J Food Health Sci

Vol. 3 Issue 2 2017 E-ISSN 2149-0473

Journal of Food and Health Science

ScientificWebJournals
(SWJ)

Journal of Food and Health Science

E- ISSN 2149-0473

Journal abbreviation: **J Food Health Sci**

is published in one volume of four issues per year by

www.ScientificWebJournals.com

Contact e-mail: jfhs@scientificwebjournals.com and ozkanozden@scientificwebjournals.com

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"Journal of Food and Health Science" publishes peer-reviewed articles covering all aspects of **Food** and **Health science** in the form of review articles, original articles, and short communications. Peer-reviewed open access journal publishes articles in **English** or **Turkish** language.

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Journal of Food and Health Science

E-ISSN 2149-0473

Journal abbreviation: **J Food Health Sci**

Vol. 3 Issue 2 Page 42-81 (2017)

Tablo of Contents/İçerik

3(2): 42-48 (2017) doi: 10.3153/JFHS17006

Journal of Food and Health Science

E-ISSN 2149-0473

ORIGINAL ARTICLE/ORİJİNAL ÇALIŞMA

FULL PAPER TAM MAKALE

THE EFFECT OF WASHING AND COOKING ON RESIDUE LEVELS OF SHRIMP TREATED WITH 4-HEXYLRESORCINOL

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Received:.09.09.2016

Accepted: 14.11.2016

Published online: 30.12.2016

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Abstract:

The aim of this study was to investigate the effects of washing with tap water and different cooking process on residue levels of shrimp treated with 4-hexylresorcinol. Dipping solutions of 4-hexylresorcinol in five different concentrations (5-10-50-100-500 ppm) were prepared using distilled water. The shrimps were dipped into the solutions for 1-5-10- 30 min. Half of the samples were washed with tap water. The residue level of 4-hexylresorcinol in all samples were analysed after frying and boiling process. The 4-hexylresorcinol residual levels in unwashed and washed raw, fried and boiled shrimp samples showed linear increases with increasing dipping time and concentration. The best results were obtained for the treatment of 5 and 10 ppm for 1-5-10-30 min.

Keywords: Shrimp, *Parapenaeus longirostris,* Blackspot, Melanosis, 4-hexylresorcinol

Introduction

The demand to fishery products is increasing each passing day because of global warming and inefficiencies in agricultural production. Capita fish consumption per person in the world weight 1960s 9.9 kg, while increased in year 2012 to 19.2 kg. Fish and fishery products play a critical role in global food security and nutritional needs of people in all world countries. In 2012, capture production of shrimp species registered a new maximum at 3.4 million tonnes and shrimp as a fishing product has one of the highest economic value of world (FAO 2014).

Deepwater pink shrimp (*Parapenaeus longirostris*) is economically important fisherys resources in Spain, France, Italy, Greece, Sicily and Tunisia, all of the East Atlantic and Mediterranean and Marmara Sea.

Fisheries products help the consumer by life maintain good health with all essential nutrients components. Shrimp is delicious seafood's and important sources of protein, low in saturated fat, vitamin B12, selenium, ω-3 highly unsaturated fatty acids (HUFA) and astaxanthin, a potent natural antioxidant for human consumption worldwide (Heu et al, 2003; Dayal Syama et al., 2013). However, this high value nutritional components shrimp is perishable due to enzymatic and microbiological spoilage during post-mortem storage (Nirmal and Benjakul 2010). Melanosis or black spots occurs in shrimp (Figure 1.), lobster and others Crustaceans during storage as a result of the action of polyphenol oxidase (PPO) on tyrosine or its derivatives (Martínez-Alvarez *et al.,* 2009). The active PPO catalyses the hydroxylation to benzoquinones of o-dihydroxyphenols. Benzoquinones react non-enzymatically with a variety of compounds, like amines, amino acids, or O_2, forming melanin's, responsible for black coloration during storage (Nirmal & Benjakul, 2010; Zamorano *et al.,* 2009).

The appearance of melanosis during storage drastically reduces their market value, nutritional value and consumer acceptability (Martínez-Alvarez *et al.,* 2005; Mayer, 2006; Erkan *et al.,* 2007). The refrigeration of shrimps or their storage on ice can slow down this process, but not inactivate it. The presence of black spots makes necessary the use of antimelanotics. Therefore, addition of chemicals is necessary to prevent melanosis in shrimps. Many studies have focused on the prevention of browning and microbial spoilage of shrimp by naturally occurring and synthetic tyrosinase inhibitors (Martínez-Alvarez *et al.,* 2007; Nirmal & Benjakul 2011a; 2011b). Sodium metabisulphite is the chemical most widely used to control the melanosis of shrimps during the storage. It acts by inactivating the enzyme polyphenoloxidase and combining with quinones to prevent their polymerization in pigmented compounds. Nonetheless, the treatment of food products with sodium metabisulphite can caused anaphylactic reactions to sulphite-sensitive individuals and bronchoconstriction to asthmatic patients. Moreover, this chemical produces an alkaline pollution of the environment that kills several aquatic species. The use of alternative compounds is necessary and is reported as good alternative 4-hexylresorcinol (Guandalini *et al.,* 1998; Montero *et al.,* 2001, 2004, 2005; Thepnuan *et al.,* 2008). This compound is considered GRAS (Generally Recognized As Safe) in many countries (Australia, Brazil, Canada, USA, etc.). 4-hexylresorcinol approved as a food additive (E586) as defined by Council Directive 89/107/EEC article 5 amending Directive 95/2/EC. Fresh, frozen and deep-frozen crustaceans to a maximum residue level of 2 mg/kg in crustacean meat. A single EC Regulation 1333/2008 on food additives has been adopted intended to replace and repeat Directives 89/107/EEC and 95/2/EC. According to Directive 2003/89/EC, 4-hexylresorcinol is not subject to allergen labelling. However, it is up to the discretion of each individual country to adopt labelling measures. According to Canada Food and Drug Regulation 1078 (1998), the residues of 4-hexylresorcinol in the edible portion of the uncooked product not exceed 1.0 mg/kg. According to China Food Additives Hygiene Standard (GB 2760-1996), 4-hexylresorcinol were classified as antioxidant. Residue level for the prevent of browning in shrimp were reported as ≤1 mg/kg (1ppm). Acceptable Daily Intake (**ADI**) "treatment of crustacean at concentrations of up to 50 mg/L, resulting in residue levels of approximately 1 mg/kg in edible portion, is not of toxicological concern" (JECFA, 1995).

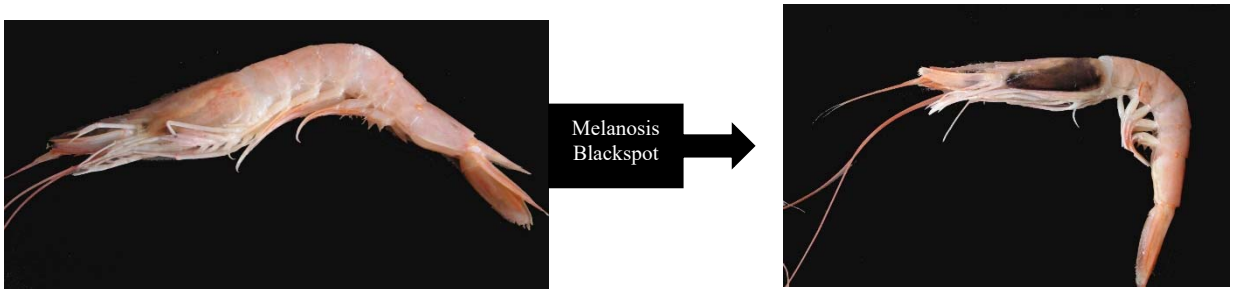


Figure 1. Malanosis/Blackspot in shrimp (Foto: Özkan Özden)

In this study were investigated the residue levels of shrimp treated with 4-hexylresorcinol in different concentration/dipping time and the effect of washing with tap water and frying and boiling process in residue levels after dipping treatment.

Materials and Methods

Samples of deep-water pink shrimp (*Parapenaeus longirostris* Lucas, 1846) was caught in the Marmara Sea (Tuzla Kayıkhanesi-Istanbul) by a commercial fishing trawler. On board, the shrimp was washed with seawater and were transported with ice (shrimp/ice = 1/1 w/w) to the laboratory. Samples of deep-water pink shrimp (2500 g for each group) were dipped for 1, 5, 10, 30 min in cold tap water (7°C) solutions of 5, 10, 50,100, 500 ppm 4-hexylresorcinol. Afterwards the shrimp was removed, each group samples divided two group. First groups were washed with tap water in 15 second, other groups non washed. Washed an unwashed shrimp samples were peeled. Samples for each groups were divided three lot. The first group of shrimp samples were not cooked. Second and third lot samples were cooked by frying (in sun flower oil at 180°C 3 min) and boiling (in boiling water for 3 min). The process was repeated in new captured shrimp samples after two days. Total 240 group samples (5 concentration (5-10-50-100-500 ppm) x 4 dipping time (1-5-10- 30 min) x first processing (unwashed and washed) x second processing (raw- frying - boiling) = 5x4x2x3=120 x 2=240 samples)) were also homogenized and stored in sterile plastic sample containers at -20°C until analysis of residues. 4-Hexylresorcinol analysis in each group sample was performed in three duplicate. The results are presented as the mean of the three measurements for each group (Figure 2).

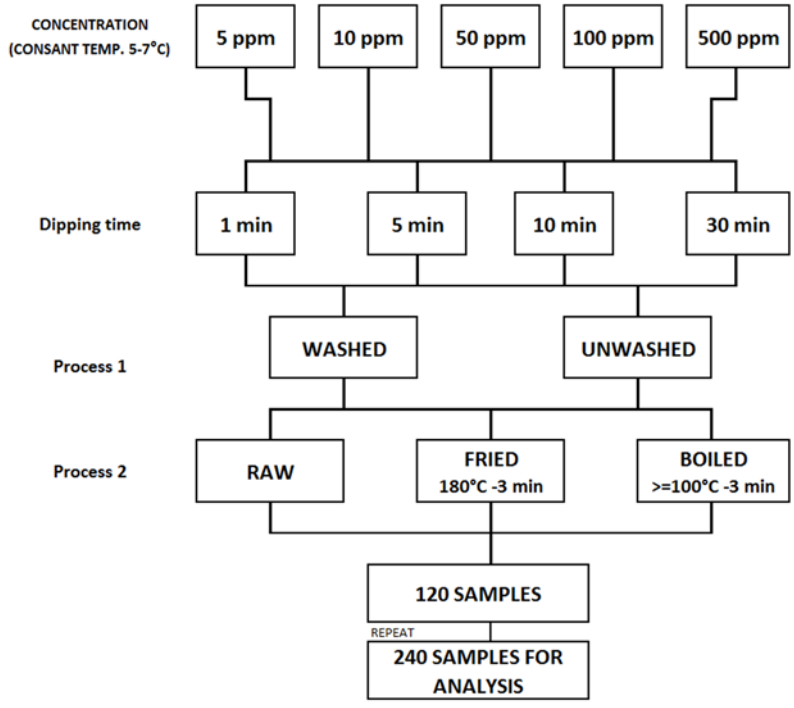


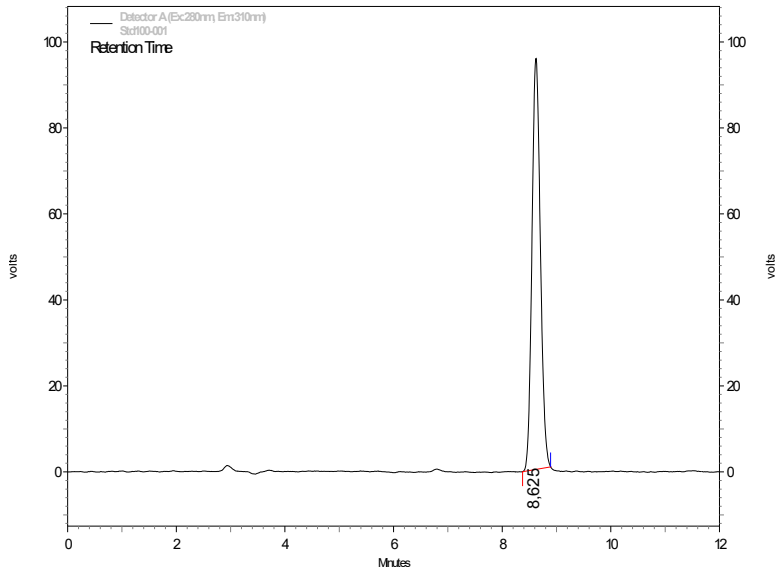
Figure 2. Full factorial experimental design for residue of 4-Hexylresorcinol in shrimp

The samples were analysed by high-performance liquid chromatography (HPLC) (Shimadzu LC 10 AT Vp series pump, Shimadzu SIL 10AD Vp cooling automatic sampling (4ºC), Shimadzu RF 10AXL fluorescence detector (FLD), Shimadzu CTO 10AV Vp, Shimadzu SCL 10A Vp, Class-Vp 6.14 - Japan) with a fluorescence detector, with the described method by Selçuk and Özden (2014a, 2014b). The concentration of 4-hexylresorcinol was measured by comparing its retention time with those of authentic standards (Acros Organics #197920250: 4-hexylresorcinol, 99%), and the 4-hexylresorcinol content was calculated on a weight basis:

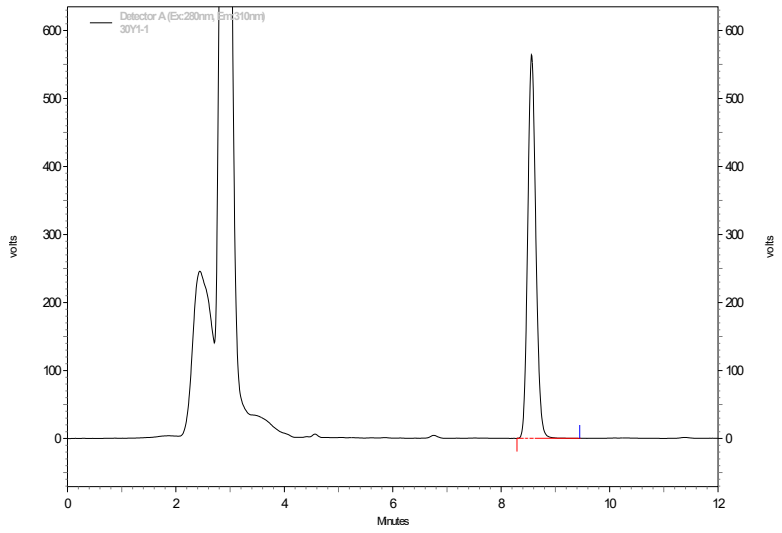
4-hexylresorcinol (mg/kg) = HPLC value read (µg/L) x 0.250 L / sample weight (g) (Figure 3.)

Statistical analysis

Statistical analysis of the data was performed in STATISTICA 7 software (StatSoft Tulsa-USA), with ANOVA post-hoc test ($p \leq 0.05$).



A. 4-hexylresercinol Standard



B. 4-hexylresercinol in Shrimp

Figure 3. 4-hexylresercinol HPLC chromatograms

Results and Discussion

In this study, the residue level in all samples showed linear increase with dipping time and concentration of dipping solutions. The residual levels of the samples treated with 4-hexylresorcinol are presented in Table 1. The residue levels of washed raw, fried and boiled samples were found lower ($p<0.05$) compared to unwashed samples in all concentrations and dipping times. According to, The Scientific Committee of Food of the European Commission and the proposal of the EU Commission were reported 2 ppm as the maximum limit for the 4-hexylresorcinol residue in the consumable portion of crustaceans (Directive 95/2/EC). The residue level in legal limit were found in shrimp samples treated with 5-10 ppm of 4-hexylresorcinol for 1-5-10-30 min and 50 ppm of 4-hexylresorcinol for 1-5 min. Additionally, the residue level in 50 ppm 4-hexylresorcinol for 10 min treated washed raw, fried and boiled shrimp samples were found below 2 ppm. The 1 ppm legal limit established for 4-hexylresorcinol residue level in Canada Food and Drug Regulation, and China Food Additives Hygiene Standard. Considering these values, in this study were found as safe treatment with 5-10 ppm of 4-hexylresorcinol for 1-5-10-30 min for unwashed shrimp samples and up to 50 ppm 1 min for washed shrimp samples. Our results showed similar with earlier scientific studies. Iyengar *et al.* (1991) found that the residue level increased with the increment in the dipping period. They detected the residue level as 0.6 mg/kg in the shrimp samples which were dipped in % 0.005 4-Hexylresorcinol solution for 1 minute. They also observed that the residue level rised to 1.0 mg/kg when they increased the dipping period to 15 minutes. Guandalini *et al.* (1998) found the residue level as 20, 42 ve 85 mg/kg in pink shrimps which were subjected to 25, 50 and 100 ppm (mg/kg) concentration of 4-Hexylresorcinol solution. Montero *et al.* (2005) reported the residue level as 2.73 ppm, 3.22 ppm, 2.57 ppm and 5.91 ppm in the 0.01% concentration of 4-Hexylresorcinol in the 2th, 4th, 7th and 5th day respectively. It was stated that the use of 4-Hexylresorcinol (0.1 % and 0.05 %) solutions can be a good alternative to commercial sulphite based products in order to prevent melanosis in Norway lobster. Besides it was concluded in the same study that the concentration of 0.05 % can prevent melanosis for 12 days (Martinez-Alvarez *et al.,* 2007). The deep-water pink shrimp samples were dipped in 0.1% 4-Hexylresorcinol solution for 3, 30 and 60 minutes and were found the level of residue in the samples as 5.5, 5.2 and 7.2 mg/kg, respectively. In the same study, after dipped in 0.25% 4-Hexylresorcinol solution for 60 minute, the level of residue was detected as 14.3, 19.3 and 22.0 mg/kg. After the washing of samples in 5 liter water for 2 minutes, the level of residue showed an increase (Mendes *et al.,* 2006).

Table 1. The level of 4-hexylresorcinol residues in deep-water shrimps (ppm in wet weight).

Dipping Concentration	Time	Unwashed Raw	Washed Raw	Unwashed Fried	Washed Fried	Unwashed Boiled	Washed Boiled
	1 min	0.08 ±0.01^{Aa}	0.05 ±0.00^{Ab}	0.09 ±0.01^{Aa}	0.06 ±0.01^{Ab}	0.07 ±0.00^{Aa}	0.06 ±0.01^{Ab}
5 ppm	5 min	0.15 ±0.01^{Ba}	0.12 ±0.02^{Ba}	0.13 ±0.01^{Ba}	0.12 ±0.01^{Ba}	0.12 ±0.01^{Ba}	0.09 ±0.01^{Bb}
	10 min	0.26 ±0.00^{Ca}	0.20 ±0.02^{Cb}	0.23 ±0.01^{Ca}	0.19 ±0.01^{Cb}	0.19 ±0.01^{Ca}	0.15 ±0.01^{Cb}
	30 min	0.32 ±0.03^{Da}	0.28 ±0.01^{Db}	0.32 ±0.01^{Da}	0.25 ±0.01^{Db}	0.32 ±0.01^{Da}	0.24 ±0.01^{Db}
	1 min	0.22 ±0.02^{Ea}	0.18 ±0.05^{Ca}	0.19 ±0.01^{Ea}	0.11 ±0.02^{Bb}	0.15 ±0.01^{Ba}	0.12 ±0.00^{Eb}
10 ppm	5 min	0.29 ±0.02^{Fa}	0.25 ±0.01^{Eb}	0.24 ±0.02^{Ca}	0.20 ±0.01^{Cb}	0.25 ±0.03^{Ea}	0.20 ±0.00^{Fb}
	10 min	0.45 ±0.03^{Ga}	0.36 ±0.03^{Fb}	0.42 ±0.01^{Fa}	0.31 ±0.00^{Eb}	0.32 ±0.01^{Fa}	0.28 ±0.00^{Gb}
	30 min	0.75 ±0.10^{Ha}	0.60 ±0.05^{Gb}	0.55 ±0.06^{Ga}	0.49 ±0.01^{Fa}	0.59 ±0.02^{Ga}	0.48 ±0.00^{Hb}
	1 min	**1.15 ±0.22^{Ia}**	0.84 ±0.09^{Hb}	0.95 ±0.08^{Ha}	0.67 ±0.10^{Gb}	**1.01 ±0.03^{Ha}**	0.71 ±0.03^{Ib}
50 ppm	5 min	**1.66 ±0.06^{Ja}**	**1.34 ±0.12^{Ib}**	**1.46 ±0.06^{Ia}**	**1.24 ±0.03^{Hb}**	**1.81 ±0.06^{Ia}**	**1.49 ±0.05^{Jb}**
	10 min	**2.12 ±0.13^{Ka}**	**1.76 ±0.12^{Jb}**	**1.96 ±0.07^{Ja}**	**1.69 ±0.12^{Ib}**	**2.27 ±0.11^{Ja}**	**1.73 ±0.06^{Kb}**
	30 min	**2.93 ±0.04^{La}**	**1.64 ±0.25^{IJb}**	**3.37 ±0.08^{Ka}**	**2.43 ±0.10^{Jb}**	**3.97 ±0.15^{Ka}**	**2.85 ±0.00^{Lb}**
	1 min	**2.33 ±0.03^{Ma}**	**2.69 ±0.20^{Kb}**	**1.97 ±0.03^{Ja}**	1.43 ±0.25^{Ib}	**2.06 ±0.07^{Ja}**	1.85 ±0.03^{Mb}
100 ppm	5 min	**2.77 ±0.12^{NLa}**	**2.64 ±0.07^{Ka}**	**2.76 ±0.29^{La}**	**2.24 ±0.17^{Jb}**	**2.77 ±0.08^{La}**	**2.05 ±0.07^{Nb}**
	10 min	**3.89 ±0.05^{Oa}**	**3.08 ±0.35^{Lb}**	**3.68 ±0.08^{Ma}**	**3.30 ±0.25^{Kb}**	**3.82 ±0.26^{Ka}**	**3.05 ±0.08^{Ob}**
	30 min	**4.87 ±0.11^{Öa}**	**4.22 ±0.14^{Mb}**	**7.01 ±0.41^{Na}**	**4.78 ±0.66^{Lb}**	**6.57 ±0.21^{Ma}**	**5.03 ±0.34^{Öb}**
	1 min	**15.90 ±0.89^{Pa}**	**12.48 ±0.42^{Nb}**	**11.46 ±0.48^{Oa}**	**9.78 ±1.02^{Mb}**	**13.81 ±0.29^{Na}**	**9.69 ±0.16^{Pb}**
500 ppm	5 min	**20.86 ±0.19^{Ra}**	**19.01 ±0.96^{Ob}**	**17.14 ±0.71^{Öa}**	**15.10 ±0.53^{Nb}**	**19.34 ±0.03^{Oa}**	**16.13 ±0.32^{Rb}**
	10 min	**24.51 ±0.14^{Sa}**	**22.38 ±0.37^{Öb}**	**25.46 ±0.30^{Pa}**	**20.93 ±2.35^{Ob}**	**25.34 ±0.49^{Öa}**	**23.08 ±0.38^{Sb}**
	30 min	**35.02 ±4.21^{Ta}**	**27.10 ±1.79^{Pb}**	**29.35 ±0.43^{Ra}**	**27.63 ±1.11^{Pb}**	**34.26 ±1.27^{Pa}**	**30.03 ±1.71^{Tb}**

Bold represents unacceptable residue levels for EU and China (grey line)

All values are the mean ± standard deviation (n = 3)-Different letters (A,B,C) in the same column indicate significant differences ($p<0.05$)-Different letters (a,b,c) in the same line indicate significant differences ($p<0.05$)

Conclusion

In all concentrations and dipping times, the residue levels of washed (raw, fried and boiled) shrimps were significantly lower ($P<0.05$) than those for unwashed shrimp samples. The legal limit (for EU and China regulation) for residue level not exceeded in shrimp samples treated with 5-10 ppm of 4-hexylresorcinol for 1-5-10-30 min and 50 ppm of 4-hexylresorcinol for 1 min only in washed shrimps.

Acknowledgements

This work was supported by the Research Found of the University of Istanbul. Project number: 4256.

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3(2): 49-58 (2017) doi: 10.3153/JFHS17007

Journal of Food and Health Science

E-ISSN 2149-0473

REVIEW ARTICLE DERLEME MAKALESİ

FONKSİYONEL BİR BESİN OLARAK TURUNÇGİLLER VE METABOLİK SENDROM İLİŞKİSİ

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Received: 22.11.2016

Accepted: 05.01.2017

Published online: 18.01.2017

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Öz:

Temel besleyici özelliklerinin yanında vücutta gösterdiği bir veya birden fazla yararlı hedef fonksiyon ile hastalık riskini azaltıp iyi hal ve sağlığı geliştirici etki gösterebilen, günlük beslenme planı içerisinde yer alıp tüketilebilen; hap, kapsül veya farklı şekillerde bir besin desteği olmayan besinlere fonksiyonel besinler adı verilmektedir. Fonksiyonel bir besin olarak turunçgiller, bileşiminde bulunan askorbik asit, folik asit, lif, pektin, potasyum, magnezyum, karotenoidler ve flavonoidler ile olumlu sağlık etkileri gösterebilmektedir. Bu bileşenlerin miktarları turunçgil çeşidine, olgunluğuna, saklama koşullarına ve işleme yöntemlerine göre değişkenlik gösterebilmektedir. Turunçgillerin sağlık üzerine olumlu etkileriyle öne çıkan bileşenleri naringin ve hesperidin flavonoidleridir. Hipertansiyon, hiperglisemi, dislipidemi, protrombotik ve proinflamatuvar faktörler, metabolik sendrom risk faktörleri arasındadır. Turunçgil flavanoidleri hiperglisemi, hiperlipidemi, hipertansiyon, inflamasyon ve vücut ağırlığı denetimi üzerine olumlu etkiler gösterebilmektedirler. Literatürde, turunçgil flavonoid ekstraktlarının kullanıldığı hayvan araştırmaları ve turunçgil tüketiminin dislipidemik etkileriyle ilgili insan araştırmaları öne çıkmaktadır. Bu nedenle günlük beslenme planında turunçgil tüketimi ve metabolik sendromla ilgili önerilerin geliştirilebilmesi için bu konuda daha fazla epidemiyolojik ve deneysel araştırmalar gereklidir.

Anahtar Kelimeler: Turunçgil, Abdominal obezite, Metabolik sendrom, Hesperidin

Abstract:

CITRUS FRUITS AS A FUNCTIONAL FOOD AND THE RELATION WITH METABOLIC SYNDROME

Functional food is a food of daily diet which have at least one target function in the body besides its' nutritional properties and can decrease disease risk and improve health and wellness. Functional food is not a pill, capsule or any type of nutritional support. Citrus fruits as a functional food have beneficial health effects related with their components such as ascorbic acid, folic acid, dietary fiber, pectin, potassium, magnesium, carotenoids and flavonoids. The amount of these components depends on type of citrus fruit, ripeness, storage conditions and process methods. The prominent components of citrus fruits are known as naringin and hesperidin flavonoids. Hypertension, hyperglycemia, dyslipidemia, protrombotic and proinflammatuary factors are the important risk factors of metabolic syndrome. Citrus flavonoids have beneficial effects on hyperglycemia, hyperlipidemia, hypertension, inflammation and body weight management. In the literatur, the studies mostly featured on the animal studies related with citrus flavonoid extracts and human studies on beneficial effects of citrus fruits consumption on dyslipidemia. Therefore, there is a need to more epidemiological and experimental studies on citrus fruits consumption recommendations in relation with metabolic syndrome.

Keywords: Citrus, Abdominal obesity, Metabolic syndrome, Hesperidin

Giriş

Metabolik sendrom (MeS), insülin direnciyle başlayan abdominal obezite, hiperglisemi, dislipidemi, hipertansiyon ve koroner arter hastalığı gibi sistemik bozukluklarının tümünü birlikte kapsayan bir endokrinopatidir (Arslan ve diğerleri, 2009). Abdominal obezitede viseral adipositlerden salgılanan leptin, rezistin, interlökin (IL) - 6, tümör nekroz faktörü (TNF) - α ve plazminojen aktivatör inhibitör (PAI) - 1 gibi metabolik olarak aktif moleküller, insülin direnciyle ilişkili olup metabolik sendrom için önemli etmenlerdir. Abdominal obeziteyle hücrelerin insülin aracılı glikoz alımı azalmakta, yağ dokusundan esterleşmemiş yağ asidi salınımı olmakta, kas ve karaciğerde yağlanma meydana gelmekte böylelikle dislipidemi ve insülin direncinin ortaya çıkması kolaylaşmaktadır (Huang, 2009).

Uluslararası Diyabet Federasyonu'nu 2005 yılında ATP III tanımlamasını güncelleyerek abdominal obezite varlığına ek olarak ATP III tanımlama listesindeki kriterlerden en az iki tanesinin daha varlığı durumunda MeS tanısı konulabileceğini belirlemiştir. Ayrıca, abdominal obezite ile diğer MeS risk faktörleri arasındaki korelasyonda etnik farklılıklara da önem verilmiştir. Buna göre bel çevresinin her etnik grubun ortalama değerlerine göre belirlenmesinin uygun olacağı belirtilmiştir (Arslan ve diğerleri, 2009). Türkiye'de Erişkinlerde Kalp Hastalığı ve Risk Faktörleri Çalışması 2003 yılı sonuçlarına göre MeS sıklığının ≥30 yaş kadınlarda %45.2, erkeklerde %27'dir. Türkiye Metabolik Sendrom Araştırması 2004 yılı sonuçlarına göre, 20 yaş ve üzerindeki erişkinlerde MeS sıklığı kadınlarda (%39.8) erkeklere (%28.0) göre daha yüksek olduğu belirlenmiştir. Türkiye Kronik Hastalıklar ve Risk Faktörleri Sıklığı Çalışması sonuçlarına göre MeS sıklığı ≥20 yaş kadınlarda %35.6, erkeklerde %16.7 olarak saptanmıştır (Sözmen, 2013).

Temel besleyici özelliklerinin yanında vücutta gösterdiği bir veya birden fazla yararlı hedef foksiyon ile hastalık riskini azaltıp iyi hal ve sağlığı geliştirici etki gösterebilen, günlük beslenme planı içerisinde yer alıp tüketilebilen; hap, kapsül veya farklı şekillerde bir besin desteği olmayan besinlere fonksiyonel besin adı verilmektedir (Coşkun, 2005). Fonksiyonel bir besin olarak turunçgiller, bileşiminde bulunan askorbik asit, folik asit, lif, pektin, potasyum, magnezyum, karotenoidler ve flavonoidler ile olumlu sağlık etkileri gösterebilmektedir (Sanofer, 2014). Turunçgil flavonoidlerinden naringin, naringenin, nobiletin, narirutin, tangeretin ve hesperidinin; anti-inflamatuvar, anti-allerjik, anti-karsinojenik, anti-diyabetik, anti-hipertansif, anti-viral, anti-oksidatif ve hipolipidemik etki gösterebildikleri bildirilmektedir (Assini, Muhvihill & Huff, 2013; Alam ve diğerleri, 2014). Bu derleme yazıda fonksiyonel bir besin olarak turunçgillerin bileşimi ve metabolik sendrom ile ilişkisinin değerlendirilmesi hedeflenmiştir.

Turunçgiller

Turunçgiller, Rutaceae familyasının Aurantoideae alt-familyasındandır. Birçok türü olmasına rağmen, tarımı yapılan en önemli turunçgiller *C. limon* (limon), *C. aurantifolia* (misket limon), *C. aurantium* (turunç), *C. sinensis* (portakal), *C. reticulata* (mandalina), *C. grandis* ya da *C. maxima* (pomelo), *C. paradisi* (greyfurt) ve *C. bergamia* (bergamot)'dır. Kökeni Güneydoğu Asya olan turunçgillerin, çağdaş anlamda üretimi 19. yüzyılda Amerika Birleşik Devletleri (ABD)`nde başlamış ve hızla yayılmıştır. Turunçgil yetiştiriciliği dünyada 40 derece kuzey enlemi ile 40 derece güney enlemi arasında yapılmaktadır. Turunçgiller dünyada en çok yetiştirilen ve tüketilen meyve grubu olarak bilinmektedir. Dünya turunçgil üretiminde 2014 yılında Çin, Brezilya ve ABD ilk üç sırada olup, Türkiye 3782 milyon ton üretimiyle sekizinci sırada yer almaktadır (Food and Agriculture Organisation, 2015). Türkiye`de 2015 yılı turunçgil üretimi ise 3975 milyon ton olup turunçgil türleri üretim oranı sırasıyla portakal (%45.7), mandalin (%29.0), limon (%18.8) greyfurt (%6.3) şeklindedir (Türkiye İstatistik Kurumu, 2016).

Turunçgiller iyi bir C vitamini kaynağı olmasının yanı sıra lif, potasyum, folat, kalsiyum, tiamin, niasin, B_6 vitamini, fosfor, magnezyum, bakır, riboflavin, pantotenik asit ile karotenoidler ve flavonoidler gibi çeşitli fitokimyasalları içermekte ve bu bileşenlerin miktarları turunçgil çeşidine, olgunluğuna, saklama koşullarına, işleme yöntemlerine göre değişkenlik gösterebilmektedir. Ortalama 100 gram portakal, greyfurt, mandalina ve limon için C vitamini değerleri sırasıyla 53-88 mg, 31-61 mg, 27-72 mg, 29-61 mg arasında değişirken, A vitamini değerleri ise sırasıyla 17 µg, 58 µg, 46-144 µg, 2-22 µg şeklinde belirtilmiştir (Turner & Burri, 2013).

Turunçgil Flavonoidleri

Flavonoidler, içerdikleri karbon halkasındaki değişimlere göre flavonlar, flavanoller, flavanonlar,

kateşinler, antosiyanidinler ve isoflavonlar şeklinde altı gruba ayrılabilir (Coşkun, 2005). Flavonoid iskelet yapısı, iki aromatik halka ve bunlara bağlı piron veya hidroksipiron halkalarından oluşmaktadır. Piron halkasına sahip olan flavon, hidroksipiron halkasına sahip olan flavanon olarak adlandırılmaktadır. Turunçgillerde bulunan flavanon türleri, naringin, naringenin, narirutin, hesperidin, eriositrin, taksifolin, neohesperidin, neoeriositrin ve poncirindir. Naringin, narirutin ve naringin glikozidleri en fazla greyfutta bulunurken hesperidin ve hesperidin glikozidleri portakal ve mandalinada; eriositrin ve hesperidin ise limon ve misket limonda yaygındır (Gattuso, Barreca, Gargiulli, Leuzzi & Caristi, 2007). Turunç kabuğu da hesperidin, neohesperidin, naringin ve tangeretin gibi flavonoidleri içermektedir. Bunun yanında alkoloid bileşikler sinefrin ve oktopamin, turunçta bulunan sırasıyla epinefrin ve norepinefrin yapısına benzeyen adrenerjik aktif bileşenlerdir (Peixoto ve diğerleri, 2012). Tablo 1'de turunçgillerin flavonoid içerikleri gösterilmiştir (Gattuso, Barreca, Gargiulli, Leuzzi & Caristi, 2007). Bazı turunçgillerde bir acı tat varlığı söz konusudur. Bu acı tat flavonoidler (naringin, neohesperidin) ve limonoidler (limonin, nomilin) ile ilgilidir. Naringin, acı tat veren bir flavanon glikozit olup greyfurt, pomelo ve turunçta bulunmaktadır. Meyve olgunluğunun artması ile birlikte naringin miktarında azalış olduğu bildirilmektedir (Yalım, Özdemir & Ekiz, 2004).

Turunçgiller ve Metabolik Sendrom İlişkisi

Turunçgiller ve metabolik sendrom arasındaki ilişki obezite, dislipidemi, hipertansiyon, hiperglisemi başlıkları altında irdelenebilmektedir. Bu konudaki insan ve hayvan araştırmaları Tablo 2 ve 3'te özetlenmiştir.

Obezite üzerine etkileri

Obezite, alınan enerjinin harcanan enerjiden fazla olması durumunda vücut yağ kütlesinin, yağsız vücut kütlesine oranının artması ile karakterizedir. Obezitenin önlenmesi ve tedavisinde fonksiyonel besinler ve bileşenler arasındaki ilişkiyle ilgili çalışmalar yapılmaktadır (Choudhary & Grover, 2012). Fonksiyonel bir besin olarak turunçgillerden limon polifenollerinin beyaz adipoz doku ve lipit metabolizmasına olan etkisinin araştırıldığı bir çalışmada erkek fareler düşük yağlı beslenen, yüksek yağlı beslenen ve diyetlerine limon polifenollerinin eklendiği yüksek yağlı beslenen olmak üzere üç gruba ayrılarak 12 hafta izlenmiştir. Buna göre, limon polifenollerinin farelerdeki ağırlık kazanımını, yağ depolanma yeteneğini, hiperlipidemiyi, hiperglisemiyi ve insülin direncini baskıladığı görülmüştür. Limon polifenolü verilen grupta peroksizom proliferatör ile aktive olan reseptör (PPAR) - α seviyeleri ve karaciğer ile beyaz adipoz dokuda asil CoA oksidaz seviyelerinde artış ile yağ oksidasyonu üzerine etkili olduğu belirlenmiştir (Fukuchi ve diğerleri, 2008). Sıçanlarda yapılan altı haftalık bir çalışmada ise ek naringenin verilmesi adipoz dokudaki adiposit ve trigliserit içeriğini düşürürken karaciğerde PPAR-α, karnitin palmitoil transferaz (CPT) - 1 ve mitokondriyal katlanmamış protein (UCP) - 2 proteinin gen ekspresyonunu önemli derecede yükseltmiştir (Alam ve diğerleri, 2014). Naringeninin, glikoz ve lipit metabolizmasındaki rolünü incelemek için 19 dişi fare ile yapılan 11 haftalık bir araştırmada bir gruba normal diyet verilirken diğer gruba %3 oranında naringenin içeren diyet verilmiştir. Naringenin verilen grupta kontrol grubuna göre postprandiyal glikoz seviyesi ile insulin seviyesinin daha düşük olduğu belirlenmiştir. Aynı zamanda intraabdominal ve subkutan yağlanma ile monosit kemotaktik protein (MCP) - 1 ve IL-6 düzeylerinde azalış, hepatik steatozda iyileşme olduğu belirlenmiştir (Ke ve diğerleri, 2015). Naringeninin, 3T3-L1 yağ hücrelerinden adiponektin salınımını destekleyici MCP-1 ile yağ dokudaki artışla ilişkili inflamatuvar değişikleri iyileştirebileceği bildirilmektedir (Alam ve diğerleri, 2014). Diğer taraftan stearoil-koenzim A desaturaz (SCD) - 1, insanlarda tekli doymamış yağ asidi sentezini sınırlayıcı basamağını katalizlemesi ve trigliserid üretiminde rol alması sebebiyle metabolik ve inflamatuar hastalıkların patogeneziyle ilişkilendirilmektedir (Peter ve diğerleri, 2009). Erkek rat karaciğer (HepG2) hücrelerinde dört hafta süren bir çalışmada, hesperidin ve nobiletinin SCD-1 seviyesindeki azalışla ilintili olarak plazma lipid seviyelerini düşürdüğü, glikoz toleransını iyileştirdiği ve obezite derecesini azalttığı gösterilmiştir (Nichols, Jackson, Manthey, Shukla & Holland, 2011). Bunun yanında 50 yetişkin birey ile on iki hafta yapılan çift kör bir çalışmada turunçta bulunan p-sinefrinin (50 mg/gün), naringin (600 mg/gün) ve hesperidin (100 mg/gün) ile birlikte dinlenme metabolik hızını artırdığı belirtilmiştir (Stohs ve diğerleri, 2011). Turunç ve p-sinefrin ile ilgili derleme bir çalışmada ise on iki hafta turunç ekstratı ve/veya p-sinefrin alımının metabolik hızı artırdığı ancak güvenli tüketim miktarlarının belirlenebilmesi için daha fazla çalışmaya ihtiyaç olduğu bildirilmiştir (Stohs, Preuss & Shara, 2012).

Greyfurt tüketiminin obezite ve metabolik sendrom ile olan ilişkisinin araştırıldığı bir çalışmada ise 91 obez birey rastgele gruplandırılarak plasebo kapsül ve 207 ml elma suyu, greyfurt kapsülü ve 207 ml elma suyu, plasebo kapsül ve 237 ml greyfurt suyu, günde 3 kez her öğünden önce yarım greyfurt tüketmesi sağlanmıştır. Buna göre, greyfurt tüketenlerde en fazla olmakla birlikte, greyfurt kapsülü ve greyfurt suyu tüketenlerde vücut ağırlığı kaybının plaseboya göre daha fazla olduğu saptanmıştır (Fujioka, Greenway, Sheard & Ying, 2006). ABD Ulusal Sağlık ve Beslenme Araştırması 2003-2006 yılı sonuçlarına göre %100 portakal suyu tüketen >19 yaş yetişkin bireylerin tüketim ortalamasının 210 ml/gün olduğu ve %100 portakal suyu tüketenlerin tüketmeyenlere göre daha düşük beden kütle indeksi (BKİ), total kolesterol, düşük dansiteli lipoprotein (LDL) değerleri ile %21 daha az obezite riskinin olduğu belirlenmiştir (O'Neil, Nicklas, Rampersaud & Fulgoni, 2012).

Dislipidemi üzerine etkileri

Dislipidemi, obezitenin önemli komorbiditelerinden biri olup aterosklerotik hastalıkların da majör risk faktörlerindendir. MeS patofizyolojisinde dislipidemi merkezi bir role sahiptir. Dislipidemi durumunda serbest yağ asidi salınımındaki artış ve çok düşük dansiteli lipoprotein (VLDL) üretimi ile yüksek dansiteli lipoprotein (HDL) katabolizmasındaki artış, insülin direnci oluşumunu etkilemektedir (Kolovou, Anagnostopoulou & Cokkinos, 2005). Ayrıca dislipidemi ile birlikte serbest radikallerin üretimi artmakta ve antioksidan enzimlerin aktiviteleri de azalmaktadır. Normal diyet, yüksek kolesterollü diyet ve %0.08 hesperidin ilaveli yüksek kolesterollü diyet olmak üzere üç gruba ayrılan 30 ratın yirmi sekiz gün izlendiği bir çalışmada, hesperidin ilaveli grupta, kolesterol (%34) ve LDL'de (%52) düşüş, HDL (%22), antioksidan enzimler olan süperoksit dismutaz (SOD), katalaz (CAT), glutatyon peroksidaz (GSH-P_x) ve glutatyon s-transferaz (GST) seviyelerinde artış olduğu belirlenmiştir (El-Shafey & Abd-Ellah, 2014). Naringeninin metabolik etkileri ve ateroskleroza etkisini inceleyen 12 haftalık bir çalışmada ise yüksek yağlı-yüksek kolesterollü ve düşük yağlı-yüksek kolesterollü beslenen 8-12 haftalık hiperkolesterolemik erkek farelerin diyetlerine eklenen %3 naringeninin hepatik lipogenezisi azaltıp yağ asidi oksidasyonunu artırarak hepatik steatozda ve aşırı hepatik VLDL salgılanmasını azaltarak dislipidemide iyileşme sağladığı belirtilmiştir (Assini ve diğerleri, 2013). Portakal suyu tüketimi ile ilgili bir çalışmada, normal trigliserit, yüksek total kolesterol ve LDL kolesterol, düzeylerine sahip 25 bireye dört hafta boyunca üç öğüne bölünmüş olarak toplam 750 ml portakal suyu verilmiştir. Çalışma sonucuna göre, HDL, trigliserit ve folat seviyelerinde sırasıyla %21, %30 ve %18 oranında artış, LDL/HDL oranında %16 oranında düşüş belirlenmiştir (Kurowska ve diğerleri, 2000). Uzun süre (>12 ay) portakal suyu tüketiminin etkilerini inceleyen bir araştırmada ise hiperkolesterolemisi olup portakal suyu tüketenlerin ortalama 480 ml/gün (26.4 mg hesperidin ve 7.4 mg naringenin) portakal suyu tükettiği ve bu bireylerde tüketmeyenlere göre serum total kolesterolde %5, LDL'de %12 ve ApoB'de %12 düşüş olduğu belirlenmiştir (Aptekmann & Cesar, 2013). Randomize kontrollü çapraz bir çalışmada ise 50-65 yaşlarında 24 erkek bireye dört hafta, 500 ml/gün portakal suyu, 500 ml/gün hesperidin eklenmiş (292 mg) kontrol içeceği ile 500 ml/gün plasebo içecek verilerek portakal suyu ve hesperidin eklenmiş kontrol içeceğinin anti-inflamatuvar ve anti-aterojenik etkiler gösterdiği belirlenmiştir (Milenkovic, Deval, Dubray, Mazur & Morand, 2011). Dört hafta boyunca plasebo, naringin (500 mg/gün) ve hesperidin (800 mg/gün) verilen hiperkolesterolemik 194 birey ile yapılan bir çalışmada ise total kolesterol, HDL, LDL ve trigliserit değerlerinde bir farklılık belirlenmemiştir (Demonty ve diğerleri, 2010). Hiperkolesterolemik ve sağlıklı 26 birey ile altı ay yürütülen çift kör plasebo kontrollü bir çalışmada ise turunçgil ekstraktı (TE) ve C vitamini ile trigliserit düzeylerinde azalış ve lipoprotein oksidasyonunu gecikme sağlamıştır (Vinson & Jang, 2001).

Hipertansiyon üzerine etkileri

Portakal suyu ve önemli flavonoidlerinden olan hesperidinin kan basıncı üzerine olan etkisinin araştırıldığı 50-65 yaş grubu kilolu 24 erkek bireye dört hafta 500 ml/gün portakal suyu, 500 ml/gün hesperidin eklenmiş (292 mg) kontrol içeceği ile 500 ml/gün plasebo içecek verilen randomize kontrollü çapraz çalışmada, portakal suyunun diastolik kan basıncını düşürdüğü ve postprandiyal mikrovasküler endotel reaktiviteyi arttırdığı belirlenmiştir (Morand ve diğerleri, 2011). Taze portakal suyunun 22 sağlıklı birey üzerinde kan basıncına olan etkisinin araştırıldığı bir diğer çalışmada ise dört hafta bireylere günde iki kez 500 ml portakal suyu verilerek kan basıncında ortalama 3-4 mmHg azalış olduğu belirlenmiştir

(Asgary & Keshvari, 2013). Proliferasyon ve migrasyonu düzenleyen vasküler düz kas hücreleri (VSMCs), ateroskleroz ve hipertansiyonun patogenezinde oldukça öneme sahiptir. Naringenin, TNF-α ile uyarılan VSMCs proliferasyonu ile migrasyonunu inhibe ettiği ve TNF-α'nın uyardığı artmış reaktif oksijen türlerini bloke ettiği bildirilmektedir (Alam ve diğerleri, 2014).

Hiperglisemi üzerine etkileri

Hiperglisemi ve insülin direnci, MeS fizyopatolojisinde oldukça önemli bir role sahiptir. Beş haftalık 30 adet tip 2 diyabetik erkek fare ile yapılan bir araştırmada bir gruba normal diyet, bir gruba 0.2 g/kg hesperidin ve bir gruba 0.2 g/kg naringin eklenen diyet verilmiştir. Beş haftanın sonunda naringin ve hesperidin eklenen gruplarda kontrol grubuna göre plazma glikoz, serbest yağ asidi, trigliserit, total kolesterol, hepatik trigliserit ve kolesterolde azalış belirlenirken fekal trigliserit ve kolesterolde artış belirlenmiştir. Bu flavonoidlerin, hepatik glukokinaz aktivitesi ve hekzokinaz aktivitesinin artışında, hepatik-3-hidroksi-metil CoA (HMG-CoA) redüktaz ve asil CoA kolesteril asil transferaz (ACAT) aktivitesi ile glikoz-6-fosfataz, fruktoz-1,6-bifosfataz ve glikojen fosforilazın azalışında etkili olduğu aynı zamanda adipoz dokuda GLUT-4 ekspresyonunu artırdığı saptanmıştır (Jung, Lee, Park, Kang & Choi, 2006). Naringenin ve hesperidinin antidiyabetik etkisi, adipoz dokuda PPAR-γ ekspresyonu ve adiponektin seviyelerindeki artışla da ilişkilendirilmektedir. Erkek ratlarla yapılan bir çalışmada, ilk olarak iki hafta süresince bir gruba normal diyet verilirken bir gruba yağlı diyet/streptotozin verilerek tip 2 diyabet gelişimi indüklenmiştir. Daha sonra dört hafta süresince diyabetik ratlardan bir gruba diyabetik diyet, bir gruba 50 mg/kg hesperidin eklenmiş diyabetik diyet, bir gruba da 50 mg/kg narigenin eklenmiş diyabetik diyet verilmiştir. Çalışma sonucunda hesperidin ve naringenin eklenmiş gruplarda HbA1c düzeylerinde artış plazma insülin düzeylerinde ise azalış saptanmıştır (Mahmoud, Ahmed, Abdel-Moneim & Ashour, 2013).

Tablo 1. Turunçgillerin flavonoid içerikleri

Table 1. Flavonoid contents of citrus fruits

Flavonoidler (mg/100mL)	Portakal Suyu (C.Sinensis)	Greyfurt Suyu (C.paradisi)	Mandalina Suyu (C.reticulata)	Misket Limon Suyu (C.aurantifolia)	Limon Suyu (C.limon)	Bergamot Suyu (C.bergamia)	Turunç Suyu (C.aurantium)
Eriositrin	0.31	0.41	0.31	0.29	16.7	-	-
Neoeriositrin	0.59	0.32	0.05	-	-	1.38	0.77
Hesperidin	28.6	0.93	24.3	1.77	20.5	-	-
Neohesperidin	-	1.21	-	-	-	1.60	0.87
Naringin	21.3	23.0	-	-	-	2.23	1.97
Naringenin	-	2.70	-	-	-	-	-
Narirutin	5.2	7.60	3.92	-	-	-	
Nobiletin	0.33	0.15	0.23	0.52	-	-	-
Tangeretin	0.04	0.12	0.26	0.18	-	-	0.08
Luteolin	-	-	-	0.61	0.08	-	-
Taksifolin	0.03	-	-	0.04	-	-	-
Poncirin	1.04	1.26	-	-	-	6.41	

Tablo 2. Turunçgiller ve MeS ile İlgili Hayvan Araştırmaları

Table 2. Animal Studies on Citrus Fruits and MeS

Araştırma planı	Denekler	Sonuç	Kaynak
12 hafta -düşük yağlı diyet -yüksek yağlı diyet - yüksek yağlı limon polifenolü (%0.5) eklenen diyet	C57BL/6J erkek fare	Limon polifenolü eklenen diyet: -Ağırlık ↑ -Hiperlipidemi, hiperglisemi ve insülin direncini baskılama -PPAR-α seviyesi↑ -Karaciğer ve beyaz adipoz dokuda asil CoA oksidaz ↑	(Fukuchi ve diğerleri, 2008)
11 hafta -normal diyet -naringenin(%3) eklenen diyet	Overektomize dişi fare	Naringenin eklenen diyet: -Tokluk glukoz seviyesi ve insülin ↓ -İntra-abdominal ve subkutan yağlanmada %50 ↓ -Lipogenesiz ve glukoneogenez ↓ -Hepatik steatozda iyileşme	(Ke ve diğerleri, 2015)
12 hafta -yüksek yağlı-yüksek kolesterollü (%3) naringenin eklenmiş diyet -düşük yağlı yüksek kolesterollü (%3) naringenin eklenmiş diyet	*Ldlr*$^{-/-}$ erkek fare	Naringenin: Hepatik lipogenezisi ↓ -Yağ asidi oksidasyonunu ↑ -TNF-α ve IL-1 ↓ -Hepatik steatozda iyileşme -Apo B100 ↓	(Assini J. M. ve diğerleri, 2013)
2 hafta -normal diyet -0.2g/kg hesperidin eklenen diyet -0.2g/kg naringin eklenen diyet	tip 2 diyabetik erkek fare	Naringin ve hesperidin: -Plazma glukoz seviyesinde ↓ -Bağırsaktan glukoz absorbsiyonunu ↓ -İnsülin salınımı ↑ -Adipoz dokuda GLUT-4 ekspresyonunu ↑ -HMG-CoA redüktaz aktivitesinde -Plazma kolesterol seviyeleri ↓	(Jung, Lee, Park, Kang & Choi, 2006)
4 hafta -normal diyet -yüksek kolesterollü diyet -%0.08 hesperidin eklenmiş yüksek kolesterollü diyet	Swiss albino rat	Hesperidin eklenen diyet: -Total kolesterol (%34), LDL (%52) ↓ -HDL (%22) ve trigliserit ↑ -SOD, CAT, GSH-Px ve GST ↑	(El-Shafey & Abd-El-lah, 2014)

↑: artış, ↓: azalış

Tablo 3. Turunçgiller ve MeS ile İlgili İnsan Araştırmaları

Table 3. Human Studies on Citrus Fruites and MeS

	Araştırma Planı	Denekler	Sonuç	Kaynak
Portakal suyu	ABD Ulusal Sağlık ve Beslenme Araştırması 2003-2006 ortalama 210 mL/gün portakal suyu	8861 birey (>19 yaş)	%100 portakal suyu tüketimi A vitamini, C vitamini, folat, potasyum ve magnezyum düzeyleri ↑ Obezite riski %21 ↓	(O'Neil, Nicklas, Rampersaud & Fulgoni, 2012)
Portakal suyu	4 hafta 3 öğüne bölünmüş 750 mL portakal suyu	25 hiperkolesterolemili birey (55 ±11 yaş)	HDL (%21), trigliserit (%30) ↑, folat (%18) ↑ LDL (%16) ↓ Homosistein →	(Kurowska ve diğerleri, 2000)
Portakal suyu	>12 ay ortalama 480 mL/gün portakal suyu	129 sağlıklı ve hiperkolesterolemili birey (18-66 yaş)	Sağlıklı bireyler: %11, LDL (%18) ve ApoB (%12) ↓ hiperkolesterolemik bireyler: LDL (%5) ve ApoB (%12) ↓ Her iki grup: C vitamini ve folat ↑ Homosistein, HDL, Apo A1→	(Aptekmann & Cesar, 2013)
Portakal suyu	4 hafta 2x500 ml/gün portakal suyu	22 sağlıklı birey (18-59 yaş)	Bireylerin kan basınçlarında ortalama 3-4 mmHg ↓	(Asgary & Keshvari, 2013)
Portakal suyu-Hesperidin	4 hafta 500 mL/gün portakal suyu 500 mL/gün hesperidin eklenmiş içecek 500 ml/gün plasebo içecek	24 kilolu birey (50-65 yaş)	Vasüler adezyon molekülleri ve yağ taşınmasında etkili gen kont-rolü ile anti-inflamatuvar ve anti-aterojenik etki	(Milenkovic, Deval, Dubray, Mazur & Morand, 2011)
Portakal suyu-Hesperidin	4 hafta 500 ml/gün portakal suyu 500 ml/gün hesperidin eklenmiş içecek 500 ml/gün plasebo içecek	24 kilolu birey (50-65 yaş)	Portakal suyu verilen grup: Diastolik kan basıncı ↓ Mikrovasküler reaktiviteyi iyileştirici etki	(Morand ve diğerleri, 2011)
Hesperidin-Naringin	4 hafta (çapraz çalışma) Plasebo Naringin kapsül (500 mg/gün) Hesperidin kapsül (800 mg/gün)	194 hiperkolesterolemik birey (18-75 yaş)	Her iki grup: Kolesterol, HDL, LDL ve trigliserit →	(Demonty ve diğerleri, 2010)
Hesperidin-Naringenin-p-Sinefrin	12 hafta Plasebo p-sinefrin (50 mg/gün) p-sinefrin (50 mg/gün)+naringin (600 mg/gün) p-sinefrin (50 mg/gün)+naringin (600 mg/gün)+hesperidin 100 mg/gün) p-sinefrin (50 mg/gün)+naringin (600 mg/gün)+hesperidin 1000 mg/gün)	5x10 birey	Tüm gruplar: Kalp atım hızı → Kan basıncı → p-sinefrin (50 mg/gün)+naringin (600 mg/gün) verilen grup: metabolik hız ↑	(Stohs ve diğerleri, 2011)
TE-Vitamin C-Vitamin E	2 ay (çapraz çalışma) Placebo Vitamin C (2x500 mg) Vitamin E (2x400 IU/gün) Vitamin C (3x330 mg + TE (3x 900 mg TE)	26 hiperkolesterolemik ve sağlıklı birey (53±10 yaş)	TE + Vitamin C: Trigliserit ↓ Lipoprotein oksidasyonunda gecikme	(Vinson & Jang, 2001).

Sonuç

Turunçgiller, iyi bir C vitamini kaynağı olarak bilinmesinin yanısıra lif, potasyum, folat, karotenoidler, flavonoidler gibi çeşitli fitokimyasallar bakımından da iyi kaynaklardır ve bu bileşenlerin miktarları turunçgil çeşidine, olgunluğuna, saklama koşullarına, işleme yöntemlerine göre değişkenlik gösterebilmektedir. Turunçgillerin sağlık üzerine olumlu etkileriyle öne çıkan bileşenleri naringin ve hesperidin flavonoidleridir. Hipertansiyon, hiperglisemi, dislipidemi, protrombotik ve proinflamatuvar faktörler, MeS risk faktörleri arasındadır. Turunçgil flavanoidlerinin hiperglisemi, hiperlipidemi, hipertansiyon ve vücut ağırlığı denetimi üzerine olumlu etkileri ile anti-inflamatuvar ve antioksidan etkileri söz konusudur. Literatürde, turunçgil flavonoid ekstraktlarının kullanıldığı hayvan araştırmaları ve turunçgil tüketiminin dislipidemik etkileriyle ilgili insan araştırmaları öne çıkmaktadır. Bu nedenle günlük beslenme planında turunçgil tüketimi ve metabolik sendromla ilgili önerilerin geliştirilebilmesi için bu konuda daha fazla epidemiyolojik ve deneysel araştırmalar gereklidir.

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3(2): 59-66 (2017) doi: 10.3153/JFHS17008

Journal of Food and Health Science

E-ISSN 2149-0473

REVIEW ARTICLE DERLEME MAKALESİ

FİTALAT ESTERLERİ VE SU ÜRÜNLERİ TÜKETİMİNDEKİ YERİ

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Received: 14.11.2016

Accepted: 17.01.2017

Published online: 04.03.2017

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Öz:

Teknolojinin gelişmesi ile birlikte kullanımı artan birçok organik ve inorganik kirletici ile kimyasal kontaminantlar hem biyolojik sistemi hem de insan sağlığını tehdit edecek boyutlara ulaşmaktadır. Fitalatlar plastik malzemelere dayanıklılık ve esneklik vermesi amacıyla kullanılan insan yapımı bileşiklerdir. Gıda ambalajları, oyuncak, kozmetik, ev malzemeleri, medikal aletler gibi birçok alanda plastik materyallerin kullanıldığı düşünüldüğünde fitalatlara ne kadar çok maruz kaldığımız ortaya çıkmaktadır. Birçok organik kirletici gibi fitalatlar da yağda çözülmekte ve su, hava ve organik yapılar sayesinde taşınabilmektedir. Plastik materyallere kovalent bağ ile bağlanmadıkları için ürünlerden çevreye yayılımı kolay olmaktadır. Sağlık riski taşıması nedeniyle gıda ile temas eden materyallerde plastik kullanımı ve fitalat esterleri içeriği yasal düzenlemelerle sınırlandırılmıştır. Su ürünleri kalıcı toksik bileşiklerin biyoakümülasyonu açısından güvenilir bir indikatördür ve insanların maruz kalma düzeyinin tespit edilmesinde kullanılabilmektedir. Gerçekleştirilen çalışmalarda diğer gıda ürünlerinde olduğu gibi su ürünlerinin de çeşitli oranlarda fitalat esterleri içerdiği ortaya konulmuştur. Farkında olmadan yoğun miktarda maruz kaldığımız fitalat ve fitalat esterleri konusunda üretici ve tüketicilerde bilinçlenme sağlanması gerekmektedir.

Anahtar Kelimeler: Fitalat, Fitalat esterleri, DEHP, BBP, Su ürünleri

Abstract:

PHTHALATE ESTERS AND THEIR STATUS IN SEAFOOD CONSUMPTION

Many organic and inorganic pollutants and chemical contaminants, which are increasingly used together with the development of technology, are threatening both biological system and human health. Phthalates are man-made compounds which are used for imparting durability and flexibility to plastic materials. Considering the use of many inland plastic materials such as food packages, toys, cosmetics, household materials, medical equipment, it is obvious that human are exposed to high levels of phthalates. Like many organic pollutants, phthalates are dissolved in oil and transported by water, air and organic structures. The propagation of phthalate esters from products to the environment is easy due to not being covalently bonded to plastic materials. The use of plastics in food contact materials is limited by legal regulations considering health risks of phthalate esters. Aquaculture is a reliable indicator of bioaccumulation of persistent toxic compounds and can be used to determine the exposure level of humans. Studies have shown that fish products contain phthalate esters in various proportions as in other food products. It is important to raise consciousness of producers and consumers about phthalate and phthalate esters which we have been exposed intensively.

Keywords: Phthalate, Phthalate esters, DEHP, BBP, Seafood

Giriş

Ülkemizde ve dünyada bir çok gıdanın ambalajlanmasında kullanılan plastikler yüksek molekül ağırlıklı organik moleküllerden ya da polimerlerden oluşmaktadır. Polivinil klorür (PVC) polimeri gibi kolay kırılabilen plastiklere esneklik ve dayanıklılığını arttırmak amacıyla yüksek miktarda plastikleştirici (yumuşatıcılar, plastifiyan) madde eklenmektedir. Ticari olarak en yaygın kullanılan plastikleştirici fitalattır. Alkil zincirinin alkol bazlı olması nedeniyle gıda ambalajları, oyuncaklar, kozmetik, farmasotik, çözgen, yapıştırıcı, boru hatları, boyalar, insektisitler, temizlik materyalleri gibi bir çok alanda kullanılmaktadır (Schettler, 2006). Gıda ile temas eden materyallerde kullanımı sınırlı da olsa fitalatların gıda içerisine nüfuz etmesi söz konusudur. Yağda çözünen bir madde olması nedeniyle özellikle yağlı gıdalarda, yağ ve sos içerisinde depolanmış gıda ürünlerinde sağlık riski oluşturacak boyutlara ulaşabilmektedir.

Özellikle PVC materyallerinde fitalat ya da fitalik asit esterleri kullanılmakta, yoğun miktarda ve yaygın kullanımı nedeniyle de doğada her yerde bulunabilmektedir. Renksiz, kokusuz ve uçucu bileşiklerdir. Yağda çözünürlükleri yüksektir. Polimer matrikse kovalent bağ ile bağlanmadıkları için ürünlerden çevreye yayılımı kolay olmaktadır. Yüzey sularında, yer altı sularında, içme suyunda, suda ve sedimentte gerçekleştirilen çalışmalarda fitalat esterleri tespit edilmiş, özellikle DEHP (dietilhekszil fitalat) ve DBP (dibütil fitalat) varlığı dikkat çekici olmuş ve mevsim değişikliklerinden etkilendiği gözlenmiştir (Yuan ve ark., 2002; Sirivithayapakorn ve Thuyviang, 2010; Liu ve ark., 2013; 2014). Gıda zincirine bulaşma; süzülme, buharlaşma, aşınma, göç etme gibi yollarla olmaktadır (Wittasek ve ark., 2011). İnsanlar başta gıda ürünleri olmak üzere sindirim yoluyla, deri temasıyla, solumayla ve damar içi medikal aletler nedeniyle fitalatlara maruz kalmaktadır. Hatta DBP ve DEHP'in oyuncaklarda ve gıda ile temas eden materyallerde yaygın kullanımı nedeniyle çocukların daha yüksek fitalat dozlarına maruz kaldığı bilinmektedir (Alves ve ark., 2016). Gıda ile sindirilmesi, su içilmesi, toz/toprak, hava solunumu ve deri teması gibi yollarla vücuda alınan fitalat miktarı günde 70µg/kg olarak bildirilmektedir (Net ve ark., 2015).

Fitalat esterleri

Fitalik asit diesterleri yaygın olarak fitalatlar adı altında bilinmektedir. İnsan yapımı organik bileşiklerdir. Benzer kimyasal özelliklere sahip olmalarına rağmen her bir fitalat esterinin kendine özgü fiziksel ve kimyasal karakteristikleri olup, biyolojik organizmaları farklı şekillerde etkilemektedir (Kamrin, 2009). Sıvı, katı ya da viskoz yapıda, düşük buhar basıncına sahip kimyasallardır. Homojen bir yapı sağlamak amacıyla polimerlerle fiziksel interaksiyon içerisinde bulunmakta, kimyasal olarak bağlanmadığı için polimeri kolay terk ederek ortama yayılmaktadır. Yaygın olarak kullanılan fitalat esterler Tablo 1'de sunulmuştur.

Fitalatlar yüksek molekül ağırlıklı (YMA) ve düşük molekül ağırlıklı (DMA) olarak iki grupta sınıflandırılmaktadır. PVC plastikleştiricisi olarak yaygın kullanılan DEHP gibi YMA olan fitalatlar az biyoakümülasyon faktörüne sahiptir. Diğer YMA fitalatlar BBzP, DiNP, DnOP ve DiDP'dir. Yüksek biyoakümülasyon faktörüne sahip DMA fitalatlar ise DBP, DEP, DiBP'dir. DMA sıcaklığa dayanıklıdır, ancak YMA dayanımı daha azdır. Her iki form da stabil değildir (Johns ve ark., 2015; Mariana ve ark., 2016).

Gıda ile temas eden maddelerde kullanımına izin verilen bazı fitalat esterlerinin özellikleri aşağıda sunulmuştur.

DEHP: Yıllık üretimi iki milyon tondan fazla olan DEHP en yaygın kullanılan fitalat esteridir. Üretilen DEHP'nin %90'dan fazlası PVC için plastikleştirici ajan olarak kullanılmaktadır. Gıda üretim ekipmanlarında, gıda ambalajlarında, yapıştırıcılarda, medikal ürünlerde, oyuncaklarda ve PVC'nin yer aldığı tüm alanlarda bulunmaktadır. DEHP her yerde bulunabilen çevresel bir kontaminanttır. Polimerlere kovalent bağla bağlanmaz. Hegzan ve mineral yağlarda çözülmektedir, hidrofobiktir.

DEP: Birçok kozmetik ürün formülasyonunda, aspirin kaplamasında, dental materyallerde, gıda ve farmakolojik paketlerde yapıştırıcı, plastikleştirici ve yüzey kaydırıcı olarak kullanılmaktadır. Bu nedenle en çok deri teması ve sindirim yoluyla vücuda alınmaktadır. Alkol, eter, aseton, benzen içerisinde çözülmektedir.

BBP: Gıda ambalajları, boyalar, deodorant, mobilya döşeme, sentetik deri malzemelerinde kullanılmaktadır. BBP'ye en çok gıdalar, özellikle yağ içeriği yüksek olan ürünler ile maruz kalınmakla beraber hava ve sudan da geçiş olmaktadır.

DiDP: Ev eşyaları ve okul malzemeleri en çok kullanıldığı alanlardır. Son yıllarda yapılan çalışma-

larda DiDP'nin oyuncaklarda kullanılmadığı, gıdalarda ise önemsenmeyecek düzeyde olduğu belirtilmiştir (Kamrin, 2009).

DiNP: Yan zinciri 8 ile 10 karbondan oluşmuş fitalatların karışımıdır. Ev eşyaları, gıda ambalajları, oyuncaklar, kıyafetler, boyalar, eldivenler vb. içerisinde yer almaktadır. Solunum, sindirim ve deri teması ile vücuda alınmaktadır.

Fitalat esterleri ve sağlık

Her alana yayılan plastik materyallerden insana nüfuz eden kimyasal maddelerin tespit edilmesi ve sağlık üzerine etkilerinin ortaya konulması önem arz etmektedir. Özellikle bisfenol-A üzerine eğilen araştırmacılar, son yıllarda fitalat esterlerinin sağlık üzerine olumsuz etkileri üzerine odaklanmışlardır. Fitalat esterleri kısa yarılanma ömrüne sahiptir ve monoesterlere, ileri oksidasyon ürünlerine ve glukronik konjugatlara metabolize olmakta ve birincil olarak üre ile atılmaktadır (Swan ve ark., 2015). Fitalat metabolitlerinin insan tükürüğü, amniotik sıvı, anne sütü gibi sıvılara geçişi araştırmacılar tarafından izlenmektedir (Jeddi ve ark., 2016).

Fitalat esterleri endokrin-bozucu ajandır. Vücutta moleküler hedeflere bağlanmakta ve hormonal foksiyonlara müdalahe etmektedir (Marina ve ark., 2016). Fitalat esterlerinin karaciğer, böbrek ve üreme sistemine zarar verdiği bildirilmiştir. DEP'e maruz kalınması ile erkek üreme sisteminde DNA yapısında hasarın ortaya çıkması arasında ilişki belirlenmiştir (Duty ve ark., 2003). DEHP endokrin sisteme zarar vermekte, prematüre doğumlara ve astıma sebep olmaktadır. Fitalat konsantrasyonundaki artış ile birlikte koroner kalp hastalıkları riskinin de arttığı bildirilmiştir (Olsen ve ark., 2012).

Fitalatlar özellikle çocukların üreme sistemine, nörolojik ve ergenlik dönemi gelişimine zarar vermekte ve obezite sorunu yaratmaktadır. Ayrıca ev tozlarında bulunan fitalatlar alerji riskini arttırmaktadır (Ait Bamai ve ark., 2016). DEHP ve DEP çocuklarda astıma (Ku ve ark., 2015), BBP ise rinit ve egzema oluşumuna yol açmaktadır (Bornehgag ve ark., 2004). Ellerini ağızlarına almaları, ağırlıklarına göre geniş yüzey alanlarına sahip olmaları, yüksek metabolik hızları ve maruz kaldıkları fitalat içerikli oyuncaklar nedeniyle çocuklar, büyük aile bireylerine oranla daha fazla fitalata maruz kalmaktadır (Frederiksen ve ark., 2007; Kim ve Park 2014; Ait Bamai ve ark., 2015). Gerçekleştirilen çalışmalar ışığı altında, başta çocuklar olmak üzere tüm bireylerin hayatını olumsuz etkileyen fitalat esterlerinin kullanımına yönelik yasal düzenlemeler yapılmaktadır.

Yasal düzenlemeler

Yarattığı sağlık riskleri nedeniyle Birleşik Devletler Çevre Koruma Ajansı (United States Environmental Protection Agency, USEPA) fitalat esterlerini "endişe yaratan kimyasallar" (chemicals of concern) listesine eklemiştir (USEPA, 2007). Avrupa Kimyasal Ajansı (European Chemical Agency, ECHA) tarafından 10 fitalat diesteri kanser yapıcı, mutajenik veya üreme için toksik (Carcinogenic, Mutagenic, Reprotoxic, CMR) olarak reprotoksik 1B kategorisinde sınıflandırılmıştır. Bu sınıflandırma 4 ile 1 arasında yapılmakta ve 1 en tehlikeli grup olarak değerlendirilmektedir. DEHP, DiBP, DnBP ve BBzP reprotoksik 1B sınıfında yer almaktadır. Bu durum göz önüne alınarak gıda ile temasta olan materyallerle ilgili yasal düzenlemeler yapılmaktadır (Cariou ve ark., 2016).

Avrupa Birliği EU No10/2011 gıda ile temas eden materyallerde plastik kullanımının düzenlenmesi konusunda kullanım amacına göre plastikleştirici ise %30 oranında, teknik destek ajanı ise %0.1'den az miktarda fitalat kullanımına izin vermektedir.

Gıdalar ise yağ içeriğine göre sınıflandırılmıştır;

1. Yağlı gıdalar (fitalat yağda ve alkolde kolay çözülmektedir)
2. Yağsız gıdalar (fitalat su içerisinde zor çözülmektedir)
3. Bebek mamaları

Belirtilen sınıflandırma içerisinde 5 fitalat esterinin (DEHP, DBP, BBP, DIDP ve DINP) kullanımına izin verilmiştir. Sadece BBP, DIDP ve DINP fitalatların yağsız gıda kategorisindeki bebek mamalarında tek kullanımlık conta ve streç film materyali için plastikleştirici ajan olarak kullanımına izin verilmektedir. En sınırlı kullanım alanı olan DBP ve DEHP yağsız gıdalarda plastikleştirici ve destekleyici ajan olarak sırasıyla SML (spesifik migrasyon limiti) 0.3 mg/kg ve 1.5 mg/kg değerinde kullanılmalıdır. Avrupa Birliği Gıda Güvenliği Otoritesi (European Food Safety Authority, EFSA)'ne göre 60 kg bir birey için günlük tolere edilebilir fitalat değeri Tablo 2'de verilmiştir.

Ülkemizde fitalat esterlerinin gıda ile temas eden ürünlerde kullanımı Türk Gıda Kodeksi ile sınırlandırılmaktadır (Tablo 3) (Anonim, 2008). Kodeksimiz Avrupa Birliği yasal düzenlemeleri ile uyum göstermektedir. Spesifik migrasyon limiti

için "fitalatlar için spesifik migrasyon limitlerinin doğrulanması sadece gıda benzerleri ile gerçekleştirilir. Ancak, spesifik migrasyon limitinin doğrulanması, gıdanın madde veya malzeme ile henüz temas etmediği ve fitalat için spesifik migrasyon ön testinde bulunan miktarın istatistiksel olarak önemli olmadığı veya tespit limitinden büyük ya da eşit olduğu durumlarda, doğrudan gıda ile yapılabilir." ifadesi yer almaktadır.

Tablo 1. Yaygın olarak kullanılan fitalat esterler

Table 1. Commonly used phthalate esters

Kısa Adı	**Yaygın Adı**	**CAS numarası**
BBzP	Bütilbenzil fitalat	CAS 85-68-7
DnBP	di-*n*-bütil fitalat	CAS 84-74-2
DEHP	di-(2-etilhekzil) fitalat	CAS 117-81-7
DEP	Dietil fitalat	CAS 84-66-2
DiBP	di-izobütil fitalat veya di-2-metilpropil fitalat	CAS 84-69-5
DiDP	di-izodesil fitalat	CAS 26761-40-0
DiNP	di-izononil fitalat	CAS 28553-12-0
DMP	di-metil fitalat	CAS 131-11-3
DnHP	di-*n*-hekzil fitalat	CAS 84-75-3
DnOP	di-*n*-oktil fitalat	CAS 117-84-0
DnPP	di-*n*-pentil fitalat	CAS 131-18-0

Tablo 2. Fitalat esterleri için kabul edilen günlük alım miktarı (EFSA, 2005)

Table 2. Acceptable daily intake of phthalate esteres (EFSA, 2005)

Fitalat Esterleri	**TDI (Tolerable Daily Intake) tolere edilen günlük alım miktarı µg/kg vücut ağırlığı/gün**	**60 kg birey için günlük tüketim sınırı**
DEHP	50	3 mg
DBP	10	0.6 mg
BBP	500	30 mg
DINP	150	9 mg
DIDP	150	9 mg

Tablo 3. Fitalat esterleri ile ilgili Türk Gıda Kodeksi'nde yer alan düzenlemeler

Table 3. Table 3. Regulations of phthalate esters in Turkish Food Codex

Fitalat Esterleri	**Kullanım Alanı**	**SML (Spesifik Migrasyon Limiti)**
DEHP	a) kullanımı tekrarlanan ve yağsız gıdalarla temas eden madde ve malzemelerde plastikleştirici olarak, (b) son üründe % 0.1'e kadar teknik yardımcı madde olarak kullanılır.	1.5 mg/kg gıda benzeri
DBP	(a) kullanımı tekrarlanan ve yağsız gıdalarla temas eden madde ve malzemelerde plastikleştirici olarak, (b) son üründe poliolefinlerde % 0.05'e kadar olan derişimlerde teknik yardımcı madde olarak kullanılır.	0.3 mg/kg gıda benzeri
BBP	a) kullanımı tekrarlanan madde ve malzemelerde plastikleştirici olarak, (b) TGK-Bebek Formülleri, TGK-Devam Formülleri ve TGK-Bebek ve Küçük Çocuk Ek Gıdaları tebliğlerinde tanımlanmış olanlar hariç yağsız gıdalarla temas eden tek kullanımlık madde ve malzemelerde plastikleştirici olarak, (c) son üründe % 0.1'e kadar teknik yardımcı madde olarak kullanılır.	30 mg/kg gıda benzeri
DiNP birincil doymuş dallanmış C8-C10 alkollerle, %60'dan fazla C9'lu		9 mg/kg gıda benzeri
DiDP birincil doymuş C9-C11 alkollerle, %90'dan fazla C10'lu		9 mg/kg gıda benzeri

Tüm gıdalarda olduğu gibi su ürünlerinde de fitalat esterleri düzeyinin belirlenmesi ve sağlık riski yaratacak boyutlar ile karşılaştırılması gerekmektedir. Sağlık açısından bu kadar ciddi sorun yaratabilecek olan fitalat esterlerine maruz kalınma düzeyleri üretici, tüketici ve düzenleyiciler tarafından bilinmesi gereken önemli bir husustur.

Su ürünleri ile ilgili çalışmalar

Fitalat esterlerinin yoğun kullanımı ve çevreye kolay yayılması nedeniyle su ve sedimentte birikmekte, bu durum su canlılarına da yansımaktadır. Atlantik Okyanusu kıyılarında yapılan bir çalışmada balık örneklerinin yanısıra yengeç (*Callinectes sapidus*), clam (Merccnaria), istiridye (*C.ariakensis*) ve beyaz karides (*Litopenaeus vannamei*) fitalat esterleri içeriği açısından incelenmiştir (Munshi ve ark., 2013). DEHP, BBP ve DBP oranları sırasıyla balıklarda 1.1, 0.22 ve 0.14 µg/g, kabuklu su ürünlerinde 1.2, 0.13 ve 0.09 µg/g olarak belirlenmiştir. Çalışmada kullanılan örneklerin fitalat içeriğinin düşük olduğu, birçok örneğin tespit sınırının altında değerler verdiği belirlenmiştir.

Fitalat esterleri balık gibi vertebralarda kolaylıkla metabolize edilmekte ve atılmaktadır (Barron ve ark., 1995). Fourgous ve ark. (2016) Akdeniz lagunlarından temin ettikleri 117 adet yılan balığı (*Anguilla anguilla*) kas dokusunda fitalat metabolitlerini tespit etmişlerdir. Numunelerin %70'inden fazlasında 9 fitalat metabolitine rastlanmıştır. Mart ve Haziran aylarında belirlenen fitalat esterleri değerlerinin Ekim ayından daha yüksek çıktığı bildirilmiştir. Bunun sebebi olarak da çevresel salımdaki mevsimsel değişiklikler ve/veya balıklar tarafından fitalatların metabolize edilmesi ileri sürülmüştür.

Son yıllarda tüm dünya genelinde fitalat esterlerinin gıdalar içerisinde yer alması ile ilişkili çalışmalara yoğunluk verilmiştir. Farklı ülkeler tüketime sunulan birçok gıda çeşidinde fitalat esterleri analizi yapmış, maruz kalınma düzeylerini belirlemiştir. Ancak görüleceği gibi su ürünleri ile ilgili çalışmalar toplam gıdalar içerisinde küçük bir yer almakta ya da genel olarak et sınıflandırması adı altında göz ardı edilmektedir.

Fierens ve ark. (2012a) ev ortamında salmon balığını farklı şekillerde pişirmiş ve fitalat içeriğini tespit etmiştir. Hammadde olarak kullandığı salmon balığında 153.85 µg/kg DEHP, 8.08 µg/kg DnBP, 5.8 µg/kg DiBP, 1.36 µg/kg BBP ve diğer fitalatları belirlemişlerdir. Özellikle alüminyum folyo ile ızgarada pişirme sonrasında 4253 µg/kg DEHP, 9.62 µg/kg DnBP seviyelerine çıkıldığı görülmüştür. Kızartma işlemi ile fitalat içeriğinde hafif bir yükselme tespit edilmiştir.

Belçika marketlerinde satışa sunulan gıda örnekleri içerisinde en yüksek fitalat %81 ile DEHP, %75 ile DiBP, %69 ile DnBP ve %58 ile BBP ile ulaşılmıştır (Fierens ve ark., 2012b). İncelenen 18 adet balık ve balık ürünlerinde medyan DEHP 86 µg/kg ve DEP 0.6 µg/kg belirlenmiştir. Aynı yıl bir başka araştırmacı tarafından Belçika'da yapılan çalışmada ise okul öncesi ve yetişkin bireylerde DEP, DEHP, BBP ve DnBP açısından sağlık riski bulunmadığı, bu fitalatların günlük tolere edilebilir sınır değerlerinin çok altında olduğu belirlenmiştir (Sioen ve ark., 2012). Ancak yapılan ikinci çalışmada su ürünlerine yer verilmemiştir.

Kamboçya'nın üç farklı yerleşim yerinde tüketilen ürünlerde fitalat analizleri gerçekleştirilmiş ve tüm gıda ürünlerinde en yüksek değere DEHP ve DiBP ile ulaşılmıştır (Cheng ve ark., 2016). Bunun sebebi olarak da PVC içerisinde plastikleştirici olarak yaygın kullanılması nedeniyle direkt salınım, sızma, evaporasyon, aşınma ve migrasyon yolları ile gıda zincirine dahil olması öne sürülmektedir.

Çeşitli gıdalarda fitalat analizinin yapıldığı bir çalışmada 3 adet su ürününde tespit edilen fitalat esterlerinin medyan değerleri sırasıyla DEHP 96 ng/g, DiBP 9.59 ng/g, DBP 4.41 ng/g, DEP 3.23 ng/g ve DMP 0.47 ng/g'dır. Analize alınan tüm gıdalar dikkate alındığında Çin'de tüketime sunulan gıdalar içerisinde en yüksek fitalat esteri DEHP'ye ait olarak belirlenmiştir (Guo ve ark., 2012).

ABD New York'ta satışa sunulan gıdalar içerisinde toplam 5 adet su ürününde çalışma yapılmıştır. Plastik kapakla sarılmış köpük tabak içerisinde salmon, kağıt içerisinde ton balığı ve karides, metal kaplar içerisinde doğranmış istiridye ve sardalya örneklerinde fitalat analizli yapılmıştır. Örneklerin %80'inde DEHP belirlenmiş ve konsantrasyonu 39.6ng/g olarak tespit edilmiştir. DBP örneklerin %40'ında 0.7 ng/g, DEP %60 oranı ile 0.86 ng/g, DMP %40 ile 0.1 ng/g, BBP %40 ile 0.1 ng/g, DiBP %40 ile 0.1 ng/g medyan değerleri tespit edilmiştir (Schecter ve ark., 2013).

Fitalatların gıda ile teması gıdanın üretildiği PVC boru hattından başlayarak paketlendiği ambalajlar, kağıt ve plastikler için kullanılan yapıştırıcılar, şişe kapaklarındaki mühürler, etiket boyalarına kadar çok çeşitli alanlarda gıda ile temasa geçebilmektedir. Tsmura ve ark. (2001) DEHP içeren el-

divenlerin yasaklanması sonrası gıdalarda yaptıkları fitalat analizlerinde DEHP ve BBP seviyelerinin önemli oranda azaldığını tespit etmişlerdir.

Sonuç

Tarım bakanlığı verileri kişi başına yıllık su ürünleri tüketimini 2015 yılı için 6.2 kg olarak belirtmektedir. Su ürünlerinin az tüketilmesi, beraberinde fitalat tüketiminin de az olacağı anlamı taşısa da, her bir gıda ürününden sindirim yoluyla vücuda alınan toplam fitalat esterleri düşünüldüğünde kümülatif etki nedeniyle sorunlar yaşanabileceği unutulmamalıdır. Bir birey aynı gün içerisinde birçok fitalata maruz kalmaktadır. Ayrıca yukarıda da belirtildiği gibi fitalat esterleri sadece sindirim değil, solunum ve deri teması gibi yollarla da vücudumuzda birikmektedir.

Gıda ürünlerinin raf ömrünün belirlenmesinde genelde gıdanın bozulmasına, tüketilemeyecek sınıra ulaşmasına göre değerlendirmeler yapılmaktadır. Oysa ki özellikle yağlı ve soslu ürünlerde gıda maddesine kimyasal madde geçişi de göz önünde bulundurulmalıdır. Birçok plastik materyalin içerisinde fitalat olmasına rağmen bu bilgi etikette yer almamaktadır. Farkında olunmadan yoğun miktarda maruz kaldığımız fitalat ve fitalat esterleri konusunda üretici ve tüketicilerde bilinçlenme sağlanması gerekmektedir. Yasal sınırlamalar bulunmasına rağmen düzenli kontroller yapılarak, fitalat esterlerinin kullanım oranları ve gıda ürünlerine nüfuz etmeleri takip edilmelidir.

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3(2): 67-74 (2017) doi: 10.3153/JFHS17009

Journal of Food and Health Science

E-ISSN 2149-0473

ORIGINAL ARTICLE/ORİJİNAL ÇALIŞMA

FULL PAPER TAM MAKALE

THE FACILITIES OF SPRAY DRIED HONEY POWDER USE AS A SUBSTITUTE FOR SUGAR IN COOKIE PRODUCTION

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Received:.01.01.2017

Accepted: 06.02.2017

Published online: 21.03.2017

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Abstract:

The cookie stands out as a high sugar content product. Recently, with the discussion of the adverse effects of sugar on health, a high number of food materials have been used as a sugar substitute. One of these is honey which was also used as sweeteners in the past. Honey might be regarded as a good alternative due to its natural origin, and its high content of vitamins, minerals and antioxidants. In this study, the mixture of honey maltodextrin (60/40%) resulting from spray-drying was incorporated in different proportions (0, 20, 40, 60, 80, 100%) instead of sugar, so the target was both to minimize the negative effect of sugar on health and to create a functional food product, enriched by nutrients. In the cookies produced, some physical, sensory, chemical and nutritional properties were investigated. With the substitution of honey powder, the diameter of the cookies and spread ratio decreased, it was found that the thickness values did not change. In addition, the hardness and a^* values have increased and L^* and b^* values have decreased. In terms of chemical properties; moisture, ash, mineral, total phenolic contents increased with the increasing amount of honey powder but there were not significant changes in water activity, crude protein, crude fat values of cookie samples. Thus, energy values were decreased. Consequently, it was found that substitution of sugar with 100% honey powder is suitable to improve cookies chemical and nutritional characteristics and up to 60% is suitable to protect sensory and physical properties.

Keywords: Honey powder, Nutrition, Cookie, Substitution, Sugar

Introduction

Biscuits and cookies have amazingly become one of the most desirable desserts for both youth and old people owing to low manufacturing cost, more convenience, variety in taste, crispiness, digestibility and longer shelf life (Akubor, 2003; Hooda and Jood, 2005; Hussain *et al*., 2006; Jayasena and Nasar- Abbas, 2011; Demir, 2014). Most bakery products can basically be enriched and fortified (Indrani *et al.,* 2007). A large variety and quantity of materials is produced industrially in powder form (Fitzpatrick *et al*., 2004; Fitzpatrick *et al*., 2007). Recently, additives have come into common usage in the baking industry. Lots of artificial sweeteners, which are sweeter than sucrose and nontoxic, have been developed and identified to substitute of sugar. During development of sugar-free formulations, the use of both an alternative sweetener and a bulking agent is employed (Savitha *et al*., 2008).

Honey, a natural biological product evolved from nectar and of great benefit to human beings both as food and medicine (Hebbar *et al*., 2003), contains high sugar such as fructose and glucose (80-90%) (Bogdanov, 2011; Satvihel *et al*., 2013), and water, in addition to small quantities of proteins, minerals, organic acids, and vitamins (Hebbar *et al*., 2003). It is consumed due to its unique aroma and taste as well as its numerous health-promoting properties (Alvarez-Suarez *et al*., 2010; Samborska *et al*., 2015). Honey in its natural form has several disadvantages as a result of high density and viscosity which cause difficulties in transportation and dosage (Cui *et al*., 2008; Hebbar *et al*., 2008; Samborska *et al*., 2015), and also leading to problems in mass production operations (Cui *et al*., 2008; Samborska and Czelejewska, 2014). It can change its properties as a result of cristalization (Shi *et al*., 2013), which may contribute to the development of osmophile yeast and fermentation (Bhandari *et al*., 1999; Hebbar *et al*., 2008; Samborska *et al.,* 2015).

Production of honey dry powder is difficult mainly because of the high content of sugars and organic acids (Truong *et al*., 2005; Rodriguez-Hernandez *et al*., 2005; Zareifard *et al*., 2012; Murugesan and Orsat, 2012; Samborska *et al*., 2015). Dried honey, like the powders can be used for direct consumption, applied as an additive to a range of food products such as beverages, yogurts, snacks, sauces, edible coatings, as well as dietary supplements. The use of dried honey as an additive for some bakery products enhance their attractiveness, improves their flavour, aroma, color, texture and helps to maintain high product quality (Samborska and Bienkowska, 2013). The honey powder is frequently produced by adding ingredients such honey, anti-caking agent, emulsifier, and filler materials of high molecular weight to increase glass transition temperature of a mixture and to minimize the problem during drying (difficult to dry and sticky) (Bhandari and Howes, 1999). The filler materials used are carbohydrate group such as starch, maltodextrin, carboxy methyl cellulose, arabic gum, and protein group such as gelatin (Barbosa-Cánovas *et al*., 2005). Honey powder with its low moisture content has the capability to be easily mixed with other ingredients apart from other advantages including convenience, ease of handling, reduced storage space, sanitation and storage for a longer period. Various methods of drying honey have been used such as spray drying, vacuum drying, tunnel drying and solidification into blocks by crystallization (Cui *et al*., 2008). Nevertheless, drying of honey poses many problems such as low recovery rates because of its high sugar content (Wang and Langrish, 2009) and also utilization of at least 50-70% of additives to obtain a dried powder (Cui *et al*., 2008). Spray drying of high sugar content liquids like honey involves the use of additives that serve as drying agents such as maltodextrin and gum Arabic (Cano-Chauca *et al*., 2005; Wang and Langrish, 2009). The conversion of liquid honey into powder form by spray drying may have the problems of hygroscopicity and stickiness which is mainly because of the presence of a high proportion of low-molecular-weight sugars in honey (Adhikari *et al*., 2007). The sticky problem leads to important economic loss and operating problems during drying, and so limits the application of spray drying for food and pharmaceutical materials (Maa *et al*., 1998; Boonyai *et al*., 2004).

Honey, which is one of widely consumed foods, has considerable nutritional properties with respect to sugar. In this study, honey, a natural source of sugar, was used in the production of cookies as a replacement of sugar. For this purpose, honey was produced in granulated form and the experiment was carried out with mixture of the granulated form of honey and maltodextrin as a carrier (60-40% v/w) using a spray-dryer unit. Then, the obtained honey powder (HP) was used as a replacement of sugar in different levels (0, 20,

40, 60, 80 and 100%) for the production of cookies. With the present study, it was aimed to determine the effect of HP addition on the physical, chemical, nutritional and sensory properties of the cookies.

Materials and Methods

Materials: Wheat flour, sodium bicarbonate and ammonium bicarbonate were obtained from Golda Biscuit and Food Industry A.Ş. (Karaman, Turkey). All-purpose shortening, skimmed milk powder, salt, sugar and flower honey were procured from local market in Konya, Turkey. High-fructose corn syrup (HFCS-F55) and maltodextrin (Dry MD-01915) were purchased from Cargill (Turkey). The samples were kept at +4°C till the analysis.

Honey powder production: Honey and maltodextrin (as a carrier) (60-40% v/w) was spray dried by Niro-Atomizer laboratory type pilot drying unit in the plant of Enka Dairy and Food Products Co., Konya, Turkey. The procedure took 60 min with an inlet air temperature of 200ºC and an outlet air temperature not exceeding 70ºC. Particles sizes were in the range of 5-25 µm.

Production of cookies: The cookies were prepared by modifying method 10-54.01 of AACCI (AACCI, 2000). Following recipe was used for the preparation of cookies in Table 1. HP was used as a replacement of sugar in different levels (0, 20, 40, 60, 80 and 100%) for the production of cookies. All ingredients used for cookie preparation were kept at room temperature. Cookie dough was mixed in Kenwood mixer (Kenwood KMX-50, United Kingdom). The dough was sheeted to a thickness of 5 mm and cut into round shapes using a 55 mm diameter dough cutter. The dough was transferred to aluminum trays and placed in a baking oven (LG MP-9485S, Seoul, Korea). These were baked at 160ºC for 10 min. Afterwards the cookie samples were allowed to cool at room temperature (22ºC) and these samples were packaged in polyethylene bags, until used.

Analysis methods: The AACC International methods were used for the determination of moisture (method 44-19.01), ash (method 08-01.01), crude protein (method 46-12.01) and crude fat (30-25.01) contents (AACCI, 2000). Water activity was measured with an Aqualab apparatus (Decagon Devices Inc., Model series 3TE, USA). Pure water (1.000 ± 0.003%) was used as standard for equipment calibration.

A digital micrometer (0.001 mm, Mitutoyo, Minoto-Ku, Tokyo, Japan) was used to measure the dimensions (diameter and thickness) of the cookie samples (AACCI method 10-54.01). The spread ratio was found using the following formula;

Spread ratio = Diameter (D) / Thickness (T)

The hardness of cookie samples after baking was measured in Newton's by a texture analyzer (TA-XT plus, Stable Microsystems, UK) equipped with 3-point bend ring. Three cookies were selected randomly and applied to the base of analyzer. Settings included pre-test speed of 1mm/s, test speed of 3mm/s, post-test speed 10mm/s, distance 5 mm, trigger force 50g and load cell: 30 kg.

Carbohydrate values are calculated; CHO %=100 – (moisture % + crude protein % + crude fat % + ash %). Energy values are calculated; energy (kcal/100 g) = [4 x (CHO % + crude protein %) + 9 x (crude fat %)] (Karaağaoğlu *et al*., 2008).

Table 1. Formulation of cookies

Ingredients	Control Weight (g)	20% HP	40% HP	60% HP	80% HP	100% HP
Wheat flour	100					
Sugar	42.0	33.6	25.2	16.8	8.4	0
Honey powder (HP)	0	8.4	16.8	25.2	33.6	42
All- purpose shortening	40.0					
High fructose corn- syrup	1.5					
Salt (NaCl)	1.25					
Skimmed milk power	1.0					
Sodium bicarbonate	1.0					
Ammonium bicarbonate	0.5					
Deionized water	Variable (13-17 mL)					

Color measurement was performed using Hunter Lab Color Quest II Minolta CR 400 (Konica Minolta Sensing, Inc., Osaka, Japan). The color measurements were determined according to the CIELab color space system (Francis, 1998). Color was expressed as *L** (100 = white ; 0 = black), *a** (+, redness ; -, greenness), and *b** (+, yellowness ; -, blueness).

The mineral (Ca, Fe, K, Mg, Mn, P and Zn) contents of the raw materials and cookie samples were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) (Vista series, Varian International AG, Switzerland) with an automatic sampler system. Approximately 0.5 g of the sample was put into a burning cup, and 5 mL of HNO_3 +5 mL H_2SO_4 was added. The samples were incinerated in a microwave oven (Mars 5, CEM Corporation, USA). The solution was diluted to 100 mL with water. Concentrations were determined by ICP-AES (Bubert and Hagenah, 1987).

Total phenolic content (TPC) was determined using the Folin-Ciocalteau method (Singleton and Rossi 1965). The TPC was used a Hitachi-U1800 spectrophotometer (Hitachi High-Technologies, Tokyo, Japan). The results were expressed as µg gallic acid equivalents per g sample.

Sensory evaluation of cookies: The sensory evaluation was performed by a panel of panelists, consisting of scientific staff of the department of Food Engineering, Faculty of Engineering and Architecture, University of Necmettin Erbakan, chosen for their experience in the sensory analysis of food. Cookie samples were evaluated by ten panelists, who are familiar with the characteristics of cookies. Ages ranged from 21 to 55. Seven of them were females. All panelists were non-smokers. Instructions were given in full to panelists beforehand. The samples were brought to room temperature before testing. The samples were coded with letters and the order of sample presentation was completely randomized for serving to the panelists to guard against any bias. The panelists cleansed their palates with water before rating each sample. The panelists were asked to score the cookie in terms of color, taste odor, appearance and overall acceptability using a hedonic scale. Each feature, a score between 1 and 5 (5: very good; 4: good; 3: moderate; 2: poor; 1: very bad) to be evaluated over 5points.

Statistical analysis: A commercial software program (Tarist, version 4.0; Izmir, Turkey) was used to perform statistical analyses. Data were assessed by analysis of variance. Means that were statistically different from each other were compared using Tukey-Q tests at 5% confidence interval. Standard deviations were calculated using the same software.

Results and Discussion

Analytical results: The investigated characteristics of honey powder were: *L** values 93.37 ±0.47, *a** values -0.68 ±0.03, *b** values 9.80 ±0.14, moisture 3.47 ±0.05%, ash 0.23 ±0.01%, water activity 0.30 ±0.01, total phenolic content 0.58 ±0.01 µg GAE/g, calcium 24.93 ±0.6 mg/100g, iron 1.57 ±0.04 mg/100g, potassium 76.52±1.26 mg/100g, magnesium 12.71 ±1.70 mg/100g, manganese 0.25 ±0.01, phosphorus 122.63 ±3.49 mg/100g and zinc 0.54 ±0.01 mg/100g. Also, the approximate composition of wheat flour used in this study was *L*, a*, b* values* 93.14 ±1.42, -0.72 ±0.17, 9.20 ±0.35 respectively, moisture 12.15 ±1.07%, ash 0.59 ±0.01%, crude protein 10.48 ±0.11%, crude fat 0.45 ±0.08%, water activity 0.51 ±0.04 and total phenolic content 0.66 ±0.03 µg GAE/g.

Physical properties of cookies: The effect of HP on physical characteristics of cookies including diameter, thickness, spread ratio, hardness and color (*L**, *a** and *b**) were given in Table 2. According to the Table 2, the addition of HP to the cookie samples resulted in a slight increase in the product thickness values. However, the cookie samples did not have any significant effect ($P < 0.05$) thickness values. Also, cookie diameter values decreased as levels of HP and this led to a reduction in spread ratio. The lowest spread ratio (7.24 ± 0.12) and the highest hardness (45.17 ± 0.16) values were obtained for the cookies made up with 100% HP. The lowest hardness values were determined for control group. According to these results, the use of HP led to more compact cookie dough and cookies with harder characteristics. Demir (2014) reported that pekmez powder increased hardness of cookies. Color values of cookies were presented in Table 2. According to the Table 2; the brightness (*L**) values of cookies produced with 100% S (control group) were found higher. There were slightly decrease *L** values and increase *a** values with HP addition, but the differences were not statistically significant. Also, the lowest *b** values were determined in the cookies made with 100% HP. Demir (2014) reported that *L** (brightness) values of cookies declined and *a** (redness) and *b** (yellowness) values raised after the replacement of sugar with pekmez powder.

Table 2. Physical, textural and color properties of cookie samples (mean values±SD)[1].

Samples[2]	Diameter (D) (mm)	Thickness (T) (mm)	Spread ratio (D/T)	Hardness (N)	Color values		
					L*	a*	b*
Control (100% S)	64.72±0.40^{a}	8.38±0.24^{a}	7.72±0.17^{a}	28.27±7.14^{c}	70.52±1.30^{a}	3.49±0.13^{b}	27.25±0.28^{a}
80% S : 20% HP	63.56±0.54^{ab}	8.29±0.29^{a}	7.67±0.20^{ab}	30.73±1.03^{c}	66.32±0.96^{b}	4.82±0.18^{a}	27.14±0.44^{a}
60% S : 40% HP	62.57±0.83^{bc}	8.51±0.16^{a}	7.36±0.24^{abc}	32.98±5.04^{bc}	65.86±0.36^{b}	4.88±0.19^{a}	27.09±0.91^{a}
40% S : 60% HP	62.97±0.21^{bc}	8.31±0.08^{a}	7.58±0.10^{abc}	33.48±1.54^{abc}	65.75±0.74^{b}	4.97±0.76^{a}	26.59±0.64^{ab}
20% S: 80% HP	62.13±0.49^{c}	8.62±0.10^{a}	7.21±0.14^{c}	41.31±2.43^{ab}	64.77±1.10^{b}	5.00±0.24^{a}	26.08±0.89^{ab}
100% HP	62.09±0.01^{c}	8.57±0.14^{a}	7.24±0.12^{c}	45.17±0.76^{a}	64.01±0.94^{b}	5.16±0.16^{a}	25.23±0.70^{b}

[1] The means with the same letter in column are not significantly different ($P<0.05$). [2]S: Sugar, HP: Honey Powder

Table 3. Some chemical characteristics of cookie samples (mean values±SD)[1].

Samples[2]	Moisture %	Water activity (aw)	Ash (%)	Crude Protein (%)	Crude Fat (%)	Carbohydrate (%)	Energy (kcal/ 100g)	Total phenolic content (µg GAE/g)
Control (100% S)	2.99±0.02^{d}	0.24±0.05^{a}	1.09±0.01^{f}	6.32±0.06^{a}	18.46±0.49^{a}	71.15±0.56^{a}	475.97±2.38^{a}	0.65±0.02^{e}
80% S : 20% HP	3.68±0.25^{c}	0.20±0.03^{a}	1.12±0.01^{e}	6.34±0.06^{a}	18.05±0.64^{a}	70.81±0.84^{a}	471.01±2.19^{ab}	0.85±0.02^{d}
60% S : 40% HP	3.99±0.01^{b}	0.21±0.03^{a}	1.18±0.01^{d}	6.33±0.08^{a}	18.22±0.28^{a}	70.29±0.35^{a}	470.40±1.44^{ab}	0.92±0.02^{c}
40% S : 60% HP	4.16±0.07^{ab}	0.22±0.01^{a}	1.22±0.01^{c}	6.35±0.06^{a}	18.33±0.42^{a}	69.94±0.41^{a}	470.14±2.42^{ab}	1.00±0.02^{b}
20% S: 80% HP	4.33±0.04^{a}	0.20±0.01^{a}	1.26±0.01^{b}	6.34±0.08^{a}	18.27±0.69^{a}	69.81±0.63^{a}	468.99±3.30^{ab}	1.07±0.03^{ab}
100% HP	4.49±0.01^{a}	0.22±0.01^{a}	1.31±0.01^{a}	6.34±0.05^{a}	18.34±0.78^{a}	69.52±0.81^{a}	468.50±3.96^{b}	1.17±0.03^{a}

[1] The means with the same letter in column are not significantly different ($P<0.05$). Values are dry weight basis. [2]S: Sugar, HP: Honey Powder

Table 4. Mineral content (mg/100g) of cookie samples (mean values±SD)[1].

Samples[2]	Ca	Fe	K	Mg	Mn	P	Zn
Control (100% S)	31.28±1.4^{f}	1.58±0.01^{f}	149.89±0.78^{f}	28.90±1.03^{f}	0.60±0.01^{f}	216.04±7.17^{f}	0.95±0.01^{f}
80% S : 20% HP	33.66±0.1^{e}	1.87±0.01^{e}	159.18±0.66^{e}	31.06±0.26^{e}	0.66±0.01^{e}	238.08±0.89^{e}	1.03±0.03^{e}
60% S : 40% HP	37.68±0.2^{d}	2.05±0.03^{d}	175.21±1.48^{d}	32.89±0.80^{d}	0.73±0.01^{d}	262.54±4.12^{d}	1.15±0.01^{d}
40% S : 60% HP	41.73±0.3^{c}	2.24±0.02^{c}	188.96±0.72^{c}	35.73±0.21^{c}	0.79±0.01^{c}	278.22±1.52^{c}	1.28±0.04^{c}
20% S : 80% HP	44.32±0.7^{b}	2.45±0.03^{b}	213.83±2.08^{b}	37.96±0.16^{b}	0.91±0.01^{b}	304.77±0.91^{b}	1.42±0.02^{b}
100% HP	47.99±0.7^{a}	2.58±0.02^{a}	226.73±1.47^{a}	40.80±0.12^{a}	1.03±0.02^{a}	328.50±4.81^{a}	1.58±0.04^{a}

[1] The means with the same letter in column are not significantly different ($P<0.05$). Values are dry weight basis. [2]S: Sugar, HP: Honey Powder

Chemical properties of cookies: Moisture and water activity of cookie samples were given in Table 3. Moisture and water activity values of the cookie samples ranged between 2.99 ±0.02 - 4.49 ±0.01 and 0.20 ±0.01 - 0.24 ±0.05 respectively. According to Table 3, there were not statistically significant changes in water activity, while moisture values significantly changed when sugar was replaced by HP (P<0.05). The moisture content of cookies in control group produced with only sugar (100% S) as sweetener were higher than the other cookie samples, and moisture content of the cookies increased when HP was used instead of sugar.

Also, some chemical properties of cookie samples were given Table 3. Ash values of cookie samples ranged between 1.09 ±0.01 and 1.31 ±0.01 respectively. Ash values significantly changed when HP incorporated to the cookies. The highest ash values were determined in the cookies made with 100% HP, while cookies of control group had the lowest ash content. This was an expected result, because honey is a very rich nutrient product. Crude protein, crude fat and carbohydrate content were not statistically significant changes. Honey protein values are low, but protein quality is high (Alvarez-Suarez *et al*., 2010).

Carbohydrate content of the cookie samples ranged between 69.52 ±0.81 and 71.15 ±0.56. Also energy values were changed from 468.50 ±3.96 to 475.97 ±2.38. The highest energy values were determined in the cookies made with 100% S (control group). Total phenolic content were changed ranged from 0.65 ±0.02 to 1.17 ±0.03. There were statistically significant changes (P< 0.05). The highest total phenolic content was determined in the cookies made with 100% HP, while cookies of control group had the lowest total phenolic content. However, honey powder has high total phenolic content, but in the spryer dryer, some phenolic content may effect from temperature.

Mineral content of cookies: The changes in mineral content in cookie samples as a result of HP addition are given in Table 4. According to Table 4, depending on HP addition levels Ca, Fe, K, Mg, Mn, P and Zn showed increasing trend. In other words, the replacement of S with HP and increasing the ratios of this replacement raised mineral content of the cookie samples. Cookie samples containing 100% S (control) had the lowest values of Ca, Fe, K, Mg, Mn, P and Zn minerals. According to the control, Ca, Fe, K, Mg, Mn, P and Zn contents (mg/100g) increased from 31.28 ±1.40, 1.58 ± 0.01, 149.89 ± 0.78, 28.90 ±1.03, 0.60 ± 0.01, 216.04 ±7.17 and 0.95 ±0.01 to 47.99 ±0.70, 2.58 ±0.02, 226.73 ±1.47, 40.80 ±0.12, 1.03 ±0.02, 328. 50 ±4.81 and 1.58 ±0.04 in cookie sample containing 100% HP, respectively. This was an expected result. It was reported by many studies that honey is a very rich source of major and minor minerals (Alvarez-Suarez *et al*., 2010).

Sensory properties of cookies*:* The sensory scores of cookie samples were presented in Table 5. The highest addition level of HP (100% HP) decreased all sensory scores of cookie samples compared to control group (100% S). According to the results, the cookies containing 80% S:20% HP combination had the highest scores for taste. HP addition decreased odor score of cookie. But this decrement was not found significant (P< 0.05). Also, the samples containing HP levels more than 60% had lower scores. Overall acceptability score of cookie containing high HP was assessed with lower sensory scores than containing high S by the panelist. In conclusion, the most preferred cookies in terms of sensory properties were the ones containing 60% HP and 40% S.

Table 5. Sensory properties of cookie samples (mean values±SD)[1].

Samples[2]	Taste	Color	Odor	Appearance	Overall Acceptability
Control (100% S)	4.50±0.45^{ab}	4.50±0.50^{a}	4.20±0.50^{a}	4.90±0.22^{a}	4.60±0.42^{a}
80% S : 20% HP	5.00±0.45^{a}	4.80±0.45^{a}	4.70±0.45^{a}	4.80±0.45^{ab}	4.70±0.45^{a}
60% S : 40% HP	4.60±0.55^{a}	4.90±0.22^{a}	4.20±0.45^{a}	4.50±0.50^{ab}	4.70±0.45^{a}
40% S : 60% HP	4.00±0.45^{bc}	4.00±0.71^{a}	4.20±0.45^{a}	4.20±0.45^{ab}	4.10±0.74^{ab}
20% S : 80% HP	3.90±0.55^{c}	2.90±0.55^{b}	4.00±0.71^{a}	4.10±0.55^{b}	3.50±0.87^{b}
100% HP	3.90±0.55^{c}	2.40±0.55^{b}	4.00±0.71^{a}	4.10±0.55^{b}	3.10±0.74^{b}

[1] The means with the same letter in column are not significantly different ($P<0.05$). [2]S: Sugar, HP: Honey Powder

Conclusion

Sugar-free or reduced-sugar foods are very popular in the World. Cookies contain large amounts of sugar and fat and are usually avoided by dieters. Therefore the creation of low-fat and/or sugarless cookies is a challenge for the bakery industry. In this study, the use of powdered form of honey instead of sugar in cookies was investigated. According to the results, moisture, ash, mineral, total phenolic contents increased with the increasing amount of honey powder but there were not significant changes in water activity and crude fat values of cookie samples. Also, carbohydrate values decreased, descriptively. Thus, energy values were decreased. As a result, HP was successfully incorporated in to cookie formulation. It was found that substitution of sugar with 100% honey powder is suitable to improve cookies chemical and nutritional characteristics and up to 60% is suitable to protect sensory and physical properties.

Acknowledgements

This research was summarized from *Master Thesis (*Necmettin Erbakan University the Graduate School of Natural and Applied Science Department in Food Engineering) by Mehmet KILINÇ.

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3(2): 75-81 (2017) doi: 10.3153/JFHS17010

Journal of Food and Health Science

E-ISSN 2149-0473

ORIGINAL ARTICLE/ORİJİNAL ÇALIŞMA

FULL PAPER TAM MAKALE

INVESTIGATION OF THE PRESENCE AND VIRULENCE TRAITS OF VANCOMYCIN-RESISTANT *ENTEROCOCCUS* IN WATER SAMPLES

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Received: 16.01.2017

Accepted: 27.02.2017

Published online: 24.03.2017

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Abstract:

The aim of this study was to determine the incidence of vancomycin-resistant enterococci (VRE) in tap and artesian well waters and to detect the vancomycin resistance genes and virulence genes of the isolates obtained from the samples. For this purpose, 200 samples (119 tap and 81 artesian well waters) were collected from several water supplies during November 2013 and June 2015 period in Bursa province. Seven isolates were recovered from artesian well waters and confirmed as *Enterococcus* by PCR method. *E-test* performed for vancomycin and teicoplanin MIC values indicated that only two isolates had the intermediate-level (8 µg/mL) resistance to vancomycin. No resistance was observed to teicoplanin in any of these isolates by *E-test*. All of 7 isolates were tested for vancomycin resistance genes (*van*A, *van*B and *van*C) and virulence genes (*gel*E, *agg, esp* and *ace*). The results showed that enterococci isolates had no these genes. The present study suggested that the presence of the intermediate level VRE in artesian well waters and, also the waters from environmental supplies near human and animal niches could be play a role as potential reservoirs for enterococci having several types of resistance to vancomycin. Also, vancomycin resistant strains can be possible the spread in environment and also the transmission to human and animals through contaminated water sources.

Keywords: Water, Artesian well water, Enterococci, Vancomycin resistance, Virulence

Introduction

Enterococci are natural inhabitants of humans and animals gastrointestinal tract but are also appeared in the water, soil, plants, and food (Strateva *et al.,* 2016). Besides being the hygiene quality indicator for water, enterococci have been proposed as indicator bacteria for antimicrobial resistance (Boehm & Sassoubre 2014). Vancomycin is a antibiotic, strongly affects Gram-positive bacteria for the treatment of serious, life-threatening infections, when other antibiotic treatment did not work (Varela *et al.,* 2013). Vancomycin resistance in enterococci have been occured all over the world since 1986 (Cetinkaya *et al.,* 2013). Vancomycin resistance was described six phenotypes as *van*A, *van*B, *van*C, *van*D, *van*E and *van*G. *Van*A type strains possess high-levels of resistance to vancomycin and teicoplanin. *Van*B and *van*C genes generate low-level resistance to vancomycin. *Van*C phenotype differs from others to its species-specific characteristic. It has seen in only *Enterococcus casseliflavus* and *E. gallinarum* strains (Messi *et al.,* 2006).

Enterococci is a well known bacteria to have various virulence factors associated with hospital infections. Enterococcal surface protein, encoded by the *esp* gene has been related contributing with colonization of urinary tract, and biofilm formation. The collagen-binding protein gene, *ace*, is involved in attachment and colonization of renal tissue in animal models (Sidhu *et al.,* 2014). The aggregation substance (*agg*) takes a part on adhesion to eucaryotic cells and extracellular matrix proteins. Gelatinase, encoded by *gel*E gene, hydrolyses diverse biological peptides such as gelatin, collagen and casein. Another virulence trait cytolysin (*cyl*) is an extracellular toxin, which lyses array of procaryotic and eucaryotic cells (Buyukyoruk *et al.,* 2014). Due to its antimicrobial resistance and virulence factors, enterococci has been not considered generally recognised as safe (GRAS) bacteria and has been known as emerging pathogen of humans. Enteroccocci plays a potential role in hospital associated infections (Cariolato *et al.,* 2008). *Entrococcus* species has advanced active gene transfer mechanisms for transmission of antibiotic resistance and virulence factor genes by plasmids (Chajecka-Wierzchowska *et al.,* 2016). Habitats such as water, soil and food are considered as possible reservuars of antimicrobial resistance and virulence genes of *Enterococcus* strains (Sidhu *et al.,* 2014).

The presence of enterococci in aquatic environments can lead to infection, when water is utilized for drinking water production, recreational activities, irrigation or shellfish harvesting. Treatment of individual diseases, caused by antimicrobial resistant bacteria, with drugs is trouble. Enterococci have a natural tendency to transmit antimicrobial resistance genes to other bacteria species by mobile genetic elements (Servais & Passerat 2009**)**.

The objective of this study was to estimate the frequency of vancomycin-resistant enterococci (VRE) contamination in tap and artesian well waters from various sources and to investigate its virulence traits and vancomycin resistance gene profiles.

Materials and Methods

Water Sampling

A total of 200 water samples including 119 tap and 81 artesian well waters (unchlorinated) were collected from different sources in Bursa province between November 2013 and June 2015. Seasonal distribution of sample numbers was 31, 60, 39 and 70 in autumn, winter, spring and summer, respectively. Tap waters were taken from taps in public places (university, schools, cemetery, mosque, fountain) and from indoor taps. On the other hand, artesian well water samples were provided from artesian pumps and taps without being connected to public water system and supplied from villages and their neighbourhoods. Samples were taken in 1000 mL sterile glass bottle and transported to the laboratory under refrigerated conditions. All bacteriological analyses were carried out on the same day.

The Isolation and Presumptive Identification of Enterococci

100 mL water sample was shaken well to mix and filtered through membrane filter (pore size, 0.45 µm; diameter, 0.47 mm) and filter page was placed in Enterococcal Broth supplemented with 6 µg/mL vancomycin at 37 ºC for 24 h. A loopful from each enrichment was streaked on Enterococcal Agar supplemented with vancomycin (6 µg/mL) and plates were incubated at 37 ºC for 24 h. Typical black colonies were described as presumptive vancomycin-resistant *Enterococcus* spp., and the isolates were preserved in Brain Heart Infusion broth containing 30% glycerol at –80 °C for further analyses (Cortes *et al.,* 2006).

PCR Analysis of Enterococcus spp. Isolates

DNA extraction was performed using by Chelex 100 (Sigma Aldrich, USA). *Enterococcus* spp. specific primer was used to amplify *tuf* gene during the confirmation procedure of the isolates. The presence of vancomycin resistance phenotyping genes (*van*A, *van*B and *van*C) and virulence trait genes (*gel*E, *ace, agg* and *esp*) in the isolates were investigated. While the *van*A and *van*B genes were detected by multiplex-PCR technique, the detection of the other genes was performed using classical PCR method. The sequence of primers used in this study is summarized in Table 1. Briefly, samples (1 µl) of each extract were amplified in 25 µl of reaction mixture containing 10 mM Tris-HCl, pH 8.9, 22 mM KCl, 1.8 mM $MgCl_2$, 200 µM each of dNTPs, 0.5 mM each primer and 1.25 U of Hot Start Taq DNA polymerase. PCR amplification procedure of each gene was performed by using thermal cycler (Runik SCM 96G) according to description of references shown in Table 1.

Determination of Vancomycin and Teicoplanin MICs

The minimum inhibitory concentrations (MICs) of vancomycin and teicoplanin were determined by *E-test* according to the CLSI guidelines (CLSI, 2014). Each isolate was cultured on blood agar and then a bacterial suspension equal to 0.5 McFarland turbidity standards in Mueller Hinton Broth was prepared and inoculated onto Mueller Hinton Agar plates. After incubation at 35-37°C for 24 h, MICs are measured on the test strip scale where the zone of inhibition intersect the strip. The isolates that had MICs of ≥32 µg/mL were considered resistant for both antibiotics, MICs of 8-16 µg/mL and 16 µg/mL intermediately resistant, and MICs of ≤4 µg/mL and ≤8 µg/mL susceptible to vancomycin and teicoplanin, respectively. *Enterococcus faecalis* ATCC 29212 was used as the control microorganism.

Table 1: List of oligonucleotide primer sequences used in this study

Gene	Product size (bp)	Oligonucleotid sequences (5′-3′)	Reference
tuf	112	TACTGACAAACCATTCATGATG AACTTCGTCACCAACGCGAAC	Ke *et al.,* 1999
*van*A	1030	CATGAATAGAATAAAAGTTGCAATA CCCCTTTAACGCTAATACGATCAA	Evers *et al.,* 1993
*van*B	433	GTGACAAACCGGAGGCGAGGA CCGCCATCCTCCTGCAAAAAA	Handwerger *et al.,* 1992
*van*C	822	GGTATCAAGGAAACCTC CTTCCGCCATCATAGCT	Dutka-Malen *et al.,* 1995
agg	1553	AAGAAAAAGAAGTAGACCAAC AAACGGCAAGACAAGTAAATA	Eaton & Gasson, 2001
esp	432	TTACCAAGATGGTTCTGTAGGCAC CCAAGTATACTTAGCATCTTTTGG	Shankar *et al.,* 1999
*gel*E	402	AGTTCATGTCTATTTTCTTCAC CTTCATTATTTACACGTTTG	Mannu *et al.,* 2003
ace	320	AAAGTAGAATTAGATCCACAC TCTATCACATTCGGTTGCG	Mannu *et al.,* 2003

Results and Discussion

Vancomycin-resistant enterococci are now recognized as a major cause of nosocomial infections. The presence of VREs in aquatic environments results from urban sewage or livestock faecal material contamination (Nam *et al.*, 2013). In the present work, presumptive vancomycin-resistant enterococci isolates were obtained from 11 of 200 water samples. 7 out of these isolates were confirmed as *Enterococcus* spp. by PCR. All of 7 confirmed isolates were obtained from artesian well water samples. None of the tap water samples were observed to be contaminated with vancomycin-resistant enterococci. The samples contaminated with *Enterococcus* spp. were collected from different water supplies in west (3 samples), south-east (2 samples) and south-west (2 samples) sides of Bursa province. The sampling time of *Enterococcus* positive isolates is summarized in Table 2. A study conducted in Turkey showed that 13 (23%) out of 57 enterococci from different soil and water samples, animals, raw vegetables and fruits were of intermediate resistance to vancomycin (Oryaşın *et al.*, 2013). Zdragas *et al.* (2008) reported that 35 vancomycin gene-negative strains from seawater in Northern Greece had low-level vancomycin resistance but not high-level VRE.

MIC quantity survey showed that only 2 out of 7 isolates were resistant in the intermediate level (8 µg/mL) to vancomycin. Therefore, the contamination rate of vancomycin-resistant enterococci was considered to be 2.5% (2/81) in artesian well water samples. On the other hand, one isolate had an MIC value of 6 µg /mL and four isolates to MIC of 4 µg /mL, and also these 5 isolates were regarded as susceptible to vancomycin, which is in accordance with reports by other authors. Said *et al.* (2015) suggested that 85 enterococci isolates from 64 wastewater and 50 surface-water samples was susceptible to vancomycin. Vancomycin-susceptible enterococci strains from waters used for human and animal drinking has also been reported from Portugal during 2006 and 2008 (Macedo *et al.*, 2011). Similarly, no VRE were detected in surface waters by Rathnayake *et al.* (2012), in unchlorinated water samples by Wilson & McAfee (2002) and in river samples, municipal and hospital wastewaters by Servais & Passerat (2009). Conversely, a study performed by Varela *et al.* (2013) demonstrated the detection of vancomycin-resistant enterococci from hospital and urban wastewater samples. Again, the VRE prevalence was recorded as 12.9% in the aquatic environmental samples in Korea by Nam *et al.* (2013) and as 25.6% in superficial water samples by Messi *et al.* (2006). Resistance to teicoplanin was not found in any of the *Enterococcus* spp. isolated in our study (Table 2). Some previously published reports also suggested the susceptibility to teicoplanin of enterococci isolates from drinking waters (Macedo *et al.*, 2011), wastewater and surface water samples (Said *et al.*, 2015) and river samples, municipal and hospital wastewaters (Servais & Passerat, 2009).

Table 2: MIC results of presumptive VRE isolates

			Antimicrobial MICs (µg/mL)	
Sample origin	Sampling time	Sample no	Vancomycin	Teicoplanin
Artesian well water	December 2013	29	4	1.0
Artesian well water	June 2014	133	8	0.50
Artesian well water	July 2014	149	8	1.50
Artesian well water	July 2014	151	4	0.75
Artesian well water	March 2015	163	4	0.125
Artesian well water	April 2015	174	6	1.50
Artesian well water	June 2015	199	4	1.0

All of two intermediate-level vancomycin-resistant enterococci and five vancomycin-susceptible isolates were also analysed for the presence of vancomycin resistance genes (*van*A, *van*B and *van*C) and virulence genes including gelatinase (*gel*E), aggregation substance (*agg*), enterococcal surface protein (*esp*), collagen binding protein (*ace*). The results indicated that neither *van*A, *van*B, and *van*C genes nor *gel*E, *agg*, *esp* and *ace* genes were found in any of the seven isolates. In comparison to our work, studies performed by Nam *et al.* (2013) showed that sixty-three and one of 64 enterococci colonies, which were positive for van genes, had the *van*C-2/3 genotype and the *van*C-1 genotype, respectively. The same authors reported the absence of the *van*A and *van*B types which is in line with our observations. Contrary to our findings regarding virulence genes, Rathnayake *et al.* (2012) noticed the presence of *esp* and *gel*E genes in *E. faecalis* and *E. faecium* water isolates. Recently, *gel*E, *efa*A, *ace* and *asa*1 genes were reported to occur in *Enterococcus* isolates from surface waters (Sidhu *et al.*, 2014). A study made by Messi *et al.* (2006) suggested that 3 (0.7%) isolates from superficial waters belonged to the *van*A, 53 (13.7%) to the *van*B and 43 (11.1%) to the *van*C phenotype.

Conclusion

People contact to water from different sources every day. As well as artesian well water do not drink to people, it is generally used on irrigation in agriculture or washing in particularly rural areas. These play an important role for transmission of enterococci to human hands, skin or stuff. In this way, antibiotic resistance genes and virulence traits in enterococci can carry over big areas. The present study revealed that only two isolates from artesian well waters were intermediately resistant to vancomycin, and none of the isolates were positive for the vancomycin resistance genes (*van*A, *van*B and *van*C) and virulence genes (*gel*E, *ace*, *agg* and *esp*). But still, it must be considered that these water sources could act as a reservoir for resistant bacteria. Prevention efforts against the risk of spread to and transmission of these genetic determinants in the environment must be focused on the prudent use of antimicrobial agents in healthcare and livestock production.

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