using concentrations of total polyphenols and flavonoids, ferric reducing antioxidant power (FRAP) assay, and total antioxidant status (TAS) in extracts of propolis from different provinces of Türkiye prepared with water, ethanol, dimethyl sulfoxide (DMSO), glycerol and acetone. Propolis samples were lyophilized in the same solvents except for that glycerol and acetone. Total concentrations of polyphenols and flavonoids, FRAP, and TAS of both normal and lyophilized extracts were found be consistent when compared with each other. After extraction of propolis and evaluation of the total polyphenol and flavonoid content and antioxidant capacity, we concluded that it is mostly dissolved in DMSO, and after that in ethanol, acetone, glycerol respectively, and the least in water according to our extraction and analysis methods.

Keywords: Propolis, Flavonoids, Polyphenols, Solubility, Different solvents

Introduction

Propolis is a resinous, sticky, natural complex mixture collected by honeybees from various plant sources (Burdock, 1998). It has a characteristic smell and colours changing from yellow, green, red to dark brown (Burdock, 1998; Orsatti et al., 2010). Propolis contains more than 300 kinds of chemical compounds such as polyphenols (flavonoids, phenolic acids, and their esters, phenolic aldehydes, alcohols, and ketones), sesquiterpene quinones, coumarins, steroids, amino acids, and inorganic compounds (Bankova et al., 2000). In recent years, propolis has gained quite popularity in the food and beverage industries in order to prevent many diseases such as cardiovascular disease, diabetes, and cancer and to protect health (Banskota et al., 2000).

Propolis has a wide range of biological activities such as antioxidant (Nagai et al., 2003; Kumazawa et al., 2004; Mohtar et al., 2020; Peixoto et al., 2021), antifungal, antibacterial, antiviral (Kujumgiev et al., 1999), antiproliferative (Banskota et al., 2002), cytotoxic (Banskota et al., 2000), immunomodulator (Orsolic et al., 2004), antimicrobial (Arslan et al., 2012), anti-inflammatory (Barlak et al., 2015), radioprotective (Benkovic et al., 2008), hepatoprotective (Banskota et al., 2000), preventive and protective effects against DNA damage (Aliyazicioglu et al., 2011).

It has been suggested that the compounds primarily responsible for the biological activities of propolis are phenolic compounds such as flavonoids (Havsteen, 2002). It has been shown that antioxidant activity, which is one of the most important biological activities of flavonoids in propolis, provides protection against lipid peroxidation in the cell membrane, thanks to its ability to scavenge free radicals (Pinchuk and Lichtenberg, 2002).

Since propolis cannot be used in its raw form, it should be purified by extraction with solvents. In this process, inert substances should be removed, and polyphenolic fractions should be protected (Pietta et al., 2002). Propolis extraction methods may affect the activity of propolis, as the use of different solvents may dissolve and extract different compounds in propolis (Sforcin, 2007).

The main purpose of this study is to compare the total phenolic contents and antioxidant activities of normal and lyophilized extracts of propolis, which is collected from different provinces of Türkiye, with the five different solvents determined as water, ethanol, dimethyl sulfoxide (DMSO), glycerol, and acetone.

Materials and Methods

Propolis Origin

Propolis was formed by mixing propolis samples supplied by Fanus Food Company (Trabzon, Türkiye) from different provinces of Türkiye.

Preparation of Water, Ethanol, Dimethyl Sulfoxide (DMSO), Glycerol and Acetone Extracts of Propolis

First, the propolis sample was frozen at -20°C and grated. The grated propolis sample was refrozen at -20°C and was ground in a blender (Arzum AR1002). With our own extraction method, 500 mg of ground propolis were dissolved in 20 mL of pure water, ethanol (Riedel-de Haën), DMSO (Carlo Erba), glycerol (Merck), or acetone (Merck) at 150 rpm and 60°C with the aid of a shaker incubator for 24 hours. After incubation, each extract was centrifuged at 2057 *g* for 10 minutes and filtered through filter paper. Collected supernatants were stored at 4 °C in the dark for further studies. The final concentration of each propolis extract including water extract of propolis (WEP), ethanol extract of propolis (EEP), DMSO extract of propolis (DEP), glycerol extract of propolis (GEP), and acetone extract of propolis (AEP) was adjusted to 25 mg/mL (stock solution). A proportion of 5 mL of the water, ethanol, and DMSO extracts were kept at -80°C for 30 minutes and lyophilized for 6 hours. 5 mL solvent (water, ethanol, or DMSO) was added to those extracts to obtain dissolved lyophilized extracts.

Determination of Total Polyphenol Content

Total polyphenol content was determined spectrophotometrically by modifying the Folin-Ciocalteu colorimetric method and adapting this method to a 96-well microplate reader (Lotito and Frei, 2004). 12.5 µL of diluted (1:50 with deionized water) propolis extracts were mixed by adding 62.5 µL of Folin-Ciocalteu reagent (Sigma) (1:10) and 125 µL of sodium carbonate (Lancaster) (20 %, w/v) into a 96-well microplate. After 30 minutes of incubation at room temperature and in the dark, absorbance was read at 700 nm on the microplate reader (Tunable VERSAmax microplate reader, USA). Gallic acid (Sigma) was used as a standard in drawing the calibration curve. Total polyphenol contents were stated as mg Gallic acid (GA)/g propolis.

Determination of Total Flavonoid Content

Total flavonoid content was determined spectrophotometrically by modifying the aluminium nitrate colorimetric method (Park et al., 1997). 20 µL of diluted (1:20 with deionized water) propolis extracts were mixed by adding 172 µL of 80 % ethanol, 4 µL of 10 % aluminium nitrate (Fluka) and

4 µL of 1 M aqueous potassium acetate (Merck) into a 96-well microplate. After 40 minutes of incubation at room temperature and in the dark, absorbance was read at 415 nm on the microplate reader. Quercetin (Fluka) was used as a standard in drawing the calibration curve. Total flavonoid contents were stated as mg Quercetin (Q)/g propolis.

Determination of Fe^{3+} (Ferric) Reducing Antioxidant Power (FRAP)

The reducing antioxidant power was determined spectrophotometrically according to the method applied by Oyaizu (1986) based on ferric to ferrous ion reduction at low pH (Oyaizu, 1986). To 40 µL of diluted (1:100 with deionized water) propolis extract in 1.5 mL of microtube (Eppendorf) was added 100 µL of 0.2 M sodium phosphate buffer (Merck) (pH 6.6) and 100 µL of 1% potassium ferricyanide (Lancaster) and mixed. The mixture was incubated at 50 °C for 20 minutes and cooled to room temperature. Then, 100 µL of 10% trichloroacetic acid (ABCR) was added to the mixture and centrifuged at 3000 g (Thermo micromax SN: 8035/2) for 10 minutes. 100 µL of the upper phase was taken and transferred to a 96-well plate. The transferred phase was mixed with 100 µL of deionized water and 20 µL of 0.1% $FeCl_3$ (Sigma) in a 96-well plate. It was incubated for 5 minutes at room temperature in the dark and absorbance was read at 700 nm on the microplate reader. Trolox (Fluka) was used as a standard in drawing the calibration curve. Antioxidant potentials of propolis were stated as mg Trolox (Tro)/g propolis.

Determination of Total Antioxidant Status (TAS)

The total antioxidant status was determined according to the colorimetric method applied by Erel (2004). TAS was measured using the TAS kit (Rel Assay Diagnostics, Cat No: RL001) and the results were stated in mmol Trolox (Tro)/100 g propolis.

Results and Discussion

Total Phenolic Contents and Antioxidant Activities of Propolis Extracts

Total polyphenol content, total flavonoid content, ferric reducing antioxidant power (FRAP) and total antioxidant status (TAS) of all normal and lyophilized extracts (DEP, EEP, AEP, GEP, WEP, lyophilized dimethyl sulfoxide extract of propolis (LDEP), lyophilized ethanol extract of propolis (LEEP), and lyophilized water extract of propolis (LWEP)) were determined and the results were stated as mg GA/g propolis, mg Q/g propolis, mg Tro/g propolis and mmol Tro/100 g propolis, respectively. These results were given in Table 1 and were found to be consistent with each other in terms of both the amount of phenolic compounds and antioxidant activity.

As we cannot use propolis in the natural state, it must be refined by extraction using solvents (Pietta et al., 2002). Since different solvents should solve various compositions of propolis in different amounts, the contents of the WEP, EEP, DEP, GEP, and AEP would be different in quality and/or quantity. In most studies, the solvents chosen to dissolve propolis are not used purely, but diluted with water from 15% to 95%, and these diluted extracts have been studied (Schnitzler et al., 2010; Silva et al., 2012; Frozza et al., 2013; Siripatrawan et al. 2013; Wang et al., 2014; Cruz et al. 2021). The reason we used pure solvents was to determine which solvent would achieve the best solubility.

Silva et al. (2012) studied polyphenolic and flavonoid contents of propolis, by preparing hydro-alcoholic, methanol, and water extracts of propolis for every region (Bragança, Coimbra, and Beja). Polyphenol and flavonoid contents of hydro-alcoholic extracts were found to be considerably high as compared to methanol and water extracts. Total phenolic (277.17 ±7.50) and flavonoid (142.32 ±4.52) contents of Bragança propolis were determined to be of quite a high concentration (mg/g), and Coimbra and Beja propolis followed them respectively. Alencar et al. (2007) found that ethanol extract of Brazilian red propolis includes 232 ±22.3 mg/g polyphenol and 43 ±1.0 mg/g flavonoid. In another study, Frozza et al. (2013) found that hydro-alcoholic extract of Brazilian red propolis includes 151.55 ±1.95 mg/g polyphenolic composition as a dry extract. This difference came from the various methods of extraction, and geographical localization as well.

In addition, each researcher works with different solvents, at different absorbance values, at different concentrations, and by modifying the methods, they apply in various ways. Therefore, this affects the amount of polyphenols and flavonoids in propolis extract. For this reason, all these criteria will also affect the antioxidant activity of propolis. For this reason, it seems difficult to make a clear comparison of the differences between the methods in the studies.

The antioxidant activities of propolis samples from different geographical regions (Argentina, Australia, Brazil, Bulgaria, Chile, China (Hebei, Hubei and Zhejiang), Hungary, New Zealand, South Africa, Thailand, Ukraine, Uruguay, United States and Uzbekistan) were compared by Kumazawa et al. (2004). EEP originated from Argentina, Australia, China, Hungary, and New Zealand had comparatively powerful antioxidant activity and stood in correlation with the total polyphenol and flavonoid contents. But Thailand propolis was found to have the lowest values (Kumazawa et al., 2004). Kumazawa et al. (2004) determined that the polyphenol content

of the ethanolic extract of European and Chinese propolis was ranged from 200 to 300 mg GA/g propolis.

It is suggested that a single constituent of propolis does not have more powerful activity than complete extract and therefore the general biological qualities of propolis emanated from the natural combination of its constituents (Sforcin, 2007). For that reason, instead of isolating the constituents of extracts used in our study and examining their effects separately, we preferred using the whole sample.

In our study, the total polyphenol and flavonoid contents of propolis with DEP were found to be higher than EEP, AEP, GEP, and WEP. Also, DEP was found to have more FRAP capacity and were at a higher level in terms of TAS than EEP AEP, GEP, WEP (Table 1).

Total polyphenol content in the LEEP was found to be higher than as in the LDEP and LWEP. Total flavonoid content, FRAP, and TAS in the LDEP was also found to be higher than as in the LEEP and LWEP (Table 1).

When we used lyophilized propolis, we aimed to separate organic compounds from the resin available and to see the difference between lyophilized and non-lyophilized samples of propolis. But according to the results of our analysis of contents and antioxidant tests, it has been found that there were no great differences between lyophilized and non-lyophilized samples of propolis in terms of content and antioxidant capacity. TAS and FRAP methods to extracts were found to be proportionate to the amounts of polyphenol and flavonoid contents.

The materials in propolis mainly lipophilic compounds. Because it is easy to extract lipophilic compounds by using ethanol, that of EEP is well known and interest greatly (Nakajima et al., 2007). Although using EEP is prevailing, research about WEP has increased in number (Mani et al., 2006).

The WEP has a good antioxidant activity due to its high phenolic compound content. It has been reported that the water extract of propolis has hepatoprotective effect on both chemical and immunological liver injury models, inhibits platelet aggregation, and shows antiviral and anti-inflammatory activity (Nagai et al., 2003; Mani et al., 2006).

Nakajima et al. (2007), in a study they conducted, revealed that water extract of Brazilian green propolis and caffeoylquinic acid derivatives had neuroprotective effects on retinal damage *in vitro* and that these effects were due to their antioxidant properties (Nakajima et al., 2007).

In another study by Nakajima et al. (2009) where they prepared Brazilian WEP and EEP, water extract of royal jelly and ethanol extract of pollen, comparing radical scavenging activity of hydrogen peroxide, superoxide anion and hydroxyl through different antioxidant capacity methods, the antioxidant capacity was found to be in WEP, EEP and ethanol extract of pollen, respectively (Nakajima et al., 2009).

When Laskar et al. (2010) compared various antioxidant determination methods, they suggested that the water extract of Indian propolis is more effective than the ethanolic extract, because of its high polyphenol content, and that it can be used in the prevention of various diseases related to free radicals (Laskar et al. 2010).

DEP is used to some extent in cell culture studies (Azarshinfam et al., 2021; Liao et al., 2021). Studies on the extraction of propolis with glycerol (Thamnopoulos et al., 2018) and acetone (Kheiri et al., 2011) are very few, and antioxidant activity studies have not been conducted in any of them. Therefore, in addition to DEP, AEP and GEP were also included in this study.

Table 1. Total phenolic contents and antioxidant activities of propolis extracts (Arithmetic mean ± SD, n=3)

	DEP	EEP	AEP	GEP	WEP	LDEP	LEEP	LWEP
Total polyphenol content (mg GA/g propolis)	141.2 ±9.99	122.7 ±6.37	100.0 ±8.49	88.0 ±7.75	19.7 ±0.29	136.8 ±4.04	142.0 ±1.41	18.2 ±1.15
Total flavonoid content (mg Q/g propolis)	55.3 ±6.63	47.8 ±8.66	47.3 ±6.43	23.3 ±1.91	1.3 ±0.12	63.5 ±7.07	54.2 ±4.86	2.4 ±1.02
Ferric reducing power (mg Tro/g propolis	273.8 ±11.62	236.9 ±13.92	221.3 ±14.11	141.8 ±18.97	26.2 ±8.57	287.1 ±8.74	232.9 ±19.23	24.0 ±5.55
Total antioxidant status (mmol Tro/100 g propolis)	248.5 ±5.10	233.1 ±1.99	157.5 ±11.06	159.8 ±5.73	15.4 ±5.39	242.9 ±13.48	238.3 ±10.1	26.0 ±1.12

DEP: DMSO extract of propolis; EEP: ethanol extract of propolis; AEP: acetone extract of propolis; GEP: glycerol extract of propolis; WEP: water extract of propolis; LDEP: lyophilized DMSO extract of propolis; LEEP: lyophilized ethanol extract of propolis; LWEP: lyophilized water extract of propolis

Conclusion

As a result; after extraction of propolis using water, ethanol, DMSO, glycerol, and acetone as solvents, evaluated the total polyphenol and flavonoid content and antioxidant capacity, we concluded that it is mostly dissolved in DMSO, and after that in ethanol, acetone, glycerol respectively, and the least in water.

In the light of all this information, propolis can be a natural raw material source for various sectors such as food industry, medicine and cosmetics, thanks to its solubility in various solvents.

Compliance with Ethical Standard

Conflict of interests: The author declares that for this article they have no actual, potential or perceived conflict of interests.

Ethics committee approval: Author declare that this study does not include any experiments with human or animal subjects; therefore, no ethics committee approval is needed.

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COVID-19 phobia, mindful eating, eating habits and body weight change among university students during pandemic: A pilot study

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ABSTRACT

To evaluate the effects of the COVID-19 phobia on mindful eating, eating habits and body weight change among university students. This study was carried out with 385 university students who have been in social isolation at home for at least 2 months. The online survey was constituted via an internet-based questionnaire on Google forms. The survey includes demographics features, body weight, eating habits, change in appetite and consumption of food/food groups. COVID-19 Phobia Scale (C19P-S) and the Mindful Eating Questionnaire (MEQ) were used. Results: Both males and females had similar scores from C19P-S and MEQ. Body weight and BMI of students significantly increased during the pandemic period ($p<0.05$). The percentage of being underweight decreased and being pre-obese/obese increased during the pandemic period. The percentage of the increase in the consumption of sweets, toffees, candies and foods with sugar was high in both groups (male: 48.2%; female: 47.1%). The most increased consumption of food groups was fruit, and also fast food was the most decreased among the consumption of food groups in this study. In addition, a negative correlation was found between C19P-S and MEQ scores ($r=-0.214$, $p<0.001$). And also there was a negative correlation between the MEQ subscale of emotional eating scores and psychological, psycho-somatic and social subscales of C19P-S ($p<0.01$). C19P-S scores had significantly higher in those with increased or decreased appetite and MEQ scores had significantly higher in those who have decreased or unchanged appetite. COVID-19 phobia can affect mindful eating and eating habits during the social isolation/pandemic period in university students. It is important to increase mindful eating during the pandemic, to provide adequate and balanced nutrition, to reduce the risk of disease and to affect the course of the disease positively.

Keywords: COVID-19 phobia, Mindful eating, Eating habits, Body weight change

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (generally known as COVID-19) emerged in Wuhan, China in December 2019 and spread throughout the world. The World Health Organization (WHO) declared COVID-19 as a pandemic on 11 March 2020 (WHO, 2021a; WHO 2020). COVID 19 is a fatal and highly infectious disease, which has several symptoms such as fever, cough, shortness of breath, muscle ache, sore throat, chest pain, diarrhea, nausea and vomiting (Chen et al., 2020). Deaths from COVID 19 are still increasing, and the disease is not yet controlled around the world (Arpaci et al., 2020). After the rapid spread of COVID-19, many measures were taken to protect people's health all over the world. In Türkiye, many preventive precautions such as the closure of schools, shopping centers, entertainment venues, sports halls, curfew and travel restrictions have been taken (Karslıoğlu et al.,2020) University students started to join online education and quarantined at home in March 2020 (Karslıoğlu et al., 2020; Türk Tabipler Birliği, 2020)

This new disease caused fear and anxiety. Because of COVID-19, due to fatal disease, people can worry, fear and panic about not only their own health, but also the health of their families and the people around them (CDC, 2021). It was reported that all of these social restrictions caused fear, stress, panic, anxiety and depression in students, too (Peters et al., 2020). Especially the pandemic period, in which social isolation was aimed, affected the lives of individuals negatively. Individuals in pandemic period have been shown to have a high frequency of psychological diseases (depression, stress, insomnia etc) (Brooks et al., 2020; Ahorsu et al., 2020).

Staying at home constantly can also affect people's eating habits. People, who are social creatures, may change their eating habits due to increased fear and anxiety caused by the social isolation (Abbas and Kamel, 2020). As it is known, psychological states (such as anxiety, fear, stress, sadness) of individuals can negatively affect their eating behaviors (Framson et al., 2009). When people are stressed, they often change their calorie intakes by either increasing or decreasing (Dallman, 2010). Emotional status like stress is related with higher energy, fat, carbohydrate, and protein intakes (Moynihan, 2010). Qurantine period can induce stress, and this stress might cause overeating and higher consumption of carbohydrate rich foods which have positive effect on mood (Muscogiuri et al., 2020).

Therefore, more attention should be paid to a healthy diet and ideal body weight during this period (Muscogiuri et al., 2020). It has been reported that healthier food choices can be made by increasing the attention to eating behavior and reducing the sensitivity to thoughts and feelings during food consumption (Omiwole et al., 2019; Baer et al., 2005). Mindfulness is associated with many positive health outcomes, including decreasing anxiety and preventing eating disorders (Allen et al., 2006). Mindful eating has been described as noticing how and why eating behavior occurs rather than what is eaten (Köse et al., 2016). Mindful eating may not only reduce food cravings (Alberts et al., 2012) but also be effective in body weight control (Framson et al., 2009; Forman et al., 2009) .

To our knowledge, this study is the first study, which examined the association of COVID-19 phobia with mindful eating in our country. We hypothesized that-COVID-19 Phobia increases mindful eating and affects eating habits negatively during pandemic period. Therefore, in this study, it was aimed to evaluate the effects of COVID-19 phobia, mindful eating, eating habits and body weight change in university students, an important risk age group, who underwent social isolation to protect themselves against the epidemic.

Materials and Methods

Materials

This cross-sectional study was carried out between March and July 2020 in the city center of Ankara and Antalya, Türkiye. In this study, 329 females and 56 males; totally 385 university students were participated. The mean age of the male and female was 21.8 ±2.09 and 20.9 ±1.74 years, respectively ($p<0.05$). Students who have been in social isolation at home for at least 2 months due to the COVID-19 outbreak were included in the study. Despite contacted with approximately 1200 students, 32.0 % percentage of the students returned.

Methods

The online survey was constituted via an internet-based questionnaire on Google forms. Online approval was obtained from each student to participate in the study. The self-report survey includes demographics features (e.g., age, gender, education, and status), body weight, height, eating habits, change in appetite and consumption of food/food groups. It was questioned whether the body weight and eating habits of the participants changed before the pandemic and during pandemic period. A questionnaire including the change in appetite of the individuals and the consumption of food groups was prepared. COVID-19 Phobia Scale (C19P-S) and the Mindful Eating Questionnaire (MEQ) were used for the assesment of COVID-19 phobia and mindful eating habits re-

spectively. The body mass index (BMI) was calculated by dividing body weight (kg) by height (m^2). After this calculation, BMI was classified as four groups. (1: Underweight (<18.5 kg/m^2), 2: Normal (18.5–24.9 kg/m^2), 3: Pre-obesity (25.0–29.9 kg/m^2), 4: Obese (≥30.0 kg/m^2) (WHO, 2021b)

COVID-19 Phobia Scale (C19P-S)

COVID-19 Phobia Scale (C19P-S) was developed, Turkish validity and reliability of the scale in was made by Arpacı et al. (Cronbach alpha: 0.925) (Arpacı et al., 2020). This scale includes 20 items. The scale consists of 4 sub-factors: psychological, psycho-somatic, economic and social. A five-point Likert-type scale (from 1"strongly disagree" to 5 "strongly agree") was used to evaluate the levels of COVID-19 phobia. The total score of scale ranges from 20 to 100. A higher total score indicates a greater phobia level.

Mindful Eating Questionnaire (MEQ)

Mindful Eating Questionnaire (MEQ) was developed by Framson et al (Framson et al., 2009) and adapted into Turkish by Köse et al. (Cronbach alpha: 0.733) (Köse et al., 2016). This scale includes 7 subscales: disinhibition, emotional eating, eating control, mindfulness, eating discipline, conscious nutrition and interference. A five-point Likert-type scale (1: never, 2: rarely, 3: sometimes, 4: often, 5: usually) was used to assess the mindful eating level. In this scale, 10 items (1, 7, 9, 11, 13, 15, 18, 24, 25 and 27) are scored straight; other 20 items are scored reserve. Higher scores indicate more mindful eating level.

Statistical Analysis

The data obtained were imported into Microsoft Excel, and The Statistical Package for the Social Sciences (version 24.0) software was used for all the analyses. The evaluation of the demographic characteristics, dietary patterns and obesity of students was based on numbers and percentages. The *t*-test (paired samples) was used to evaluate body weight before and during pandemic period. The means and SDs of each subscale and total scores of COVID-19 Phobia Scale and Mindful Eating Questionnaire of participants according to BMI classification during pandemic period were measured. The One-Way Anova was used for COVID-19 phobia and mindful eating of participants according to BMI classification during pandemic period. The Spearman test was used to evalulate the relationship between COVID-19 phobia and mindful eating of students. Results were considered statistically significant at p-values <0.05 for all analyses.

Results and Discussion

Public health emergencies can have many psychological effects on individuals, which can be expressed as anxiety, fear, and worry (Mei et al., 2011; Gritsenko et al.,2020; Ornell et al., 2020) and to understand the psychological effects of a pandemic, the emotions such as fear and anger, must be considered and observed (Arpaci et al., 2020; Cao et al.,2020; Butler and Barrientos, 2020; Ammar et al., 2020). Social isolation is a very important public health emergency objective to protect against the COVID-19 (Muscogiuri et al, 2020). Being forced to stay indoors due to the COVID-19 pandemic for a long time can eventually lead to anxiety and stress conditions that can cause change in mindfulness, eating habits,body weight change of healthy individuals(Abbas and Kamel, 2020; Framson et al., 2009; Dallman, 2010; Moynihan et al., 2010; Muscogiuri et al, 2020). The evaluation of body weight of individuals before and during the pandemic period was given in Table 1. Before pandemic, 12.7% of the participants are underweight, 70.9% are normal and 16.4% are overweight according to BMI classification. In present study, the mean body weight (kg) was 60.6±11.56 before the pandemic but was 61.4 ±12.13 during the pandemic ($p<0.001$). Also the mean BMI of students significantly increased during the pandemic period (22.2 ±3.43 kg/m^2) compared to before the pandemic period (21.9 ±3.28 kg/m^2) (Table 1). Similar to our study, it was found that COVID-19 pandemic was greatly associated with increased weight gain (Pellegrini et al., 2020; Reyes-Olavarría et al., 2020). Higher BMI is now also thought to be a risk factor for COVID-19 mortality (Klang et al., 2020). That's why we can say that pandemic period can lead to COVID-19 phobia conditions that can cause increased weight gain and change eating habits of healthy students in this study.

Ammar et al. (Ammar et al., 2020) stated that that anxiety, boredom or COVID-19 phobia could cause negative changes in eating behavior. In a study, 82 % of participants reported an increase in the amount of unhealthy food during pandemic (Robinson et al., 2021). Further, constantly hearing or reading about the epidemic without a break during quarantine can be stressful. Only 18.4 % of students stated their appetite decreased but near the half of students (42.9 %) reported that their appetite increased during pandemic period (data not shown in table) in present study. Consequently, the stress pushes people toward overeating, mostly looking for sugary foods (Muscogiuri et al., 2020). Changes in consumption of food groups and certain foods in participants during the pandemic period were shown in Table 2. Similarly, the percentage of the increase in the consumption of sweets, toffees, candies and foods with sugar was high in both groups (male: 48.2 %; female: 47.1 %) in this study (Table 2). During this pandemic period, it is important to take care of nutritional habits, following a healthy and balanced nutritional pattern containing a high amount of minerals, antioxidants, and vitamins. It

was reported that fruit and vegetables supplying micronutrients can boost immune function (Abbas and Kamel, 2020; Muscogiuri et al., 2020; Butler and Barrientos, 2020) It was found that the most increased consumption of food groups was fruit, and also fast food was the most decreased among the consumption of food groups in this study (Table 2). This may be due to the social isolation and lack of access to fast food because of the COVID phobia in this age group.

Stress and emotional status influence eating behavior. To many, stress and negative mood can induce loss of appetite and hypophagia (Macht,2008). However negative emotions and stress cause them to eat more and increase emotional eating (Van Strien,2018) So mindful eating is important to reduce food cravings (Alberts et al., 2012) and is associated with preventing eating disorders and obesity (Allen et al., 2006; Alberts et al., 2012). Mindful eating has been described as noticing how and why eating behavior occurs rather than what is eaten (Köse et al., 2016), and it is also effective in body weight control (Framson et al., 2009; Alberts et al., 2012) Similarly, in present study those who stated that his/her appetite was decreased or not changed had the significantly higher score from MEQ than increased appetite (p: 0.021). Evaluation of COVID-19 phobia and mindful eating of participants according to obesity during the pandemic period was shown in Table 3. Furthermore, total MEQ scores were the highest in underweight ($p<0.001$) (Table 3). Total and subscales scores of C19P-S (except social subscale) had not significantly difference according to the BMI classification ($p>0.05$). Spearman correlation matrix of the relationship between ages, body weight, BMI, total C19P-S with MEQ scores were given Table 4. Total C19P-S scores was negatively associated with total MEQ scores (r:-0.203 $p<0.001$). There was a significant negative correlation between total MEQ scores and body weight/BMI during pandemic ($p<0.001$). There was a significant positive correlation between total MEQ scores and age ($p<0.001$). The percentage of being underweight decreased, but being pre-obesity/obese increased during the pandemic period (Table 1).Therefore, mindful eating is important not only for the prevention of obesity, but also for the adequate and balanced nutrition (Butler and Barrientos,2020; Van Strien,2018) . Total C19P-S scores was negatively associated with total MEQ scores (r:-0.203 $p<0.001$) in this study (Table 4). In addition, Total C19P-S score was related negatively MEQ subscales (disinhibition, emotional eating, eating control and interference). Spearman correlation matrix of the relationships between C19P-S and MEQ subscales were given Table 5. Total C19P-S score was related negatively disinhibition, emotional eating, eating control and interference subscale scores of MEQ. Total MEQ score was related negatively C19P-S subscales (psychosomatic, social and economic) ($p<0.01$). (Table 5). Increased COVID-19 phobia might cause decreasing mindfulness.

Table 1. Evaluation of body weight in individuals before and during the pandemic period

	Total (n:385)		
	Before pandemic period	During pandemic period	
Variables	**X ±SD**	**X ±SD**	**p**
Body weight (kg)	60.6±11.56	61.4±12.13	**<0.001**
BMI (kg/m^2)	21.9±3.28	22.2±3.43	**<0.001**
Obesity classification	**n (%)**	**n (%)**	
Underweight	49 (12.7)	39 (10.1)	
Normal	273 (70.9)	268 (69.6)	
Pre-obesity /Obese	63 (16.4)	78 (20.3)	

BMI: Body mass index

Table 2. Changes in consumption of food groups and certain foods in participants during the pandemic period

	Male (n:56)			Female (n:329)		
	Increased	Decreased	Not changed	Increased	Decreased	Not changed
Food and food groups	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Bread and types of bread	26 (46.4)	7 (12.5)	23 (41.1)	126 (38.3)	65 (19.8)	38 (41.9)
Rice/bulgur/ pasta	28 (50.0)	14 (25.0)	14 (25.0)	133 (40.4)	55 (16.7)	141 (42.9)
Milk and milk products	28 (50.0)	5 (11.4)	23(41.1)	168 (51.1)	39 (11.9)	122 (37.1)
Meat and meat products	33 (58.9)	6 (10.7)	17 (30.4)	168 (51.1)	40 (12.2)	121 (36.8)
Legumes	24 (42.9)	10 (17.9)	22 (39.3)	102 (31.0)	48 (14.6)	179 (54.4)
Vegetables	31 (55.4)	9 (16.1)	16 (28.6)	182 (55.3)	28 (8.5)	119 (36.2)
Fruits	33 (58.9)	6 (10.7)	17 (30.4)	209 (63.5)	35 (10.6)	85 (25.8)
Sweets, toffees, candies and foods with sugar	27 (48.2)	7 (12.5)	22(39.3)	155 (47.1)	70 (21.3)	104 (31.6)
Fast foods	10 (17.9)	39 (69.6)	7 (12.5)	32 (9.7)	244 (74.2)	53 (16.1)

Table 3. Evaluation of phobia of COVID-19 and mindful eating of participants according to BMI classification during pandemic period

	Underweight (n:39)	Normal (n:268)	Pre-obesity/Obese (n:78)	p
MEQ Subscales	**X ±SD**	**X ±SD**	**X ±SD**	
Disinhibition	18,8 ±2.67[a]	16.6 ±3.77[b]	15.8 ±3.29[b]	**<0.001**
Emotional Eating	18.0 ±3.68[a]	16.2 ±4.31[a.b]	15.2 ±4.75[b]	**<0.001**
Eating Control	16.4 ±3.71[a]	14.3 ±3.73[b]	14.2 ±3.60[b]	**<0.001**
Mindfulness	14.8 ±1.85[a]	15.9 ±1.96[b]	15.7 ±1.87[b]	**<0.001**
Eating Discipline	12.6 ±2.52	13.0 ±2.69	13.2 ±2.36	0.488
Conscious Nutrition	15.8 ±2.13	15.7 ±2.66	15.3 ±2.81	0.523
Interference	7.4 ±1.58	6.9 ±1.77	7.2 ±1.39	0.153
Total MEQ scores	100.7 ±11.66[a]	95.0 ±13.47[b]	92.9 ±13.67[b]	**<0.001**
C19P-S Subscales	**X ±SD**	**X ±SD**	**X ±SD**	**p**
Psychological	18.8 ±4.62	19.2 ±4.77	19.3 ±4.04	0.884
Psycho-Somatic	8.4 ±3.11	9.1 ±3.21	9.2 ±2.98	0.337
Social	12.0 ±3.12[a]	13.3 ±3.66[a.b]	13.9 ±3.42[b]	**<0.001**
Economic	7.4 ±2.34	7.9 ±2.57	8.5 ±2.37	0.067
Total C19P-S scores	46.8 ±10.40	50.1 ±11.22	51.9 ±9.42	0.550

MEQ: Mindful Eating Questionnaire, C19P-S: COVID-19 Phobia Scale

Furthermore, pandemic period may lead to emotional disturbance and may be a risk factor for the development of COVID-19 phobia in university students. Students are generally sociable beings, and this period of social isolation might have forced them to cope with the growing fear and anxiety (Abbas and Kamel,2020). A survey indicated that college students were afflicted with experienced mild and severe anxiety because of the COVID-19 outbreak (Cao et al.,2020). Another study reported that students were depressed, exhausted, nervous and angry due to COVID-19 (Gritsenko et al.,2020). This study indicated that both males and females had similar scores from C19P-S ($p>0.05$). But there was a significantly difference in perceived appetite. Those who stated that his/her appetite was increased or decreased had the significantly higher score from C19P-S than unchanging appetite ($p<0.001$). It can be said that there is a relationship between appetite change and COVID-19 phobia. COVID-19 phobia may have influence on weight-related behaviors such as appetite change among university students.Providing reduced covid phobia can increase mindful eating in students and may have positive effects on body weight change.

Table 4. Spearman correlation matrix of the relationship among age, body weight, BMI, total C19P-S and MEQ scores

	1	2	3	4	5
1.Age (years)	1				
2.Body weight during pandemic (kg)	0.202*	1			
3.BMI during pandemic (kg/m^2)	0.182*	0.861*	1		
4.Total C19P-S scores	0.074	0.016	0.050	1	
5.Total MEQ scores	0.191*	-0.202*	-0.198*	-0.203*	1

*p<0.001, MEQ: Mindful Eating Questionnaire, C19P-S: COVID-19 Phobia Scale

Table 5. Spearman correlation matrix of the relationships between C19P-S and MEQ subscales

C19P-S and MEQ Subscales	1	2	3	4	5	6	7	8	9	10	11	12	13
1.Psychological	1												
2.Psycho-Somatic	0.457**	1											
3.Social	0.690**	0.562**	1										
4.Economic	0.345**	0.543**	0.446**	1									
5.Total C19P-S scores	0.795**	0.771**	0.858**	0.669**	1								
6.Disinhibition	-0.074	-0.145**	-0.200**	-0.150	-0.192**	1							
7.Emotional Eating	-0.147**	-0.113*	-0.247**	-0.096	-0.221**	0.618**	1						
8.Eating Control	0.015	-0.081	-0.134**	-0.104*	-0.108*	0.487**	0.345**	1					
9.Mindfulness	-0.066	-0.094	-0.061	0.030	-0.079	0.041	0.096	0.139**	1				
10.Eating Discipline	0.002	-0.081	-0.072	-0.018	-0.067	0.269**	0.279**	0.341**	0.293**	1			
11.Conscious Nutrition	-0.024	-0.017	-0.096	-0.080	-0.091	0.400**	0.269**	0.398**	0.092	0.296**	1		
12.Interference	-0.105*	-0.145**	-0.186**	-0.121*	-0.173**	0.469**	0.461**	0.347**	0.116*	0.411**	0.338**	1	
13.Total MEQ scores	-0.085	-0.144**	-0.228**	-0.138**	-0.214**	0.788**	0.753**	0.713**	0.228**	0.563**	0.592**	0.627**	1

*p<0.05, ** p<0.01, MEQ: Mindful Eating Questionnaire, C19P-S: COVID-19 Phobia Scale

The best recommendation for staying healthy during pandemic is to follow the general health advice such as eating a balanced diet with mindful eating and managing stress (Muscogiuri et al., 2020). Patients with anxiety/depression were strongly associated with weight gain and resulted in being the more relevant factor in predicting increase in body weight, after adjusting for consuming unhealthy foods (Pellegrini et al., 2020).

Present study has some limitations. Firstly, despite contacted with approximately 1200 students, 32.0 % percentage of the students returned. This may be due to the online survey forms that are frequently conducted during this period. Secondly, the parameter evaluating the anxiety state of the participants can be added to the study. In our knowledge, there exists no study about COVID-19 phobia and mindful eating. We think this is important in terms of guiding future studies.

Conclusion

COVID-19 phobia can affect mindful eating and eating habits during the social isolation/pandemic period in university students. COVID-19 phobia, which increases due to the extension of the pandemic, is expected to affect students' mindful eating negatively. It is important to increase the mindful eating during the pandemic, to provide adequate and balanced nutrition, to reduce the risk of disease and to affect the course of the disease positively.

Compliance with Ethical Standard

Conflict of interests: The author declares that for this article they have no actual, potential or perceived conflict of interests.

Ethics committee approval: This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving study participants were approved by the Clinical Research Ethics Committee of Akdeniz University (Project number:KAEK-446). (24/06/2020). The participants consented to participate in the study, with a digital informed consent form.

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Çanakkale Boğazı'ndan toplanan deniz marulu (*Ulva rigida*)'nun mevsimsel besin içeriğinin belirlenerek salata ve çorba olarak değerlendirilmesi

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ÖZ

Bu çalışmanın amacı, deniz marulu (*Ulva rigida*)'nun besin içeriğinde meydana gelen mevsimsel farkları saptayarak farklı tüketim tercihlerine göre duyusal özelliklerini belirlemektir. Bu sebeple, Çanakkale Boğazı'ndan mevsimsel olarak toplanan *U. rigida*'nın hem yaş hem de kuru örneklerinin ham besin bileşimi, amino asit ve yağ asidi içerikleri belirlenmiştir. Bulgulara göre; *U. rigida* yüksek protein ve amino asit içeriğine sahipken, yaş örneklerde % 0.46-0.85, kuru örneklerde ise % 1.81-4.53 arasında değişen düşük ham yağ oranına sahip olduğu saptanmıştır. Sonrasında her mevsim elde edilen örneklerden alg salatası ve alg çorbası elde edilmiştir. Yapılan duyusal analizlere göre en beğenilen tüketim tercihi tüm gruplar içinde alg salatası olarak tespit edilmiştir. Ayrıca, her iki tüketim tercihi de ilkbahar ve sonbahar örneklerinde en yüksek puanları almıştır. Sonuç olarak, *Ulva rigida* tüketiciler tarafından beğenilen ve besin içeriği ilkbaharda yüksek bulunan bir gıda kaynağıdır.

Anahtar Kelimeler: Amino asit, Yağ asidi, Yosun, Besin bileşimi, Gıda

ABSTRACT

Evaluation of sea lettuce (*Ulva rigida*) collected from Çanakkale Strait as salad and soup by determining the seasonal nutritional content

The main aims of this study are determining seasonal chemical composition of green seaweed and evaluating usage of a food source with different processing techniques. For this purpose, *Ulva rigida* were collected from Çanakkale Strait, Türkiye and amino acid, fatty acid and proximate composition were determined seasonally in both fresh and dried samples. According to results; *Ulva rigida* contains high protein and amino acids content whereas it has low fat content differs in the range of 0.46-0.85 % in fresh samples and 1.81-4.53 % in dried ones. In the following, the obtained algae samples in the all seasons were processed into salad and algae soup. Most favorite consuming options determined as algae salad among groups. Besides, two consuming options were scored highest in spring and autumn. In a conclusion, *Ulva rigida* is evaluated a desirable aquatic food source by the consumers, especially in spring.

Keywords: Amino acid, Fatty acid, Seaweed, Proximate composition, Food

Giriş

Günümüzde artan gıda talebini karşılamak için araştırıcılar alternatif kaynaklara yönelmişlerdir. Makroalgler (deniz yosunları) ön plana çıkan denizel gıda kaynaklarındandır (Berik ve Çankırılıgil, 2020; Mchugh, 2003; Sørensen ve ark., 2019). Makroalglerin gıda olarak kullanımları Japonya'da IV., Çin'de V. yüzyıla dayanmaktadır. Türkiye'de makroalg tüketimi az olmasına karşın yurtdışında özellikle Çin, Japonya, Kore, Endonezya, Malezya, Fransa, Amerika, Kanada ve İskoçya'da tüketilmektedir (Mchugh, 2003). Amerika Birleşik Devletleri ve Avrupa'ya gerçekleştirilen göçler, makroalg tüketimine olan talebi bu kıtalara da taşımıştır (Turan, 2007). Pek çok çalışma makroalglerin yüksek protein, esansiyel amino asitler, algal polisakkaritler ve mineral içerikleri ile besleyici denizel gıda kaynakları olduğunu bildirmektedir (Ak ve ark., 2015; Lorenzo ve ark., 2017; Vizetto-Duarte ve ark., 2016). Makroalglerin doğal olarak toplanmalarının yanı sıra, yetiştiriciliği de yapılmaktadır (Ak ve ark., 2015). Dünya çapında 2018 yılında toplam 32.4 milyon tonluk alg üretimi gerçekleştirilmiş olup bu miktarın büyük çoğunluğunu makroalg türleri oluşturmaktadır. Elde edilen makroalgler ağırlıklı olarak gıda tüketiminde kullanılmakta olduğu gibi aynı zamanda çeşitli endüstri kollarında hammadde olarak da kullanılmaktadırlar (FAO, 2020). Bu özellikleri ile makroalgler pek çok türü gıda olarak kullanılabilen denizel kaynaklardır. Özellikle Türkiye denizlerinde geniş yayılım gösteren *Ulva*, *Porphyra*, *Gelidium*, *Rhodymenia*, *Laurencia* gıda olarak tüketilebilir türlerdir (Cirik ve Cirik, 2017).

Bu çalışmada, hem gıda olarak çok eski çağlardan beri kullanım alanı olan hem de kozmopolit bir alg olan *Ulva rigida* tercih edilmiştir. Deniz marulu olarak da adlandırılan *U. rigida* özellikle denizlerin sığ ve kayalık bölgelerinde azot ve fosfor gibi besleyici elementlerin bol olduğu kısımlarda doğal olarak yayılım gösteren kozmopolit bir türdür (Cirik ve Cirik, 2017). Yüksek Biyolojik değerleri; *Ulva* türlerinin tüketimini de en az balık ve çift kabuklular kadar cazip kılmaktadır (Berik ve Çankırılıgil, 2020; Ortiz ve ark., 2006). *Ulva* türleri yüksek miktarlarda n-3 PUFA, α-linolenik, B12 vitamini, faydalı iz elementler ve ulvan adı verilen oldukça kompleks yapıdaki karbohidratları içermektedirler (Alves ve ark., 2013; Berik ve Çankırılıgil, 2020; Girao ve ark., 2012). Besin içeriği açısından zengin bir tür olan *Ulva rigida*, tallusları tatlı suyla yıkanıp kurutularak veya taze olarak salatalarda ve çorbalarda tüketilebilmektedir (Ova Kaykaç, 2007). Ancak her alg gibi *Ulva* türlerinin de besleyiciliği doğal yayılım alanı ya da kültür ortamındaki besin tuzu, su sıcaklığı, tuzluluk, ışık ve mevsim gibi birçok faktöre bağlı olarak değişmektedir (Ak ve ark., 2012; Dawes, 1998; Öztaşkent ve Ak, 2021). Bu sebeple, çalışmamızda Çanakkale Boğazı'ndan toplanan *Ulva rigida* örneklerinin mevsimsel olarak besin içeriği (ham besin bileşimi, amino asitler ve yağ asitleri)'nde meydana gelen değişimler incelenmiştir. Ayrıca her mevsimde toplanan makroalglerin taze formlarından salata; kurutulmuş formlarından çorba hazırlanarak tüketici beğeni analizleri gerçekleştirilmiştir. Makroalglerden üretilen gıdaların, büyük bir dışalım gücü bulunduğu düşünülürse bu çalışma, sonradan yapılacak çalışmalara ve dolayısıyla ülke ekonomisine katkı sağlayacağını öngörmekteyiz.

Materyal ve Metot

Örnekleme ve İşleme

Çalışma ana materyali deniz marulu, *Ulva rigida* (C. Agardh, 1823) olarak seçilmiştir. Bu çalışmada, toplam 30 kg deniz marulu kullanılmıştır. *Ulva rigida* örnekleri Çanakkale Boğazı'ndaki Kepez ilçesi sahilinden (40° 6' 14.68"K, 26° 23' 45.81"D) mevsimsel olarak 2011-2012 yılları arasında toplanmıştır. Örneklemeler ocak, nisan, temmuz ve ekim aylarında gerçekleştirilmiştir. Örneklemeler sırasında alglerin toplandığı istasyonda suyun tuzluluğu yıl boyunca ‰ 24-26 arasında tespit edilmiştir. Su sıcaklığı ise kış mevsiminde 8°C, ilkbahar mevsiminde 13°C, yaz mevsiminde 20°C ve sonbahar mevsiminde 13°C olarak tespit edilmiştir. Hava sıcaklıkları ise kış mevsiminde 4°C, ilkbahar mevsiminde 12°C, yaz mevsiminde 24°C ve sonbahar mevsiminde 16°C olarak ölçülmüştür. Örnekleme istasyonu ve Akdeniz'deki *Ulva rigida* dağılımı Şekil 1'de görülmektedir.

Elde edilen algler, deniz suyu ile dolu taşıma kaplarında Çanakkale Onsekiz Mart Üniversitesi Deniz Bilimleri ve Teknolojisi Fakültesi İşleme Teknolojisi Laboratuvarı' na uygun koşullarda getirilmiş ve çalışmalar hemen başlatılmıştır. Öncelikle, algler talluslarında taşıdıkları epifit bitkiler ve sucul omurgasızlar gibi istenmeyen canlılardan temizlenmişlerdir. Sonrasında ise, algler ‰ 25'lik tuzlu suda yumuşak uçlu fırça ile fırçalanarak tüm kum taneciklerinden temizlenmişlerdir. Temizlenmiş alg tallusları ikiye ayrılarak yaş ve kuru olarak analizlere tabi tutulmuşlardır. Kurutma işlemi 40°C'deki etüvde 48 saat sürmüştür. Elde edilen yaş ve kurutulmuş örneklere kimyasal analizler uygulanmıştır.

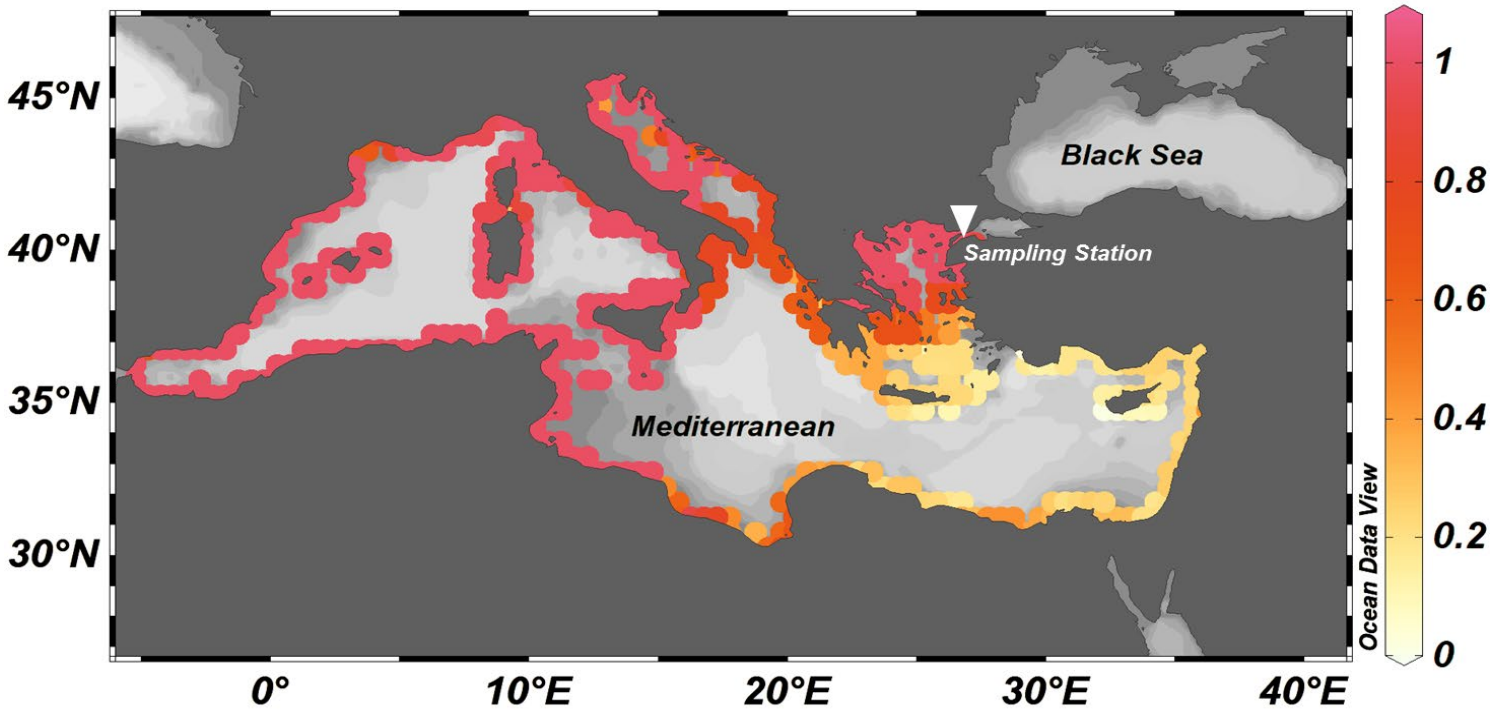
Ayrıca yaş örneklerden salata, kuru örneklerden ise çorba yapılarak duyusal özellikleri belirlenmiştir. Deniz marulundan salata yapmak için; temizlenen alg talluslarının tek tek üzerine 15 ml susam yağı ve 2 gr kaya tuzundan oluşan karışım mutfak fırçası ile sürülmüş ve ardından her birine 5 (250 g) yaprak gelecek şekilde rulo yapıldıktan sonra 10 dakika marine olması beklenmiştir. Marine işlemi tamamlanınca rulolar düzleştirilmiş ve sıcak teflon tavada 165°C'de 3 dakika boyunca kızartılmış ve ardından 0,5 cm kalınlığında parçalara dilimlenerek servis edilmiştir. Deniz

marulu çorbası için öncelikle 1 L önceden hazırlanmış tavuk suları kaynatılmış ve üzerine 100 g kurutulmuş makroalg yaprakları ilave edilerek karıştırılmıştır. Makroalg yapraklarının yumuşamasının ardından çorba karışımının kaynaması sonlandırılmıştır. Ardından 15 mL susam yağı, 100g kıyılmış yeşil soğan, 5 g kaya tuz ve 5 g karabiber çorbaya ilave edilerek, karıştırılmış ve servis edilmiştir. Alglerin hazırlanması sırasında kullanılan kaya tuzu, karabiber, taze soğan, zeytinyağı ve susam yağı yerel marketlerden temin edilmiştir.

Besin Bileşiminin Belirlenmesi

Alglerin su (nem) içerikleri Horwitz (2000)'e göre gerçekleştirilmiştir. Homojenize edilmiş örnekler darası alınan petrilere 5 g tartılarak (16-18 saat) 105°C deki etüvde kurutulmuştur. Kurutulan örnekler tartılarak % su oranı hesaplanmıştır. Ham protein analizi Kjeldahl metoduna göre gerçekleştirilmiştir (AOAC, 2000). Homojenize edilen örneklerden 0.5 g tartılarak 20 mL H_2SO_4 ile 420°C'de yakılmıştır. Yakma işlemi sonrasında elde edilen örneklere 50 mL saf su eklenerek 50 mL NaOH ile distile edilmişlerdir. Filtratlar 0,1 N HCl ile titre edilerek elde edilen değerler AOAC (2000) metoduna göre hesaplanmıştır.

Yağ analizi Folch ve ark. (1957)'nin uyguladığı yöntem esas alınarak yapılmıştır. Homojenize örneklerden 5 g tartılarak üzerlerine 20 mL metanol/kloroform (1:2) eklenmiş ve yağ ekstraksiyonu gerçekleştirilmiştir (12 saat, 25°C). Elde edilen çözeltiler süzülerek 60°C'lik su banyosunda rotary evaporatör (IKA RV10 basic) kullanılarak distile edilerek son tartımları yapılmıştır. Kül tayininde ise 2 g ağırlığındaki homojenize örnekler porselen krozelere alınarak 550°C'de 5 saat süre ile yakma işlemine tabii tutulmuşlardır. Yakma işlemi sonrasında örneklerin son tartımları yapılmış ve sonuçlar Horwitz (2000) metoduna göre hesaplanmıştır.



Şekil 1. Deniz marulu (*Ulva rigida*)'nun Akdeniz'deki yayılım haritası ve örnekleme istasyonu. Yayılım datası Aqua-maps'dan temin edilmiş olup (Kaschner ve ark., 2016) Ocean Data View ile görselleştirilmiştir (Schlitzer, 2020).

Figure 1. Distribution of *Ulva rigida* in the Mediterranean and the sampling station. The distribution data were obtained from Aquamaps (Kaschner et al., 2016), and the map was visualized with Ocean Data View (Schlitzer, 2020).

Amino Asit Kompozisyonunun Belirlenmesi

Alg örnekleri 6 N HCl ile 110°C'deki etüvde 24 saat hidrolize edilmişlerdir. Hidrolizi yapılan örnekler 0.20 µm PTFE şırınga filtreden süzülmüş ve HCl, evaporatörde yüksek vakum altında 60°C'de uçurularak; geriye kalan kalıntı pH'ı 2.2 olan sodyum sitrat tampon çözeltisiyle (0.1 M, pH 2.2) seyreltilmiştir (Srivastava ve ark., 2006). Hidroliz işlemi tamamlanmış örneklerin aminoasit miktarlarının tespiti için EZ: faast GC/FID Free (Physiological) amino asit kitleri kullanılmıştır (Badawy ve ark., 2008). Hazırlanan ve türevlendirilen örnekler 2.0 µL halinde GC'ye (Finnigan Trace GC Ultra AI 3000 Thermo Finnigan analyzer) enjekte edilerek analiz edilmişlerdir. Analizlerde sabit faz olarak Zebron Zebron™ ZB-HAAC GC kolon (10 m x 0.25 mm) kullanılmış olup, mobil faz olarak helium seçilmiştir. Akış hızı 1.0 mL/dk, alev iyonizasyon dedektörü (FID) sıcaklığı ise 320°C olarak ayarlanmıştır. İnternal standart (IS) olarak Norvaline kullanılmış ve konsantrasyonu örneklerde 200 nmol/mL olacak şekilde hazırlanmıştır. Kolon fırınının sıcaklığı 110°C'den 320°C'ye kadar 35°C/dakika olacak şekilde artırılmıştır. Elde edilen pikler standartlar ile karşılaştırılarak, amino asit miktarları g/mg olarak hesaplanmıştır. Hesaplamalarda, 19 adet amino asidin tayini yapılmış olup, diğer amino asit ve benzeri yapıların (4-Hidroksiprolin, hidroksilizin, sarkosin, α-aminobutirik asit, β-aminoisobutirik asit, allo-İsolösin, thioprolin, α-aminoadipik asit, aminopimalik asit, ornitin, glisin-prolin, proline-hidroksiprolin, sistatiyonin) toplam miktarları verilmiştir.

Yağ Asidi Kompozisyonunun Belirlenmesi

Yağ asidi analizinde öncelikle Folch ve ark. (1957)'na göre elde edilen ham yağ örneklerinin esterleştirme işlemi yapılmıştır. Bunun için 0.15 g ham yağ numunesi balonda tartılmış ve 5 mL metanolik 0.5 N NaOH ilave edilmiştir. Kaynama taşı atılarak soğutucu bağlanmış su banyosunda 15 dakika kaynatılarak sabunlaştırılmıştır. Soğutucunun üzerinden 5 mL BF_3 reaktifi katıldıktan sonra 5 dakika daha kaynatılmıştır. Daha sonra numuneye 2 mL heptan ilave edilmiş ve 1 dakika daha kaynatılmıştır. Soğutucu çıkarılmış ve örnek olarak 25 mL lik balon jojeye alınmıştır. Balon doymuş NaCl ile çalkalanarak, oluşan üstteki heptan fazından mikro pipetle 1-2 mL alınarak cam viale aktarılmıştır. İçine birkaç adet kristal anhidrik Na_2SO_4 atılmıştır. Bu solüsyondan enjektörle örnek alınarak MS dedektöre sahip gaz kromatografisine (Thermo GS-MS Finnigan Trace DSQ) 2.0 µL enjekte edilmiştir (IUPAC, 1978). Analizlerde ZB-WAX 30 m kolon sabit faz olarak kullanılırken, hidrojen gazı mobil faz olarak tercih edilmiştir. Enjeksiyon bloğu sıcaklığı 200°C, dedektör sıcaklığı 200°C, ilk fırın sıcaklığı ise 100°C olarak seçilmiş olup sıcaklık artışı 1°C/dakika olarak ayarlanmıştır. Gaz kromatografisinde okunan değerler Xcalibur adlı programda değerlendirilmiştir.

Duyusal Özelliklerin Belirlenmesi

Duyusal analizlerde, her mevsim toplanan yaş makroalgler ile yapılan salata ve kurutulmuş makroalgler kullanılarak üretilen çorbaların tüketilebilirliğinin ölçülmesi amacıyla tüketici beğeni analizi gerçekleştirilmiştir. Analiz için yaşları 18-60 yaş arasında değişen ÇOMÜ Deniz Bilimleri ve Teknolojisi Fakültesi öğrencileri ve personeli hedef tüketici kitlesi olarak seçilmiştir. Yaş örneklerden üretilen salatanın analizinde her mevsim 78 kişi denemeye alınmış, kurutulmuş makroalglerden üretilen çorbanın analizi için her mevsim 82 kişi olacak şekilde duyusal analizler gerçekleştirilmiştir. Salatanın ve çorba; görünüş, tat, koku ve genel beğeni kriterleri üzerinden değerlendirilmiş ve her iki ürünün değerlendirilmesi 7 noktalı hedonik skala üzerinden gerçekleştirilmiştir. Bu skala, tüm özellikler için, "hiç beğenmedim" (1) ile "çok beğendim" (7) değerleri arasında puanlanmıştır. Analizde ayrıca panelistlerin her bir nitelik için varsa düşüncelerini belirtmeleri istenmiştir (Meilgaard ve ark., 1999).

İstatistiksel Analizler

Araştırmada alglerin besin kompozisyonu, amino asit ve yağ asidi içeriklerin mevsimsel farklılıkların tespiti amacıyla tek yönlü varyans analizi (ANOVA) uygulanmıştır. Verilerin tek yönlü varyans analizi (ANOVA) için uygunluğu Anderson–Darling (normal dağılım için) ve Levene eşit varyans (homojen dağılım için) testleri uygulanarak belirlenmiştir. Salata ve çorba ürünlerine ait duyusal tüketici beğenilerinin, mevsimsel farklılıklarının tespiti amacıyla parametrik olmayan analizlerden Kruskal-Wallis analizi uygulanmıştır. İstatistikî analizlerde Minitab 17 ve IBM SPSS Statistics 21 istatistik paket programları kullanılmıştır.

Bulgular ve Tartışma

Besin Bileşimi

Ulva rigida'nın besin kompozisyonu "su (nem), protein, yağ ve kül" Tablo 1.'de verilmiştir. Sonuçlara göre yaş örneklerde en yüksek su değeri kış mevsiminde (%83.89) saptanmıştır ($P<0.05$). Makroalglerin su içerikleri yaş ağırlıkta %80-90 arasında değişmektedir (García-Casal ve ark., 2007). Kurutulmuş örneklerde ise nem içeriği %8.49-12.23 arasında tespit edilmiştir. Örneklere uygulanan kurutma işlemi sonrasında alglerin su (nem) oranları %85.31 ile %88.76 arasında azalmış olup en düşük değer ilkbahar mevsiminde (%8.49) saptanmıştır. Diğer aylarda tespit edilen su (nem) içeriklerinin ise istatistiksel olarak birbirlerinden farksız olduğu görülmektedir ($P>0.05$). Kurutulmuş örneklerin protein, yağ ve kül içerikleri ise yapılan kurutma işleminden

sonra azalan su oranına bağlı olarak nispi olarak artış göstermiş olup, en yüksek değerler ilkbahar mevsiminde saptanmıştır (P>0.05).

Yaş ve kuru örneklerde en yüksek protein içeriği ilkbahar mevsiminde toplanan örneklerde tespit edilmiş ve bunu sırasıyla yaz, sonbahar ve kış mevsimi izlemiştir (P<0.05). Yaz mevsiminde ışık şiddetinin ve su sıcaklığının artması ile deniz suyunda meydana gelen aşırı alg çoğalmaları nedeniyle sular besin tuzu bakımından fakirleşmektedir (Cirik ve Cirik, 2017). Bunun sonucunda da sudaki azot miktarı azalmakta ve dolayısıyla alglerin yapılarındaki protein oranı da düşük tespit edilebilmektedir. Benzer şekilde, birincil üretimde en yoğun üretim su sıcaklığının yükseldiği ilkbahar aylarında görülürken ortamdaki azot ve fosfatın bu üretimle azalmasıyla alglerin protein içerikleri de azalmaya başlamaktadır. Bu nedenle kış aylarında hem ışık, hem de sıcaklık alglerin fotosentez hızını etkilediği için protein içerikleri de düşük çıkabilmektedir. Alglerin protein içerikleri bulundukları ortamın tuzluluğuna ve mevsimsel değişimlere bağlı olarak değişim gösterebilmektedir (Lobban ve Harrison, 1994). Bu nedenle aynı bölgelerde toplanan aynı türe ait örneklerin içerikleri farklılıklar göstermiştir.

Çalışmamızda elde edilen örneklerin yağ içerikleri yaş örneklerde % 0.46 ±0.05 – 0.85 ±0.02 arasında, kuru örneklerde ise % 1.81 ±0.21 – 4.53 ±0.60 arasında birbirlerine yakın değerlerde tespite edilmesine karşın mevsimler arasında istatistiksel farklar tespit edilmiştir (P<0.05). Hem yaş ve hem de kuru örneklerde en yüksek yağ oranı yaz mevsiminde tespit edilirken, en düşük yağ oranı kış mevsiminde tespit edilmiştir. Alglerin yağ içerikleri ise diğer deniz ürünlerine göre oldukça düşük olup, genellikle tüm alg türlerinde %1-5 arasında değişmektedir (Ivanova ve ark., 2013). *Ulva* türleri ile yapılan başka çalışmalarda da ham yağ oranı çalışmamız sonuçları ile benzer bulunmuştur (Ak, 2015; Ivanova ve ark., 2013; Ortiz ve ark., 2006).

Örneklerin ham kül oranları yaş ve kuru örneklerde bahar aylarında en yüksek değerde tespit edilmiştir (P<0,05). İlkbahar aylarında, kar sularının erimesi ve yağışların başlaması gibi çevresel etkilerle denizel ortama taşınan besin tuzu miktarı artmaktadır. Makroalgler azot başta olmak üzere bu besin tuzlarını vakuollerinde depolamaktadırlar (Lobban ve Harrison, 1994). Bu nedenle, protein prosesine benzer bir şekilde, ham kül miktarı bahar aylarında yüksek miktarda bulunurken, daha sonra besin tuzlarının kullanımlarına bağlı olarak azalmaktadır (Topçu ve Ak, 2013). En düşük ham kül miktarı ise yaş örneklerde yaz ayında, kurutulan örneklerde ise kış ayında tespit edilmiştir (P<0.05). Kuru ve yaş örnekler arasındaki bu farklılık, kış mevsiminde elde edilen yaş örneklerdeki yüksek su miktarının kurutma işlemi sebebiyle yaşanan su kaybı ile bazı besin tuzlarının kaybedilmiş olması ile açıklanabilir.

Amino Asit Kompozisyonu

Ulva rigida yaş ve kuru örneklerinin mevsimlere bağlı amino asit değişimi Tablo 2 ve Tablo 3'de verilmiştir. Yapılan bu çalışmada, farklı mevsimlerde toplanan makroalglerin amino asit değişimleri incelenmiştir. Triptofan hiçbir grupta tespit edilememiştir. Yüksek ısı ve düşük pH'ya karşı hassas olan triptofan ön yakma işlemi nedeniyle kaybedilebilmektedir (Çankırılıgil ve ark., 2020). Triptofan gibi asit hidrolizinden etkilenen asparajin (ASN) ve glutamin (GLN) ise, sırasıyla aspartik asit (ASP) ve glutamik asite (GLU) dönüşmüştür. Bu amino asitlerin bulgularda verilen miktarları, dönüştükleri amino asitlerin (ASP+ASN ve GLU+GLN) toplamları şeklinde sunulmuştur. Aspartik asit ve glutamik asit alglere karakteristik tadını veren amino asitler olup (Yaich ve ark., 2011), çalışmamızda da en yüksek miktarlarda tespit edilen aminoasitlerdir. Ortiz ve ark. (2006) da çalışmalarında iki farklı alg türünün aminoasit kompozisyonunu karşılaştırarak bu türlerden *Ulva lactuca*'nın glutamik asit açısından zengin olduğunu ve bunu aspartik asit, alanin, lösin ve fenilalaninin takip ettiğini bildirmişlerdir. Çalışmamızda, esansiyel amino asitlerden isolösin, lösin, lizin, fenilalanin, tireonin ve valin tüm gruplarda tespit edilmiştir. Metiyonin ise yaz örneklerinde hiç tespit edilememiş; kış mevsiminde ise sadece kurutulan örneklerde tespit edilememiştir. Kuru örneklerdeki bu değişimin sebebinin, zaten düşük miktarlarda bulunan metiyoninin su çıkışı ile kaybedilmesi olduğu düşünülmektedir. Ayrıca, makroalglerin asparik asit ve glutamik asit bakımından zengin olduğu, ancak metiyonin ve histidin açısından fakir olduğu çeşitli çalışmalarda bildirilmiştir (Černá, 2011; Dawczynski ve ark., 2007).

Tablo 1. Deniz marulu (*Ulva rigida*)'nun mevsimsel besin bileşimi

Table 1. Seasonal proximate composition of sea lettuce (*Ulva rigida*)

	Kış	İlkbahar	Yaz	Sonbahar
Yaş Örnek				
Su (nem) (%)	83.89 ±1.37[a]	75.55 ±0.88[c]	78.44 ±0.65[b]	80.60 ±0.83[b]
Ham Protein (%)	8.55 ±0.30[d]	13.60 ±0.57[a]	11.34 ±0.62[b]	9.52 ±0.59[c]
Ham Yağ (%)	0.46 ±0.05[c]	0.85 ±0.02[a]	0.64 ±0.01[b]	0.60 ±0.02[b]
Ham Kül (%)	5.40 ±0.07[c]	6.73 ±0.30[a]	4.60 ±0.09[d]	6.16 ±0.05[b]
Kurutulmuş Örnek				
Su (nem) (%)	12.23 ±0.94[a]	8.49 ±0.08[b]	10.73 ±0.58[a]	11.84 ±0.62[a]
Ham Protein (%)	15.78 ±0.63[d]	25.98 ±0.12[a]	20.65 ±1.03[b]	17.93 ±0.15[c]
Ham Yağ (%)	1.81 ±0.21[d]	4.53 ±0.60[a]	3.32 ±0.04[b]	2.19 ±0.03[c]
Ham Kül (%)	24.36 ±0.64[c]	28.54 ±0.06[a]	21.63 ±1.01[c]	25.88 ±0.09[d]

Veriler ortalama ±Standart hata olarak verilmiştir. Aynı satırdaki farklı harfler istatistiki farkları göstermektedir (P<0,05)

Tablo 2. *Ulva rigida* yaş örneklerinin mevsimsel amino asit kompozisyonu (mg/g)

Table 2. Seasonal amino acid composition of non-dried *Ulva rigida* specimens (mg/g)

Amino Asitler	Kış	İlkbahar	Yaz	Sonbahar
Esansiyel Amino Asitler				
Triptofan (TRP)	T.E.	T.E.	T.E.	T.E.
İsolösin (ILE)	5.92 ±0.54[b]	7.43 ± 0.10[a]	1.49 ±0.16[c]	7.64 ±0.36[a]
Lösin (LEU)	7.76 ±0.89[b]	10.97 ±0.20[a]	0.96 ±0.01[c]	10.71 ±0.58[a]
Lizin (LYS)	6.33 ±0.47[b]	12.54 ±0.73[a]	0.84 ±0.18[c]	8.18 ±0.22[b]
Metionin (MET)	1.07 ±0.36[bc]	3.86 ±0.67[a]	T.E.	1.77 ±0.19[b]
Fenilalanin (PHE)	4.50 ±0.31[a]	6.03 ±0.32[a]	0.65 ±0.02[b]	6.00 ±0.72[a]
Tireonin (THR)	4.58 ±0.87[a]	5.12 ±0.10[a]	0.49 ±0.04[b]	4.59 ±0.09[a]
Valin (VAL)	8.84 ±0.39[a]	8.30 ±0.65[a]	1.49 ±0.35[b]	8.36 ±0.41[a]
Toplam EAA	42.34 ±1.24[c]	57.33 ±1.36[a]	8.77 ±0.51[d]	50.05 ±1.44[b]
Esansiyel Olmayan Amino Asitler				
Histidin (HIS)	3.34 ±0.26[a]	3.07 ±0.54[a]	2.85 ±0.20[a]	2.79 ±0.06[a]
Alanin (ALA)	5.73 ±0.38[b]	7.08 ±0.03[a]	1.06 ±0.02[c]	6.77 ±0.27[a]
Aspartat + Asparjin (ASP+ASN)	40.14 ±0.97[a]	26.83 ±2.83[b]	24.61 ±0.82[b]	24.04 ±2.25[b]
Glutamat + Glutamin (GLU+GLN)	14.75 ±2.49[b]	26.69 ±0.60[a]	1.38 ±0.00[c]	25.05 ±1.00[a]
Glisin (GLY)	4.64 ±0.25[b]	9.53 ±0.34[a]	0.76 ±0.04[c]	8.48 ±0.54[a]
Prolin (PRO)	6.38 ±0.69[b]	10.66 ±1.05[a]	0.70 ±0.01[c]	9.84 ±0.91[a]
Serin (SER)	3.87 ±0.66[a]	4.86 ±0.75[a]	0.48 ±0.02[b]	4.36 ±0.30[a]
Tirosin (TYR)	3.10 ±0.77[a]	4.51 ±0.16[a]	0.60 ±0.03[b]	3.80 ±0.40[a]
Sistin (C-C)	1.75 ±0.28[a]	1.57 ±0.27[a]	T.E.	1.24 ±0.16[a]
Toplam NEAA	80.35 ±1.81[b]	91.73±2.01[a]	29.58±1.13[c]	83.60±1.71[b]
EAA/NEAA	0.53	0.62	0.30	0.60
Toplam Amino Asitler	126.03 ±3.25[b]	152.15 ±3.11[a]	41.20 ±1.57[c]	136.44 ±3.64[b]
Diğer Amino Asit Benzeri Yapılar	28.34[a]	26.11[a]	15.46[c]	21.40[b]

Veriler ortalama ±Standart hata olarak verilmiştir. Aynı satırdaki farklı harfler istatistiki farkları göstermektedir (P<0.05).
T.E.; tespit edilemedi, EAA; esansiyel amino asitler, NEEA; esansiyel olmayan amino asitler, OAA; diğer amino asitler.

Tablo 3. *Ulva rigida* kuru örneklerinin mevsimsel amino asit kompozisyonu (mg/g)

Table 3. Seasonal amino acid composition of dried *Ulva rigida* specimens (mg/g)

Amino Asitler	Kış	İlkbahar	Yaz	Sonbahar
Esansiyel Amino Asitler				
Triptofan (TRP)	T.E.	T.E.	T.E.	T.E.
İsolösin (ILE)	7.58 ±1.40^{b}	20.09 ±1.56^{a}	1.49 ±0.38^{c}	11.40 ±0.93^{b}
Lösin (LEU)	12.37 ±0.29^{b}	33.04 ±0.12^{a}	1.66 ±0.10^{c}	15.80 ±2.19^{b}
Lizin (LYS)	10.07 ±0.57^{b}	25.98 ±2.30^{a}	0.82 ±0.06^{c}	14.23 ±0.96^{b}
Metionin (MET)	T.E.	5.12 ±0.28^{a}	T.E.	5.13 ±0.66^{a}
Fenilalanin (PHE)	7.37 ±0.86^{b}	18.76 ±0.76^{a}	1.04 ±0.12^{c}	8.96 ±1.02^{b}
Tireonin (THR)	5.42 ±0.39^{b}	13.73 ±1.57^{a}	0.90 ±0.11^{c}	8.14 ±0.24^{b}
Valin (VAL)	9.57 ±0.65^{c}	25.01 ±1.39^{a}	1.65 ±0.14^{d}	14.87 ±0.30^{b}
Toplam EAA	57.61 ±1.26^{c}	150.14 ±2.26^{a}	9.38 ±0.57^{d}	83.76 ±1.89^{b}
Esansiyel Olmayan Amino Asitler				
Histidin (HIS)	5.24 ±0.54^{ab}	8.41 ±1.83^{a}	1.82 ±0.20^{b}	5.23 ±0.42^{ab}
Alanin (ALA)	8.30 ±0.81^{b}	20.59 ±0.09^{a}	1.88 ±0.21^{c}	10.33 ±0.98^{b}
Aspartat + Asparjin (ASP+ASN)	27.81 ±0.38^{b}	47.21 ±5.28^{a}	10.89 ±1.03^{c}	54.94 ±3.28^{a}
Glutamat + Glutamin (GLU+GLN)	22.74 ±2.88^{b}	70.70 ±6.35^{a}	2.88 ±0.22^{c}	39.44 ±1.18^{b}
Glisin (GLY)	7.03 ±0.42^{c}	23.48 ±1.42^{a}	0.52 ±0.10^{d}	11.50 ±1.11^{b}
Prolin (PRO)	10.17 ±0.77^{b}	25.11 ±1.60^{a}	1.25 ±0.03^{c}	13.91 ±0.92^{b}
Serin (SER)	4.71 ±0.43^{c}	14.27 ±0.17^{a}	1.67 ±0.15^{d}	8.21 ±0.39^{b}
Tirosin (TYR)	3.56 ±0.23^{b}	12.10 ±0.83^{a}	0.48 ±0.08^{c}	5.22 ±0.50^{b}
Sistin (C-C)	0.53 ±0.09^{bc}	5.12 ±0.32^{a}	T.E.	1.27 ±0.18^{b}
Toplam NEAA	84.84 ±2.18^{c}	218.59 ±4.29^{a}	20.58 ±0.76^{d}	144.82 ±3.08^{b}
EAA/NEAA	0.68	0.69	0.46	0.58
Toplam Amino Asitler	147.69 ±2.62^{c}	377.05 ±6.36^{a}	31.78 ±1.28^{d}	233.82 ±3.49^{b}
Diğer Amino Asit Benzeri Yapılar	29.19^{b}	47.28^{a}	19.88^{c}	40.49^{a}

Veriler ortalama ±Standart hata olarak verilmiştir. Aynı satırdaki farklı harfler istatistiki farkları göstermektedir (P<0.05). T.E.; tespit edilemedi, EAA; esansiyel amino asitler, NEEA; esansiyel olmayan amino asitler, OAA; diğer amino asitler.

Yapılan analizde yaş ve kuru numunelerde toplam amino asit miktarı en yüksek ilkbahar mevsiminde en düşük ise yaz mevsiminde tespit edilmiştir (P<0.05). Yaz mevsimi amino asit içeriği yönünden diğer mevsimlere nazaran oldukça düşük bir içeriğe sahiptir (P<0.05). Yaz örneklerinin protein oranları diğer mevsimlerle benzer bulunurken, aminoasit oranları oldukça düşük tespit edilmiştir. Yüksek protein oranına rağmen, total amino asit içeriğinin düşük çıkması bahar aylarında alglerin vakuollerinde azot başta olmak üzere çeşitli besin tuzlarını biriktirmesi ile açıklanabilir. Algler azotlu bileşikleri (nitrit, nitrat, üre, amonyak) sucul ortamdan almakta (Hanisak, 1983; Topçu ve Ak, 2013) ve bunları kofullarında depolayabilmektedirler (Lobban ve Harrison, 1994). Bu azotlu bileşikler amonyum formuna indirgenerek kloroplastlarda amino asit sentezinde kullanılmaktadır. Ortamdaki amonyum konsantrasyonu arttıkça alglerin amonyum alımı da artmaktadır (Lobban ve Harrison, 1994; Topçu ve Ak, 2013). Bahar aylarında, yağmur suları ile ortamdaki miktarı artan bu azotlu bileşikler ve besin tuzlarının amino asit sentezinde kullanıldığı söylenebilir. Bu sebeple protein ve amino asit miktarlarında artış saptanmıştır. Yaz aylarında amino asit miktarının düşük tespit edilme sebebi ise protein olmayan azotlu bileşiklerin var olmasına rağmen amino asit sentezinde kullanılacak kadar yeterli olmamaları olarak açıklanabilir. Ayrıca, Lobban ve Harrison (1994) alglerdeki amino asitlerin ihtiyaç halinde mitokondride enerji üretimi için kullanılabildiğini belirtmektedir. Protein oranının amino asit miktarına kıyasla fazla bulunmasıın sebebi ise; Kjeldahl metodunda protein oranının toplam azot miktarı baz alınarak hesaplanması olarak açıklanabilir. Protein olmayan diğer azotlu bileşiklerin miktarı protein hesaplamalarını etkilemektedir. Tablo 2 ve 3'de diğer amino asit benzeri yapıların miktarları görülmektedir. Bu maddeler, yaz örneklerinde toplam amino asitlerin yaklaşık %29-40'ını kapsarken, bu oran diğer mevsimlerde %11-18 arasında bulunmuştur.

Yağ Asidi Kompozisyonu

İnsan beslenmesinde önemli besin öğelerinden birisi de, yağ asitleridir (Hosomi ve ark., 2012). Yapılan çalışmalar çoklu doymamış yağ asitleri ağırlıklı beslenmenin, pek çok ciddi rahatsızlığa karşı koruyucu etki yaptığını göstermektedir (Ellulu ve ark., 2015). Makro algler faydalı çoklu doymamış yağ asitleri açısından oldukça zengin gıda kaynaklarıdır (Rodrigues ve ark., 2015). Çalışmamızda deniz marulu (*Ulva rigida*)'nun mevsimsel yağ asidi içeriği belirlenmiş olup; yaş ve kuru numunelerdeki yağ asidi içerikleri Tablo 4 ve Tablo 5'de verilmiştir. Analizde 32 adet yağ asidi piki tespit edilmiş

ve piklerin tümü tanımlanmıştır. Bu yağ asitlerinden 14 adedi doymuş yağ asitlerini (SFA), 18 adedi ise doymamış yağ asitlerini (UFA) oluşturmaktadır. Yaş makroalg örneklerinde doymuş yağ asitlerinin toplamı, deniz suyu sıcaklığının arttığı yaz aylarında azaldığı; kış mevsiminde ise arttığı tespit edilmiştir (P<0.05). Palmitik asit (C16:0) doymuş yağ asitleri içerisinde en baskın yağ asitidir ve doymuş yağ asitleri toplamının %60-70'ini oluşturmaktadır. Alg örneklerinin tekli doymamış yağ asitleri toplamı ise yaz ve sonbahar mevsimlerinde en yüksek, ilkbaharda ise en düşük miktarlarda tespit edilmiştir. *Ulva rigida* örneklerinde en yüksek miktarda bulunan tekli doymamış yağ asidi palmitoleik asit (C16:1) olarak tespit edilmiştir. Palmitoleik asit en yüksek değerine yaz mevsimi yaş örneklerinde (%16.80 ±0.97) ulaşmıştır. Palmitoleik asidi, oleik asit ve nervonik asit izlemektedir. Çoklu doymamış yağ asitleri (PUFA) kompozisyonu incelendiğinde ise; yaz mevsiminde en yüksek oranda, kış mevsiminde ise en düşük oranlarda olduğu tespit edilmiştir (P<0.05). Makroalgler düşük miktarda ham yağ içermektedirler. Ancak bu yağ çoklu doymamış yağ asitleri (PUFA) açısından oldukça zengindir (Kumari ve ark., 2010). Benzer çalışmalarda *Ulva* türlerinin düşük lipid içeriğine karşın yüksek PUFA içerdiğini göstermektedir (Ivanova ve ark., 2013; Ortiz ve ark., 2006). Besin niteliği bakımından değerini arttıran önemli bir özelliktir. Bu grupta ise alfa-linolenik asit (C18:3 n–3) ve dokosaheksaenoik asit (C22:6 n–3) baskın yağ asitleri olarak göze çarpmaktadır. Sulardaki birincil üreticiler olarak alg türlerinin; insanlar için gıdalarla alınması elzem olan esansiyel yağ asitlerinden linoleik asit ve α-linolenik asiti yüksek oranlarda sentezledikleri bilinmektedir (Santos ve ark., 2017; Singh, 2005).

Yaş makroalg örneklerinde PUFA/SFA oranları farklı mevsimlerde 0,17-0,86 arasında değişirken omega-6:omega-3 (n–6/n–3) oranı ise 0.51-0.96 arasında değişim göstermektedir. Kuru örneklerde ise, PUFA/SFA oranları 0.10-0.52, n–6/n–3 oranı ise 0.63-1.31 arasında belirlenmiştir (Tablo 4, 5). Algler kurutulduktan sonra yaş örneklere göre doymuş yağ asitleri toplamında istatistiki olarak artış saptanırken, doymamış yağ asitlerinde azalma tespit edilmiştir (P<0.05). Buna bağlı olarak MUFA, PUFA, n-6 ve n-3 miktarları ile PUFA/SFA ve n–6/n–3 oranlarının da azaldığı tespit edilmiştir.

Duyusal Özellikler

Deniz marulunun gıda olarak tüketilebilirliğini belirlemek için duyusal analiz yapılmıştır. Mevsimsel olarak toplanan yaş ve kurutulmuş makroalglerden sırasıyla salata ve çorba yapılmış ve söz konusu ürünlere tüketici beğeni testi uygulanmıştır. Duyusal analizlere ait sonuçlar Tablo 7'de görülmektedir. Yaş örneklerden elde edilen salataların görünüş, lezzet, tekstür ve genel beğeni parametreleri incelenmiş ve analiz sonuçlarına göre ilkbahar mevsimi tüm duyusal kriterlerde 6'nın üzerinde değerler alarak salatanın en beğenildiği mevsim olmuştur. Salatada ilkbahar mevsimini sonbahar mevsimi takip etmektedir. Sonbahar mevsiminde toplanan örneklerde ise tekstür kriteri (5.3) dışında, tüm kriterler 6 ve üzerinde skor almışlardır. Yapılan istatistiki analiz sonuçlarına göre, duyusal değerler mevsimler arasında önemli bir farklılık göstermektedir (P<0.05). Kurutulmuş makroalg örneklerinden elde edilen çorbalar, salatalara göre genel olarak daha yüksek duyusal sonuçlar almıştır. Salatada olduğu gibi en yüksek sonuçlar ilkbahar ve sonbahar mevsimlerinde toplanan örneklere aittir. Tüketici beğeni testi kişi frekans grafikleri Şekil 2 ve Şekil 3'de görülmektedir. Tüketici beğeni analizi puanları frekans yönünden incelendiğinde, makroalg salatası örneklerinde en yüksek beğeni puanı olan 7; görünüş, lezzet, tekstür ve genel beğeni kriterlerinde sırasıyla ilkbahar (38 panelist; %49), ilkbahar ve sonbahar (28 panelist; %36), sonbahar (24 panelist; %31) ve sonbahar (24 panelist; %31) mevsimlerinde almıştır. Bir diğer yüksek puan olan 6 ise yine ilkbahar ve sonbahar mevsimlerinde duyusal kriterlerde en yüksek frekansta tercih edilmişlerdir. İlkbahar ve sonbahar mevsimlerinde 6 ve 7 puan tüm duyusal kriterler içinde %50'den fazla sayıda tercih edilen puanlamalar olmuşlardır. Alg çorbasında ise, görünüş ve tat ilkbahar mevsiminde; koku ve genel beğeni ise sonbahar mevsiminde 6 ve 7 puanla yüksek olarak bulunmuştur.

Sonuç

Makroalgler önemli makro ve mikro besinler açısından zengindir. Pek çok ülkede nitelikli biyolojik içerikleri nedeniyle gıda kaynağı olarak kullanılmaktadırlar. Bu çalışmada incelenen tüm parametreler; deniz marulunun yaş ve kuru olarak tüketilebilirliğini doğrudan etkilemektedir. Sonuç olarak ham besin bileşimi, gerek amino asit, gerekse yağ asidi içeriği yönünden *Ulva rigida*, karasal kaynaklı gıdalarla yarışabilecek niteliklere sahiptir. Özellikle bahar aylarında söz konusu bu parametreler açısından makroalgler daha da zenginleşmektedir. Türkiye denizlerinde yaygın olan fakat geleneksel mutfağımızda olmayan makroalgler daha fazla ilgiyi hak etmektedir. Asya ülkeleri başta olmak üzere pek çok ülkede salata, sos, çorba vb. birçok yemekte vazgeçilmez gıda maddeleridir. Bu amaçla yaş alglerden üretilen salata ile kuru algler kullanılarak elde edilen çorba tüketiciler tarafından beğeni kazanmış olup özellikle *Ulva rigida*'nın besin içeriğinin en zengin olduğu bahar aylarında en yüksek puanları kazanmışlardır. Bu sonuçlar sağlıklı beslenmek isteyenlerin ve özellikle et yemeyenlerin su bitkileri tüketimine yatkın olabileceğini; yemek listelerinde makroalg bulunmamasının tanıtım eksikliğinden kaynaklandığını düşündürmektedir. Ayrıca, söz konusu türün biyokimyası ile ilgili Çanakkale Boğazı kaynaklı

çalışmalar oldukça sınırlıdır. Bu çalışmalar ile sektörde yeni arayışlar içerisinde olan girişimci ve temsilciler denizlerimizde hazır bulunan bu türü işleyerek su ürünleri işleme sektörüne de katkı sağlayacaklardır.

Tablo 4. *Ulva rigida* yaş örneklerinin mevsimsel yağ asidi kompozisyonu (%)

Table 4. Seasonal fatty acid composition of non-dried *Ulva rigida* specimens (%)

Yağ asitleri	Kış	İlkbahar	Yaz	Sonbahar
C10:0	0.35 ±0.04^{a}	0.36 ±0.02^{a}	T.E.	T.E.
C11:0	0.09 ±0.01^{a}	0.06 ±0.01^{b}	0.06 ±0.01^{b}	T.E.
C12:0	5.49 ±0.27^{a}	3.34 ±0.17^{c}	2.38 ±0.12^{d}	4.50 ±0.25^{b}
C13:0	4.85 ±0.03^{a}	3.03 ±0.01^{b}	1.80 ±0.01^{c}	1.08 ±0.02^{d}
C14:0	5.33 ±0.48^{a}	3.62 ±0.36^{b}	2.61 ±0.25^{c}	3.71 ±0.23^{b}
C15:0	0.42 ±0.01^{a}	0.42 ±0.02^{a}	0.28 ±0.00^{b}	T.E.
C16:0	38.04 ±0.04^{a}	31.37 ±0.65^{c}	28.41 ±0.60^{d}	33.67 ±0.98^{b}
C17:0	0.56 ±0.04^{a}	0.19 ±0.02^{c}	0.36 ±0.04^{b}	0.23 ±0.02^{c}
C18:0	2.20 ±0.03^{a}	1.80 ±0.02^{b}	1.64 ±0.01^{c}	1.52 ±0.01^{d}
C20:0	2.42 ±0.42^{a}	0.89 ±0.00^{b}	0.72 ±0.01^{b}	1.85 ±0.19^{a}
C21:0	0.58 ±0.01^{a}	0.43 ±0.01^{b}	0.18 ±0.01^{c}	0.13 ±0.01^{d}
C22:0	2.78 ±0.03^{a}	1.84 ±0.02^{b}	0.74 ±0.01^{d}	0.80 ±0.00^{c}
C23:0	0.18 ±0.02^{a}	0.08 ±0.02^{b}	0.04 ±0.02^{c}	T.E.
C24:0	0.94 ±0.01^{a}	0.80 ±0.04^{b}	0.50 ±0.01^{c}	0.40 ±0.02^{d}
∑SFA	**64.24 ±0.72^{a}**	**48.23 ±0.99^{b}**	**39.72 ±0.86^{c}**	**47.88 ±0.39^{b}**
C14:1	0.88 ±0.01^{a}	0.60 ±0.02^{b}	0.28 ±0.02^{c}	0.10 ±0.03^{d}
C15:1	0.20 ±0.02^{a}	0.14 ±0.03^{b}	T.E.	T.E.
C16:1	14.52 ±0.53^{a}	12.03 ±0.96^{b}	16.80 ±0.97^{a}	14.48 ±1.01^{a}
C17:1	0.54 ±0.03^{c}	0.55 ±0.09^{c}	1.40 ±0.18^{b}	2.25 ±0.25^{a}
C18:1	4.27 ±0.04^{c}	4.40 ±0.02^{bc}	5.11 ±0.20^{b}	8.07 ±0.59^{a}
C20:1	1.85 ±0.03^{a}	0.90 ±0.02^{b}	0.85 ±0.02^{c}	0.45 ±0.01^{d}
C22:1	0.87 ±0.14^{c}	2.08 ±0.21^{a}	1.51 ±0.16^{b}	2.34 ±0.20^{a}
C24:1	1.44 ±0.10^{a}	0.38 ±0.06^{b}	T.E.	T.E.
∑MUFA	**24.57 ±0.38^{b}**	**21.09 ±0.71^{c}**	**25.95 ±0.92^{ab}**	**27.69 ±1.24^{a}**
C18:2*n*–6	2.06 ±0.04^{b}	6.64 ±0.30^{a}	7.18 ±0.22^{a}	6.72 ±0.23^{a}
C18:3*n*–6	0.57 ±0.13^{c}	1.53 ±0.17^{b}	4.60 ±0.26^{a}	4.67 ±0.43^{a}
C18:3*n*–3	3.13 ±0.47^{c}	6.26 ±0.32^{b}	8.84 ±0.19^{a}	3.53 ±0.27^{c}
C20:2	0.72 ±0.04^{c}	2.70 ±0.27^{a}	1.47 ±0.26^{b}	0.12 ±0.02^{d}
C20:3*n*–6	0.50 ±0.06^{a}	0.61 ±0.11^{a}	0.25 ±0.02^{b}	0.17 ±0.02^{b}
C20:3*n*–3	0.90 ±0.07^{b}	0.91 ±0.24^{b}	1.58 ±0.13^{a}	0.53 ±0.03^{c}
C20:4*n*–6	0.21 ±0.03^{b}	0.22 ±0.02^{b}	1.54 ±0.10^{a}	0.23 ±0.03^{b}
C20:5*n*–3	0.52 ±0.04^{a}	0.46 ±0.02^{a}	0.17 ±0.02^{c}	0.32 ±0.04^{b}
C22:2	0.52 ±0.04^{c}	2.72 ±0.07^{a}	1.43 ±0.11^{b}	0.23 ±0.03^{d}
C22:6*n*–3	2.06 ±0.23^{c}	8.64 ±0.39^{a}	7.27 ±0.38^{b}	7.91 ±0.33^{ab}
∑PUFA	**11.19 ±1.03^{c}**	**30.69 ±1.54^{a}**	**34.33 ±1.58^{a}**	**24.44 ±1.28^{b}**
PUFA/SFA	**0.17**	**0.64**	**0.86**	**0.51**
∑*n*–6	**3.34 ±0.22^{d}**	**9.00 ±0.5^{6}c**	**13.57 ±0.18^{a}**	**11.79 ±0.68^{d}**
∑*n*–3	**6.61 ±0.80^{c}**	**16.27 ±0.80^{a}**	**17.86 ±0.33^{a}**	**12.30 ±0.57^{b}**
***n*–6/*n*–3**	**0.51**	**0.55**	**0.76**	**0.96**

Veriler ortalama ±Standart hata olarak verilmiştir. Aynı satırdaki farklı harfler istatistiki farkları göstermektedir (P<0.05). T.E.; tespit edilemedi, SFA; doymuş yağ asitleri, MUFA; tekli doymamış yağ asitleri, PUFA; çoklu doymamış yağ asitleri, n–3; omega 3 yağ asidi, n–6; omega 6 yağ asidi.

Tablo 5. *Ulva rigida* kuru örneklerinin mevsimsel yağ asidi kompozisyonu (%)

Table 5. Seasonal fatty acid composition dried *Ulva rigida* specimens (%)

Yağ asitleri	Kış	İlkbahar	Yaz	Sonbahar
C10:0	T.E.	T.E.	T.E.	T.E.
C11:0	T.E.	T.E.	T.E.	T.E.
C12:0	6.29 ±0.27^{a}	3.78 ±0.57^{c}	2.92 ±0.16^{c}	4.78 ±0.16^{b}
C13:0	5.55 ±0.29^{a}	4.33 ±0.25^{b}	2.86 ±0.30^{c}	2.22 ±0.18^{c}
C14:0	5.85 ±0.28^{a}	4.40 ±0.42^{b}	4.29 ±0.08^{b}	5.28 ±0.16^{a}
C15:0	1.43 ±0.36^{a}	1.16 ±0.21^{a}	0.94 ±0.09^{a}	1.03 ±0.13^{a}
C16:0	40.20 ±1.05^{a}	33.80 ±0.93^{b}	30.50 ±1.69^{c}	34.24 ±0.75^{b}
C17:0	0.42 ±0.04^{ab}	0.22 ±0.03^{b}	0.54 ±0.21^{a}	0.24 ±0.03^{b}
C18:0	2.33 ±0.30^{a}	2.26 ±0.11^{a}	2.45 ±0.13^{a}	2.18 ±0.34^{a}
C20:0	2.49 ±0.17^{a}	1.42 ±0.17^{b}	1.99 ±0.40^{ab}	2.04 ±0.27^{ab}
C21:0	1.05 ±0.08^{a}	0.77 ±0.09^{b}	0.33 ±0.04^{d}	0.57 ±0.06^{c}
C22:0	2.99 ±0.31^{a}	2.23 ±0.11^{b}	1.14 ±0.14^{c}	1.86 ±0.17^{b}
C23:0	0.64 ±0.20^{b}	0.35 ±0.11^{b}	0.27 ±0.11^{b}	1.28 ±0.25^{a}
C24:0	2.25 ±0.27^{a}	1.60 ±0.32^{bc}	2.18 ±0.12^{ab}	1.33 ±0.12^{c}
∑SFA	**71.49 ±0.99^{a}**	**56.33 ±1.65^{b}**	**50.41 ±1.56^{c}**	**57.04 ±0.70^{b}**
C14:1	1.21 ±0.15^{a}	0.85 ±0.19^{a}	0.35 ±0.19^{b}	0.26 ±0.09^{b}
C15:1	0.24 ±0.08^{a}	0.20 ±0.06^{a}	0.23 ±0.11^{a}	0.21 ±0.07^{a}
C16:1	12.74 ±0.59^{b}	10.82 ±0.68^{c}	15.05 ±0.34^{a}	13.37 ±0.39^{b}
C17:1	0.66 ±0.14^{c}	0.64 ±0.14^{c}	1.72 ±0.35^{b}	3.87 ±0.27^{a}
C18:1	2.35 ±0.24^{b}	2.15 ±0.21^{b}	2.88 ±0.18^{a}	3.01 ±0.13^{a}
C20:1	0.72 ±0.10^{a}	0.54 ±0.12^{ab}	0.62 ±0.15^{a}	0.31 ±0.03^{b}
C22:1	0.79 ±0.15^{c}	1.46 ±0.08^{ab}	1.37 ±0.15^{b}	1.75 ±0.09^{a}
C24:1	2.48 ±0.13^{a}	1.50 ±0.19^{b}	1.38 ±0.11^{b}	2.13 ±0.22^{a}
∑MUFA	**21.20 ±0.31^{b}**	**18.17 ±0.95^{c}**	**23.61 ±0.36^{a}**	**24.92 ±0.33^{a}**
C18:2*n*–6	2.13 ±0.30^{b}	7.11 ±0.34^{a}	7.76 ±0.45^{a}	7.05 ±0.64^{a}
C18:3*n*–6	T.E.	0.34 ±0.06^{c}	1.61 ±0.24^{b}	1.97 ±0.06^{a}
C18:3*n*–3	1.57 ±0.29^{b}	4.80 ±0.32^{a}	5.11 ±0.19^{a}	1.59 ±0.40^{b}
C20:2	0.54 ±0.08^{c}	3.03 ±0.31^{a}	1.69 ±0.30^{b}	1.43 ±0.12^{b}
C20:3*n*–6	0.25 ±0.08^{a}	0.27 ±0.05^{a}	0.26 ±0.14^{a}	0.19 ±0.05^{a}
C20:3*n*–3	0.40 ±0.02^{b}	0.63 ±0.13^{a}	0.39 ±0.09^{b}	0.19 ±0.05^{b}
C20:4*n*–6	0.18 ±0.03^{b}	0.18 ±0.05^{b}	0.77 ±0.04^{a}	0.13 ±0.04^{b}
C20:5*n*–3	0.58 ±0.08^{a}	0.41 ±0.15^{ab}	0.30 ±0.03^{b}	0.19 ±0.03^{b}
C22:2	0.19 ±0.06^{b}	1.91 ±0.25^{a}	1.59 ±0.15^{a}	0.13 ±0.03^{b}
C22:6*n*–3	1.47 ±0.07^{b}	6.82 ±0.97^{a}	6.50 ±0.33^{a}	5.17 ±1.04^{a}
∑PUFA	**7.31 ±0.84^{c}**	**25.50 ±2.45^{a}**	**25.98 ±1.39^{a}**	**18.05 ±0.98^{b}**
PUFA/SFA	**0.10**	**0.45**	**0.52**	**0.32**
∑*n*–6	**2.55 ±0.37^{c}**	**7.90 ±0.45^{b}**	**10.41 ±0.67^{a}**	**9.33 ±0.57^{a}**
∑*n*–3	**4.02 ±0.46^{c}**	**12.66 ±1.45^{a}**	**12.29 ±0.44^{a}**	**7.15 ±0.69^{b}**
***n*–6/*n*–3**	**0.63**	**0.63**	**0.85**	**1.31**

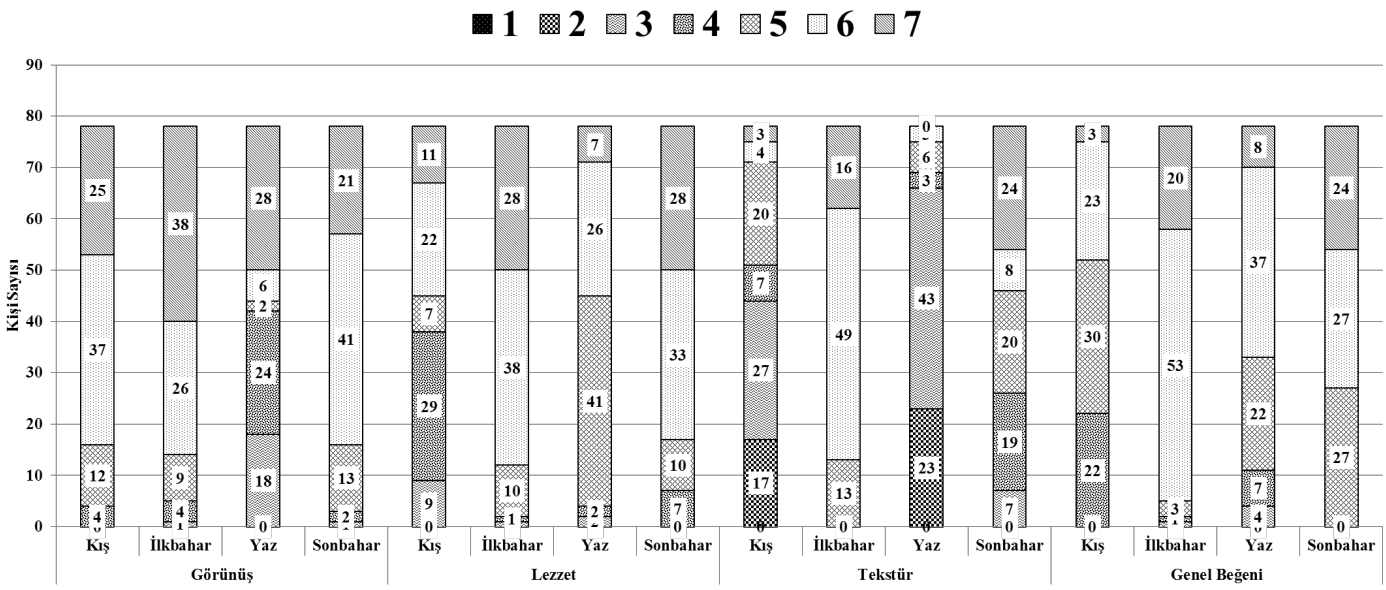
Veriler ortalama ±Standart hata olarak verilmiştir. Aynı satırdaki farklı harfler istatistiki farkları göstermektedir (P<0,05). T.E.; tespit edilemedi, SFA; doymuş yağ asitleri, MUFA; tekli doymamış yağ asitleri, PUFA; çoklu doymamış yağ asitleri, n–3; omega 3 yağ asidi, n–6; omega 6 yağ asidi.

Tablo 6. *Ulva rigida*'dan elde edilen alg salatası ve alg çorbasının duyusal beğeni testi sonuçları

Table 6. Sensorial appreciation of algae salad and algae soup prepared by *Ulva rigida*

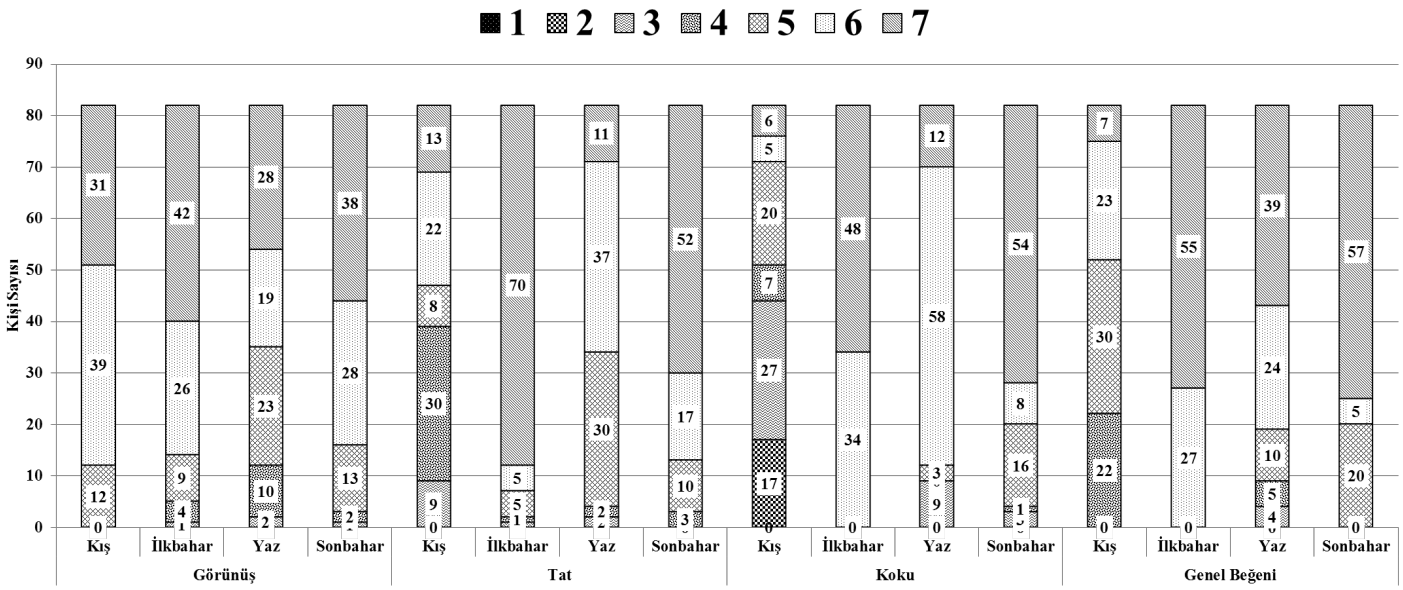
Duyusal Kriterler	Kış	İlkbahar	Yaz	Sonbahar
Alg Salatası (Yaş örnek)				
Görünüş	6.1 ±0.8^{a}	6.2 ±0.9^{a}	5.0 ±1.7^{a}	6.0 ±0.8^{a}
Lezzet	5.0 ±1.3^{b}	6.2 ±0.8^{a}	5.4 ±0.8^{b}	6.1 ±0.9^{a}
Tekstür	3.7 ±1.4^{b}	6.0 ±0.6^{a}	3.0 ±1.0^{b}	5.3 ±1.4^{a}
Genel Beğeni	5.1 ±0.9^{a}	6.2 ±0.7^{a}	5.5 ±1.0^{a}	6.0 ±0.8^{a}
Alg Çorbası (Kuru örnek)				
Görünüş	6.2 ±0.7^{a}	6.3 ±0.9^{a}	5.7 ±1.1^{a}	6.2 ±0.9^{a}
Tat	5.0 ±1.3^{b}	6.7 ±0.7^{a}	5.6 ±0.8^{ab}	6.4 ±0.8^{a}
Koku	3.8 ±1.5^{b}	6.6 ±0.5^{a}	5.8 ±1.1^{a}	6.3 ±1.1^{a}
Genel Beğeni	5.2 ±0.9^{b}	6.7 ±0.5^{a}	6.1 ±1.1^{a}	6.5 ±0.9^{a}

Aynı satırdaki farklı harfler istatistiki farkları göstermektedir (P<0.05).



Şekil 2. Salata örneklerine ait duyusal beğeni analizi kişi frekansı grafiği. 1 ile 7 arasında değişen her bir puanı veren kişi sayısı (frekans) grafik üzerinde ilgili puan segmentinde gösterilmiştir.

Figure 2. Frequency graph of algae salad's sensorial appreciation test. The person count (frequency) who gave each score varying between 1 and 7 was shown in the relevant score segment on the graph.



Şekil 3. Çorba örneklerine ait duyusal beğeni analizi kişi frekansı grafiği. 1 ile 7 arasında değişen her bir puanı veren kişi sayısı (frekans) grafik üzerinde ilgili puan segmentinde gösterilmiştir.

Figure 3. Frequency graph of algae soup's sensorial appreciation test. The person count (frequency) who gave each score varying between 1 and 7 was shown in the relevant score segment on the graph.

Etik Standart ile Uyumluluk

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

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Açıklama: -

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The effect of cooking methods on the formation of volatile N-nitrosamine in sausages with different contents

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ABSTRACT

The study's main aim is to assess the levels of volatile N-nitrosamine (VNA) in sausages based on different brands, ingredients, and cooking methods. The effects of sausage composition and cooking method on VNA formation, which caused this difference, were studied. For this, sausages from 17 different brands and 3 different ingredients (beef, chicken, and turkey) were bought from the market, cooked using 3 different techniques (frying, boiling, and microwave), and their VNA levels were determined by GC-MS (Gas chromatography-mass spectrometry). The study found different levels of VNA in all sausages. NDMA, NDEA, NDPA, NPYR, and NPIP were found in more than 70% of the samples, according to the study's findings. In the samples, the most NDMA, NPYR, and NPIP formation were observed. Total VNA levels in the study ranged from 0.18 to 109.28 ppb.

Keywords: Volatile nitrosamines, GC-MS, Sausage, Carcinogen

Introduction

Cured, heat-treated, or emulsified meat products are essential food industry products that serve consumers. Emulsified meat products like sausage and salami are popular because they are delicious and convenient. These products, in addition to their benefits, pose a risk due to the carcinogenic nitrosamines they contain. The fact that nitrite gives color (Horsch, 2013) to meat products and is an effective antimicrobial against *C. Botulinum* (Archer, 2002) and both gram-positive and gram-negative microorganisms (Horsch, 2013) makes it an essential additive for meat products. However, in spite of these advantages of nitrite, it is also known that nitrosamines formed in meat products can pose a risk and danger to consumer health according to the International Cancer Agency (IARC) (IARC, 2010). It is known that high temperature, long processing time and protein oxidation affect VNA formation in products. (Lu et al., 2022).

In sausages, Jo et al.(2003), found nitrosamines in concentrations ranging from 10 to 40 µg/kg. Cintya et al., (2019) on the other hand, found no NDEA in any of the meat products they purchased from the market in their current study of 5 different brands of sausage, smoked meat, burger, and canned meat samples. NDMA and NPYR were found in approximately 90% of fried, grilled, and smoked sausages purchased in Estonian supermarkets. Fried poultry meat with red pepper contains 24.42 µg/kg Nitrosamine, according to the same study (Yurchenko and Mölder, 20007). In a different study, NDMA levels in market-provided products such as dry-cured pork, cooked pork, mortadella (an Italian sausage), and bresaola (Italian pastrami) were all found to be between 0.3 and 1.1 g/kg (Sannino and Bolzoni, 2013). Yuan et al. (2015) investigated the nitrosamine levels of 28 different meat products purchased from the market, based on the cooking methods followed, and found that the highest nitrosamine levels were found in grilled sausages, with nitrosamine levels ranging from 0.42 to 51.018 ppb. The researcher claimed in another study examining the maturation process in terms of nitrosamine formation that three volatile N-nitrosamine (VNA) derivatives (NDMA, NDEA, and NPYR) were formed and the amount increased in the process. The chemical and microbiological reactions that occur during the ripening process are the reason for this situation (Xiao et al., 2018). According to research, cured, salted, or emulsified meat products are risky in VNA, especially when fried or grilled (Lee, 2019). While heat treatment applications (cooking time, method, and temperature) greatly affect the process, nitrate, nitrite, primary, secondary and tertiary amines, amides, proteins, peptides, amino acids, different precursors, and microbial activity are required for nitrosamine formation (Yurchenko and Mölder, 20007). In this context, it is of great significance for public health that the production of this type of processed food is carried out carefully in terms of the amount of additives and processing time (Özbay et al., 2019).

VNA are potential carcinogens whose exposure should also be evaluated. The tolerable intake dose of NDMA has been determined to be 96 ng/day (Anon, 2019a). Another volatile toxic N-nitrosamine, NDBA, has been related to the development of tumors in the liver and esophagus. The urinary bladder has been identified as the location where NDBA has the strongest cancer effect. While NDEA causes tumors in the liver and esophagus (EPA, 2016), 26.5 ng/day has been reported as a tolerable intake amount for NDEA (Anon, 2019a). In terms of acute toxicity, a single oral dose of NMEA with an LD50 of 90 mg/kg in mice has been reported. The genotoxic and mutagenic effects of NPYR and its causation of liver tumors have been detected in in vivo and in vitro studies (EPA, 2016). The European Union, on the other hand, recently suggested that the use of nitrates and nitrites causes the formation of nitrosamines, and in this respect, their use with ascorbic acid will reduce the amount (Anon, 2019b). In addition, it is important to use natural alternative nitrite and nitrate sources or to reduce the amount of nitrite-nitrate used in meat products. (Flores and Toldra, 2021).

In this study, seven different nitrosamines among the nitrosamines, which have nearly three hundred types in matrices such as air, water, soil, and food, were studied together with seven different nitrosamines, which are considered to be possible carcinogens by the International Agency for Research on Cancer (IARC), and whose carcinogenic effects are emphasized by the United States Environmental Protection Agency (EPA). These nitrosamine derivatives are N-nitrosodimethylamine (NDMA), N-nitrosodiethylamine (NDEA), N-nitrosodine-butylamine (NDBA), N-nitrosopiperidine (NPIP) N-nitrosopyrrolidine (NPYR) N-nitrosodine propylamine (NDPA) and N-nitrosomethylethylamine (NMEA) and their levels were determined in sausage samples with different contents and cooking methods of different brands by GC-MS (Gas Chromatography-Mass Spectrometer) device.

With the study, it was found what level of VNA was formed in sausage consumption depending on different cooking and content preferences. Depending on this situation, a viewpoint can be developed to improve consumption preferences.

Materials and Methods

Chemicals and Standards

All chemicals used in the analysis were of analytical purity. A nitrosamine standard mixture in dichloromethane (2000

mg/L) containing *N*-Nitrosodiethylamine (NDEA), *N*-Nitrosopiperidine (NPIP), *N*-Nitrosomethylethylamine (NMEA), Nitrosopyrrolidine (NPYR), *N*-Nitrosodi-*n*-propylamine (NDPA), *N*-Nitrosodimethylamine (NDMA), *N*-Nitrosodibutylamine (NDBA), was obtained from Sigma Aldrich (EPA 521 Nitrosamine Mix, 2000 ppm, Sigma-Aldrich, St Louis, USA). Different concentrations N-nitrosamine standards in were stored at -18 ºC. N-nitrosamines are potential carcinogens, so studies have been done carefully.

Materials

Sausages determined as the sample of analysis has been taken from the markets in Aksaray province. For this purpose, samples of 17 different samples has been purchased in order to examine the effect of different cooking methods on the formation of N-nitrosamine, these sausages were subjected to different cooking processes. Uncooked sausage samples were analyzed as a control group. Thus, sausage samples belonging to 17 different brands were cut into approximate sizes and analyzed by cooking with 1 control (uncooked) and 3 cooking methods (microwave, frying, boiling). Thus, 68 samples (17x4) belonging to 3 different cooking groups and a control group of 17 brands of sausages were analyzed. The study was completed in two parallels. (n=136)

The cooking process parameters were determined by preliminary tests. Accordingly, the parameters in which the sausages were cooked without burning were recorded. Sausage cooking parameters are shown in Table 1.

Table 1. Susage cooking parameters

Cooking Type	Time (min)	Ambient	Temperature
Boiling	10	Boiling water in a steel pot	Water, 100°C
Frying	4	Hot sunflower oil in a teflon pan	Sunflower oil, 200°C
Microwave	3	600 W with microwave energy in porcelain bowl	-

Methods

Determination of N-nitrosamines

Extraction of N-nitrosamines was done using method of Özbay and Şireli (2021a). Sausage samples were broken and homogenized with a blender (Philips, HR1316/00, Istanbul, Türkiye), after 20 g sample was weighed with a precision scale (KERN, ABJ 220-4NM, Ballingen, Germany). After 40 ml DClM (Dichloromethane) (Merck, Dichlotomethane for gas chromatography, Darmstadt, Germany) was added to it. The samples were then kept in an ultrasonic water bath (VWR, Ultrasonic Cleaner - USC-TH, Leicestershire, England) for 15 minutes. At the end of the period, the samples were filtered with filter paper (S & H Labware, Ø125 mm, Ankara, Türkiye) and the filtrate was collected. The samples were then treated with DClM (40 ml) again in the water bath for 15 minutes. The collected filtrate was transferred to the rotary evaporator (Heidolph, Hei-VAP Advantage, Schwabach, Germany) and the solvent (DClM) in it was removed. The extract was collected with 1 ml of methanol (Merck, Methanol for chromatography, Darmstadt, Germany), filtered (0.45 µm, Sartorius Stedim, Göttingen, Germany) and taken into glass vials. The samples taken into the vial were homogenized by vortexing (VELP Scientifica, Velata, İtaly). Samples kept in vials were kept closed and parafilm wrapped in refrigerator until analysis.

GC (Agilent Technologies, 7890A, Santa Clara, United States) device and integrated mass spectrometer - MS (Agilent Technologies, 5975C, Santa Clara, United States) detector were used for volatile N-nitrosamine analysis. DB624 (Agilent, Santa Clara, USA) capillary column (30 m, 0.25 mm I.D and 1.40 µm) was used as the GC-MS column. Chromatographic conditions were as follows: inlet temperature, 180°C; inlet mode, pulsed split-less, septum purge flow: 3 mL/min, using helium (purity ≥ 99.999%) as carrier gaz. The oven temperature was programmed as follows: start temperature of 60°Cfor 2 min, then increased to 120°Cfor 2 min, at a rate of 20°Cper minute followed by a further increase to 220°Cat a rate of 20 °C per minute. Finally isothermally at 220°Cfor 2 min. The conditions set for the mass spectrometer were as follows: transfer line temperature, 180°C, electron impact ionization mode at 70 eV; scan range from m/z 40 to 200. The time for solvent delay was set to 5 min. The retention times and qualifier ions are shown in Table 2. A representative chromatogram from GC analysis is shown in Figure 1.

Table 2. Retention times, molecular weight, qualifier ions for the detection in MS SIM mode applied method for the 7 VNAs using GC-MS

Compound	Weight (M/W)	Rt (min)	Qualifier ions (m/z)
NDMA	74	5.441	74.1 / 84.0 / 86.0
NMEA	88	6.82	88.1 / 71.1 / 73.1
NDEA	102	7.839	102.1 / 71.1 / 73.1
NDPA	130	10.071	70.1 / 113.1 / 130.1
NPYR	100	10.426	100.1 / 71.1 / 85.1
NPIP	114	10.909	114.1 / 71.1 / 85.1
NDBA	158	12.664	84.1 / 99.1 / 116.1

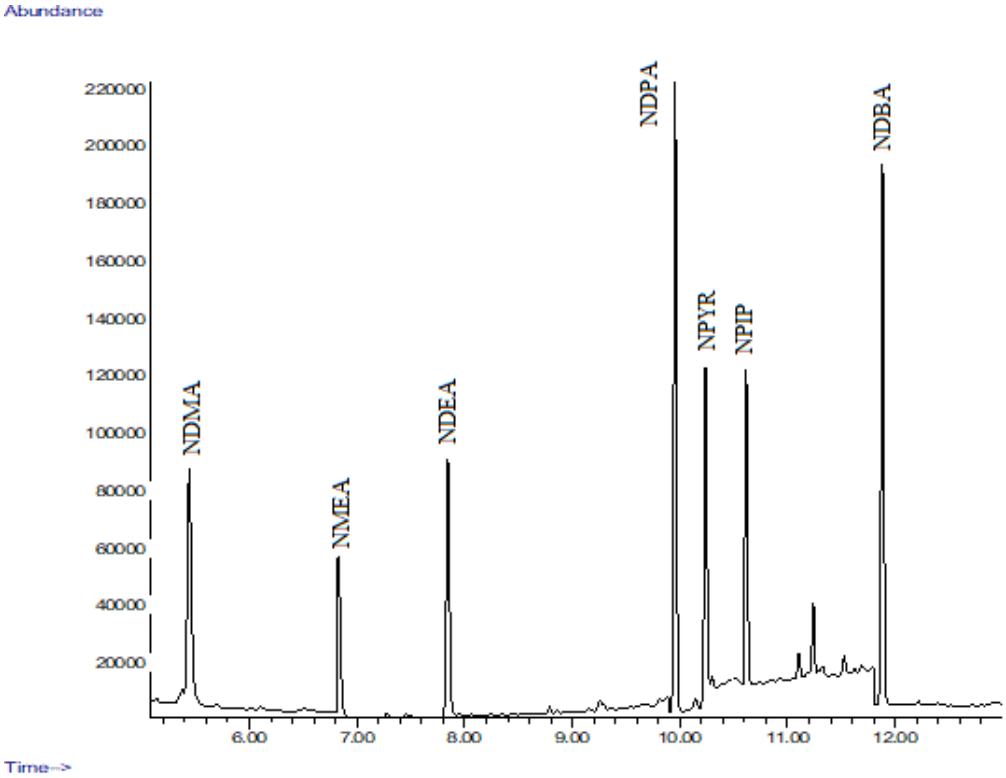

Fig. 1 GC-MS chromatography showing seperation of N-nitrosamines

To calibrate the gas chromatography mass spectrometry (GC-MS) chromatogram, 7 different standard solutions were prepared, which covered the concentration range 0.5 to 75 µg/mL. The lowest detectable concentrations for NAs were established between 0.06 and 0.17 μg/mL. The limit of quantification (LOQ) was calculated as 3 × LOD (between 0.21 and 0.56 μg/mL).

For the recovery experiment, a sample with low content of N-nitrosamines was chosen and fortified with 3 different levels of standard solutions. Recoveries were found to be between 87.70% and 95.82%. Validation of GC-MS method was carried out by following Eurochem method validation steps (Magnusson and Örnemark, 2014). Validation study results are shown in Table 3.

Table 3. Method validation results

Compounds	Calibration (µg/L)	Linearity (r^2)	LOD (µg/kg)	LOQ (µg/kg)	Recovery (%)	Reproducibility (%RSD)
NDMA	0.5 - 75	0.999225	0.11	0.36	87.70	3.5342
NMEA	0.5 - 75	0.989719	0.12	0.40	92.90	2.0331
NDEA	0.5 - 75	0.998683	0.11	0.37	90.02	8.1090
NDPA	0.5 - 75	0.979911	0.10	0.32	87.44	5.0704
NPYR	0.5 - 75	0.999627	0.06	0.21	94.5	3.4497
NPIP	0.5 - 75	0.995153	0.17	0.56	93.9	4.5262
NDBA	0.5 - 75	0.994560	0.08	0.26	95.82	5.0957

Statistical Analysis

One Way ANOVA was applied to the data by using SPSS (Statistical Package for the Social Science) 15.0-licensed program for the statistical analysis of all samples. Thus, 136 samples of 17 brands were analyzed in the study.

Results and Discussion

NDMA, NDEA, NDPA, NPYR, and NPIP were found in more than 70% of the samples, according to the study's findings. In the samples, the most NDMA, NPYR, and NPIP formation were observed. Kaban et al. (2021), found the most NDMA, NPYR and NPIP in heat-treated sausages similar to our study. They reported that NPIP occurs at the highest level. NDMA, NPIP, and NPYR are mainly formed in sausages, according to Lee et al. (2019). According to Campillo et al. (2011), the most common volatile N-nitrosamine derivatives in processed meat products are NDMA and NPIP, which is consistent with previous studies. While in another study, 7.86–29.11 ppb level of VNA was detected in salami collected from the market (Özbay and Şireli, 2021b), while in this study, a varying level of VNA was found at the level of 8.45-65.2 ppb. This situation was interpreted as a cooking relationship with VNA formation. Similarly, in a study examining VNA in raw meat products, NDPA, NDBA and NPIP were detected in almost all samples (Sun et al., 2020). The fact that VNA formation and levels are affected by very complex processes is thought to be the reason for these differences.

The VNA values of cooked sausages ranged from 0.9 to 109.28 ppb, according to the findings of the study. In their review analysis, Gushgari and Halden (2018) found that 118 different processed meat products included a variable amount of total VNA ranging from 0.1 to 121 ppb. Kaban et al. (2021) reported that they detected NPIP at the level of 5.19 – 16.40 ppb in heat-treated sausage, followed by the formation of NDMA and NPYR. Cintya et al. (2019) evaluated non-volatile N-nitroso-thiazolidine-4-carboxylic acid (NTCA) in 20 samples of ready-to-eat sausage, smoked meat, burger, and canned meat purchased from the market. N-nitrosamine derivatives such as N-nitroso-2-methyl thiazolidine-4-carboxylic acid were detected in high concentrations (NMTCA). The amount of NTCA in the samples varies between 500-4227 ppb, while NMTCA varies between 20-990 ppb.

Table 4 shows the statistical relationship between the VNA level in sausages and cooking, brand, and content. The table shows that there is a significant relationship between cooking methods and VNA formation (P<,005). In the same table, it can be seen that the brand has no significant effect on VNA formation (P>,005). According to the table, the sausage content only has an effect on the formation of NDPA. In addition to these findings, it was found that veal sausage contained higher VNA than other ingredients when the average VNA levels in Table 5 were evaluated. Turkey sausage and chicken sausage come after beef sausage. The study obtained results in parallel with the study of Moradi et al (2021). In both studies, meat sausages contained significantly higher VNA compared to chicken sausage.

In the Table 5, it is seen that frying causes higher VNA amount in terms of cooking methods. Next comes the boiling process. The fact that microwave cooking is the cooking method that remaining of the lowest level of VNA formation is observed. The effects of content and cooking method on the average VNA formation separately are shown in Figure 2 and Figure 3, respectively.

Table 4. Statistical analysis results

	NDMA	NMEA	NDEA	NDPA	NPYR	NPIP	NDBA	Total NAs
Cooking (Raw, fried, boiled, microwave)	.021	.002	.000	.002	.005	.001	.000	.000
Brand	.021	.272	.535	.017	.124	.004	.200	.040
Contents (Beef, turkey, chicken)	.158	.050	.038	.001	.030	.062	.024	.007

Means in the same row with different superscripts are significantly different (P<.005)

Table 5. Average volatile N-nitrosamine results by cooking and content (ppb)

Content	NDMA	NMEA	NDEA	NDPA	NPYR	NPIP	NDBA	Total NAs
Beef Sausage	2.6996	2.6279	3.3946	3.4283	2.6129	2.6450	2.5396	19.9479
Turkey Sausage	0.7015	0.5335	1.4315	2.0390	0.7195	1.7010	0.8100	7.9360
Chicken Sausage	1.3929	0.4087	1.2287	0.6258	1.0796	0.9317	1.0300	6.6975
Cooking Type								
Raw Sausage	0.4118	0.2659	0.3859	0.3700	0.4876	0.4371	0.2241	2.5824
Fried	3.5106	3.9765	4.7953	3.6082	3.1912	3.5594	3.6271	26.2682
Boiled	2.2571	0.6494	2.1188	2.5265	1.7876	2.0347	1.8594	13.2335
Microwave	0.4235	0.0229	0.9112	1.6176	0.5929	1.0194	0.2818	4.8694

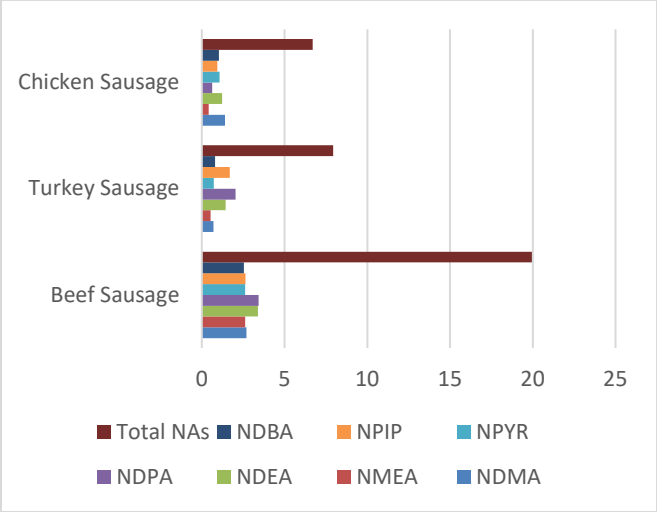

Figure 2. Average volatile N-nitrosamine levels (ppb) depending on sausage content

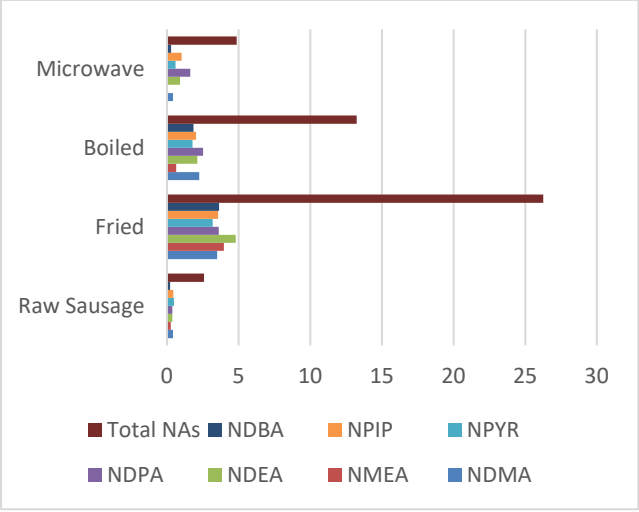

Figure 3. Average volatile N-nitrosamine levels (ppb) depending on cooking method

According to the study's findings, total VNA levels in fried sausage range from 8.24 to 109.28 ppb. The highest value was obtained from fried beef sausage, while the lowest level was obtained from turkey sausage. All of the fried samples revealed NPYR, NMEA, NDPA, NPIP, and NDBA. Similarly, Gloria et al (1997) found NDMA, NDEA, NDBA, NDEA, NPIP, and NPYR in the samples they examined in their study by frying 37 processed meat products from the market, such as bacon and its derivatives. They also found NPYR at levels ranging from 7 to 25 ppb in all of the products they examined. In another study, NPYR was found to be higher (5.02 ppb) than uncooked sausage (3.97 ppb) after frying dry cured sausages. In parallel, researchers reported that they detected NPYR in 90% of 386 (raw, fried, smoked, canned) processed meat products. In the same study, the highest volatile VNA level belonged to fried meat, then grilled meat and finally cured meat (Yurchenko ve Mölder, 2007). In general, the fact that frying processes contain high levels of volatile N-nitrosamines exhibits parallelism with this research.

Boiling is another cooking method used in the study. During the boiling process, a total of 3.67–58.24 ppb VNA was produced in the sausages. When compared to frying, boiling provides a smaller amount of total VNA. After boiling the sausages, Li et al. (2012) determined the formation of NDMA, NDEA, and NPYR volatile N-nitrosamine derivatives. Yurchenko and Mölder (2007) found similar results with the study. They analyzed the volatile N-nitrosamine levels of sausages purchased directly from the market, fried, grilled, smoked etc., and found NDMA and NPYR in approximately 90% of all samples.

Microwave cooking is another method that has been studied. The microwave cooking process yielded the lowest results of all the cooking methods. According to the findings, the average total VNA level in sausages cooked in the microwave ranged from 0.9 to 17.06 ppb. In comparison to frying and boiling, microwave cooking yields lower total VNA levels. In microwaved sausage samples, Li et al. (2012) found 1.02 ppb NDMA, 0.18 ppb NDEA, and 3.76 ppb NPYR. In the same study, lower levels of volatile N-nitrosamine formation in microwave cooking were found to be similar to those found in other heat treatments (boiling, frying).

Important data was recorded on volatile N-nitrosamine formation at various levels during cooking methods. Many parameters, including heat treatment time, temperature, and method, ambient temperature, nitrosamine precursor concentration, salt concentration, and pH, are found to affect the complex processes that change the formation of VNA in this process (Gençcelep, 2010; Herrmann et al., 2015; Honikel, 2008; Özçelik, 1982). The fact that chemical and physical factors have a big impact on VNA formation is regarded to be the main reason why the amount varies by method and application. Researchers have reported detecting volatile N-nitrosamine results on a wide range of scales in several studies (Lee, 2019; Gushgari and Halden, 2018).

Conclusion

In the research, sausage samples belonging to 17 different brands obtained from the market were evaluated in terms of possible carcinogenic VNA levels. The VNA values of the sausages varied widely, depending on the different ingredients, brands and cooking methods.

As a crucial result of the study, it is possible to conclude that each cooking method increases the level of VNA in the sausage. As for cooking methods, frying has also been found to increase the risk of VNA formation. Microwave cooking might be considered a healthier option for sausage consumption as a cooking method. Furthermore, it is possible to assert that the sausage's content leads to the formation of VNA. While veal sausage has a higher overall VNA content, turkey and chicken sausage come in second and third, respectively. In this regard, the method of cooking and the composition of the sausage will help to limit the risks of sausage consumption. The formation of VNA in all sausages, albeit at varying levels, is also a significant result of the study.

In the future, it will be important to detect VNA derivatives by conducting similar studies for other foods that are considered as risk groups (ripened cheeses, pickles, alcoholic beverages, etc.). Furthermore, alternatives to these foods' consumption processes could have a positive impact on public health.

Compliance with Ethical Standard

Conflict of interests: The author declares that for this article they have no actual, potential or perceived conflict of interests.

Ethics committee approval: Author declare that this study does not include any experiments with human or animal subjects; therefore, no ethics committee approval is needed.

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Makroalglerin mineral içeriği ve insan sağlığı için kullanım olanakları

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ÖZ

Makroalgler çok eski zamanlardan beri insanlar tarafından gıda, gıda takviyesi, hayvan yemi, gübre ve ilaç olarak kullanılmaktadır. Birçok kara bitkisinde olduğu gibi makroalgler de insan sağlığı için çok önemli inorganik ve organik maddeler içerir. Makroalgler protein, lipit, karbonhidrat ve mineral madde içeriklerinden dolayı geleneksel olarak kullanılan bir gıda maddesi olmasının yanında "biyoaktif maddeler", "makroalg mineral katkıları", "ilaç ve kozmetik hammaddesi" gibi fonksiyonel kullanımlar açısından da büyük bir potansiyele sahiptir. Deniz suyundaki mineralleri absorbe eden makroalgler, başta demir ve iyot olmak üzere zengin bir mikro element ve kalsiyum, potasyum, magnezyum olmak üzere iyi bir makro element kaynağıdır. Bazı makroalglerin mineral düzeyleri kara bitkilerinden daha yüksek olabilmektedir. Bilimsel veriler, makroalg kaynaklı bazı minerallerin insanlarda biyoyararlılığının, kayaçlardan elde edilen minerallere göre daha yüksek olduğunu ortaya koymuştur. Minerallerin insan sağlığı açısından öneminin anlaşılmasıyla, yüksek esansiyel element içeriğine sahip olan makroalglerin mineral kaynağı olarak kullanımına ilgi son yıllarda artmıştır. Makroalglerin mineral kompozisyonları lokasyon, mevsim, suda kalış süresi, türün fizyolojisi gibi faktörlerin yanında, deniz suyundaki element miktarı, ışık şiddeti ve tuzluluk gibi çevresel koşullara bağlı olarak da değişebilmektedir. Bu nedenle, insanların günlük mineral ihtiyaçlarını karşılamada kullanılacak alg ürünlerinin hangi oran ve miktarlarda tüketilebileceği ve potansiyel sağlık riskleri konularında yeni araştırma ve yaklaşımlara ihtiyaç duyulmaktadır. Bu çalışmada, makroalglerin mineral içerikleri, minerallerin insan sağlığı açısından önemi ve alglerin mineral kaynağı olarak kullanım potansiyelleri incelenmiştir.

Anahtar Kelimeler: Makroalgler, Mineral, Mikro elementler, Sağlık, Fonksiyonel gıda

ABSTRACT

Mineral content of macroalgae and possible uses for human health

Seaweeds have been used since ancient times as food, food additives, fertilizer, and a source of medicine. Like terrestrial plants, seaweeds contain many inorganic and organic substances which can beneficial to human health. Seaweeds have great potential as "bioactive compounds for functional use, "algae mineral supplements", "pharmaceuticals and cosmetics" and in addition to their potential of good sources of minerals, trace elements, proteins, lipids, and carbohydrates as traditional food. Due to the mineral absorption ability of macroalgae from the seawater, many species are a perfect source of some trace elements such as iron and iodine and a good source of some macro minerals such as calcium, phosphate, and magnesium. In some cases, the mineral content of the seaweeds may be higher than that of land plants. Scientific data show that the bioavailability of algae minerals is higher than rock-based minerals for humans. In recent years, the potential use of seaweed minerals as "algae mineral supplements" gained attention due to their rich elemental composition and the importance of minerals for human health. Mineral composition of seaweeds may vary according to locality, season, residence time, species physiology, and environmental conditions such as level of elements in seawater, light intensity, and salinity. Thus, new approaches and researches are needed on how much seaweeds can be consumed daily and their potential health risks. In this study, the mineral contents of seaweeds, the importance of minerals for human health, and potential uses of algae minerals were investigated.

Keywords: Macroalgae, Minerals, Trace elements, Health, Functional food

Giriş

Su yosunları olarak da bilinen makroalgler, denizel ortamlarda herbivor beslenen hayvanların besinlerini oluşturma, suya oksijen sağlama, sucul canlılara korunma ve üreme ortamı oluşturma gibi çok önemli rollere sahiptir. Makroalgler binlerce yıldan bu yana özellikle uzak doğu ülkelerinde insan gıdası, hayvan yemi ve gübre olmak üzere çok çeşitli amaçlarla kullanılagelmektedir. Özellikle Çin, Japonya ve Kore gibi ülkelerde geleneksel olarak tüketilen yeşil, kahverengi ve kırmızı alglerin yüksek oranlarda kullanımı son yıllarda Avrupa, Güney Afrika ve Amerika kıtasındaki birçok ülkede de hızla yaygınlaşmaktadır (Dawes, 1998; McHugh, 2003). Makroalglerin taze, kurutulmuş veya besin katkısı şeklinde insan gıdası olarak tüketimi ülkeler arasında farklılıklar göstermekte olup, Japonya'da 2010-2014 yılları arasındaki kişi başı makroalg tüketiminin 9.6-11.0 g makroalg / gün olduğu rapor edilmiştir (MHLW, 2014). Yapılan çok sayıda bilimsel araştırma makroalglerin geleneksel gıda maddesi olması yanında, fonksiyonel gıda olma potansiyelinin de çok yüksek olduğunu göstermektedir (Mendes ve ark., 2009; Harnedy ve FitzGerald, 2011; Pangestuti ve Kim, 2011; Cornish ve ark., 2015; Fleurence ve Levine, 2016). Son yıllarda "fonksiyonel gıda", "makroalg mineral karışımları", "ilaç ve kozmetik hammaddesi" ve "biyoaktif maddeler" olarak kullanımları yaygınlaşmış durumdadır. Makroalglerden elde edilen alginat, agar, karragenan, fukoidan ve çeşitli biyoaktif maddeler (antibakteriyal, antiviral, antifungal vb.) çok fazla talep görmektedir. Makroalglerin lif içeriğinin yüksek olması ve diyabet, obezite, kalp rahatsızlıkları, kanser gibi rahatsızlıkların düşük lif alımıyla ilişkilendirilmesi de makroalglere olan ilgiyi artırmıştır (Southgate, 1990). Buna bağlı olarak makroalg ve mikroalg ürünlerine talep giderek artmakta (Wells ve ark., 2016) ve artan ihtiyacı karşılamak için denizlerdeki doğal popülasyonların hasadının yanında birçok türün kültürü de yapılmaktadır. Dünyada 2006-2015 yılları arasında doğadan toplama ve kültür yoluyla yapılan makroalg üretiminde en fazla paya sahip olan ülkeler ve üretim miktarları (ton yıl^{-1}) Tablo 1'de verilmiştir (FAO, 2018). Tablo 1'de görüldüğü gibi 2015 yılı dünya makroalg üretim verilerine göre, kültür yoluyla yapılan makroalg üretiminin % 85.8'i Çin ve Endonezya tarafından, doğal hasat yoluyla yapılan toplam makroalg üretiminin % 78'i ise sırasıyla Şili, Çin, Norveç ve Japonya tarafından gerçekleştirilmiştir (FAO, 2018). Toplam 30.45 milyon ton yıl^{-1} olan 2015 yılı dünya makroalg üretiminin (doğadan toplama+kültür) % 46.6'sı Çin tarafından gerçekleştirilmiştir (FAO, 2018). Dünyada 2012 yılında doğal ve kültür yoluyla yapılan makroalg üretiminin % 38'nin insanlar tarafından doğrudan gıda olarak tüketildiği kaydedilmiştir (FAO, 2014). Ülkemiz kıyılarında makroalglerin taksonomisi ve dağılımları konusunda yapılmış birçok çalışma bulunmakta olup, son yıllarda biyokimyasal içerikleri konusundaki çalışmalarda da artış olmuştur. Ancak, ülkemizde makroalglerin gıda olarak tüketimi henüz yaygın olmayıp, ticari üretimi yapılmamaktadır. Makroalg üretimi konusunda az sayıda da olsa deneysel çalışmalar bulunmakla birlikte, dünyadaki genel yaklaşıma paralel olarak ülkemizde de uygun makroalg kültür alanları ve üretim potansiyelinin araştırılması son derece önem arz etmektedir.

Makroalgler protein, lipit, doymamış yağ asitleri, vitamin ve çeşitli biyoaktif maddeler yönünden zengin olmalarının yanında bilinen en zengin mineral içeriğine sahip besinlerden biridir. Deniz suyu ile sürekli temasta olmalarından ve mineralleri doğrudan almalarından dolayı, özellikle mineral içerikleri karasal bitkilere göre daha yüksektir. Kuzeydoğu Akdeniz kıyılarından toplanan kırmızı alg, *Jania rubens*'in toplam mineral içeriğinin kuru maddede % 51.63'e kadar yükselebildiği (Polat ve Özoğul, 2013), Hindistan kıyılarından toplanan 18 makroalg türünün toplam mineral içeriğinin ise % 20 ile % 50 arasında değiştiği ortaya konmuştur (Barot ve ark., 2019). Makroalglerin mineral içeriği türe, türün fizyolojik durumuna, lokalite ve mevsim gibi faktörlere göre değişiklikler gösterebilmektedir (Nelson ve ark., 2002; Kostetsky ve ark., 2004). Khairy ve El Sheikh (2015), Akdeniz kıyılarında üç makroalg türünde minerallerin mevsimsel değişimini incelemiş, en yüksek kalsiyum, sodyum, potasyum ve magnezyum içeriğinin yaz mevsiminde, en düşük değerlerin ise genellikle sonbaharda bulunduğunu bildirmiştir. Endonezya'da hasat edilen yeşil, kahve ve kırmızı alglerin demir ve çinko içeriklerinin Japonya'daki *Porphyra, Ulva intestinalis, Sargassum fusiformis*'den düşük olduğu, bu sonuçlara bakılarak sıcak ekvatoral bölgelerdeki alglerin mineral düzeylerinin yüksek enlemlerde bulunanlara göre daha düşük olabileceği öne sürülmüştür (Wells ve ark., 2016). Ancak, mineral içeriğinin mevsimsel ve bölgesel dağılımı ile ilgili genelleme yapmak için bilgiler nispeten sınırlıdır (Cabrita ve ark., 2016). İnsanların yetersiz mineral alımı nedeniyle çeşitli sağlık problemleriyle karşılaşma riskleri, makroalglerin biyoyararlılığı yüksek mineral kaynağı olarak incelenmesinin önemini ortaya koymaktadır. Bu çalışmada makroalglerin mineral içerikleri incelenerek, mineral kaynağı olarak insan sağlığı açısından önemi ve kullanım potansiyellerinin değerlendirilmesi amaçlanmıştır.

Tablo 1. Dünyada 2009-2015 yılları arasında doğadan toplanan ve kültürü yapılan makroalg üretiminde en fazla paya sahip olan ülkeler ve üretim miktarları (ton/yıl) (FAO, 2018 den uyarlanmıştır)

Table 1. World annual cultured and wild (natural harvest) macroalgae production by countries in 2009-2015, weight in tonnes, (adopted from FAO, 2018)

Ülkeler *Countries*	**Doğadan toplanan makroalg miktarı (ton yıl^{-1})** *Amount of macroalgae collected from nature (ton year^{-1})*						
	2009	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**
Şili	368.032	368.580	403.496	436.035	517.929	417.331	345.704
Çin	276.170	246.620	274.060	257.640	283.010	245.550	261.770
Norveç	160.361	158.516	152.382	140.998	154.150	154.230	147.391
Japonya	104.103	97.231	87.779	98.514	84.498	91.601	93.300
Dünya Toplam Üretimi	1.113.127	1.071.940	1.116.820	1.127.014	1.289.563	1.199.111	1.087.468
	Kültür yoluyla üretilen makroalg miktarı (ton yıl^{-1}) *Amount of cultured macroalgae (ton year^{-1})*						
	2009	**2010**	**2011**	**2012**	**2013**	**2014**	**2015**
Çin	10.495.995	11.092.270	11.549.555	12.832.060	13.561.445	13.326.315	13.924.535
Endonezya	2.963.556	3.915.017	5.170.201	6.514.854	9.298.474	10.076.992	11.269.341
Filipinler	1.739.995	1.801.272	1.840.833	1.751.071	1.558.378	1.549.576	1.566.361
G. Kore	858.659	901.672	992.283	1.022.326	1.131.305	1.087.048	1.197.129
Japonya	456.426	432.796	349.737	440.754	418.365	373.908	399.300
Dünya Toplam Üretimi	17.337.986	18.992.284	20.785.191	23.555.401	26.862.752	27.354.942	29.363.158

Makroalglerin Mineral İçeriği

Makroalgler genellikle, deniz suyu ile benzer mineral düzeylerine sahip olup, deniz suyundaki mineral içeriğine göre bu değerler de farklılık gösterebilmektedir (Cotas ve ark., 2020). Makroalglerin kalsiyum, magnezyum, sodyum, fosfor, potasyum gibi makroelementler ile çinko, demir, manganez, iyot, molibden, selenyum, bakır gibi mikro element içeriklerini belirlemek üzere birçok çalışma yapılmıştır (Ruperez, 2002; Polat ve Özoğul, 2009; Khairy ve El Sheikh, 2015). Makroalglerin mineral içerikleri ile ilgili genellemeler yapmak, içeriklerinin mevsimsel, coğrafik ve taksonomik varyasyonlar göstermesi nedeniyle oldukça güç olmakla birlikte, yapılan çalışmalar makroalglerin özellikle başta demir ve iyot olmak üzere zengin bir mikroelement kaynağı olduğunu göstermiştir. Makroalglerin mineral içeriği birçok meyve ve sebzeye göre daha yüksektir (Zaragoza, 2015; Cabrita ve ark., 2016). Khairy ve El sheikh (2015), kalsiyumun alglerdeki en önemli element olduğunu ve kara bitkilerine göre daha fazla kalsiyum biriktirdiklerini bildirmiştir. Yeşil alglerin, kırmızı ve kahverengi alglere göre daha fazla kalsiyum içerdiği rapor edilmiştir (El-Said ve El-Sikaily, 2013). Buna karşın, kırmızı ve kahverengi algler, yeşil alglere göre daha yüksek konsantrasyonlarda Na, K ve Zn biriktirme eğilimindedir (Circuncisão ve ark., 2018). Barot ve ark. (2019), Hindistan kıyılarından topladıkları kahverengi alglerde kalsiyum ve potasyumun, kırmızı alglerde ise sodyum ve demirin yüksek düzeylerde bulunduğunu bildirmiştir. Biancarosa ve ark.(2018) tarafından 21 makroalg türünde yapılan çalışmada, en yüksek kalsiyum içeriği 30 g kg^{-1} kuru madde olarak kahverengi alglerden *Fucus vesiculosus'*da bulunmuştur. Benzer olarak, Panayotova ve Stancheva (2013), kahverengi alglerde kalsiyum ve potasyumun, yeşil alglerde ise magnezyum ve sodyumun daha yüksek oranlarda bulunduğunu bildirmiştir (Tablo 2). Ruperez (2002) ise kahverengi alglerde makroelementlerin daha yüksek bulunduğunu, en yüksek düzeyde bulunan elementin potasyum olduğunu belirtmiştir (Tablo 2). Cabrita ve ark. (2016) Portekiz kıyılarında 15 makroalg türünün mineral içeriğini incelediği çalışmada, en yüksek kalsiyum (49.76 g kg^{-1} kuru madde) ve magnezyumu (19.54 g kg^{-1} kuru madde) yeşil alglerde bulmuş olup, bu değerler Ekşi ve Özen (2012) tarafından kivi meyvesi (214 mg kg^{-1} Ca, 123 mg kg^{-1} Mg) için, Kadiri ve ark. (2015) tarafından domates (7.49 mg kg^{-1} Ca, 1.32 mg kg^{-1} Mg) için bildirilen değerlerden daha yüksektir. Panayotova ve Stancheva (2013) ise, makroalglerin en önemli kalsiyum ve fosfor kaynaklarından biri olduğunu ve içeriklerinin elma, portakal, havuç ve patates gibi bitkilerden daha yüksek olduğunu bildirmiştir.

Tablo 2. Farklı makroalg türlerinin mineral içerikleri

Table 2. Mineral composition of different macroalgae species

Grup *Group*	Tür *Species*	Ca	Mg	Na	K	Fe	Zn	I	Mn	Ref.
		mg 100 g^{-1} kuru ağırlık mg 100 g^{-1} dry weight								
Kırmızı Algler	*Chondrus crispus*	420±22	732±6	4270±62	3184±0	3.97±0.11	7.14±0.13	22.1±0.7	1.32±0	1, 2
	*Gelidium crinale**	8.68±0.70	2.31±0.28	5.32±0.27	5.57±0.11	89.27±2.10	3.80±0.30		19.3±0.40	3
	Halymenia floresia	730 ±283.4	900 ± 162.7	5540 ± 763.5	5960 ± 861.3	14.34 ± 0.39	2.11 ± 0.01	-	-	4
	Jania rubens	14790.3±1.46	2228.08 ± 2.09	1215 ± 1.00	627.33 ± 1.52	84.93 ± 0.89	1.35 ± 0.12		1.23 ± 0.15	5
	Porphyra tenera	390±17	565±11	3627±115	3500±71	10.3±0.41	2.21±0.17		2.72±0	1
	Porphyra spp.	525.0 ±1.41	261.75±1.06	348.75±1.06	1395.0±4.24	12.28±0.32	2.79±0.1	-	2.26±0.04	6
Kahverengi Algler	*Ascophyllum nodosum*	984.73±47.26	867.82±12.01	4575±50.05	3781.35±13.4	13.34±0.90	-	-	1.96±0.69	7
	Bifurcaria bifurcate	996.42±12.83	528.04±8.25	1836.82±52.12	9316.28±101.9	-	-	-	-	7
	*Cystoseira barbata**	21.40±2.40	4.14±0.71	1.92±0.04	11.93±2.51	33.43±3.43	1.43±0.29		4.77±0.35	3
	*Cystoseira crinata**	19.93±1.60	4.22±0.19	5.16±0.54	17.95±0.69	6.10±0.92	1.63±0.06		1.60±0.26	3
	Fucus vesiculosus	938±7	994±13	5469±60	4322±46	4.20±0.17	3.71±0.37	-	5.50±0.11	1
	Laminaria digitata	1005±5	659±6	3818±43	11.579±128	3.29±0.54	1.77±0.44	170 ±5.5	<0.5	1, 8
	Saccorhiza polyschides	1120 ± 94.2	560 ± 32.4	2650 ± 176.9	10170 ± 112.4	25.12 ± 1.52	8.57 ± 0.39	-	-	4
	Undaria pinnatifida	931±38	1181±34	7064±166	8699±144	7.56±1.13	1.74±0	26 ± 2.4	0.87±0	1, 8
Yeşil Algler	*Codium* spp.	820 ± 253.6	410 ± 18.8	3960 ± 106.8	4500 ± 182.4	45.14 ± 0.82	0.78 ± 0.14	-	-	4
	*Chaetomorpha linum**	7.30±0.28	2.50±0.25	3.09±0.31	15.44±0.97	105.97±3.95	1.87±0.21		29.4±3.56	3
	*Ulva rigida**	5.52±0.79	7.85±0.70	5.90±0.09	4.60±0.28	24.53±3.01	1.30±0.26		3.30±0.10	3
	Ulva lactuca	574.6-558.1	825.8-842.4	92.20-93.63	77.32-77.67	163.8-167.8	10.06-11.75	12.9±0.5	7.33-7.44	9
	Ulva spp.	1030 ± 321.5	2040 ± 34.7	3670 ± 216.4	3770 ± 114.6	36.28±0.49	1.91 ± 0.65	2.60±0.2	-	4

* Na, Mg, Na ve K için birim mg g^{-1} kuru ağırlık'tır.

(1) Ruperez, 2002; (2) Nunes et al., 2019; (3) Panayotoda ve Stancheva, 2013; (4) Garcia ve ark., 2016; (5) Dixit ve Reddy, 2017; (6) Admassu ve ark., 2018; (7) Lorenzo ve ark., 2017; (8) Kolb ve ark., 2004; (9) Devi et al. 2015.

Makroalglerin çinko, demir, manganez, iyot, molibden, selenyum ve bakır gibi mikroelement içeriği ile ilgili olarak yapılan çalışmalarda, türlerin birbirlerinden çok farklı içeriklere sahip olduğu görülmektedir (Tablo 2). Anantharaman ve ark. (2010), Hindistan'ın güneydoğu kıyılarında Chlorophyceae, Phaeophyceae ve Rhodophyceae türlerinde demir içeriğinin 17.83-60.45 ppm arasında değiştiğini, en yüksek demir içeriğinin yeşil alglerden *Halimeda macrolaba*'da bulunduğunu kaydetmişlerdir. Aynı araştırmada magnezyum 32.91-181.5 ppm, mangan 0.73-4.03 ppm aralıklarında bulunmuştur. Se-Kwon Kim (2012) iyot gibi halojenlerin makroalglerde kara bitkilerine göre 10-20 kat daha fazla bulunabileceğini bildirmiştir. Kahverengi algler, diğer gruplara göre daha fazla iyot biriktirmektedir (Biancarosa ve ark., 2018). Makroalglerde demir (3501 mg kg^{-1}) ve iyot (957.6 mg kg^{-1}) en yüksek düzeylerde bulunan mikroelementler olup, bunları çinko (153.62 mg kg^{-1}) ve manganez (392.7 mg kg^{-1}) takip etmektedir, selenyum (2.68 mg kg^{-1}) ve kobalt (1.96 mg kg^{-1}) ise çok daha düşük düzeylerde bulunmaktadır (Cabrita ve ark., 2016). Bu mikroelement değerleri Leterme ve ark. (2006) tarafından muz, patates, mısır ve nar gibi bitkiler için verilen değerlerden daha yüksektir. Yeşil alglerden *U. intestinalis'*de 5800 mg kg^{-1} düzeyinde bulunan demirin, 2 - 4 mg 100g^{-1} aralıklarında demir içeren yeşil sebze, baklagil, fındık ve tahıllar gibi kara bitkilerinden daha yüksek olduğu görülmektedir (Coultate, 1996; Biancarosa ve ark., 2018). Tahıllarda ve yağlı tohumlarda düşük düzeylerde bulunan iyot, makroalglerden *Laminaria digitata'*da 10000 mg kg^{-1} ve *Saccharina latissima'*da 4600 mg kg^{-1} gibi yüksek düzeylerde bulunabilmektedir (Biancarosa ve ark., 2018).

Araştırmalar, makroalglerin yüksek düzeyde mineral madde içerdiğini ve alg minerallerinin gıda katkı maddesi ve besin desteği olarak kullanımına olan ilginin son yılllarda arttığını göstermektedir. Ancak, makroalglerin potansiyel kullanımları ile ilgili çalışmalar son yıllarda artmış olmakla birlikte, kara bitkileriyle karşılaştırıldığında halen daha fazla çalışmanın yapılmasına ihtiyaç olduğu görülmektedir.

Minerallerin İnsan Sağlığı İçin Önemi ve Makroalg Minerallerinin Kullanımı

Mineraller, insan vücudunda metabolizma ve yaşamsal fonksiyonların sürdürülebilmesi için belli düzeylerde bulunması gereken inorganik maddelerdir. Bu maddeler, biyokimyasal reaksiyonlarda enzim kofaktörü olarak görev alır (Güngör, 2003). Ayrıca, mineraller vücutta ozmotik dengenin korunması, kasların uyarılması, sinir impulslarının iletimi gibi olaylarda rol oynamanın yanı sıra iskelet, diş, hemoglobin, hormon ve enzimlerin yapısında yer alır. Minerallerin yeterli düzeyde alınması, başta kanser, kardiyovasküler rahatsızlıklar olmak üzere, alzheimer, erken yaşlanma ve beslemeye bağlı hastalıkların önlenmesinde önemli katkılar sağlayabilmektedir (Fenech ve Ferguson, 2001). Bu nedenle, vücudun

sağlıklı kalabilmesi ve temel fonksiyonlarını sürdürebilmesi için birçok mineralin düzenli olarak alınması gerekmektedir.

İnsan sağlığı için önemli bazı mineraller göz önüne alındığında, kalsiyum insan vücudunda en fazla bulunan mineral olup, kemik ve dişlerin yapısında yer almaktadır. Kasların kasılması, hormon salgılanması gibi olaylarda da kalsiyuma ihtiyaç duyulmaktadır. Ayrıca, kalsiyum kanın pıhtılaşmasında da görev almaktadır (Kılıç ve ark., 2002). Kalsiyum, makroalglerde en yüksek düzeyde bulunan elementlerden biridir. Swarnalatha (2018) makroalglerde kalsiyum fosfat olarak bulunan kalsiyumun biyolojik olarak kullanılabilirliğinin, sütte kalsiyum karbonat olarak bulunan formundan daha fazla olduğunu bildirmiştir. Assoumani (1997), alglerden sağlanan kalsiyumun kireçtaşından elde edilene göre büyüme ve biyolojik kullanılabilirlik yönünden daha avantajlı olduğunu, Kaats ve ark. (2011) ise, alg kalsiyumu kullanımının kalsiyum karbonat ve kalsiyum sitrat gibi tuzların kullanımına göre üstünlük gösterdiğini bildirmiştir. Araştırıcılar, alg kalsiyumu ile muamele edilen insan osteoblast hücre kültüründe alkalin fosfataz aktivitesinin, kalsiyum karbonat ve kalsiyum sitrat uygulamasına göre önemli düzeyde arttığını belirtmişlerdir. Brown ve ark. (2014), kırmızı alglerden *Lithothamnion calcareum* ekstraktının osteoartrit hastalarında olumlu etkiler sergilediğini, uygulama sonrasında eklem ağrılarında azalma ve hareketlilikte artış olduğunu bildirmiştir. Japonya'da geleneksel beslenmenin alerjik reaksiyonları önlemedeki etkileri konusunda yapılan bir çalışmada, besinlerle yüksek düzeyde makroalg kalsiyum, magnezyum ve fosforu alımının alerjik rinit oluşumunu azaltabileceği rapor edilmiştir (Miyake ve ark., 2006).

Sodyum elementi, sinir ve kas fonksiyonlarının uyarılması, hücredeki sıvı dengesi ve diğer besinlerin emiliminde görev alır. Potasyum ise, adale kasılmasında sinir impulslarının iletilmesinde ve enzim faaliyetlerinde rol oynar (Güngör, 2003). Bunların yanında, kalp ritminin düzenli olmasında, protein sentezi, karbonhidrat yıkımı ve kanın pH dengesinin sağlanmasında görev almaktadır. Makroalgler düşük Na/K oranına (0.33-1.34) sahip oldukları için makroalg içeren diyetlerin yüksek tansiyon problemi olan kişilerde tansiyon riskini azaltabileceği bildirilmiştir (Ruperez, 2002).

Magnezyum, birçok kimyasal reaksiyonda, enzim aktivasyonunda ve kalbin düzenli atmasında görev alır (Ensminger ve ark., 1995). Ayrıca, kasların güçlenmesi, protein sentezinde, hücre büyüme ve yenilenmesinde önemli rol oynar. İnsan vücudunun günlük magnezyum ihtiyacının 280-350 mg olduğu bildirilmektedir (Görmüş ve Ergene, 2004). Makroalg kalsiyum ekstratındaki magnezyumun, yumurtalığı alınmış sıçanlarda kalsiyum dengesi ve kemik yapısına etkisi incelenmiş, makroalg kalsiyum ekstraktının sentetik kalsiyum ve magnezyum desteğine kıyasla kemik sağlığı için etkili bir kalsiyum ve magnezyum kaynağı olduğunu belirlenmiştir (Bae ve ark., 2011). Frestedt ve ark (2009), makroalglerden elde edilen, kalsiyum ve magnezyumca zengin doğal bir multimineral olan aquamin adlı maddenin osteoartrit hastalarında semptomları ve antiinflamasyon ilaç ihtiyacını azalttığını, potansiyel tedavi edici etkisinin olduğunu rapor etmişlerdir.

Çinko, 300'den fazla enzimin fonksiyonu için gerekli olan en önemli elementlerden birisidir. İnsanlardaki çinkonun % 85'i kas ve kemik dokuda, % 11'i deri ve karaciğerde, % 4'ü ise diğer dokularda bulunur (Mišurcová ve ark., 2011). Beyinde de yüksek düzeyde çinko bulunmaktadır (Tapiero ve Tew, 2003). Ayrıca, protein ve nükleik asit sentezi, DNA sentezi, hormonların depolanması ve salınımı, nörotransmisyon, büyüme ve gelişme gibi birçok metabolik olayda yer alır ve kemik gelişimi ile ilgili birçok hormonun yapısına katılır (Salgueiro ve ark., 2002). Yetersiz düzeyde çinko içeren topraklarda yetişen ürünlerde çinko eksikliği görülebilir. Çinko aynı zamanda, bir antioksidan gibi etki etmekte olup, immün sistemin gelişmesi için en önemli minerallerden biridir (Rink ve Haase, 2007; Akdeniz ve ark., 2016). Özellikle düşük proteinli ve yüksek fitat içeren besinlerle beslenen insanlarda sıklıkla karşılaşılan çinko yetersizliği epiderma, sinir sistemi, gastrointestinal sistem, kemik ve üreme sistemi üzerinde olumsuzluklara neden olur (Salgueiro ve ark., 2002; Tapiero ve Tew, 2003). Günlük alınması önerilen çinko miktarı 15 mg'dır (Ruperez, 2002). Ülkemizde tarım arazilerinin yaklaşık % 50'sinin yararlı çinko yönünden fakir olması, bitkilerde çinko eksikliğine neden olmakta ve bunun sonucunda tahıla dayalı beslenmenin yoğun olduğu bölgelerde insanlarda bazı sağlık sorunları ortaya çıkmaktadır (Yılmaz ve Sonkaya, 2018). Mineral içeriği yönünden karasal bitkilere göre daha zengin olan makroalglerin (Circuncisão ve ark., 2018; Cotas ve ark., 2020) beslenmede kullanımı bu eksikliğin giderilmesine katkı sağlayacaktır.

Demir, vücutta hayati fonksiyonları olan bir elementtir. Vücuttaki demirin %60-70'i hemoglobine bağlı olarak eritrositlerde, % 10'u demir içeren enzimlerde, miyoglobinde ve sitokromda, geri kalanı ise hemosiderin ve ferritin olarak depolanır (Lieu ve ark., 2001). Hemoglobin tarafından akciğerlerden dokulara oksijen taşınmasının yanında, DNA sentezi ve elektron taşınımı gibi son derece önemli metabolik işlemlerde rol oynayan enzimlerin yapısında yer alır (FAO/WHO, 1998; Lieu ve ark., 2001; Puntarulo, 2005). Demirin yetersiz alınması özellikle bilişsel performans, fiziksel gelişim, immün sistem ve gastrointestinal sistemde bozulma gibi hayati fonksiyonlarda aksamalara ve rahatsızlıklara yol açabilir (Walker, 1998; WHO, 2001). Günlük alınması önerilen demir miktarı 10-18 mg'dır (Ruperez, 2002). Günlük alınması önerilen miktar (RDA) dikkate alındığında, 8 g kurutulmuş *Ulva* spp.

alımının Fe ihtiyacını % 78, aynı miktarda *Porphyra* spp. alımının % 16 oranında karşılayabileceği bildirilmiştir (Circuncisão ve ark., 2018).Yeşil alglerden *Ulva reticulata* eklenmiş çikolatada demir biyoyararlığının %21 düzeylerine ulaştığı, bu değerin sade çikolatadaki değerden %10 daha yüksek olduğu belirlenmiştir. Çalışmada, hemoglobin, serum demiri ve serum ferritin düzeylerinde de önemli artışlar olduğu bulunmuştur (Banu ve Mageswari, 2015). Yapılan çalışmalar, makroalg tüketiminin anemik topluluklarda ve veganizm gibi düşük demir tüketen kişilerde demir düzeylerini iyileştirebileceğini ortaya koymuştur (Circuncisão ve ark., 2018).

Mangan, özellikle yüksek enerji gereksinimi olan beyin ve retinada yoğunlaşmış olarak bulunur ve esansiyel bir mikroelementtir. Kemik doku, karaciğer ve böbrekler manganı yüksek düzeyde içerirler (Aschner ve Aschner, 2005). Mangan, protein, lipit ve karbonhidrat metabolizması ile ilişkili bir mineral olup, özellikle normal immün fonksiyonu, kan şekerinin düzenlenmesi ve beyin fonksiyonları için gereklidir (Takeda, 2003). Mangan eksikliği çeşitli hastalıklara (osteoporoz, epilepsi, gelişme geriliği, yetersiz kemik gelişimi, glikoz, lipit ve protein metabolizmasında aksamalar) neden olabilmektedir (Aschner ve Aschner, 2005; Nkwenkeu ve ark., 2002). Mangan yetersizliği kadar aşırı alımına bağlı toksik özelliği de son derece önemli olup, aşırı mangan alımı nöropsikiyatrik belirtilere, Parkinson ve benzeri hareketlerle ilgili fonksiyon bozukluklarına neden olabilmektedir (Cersosimo ve Koller, 2006; Nkwenkeu ve ark., 2002). Chen ve ark. (2018) yetişkinler için günlük 2-5 mg mangan dozunun yeterli ve güvenli olduğunu bildirmiştir. Günlük alınması önerilen düzeyler (RDA) bağlamında, 8 gr kurutulmuş *Ulva* spp. alımının Mn ihtiyacını % 49, aynı miktarda *Porphyra* spp. alımının ise % 10 oranında karşılayabileceği bildirilmiştir (Circuncisão ve ark., 2018).

İyot, büyüme ve gelişme ile birçok fizyolojik olayı kontrol eden tiroit hormonunun üretiminde rol oynar (Güngör, 2003). Tiroit hormonunun gebeliğin 15. haftasından 3 yaşa kadar bebeklerde beynin ve merkezi sinir sisteminin gelişmesinde önemli rolü vardır (FAO/WHO, 1998; Zimmermann ve Crill, 2010). İyot eksikliği hipotiroit ve beyin gelişiminde hasara neden olabilmektedir (Laurberg ve ark., 2010). Başta Avrupa ve Amerika olmak üzere dünya genelinde yaklaşık 1.9 milyar insanda iyot yetersizliği söz konusudur (De Benoist ve ark., 2003). İnsanda sağlıklı tiroit fonksiyonları için kilogram başına günlük 2 µg olmak üzere, bir yetişkinin günde yaklaşık 140 µg alması gerekmektedir (Mouritsen ve ark., 2013). Bu bağlamda makroalgler mükemmel bir iyot kaynağı olarak öne çıkmakta ve yüksek iyot içerikleri nedeniyle halen kahverengi alglerden *Sargassum, Laminaria, Ecklonia, Macrocystis,* kırmızı alglerden *Gracilaria, Palmaria* yeşil alglerden *Enteromorpha* ve *Ulva* gibi cinsler doğal iyot katkı maddesi üretiminde kullanılmaktadır (Mišurcová ve ark., 2011). Miyai ve ark. (2008), 7-10 gün süresince günlük 15-30 g *Saccharina japonica* verilen bireylerde üriner iyot düzeyinin kısa sürede hızla arttığını rapor etmiştir. Kahverengi alglerden *Sargassum vachellianum* ve kırmızı alglerden *Gracilaria lemaneiformis* türlerinde iyot düzeyleri sırasıyla 47.51 mg / 8g ve 34.09 mg / 8g olarak belirlenmiş olup, bu algler kuru madde olarak günde 8 g alındığında, alınması önerilen iyot miktarının çok daha üzerinde iyot alınmış olacağı bildirilmiştir (Mišurcová ve ark., 2011). Bu nedenle, insanlarda iyot takviyesi olarak kullanılacak olan makroalg türünün içerdiği iyot düzeyinin iyi tespit edilmesi ve ihtiyacın karşılanmasında uygun dozun kullanımı son derece önemlidir. İyot içeriği yüksek olan makroalglerin fazla tüketiminin sağlık problemlerine neden olabileceği, yetersiz iyot alımı kadar aşırı alımının da olumsuz etkilerinin olabileceği göz önüne alınmalıdır. Bu nedenle, her bir türün farklı mevsimlerdeki iyot içeriklerinin araştırılması oldukça önemlidir. Farklı iyot formlarının suda çözünebilir olması ve uçucu özellik gösterebilmesi nedeniyle iyot kaynağı olarak kullanılacak makroalglerin işlenmesinde bu hususlar dikkate alınmalıdır. Örneğin, Nitschke ve Stengel (2016), makroalglerden *Alaire esculenta*, *Palmaria palmata* ve *Ulva intestinalis*'in yıkanması ve kurutulması sonrasında önemli bir iyot kaybının olmadığını, ancak kaynatma ve kurutma sonrasında iyot düzeyinin %14-75 oranında azaldığını kaydetmiştir.

Diğer önemli bir mikroelement olan selenyum, vücut dokularının oksidatif strese karşı korunması, enfeksiyonlara karşı savunma sağlanması, büyüme ve gelişmenin düzenlenmesinde rol oynar. Selenyum içeren enzimler stres, enfeksiyon yada doku yaralanmalarında, vücudu hidrojen peroksit yada serbest radikallerin zararlı etkisinden korur (FAO/WHO, 1998). Selenyum, iyot ile birlikte tiroit metabolizmasında da rol oynamakta olup, tiroid hormonunun düzenli çalışması için gereklidir. Bu nedenle, insanlarda selenyum eksikliği tiroid metabolizmasını da etkilemektedir (Cann ve ark., 2000). Makroalgler selenyum ve iyot yönünden zengin olduğundan (Cann ve ark., 2000; Lossow ve ark., 2019), uygun miktarlarda tüketimi bu elementlerin eksikliğinden kaynaklanan problemlerin giderilmesine katkı sağlayacaktır. Günlük alınması önerilen düzeyler (RDA) kapsamında insanlarda 8 g kurutulmuş *Ulva* spp. alımının Se ihtiyacını % 29, aynı miktarda *F.vesiculosus* alımının ise % 15 oranında karşılayabileceği bildirilmiştir (Circuncisão ve ark., 2018).

Makroalglerin güçlü biyoadsorbsiyon ve biyoakümülasyon kapasitelerinden dolayı mineral içerikleri kara bitkilerine göre 10 ila 100 kat daha yüksektir (Circuncisão ve ark., 2018). Birçok makroalg türünün kalsiyum, magnezyum, çinko, demir ve iyot gibi mineral içeriklerinin birçok kara bit-

kisine göre daha yüksek düzeylerde bulunması bu görüşü desteklemekte olup (Ruperez, 2002; Swarnalatha, 2018; Panayotoda ve Stancheva, 2013; Circuncisão ve ark., 2018), makroalglerin insanların mineral ihtiyacını karşılamak için iyi bir kaynak olduğunu göstermektedir. Ancak, özellikle bazı minerallerin günlük alınması tavsiye edilen miktarlardan daha fazla alınması durumunda çeşitli sağlık problemlerine yol açabileceği de bilinmektedir. Diğer taraftan, bulundukları ortamdaki ağır metalleri yapılarında biriktirebilmeleri nedeniyle özellikle kirleticilerle kontamine olmuş ortamlardan elde edilen makroalg tüketiminin ciddi sağlık problemlerine yol açabileceği de belirtilmektedir (Cherry ve ark., 2019). Makroalgler elde edildiği ortama bağlı olarak çeşitli ağır metalleri (kurşun, civa, arsenik, kadmiyum, kromiyum, nikel, vanadyum vb.), pestisitleri, radyoaktif izotop, dioksin ve bazı antibesinsel maddeleri (lektin, florotanin, fitik asit, antienzimler vb.) içerebilirler (Garcia-Vaquero ve Hayes, 2016; Polat ve ark., 2018; Circuncisão ve ark., 2018). Bu nedenle, makroalglerin insan sağlığı için kullanım potansiyelinin araştırılmasının yanında, özellikle gıda ve yem amaçlı kullanımlarından önce gerekli testlerden geçirilmesi, hangi makroalgin ne oranlarda tüketilebileceği ve neden olabilecekleri risklerin de araştırılması son derece önem arz etmektedir.

Sonuç

Makroalgler, insanlarda metabolizmanın sağlıklı çalışabilmesi için gereksinim duyulan ve eksikliğinde ciddi sağlık problemlerine yol açan birçok mineral yönünden zengin bir kaynaktır. Yüksek mineral madde düzeyleri nedeniyle fonksiyonel gıda olarak nitelendirilen makroalgler, başta demir ve iyot olmak üzere birçok minerali karasal besinlere göre çok daha yüksek düzeylerde içerirler. Diğer önemli bir husus da makroalg minerallerinin biyoyararlılığının, kayaçlardan elde edilen minerallere göre daha yüksek olmasıdır. Halen, makroalg mineralleri tüm dünyada büyük ilgi görmektedir. Makroalglerin mineral içerikleri türe, türün fizyolojik durumuna, mevsime, suda kalma süresine ve lokaliteye göre değişebildiğinden, daha ayrıntılı çalışmaların yapılarak en uygun türlerin, üretim ortamlarının ve hasat zamanının belirlenmesi gibi bilgilerin elde edilmesine ihtiyaç bulunmaktadır. Tüm bunlara ek olarak, alg minerallerinin en etkin yöntemlerle elde edilmesi ve bunların kullanıldığı deneysel çalışmaların yapılarak sonuçların diğer mineral kaynaklarıyla karşılaştırılması da, alglerin mineral kaynağı olarak kullanımının yaygınlaşmasında önemli olacaktır. Dünyadaki gelişmelere paralel olarak ülkemiz kıyılarındaki kullanılabilir makroalg türlerinin tespit edilmesi, makroalglerin kültüre alınma potansiyelinin araştırılması ve biyoaktif bileşenlerinin elde edilmesi ile ilgili stratejilerin belirlenmesi, bu doğal kaynakların etkin ve sürdürülebilir kullanımı için önem arz etmektedir.

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 izne tabii değildir.

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Evaluation of prebiotic, probiotic, and synbiotic potentials of microalgae

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ABSTRACT

Microalgae can be considered an alternative food ingredient thanks to their nutritional composition and bioactive molecules. Microalgae are considered a rich source of sulfated and non-sulfated polysaccharides, and certain types of polysaccharides vary depending on their taxonomic groups. It is thought that valuable bioactive compounds possessed by algae biomass can increase the vitality of probiotic bacteria by stimulating their growth and being a good source for lactic acid production. Probiotics are defined as living, microbial dietary supplements that beneficially affect the human organism with their effects on the intestinal tract when they are consumed adequately. Prebiotics are indigestible or poorly digested food ingredients that stimulate the growth or activity of probiotic bacteria. Synbiotic is a term that expresses the union of probiotics and prebiotics to exert health benefits on humans. Spirulina and Chlorella are good sources of protein and polysaccharides or oligosaccharides that have been suggested as potential prebiotic candidates. These microalgae are thought to have a stimulating effect on the growth of probiotic bacteria. In this study, synbiotic efficacy and prebiotic activity of microalgae on probiotic microorganisms will be discussed and their potential in this area will be revealed.

Keywords: Microalgae, Probiotics, Prebiotics, Bioactive compounds

Introduction

Algae are the primary producers that synthesize organic molecules using luminous energy and carbon dioxide, and they play an important ecological role in the food chain in aquatic environments. Microalgae are microscopic organisms that live in a wide variety of habitats such as marine, freshwater, and extremely salty environments as well as moist soils and rocks (Yalçın Duygu, 2019). These photosynthetic species, encompass several different phyla and classes of organisms that constitute the multi-cellular structure of lengths up to 60 m (Macroalgae) and unicellular organisms with the size of 0.2 µm (Microalgae) (Camacho et al., 2019). Depending on the species, microalgae biomass contains a wide range of biologically active compounds such as proteins, lipids, polyunsaturated fatty acids (PUFAs), pigments, vitamins, and minerals (Schlagermann et al., 2012). Research on the biotechnological usage areas of these microalgae compounds, which were discovered by many studies on microalgae, has been concentrated on these organisms. Nowadays, microalgae are used as raw materials in many areas such as the pharmaceutical and cosmetic industry, health foods, formulation of food and feed, fertilizer, and wastewater treatment due to their high reproduction rate and biochemical structures (Gouveia et al., 2006; Lum et al., 2013; Wang et al., 2015). Eukaryotic microalgae and cyanobacteria are seen as promising organisms for fuel production (Culaba et al., 2020). Studies show that microalgae have anti-inflammatory, antioxidant, anticancer, antimicrobial, and anti-obesity capacities as well as hypocholesterolemic characteristics (Priyadarshani & Rath, 2012). It is thought that the carbohydrates and proteins that algae biomass possess can be a good source for lactic acid production. The lack of lignin in algae biomass compared to plant biomass provides an additional advantage to algae for lactic acid production (Nguyen et al., 2012). In this context, in recent studies, there has been a tendency to add algae biomass to fermented products to increase functional product and nutritional properties by encouraging the viability of probiotics (Varga et al., 2002). Adding algae and probiotics together promotes growth and increases the viability and acid production of probiotic bacteria such as *Lactobacillus* and *Bifidobacterium* (Webb, 1982). Studies have shown that adding algae and lactic acid bacteria to fermented foods can create new opportunities in taste, color, texture, and quality (Omar et al., 2019).

Probiotics refer to beneficial and viable microorganisms that maintain or improve the host's health when adequately consumed (Gupta et al., 2017). This group of bacteria is either natural inhabitants of the gut or can be found in fermented foods (Sornplang & Piyadeatsoontorn, 2016). The growth and activities of probiotics are induced by prebiotics. Prebiotics are selectively fermented by particular strains of resident intestinal microbiota and provide a targeted increase in specific bacteria that deliver health benefits to the host (Wilson & Whelan, 2017). The relationship between prebiotics and probiotics is referred to as synbiotics. As a result, probiotics and prebiotics work together to encourage well-being and the creation of new products. Varieties of algal species have been used in the food and pharmaceutical industries. The microscopic descriptions of these algal species, as well as their prebiotic properties, are described (Gupta et al., 2017). The main purpose of this review is to demonstrate the findings that microalgae can increase the growth and viability of probiotics thanks to their valuable bioactive compounds and examine their potential resources as prebiotic and synbiotic.

Probiotics

Probiotics are described as "nonpathogenic living microorganisms that provide health benefits to the host when adequately consumed" by FAO/WHO (Plaza-Diaz et al., 2019; Sun & Yoon, 2011). These bacteria can be originated from either the gut microbial flora or fermented food. Probiotics consist of lactobacilli *(L. acidophilus, L. amylovorus, L. bulgaricus, L. crispatus, L. casei, L. gasseri, L. helveticus, L. johnsonii, L. pentosus, L. reuteri, L. paracasei, L. plantarum, L. rhamnosus),* Lactococcus species *(Lactococcus lactis, L. lactis, L. reuteri, L. rhamnosus, L. casei, L. acidophilus, L.curvatus, L. plantarum),* Bifidobacterium species *(B. animalis, B. breve, B. infantis, B. bifidum, B. lactis, B. catenulatum, B. longum, B. adolescentis),* Pediococcus species *(P. acidilactici and P. pentosaceus),* Saccharomyces species *(S. cerevisiae, S. boulardii),* and some Streptococcus species *(S. thermophilus, S. sanguis, S. oralis, S. mitis, and S. salivarius*). Probiotics have been known to regulate dysbiosis of gut microflora caused by the overuse of antibiotics or prevent inflammatory bowel diseases, diarrhea, irritable bowel syndrome, gluten intolerance, gastroenteritis, *Helicobacter pylori* infection, prostatitis, colon cancer, urogenital tract disorders, virus infections, and allergies (Kahraman Ilıkkan, 2020; Plaza-Diaz et al., 2019; Sun & Yoon, 2011). This group of bacteria strengthens tight junctions and prevents the entrance of pathogens to host cells. Probiotics also modulate and reinforce the immune system through mucus production, short-chain fatty acid (SCFA) synthesis, macrophage activation, secretory IgA production, stimulation of cytokines, which include chemokines, interferons, interleukins, lymphokines, and tumor necrosis factors

(Rodríguez-Lagunas et al., 2017). Therefore, probiotics are also called "immunobiotics".

Prebiotics

Prebiotics were first defined in 1995 as "nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and /or activity of one or a limited number of bacteria in the colon, thus improving host health" (Carlson et al., 2018). In 2010 the International Scientific Association for Prebiotics and Probiotics (ISAPP) modified that definition: "a selectively fermented ingredient that results in specific changes in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health" (Gibson et al., 2010).

Prebiotics are health-beneficial substrates that are special functionally. These are non-digestible or low digestible foodstuffs (Gupta et al., 2017). Prebiotics are selectively fermented by the intestinal microbiota. Fermentation brings many benefits to the host. These benefits can be listed as follows; increase in the production of short-chain fatty acids, increase in fecal mass, decrease in end products with nitrogen and fecal enzymes, a moderate decrease in colonic PH, and contribution to the development of the immunological system (by providing immune modulation with increased intestinal-specific immunoglobulins) beneficial to the host. Prebiotics promote the growth of beneficial bacteria such as *Bifidobacteria, Lactobacilli*, and *Eubacteria*, which are critical for human health. Selective stimulation of the growth activity of intestinal bacteria is effective in maintaining health (Torun & Konuklugil, 2020; Wilson & Whelan, 2017).

Inulin, oligofructose, galactooligosaccharides, and lactulose are examples of commonly used prebiotics. The prebiotic activity has been demonstrated in vitro and in vivo for a variety of polysaccharides from different sources (O'Sullivan et al., 2010). Prebiotics such as inulin and pectin have a lot of health benefits, including reducing the frequency and length of diarrhea, relieving discomfort and other symptoms associated with irritable bowel syndrome, and preventing colon cancer (Pandey et al., 2015).

Synbiotics

Synbiotic is a term that expresses the union of probiotics and prebiotics to exert health benefits on humans (Pandey et al., 2015; Sataloff et al., 2016). Studies on synbiotics have shown that this association between probiotics and prebiotics increases the persistence and survival of probiotics in the gastrointestinal tract (GIT) and this is the main aim of this union (Omar et al., 2019; Sataloff et al., 2016). The most common prebiotics are fructooligosaccharides (FOS), galactooligosaccharides (GOS), and trans-galactooligosaccharides (TOS). However, synbiotics commonly include GOS, fructans (FOS and inulin) as prebiotics, and *Bifidobacterium, Lactobacilli, S. boulardii, B. coagulans* as probiotics (Davani-Davari et al., 2019; Pandey et al., 2015). Recently, many synbiotic formulas are available in pharmacy markets, but, the discovery of new prebiotics from algae will be an alternative source for the synbiotics (Gupta et al., 2017). Prebiotic potentials of microalgae such as *Spirulina spp*., *Tetraselmis* species, *Dunaliella salina,* and *Chlorella* spp. have been evaluated in many types of research (Table 1). Therefore, the utilization of microalgae together with probiotics will be an emerging approach in terms of synbiotics (Omar et al., 2019).

General Characteristics of Micro and Macroalgae

Algae are unicellular or multicellular photosynthetic organisms containing chlorophylls and other pigments. They transform luminous energy into chemical energy through photosynthesis and perform the critical functions of the energy cycle in natural ecosystems. Algae generally store this energy as starch and carbohydrate. The efficient energy transformation of algae forms an important food network in aquatic ecosystems (Tipnee S., Ramaraj R., 2015). They are grouped based on the pigment types used for photosynthesis, cell wall structure, and carbohydrate compound types stored for energy. Based on the pigments they have, they can also be divided into three groups brown (Phaeophyceae), red (Rhodophyceae), and green (Chlorophyceae) algae (Dawczynski et al., 2007). Today, the number of algae species in the world is estimated to be around ten million, and microalgae constitute the majority of these species. Cyanobacteria differ from other bacteria because they carry out oxygen photosynthesis. As a result of the cyanobacteria's release of oxygen through photosynthesis, they ensured the survival of other life forms on Earth (Chu, 2012). Approximately 70% of algae live in water (seas, lakes, or rivers), but they can also be found in terrestrial environments (lands, trees, and rocks). Algae generally live in environments at 20°C to 30°C, but some strains can live at lower temperatures or in areas full of ice, at higher temperatures (70°C) in spring water, in salty environments, and in lakes and seas where the light intensity level is low and pressure level is high. Different factors such as substrate, temperature, light, turbidity, saltiness, pH, O_2 and CO_2 rates, nutrient salts, oligo-elements, and vitamins affect the distribution of algae to the physical and chemical changes in the environment. The primary and secondary metabolites produced by macro and microalgae include minerals, vitamins, proteins, amino acids, carbohydrates, long-chain polyunsaturated fatty acids, steroids, dietary minerals, halogenated compounds, polyketides, and diverse antioxidants, etc., all of which are used in different fields of industries (Hunt et al., 2010;

Chandini et al., 2008). These industries include the food sector, cosmetics, aquaculture, drug industry, wastewater treatment, and production of anti-tumor, anti-bacterial, and antiviral compounds (Borowitzka & Borowitzka, 1988; Cohen, 1999; Hosikian et al., 2010; Pal et al., 2014). In addition, algae are well known for their renewable bioenergy potentials.

Algae are divided into microalgae and macroalgae based on their sizes. The dimensions of microalgae are expressed in microns, while macroalgae have sizes ranging from 1-2 cm to 40-60 m depending on the species. Macroalgae constitute significant elements of algae groups in aquatic habitats. Moreover, they form environments for feeding, sheltering, and reproducing aquatic animals (Ak et al., 2011). Macroalgae are used in the food and medical sectors but they are also raised in different regions of the world for the extraction of phycocolloids. Macroalgae are rich sources of biologically active metabolites such as minerals, vitamins, protein, and carbohydrates (Chandini et al., 2008). Most of the studies of macroalgae consist of evaluations regarding the amount of macroalgal biomass, the relationship between macroalgal growth and food material, the effects of extreme growth due to external factors, and relative effects that emerge in the environment. Moreover, evidence indicates that macroalgal biomass has the potential for the production of different fluids and solid biofuels through similar conversion (Grayburn et al., 2013). Macroalgae are also rich in polysaccharides that can be utilized as functional compounds of potential prebiotics for both human and animal health applications (O'Sullivan et al., 2010). The prebiotic and indirect probiotic activity of macroalgae compounds can be classified as alginates, laminarin, fucoidans, carrageenan, agar, and porpiran (Torun & Konuklugil, 2020).

Microalgae comprise proteins, amino acids, antioxidant components, Fe and Ca, unsaturated fatty acids, vitamins (A, B2, B6, B8, B12, E, and K). Microalgae are also known and used as a therapeutic and functional food (Gyenis et al., 2005). Some microalgae are a potential source of long-chain polyunsaturated fatty acids (LC-PUFA), particularly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which are implied to be useful in preventing cardiovascular disease (Arterburn et al., 2008). Microalgal lipids can be divided into two groups according to their structures. These are defined as follows; polar lipids (phosphoglycerides, glycosylglycerides, and sphingolipids) and nonpolar lipids (acylglycerols, sterols, free fatty acids, wax, and steryl esters) (Borowitzka & Moheimani, 2013). Although the amounts and types of microalgal lipids vary according to the species of algae, environmental factors, and growth conditions, these lipids play important roles in microalgal metabolism. For many microalgae species, the amount of lipid in dry biomass has been determined between 20-50% (Chen et al., 2018; Mata et al., 2010). The carotenoids in microalgae are derived from 5-carbon isoprene units that are enzymatically polymerized to form highly conjugated 40-carbon structures. Microalgal carotenoids are used as an additive for animal feeds, natural food colorants, and cosmetics. Carotenoids have therapeutic value with their anti-inflammatory and anti-cancer activities due to their antioxidant properties, and they also act as B-carotene provitamin A from a nutritional perspective (Chu, 2012).

Carbohydrates have two basic functions in microalgae cells, structural components in cell walls and storage components within the cell. As storage compounds, carbohydrates provide the energy needed for the metabolic processes of organisms (Kromkamp, 1987). Although carbohydrates in algal biomass have the lowest energy content compared to other organic compounds such as protein and lipid (Markou et al., 2012). The carbohydrate content of microalgae in biomass depends on the microalgae species, the growth, and environmental conditions. Algal carbohydrates consist of an extensive category of sugars (monosaccharides) and their polymers (di-, oligo- and polysaccharides), with the most common carbohydrates being glucose, rhamnose, xylose, and mannose (Mussgnug et al., 2010; Nakamura et al., 2005). The polysaccharides that microalgae possess are complex and heterogeneous macromolecules composed of different monosaccharides and sometimes glucuronic acid and sulfate groups. These molecules could be used as prebiotics and probiotics (Sathasivam et al., 2019). Polysaccharides such as laminarin, alginate, and poly-mannuronic acid obtained from micro and macroalgae living in aquatic environments act as prebiotics. Microalgae are considered a rich source of sulfated and non-sulfated polysaccharides and certain types of polysaccharides vary depending on their taxonomic groups.

Microalgae as Probiotics

The emergence of microbiota resistance to antibiotics and traditional drugs has led scientists to find alternative remedies to this issue. Nano-encapsulated multiplex supplements are costly to manufacture, therefore commercially expensive and inconvenient to use (Panghal et al., 2018). With the increase in the human population, the shortage of natural resources has made aquaculture a substantial sector. However, disease outbreaks have become an important problem in this sector and cause economic losses. Chemotherapeutic drugs used for the treatment of these types of diseases are both expensive and some have negative effects on the aquatic environment. Studies have begun to control infectious disease factors and to find alternative therapeutic agents that are environmentally friendly, and the use of probiotics as a food ingredient for the

solution to this situation is considered promising (Camacho et al., 2019).

Table 1. Summary of studies conducted with microalgae on probiotics or fermentative products

Microalgae	Prebiotic	Probiotics/ Products	Effects	Reference
S. platensis 945	Extracellular products	*L. lactis* C2, *S. thermophilus* LO1, *L. casei* YK3, *L.acidophilus* JL2, and *L. bulgaricus* YL1	Growth promotion	(Parada et al., 1998)
S. platensis	Biomass	*S. thermophilus* TH4, *L. lactis* C2, and *L. delbrueckii* YL1	Growth promotion	(De Caire et al., 2000)
S. platensis	Biomass	*L. casei* MTCC1423, *Lactobacillus acidophilus* MTCC447, and *S. thermophilus* MTCC1938	Growth promotion	(Bhowmik et al., 2009)
S. platensis and *C. vulgaris*	Biomass	Yoghurt (*Lactobacillus acidophilus* LA-5, *Bifidobacterium lactis* BB-12, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Streptococcus thermophilus*)	Growth promotion	(Beheshtipour et al., 2012)
S. platensis and *C. vulgaris*	Biomass	*L. plantarum* and *E. faecium*	Growth promotion	(Gyenis et al., 2005)
C. vulgaris	Biomass	*L. brevis*	Growth promotion	(Scieszka & Klewicka, 2020)
S. maxima	S. maxima-derived modified pectin	Gut microbiota	Promoted *Bacteroidetes*	(Chandrarathna et al., 2020)
S. platensis	Biomass	*L. acidophilus* /Cheese	Growth promotion	(Mazinani et al., 2016)
Isochrysis galbana	Biomass	*Lactic acid bacteria*	Growth promotion	(Nuño et al., 2013)
Arthrospira platensis (Spirulina) and *Chlorella vulgaris*	Biomass	*Bifidobacterium animalis* and *Lactobacillus casei*	Growth promotion	(Leal et al., 2017)
S. platensis	Biomass	Yoghurt	Growth promotion of total lactic acid bacteria in yoghurt	(Agustini et al., 2017)

The trace elements, vitamins, and bioactive compounds of the microalgae biomass have the characteristics of promoting the growth of the desired bacteria. Some types of microalgae have qualities that can increase the viability of probiotics by stimulating the growth of desired probiotic bacteria in yogurt and fermented milk (Beheshtipour et al., 2012). It has been reported that co-culturing microalgae and probiotics together can stimulate growth and increase the viability of probiotics in the products and also in the gastrointestinal tract due to their alkaline properties and effective compounds (Parada et al., 1998). Parada et al. (1998) demonstrated this statement that by adding products from *S. platensis* to the culture medium, it acted as a nitrogen-depleting photo-autotropic microorganism and releases exopolysaccharides as well as other compounds. This latter case could be responsible for the stimulating effect on LAB. In the other study, it was revealed that using *S. platensis* as algae biomass, significantly stimulated the growth of thermophilic bacteria and acid production (Varga et al., 1999). *C. vulgaris* and *S. platensis* were used together in many studies conducted on the increase in the health and biochemical properties of probiotic bacteria found in yogurt and milk. As a result, the viability of probiotic bacteria in the nutrient medium using these two microalgae were found to be significantly higher (Beheshtipour et al., 2012; Gyenis et al., 2005).

Microalgae as Prebiotics

Microalgae synthesize carbohydrates through photosynthesis, and the chemical profile of carbohydrates (mainly starch and cellulose) varies from species to species. The procedure of partial hydrolysis of polysaccharides in the microalgae cell wall is widely used in the food and feed industry. Studies have shown that microalgal oligosaccharides cannot be com-

pletely fermented by the regular intestinal microbiota of humans or animals. However, it is stated that they stimulate the growth and activity of specific beneficial bacteria present in the colon and act like prebiotics (Camacho et al., 2019). The use of microalgae as prebiotics by the food industry has been limited to dairy products. Technological advances could open up an opportunity to develop prebiotics from microalgae for application to foods fermented with lactic acid other than yogurt or cheese.

Polysaccharides and oligosaccharides promote compounds with potential health benefits, notable for prebiotic applications. Algal-derived polysaccharides have prebiotic potential, which has been used for decades to improve animal and human health. Depending on the taxonomic groups of algae, their polysaccharide types are different, and algae are considered a rich source of sulfated polysaccharides. The main function of these high molecular weight polysaccharides is that they are rich in hydroxyl (OH) groups and making them hydrophilic (Gupta et al., 2017). Some microalgae such as *Arthrospira platensis* can stimulate the growth of beneficial bacteria such as *Streptococcus thermophiles*, *Lactobacillus casei,* and *L. acidophilus* (Parada et al., 1998). In the study conducted by (Liu et al., 2015), it was shown that the biomass obtained from microalgae *Chondrus crispus* has prebiotic properties.

Some Algal Species with Probiotic and Prebiotic Potential

Several algal species have been used in the food and pharmacological industries. Recently, various research studies on the prebiotic and probiotic properties of microalgae have shown that aqueous algae extract from *Spirulina platensis, Chlorella, Dunaliella salina, Chlorococcum* are potential sources.

Spirulina

Spirulina is a filamentous blue-green alga, commonly found in many freshwater environments. *Spirulina* has been consumed as food or health food since ancient times due to its high nutritional value. *Spirulina* is produced on a large scale in outdoor pools for commercial purposes to be used as a nutritional supplement in some countries such as Thailand, China, the United States, and India. *S. platensis* contains about 4-7% lipids, 13.6% carbohydrates (glucose, rhamnose, mannose, xylose, and galactose), 78% high-quality proteins, vitamins (A, B2, B6, E, H, and K, more vitamin B12), all essential minerals, trace elements, as well as enzymes and some natural pigments (Parada et al., 1998; Shekharam et al., 1987). *Spirulina* contains essential fatty acids such as γ -linolenic acid (GLA) and linoleic acid (LA). *S. platensis* depletes nitrogen in the growth medium and releases extracellular carbohydrates and other growth agents responsible for stimulating the growth of lactic acid-producing strains. *Spirulina* biomass has a stimulating effect during fermentation and storage of *Lactobacillus casei, Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus bulgaricus,* and *Bifidobacterium spp.* (Gupta et al., 2017). The stimulating effect of the extract obtained from *S. platensis* on the growth of three probiotic bacteria (*L. bulgaricus, L. lactis,* and *B. longum*) was shown in the study conducted by Pascal et al. (2011). Galactose and xylose, which are characterized by algal biomass, formed oligosaccharides that function as prebiotic compounds to stimulate probiotic bacteria (Pascal et al. 2011).

Chlorella

Chlorella is another microalga that has been cultured for the commercial production of healthy food. *Chlorella vulgaris* is a single-celled green alga and is widely distributed in freshwater, marine, and terrestrial environments. Thanks to its high photosynthetic property, *Chlorella* can grow rapidly under autotrophic, myxotrophic, and heterotrophic conditions. All these features made it one of the first microalgae to be considered for commercial production and large-scale cultivation (Ru et al., 2020). *Chlorella* is a good source of nutrients such as protein %61.6, lipid %12.5, carbohydrates % 13.7, elements (selenium, magnesium, phosphorus, zinc, calcium, and aluminum), and vitamins (ascorbic acid, thiamine, B1, B2, B6, D, E, and K) (Blas-Valdivia et al., 2011). *Chlorella*, contains β-glucan, an active immunostimulator, and has other useful impacts in scavenging free radicals and reducing blood lipids. It also produces an additional product called "Chlorella Growth Factor" as an agent to improve the growth of lactic bacteria (Chu, 2012).

The most abundant polysaccharide in *C. vulgaris* is starch and cellulose, consisting of amylose and amylopectin, and together with sugars, they act as an energy store for cells. Additionally, one of the most important polysaccharides is β-1,3-glucan, namely as a free radical scavenger with many health and nutritional benefits (Lee et al., 2007). Several studies on the health benefits of consuming *Chlorella* have shown that it can lower blood sugar levels, increase hemoglobin concentrations, and act as hypo-cholesterolemic and hepatoprotective agents during malnutrition (Jeong et al., 2009). *Chlorella* has been reported as an important source of polysaccharides or oligosaccharides that have been suggested as potential prebiotic candidates. Industrially, *Chlorella* has been successfully added to yogurt and cheeses (Jeon, 2006; Beheshtipour et al., 2012).

Conclusion

Probiotics, prebiotics, and synbiotics have all been described and evaluated in terms of their systemic impact on the host's health, metabolism, and immune system. However, studies on new sources such as probiotics and prebiotics are still ongoing. Microalgae have been studied as one of these new sources. Although many studies have revealed that microalgae have a prebiotic effect thanks to the oligo- and polysaccharides contents and it improves the intestinal microflora, there are very few studies in which algae are used together with probiotics as a food product. Microalgae can be potential sources in this regard and it is beneficial to increase the research on the probiotic properties to find more scientific evidence.

Compliance with Ethical Standard

Conflict of interests: The author declares that for this article they have no actual, potential, or perceived conflict of interests.

Ethics committee approval: The author declares that this study does not include any experiments with human or animal subjects; therefore, no ethics committee approval is needed.

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Type of manuscript	Page	Abstract word limit	Reference limit
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....(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).

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Table 2.

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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.
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