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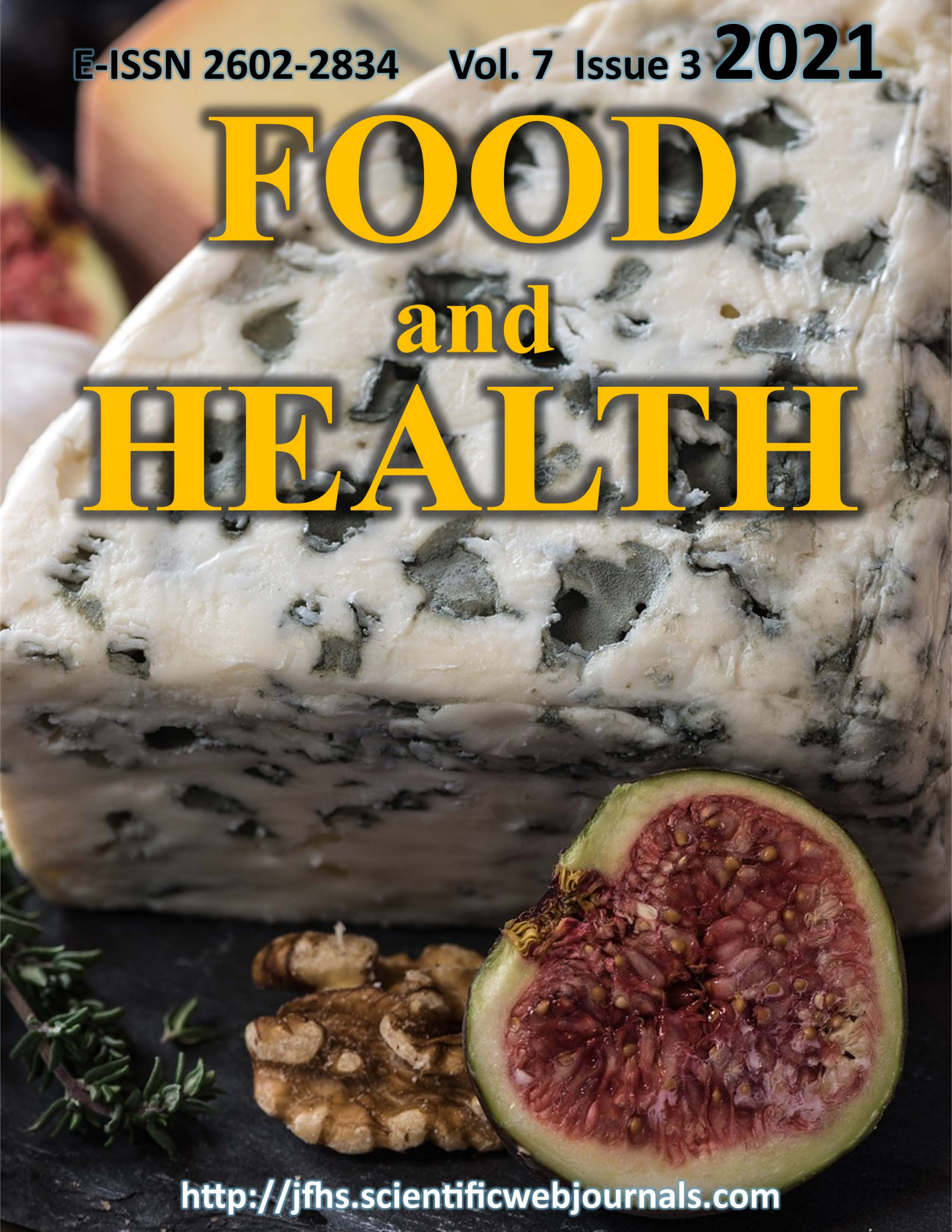

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"Food and Health" journal will publish peer-reviewed (double blind) articles covering all aspects of **food science and their health effect** in the form of original research articles (full papers and short communications), and review articles. Their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

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

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Content

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Essential oil profile of six spontaneous hybrids from male sterile *Salvia officinalis* L.

Nadire Pelin BAHADIRLI

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ABSTRACT

Herbal medicines and beverages have started to take an essential place in our daily lives. *S. officinalis* is one of the most used herbal tea species in the sage family. Secondary metabolites, especially essential oils, plays an important role in its biological properties. *S. officinalis* essential oil is mostly rich in camphor and thujone, which of these compounds could be toxicological. In the present study, six spontaneous hybrid plants and their parents were analyzed for their essential oil contents. Male sterile *S. officinalis* were used as maternal plant, *S. fruticosa* and *S. aramiensis* were probable paternals where they were cultivated nearby. Grown plants were analyzed by gas chromatography-mass spectrometry. Essential oil compounds were used to identify their relation to each other. The main components of *S. officinalis* were thujone (40.97%), 1,8-cineole (24.65%) and camphor (19.37%). 1,8-cineole content of hybrid genotypes were varied between 35.13-64.92%. Camphor level of hybrids were varied in lower levels as between 2.92-26.35% while thujone content were very low compared to the maternal *S. officinalis* as 0.95-6.83%.

Keywords: Anatolian sage, Breeding, Greek sage, Hatay sage, Hybrid, Principle component

Introduction

Essential oils are complex combinations of volatile, organic compounds that provide the flavor and fragrance of a plant (Tisserand and Yound, 2014). Essential oils had proficiency in the prevention and cure of various diseases and worked as an antiviral, antibacterial, antioxidant, antidiabetic, and anticancer agent (Tanu and Harpreet, 2016). Many of the herbal products contain essential oils besides other biological constituents.

The genus *Salvia*, with approximately 1000 species, is an important genus regarding secondary metabolite contained species in the Lamiaceae family. The genus is widely distributed from the Far East, through Europe and across to the New World (Kintzios, 2000). Flora of Turkey represented by 100 species, seven varieties of the genus *Salvia* (Kusaksiz, 2019). Secondary metabolites of the genus have been studied to determine its antioxidant, antimicrobial, anti-Alzheimer, anticancer and insecticidal properties (Pavlidou et al., 2004; Senel et al., 2010; Exarchou et al., 2015; Sarrou et al., 2016). *Salvia* species has a great value in cosmetic, food and pharmaceutical industries (Carović-Stanko et al., 2016). The amount of trade for nature collected medicinal and aromatic plants are difficult to find, especially in underdeveloped countries. Commercially used *Salvia* species from Turkey are *S. coccinea*, *S. farinacea*, *S. microphylla*, *S. officinalis*, *S. officinalis* 'Incterina', *S. officinalis* 'Purpurascens', *S. officinalis* 'Tricolor', *S. splendens*, *S. x superba* and *S. transylvanica* (Karabacak, 2009). In Turkey, total sage cultivation (species not mentioned) is nearly 412 ha (Karik and Tunctürk, 2019). *S. fruticosa* and *S. officinalis* are the main species that cultivated and exported. Most of the *S. fruticosa* still collect from nature; an also small amount of cultivation has been producing for both *S. officinalis* and *S. fruticosa* (Arslan, 2014). *S. fruticosa*, in 2019, was exported that the amount of 500 tonnes (Kusaksiz, 2019). Three *S. officinalis* varieties (Erada TJ, Güripek and Elif) and one *S. fruticosa* variety (Karık) were recorded (Anonymous, 2020). Cultivation of registered varieties are essential to obtain standardized leaf and essential oil. Essential oil standards of *S. officinalis* were published in ISO 9909:1997. This report dedicated that essential oil composition of *S. officinalis* L. should contain α-thujone (18.0-43.0%), camphor (4.5-24.5%), 1,8-cineole (5.5-13.0%), *β*-thujone (3.0-8.5%), *α*-humulene (≤12.0%), *α*-pinene (1.0-6.5%), camphene (1.5-7.0%), limonene (0.5-3.0%), bornyl acetate (≤2.5%), linalool and bornyl acetate (≤1.0%). Herbal monograph of *Salvia officinalis* was reported from the European Medicines Agency (EMA, 2016). Extensive ranges for compounds could be seen in the report. *S. officinalis* know with its high content of thujone, and thujone reported to be neurotoxic. In the European Union herbal monograph on *S. officinalis* L. suggested that chemotypes with low content of thujone should be preferred (EMA, 2016). New varieties of sage with a high leaf and essential oil yields, also resistant to diseases should be developed.

Salvia species from Turkey's flora are insect-pollinated and outcrossing. There are several studies revealed hybridization in nature (Hedge, 1982). In the Flora of Turkey, Davis (1982) stated that many *Salvia* species create hybrids in the natural flora of Turkey. Hybrids between *S. suffruticosa* × *S. bracteata* named *S.*×*spireaefolia*; and also, from Iranian flora hybridization between *S. suffruticosa*×*S. hydrangea* were reported (Davis, 1982). Furthermore, hybrids between S. *ceratophylla* and *S. aethiopis, S. cyanescens* and *S. candidissima* were reported (Davis, 1982). Flower type (pin, thrum and homestyle) seen as the biggest obstacle for interspecific crossing (Haque and Ghoshal, 1981). In that study, during three years, fourteen *Salvia* species (*S. coccinea*, *S. splendens*, *S. farinacea*, *S. hispanica*, *S. grahamii*, *S. pratensis*, *S. taraxacifolia*, *S. aegyptica*, *S. tilifolia*, *S reflexa*, *S. glutinosa*, *S. verbenaca*, *S. hormium*, *S. lucantha*) were crossed and only in three species positive results were obtained.

S. officinalis, *S. fruticosa* and *S. aramiensis* present in the same section of the genus (Dogan et al., 2008). Natural hybrids from the flora of Croatia were recorded and analyzed with molecular markers (Radosavljevic et al., 2019; Rivera et al., 2019). Spontaneous hybridization between *Salvia officinalis* and *S. lavandulifolia* and *S. officinalis* and *S. fruticosa*, *S. fruticosa* and *S. tomentosa* in the cultivated areas were reported in different researches (Sanchez Gomez et al., 1995; Evropi-Sofia, 2013; Herraiz-Penalver et al., 2015; Bahtiyarca Bagdat et al., 2017). Male sterility of *S. officinalis* sourced from partially and completely undeveloped microspores were reported from the study of Linnert (1955). Essential oils and herb yield were the primary purposes of these studies. Artificial hybridization between *S. officinalis*, *S. fruticosa* and *S. tomentosa* were done by Putiesky et al. (1990), and cultivar called Neve Ya'ar No:4 were recorded (Dudai et al., 1999). Furthermore, artificial hybridization between *S. fruticosa*, *S. officinalis* and *S. aramiensis* were studied (Bahadirli and Ayanoglu, 2019). In these studies, essential oil content and rate of compounds were found in the middle of the parent plants while in some of them higher contents were observed.

The aim of this study was to identify essential oil content and compounds of the spontaneous hybrids from the seeds of male sterile *S. officinalis* that cultivated nearby *S. fruticosa* and *S. aramiensis*. Furthermore, to reveal their relations with parents by principle component analysis.

Materials and Methods

Plant Material

The seeds of the plant materials used for this study came from experimental field from Department of Field Crops, Hatay Mustafa Kemal University where *S. officinalis*, *S. fruticosa* and *S. aramiensis* were cultivated in nearby plots. Flowering started at the late of March in *S. fruticosa*, beginning of April for *S. officinalis* and mid of April for *S. aramiensis*. In both *S. officinalis* and *S. fruticosa* flowering continue almost two months and flowering overlap in three of the species in the study. To prove the male sterility of *S. officinalis* firstly anthers were removed and examined with triphenyl tetrazolium for vitality of pollens. Secondly, some of the flower stems were covered with net to detect if there is any self-pollination. *S. officinalis* seeds were collected during summer season in 2018. Most of the collected seeds were empty (without any embryo). Selected seeds primed in 500 ppm GA_3 solution for 24 hours before placing in petri-dishes. The germination gen-erally starts in seven days to one month. After germinations of the seeds (3-5 cm), the plants were sown in a plastic viol and placed in a green house. Planting material comprise peat and perlite mix (1/3). When the seedlings grow up to 20 cm, the seedlings planted in plastic pots. During summer time seedlings were placed outside of the green house and watered when needed. Grown hybrid plants were harvested in late July and air dried in drying oven at 35 °C.

Essential Oil Extraction

Dry leaves were hydro-distilled for 3 hours with using Clevenger-type apparatus. Essential oil ratio was calculated as the mean value from dry plant material weight and expressed in g/100 g dry weight (%). Essential oils were kept in amber vials at +4 °C for further analysis.

Essential Oil Analyses

The essential oils were determined according the method described by Bahadirli and Ayanoglu (2019). Seperations and determination of the essential oil components were done by GC-MS (Gas Chromatography Mass Spectrometry) device Thermo Scientific ISQ Single Quadrupole. Approximately 5 µl of essential oil was dissolved in a 2 ml cyclohexane for GC-MS injection. Separation of the essential oils were carried out by a TG-Wax MS (5% Phenyl Polysilphenylene-siloxane, 0.25 mm inner diameter * 60 m length, 0.25 µm film thickness) column. The ionization energy was calibrated as 70 eV, and the mass interval was m/z 1.2- 1200 amu. The scan mode was used as the screening more in data collection. MS transfer line temperature was 250°C, MS ionization temperature was 220°C, and whereas colon temperature was 50°C at the beginning, then it was increased up to 220°C with 3°C/min rate. The structure of each component was defined using mass spectrums (Wiley 9) with Xcalibur software. Retention indices were determined using retention times of n-alkanes (C8-C40) that were injected after the plants essential oil under the same chromatographic conditions.

Principal Component Analysis (PCA)

Comparison of Essential oils between parent species and hybrids were analyzed with PCA using XLSTAT (2009) statistics program. The compounds (PCA) that appeared in an amount higher than 1% in at least one sample were used.

Results and Discussion

The essential oil content of hybrids and their parent plants was determined. *S. officinalis* essential oil (EO) content was 2.5%, *S. fruticosa* EO content was 3.5% and *S. aramiensis* EO content was 2.14%. Essential oil rates of hybrid plants were found as follows H-1 was 2.10%, H-2 was 3.40%, H-3 was 3.0%, H-4 was 3.20%, H-5 was 1.60% and H-6 was 2.5%. *S. officinalis* × *S. lavandulifolia* hybrid essential oil content found in the middle of the parent species and essential oil ranged between 0.9-2.8% (Herraiz-Penalver et al., 2015).

Essential oil compounds were determined by GC-MS analysis and results were given in Table 1. The main components of *S. officinalis* were thujone 40.97%, 1,8-cineole 24.65% and camphor 19.37%. Thujone levels of all hybrid plants were found much lower than maternal plant *S. officinalis*, the range was 0.95-6.83%. All of the hybrid plants' 1,8-cineole range were higher than *S. officinalis* and ranged between 35.13-64.92%. Both of the *S. fruticosa* (50.27%) and *S. aramiensis* (57.76%) had higher 1,8-cineole rate than *S. officinalis*. All of the hybrids 1,8-cineole content were in the middle of the parents except H-2, 1,8-cineole was 64.92%.

Spontaneous hybrid between *S. officinalis* and *S. lavandulifolia* were investigated for essential oil composition (Sanchez Gomez et al., 1995). In the study, hybrid plants' essential oil content found as same as *S. officinalis* 0.60%. Major compounds of *S. officinalis* essential oil were α-thujone 22.82%, 1,8-cineole 15.71%, viridiflorol 10.92%, β-thujone 4.32% and camphor 4.99%, while hybrid plants' essential oil composition found as 1,8-cineole 18.01%, β-pinene 14.11%, camphor 10.80%, α-thujone 3.04% and β-thujone 0.56% (Sanchez Gomez et al., 1995).

Table 1. Essential oil compounds of hybrid genotypes and their parents

Compound Name	RI*	M	F-A	F-F	H-1	H-2	H-3	H-4	H-5	H-6
α-Pinene	1034	0.90	4.34	6.99	5.69	4.05	6.48	5.79	4.47	5.65
Camphene	1098	0.97	0.08	6.82	4.22	0.52	5.56	4.80	5.24	3.12
β-Pinene	1135	1.16	20.03	2.94	4.72	8.09	4.13	4.92	9.57	5.15
α-Myrcene	1160	1.44	1.78	1.38	2.03	2.55	1.63	2.48	2.41	3.15
α-Phellandrene	1179	0.02	1.51	Nd	0.17	0.27	0.07	0.16	0.21	0.19
α-Terpinene	1197	0.04	nd	0.07	0.06	0.15	0.12	0.08	nd	nd
Limonene	1205	0.97	2.67	1.31	1.08	0.98	1.23	1.09	1.03	1.08
γ-Terpinene	1245	0.26	0.13	0.08	0.35	0.73	0.38	0.37	0.12	0.57
1,8-cineole	1278	24.65	57.76	50.27	53.73	64.92	44.17	48.45	35.13	52.92
p-Cymene	1302	0.30	nd	1.00	0.20	0.24	0.21	0.14	0.07	0.36
1-Octen-3-ol	1457	0.06	0.07	0.06	0.06	nd	0.04	0.03	0.21	nd
Sabinene hydrate	1496	0.54	1.24	0.07	0.55	0.67	0.16	0.66	0.37	0.45
Linalool	1535	0.12	nd	0.42	0.35	0.19	0.40	0.2	0.56	0.21
Thujone	1587	40.97	nd	1.06	1.70	6.83	2.47	2.05	0.95	3.30
Valencene	1631	nd	nd	Nd	0.02	nd	0.07	0.08	nd	0.27
Caryophyllene	1662	0.88	4.80	1.07	3.01	2.04	1.38	4.40	1.14	6.67
Bornyl acetate	1700	1.17	nd	0.27	0.38	1.81	0.92	1.45	0.41	nd
α-Terpineol	1701	nd	nd	3.95	1.58	nd	3.21	nd	nd	nd
Camphor	1714	19.37	nd	18.44	15.55	2.92	23.68	16.22	26.35	11.29
α-Humulene	1721	0.14	0.55	0.28	0.31	0.46	0.47	nd	nd	nd
Borneol	1740	1.97	nd	Nd	1.96	0.79	1.70	3.19	0.58	2.43
Geranyl acetate	1760	nd	nd	Nd	nd	nd	nd	0.03	0.35	nd
Viridiflorol	2048	2.18	nd	0.67	0.12	0.06	0.34	0.60	7.24	0.27
Spathulenol	2089	nd	1.13	0.05	nd	nd	nd	nd	nd	nd
Caryophyllene oxide	2084	0.49	0.96	0.92	0.10	nd	0.15	0.45	1.03	0.60
Junipene	2365	nd	nd	0.04	0.03	nd	0.02	0.08	0.73	0.08
Total		98.60	97.05	98.16	97.97	98.27	98.91	97.72	98.17	97.76

nd= not detected, *RI= Retention Indices were calculated according to the *n*-alkanes
M=Mother plant (*S. officinalis*); F-A=Father plant (*S. aramiensis*); F-F=Father plant (*S. fruticosa*); H=hybrid plant

First artificial hybridization between *S. officinalis* and *S. fruticosa* was reported from Putievsky et al. (1990). In the study, thujone levels of hybrids found close to *S. officinalis,* while 1,8-cineole and camphor found in the middle of the parent plants. *S. officinalis* essential oil major compounds found as α-thujone 55.0%, 1,8-cineole 13.0%, β-thujone 10.0% and camphor 2.0%, *S. fruticosa* essential oil major compounds found as 1,8-cineole 48.0%, β-pinene 11.0% and camphor 8.0%. The major compounds of hybrids when *S. officinalis* used as maternal determined as 1,8-cineole 30.0%, α-thujone 27.0%, β-thujone 7.0% and β-pinene 7.0%. When *S. fruticosa* used as maternal plant hybrid, essential oils found as thujone 29.0%, 1,8-cineole 24.0%, β-thujone 7.0% and β-pinene 7.0%. Later that research, artificial hybrid between *S. officinalis* and *S. fruticosa* named Neve Ya'ar No:4 was studied for yield and essential oil characteristics (Dudai et al., 1999). Their results showed that major compounds of essential oil components were camphor (28.19%), thujone (22.20%) and 1,8-cineole (13.67%) (Dudai et al., 1999). Moldavian infraspecific hybrid *S. officinalis* cv. Miracol was analyzed and the major compounds of essential oil were found as α-thujone 21.24%, camphor 19.14% and 1,8-cineole 10.37% (Goncearuc, 2014). A spontaneous hybrid from the cultivated area of *S. officinalis* and *S. lavandulifolia* subsp. *lavandulifolia* essential oil compounds were found as estimated for essential oil compounds. 1,8-cineole rates of *S. lavandulifolia* subsp. *lavandulifolia* found between 15.5-55.1%, in *S. officinalis* 3.3-11.1% and in hybrid 12.0-34.7%. α-thujone rates in of *S. lavandulifolia* subsp. *lavandulifolia* found between 0-0.2%, in *S. officinalis* 25.4-57.2% and in hybrid 13.6-23.6%. Camphor rate in *S. lavandulifolia* subsp. *lavandulifolia* 0.8-6.8%, in *S. officinalis* 1.6-10.8% and in hybrid 1.5-4.1% (Herraiz-Penalver et al., 2015). Another spontaneous hybrid were studied for essential oil content (Bahtiyarca Bagdat et al., 2017). Main components of essential oils showed wide variations

such as α-thujone 8.32-42.46 %, β-thujone 2.02-21.39 %, 1,8-cineole 4.66-29.34 %, borneol 0.91-16.73 % and camphor 4.22-30.77%.

Principle Component Analysis (PCA) on essential oil compounds of all genotypes resulted in that high correlation was observed between EO compounds and genotypes. Thujone and camphor compounds had a negative correlation with 1,8-cineole, *α-β* pinene and camphene. Variables (F) F1 and F2 that explains 95.15% of the variations were chosen to create two-dimensional graphic and results were given in Figure 1. The figure shows the distribution of hybrids and their parents according to their essential oil compounds. S. officinalis placed far from all of the other genotypes in the figure. H-1 and H-2 were in the middle of the *S. fruticosa* while H-3, H-4, H-5 and H-6 were between *S. fruticosa* and *S. officinalis*, but close the *S. fruticosa*.

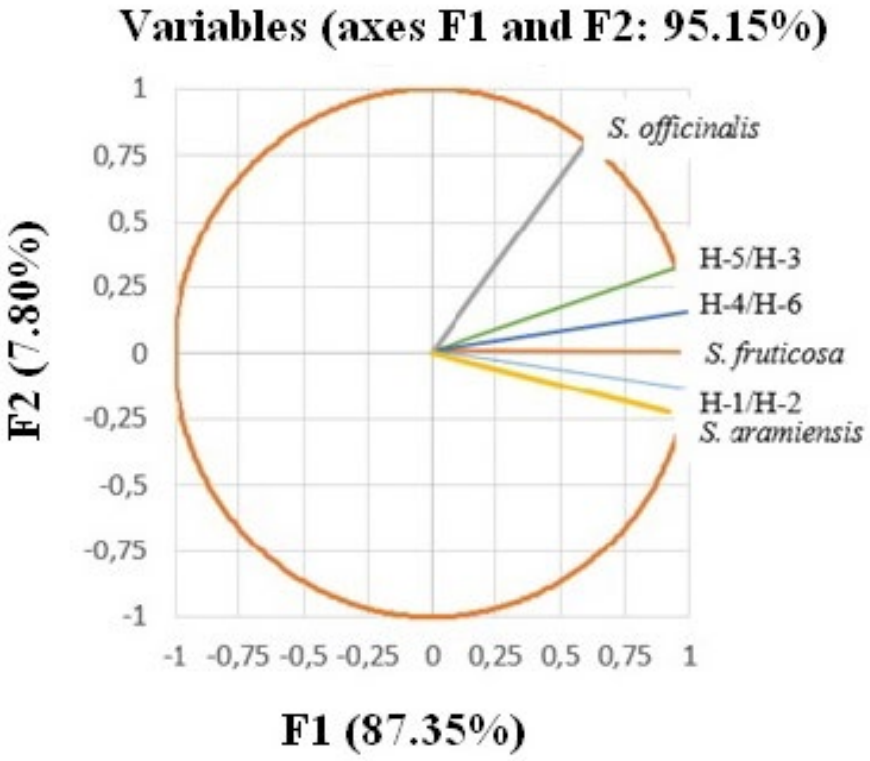

Figure 1. Hybrids and their parents' distribution by PCA according to essential oil compounds

According to the results, genotypes were distributed according to their 1,8-cineole, thujone and camphor content. Results were compatible with other studies. Jug-Ducakovic et al. (2012) were found a high negative correlation between thujone and camphor content in *S. officinalis* genotypes. Cvetkovikj et al. (2015), were analyzed 25 *S. officinalis* population according to their essential oil compounds and genotypes were distributed by high Thujone high trans-caryophyllene content. In the study of Herraiz-Penalver et al. (2015), PCA analysis separated the genotypes regarding of their thujone and 1,8-cineole rate.

Conclusion

The findings clearly illustrate that spontaneous hybridization has been occurred between *S. officinalis*, *S. fruticosa* and *S. aramiensis*. Male sterility of *S. officinalis* helped to identify the hybridization. Developing new cultivars still remains its importance, especially in medicinal and aromatic plants. New cultivars of sage with high yield, low camphor and thujone levels needed in medicinal and aromatic plant market. In the study, high 1,8-cineole with low camphor and thujone content were observed. However, camphor levels were not varied as thujone and 1,8-cineole content. *S. fruticosa* has been used mostly from collected materials from nature. Besides that, *S. officinalis* already has great value for trading. In the study, cultivated plants of *S. fruticosa* and *S. aramiensis* from the flora of Hatay were used. It is important to use existing diversity from flora. The further field trial will have established to obtain yield and patent of the genotypes.

Compliance with Ethical Standard

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

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

Funding disclosure: -

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Disclosure: -

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Incidence and contamination level of *Clostridium perfringens* in meat and meat products sold in Sakarya province of Turkey

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ABSTRACT

Totally 101 meat and meat product samples obtained from local markets and restaurants were analyzed for incidence and contamination level of *Clostridium perfringens*. The typical colonies grown anaerobically on Tryptose Sulfite Cycloserine Agar supplemented with 4-Methyliumbelliferyl (MUP) were confirmed by biochemical tests. Forty-eight of the samples (47.5%) were contaminated with *C. perfringens*. The highest incidence of the pathogen was determined in uncooked meatball samples (72.2%) followed by ground beef samples (61.3%). The incidence of *C. perfringens* in chicken meat, cooked meat döner, cooked chicken döner and emulsified meat product samples were 33.3, 33.3, 28.6 and 16.7%, respectively. Thirteen out of 101 samples (12.9%) yielded typical colonies on TSC-MUP Agar, but could not be confirmed as *C. perfringens.* Average contamination levels in sample groups ranged from 8.3 to 1.5×10^2 cfu/g, with the highest ground beef and the lowest chicken meat.

Keywords: *Clostridium perfringens*, Incidence, Meat, Meat products, Ground beef, Meatball

Introduction

Clostridium perfringens is an anaerobic, non-motile, sulfite reducing, spore-forming, Gram-positive and rod-shaped bacterium (Brynestad and Granum, 2002; Garcia et al., 2019). Spores are usually located sub-terminally and formed only in specially formulated culture media (Juneja et al., 2010). It was isolated in 1892 and named as *Bacillus aerogenes* and then *as Clostridium welchii* (Garcia and Heredia, 2011). It can grow at temperatures from 15°C to 50°C with an optimum at 45°C for most strains (Brynestad and Granum, 2002). *C. perfringens* is very common in nature and can be isolated from soil, dust, gastrointestinal systems of human and animals, on surfaces of vegetables as well as other raw and processed foods (Juneja et al., 2010). Although it has an anaerobic nature, it can grow at E_h values of +350 mV and can reduce its environment to less than -400 mV (Garcia and Heredia, 2011).

Acute diarrhea and severe abdominal pain are observed in 8-24 h following the digestion of the food harbored high number vegetative cells of *C. perfringens*. Vomiting and fever are very rare. Generally the patient recovers in 24-48 h. Death is rare and particularly seen in elderly patients (Labbe and Juneja, 2017). *C. perfringens* is classified as A, B, C, D and E according to toxin type. The type A is related food poisoning, meanwhile can cause gaseous gangrene and septicemia (Brown, 2000; McClane et al., 2012). In the European Union, *C. pefringens* caused 124 out of 160 total outbreaks occurred in 2014 (EFSA-ECDC, 2015). It is estimated that *C. perfringens* poisoning is more common since the mild cases are not reported (Juneja et al., 2010). Considering the relatively mild symptoms, the under-reported cases have been estimated as 10-fold (Mead et al., 1999). Foodborne poisoning associated with *C. perfringens* is due to improper handling and preparation of foods. A recent report revealed that improper stored and inadequate reheated meat could result in large *C. perfringens* outbreak (Mellou et al. 2019). The high number of vegetative cells (>10^6 cfu/g) multiplied in food exposed to elevated temperatures are ingested and then food poisoning occurs (Garcia and Heredia, 2011).

C. perfringens contaminated meat and meat products via fecal contamination of carcasses or contamination from other ingredients such as spices, post-processing contamination is possible as well (Juneja et al., 2010; McClane et al., 2012; EFSA-ECDC, 2015). Although *C. perfringens* can be isolated from different types of foods, it is mostly isolated from meat and meat products. Because meat and meat products are good sources of thirteen amino acids which cannot be produced by *C. perfringens* and needed for growth of this pathogen (Andersson et al., 1995). The previous studies have shown than *C. perfringens* incidence ranged from 7 to 96% in meat and meat products including ground beef (Başkaya et al., 2004; Kamber et al., 2007), chicken meat (Çakmak et al., 2006; Yıldırım et al., 2015), beef and sheep meats (Guran et al., 2014), cured raw meat products (Taormina et al., 2003), emulsified meat products (Elmalı et al., 2005) and cooked döner (Vazgecer et al., 2004). As far as we have known there is not any survey study in the literature on incidence of *C. perfringens* in meat and meat products sold in Sakarya, Turkey. Therefore, considering the survey studies may provide a better understanding the risk for foodborne pathogens, it was aimed in this study to determine the incidence and contamination level of *C. perfringens* in meat and meat products sold in Sakarya province of Turkey.

Materials and Methods

Materials

Totally 101 samples including ground beef (31), chicken meat (27), meatball (18), cooked meat döner (12), cooked chicken döner (7) and emulsified meat products (6) were collected from 57 different butcher shops, markets and fast food restaurants between April 2013 and February 2014 in Sakarya province of Turkey. Samples were transferred to laboratory in cooled conditions and kept in refrigerator until analysis.

Preparation of Samples for Analyses and C. perfringens Enumeration

C. perfringens enumeration and confirmation was performed according to the method described by Rhodehamel and Harmon (1998). Aseptically 25 g sample was transferred into a stomacher bag with 225 mL sterile peptone water (1% peptone) and homogenized (BagMixer® 400, Interscience Co., Saint Nom, France) for two minutes at low speed. Serial dilutions were prepared using peptone water. The pour plating technique was used for enumeration of *C. perfringens*. One mL-portions from 10^{-1} and 10^{-2} dilutions were transferred to sterile petri plates and Tryptose Sulfite Cycloserine (TSC) Agar (Merck, Darmstadt, Germany) containing MUP (4-Methylumbelliferyl phosphate) was poured and mixed well. After solidifying, the plates were overlaid with an additional 10 mL TSC Agar. The plates were incubated anaerobically (Anaerocult A; Merck, Darmstadt, Germany) at 37°C for 24 h. Following incubation plates were examined under 366 nm ultraviolet light (366 nm UV Lamp; Merck Darmstadt, Germany) and the black colonies with blue florescence were counted as *C. perfringens*.

Morphological and Biochemical Confirmation

The typical colonies grown on TSC Agar were picked and inoculated in Thioglycolate Broth (Merck, Darmstadt, Germany). Tubes were incubated anaerobically at 37°C for 24 h. The Thioglycolate Broth cultures of the isolates were maintained at -20°C by adding 15% glycerol. Gram-positive and sporeforming isolates were subjected to biochemical tests. *C. perfringens* was confirmed by motility-nitrate and lactose-gelatin tests (Anonymous, 1999). The active cultures of suspected isolates were stab-inoculated into Motility-Nitrate Medium (Sigma-Aldrich, St. Louis, MO, USA) and incubated anaerobically at 37°C for 24 h. The cultures grown only along the stab line in Motility-Nitrate Medium were considered non-motile, while those grown away from the stab line were considered motile. Red color formation after addition of nitrite-detection reagent (Sigma-Aldrich, St. Louis, MO, USA) showed the reduction of nitrate. If no red color observed within 15 min, zinc dust was added and color of the medium was checked after 10 min. The Lactose-Gelatin Medium (Sigma-Aldrich, St. Louis, MO, USA) tubes inoculated with active cultures were incubated anaerobically at 37°C for 24 h. The gas blisters and converting the color from red to yellow were the signs of lactose fermentation. To detect gelatinase activity, the tubes were kept at 5°C for 1 h and checked for liquefaction. In case solidification occurred, the tubes were incubated at 37°C for additional 24 h. The cultures in liquefied tubes were considered gelatinase positive.

Results and Discussion

Totally 101 samples, including ground beef, chicken meat, uncooked meatball, cooked meat döner, cooked chicken döner and emulsified meat products, were analyzed for incidence and contamination level of *C. perfringens*. Table 1 depicts incidence of the pathogen in the samples. Out of 101 samples, 48 (47.5%) were positive for *C. perfringens*. Although thirteen samples (4 ground beef, 3 uncooked meatballs, 3 chicken meat, and one emulsified meat product) yielded typical colonies on TSC Agar, these colonies could not be confirmed as *C. perfringens* by biochemical tests. This result is not surprising considering the specificity of the culture medium used in this study. Fischer at al. (2012) have mentioned that the specificity of TSC-MUP Agar was 74.5%. In other words this culture medium yielded 24.5% false negative results and non-perfringens *Clostridium* species were isolated frequently. The highest incidence of the pathogen was in uncooked meatball samples (72.2%) followed by ground beef samples (61.3%). The previous studies have shown that incidence of *C. perfringens* and/or sulfite reducing bacteria in beef meat or ground beef is ranged from 18 to 96%. *C. perfringens* incidence was reported as 18% in ground beef samples sold in small butcher shops and local markets (Kamber et al., 2007). Başkaya et al. (2004) determined sulfite reducing anaerobic bacteria in 74% of the ground beef samples. Guran et al. (2014) reported that 96% of beef meat samples were contaminated with *C. perfringens*.

The incidence of the pathogen was 72.2% in meatball samples. Meatball is produced mainly from ground beef and/or lamb meat, fat, roasted bread crumbs, salt, onion garlic and various spices. The spices used to prepare meatball may increase the microbial load of product. De Boer et al. (1985) isolated *C. perfringens* spores from 80% of 54 spices and herbs. It is well known that spices are main source of sporeforming pathogenic bacteria including *Bacillus* and *Clostridium* species and under favorable conditions their spores may germinate and multiply (Pafumi, 1986). Therefore, the spices used in meatball recipe may be the reason for high incidence of *C. perfringens* in meatball samples.

Table 1. *C. perfringens* incidence in meat and meat products

Sample type	Sample number	*C. perfringens* confirmed sample number (%)	Sample number with typical colony on TSC Agar but not confirmed (%)	Sample number with no typical colony on TSC Agar (%)
Ground beef	31	19 (61.3)	4 (12.9)	8 (25.8)
Meatball (uncooked)	18	13 (72.2)	3 (16.2)	2 (11.1)
Chicken meat	27	9 (33.3)	5 (18.5)	13 (48.2)
Meat döner (cooked)	12	4 (33.3)	0 (0)	8 (66.7)
Chicken döner (cooked)	7	2 (28.6)	0 (0)	5 (71.4)
Emulsified meat products	6	1 (16.7)	1 (16.7)	4 (66.6)
Total	101	48 (47.5)	13 (12.9)	40 (39.6)

C. perfringens incidence was confirmed only in 9 chicken meat samples (33.3%). The previous studies have shown that the incidence of this pathogen in poultry products may vary from 2.5 to 94% according to product type. Çakmak et al. (2006) detected *C. perfringens* in 70% of frozen raw ground poultry samples while 2.5% of poultry burger samples were contaminated with this pathogen. Yıldırım et al. (2015) reported that 46% of chicken leg and breast meat samples were positive for *C. perfringens.* Shaltout et al. (2017) determined *C. perfringens* in 21.6% of chicken meats samples. Higher incidence levels were reported by Guran and Oksuztepe (2013). These researchers determined that 66-94% of chicken parts were contaminated with *C. perfringens*. Incidence of this pathogen in intestinal tract of broiler chickens can be as high as 95% (Immerseel et al., 2004). Therefore it is not unexpected situation that its incidence in processed meat of poultry is high.

The incidence of *C. perfringens* in cooked beef and chicken döner samples were 33.3 and 28.6%, respectively. The previous studies revealed that the incidence of sulfite reducing Clostridia incidence is low in döner samples. Vazgecer et al. (2004) reported that sulfite reducing Clostridia were determined in the 7% of the cooked döner samples. In contrast, Bostan et al. (2011) did not determined sulfite reducing Clostridia in cooked döner samples. Spores of *C. perfringens* may survive during cooking and then if cooked foods are kept at temperatures between 12°C and 50°C they can germinate and multiply. Moreover an efficient reheating step may be required to kill vegetative cells (Jaloustre et al, 2013). Döner is a very popular meat meal which is prepared by seasoning of meat with spices and then cooking of cone-like shaped meat mass in front of cooking apparatus. Meat mass is rotated slowly during cooking and the cooked surface layer is cut as thin flakes. In this cooking process, the temperature of meat mass may be at ranges that allow survival and growth of spore-forming bacteria including pathogenic species. Considering cooking method, döner may have risk for *C. perfringens.*

C. perfringens was confirmed only in one emulsified meat product sample (16.7%). Apaydın et al. (2003) reported that 10% of bologna-type sausage samples were contaminated with *C. perfringens*. The incidence of the pathogen in emulsified meat products was determined as 22.1% by Elmalı et al. (2005). The spores of *C. perfringens* are heat resistant with 34.2 min D value at 90°C (Byrne et al., 2006). With this in mind, it can be estimated that they can well survive during production of this type of meat products.

The differences between the isolation rates of the current study and the previous ones may be attributed to number of the samples and analysis methods. The method used in this study did not contain any enrichment step. Wen and McClane (2004) reported approximately 50-fold increase in *C. perfringens* recovery when an enrichment procedure is applied using Fluid Thioglycolate medium.

Fluorogenic substrate, 4-methylumbelliferyl phosphate (MUP), added TSC Agar was used in this study for enumeration and isolation of *C. perfringens* from the meat products. MUP is a fluorogenic substrate which is highly specific for *C. perfringens*. MUP is metabolized by *C. perfringens* producing 4-methylumbelliferone which can be detected under a long wave (366 nm) UV light (Adcock and Paint, 2001). Thirteen out of 101 samples yielded typical colonies on TSC Agar supplemented with MUP. That is to say, false-positive result ratio was 12.9%. Similarly, Araujo et al. (2001) reported false-positive results on this culture medium during recovery of *C. perfringens* from groundwater samples. Despite this, the researchers found that the MUP added TSC Agar was superior to the other culture media used for detection of *C. perfringens*.

The contamination levels in the *C. perfringens* confirmed samples are shown in Table 2. Results revealed that 41.6% of the samples harbored *C. perfringens* lower than 10^1 cfu/g. More than half of the confirmed samples (52.1%) contained the pathogen in the range from 10^1 to 10^2 cfu/g. The contamination level of the pathogen was 10^2-10^3 cfu/g only in two meatball samples. The highest level detected in one ground beef sample was 2.4×10^3 cfu/g. The average counts of sample groups were ranged from 8.3 to 1.5×10^2 cfu/g. According to Turkish Food Codex Regulation on Microbiological Criteria (Anonymous, 2011), the counts of sulphite reducing anaerobic bacteria should not exceed 10^4 and 10^3 cfu/g in non-ready-to-eat and ready-to-eat foods, respectively. Based on this knowledge, it can be concluded that the samples analyzed in current study had acceptable levels of *C. perfringens* as a member of sulphite reducing anaerobic bacteria group. On the other hand, these results are generally in consistent with the published data. Kamber et al. (2007) determined levels of *C. perfringens* in ground beef samples obtained from local markets and butcher's shops as 2.75×10^2 and 6.82×10^2 cfu/g, respectively. Apaydın et al. (2003) reported the incidence of *C. perfringens* as 1-1.27 log cfu/g in bologna-style sausages. The mean number of ground poultry samples was determined as 2.6 MPN/g by Çakmak et al. (2006). On the other hand, Yıldırım et al. (2015) have stated that the mean counts of *C. perfringens* in chicken breast and leg meats were 3.21×10^3 and 1.64×10^4 cfu/g, respectively. These levels are higher than both our study and the previous studies.

Table 2. Contamination levels in *C. perfringens* confirmed samples

Sample type	Number of samples for each contamination level				Average (cfu/g)
	$<10^1$	10^1 - 10^2	10^2 – 10^3	$>10^3$	
Ground beef	6	12	0	1	1.5×10^2
Chicken meat	7	2	0	0	8.3
Meatball (uncooked)	5	6	2	0	5.8×10^1
Meat döner (cooked)	2	2	0	0	2.3×10^1
Chicken döner (cooked)	0	2	0	0	2×10^1
Heat processed meat products	0	1	0	0	1.5×10^1
Total	20 (41.6%)	25 (52.1%)	2 (4.2%)	1 (2.1%)	

C. perfringens vegetative cells higher than 10^6 cfu per gram of food are needed to result in food poisoning (Juneja et al., 2010). The numbers of the pathogen in the samples analyzed did not exceed this level. However, to control temperature during cooking and storage is a key factor to avoid *C. perfringens* poisoning. If the cooked food is cooled down slowly, kept at warm temperatures for extended periods and not reheated sufficiently before consumption to destroy vegetative cells, this critical level may be reached. Additionally, cooking may provide more favorable conditions for the growth of *C. perfringens* by increasing anaerobic environment and reducing competing spoilage organisms (Juneja et al., 2010; Kouassi et al. 2014). Moreover, cooking may cause heat shock which triggers the germination of spores (Juneja et al., 2010).

Conclusion

It may be concluded that *C. perfringens* is very common in raw or cooked meat products having regard to its confirmed presence in almost half of samples (47.5 %) analyzed in this study. The highest incidence was in uncooked meatball samples followed by ground beef samples. Although the contamination levels in samples analyzed in this study were lower than that of required for food poisoning (10^6 cfu/g), it seems that presence of this pathogen in meat and meat products is generally unavoidable. All things considered, it should be emphasized that temperature control during processing, transportation and storage is the key factor for prevention growth of *C. perfringens* and thus food poisoning caused by this pathogen. Another key thing to remember is that the temperature and time during cooking and/or reheating should be adequate to kill vegetative cells of *C. perfringens*.

Compliance with Ethical Standard

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

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

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Disclosure: -

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Evaluation of the effect of cooling on the microbiological quality of lamb carcasses

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ABSTRACT

This study aimed to determine the effects of cooling on microbiological quality of lamb carcasses. Total Aerobic Bacteria Count (TABC), *Enterobactericeae* counts and the presence of *Salmonella* spp. were investigated in accordance with the Turkish Food Codex Regulation on Microbiological Criteria and European Union Commission Regulation 2073/2005. Sampling was carried out on aseptic conditions from the surface of 25 randomly selected lambs brought to slaughterhouse. The results showed that the mean TABC were 2.24±0.087 log cfu/cm^2 before cooling and 2.41±0.061 log cfu/cm^2 after cooling (P>0.05). The mean *Enterobactericeae* count was 0.21±0.11 log cfu/cm^2 before cooling and 0.69±0.13 log cfu/cm^2 after cooling (P<0.01). Furthermore, *Salmonella* spp. were determined on 3 carcasses before cooling and one another carcass after cooling. The influence of cooling on categorisation according to the legislations presented that it could potentially improve the numbers of acceptable carcasses for TABC and *Salmonella* spp. Nevertheless, the results exhibited that the hygiene and cooling stage of the slaughter line must be re-evaluated in terms of HACCP requirements and that corrective measures/actions must be taken.

Keywords: *Enterobactericeae,* Cooling, Lamb carcass, *Salmonella* spp., Surface contamination

Introduction

Slaughter and dressing without microbial contamination of carcasses are practically impossible. The contamination originates from various sources, including fleece, viscera, equipments, other carcasses, and the hands and aprons of the personels (Hauge et al., 2011). Prerequisite programmes and the Hazard Analysis Critical Control Point System (HACCP) are applied to interrupt towards controlling carcass contamination. Fleece removal, evisceration, water washing, chilling and storage are possible critical points for the microbial contamination of carcasses during slaughter process (Milios et al., 2011).

Turkish Food Codex Regulation on Microbiological Criteria (2011), Commission Regulations (EC) No. 2073/2005 (2005) and 1441/2007 (2007) put forward microbiological performance criteria for TABC, *Enterobacteriaceae* and *Salmonella* spp. on fresh lamb carcasses. The performance criteria for TABC and *Enterobacteriaceae* are set out as a three class sampling plan; satisfactory, acceptable and unsatisfactory, according to the contamination level found in four different areas on the carcasses. The performance criteria vary based on whether the samples are obtained by excision or swabbing; swab samples are considered to represent only 20% or less of the microbial loads obtained by excision (EC471, 2001; O'Brien et al., 2007; Lenahan et al., 2010).

The Regulations commands for the use of TABC and *Enterobacteriaceae* as indicators of hygiene and faecal contamination on carcasses before cooling, however, it does not monitor the impact of cooling on carcass hygiene. Cooling is utilized as the Critical Control Point (CCP) as part of the HACCP plan to increase carcass safety (Lenahan et al., 2010).

The basis of HACCP in relation to fresh meat is that the loads of microorganisms on carcass surfaces are decreased or their growth is limited, since elimination is impossible (Sheridan, 2000). Moreover the biochemical periods and structural changes that occur in muscle during the first 24 h post mortem play a major role in the meat quality and are influenced by the cooling processes that carcasses are subjected to after slaughter (Fernandez and Vieira, 2012).

This study aimed to determine the levels of TABC's, *Enterobacteriaceae* and the presence of *Salmonella* spp. on lamb carcasses before and after cooling, and analysed the effect of cooling on carcass categorisation by Turkish Food Codex Regulation (2011).

Materials and Methods

Materials

In this study a total of 25 lamb carcasses, slaughtered at a private slaughterhouse located in Muğla province and stored at 2°C for 24 hr, were used as materials (hind shank, brisket, fore shank and neck areas).

In this study, the carcasses were chilled in conventional-commercial chillers, the temperatures measured during the cooling period was 2°C, but the air velocity and humidity of the chillers were not measured during the cooling process. In order to determine the efficiancy of cold storage, the surface and core temperatures of the carcasses were taken immediately after slaughtering process just before cooling and after 24 hours of storage period at 2°C by using thermometer (PCE-IR 100, Germany).

Sampling procedure was conducted as it is stated at ISO 17604 (2015). Sterile swab sponges (World Bioproduct SR-DRY-G, USA) were placed in sterile stomacher bags containing 10 ml of buffered peptone water (BPW) (Oxoid CM509, England) prior to sampling. Sampling was carried out on the hind shank, brisket, fore shank and neck areas of right half of the randomly selected lamb carcasses (total of 400 cm^2 for each carcass) by using a 100 cm^2 template (10x10 cm) and sponge swabs just before cooling. Similar procedure was carried out for the left half of the carccasses after cooling. After sampling, sponge swabs were brought to the laboratory in ice-boxes then the levels of TABC and *Enterobactericeae*, and the presence of *Salmonella* spp. on the samples were analysed (EC 2073/2005, 2005; TFC, 2011).

Microbiological Analysis

An additional 15 ml of BPW were added into sterile stomacher bags containing sponge swabs from each part of the carcasses samples and brought to the laboratory. Then 2 minutes of homogenisation was carried out for each swab and homogenats from one half of the sheep carcasses were put into another stomacher bag for further homogenisation again, then serial dilutions were prepared.

In order to determine the levels of TABC and *Enterobacteriaceae,* plating outs were carried out on Plate Count Agar (PCA) (Oxoid CM463, England) and Violet Red Bile Glucose Agar (VRBGA) (Oxoid CM485, England), then incubated at 30°C for 48 hr and at 37°C for 24 hr, respectively. While all of the colonies on PCA counted in order to determine the level of TABC, for *Enterobacteriaceae*, red colonies in 0.5 cm diameter or larger on VRBGA were considered as *Enterobacteriaceae* (ISO 4833-2, 2013; ISO 21528-2, 2017).

For *Salmonella* spp. isolation, pre-enrichment was carried out by adding 225 mL of BPW into the homogenates. Then the samples were incubated at 37°C for 18 ±2 hr. All of the procedures followed during inoculation, incubation and identification stages were carried out as stated in ISO 6579-1 (2017). The confirmation of the results were done by using Salmonella Latex Test (OXOID FT0203A, England) kit.

Statistical analysis

For statistical analysis SPSS version 22 (USA, 2013) was used. The effects of cooling on the levels of TABC and *Enterobacteriaceae* were analysed by using paired t test.

Results and Discussion

Strict maintenance of hygiene practices in slaughter process is important in the prevention of microbial contamination of the carcass surface in the interest of providing both meat quality and health protection (Zweifel and Stephan, 2003). The legislation suggested that microbial loads of carcass as hygiene indicators are used in slaughterhouses for evaluating the effective application of the HACCP system. TABC has been used as a general measure of the surface contamination of carcasses, whereas *Enterobacteriaceae* counts have been accepted as an indicator of fecal contamination (Zweifel and Stephan, 2003; Milios et al., 2011).

This study observed that the mean levels of TABC obtained from lamb carcasses before and after cooling were 2.24 ±0.087 log cfu/cm^2 and 2.41 ±0.061 log cfu/cm^2, respectively and there was no statistical difference between before and after cooling values (P>0.05). *Enterobacteriaceae* were isolated from the carcasses both before and after cooling. When the levels of *Enterobactericeae* were evaluated, it was determined as 0.21 ±0.11 log cfu/cm^2 before cooling and 0.69 ±0.13 log cfu/cm^2 after cooling, and the difference between the values was statistically significant (P<0,01). Cooling procedure reduced TABC on 52% and *Enterobactericeae* on 20% of the carcasses evaluated. The mean of TABC and *Enterobactericeae* (log cfu/cm^2) on lamb carcasses before and after cooling are shown in Table 1.

The mean of TABC determined from samples before and after cooling were somewhat lower than reported some previous studies (Lenahan et al., 2010; Hauge et al., 2011; Fernandez and Vieira, 2012). The mean *Enterobactericeae* observed in this study were higher than several studies (Yalçın et al., 2004; Lenahan et al., 2010), but in this study *Enterobactericeae* count were lower than Hauge et al. (2011) and Gürbüz et al. (2018). However, other studies have reported a higher rate of reduction in TABC and *Enterobacteriaceae* count on lamb carcasses than this study as a result of cooling (Gill and Jones, 1997; Yalçın et al., 2004; Hauge et al., 2011; Lenahan et al., 2010). Increases in microbial loads especially *Enterobacteriaceae* counts may have been due to regrowth or contamination just before or during the cooling process. The cooling can damage bacterial cells as a result of low temperatures and water activity values, but some cells have been shown to recover from these chill stresses (Yu et al., 2001; Borch and Arinder, 2002). Contamination of carcasses during cooling may have been caused by air, handling by personnel or carcasses touching each other.

Previous studies have shown different microbial loads due to the differences in sampling procedures such as sampling sites on carcass, size of sampling area. Furthermore fleece cleanliness, slaughtering procedures such as fleece removal technique and hygienic practices, may also affect the results (Hauge et al., 2011; Salmela et al., 2013). It has been considered that variations in TABC and *Enterobacteriaceae* levels may caused by differences in cooling parameters between studies. In this study, the carcasses were chilled in conventional chillers then the surface and core temperatures of lamb carcasses were measured before and after cooling (Table 2). The temperature measurement results were found in accordance with the temperature values specified in the regulation (Regulation of Special Hygiene Rules for Animal Food, 2011). But the other cooling parameters such as carcass spacing, air velocity and relative humidity were not recorded. On the other hand, microbial loads on carcasses were not uniform, therefore the carcas samples selected for studies could have effects on the TABC and *Enterobacteriaceae* counts determined (Lenahan et al., 2010).

Table 1. The mean of TABC and *Enterobactericeae* (log cfu/cm^2) on lamb carcasses before and after cooling

	N	Before cooling ($\bar{X} \pm S_{\bar{X}}$)	After cooling ($\bar{X} \pm S_{\bar{X}}$)	Significance
TABC	25	2.24 ±0.087	2.41 ±0.061	NS
Enterobactericeae	25	0.21 ±0.11	0.69 ±0.13	**

Table 2. The mean surface and core temperature values of lamb carcasses before and after cooling

	N	Before cooling ($\bar{X} \pm S_{\bar{X}}$)	After cooling ($\bar{X} \pm S_{\bar{X}}$)
Surface Temperature	25	25.82 ±0.62	8.43 ±1.14
Core Temperature	25	34.10 ±1.53	3.49 ±0.69

The limits for process hygiene criteria of lamb carcases given in TFC (2011) and Regulation EC 2073/2005 (2005) apply for daily mean log results at slaughterhouse level, but they can also be used for evaluation of bacterial contamination level in general. In this study, the means of TABC and *Enterobacteriaceae* did not exceed legal limits. To be able to determine performance criteria, TABC and the level of *Enterobacteriaceae* are required just before cooling by legislations. In this study, the swab criteria for lamb carcasses were calculated from excision sample values (O'Brien et al., 2007; Lenahan et al., 2010).

Based on the criteria for swabbing, 21 carcass samples were in the acceptable for TABC before cooling and 23 carcasses were after cooling. For *Enterobacteriaceae*, 19 carcasses before cooling and 15 carcasses after cooling were in the acceptable category. None of the samples were found in the unacceptable category (Table 3). When TABC was considered, the numbers of acceptable carcasses increased after cooling. However, the cooling process affected adversely the number of acceptable carcasses when *Enterobacteriaceae* levels were considered. The numbers of marginal carcasses decreased using the TABC and increased when *Enterobacteriaceae* levels were used. These results showed that cooling conditions and hygienic practices during process had the possibility to affect the extend of acceptable or unacceptable carcasses. The use of TABC and *Enterobacteriaceae* data to determine process control charts showing contamination rates have been used by several studies (O'Brien et al., 2007; Salmela et al., 2013). However, the same studies determined that sampling at different parts of during the slaughtering and cooling process to have an influence on the results. The process hygiene criteria for carcasses are evaluated at the end of the slaughtering, and do not regard any of the process that affect the contamination, or possible different contamination sources at the different parts of the slaughtering process. In order to evaluate slaughtering hygiene more efficiently at the slaughterhouse level, detailed information about the slaughtering process is needed in addition to the carcass microbial load before cooling.

Salmonella spp. were detected on 3 (12%) carcass samples before cooling and only one carcass sample different from other *Salmonella* spp. positive carcasses after cooling. The presence of *Salmonella* spp. on carcasses indicated fecal or environmental contamination during the slaughtering process. Presence of *Salmonella* spp. on a different carcass after cooling could be due to the cramped hanging of lamb carcasses, personnel contamination and temperature changes during cooling stage. That is because of lamb carcasses have high water activity and pH values, *Salmonella* spp. can easily grow on the carcasses. *Salmonella* spp. can also adapt to conditions of temperature, pH, and water activity beyond their normal growth range, posing great risks to food safety. Although *Salmonella* spp. are generally considered mesophilic in nature, some *Salmonella* spp. strains are able to grow in foods chilled at 2°C to 4°C and other can grow at temperatures of up to 54°C (Seo and Bohach, 2013). TFC (2011) and Regulation EC 2073/2005 (2005) allow that no more than 2 (c value) of 50 lamb carcasses (n value) before chill can be positive for *Salmonella* spp.. In addition, legislations recommend that a lower c value was used to reduce the presence of *Salmonella* spp.. Previous studies have shown *Salmonella* spp. on 1.5% on chilled carcasses (Duffy et al., 2001) and 0.25% on chilled carcasses (Lenahan et al., 2010). Kalchayanand et al. (2007) found the prevalance of *Salmonella* spp as 4.3% on pre-evisceration lamb carcasses. However, Salmela et al. (2013) and Gürbüz et al. (2018) did not detect any *Salmonella* spp. contamination on carcasses.

Table 3. The categorisation of before and after chill lamb carcasses for TABC and *Enterobactericeae*

Category	TABC (N:25)			*Enterobactericeae* (N:25)		
	Performance criteria for swabbing (log cfu/cm^2)	Before cooling	After cooling	Performance criteria for swabbing (log cfu/cm^2)	Before cooling	After cooling
Acceptable	<2.8	21	23	<0.8	19	15
Marginal	2.8-4.3	4	2	0.8–1.8	6	10
Unacceptable	>4.3	-	-	>1.8	-	-

Conclusion

This study presented an overview to the microbial contamination of lamb carcasses before and after cooling. The levels of TABC and *Enterobacteriaceae* on the carcasses in this study were acceptable based on the performance criteria in legislations. However, the presence of *Salmonella* spp. on carcasses and the increase in The *Enterbactericeae* level after cooling were found to be remarkable. The variation in carcass contamination levels during the cooling process indicated that this part could be used as CCP to control contamination and improve carcass hygiene. As a result, microbiological analyzes which were carried out at the end of the slaughtering process, just before cooling, in relation to the regulations, were not sufficient for evaluating microbial quality of the carcasses. Therefore, microbial contamination levels of carcasses after cooling should be added to the performance criteria.

Compliance with Ethical Standard

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

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

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Disclosure: -

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Determination of some minerals and heavy metal levels in Urfa cheese and cow's milk

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ABSTRACT

This research was carried out to determine the levels of some minerals and heavy metals in Urfa cheeses and cow's milk offered for sale in Şanlıurfa. Mineral and heavy metal levels were determined by analysing 18 Urfa cheese and 21 cow milk samples collected from retail outlets with ICP-MS. Mean mineral and heavy metal contents in Urfa cheese samples were; magnesium (Mg) 129.8 ±31.14 mg/kg, calcium (Ca) 2712.66 ±1002.4 mg/kg, potassium (K) 272.06 ±127.21 mg/kg, titanium (Ti) 7.48 ±2.63 mg/kg, zinc (Zn) 40.4 ±16.33 mg/kg, selenium (Se) 10.6 ±3.51 mg/kg. In cow milk samples, average selenium (Se) 0.22 ±0.04 mg/kg, titanium (Ti) 0.07 ±0.009, mg/kg, copper (Cu) 0.0068 ±0.01 mg/kg and chromium (Cr) was detected at the level of 0.019 ±0.001 mg/kg. Arsenic (As), cadmium (Cd), lead (Pb) and thallium (Tl) levels were below the detectable levels in Urfa cheese and cow's milk samples. As a result, it was concluded that the samples do not pose a significant danger to public health in terms of heavy metal pollution and can contribute significantly to nutrition with the mineral substances it contains.

Keywords: Cow milk, Urfa cheese, Mineral, Heavy metal, ICP-MS

Introduction

Milk is a nutrient secreted in the mammary glands of female mammals and produced to feed their offspring. Various foods obtained from milk are called "dairy products" (İstanbulluoğlu et al. 2016). Milk and dairy products contain important nutrients, which are essential for human life. Milk is highly preferred food because it has high biological values and rich with nutrients (Lıcata et al., 2004). Cheese is a dairy product produced by the coagulation of milk casein, produced in a variety of flavors and textures. For centuries, cheese is a dairy product that has an important place in the nutrition of all societies. In addition to being rich in high quality protein, fat and vitamins, it is easily digestible (Öksüztepe et al., 2013). Milk and cheese are rich in not only protein, fat and vitamins, but also macro elements such as sodium, calcium and phosporus. In addition to macro-elements milk and cheese also contains trace elements such as Zn, Fe, Cu and Se (Mendil, 2006; Altun and Paksoy, 2020). Mineral content of milk may vary depending on the genetic characteristics of the animal, the lactation period, environmental conditions, type of pasture and soil pollution (Özlü et al., 2012).

In our country, besides white cheese, tulum cheese, cheddar cheese and mihalic cheese, many local cheese varieties are also produced. These types of cheese are generally obtained by primitive techniques. Urfa cheese is also one of the local cheese. The production of Urfa cheese is mostly made in the Southeastern Anatolia Region and especially around Şanlıurfa province. Urfa cheese is usually made from sheep and goat milk. Its production is mostly done in villages using traditional methods. Urfa cheese is a semi-hard cheese matured in salt water. By adding rennet to milk, it is allowed to coagulate in about 60 minutes at 30-32 ° C. After coagulation step curd is subjected to dry salting for 12-24 hours and stored in salt water for 3-4 months. Some manufacturers boil the cheese in boiling whey for 3-5 minutes before salting. During its production, it is generally carried out using primitive tools and equipment without paying attention to hygienic rules (Atasoy et al., 2004).

Although milk and cheese have many benefits in nutrition, they can sometimes contain many environmental pollutants such as pesticides, detergents, drug residues, heavy metals, which may be dangerous for human health (Lıcata et al., 2004). Heavy metals contamination of milk and milk products can sometimes occur during the processing of milk into the product. Generally, heavy metal contamination is transmitted from environmental sources such as soil and water or feed consumed by the animal. In addition, metals in the composition of machinery and equipment used during milk storage and processing can dissolve into the product during milking. The elements that can pass from machinery and equipment to milk and cheese are Cu, Zn, Cd, As, Pb (Bakırcıoğlu et al., 2011). Heavy metals tend to accumulate in the tissues of the human body, reaching toxic values over time, causing serious health problems. Heavy metals can be transmitted to the human body through digestion, respiration and skin. Heavy metals can cause acute, subacute and chronic intoxication symptoms depending on the frequency, duration and dose taken into the body. Some of these symptoms are liver necrosis, microcytic anemia, memory retardation, speech and voice disorders (Özturan and Atasever, 2018). Different methods have been used like flame atomic absorption spectrometry (FAAS), inductively coupled plasma-mass spectrometry (ICP-MS), induced plasma-optical emission spectrometry (ICP-OES), atomic absorption spectrometry (AAS), etc. to measure mineral levels in various milk and dairy products. (Özlü et al., 2012; Öksüztepe et al., 2013; Öztürk, 2009; Temurci and Güner, 2006; İşleyici et al., 2017; Kılıçel et al., 2004). The aim of this study is to determine the levels of some minerals and heavy metals in Urfa cheeses and cow's milk offered for sale in Şanlıurfa with the ICP-MS device.

Materials and Methods

Sampling

Within the scope of the study, 18 Urfa cheese samples made from sheep's milk and 21 cow milk samples were taken from local producers in the central district of Şanlıurfa province for sale. Cheese samples were provided as 250 g and milk samples as 25 mL, they were brought to the laboratory immediately in compliance with the cold chain conditions and they were kept at -19ºC until the analysis process.

Preparation of the Samples

After the cheese and milk samples were homogenized, 1 gram of each sample was weighed and taken into the sample containers of the microwave device. 4 mL of 65% (v/v) nitric acid (HNO_3) and 2 mL of 30% (v/v) hydrogen peroxide (H_2O_2) were added with a pipette and placed in the microwave device. Cheese and milk samples were digested in microwave oven with a program whose temperature and duration were predetermined (Table 1). After microwave digestion cheese and milk samples were taken into sterile tubes after cooling, and diluted with ultrapure water (Paksoy at al. 2018).

Analysis of the Samples

Mineral and heavy metal analysis of cheese and milk samples were performed with the Agilent brand 7500ce series ICP-MS (Tokyo, Japan) device in the Mersin University Advanced Technology Education, Research and Application Center laboratory.

Quality Control

Limit of quantification (LOQ) and limit of detection (LOD) of each element was calculated as 10 times and the recovery of 12 elements (Mg, K, Ca, Ti, Zn, As, Cr, Se, Cu, Cd, Tl, Pb) in Urfa cheese and cow milk samples are shown in Table 2.

Statistical Evaluations

All data were analyzed statistically using SPSS 22.0 (SPSS Inc., Chicago, IL, USA) software.

Table 1. Burning process steps in microwave device

Stage	Temperature (°C)	Time (min)
1	90	8
2	170	10
3	210	25

Table 2. Quality control.

Element	LOQ	LOD
Mg (mg L^{-1})	48.58	4.6
K (mg L^{-1})	1013	200
Ca (mg L^{-1})	578	22
Ti (mg L^{-1})	6.8	0.1
Zn (mg L^{-1})	1.81	0.2
As (mg L^{-1})	0.18	0.01
Cr (mg L^{-1})	0.1	0.01
Se (mg L^{-1})	0.24	0.1
Cu (mg L^{-1})	0.06	0.01
Cd (mg L^{-1})	0.11	0.01
Tl (mg L^{-1})	0.2	0.02
Pb (mg L^{-1})	0.16	0.01

LOD: Limit of detection; LOQ: Limit of quantification

Results and Discussion

Within the scope of the study, the average, standard deviations, the lowest and highest levels of mineral substances and heavy metal amounts of cheese samples are shown in Table 3 and Figure 1. The average, standard deviations, the lowest and highest values of the milk samples are shown in Table 4 and Figure 1.

Trace element and heavy metal contents of milk and dairy products may vary depending on the lactation stage, the nutritional status of the animal, environmental and genetic factors, or possible contamination during production (Özturan and Atasever, 2018).

Table 3. Mineral levels of Urfa cheese samples (mg/kg).

Element	Min	Max	Mean (x ± Sx)
Mg	92.9	227.7	129.8 ±31.14
K	98.9	555.1	272.06 ±127.21
Ca	1828	6025	2712.66 ±1002.4
Ti	5.2	16.2	7.48 ±2.63
Zn	23.9	75.4	40.4 ±16.33
As	<LOD	<LOD	<LOD
Se	1.8	14.7	10.6 ±3.51
Cd	<LOD	<LOD	<LOD
Tl	<LOD	<LOD	<LOD
Pb	<LOD	<LOD	<LOD

n:18, x: Arithmetic Mean, Sx: Standard Deviation, LOD: Limit of detection

Table 4. Mineral levels of cow's milk samples (mg/kg).

Element	Min	Max	Mean (x ± Sx)
Se	0.13	0.31	0.22 ±0.04
Ti	0.05	0.08	0.07 ±0.009
Cr	<LOD	0.01	0.019 ±0.001
Cu	<LOD	0.04	0.0068 ±0.01
As	<LOD	<LOD	<LOD
Cd	<LOD	<LOD	<LOD
Tl	<LOD	<LOD	<LOD
Pb	<LOD	<LOD	<LOD

n:21, x: Arithmetic Mean, Sx: Standard Deviation, LOD: Limit of detection

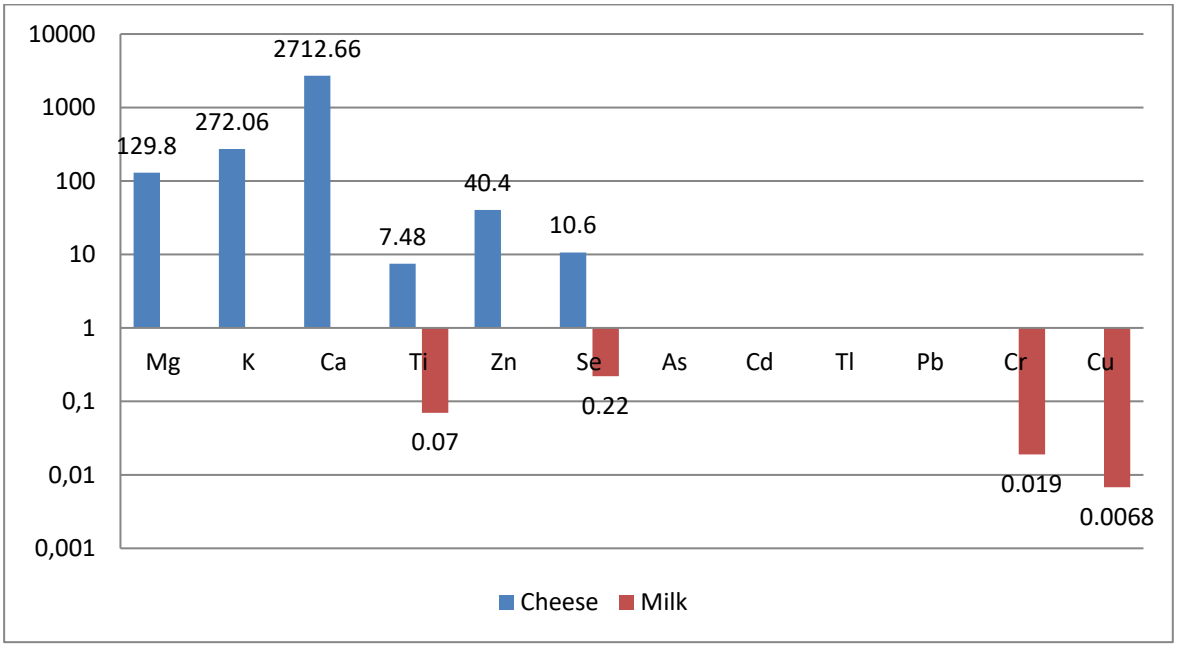

Figure 1. Mineral levels (mg/kg) of Urfa cheese and cow's milk samples.

Ca is responsible for many functions in the body such as heart rhythm, blood coagulation, hormone secretion, muscle contraction, enzyme activation in the body, and is also found in the structure of bones. Ca makes up 1.5-2% of an adult's mass. Milk and dairy products are foods rich in Ca which is one of the most important mineral substances in cheese, and its amount varies according to the type of cheese (Altun et al., 2016). In our study, the average Ca amount in Urfa cheeses is 2712.66 ±1002.4 mg/kg. The amount of Ca in Urfa cheese is lower than the amount of Ca reported by many researchers in cheeses (Demirci, 1988; Arslan et al. 1996; Mendil, 2006; Öksüztepe et al., 2013; İşleyici et al., 2017; Altun et al., 2016; Çetinkaya et al., 2016), but higher than the values found by some researchers (Ayar et al., 2006; Kirdar et al., 2015; Arslaner and Salık, 2020). According to the results of this study, it was seen that the Ca content of Urfa cheese can be a good source in human nutrition.

Mg has many functions involved in more than 300 reactions in the body. Milk and dairy products are a good source of Mg. There is approximately 100 mg of Mg in a liter of milk (Haug et al., 2007). The amount of Mg in milk varies depending on the lactation period, the animal's feeding and the treatment applied to the milk. In our study, the average amount of Mg in Urfa cheeses was 129.8 ±31.14 mg/kg. The amount of Mg in Urfa cheese was lower than the amount of Mg reported by

many researchers (Öksüztepe et al., 2013; İşleyici et al., 2017; Kılıçel et al., 2004; Altun et al., 2016; Çetinkaya et al., 2016; Arslaner and Salık, 2020; Özbek and Akman, 2016). It was higher than the values found by some researchers (Mendil, 2006; Ayar et al., 2006; Kirdar et al., 2015). The difference in the amount of Mg is thought to be due to the milk used in the production of the cheese or the techniques used in the production and the environmental conditions of the region.

K has many functions such as osmotic pressure, electrolyte balance, acid-base balance, nerve stimulation, contraction of the heart and other muscles, protein synthesis, and the conversion of glucose into glycogen (Özturan and Atasever, 2018). Milk and dairy products also contain some K. The amount of K also changes depending on the lactation period, the ration of the animal and the process applied to the milk. In our study, the average K level in Urfa cheese was 272.06 ±127.21 mg/kg. The amount of K in Urfa cheese was lower than the amount of K reported by many researchers in cheeses (Öksüztepe et al., 2013; İşleyici et al., 2017; Demirci, 1988; Arslan et al. 1996; Çetinkaya et al., 2016; Kirdar et al., 2015; Arslaner and Salık, 2020). The results close to the values found by some researchers (Mendil, 2006; Altun et al., 2016, Özbek and Akman, 2016). Park (2000) stated that significant amounts of K in cheese are lost during cheese production. The reason for the lower amount of K in Urfa cheese compared to other cheeses suggests that it may be due to production.

Zn plays a role in many physiological processes such as nucleic acid and protein synthesis, cellular replication, insulin secretion, sexual maturation, and strengthening of the immune system. High concentrations of Zn cause nausea and vomiting in children, anemia and cholesterol problems in adults (Özturan and Atasever, 2018). In our study, the average Zn level in Urfa cheeses is 40.4 ±16.33 mg/kg. The amount of Zn in Urfa cheese was higher than the amount of Zn found by many researchers in cheeses (Mendil, 2006; Öksüztepe et al., 2013; Altun et al., 2016; İşleyici et al., 2017; Çetinkaya et al., 2016; Kirdar et al. , 2015; Yalçın and Tekinşen, 2010). It was lower than the values found by some researchers (Demirci, 1988; Kirdar et al., 2015). The fact that the Zn content of Urfa cheese was higher than the Zn content reported in other cheeses suggests that it may be due to Zn contamination caused by the materials used in the production stages of Urfa cheese.

Se plays an important role in immunity, antioxidant system, DNA synthesis and DNA repair. The recommended daily intake of Se is 55 µg. It is an important source of Se in milk and dairy products (Haug et al., 2007). In our study, the average Se amounts in Urfa cheese and cow milk are 10.6 ±3.51 mg/kg, 0.22 ±0.04 mg/kg, respectively. Ayar et al. (2007) reported that they found the highest Se value in various milk and dairy products with 0.434 mg/kg in tulum cheese and then in butter with 0.315 mg/kg. In a study conducted on dairy products in Italy, the Se amount of Groviera cheese was found to be 1.10 mg/kg (Garcia et al., 2006). It has been reported that the Se amount in Bayburt civil cheese was between 0.00-723.57 µg/kg (Arslaner and Salık, 2020). In a study, it was stated that Se is kept in the casein network so it was determined in higher amounts in semi-hard cheeses than in milk (Garcia et al., 2006). Since Urfa cheese is a semi-hard cheese, the Se content was high.

Ti is a naturally occurring light mineral on earth. Taking Ti into the body in large quantities is not toxic (Şanal and Güler, 2010). Titanium dioxide has recently been used in nanotechnology applications in foods. It is also used as a food additive to increase the color brightness in milk products (Berik, 2018). In our study, the average Ti amounts in Urfa cheese and cow milk are 7.48 ±2.63 mg/kg, 0.07 ±0.009 mg/kg, respectively. Studies on Ti levels in milk and dairy products are limited. Şanal and Güler (2010) reported in their study that they found the amount of Ti in cow milk at the level of 0.31±0.23 mg/kg in the province of Hatay. Dobrzanski et al. (2005) reported that Ti content in raw milk of cows in lower Silesia and upper Silesia (USA) was 104.42 ±24.70, 54.48 ±29.40 µg/L, respectively.

Cu is required as a necessary trace element for adequate growth, cardiovascular system, lungs, neuronendocrine function and iron metabolism. Cu is an essential trace element but have dangerous effects on the human body in high concentrations. As a result of contamination, Cu can reach high levels in milk and dairy products (Özturan and Atasever, 2018). JECFA (Joint FAO/WHO Expert Comitee on Food Additives) has determined the maximum daily allowable dose for Cu as 0.5 mg/kg. In the Turkish Food Codex Regulation on Contaminants in Foodstuffs, the maximum acceptable value for Cu was not specified (Anonymous, 2011). According to the Food Codex Commission within the Food-Agriculture Organization (FAO) and World Health Organization (WHO), the maximum amount of Cu that can be found in foods is 0.1-5.0 mg/kg (FAO/WHO, 2012). In our study, the average Cu amount in cow milk was determined as 0.0068 ±0.01 mg/kg. The reported average amount of Cu in milk samples in Licata et al.'study (2004) was 1.98 µg/kg, Şimsek et al.'s (2000) study was 0.39-0.96 mg/kg, Temurci and Güner's (2006) study was 4,300 mg/L, Yüzbaşı et al.'s (2009) study was 1.7 mg/kg and Beykaya et al.'s (2019) study was 33.69 µg/kg. İnci et al. (2017) reported that the Cu content was below the detectable limit. Our results were parallel with the other studies.

Because heavy metals cause acute and chronic health problems, national and international food organizations have introduced regulations to prevent contamination. However, in the communiqué on determining the maximum levels of certain contaminants in foodstuffs of the Turkish Food Codex, no limit has been determined for milk and dairy products. A limit was set for Pb as 0.020 mg/kg.

Be a metal that can be found everywhere in nature, Cr affects carbohydrate and protein metabolism by providing insulin movement in the body. The average daily intake of Cr by a person is between 30 and 200 µg (Beykaya et al. 2019). Milk and dairy products contain low amounts of Cr (Kahvecioğlu et al., 2009). In the Turkish Food Codex Contaminants Regulation, there is no limit for the amount of Cr in foodstuffs (Anonymous, 2011). In our study, the average Cr amount in cow milk is 0.019 ±0.001 mg/kg. Beykaya et al. (2019), Licata et al. (2004), Temurci and Güner (2006) reported an average amount of Cr 31.81 µg/kg, 2.03 µg/L, 1.016 mg/L, respectively. The amount of Crin this study in cow's milk is very low and it was lower than the other studies.

Cd is considered as the most important food contaminant and it is important because it has negative effects on human health (Özturan and Atasever, 2018). In the Turkish Food Codex Contaminants Regulation, no limit is specified for Cd in milk and its products, but it has been reported that it should be between 0.05-1.00 mg/kg for some other foods (Anonymous, 2011). In the study we conducted, the amount of Cd was found below the detection limits (<LOD) in milk and cheese. Boudebbouz et al. (2020) found the Cd levels in milk samples from thirty-six regions around the world are above the standard limit of 0.0026 µg/g, that Cd levels in milk samples in 18 regions are below the standard limit (0.0026 µg/g) and in five regions Cd It has been reported that their levels are below the detectable limit. In many studies conducted to determine the level of heavy metals in cow's milk in our country, Cd was detected, even in low amounts (Yüzbaşı et al., 2009; Beykaya et al., 2019; Ay and Karayünlü, 2008). İnci et al. (2017), in line with our study, reported that the amount of Cd in raw milk collected in Aydın was below the detectable limit. In many studies related to heavy metal levels in cheeses, Cd was found even at low levels (İşleyici et al., 2017; Çetinkaya et al., 2016; Yalçın and Tekinşen, 2010; Ayar et al., 2009; Eroğlu, 2019). Öksüztepe et al. (2013) found the amount of Cd in cheese samples below the detectable limit, in parallel with our study.

The presence of Pb in milk and dairy products may from the environmental sources (atmosphere, vehicle exhausts, urban waste, etc.). Pb is a kind of neurotoxin and causes abnormal brain and nervous system functions (Kahvecioğlu et al., 2009). Pb is toxic and has negative effects on human health. The Codex Alimentarius Commission (FAO/WHO, 2012) determined the Pb amount at the level of 0.02 mg/kg for milk and dairy products. In the Turkish Food Codex Contaminants Regulation, the highest acceptable Pb value was determined as 0.020 mg/kg for milk and its products (Anonymous, 2011). Many studies have reported that Pb levels in milk samples are below the specified limits (Licata et al., 2004; Ay and Karayünlü, 2008; Beykaya et al., 2019; İnci et al., 2017). In this study the amount of Pb was found below the detection limits (<LOD) in milk and cheese. In many studies on heavy metal levels in cheeses, Pb was found even at low levels (Mendil, 2006; Yalçın and Tekinşen, 2010; İşleyici et al., 2017; Çetinkaya et al., 2016; Ayar et al., 2009; Eroğlu , 2019). Öksüztepe et al. (2013) found the Pb content in cheese samples was below the detectable limit, in parallel with our study.

As being common in nature and increasing environmental exposure today caused the increase of high As content in some products. As is also contaminated to milk and dairy products from the environment (İstanbulluoğlu et al., 2016). In our study, the amount of As was found below the detection limits (<LOD) in milk and cheese samples. In line with our results, Öksüztepe et al. (2013) in Tulum cheese, Serencam et al. (2018) in çivil cheeses did not detect As. Some researchers have identified As in milk and dairy products (İstanbulluoğlu et al.2016; Ayar et al., 2007).

TI joins the food chain by passing from soil to plants. TI is one of the most toxic metals to metabolism. Cd, Pb, Cu, Zn are both toxic. TI affects many systems (gastrointestinal, cardiovascular and urinary) as it accumulates in the body. General symptoms caused by TI are fatigue, loss of appetite, foot pain, headache, depression and hair loss (Leonard et al., 1997). The amount of TI is high in the milk and meat of animals fed in regions with high TI in the soil (Şanal and Güler, 2010). In our study, the amount of TI was found below the detection limits (<LOD) in milk and cheese. Studies on the amount of TI in animal foods are limited. Şanal and Güler (2010) reported in their study that they found the Tl amount in cow's milk at the level of 7.01 ±0.80 mg/kg in the province of Hatay. Dobrzanski et al. (2005) reported that the TI amount in the raw milk of cows in the lower Silesia and upper Silesia regions in America was (0.73 ±0.44, 0.84 ±0.81) µg/l, respectively.

Conclusion

The mineral substance levels of the samples showed that Urfa cheese and cow's milk are important foodstuffs for human nutrition. In addition, it was observed that the heavy metal levels of Urfa cheese and cow's milk did not exceed the limits specified in national and international standards. The very low

heavy metal levels of Urfa cheese and cow milk are thought to be due to the very low level of heavy metal pollution in the region. As a result, it was concluded that Urfa cheese and cow milk in Urfa province can contribute significantly to nutrition due to the minerals in its composition, and it does not pose a significant danger to public health in terms of heavy metal pollution. However, industrialisation continuously increasing may be pose a risk in terms of heavy metals contamination on milk and milk products therefore this risks should be checked proper interval time.

Compliance with Ethical Standard

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

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

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Prediction of growth kinetics of *Pseudomonas* spp. in meat products under isothermal and non-isothermal storage conditions

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ABSTRACT

The main objective of the present study was to develop and validate a new alternative modelling method to predict the shelf-life of food products under non-isothermal storage conditions. The bacterial growth data of the *Pseudomonas* spp. was extracted from published studies conducted for aerobically-stored fish, pork and chicken meat and described with two-step and one-step modelling approaches employing different primary models (the modified Gompertz, logistic, Baranyi and Huang models) under isothermal storage temperatures. Temperature dependent kinetic parameters (maximum specific growth rate 'μ_{max}' and lag phase duration 'λ') were described as a function of storage temperature via the Ratkowsky model integrated with each primary model. The Huang model based on the one-step modelling approach yielded the best goodness of fit results (RMSE = 0.451 and adjusted-R^2 = 0.942) for all food products at isothermal storage conditions, therefore, was also used to check it's the prediction capability under non-isothermal storage conditions. The differential form of the Huang model provided satisfactorily statistical indexes ($1.075 > B_f > 1.014$ and $1.080 > A_f > 1.047$) indicating reliably being able to use to describe the growth behaviour of *Pseudomonas* spp. in fish, pork and chicken meat subjected to non-isothermal storage conditions.

Keywords: Dynamic condition, Microbiological quality, *Pseudomonas* spp., growth kinetic, Predictive microbiology

Introduction

Meat is among nutrient-dense foods and is a source of protein. Fish, pork and chicken meat play an important role in meat industry; however, are highly perishable food products even when kept under refrigeration, which may result in an important economic loss (Bruckner et al., 2013; Dominguez and Schaffner, 2007; Koutsoumanis, 2001). Initial microbial quality and storage conditions have a direct effect on product shelf-life, and *Pseudomonas* spp. is one of the most abundant bacterial genera, naturally existing in fish, pork and chicken microbiota (Bruckner et al., 2013; Ghollasi-Mood et al., 2017; Lytou et al., 2016; Koutsoumanis, 2001).

Microbial load in food can be determined with traditional microbiological enumeration techniques. Even more, the results of these techniques give us only information about specific time and condition. But the growth behaviour of microorganisms depends on changing environmental factors. Therefore, the traditional enumeration techniques are not adequately practical. Predictive microbiology is a tool used to describe microbial behaviour in food. Although traditional microbiological methods have high costs and time-consuming results, these methods are still used simultaneously with predictive microbiology to describe microbial behaviour in the development of products and processes (Bovill et al., 2001).

The main objective of predictive microbiology is to predict microbial behaviour, which can prevent food spoilage as well as food-borne illnesses by employing mathematical models. Primary and secondary models are commonly used in predictive food microbiology (Whiting, 1995). For the primary models, the modified Gompertz, logistic, Baranyi and Huang models are the most popular ones describing microbial growth data as a function of time at constant environmental conditions. The secondary models indicate how obtained the growth parameters from primary models change with respect to one or more environmental or cultural factors (*e.g.*, gas composition, pH, temperature and salt level). Temperature is one of the most important environmental factors directly affecting the growth behaviour of microorganisms in foods, and its effect is widely described using the Ratkowsky model (Ratkowsky et al., 1982).

Under real life conditions, environmental factors are not always constant during the pass time for the food product reaches consumers (Zwietering et al., 1994). Therefore, dynamic models are essential to model by taking into account the changing environmental conditions which a food product really subjects to (Pérez-Rodríguez and Valero, 2013). Dynamic models considering the effect of changing temperature are important to model the effect of the temperature on microbial growth under non-isothermal conditions.

Generally, the primary and secondary models are separately fitted to the growth data and kinetic parameters, respectively and this is the most popular modelling procedure followed in the predictive food microbiology. But there are some drawbacks concerning about this modelling approach. The major drawback is to lead to be accumulation and propagation of errors due to being sequentially performed nonlinear regression two times (Huang, 2017). To avoid these disadvantages of two-step modelling approach, alternatively, a one-step modelling approach can be applied while simulating microbial data and kinetic parameters. In this approach, primary and secondary modelling for the growth and temperature (as a changing environmental factor) data is performed simultaneously. Therefore, the use of this approach frequently provides better prediction performance, lower uncertainty, more precise coefficients and robust confidence interval than the two-step modelling approach (Jewell, 2012; Martino and Marks, 2007).

In the present study, the growth behaviour of *Pseudomonas* spp. naturally existing in fish, pork and chicken microbiota were described with both two-step and one-step modelling approaches for isothermal storage conditions. The fitting capabilities of both approaches were compared and the approach which gave better fitting performance was tested under non-isothermal storage conditions.

Materials and Methods

Experimental Data

The bacterial growth data of *Pseudomonas* spp. were extracted from the published works performed for fish, pork and chicken meat (Bruckner, 2010; Bruckner et al., 2013; Koutsoumanis, 2001). While there were six isothermal storage conditions (0, 2, 5, 8, 10 and 15 °C) to simulate the bacterial growth behaviour for fish (Koutsoumanis, 2001), there were five isothermal storage conditions (2, 4, 7, 10 and 15 °C) for pork and chicken meat (Bruckner, 2010; Bruckner et al., 2013). The experimental set-ups to monitor *Pseudomonas* spp. in the targeted food products (fish, pork and chicken meat) were explained in detail in the respective studies (Bruckner, 2010; Bruckner et al., 2013; Koutsoumanis, 2001). In brief, food products were transported to the laboratory under temperature-controlled refrigeration conditions. As soon as they arrived and the initial microbiological analyses of them were performed, and they were started to keep at aerobically storage conditions. For microbiological analyses, food samples (25 g) were added aseptically to 225 mL

of 0.1% peptone water with salt (NaCl, 0.85%), and the mixture was homogenized for 60 s with a stomacher. A 10-fold dilution series of the homogenate was prepared using saline peptone diluents. Appropriate dilutions were transferred to *Pseudomonas* Agar Base with CFC supplement (Oxoid) incubating at 20-25 °C for 48 h. In the current study, data collection process for the growth curves was performed using GetData Graph Digitizer 2.26 software (www.getdata-graph-digitizer.com) by which the growth data points could be extracted accurately with one decimal precision.

Modelling

Four different primary models namely the modified Gompertz (Zwietering et al., 1990), logistic (Zwietering et al., 1990), Baranyi (Baranyi and Roberts, 1994) and Huang (Huang 2017) models were fitted with the two-step and one-step modelling approaches as they are the most used sigmoid functions that describe the bacterial growth behaviour and are defined by Eqs (1), (2), (3) and (4), respectively at constant environmental conditions:

$$y(t) = y_0 + (y_{max} - y_0).\exp\left\{-\exp\left[\frac{\mu_{max}.e}{(y_{max}-y_0)}.(\lambda - t) + 1\right]\right\} \quad (1)$$

$$y(t) = y_0 + \frac{(y_{max}-y_0)}{\left\{1+\exp\left[\frac{4.\mu_{max}}{(y_{max}-y_0)}.(\lambda-t)+2\right]\right\}} \quad (2)$$

$$y(t) = y_0 + \mu_{max}F(t) - \ln\left(1 + \frac{e^{\mu_{max}F(t)}-1}{e^{(y_{max}-y_0)}}\right) \quad (3)$$

$$y(t) = y_0 + y_{max} - \ln(e^{y_0} + [e^{y_{max}} - e^{y_0}].e^{-\mu_{max}B(t)}) \quad (4)$$

F(t) and *B*(t) are the adjustment functions that are respectively described by Baranyi and Roberts (1994) and Huang (2017):

$$F(t) = t + \frac{1}{v}\ln\left(e^{-vt} + e^{-\mu_{max}\lambda} - e^{(-vt-\mu_{max}\lambda)}\right) \quad (5)$$

$$B(t) = t + \frac{1}{4}\ln\left(\frac{1+e^{-4(t-\lambda)}}{1+e^{4\lambda}}\right) \quad (6)$$

where t is the time (h), $y(t)$ is the concentration of bacterial populations (ln CFU/g) at time t, y_0 is the initial concentration of bacterial populations (ln CFU/g), y_{max} is the maximum concentration of bacterial populations (ln CFU/g), μ_{max} is the maximum specific bacterial growth rate (1/h), λ is the duration of lag phase (h) and v is the rate of increase of limiting substrate, assumed to be equal to μ_{max}.

The Ratkowsky model (Ratkowsky et al., 1982) was employed for the determination of relationship between storage temperature and μ_{max} using the Eq. (7):

$$\sqrt{\mu_{max}} = b_1(T - T_0) \quad (7)$$

where T is the storage temperature (°C), T_0 is the notional temperature (°C), μ_{max} is the maximum specific bacterial growth rate (1/h), b_1 is the regression coefficient.

Additionally, λ was defined as a function of μ_{max} with respect to temperature using the Eq (8) (Robinson et al., 1998):

$$\lambda = \frac{b_2}{\mu_{max}(T)} \quad (8)$$

where b_2 is the regression coefficient, $\mu_{max}(T)$ is the a function of temperature, which leads λ to be defined as a function of storage temperature.

For the two-step and one-step modelling approaches, each of the parameters was calculated by means of NonLinearModel command which uses Levenberg Marquardt algorithm in the Matlab 8.3.0.532 (R2014a) software (MathWorks Inc., Natick, MA, USA). Determination of suitable starting values in nonlinear regression procedure is necessary step to estimate the accurate parameters. The starting values for the parameters, y_0 and y_{max} were selected as the minimum and maximum concentration of bacterial populations considering the entire temperature range, respectively. Randomly choosing starting points for the parameters, b_1, b_2 and T_0 might lead the estimated parameters to possible local optimal points around global one for especially the one-step modelling approach. Therefore, the starting points of these parameters were selected by using ga command which uses genetic algorithm in Global Optimization Toolbox of Matlab software for the two-step and one-step modelling approaches. Following successful iteration process for the nonlinear regression procedure, the global optimum values of the parameters were obtained.

Comparison of the Goodness of Fit of the Models

The comparison of the global models' estimation capabilities was performed by taking into consideration the root mean square error (RMSE) and the adjusted coefficient of

determination (adjusted-R^2) using Eqs. (9) and (10) respectively (Milkievicz et al. 2020):

$$\mathrm{RMSE} = \sqrt{\sum_{i=1}^{n} \frac{(\mathrm{observed_i} - \mathrm{fitted_i})^2}{n - s}} \qquad (9)$$

$$\text{adjusted-}R^2 = 1 - \left(\frac{n-1}{n-s}\right)\left(\frac{\mathrm{SSE}}{\mathrm{SST}}\right) \qquad (10)$$

where $\mathrm{observed_i}$ is the experimental bacterial growth, *n* is the number of experiments, s is the number of parameters of the model, SSE is the sum of squares of errors and SST is the total sum of squares. RMSE and adjusted-R^2 were calculated for entire data sets, which correspond to 5 for fish and 6 for pork and chicken meat considering observed and fitted values as log CFU/g.

Validation of the Global Model

Verification of the developed models in the predictive food microbiology is crucial to be reliably employed as a simulation tool. The prediction performance of the global model that gave the best fitting capability to model the growth behaviour of *Pseudomonas* spp. existing in fish, pork and chicken microbiota were assessed by considering the growth data obtained from non-isothermal storage conditions. The comparison was done considering each of the global models' corresponding the bias (B_f) and accuracy (A_f) factors (Ross, 1996) given in Eqs. (11) and (12), respectively:

$$B_f = 10^{\frac{\sum_{i=1}^{n} \log(y_{\mathrm{predicted}}/y_{\mathrm{observed}})}{n}} \qquad (11)$$

$$A_f = 10^{\frac{\sum_{i=1}^{n} |\log(y_{\mathrm{predicted}}/y_{\mathrm{observed}})|}{n}} \qquad (12)$$

where $y_{predicted}$ refers to predicted maximum growth rate (log CFU/h), $y_{observed}$ refers to experimental maximum growth rate (log CFU/h), *n* refers to the number of data.

The B_f is a measure of average variation between the predictions and observations. The model yielding B_f greater than 1 is considered as 'fail dangerous', while the model providing B_f less than 1 is considered as 'fail safe'. A value of 1 for B_f indicates that there is a perfect agreement between the predictions and observations. The A_f measures the average difference between the predictions and observations by disregarding whether the difference is positive or negative. The larger A_f value, the less accurate is the average estimate (Ross, 1996). Additionally, two validation criteria known as mean deviation (MD) and mean absolute deviation (MAD) were calculated to evaluate the prediction capability of the models for non-isothermal storage conditions, as stated by Le Marc et al. (2008). A value of MD and MAD closing to 0 shows that the prediction capability of the model is perfect.

Results and Discussion

The growth data of the *Pseudomonas* spp. existing in fish, pork and chicken meat microbiota were fitted using two-step and one-step modelling approaches, and the statistical indicates were given in Table 1. RMSE and adjusted-R^2 values presented in Table 1 indicate the overall fitting capabilities for two-step modelling approach, which means that RMSE and adjusted-R^2 values were calculated after consecutively done primary and secondary model fitting for entire data sets for each food product. The statistical indices showed that Huang model gave the best fitting performance for each food product. The fitting capability of the Baranyi model was the second. The Modified Gompertz and logistic models yielded almost the same fitting capabilities, which means that both of the primary models could not estimate the growth behaviour of *Pseudomonas* spp. as good as the Huang and Baranyi models estimated when the-wo step modelling approach was employed.

It is known that the degree of freedom while employing nonlinear regression procedure is important to decrease in uncertainty and increase in reliability of the model parameters (Huang, 2017). While doing simulation with one-step modelling approach, primary and secondary modelling is performed simultaneously considering whole experimental data set, which means that the simulation with one-step modelling approach has always higher degrees of freedom than the simulation with two-step modelling approach. Therefore, the improvement obtained from one-step modelling approach can be attributed to higher degrees of freedom in one-step modelling approach.

One-step modelling approach, an alternative way to traditionally used two-step modelling approach, was employed to quantitatively detect *Pseudomonas* spp. count. The statistical indices, RMSE and adjusted-R^2 values, showing the fitting capability of one-step modelling approach were presented for each food product in Table 1. The RMSE and adjusted-R^2 values of each of the primary models and each food product based on one-step modelling approach were calculated maximum 0.466 and minimum 0.938, respectively. These results showed that no matter which primary model was used, the one-step modelling approach gave considerably better prediction performance when the one-step modelling approach was employed. Therefore, the growth kinetics obtained from

the one-step modelling approach for each food product (fish, pork and chicken meat) and each primary model (the modified Gompertz, logistic, Baranyi and Huang models) were given in Table 2.

The Huang model based on the one-step modelling approach showed that maximum counts of *Pseudomonas* spp. were 8.1 ± 0.1, 9.5 ± 0.1 and 9.4 ± 0.1 for the fish, pork and chicken meat, respectively (Table 2), while the maximum counts were experimentally found to be of 8.30 ± 0.30, 9.8 ± 0.2 and 9.6 ± 0.2, for the fish, pork and chicken meat, respectively. This indicated that the Huang model provided suitable prediction performance for maximum counts of *Pseudomonas* spp. in each food product.

Table 1. Comparison of fitting capability of different primary models based on two-step and one-step modelling approaches

Food products	Primary models	Modified Gompertz		Logistic		Baranyi		Huang	
	Modelling approach	2-step*	1-step	2-step*	1-step	2-step*	1-step	2-step*	1-step
Fish	RMSE	0.572	0.466	0.586	0.460	0.567	0.452	0.543	0.451
	Adjusted-R^2	0.907	0.938	0.903	0.940	0.909	0.941	0.916	0.942
Pork	RMSE	0.609	0.383	0.506	0.406	0.607	0.440	0.573	0.430
	Adjusted-R^2	0.941	0.977	0.959	0.974	0.941	0.969	0.948	0.971
Chicken	RMSE	0.540	0.260	0.423	0.263	0.389	0.259	0.397	0.256
	Adjusted-R^2	0.933	0.984	0.959	0.984	0.965	0.984	0.964	0.985

RMSE: root mean square error and Adjusted-R^2: adjusted coefficient of determination, calculated overall data sets for each food product considering observed and fitted values as log CFU/g.

* RMSE and adjusted-R^2 values calculated after consecutively done primary and secondary model fitting for entire data sets for each food product.

Table 2. Kinetic parameters of *Pseudomonas* spp. in different food products using one-step modelling approach.

Food product	Primary models	y_0 (log CFU/g)	y_{max} (log CFU/g)	T_0 (°C)	b_1	b_2
Fish	Modified Gompertz	3.4 ± 0.2	8.3 ± 0.1	-8.52 ± 0.50	0.0260 ± 0.0014	2.35 ± 0.88
	Logistic	2.9 ± 0.3	8.2 ± 0.1	-8.55 ± 0.49	0.0255 ± 0.0014	1.25 ± 1.28
	Baranyi	3.3 ± 0.2	8.1 ± 0.1	-8.58 ± 0.46	0.0238 ± 0.0011	1.41 ± 0.69
	Huang	3.4 ± 0.1	8.1 ± 0.1	-8.58 ± 0.46	0.0236 ± 0.0010	1.45 ± 0.51
Pork	Modified Gompertz	3.2 ± 0.2	9.8 ± 0.2	-14.30 ± 1.25	0.0179 ± 0.0012	2.65 ± 1.04
	Logistic	2.3 ± 0.1	9.7 ± 0.2	-14.28 ± 1.30	0.0173 ± 0.0011	0.00 ± 0.00
	Baranyi	3.3 ± 0.2	9.5 ± 0.1	-14.01 ± 1.27	0.0165 ± 0.0012	1.61 ± 0.82
	Huang	3.4 ± 0.1	9.5 ± 0.1	-14.03 ± 1.24	0.0165 ± 0.0011	1.78 ± 0.64
Chicken	Modified Gompertz	3.9 ± 0.1	9.8 ± 0.2	-7.77 ± 0.37	0.0289 ± 0.0011	2.55 ± 0.65
	Logistic	3.3 ± 0.2	9.6 ± 0.1	-7.76 ± 0.37	0.0284 ± 0.0010	1.14 ± 0.96
	Baranyi	3.9 ± 0.1	9.4 ± 0.1	-7.65 ± 0.35	0.0272 ± 0.0009	1.77 ± 0.46
	Huang	4.0 ± 0.1	9.4 ± 0.1	-7.62 ± 0.35	0.0270 ± 0.0008	1.74 ± 0.36

While simulating the growth behaviour of microorganisms, accurately determining the exponential phase in which the growth rate reaches maximum value and the variations in organoleptic properties of foods also reach maxima and the lag phase in which organoleptic properties almost do not change are very important. μ_{max} and λ are the most important critical parameters to describe the growth behavior of microorganisms on food, and temperature has a key role in affecting directly both of these growth parameters (Huang, 2008). The kinetic parameters including μ_{max} and λ belonging to *Pseudomonas* spp. for each food product (fish, pork and chicken meat) and each primary model (the modified Gompertz, logistic, Baranyi and Huang models) were shown in Figure 1 and Figure 2, respectively. As it is expected, the figures demonstrate that μ_{max} increased and λ decreased because of rising storage temperature. At this point, it needs to be highlighted that the logistic model tented to yield λ smaller than other primary models (modified Gompertz, Baranyi and Huang models) no matter for which food product was. Additionally, logistic model's statistical indices about b_2, which are used to calculate λ, were higher than other models for chicken and fish, which means a weakness of the logistic model about describing λ. These results are in a good agreement with the findings reported by Tarlak, (2020) for mushroom.

Validation is an important step to check how well the developed models are working. The Huang model is the best primary model simulating the growth behaviour of *Pseudomonas* spp. in fish, pork and chicken meat, therefore, Huang model was used to test the prediction capability for the *Pseudomonas* spp. concentration under non-isothermal storage conditions (Figure 3). The statistical values for validation of the Huang model are given in Table 3. B_f and A_f were calculated maximum 1.075 and 1.080, respectively for all food products (fish, pork and chicken meat). A B_f and A_f of 1 indicates no structural deviation of the model. The B_f factor of 1.075 indicated that the model overestimates less than 7.5% whereas the A_f factor of 1.080 showed that on average the predicted value was less than 8.0% different (either smaller or larger) from the observed value for each of the food products. In addition, MD and MAD values were less than 0.39 and 0.41, respectively considering all food products (fish, pork and chicken meat). All these statistical indexes show that the Huang model can be reliably used to predict the growth behaviour of *Pseudomonas* spp. in fish, pork and chicken meat at not only isothermal but also non-isothermal storage conditions. Because the spoilage of fish, pork and chicken meat is directly linked with *Pseudomonas* spp. concentration, the one-step modelling approach could be also used for the prediction of product shelf life.

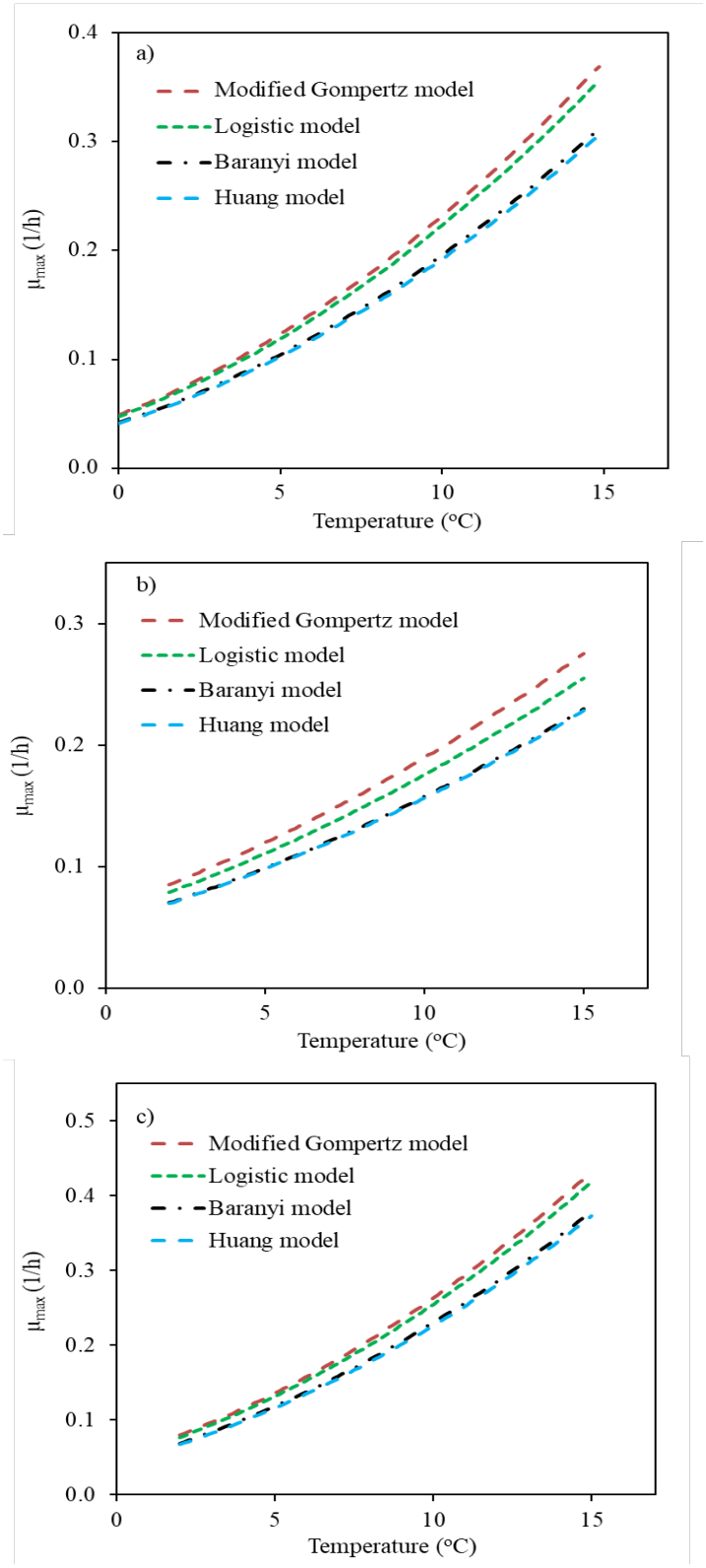

Figure 1. The effect of storage temperature on the maximum specific growth rate (μ_{max}) values obtained from one-step modelling approach for (a) fish, (b) pork and (c) chicken meat.

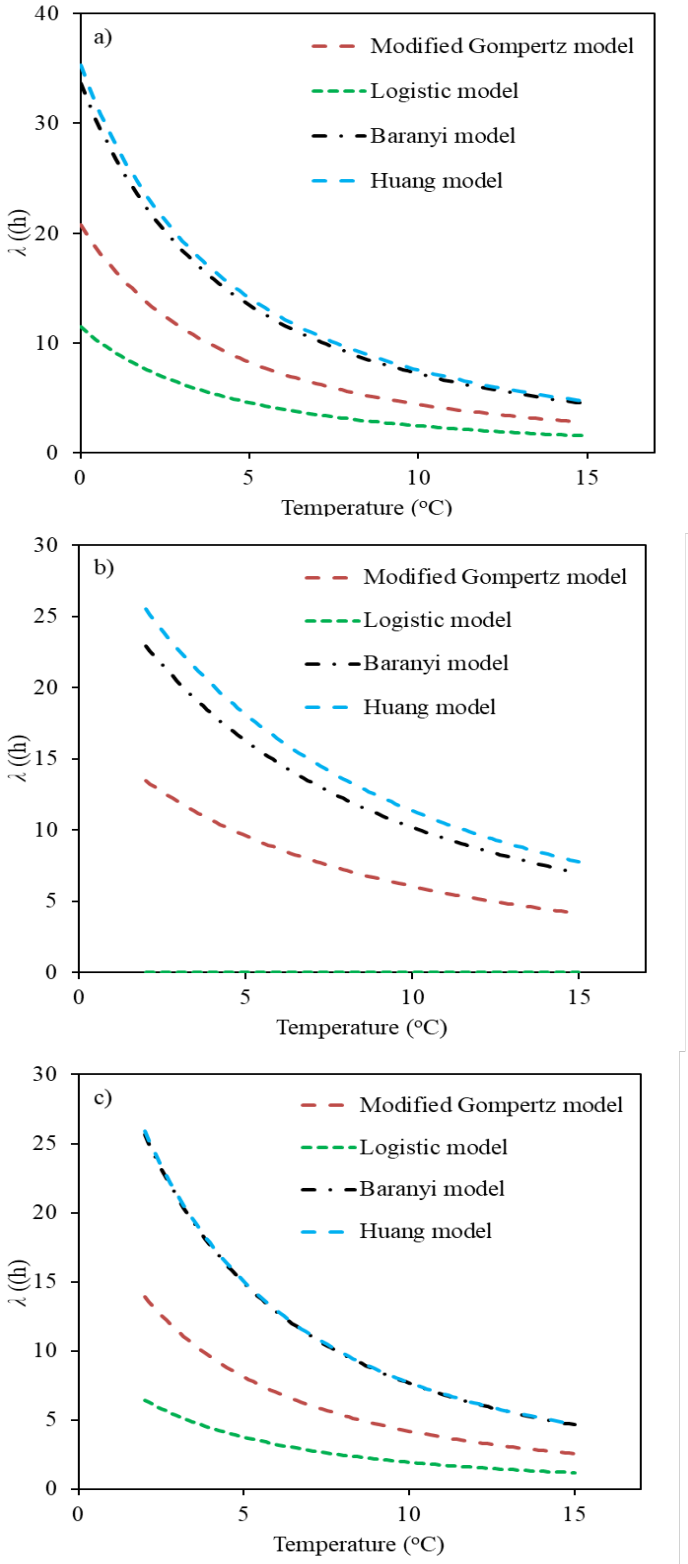

Figure 2. The effect of storage temperature on the lag phase duration (λ) values obtained from one-step modelling approach for (a) fish, (b) pork and (c) chicken meat.

Table 3. Validation criteria of one-step modelling approach based on the Huang model.

Food products	B_f	A_f	MD	MAD
Fish	1.014	1.059	0.02	0.33
Pork	1.075	1.080	0.39	0.41
Chicken	1.016	1.047	0.18	0.31

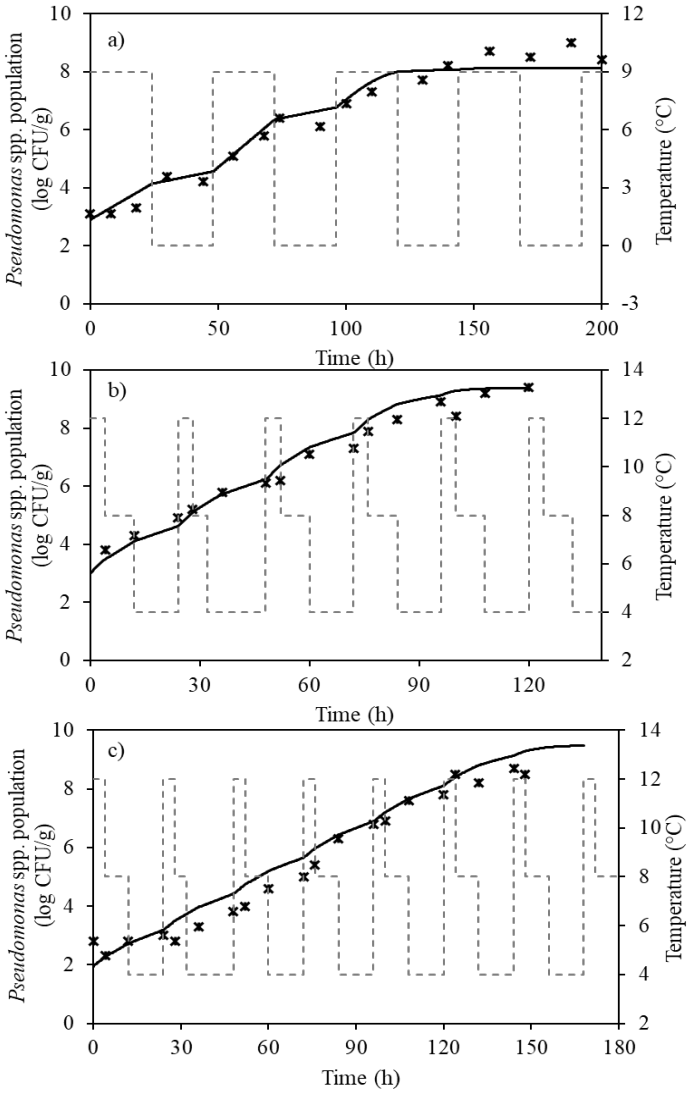

Figure 3. The prediction of *Pseudomonas* spp. concentration in (a) fish, (b) pork and (c) chicken meat subjected to non-isothermal storage conditions. Observed (*) and predicted (−) *Pseudomonas* spp. concentration. The dashed lines (--) show the changing temperature during storage.

Conclusion

No matter which primary model was used, the one-step modelling approach considerably improved the prediction capability of the models, which were published for the quantitative prediction of *Pseudomonas* spp. concentration in aerobically stored fish, pork and chicken meat. The successfully validated differential form of the Huang model merged with the Ratkowsky model provided valuable information to evaluate and simulate the growth behaviour of the *Pseudomonas* spp. in aerobically stored fish, pork and chicken meat under non-isothermal conditions in which the food products are usually subjected to during storage, delivery and retail marketing. The predictive models used in this work have a high potential to be used as a simulation tool for the meat processors to follow the microbiological quality of the food products before they reach to the consumers.

Compliance with Ethical Standard

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

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

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Food safety knowledge levels of gastronomy/culinary arts students and food workers in Southern Turkey

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ABSTRACT

This research was aimed to analyze the food safety knowledge levels between gastronomy and culinary arts students and food workers in the Çukurova Region of Turkey. A total of 155 participants (80 students, 75 food workers) have participated in this cross-sectional study. The overall knowledge scores of food workers and the students were 89.33% and 78.89% respectively, and food workers have more scores in all sections ($p<0.001$). Hygiene certification, occupation, and working establishments had a significant association with total food safety scores; however, gender, marital status, age, education level, frequency of hygiene education, working experience, monthly income had no significant association with their scores ($p>0.05$). A positive correlation was found between gender, education status, hygiene certification, and frequency of receiving hygiene education; but no correlation was found between working in any food business, graduated high schools, and age of the students ($p>0.05$). More knowledge gaps of both groups have been found about time-temperature control, holding/storage temperature, and cross-contamination. Finally, it is important to measure the reflection of the hygiene training on practices and to make it regular in ensuring food safety.

Keywords: Food safety, Food workers, Culinary art students

Introduction

Foodborne diseases and infections have become important rising public-health problems because of increased morbidity and mortality rates around the world (Taylor et al., 2015). This problem has not only negatively affected developing countries but also has effected negatively in developed countries. Moreover, foodborne diseases cause crucial social and financial problems in countries as well as their health system (Akoğlu et al., 2017; Draeger et al., 2019). Considering the main reason for these diseases around the world has shown that mass catering and food service facilities are the most frequent cause of foodborne outbreaks. Besides, the plurality of those outbreaks arises from wrong/ inappropriate applications during food handling processes (Giritlioglu et al., 2011; Taha et al., 2020). Like other countries worldwide, foodborne diseases have been an important problem in our country, which was the result of economic crises and negative effects on public health. Moreover, the distribution of foodborne diseases in our country is less known because reporting of foodborne illness to official authorities is less in our country.

Food workers are the most important key factor to prevent foodborne diseases in the way of their direct contact with the foods. Moreover, during the preparation steps in the food-products, they have an active role in transmitting the foodborne pathogens to the consumers because of their insufficient awareness level of food safety (Akoğlu et al., 2017; Alqurashi et al., 2019; Taha et al., 2020).

The main purpose of the gastronomy and culinary arts departments is to educate students on different food establishments as food and beverage management and provide knowledge to be a manager or staff in several food products operations. After graduation, students will work as managers or food workers in a number of cafeterias. On the other hand, food safety knowledge levels of food workers in the area and what kind of training should be given routinely to them are so critical to contribute to food safety in public health. Therefore, a comparative assessment of food workers and the students' knowledge and attitudes about food hygiene and safety are so essential to provide their safe food preparation and designing well training for their education/application. These factors are analyzed in the present study.

Materials and Methods

Sample Collection and Study Design

A questionnaire form was prepared to measure students' and food workers' knowledge and experience of food hygiene and safety by taking into consideration of validated previous studies (Baş et al., 2006; Giritlioglu et al., 2011; Osaili et al., 2018). Before the data collection, randomly selected participants have pre-tested the questionnaire. After this pre-test application, the questionnaire calibration was made including some questions that were transformed to be more clear and understandable for the participants (pre-test data not shown). The data were collected from gastronomy and culinary arts students and food workers with face-to-face and online interviews, from 15 February to 29 May 2020. The aim of the study and methodology was explained to the participants and those who agreed to participate in the study were asked to sign a consent form.

The questionnaire form was consisting of four sections (sections A, B, C, and D). In section (A), the determining of food workers' and the students' socio-demographic characteristics with 9 and 8 questions, respectively. In this section, the demographic characteristics of the participants, gender, age, working time, amount of the salary of the food workers; hygiene education and certification status, working experience in any food business, and schools where they were graduated were asked to the students. In section (B), the participants were given 14 questions to evaluate their level of knowledge about food hygiene and safety. In this section, there are information questions about food workers and students' kitchen hygiene, food safety, cross-contamination, food poisoning, disease-borne microorganisms, time and temperature control, and regulation about hygiene education of food workers. In response, they were asked to choose between ‘’correct’’, ‘’wrong’’ or ‘’don't know’’. The third section (C) contains 9 questions about safe food production practices. In response, they were asked to ‘’agree’’, ‘’no idea’’or ‘’disagree’’. In the last section (D), two questions were graded using a 5-point Likert scale ranging from "always ’’(4)" to "never (0)". The reason for using the Likert scale in section D is that hand washing after toilet and sneezing is very important in bacterial and viral transmission (Lund, 2015; Mehta, 2020). For this reason, it was thought to measure how often this hygiene practice was applied. The total food safety knowledge score of the participants was calculated by adding the correct answers to 25 questions in three categories (maximum score is 100) (Baş et al., 2006; Osaili et al., 2017). In sections B and D; each correct answer was given ‘’4’’ points, but other answers (wrong or don't know/any idea or disagree) were awarded ‘’0’’ points (the maximum scores for sections (B) and (D)are ‘’56’’ and ‘’36’’, respectively). In section C; always, often, sometimes, rarely and never answers were given as ‘’4’’, ‘’3’’, ‘’2’’, ‘’1’’, and ‘’0’’ points, respectively. The maximum score for section (C) is ‘’8’’.

Statistical Analysis

The IBM SPSS Statistics Version 20.0 statistical software package was used for data analysis. Categorical variables were expressed as numbers and percentages, whereas continuous variables were summarized as mean and standard deviation and as median and minimum-maximum where appropriate. A Chi-Square test was used to compare categorical variables between the two groups. For comparison of continuous variables between two groups, the Student's t-test or Mann-Whitney U test was used depending on whether the statistical hypotheses were fulfilled or not. For comparison of more than two groups, One way ANOVA or Kruskal Wallis test was used depending on whether the statistical hypotheses were fulfilled or not. P-value used in tables 3, 4, 5, and 6; Mann Whitney test (MW) result is 0.284, T-test (t) is 0.005, for Anova (A), it is 0.348, and Kruskal-Wallis (KW) is 0.325. The test statistic values and degree of freedom were not given in tables to keep the tables easy to understand. The statistical level of significance for all tests was considered to be 0.05.

Results and Discussion

The results have been analyzed in six categories. Section A: Demographic characteristics of the participants. Section B: Frequency and percentage levels of participants' safe food practices. Section C: Safe food production practices. Section D: Personal hygiene practices of the participants. Section E: Total food safety knowledge scores of the participants and section F: The relationships between demographic profiles and the scores of each section of the questionnaire.

Section A: Demographic Characteristics of Participants

Considering the food workers in Table 1: The majority of them male (84% male; 16% female). Because of tough hard-working conditions/cultural factors, females generally do not prefer working in food-production businesses. This result is in line with previous studies in Turkey (Baş et al., 2006; Çakıroğlu and Uçar, 2008; Tuncer and Akoğlu, 2020). Eighty-three percent of them were married. About 47% of food workers were between 30 and 40 years old, and 28% > 40 years old. The percentage of food workers with experience 0 and 6 years, 6–11 years, 12-20 years, and > 20 years were 23, 41, 24, and 12%, respectively. Besides, 73% of them were working in catering restaurants (seven restaurants) and 27% of them working in kebab restaurants (four restaurants). Regarding education, 32, 39, 20, and 9% of the participants had primary, secondary, high school, and higher (college/university) education levels, respectively. In our study, the number of food workers who graduated from the university is low (9%) and the result of this study is comparable with the studies performed by Tuncer and Akoğlu's (2020) results.

Table 1. Demographic characteristics of the participants (Section A)

Demographic factors		**Food Workers (*n*=75)**		**The Students (*n*=80)**	
		Freq.	**(%)**	**Freq.**	**(%)**
Gender					
Male		63	84	54	67.5
Female		12	16	26	37.5
Age(years)					
<30	18*	19	25	11	13.8
30-40	19*	35	47	19	23.8
≥40	≥20*	21	28	50	62.5
Education / Graduated High School*					
Primary school	Profession*	24	32	21	26.2
Secondary school	Anatolia*	29	39	42	52.5
High school	Open*	15	20	6	7.5
Collage (university)	Religious*	7	9	11	13.8
Hygiene certification					
Certificated		68	91	5	6.2
Not certificated		7	9	75	93.8
Hygiene education					
Once		47	63	51	63.7
2 or 3 times		21	28	15	18.8
≥4 times		7	9	14	17.5

* Questions of the students

The continuous of Table 1.

Demographic factors		Food Workers (*n*=75)		The Students (*n*=80)	
		Freq.	(%)	Freq.	(%)
Occupation	**Education status***				
Cook	Class one*	34	45.4	44	55
Assistant cook		16	21.3		
Waiter	Class two*	12	16	36	45
Others (participant, steward, manager)		13	17.3		
Working experience (years)	**Working in any food business***				
0-6	Yes*	17	23	32	40
6-12		31	41		
12-20	No*	18	24	48	60
≥20		9	12		
Monthly income(Turkish Liras)					
2500-3000		47	63		
3000-3500		24	32		
3500-4000		4	5		
Marital status					
Married		62	83		
Single		13	17		
Working area					
Catering		55	73		
Kebab restaurant		20	27		

* Questions of the students

In their study, 14% of food workers who worked in hotels had a bachelor's degree. The distribution rate of occupations for food workers; the cook was 45.4%, and 16% was waiter, respectively. The percentage of the participants who had taken food safety training (once) was 63% and 91% of them had a hygiene certificate. The training rate of the food workers was lower than the study of Tuncer and Akoğlu's study (2020). In their work, 82.3% of food processors previously joined training courses on food safety. Considering the students; 67.5% of the participants were males, 62.5% of them were ≥20 years old. In addition, 52.5% of them graduated from Anatolia High schools, 93.8% of them have no hygiene certificate, 40% of them working in a food establishment, 55% of them were in first-class (freshmen), and 45% of the second class (sophomore) in the university.

Section B: Frequency and Percentage Levels of Participants' Safe Food Production Levels

The results are shown in Table 2. As this table shows, nearly all students (98.75%) and all food workers (100%) agreed that ignoring food hygiene rules during food production caused foodborne diseases. These results are similar to a study about cookery students by Giritlioglu et al. (2011) and by a study about hospital food workers by Tokuc et al. (2009). Both studies reported (90.02 %) about this item.

Monitoring of holding temperature of hot ready-to-eat food is an important preventive method of foodborne illness. For example, *Clostridium perfringens* create heat-resistant spores that are not inactivated by cooking. Therefore, meals that are not eaten immediately but are prepared before should be cooled correctly and stored meals should be heated to at least 74°C for 15 seconds before consumption (Lund, 2015). The ideal holding temperature of hot ready-to-eat food should be more than ≥63°C (EFSA and ECDC, 2015). Our results show that 23.75% of students and 77% of food workers had accurate information about this item. Students' findings are lower but food workers' findings are higher than the previous study of Giritlioglu et al. (2011). In their study, about 48.8% of food workers knew the right temperature. In the two groups, 13% of food workers and 23.75% of students gave correct answers to the question of "bacteria don't proliferate at 90°C". And 37.5% of students had no idea about it. The majority of food workers (87%) and 38.75% of the students have incorrect information about the temperature of bacterial proliferation in foods. Our results were lower than Giritlioglu and colleagues' (2011) results; their results were 56% about this item. Proba-

ble causes of our results are lack of information about bacterial growing in ready-to-eat foods. Another question was "only ill people carry bacteria which may cause food poisoning", 25% of food workers and 3.75% of students knew the correct answers. Besides, 7.5% of students have chosen no idea about it. Both of our groups' results were lower than the previous study by Ncube et al. (2020). In their study, 78.2% of the participants knew the correct answers to this question. These findings show that participants did not understand the risk of food contamination that an unsanitized food worker posed. Like our results, some studies reported that food workers lacked adequate knowledge about the risk of contamination of food by diarrheal food workers (Clayton et al, 2002; Osaili et al., 2011). In contrast, some studies have demonstrated that food workers have good knowledge about this issue (Da Cunha et al., 2014; Pichler et al., 2014). Considering the other question that "bacterial proliferation stop at body temperature (37°C)", 12% of students and 32.5% of food workers answered this question correctly, but 15% of students have chosen ‘’no idea’’ about it. Akoğlu et al. (2017) found both of our groups’ results lower than the previous study. In their study; 89.4% of the participants gave correct answers to this item.

Improper thawing application of frozen poultry and red meat can cause food poisoning. During the thawing process, the temperature becomes permissive for bacterial growth (Marriott and Gravani, 2006). Thawing food in room temperature keeps food temperature at a danger zone for a long time. This condition fosters microbial growth, which may cause food poisoning. The methods of thawing the frozen meat/poultry are; thawing at refrigerator temperature (5°C; app. whole day), in a microwave or cold water (app. an hour by changing the water every 30 minutes) (USDA, 2013). Temperature control of the meat is an important way to prevent poisoning, like thawing in a refrigerator. In this study, 71% of food workers and 58.7% of students did not thaw frozen meat at room temperature in proportion to the 64.6% result of a recent study in Turkey (Akoğlu and Tuncer, 2017); food workers’ scores were higher but our students' scores were lower than Akoğlu and Tuncer's study (2017). Besides, both of our group's knowledge levels were higher than some previous studies such as 38.2% in China (Gong et al., 2016), 66% in Kuwait (Al-Kandari et al., 2019), and 54% in the United Arab Emirates (Taha et al., 2020). On the other hand, 87.5% of students and 74.6% of food workers knew that re-freezing of thawed foods causes foodborne diseases. However, 8.75% and 22.7% of them did not know this item, respectively. And 3.75% of students and 2.7% of food workers had no idea about this subject. Our two groups' results were lower than Akoğlu and Tuncer's (2017) reports. In their study, 92% of gastronomy and culinary arts students gave the correct answer to the aforementioned question. In contrast, our results were higher than Sani and Siow’s (2014) studies, in which about 75% of the respondents were certain about refreezing defrosted food.

Table 2. Food safety knowledge, safe food production and personal hygiene practices of the participants

Food Safety Knowledge (Section B)	The Given Answers of Food Workers and Students*					
	Correct		Wrong		Don’t know	
	Freq.	%	Freq.	%	Freq.	%
1. Failure to comply with the hygiene rules in the preparation of foods, causes foodborne illness	**75/79**	**100/98.75**	-/1	-/1.25	-/-	-/-
2. Incorrect heating of food causes foodborne illness	**75/74**	**100/92.5**	-/3	-/3.75	-/3	-/3.75
3. Foods that are ready to eat should be stored at 20°C	17/31	23/38.75	**58/19**	**77/23.75**	-/30	-/37.5
4. In ready-to-eat foods, bacteria multiply at about 90°C	65/31	87/38.75	**10/27**	**13/33.75**	-/22	-/27.5
5. Bacteria that cause food poisoning are only transmitted by sick people	56/71	75/88.75	**19/3**	**25/3.75**	-/6	-/7.5
6. Bacteria stop proliferation at body temperature (37°C)	66/42	88/52.5	**9/26**	**12/32.5**	-/12	-/15
7. Frozen chicken and red meat should be thawed at room temperature	21/27	28/33.75	**53/47**	**71/58.75**	1/6	1/7.5
8. Re-freezing of thawed foods can cause foodborne illness	**56/70**	**74.6/87.5**	17/7	22.7/8.75	2/3	2.7/3.75
9. After cooked foods cool down to 21°C at room temperature, they should be put in the refrigerator / cold storage	**72/48**	**96/60**	3/18	4/22.5	-/14	-/17.5
10. The same cutting board can be used for chopping and cutting different types of meat (red meat, chicken, fish, etc.)	69/60	92/75	**6/13**	**8/6.25**	-/7	-/8.75
11. The same cutting board can be used to chop meat and vegetables	70/66	93/82.5	**5 /9**	**7/11.25**	-/5	-/6.25
12. *Salmonella* is an important bacterium that causes food poisoning	**65/41**	**87/51.25**	4/23	5/28.75	6/16	8/20
13. Food should be served no later than two hours after preparation	**71/51**	**95/63.75**	3/19	4/23.75	1/10	1/ 2.5
14. Food production personnel must have a hygiene certificate	**75/76**	**100/95**	-/2	-/2.5	-/2	-/2.5

*Students’ answers were given after ‘’/’’

****Correct answers are given in bold type**

The continuous of table 2.

Safe Food Production Practices (Section C)	Agree		No idea		Disagree	
	Freq.	%	Freq	%	Freq.	%
1. Food safety is an important matter in the food sector. Therefore, I attending to my personal hygiene	**75/76**	**100/ 95**	-/3	-/3.75	-/1	-/1.25
2. In food production processes, I always prefer fresh raw materials	**75/78**	**100/ 97.5**	-/2	-/2.5	-/-	-/-
3. If my hands or fingers are cut, I never contact raw/cooked food.	**75/72**	**100/ 90**	-/6	-/7.5	-/2	-/2.5
4. I wear protective materials (cap, mask, and gloves) during food production to avert foodborne illness	**75/78**	**100/ 97.5**	-/-	-/-	-/2	-/2.5
5. To ensure safe food production, my working area always remains clean	**75/80**	**100/ 100**	-/-	-/-	-/-	-/-
6. I don't wear my uniform and shoes outside my workspace	**74/70**	**98.7/ 87.5**	-/3	-/3.75	1/7	1.3/ 8.75
7. Without wearing protective gloves, I don't contact raw foods (especially raw meat, eggs, chicken meat, etc.)	**75/65**	**100/ 81.25**	-/9	-/11.25	-/6	-/7.5
8. During food production processes, I don't wear jewelry such as rings, earrings, and watches	**74/79**	**98.7/ 98.75**	-/-	-/-	1/1	1.3/ 1.25
9. When I have the flu, cold or diarrhea, etc. I don't work in the production line	**75/73**	**100/ 91.25**	-/2	-/2.5	-/5	-/6.25

Personnel Hygiene Practices (Section D)	Always		Often		Sometimes		Rarely		Never	
	Freq.	%	Freq.	%	Freq	%	Freq.	%	Freq.	%
1. How often do you wash your hands after using the toilet?	75/ 80	100/ 100	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-
2. Do you wash your hands after coughing or sneezing? How often?	75/ 34	100/42.5	-/32	-/40	-/9	-/11.25	-/3	-/3.75	-/2	-/2.5

*Students' answers were given after ''/''

****Correct answers are given in bold type**

Inappropriate temperature/time control and cooling of the cooked/pre-cooked foods are the most common cause of foodborne diseases. According to the World Health Organization (WHO), nearly 75-85% of all foodborne illness results from time/temperature misuse, and 56% of them result from improper cooling. After cooked/pre-cooked foods should be kept at over 60°C to prevent the growth of food poisoning bacteria. In general, between 5°C and 63°C is considered a " danger zone" in which those bacteria can grow quickly at this temperature range. Proper cooling procedures for cooked or pre-cooked foods have been explained by the literature. Briefly; after cooking, a two-stage cooling method is commonly applied to prevent bacterial growth in the food. The first step consists of cooling the food product from 60°C to 21°C in 2 hours, instead, in the second step, cooling takes place for up to 4 hours from 21°C to 5°C. The first step just mentioned is the most critical because microbial growth of the bacteria at temperatures between 51°C and 21°C is usually faster than low temperatures (US FDA, 2017). The questions about time/temperature control and cooling were the most frequently answered incorrectly by the respondents according to some previous studies. Our food workers' results were higher than some recent studies (Gomes-Neves et al., 2014; Al-Kandari et al., 2019). In their study, food workers had less knowledge about time and temperature control (45 and 63%), respectively. However, our students' results (60%) were lower than Al- Kandari et al. (2019)'s study but over than Al-Shabib et al. (2016)'s study. However, 22.5% of students and 4% of food workers gave wrong answers to that "after cooked foods cool down to 21°C at room temperature, they should be put in the refrigerator / cold storage" in our study. Moreover, 17.5% of students had no idea about this issue. Food workers have more information/awareness about correct cooling practices than the students do. These results might be based on the working experience. Other findings are that only 16.25% of the students and 8.75% of food workers were aware that red meat and poultry meat cannot be chopped together on the same cutting board, and that 82.5% of students and 93% of food workers were not aware of it is hazardous in terms of cross-contamination. Our results are lower than the previous study by Taha et al. (2020). In their study, 39.5% of food workers were not aware of these items. The possible causes of our results might be the participants were less informed about the importance and relationships between cross-contamination and food poisoning in their training/education period. *Salmonella* is a Gram-negative bacterium that

is important food-borne pathogens and responsible for several outbreaks worldwide and this pathogen might be transmitted by food workers' lack of hygiene application (Sun et al., 2020). In our study, 52.25% of the students and 87% of food workers knew that this bacteria can cause serious food poisoning. These results were higher than the results of Alqurashi et al. (2019). In their study, 7.6% of the hospital food workers in Saudi Arabia knew that *Salmonella* is an important bacterium that causes food poisoning.

Microorganisms reproduce rapidly in foods kept in room conditions. Therefore, cooked foods that are not stored in the refrigerator deteriorate in a short time. If these foods are not consumed immediately, they should be kept in refrigerator conditions after they cool down quickly (Lund, 2015). Most of our respondents answered this item correctly. Sixty-three point eight percent of the students and 95% of food workers knew that food should be served no later than two hours after preparation. However, in the present study, almost a quarter of the students (23.75%) were unaware of this risk. Our results were higher than Osaili et al.'s (2018) study. They reported that a small part of the participants (15%) had known the correct retention temperature needed to eat. Besides, the results of the students were lower than Güven and his colleague's (2010) results. However, our food worker's results were higher than the results of Güven et al.'s (2010) study. In their study, 10.9% of food workers were not aware of this item, and 89.1% of their respondents knew that "Cooked foods should be consumed immediately or if they are to be consumed later it should be stored in the refrigerator after cooling down quickly". Also, while 95% of students and 100% of food workers knew that the staff involved in food production had a hygiene certificate before operating food businesses, they knew that a hygiene certificate was needed according to European and Turkish legislation (EC 2004; Anonymous, 2013).

Section C: Safe Food Production Practices

These results are shown in Table 2. The table indicates that almost all students (95%) and all food workers (100%) shown a high-level consideration of personal hygiene. Ninety percent of the students and 100% of food workers were aware that severe foodborne diseases can be caused by hand or finger injury. This result was in agreement with a study by Tokuc et al. (2009) and higher than the study of Al-Kandari et al. (2019). They found that almost 93.2% of the food workers in hospitals and 85% of food workers in Kuwait Restaurants were knowledgeable about the risk of touching on the food with cut hands or fingers, respectively.

The use of protective equipment is one of the most important prerequisites in the food production line. In each step of food production and service processes, In order to prevent microbial contamination, food personnel should wear masks, caps, and gloves. Nearly all students (97.5%) and all food workers (100%) stated that they used all protective types of equipment in each step of food production. Our results were parallel to previous studies conducted in Saudi Arabia and Turkey; Alqurashi et al.'s (2019), Çakiroglu and Uçar's (2008) studies. They reported similar results in their study in which 82.2% (cap), 70.6% (mask), 81% (gloves), and 82.9% of the hospital food staff and food workers put on caps, masks, and gloves all along with the food production processes, respectively. In the present study, all of the respondents (100%) indicated that they always keep clean in their working area to maintain safe food production. Also, 87.5% of the students and 98.7% of the food workers clarified that they did not dress in the work shoes and clothes outside of their working area. Our results were similar to Giritlioglu et al.(2011). In their study, 90.2% of cookery students stated that they did not use working clothes and shoes outside their workspace.

According to literature; "people's throat, nose, intestines, and feces are loaded with bacteria" thus, the human is the potential source of both saprophyte and pathogenic microorganisms in foods. Therefore, food workers play a crucial role in the transmission of respiratory (cold, angina, pneumonia and covid-19 diseases, tuberculosis, scarlet fever) and digestive (dysentery, cholera, typhoid) disease factors (Palulu, 2014; Mehta, 2020). For all these reasons, it is so important to use an apron, gloves, cap, arm sleeve, and to comply with personal hygiene rules during each step of food preparation. According to our study, most of the students (81.7%) stated that they did not touch raw food without wearing protective gloves and most of them (84.2%) stated that they did not wear jewelry during food production. Palulu (2014) reported that 97.2% of food workers put off their jewelry before starting in food handling. On the other hand, Al-Shabib et al. (2016) indicated in their study that about 75.9% of street vendors in Saudi Arabia are knowledgeable that wearing accessories in the food processing area could cause bacterial contamination. Most of the students (89%) stated that they do not deal with food when they have flu, cold, or diarrhea. Similarly, this rate had been reported as 86.2% in Al-Shabib et al.'s (2016) study. Our results indicate that the students were aware of the risk of unhealthy personnel working in the food production area and that their education had successfully taught them, that infections can be easily transmitted under this kind of condition.

Section D: Personal Hygiene Practices

These results are presented in Table 2. Handwashing is an essential behavior for providing food safety and has a crucial role in eliminating bacteria and viruses. Recently, this hygiene practice has become the most important issue due to the spreading of the Sars CoV-2 virus and the subsequent efforts to prevent the spread of the virus worldwide**.** People who are asymptomatically infected can secrete some amount of bacteria or viruses for a long time, and those with symptoms can continue to secrete the pathogenic microorganisms after getting rid of the symptoms of infection. No matter how hygienically stored, prepared, cooked, or kept food can be contaminated with food by dirty hands and dirty tools. Therefore, effective hand washing (at least 20 seconds including each part of the hands) and good hygiene practices for food workers are so crucial to prevent the risk of infection (Lund, 2015; Mehta, 2020). Table 4 shows that 100% of all respondents stated that hand washing was important after using the toilet and 100% of food workers stated that hand washing was also so important after coughing and sneezing. Considering the other studies, our food workers' results were similar to Osaili et al.'s (2017) study but higher than Tokuc et al.'s (2009) reports. Their findings were 99.2% and 93.2%, respectively in this manner. In other results about personal hygiene, 100% of food workers and 42.5% of the students indicated that they always washed their hands after coughing or sneezing, while 40% of them answered as "often". The students' results in the present study were lower than previous studies of Darko et al. (2015) and Osaili et al. (2017). In their studies, 87.2 and 91.2% of the kitchen staff knew the correct time to wash their hands, respectively. Our results indicated that the students have lower attention to hygiene –need more education about this subject- than other necessities about food safety.

Section E: Assessment of Total Food Safety Knowledge Scores among Food Workers and Students

Table 3. indicated that a statistically significant difference was found between the food safety knowledge, food safety practice, and personal hygiene total scores of the students and food workers ($p<0.001$). Between the two groups; food safety knowledge, food safety practice, and personal hygiene total scores of food workers were found to be statistically higher than the students ($p<0.001$). As seen in Table 3; the overall score of food safety knowledge aspects of food workers was 45.44 ±5.41 out of 56 points, corresponding to 81.20% of questions answered correctly. This score was 38.35 ±12.37 in Gastronomy students and corresponding to 68.48% of questions answered correctly by them. The food safety practices aspect of food workers was 35.89±0.92 out of 36 points, corresponding to 99.70% of questions answered correctly by them. However, this score was 33.55±3.34 in Gastronomy students and corresponding to 93.20% of questions in this section answered correctly by them. Personnel Hygiene Practices aspect was scored the highest percentage of correct food workers (100%), whereas this rate was measured as 87.38% in the students. Table 4. shown that between the two groups; total food safety knowledge, food safety practice, and personal hygiene scores of food workers (89.33%) were found to be higher than the students' scores (78.89%) ($p<0.001$). Total food safety knowledge level of each group [89.33% (food workers), 78.79% (the students) respectively] and total mean scores (83.94%) of both groups shown in the current study were higher than the results of some previous studies in the Middle East and European countries; Dubai and Sharjah (70%) (Taha et al., 2020), in Kuwait (70%) (Al-Kandari et al., 2019) and in Portugal (56.5%) (Gomes-Neves et al., 2014); but our students' scores were lower than Al-Shabib et al.'s (2016) study. In their study, the ''personal hygiene'' scores of the participants were found (80%) in Saudi Arabia. Also, the lower level of the students' scores than food workers about each section in the present study are so different from Akoğlu and Tuncer's study (2017) conducted in Turkey. They found higher scores about food safety knowledge levels (72.88%) of their students than previous studies of food workers. Besides, our students' food safety knowledge levels (78.79%) were higher than their scores.

Table 3. Comparison of food safety knowledge, food safety practice, and personal hygiene practices among food workers and students

	Groups			
	Food Workers (*n*=75)		Gastronomy and Culinary Arts Students (*n*=80)	
Variables	**Possible range of the scores**	**Mean ±Standard Deviation Med (Min, Max)**	**Mean ±Standard Deviation Med (Min, Max)**	**p value**
(1) Food Safety Knowledge Total Scores	0-56	45.44 ±5.41 48 (28.56)	38.35 ±12.37 36 (12.56)	**<0.001*^{t}**
(2) Food Safety Practices Total Scores	0-36	35.89 ±0.92 36 (28.36)	33.55 ±3.34 36 (24.36)	**<0.001*^{t}**
(3) Personnel Hygiene Practices	0-8	8 ±0.00 8 (8.8)	6.99 ±1.09 7 (4.8)	**<0.001*^{MW}**

***p-value <0.05** is significantly different

p-value used in the tables; Mann Whitney test result is 0.284^{MW} and T-test is 0.005^{t}

Table 4. Comparison of total food safety knowledge scores of food workers and the students

Groups	Mean±SD	Median	Min-Max	p value
Food workers, *n=75*	89.33 ±5.36	92.0000	72-94	<0.001*[t]
Students, *n=80*	78.89 ±13.14	79.5000	41-94	
Total	83.94 ±11.39	88.0000	41-87	

*p-value<0.05 is significantly different.
p-value used in the tables; T-test is 0.005[t]

Section F: Relationships between Demographic Profiles and Participants' Food Safety Knowledge Levels

Table 5. indicated that for food workers, gender, marital status, age, education level, frequency of hygiene education, working experience, monthly income had no significant association with total food safety knowledge (p>0.05). Similarly, some recent researches have reported no correlation was found between gender and food safety knowledge of food workers (Osaili et al., 2018; Taha et al. 2020). They did not found a positive correlation between marital status and occupation and food safety knowledge of food workers. The possible causes of our results may be depending on participants; male or female, married or single, participants are all working under similar conditions as food workers. On the other hand, in our study; hygiene certification, occupation, and working establishments had a significant association with total food safety scores of the food workers (p<0.05). This result is parallel to the study of Rebouças et al. (2017). In their study, the restaurant staff had more personal hygiene information, better attitudes, and practices compared to the chief cooks and business managers, and the level of knowledge of the chief cooks and managers was insufficient in Brasil. The possible reasons for our results may be that food production personnel (especially head-cook/cook) are more aware of hygiene practices than other personnel (waiter, dishwasher, manager,.. etc). Besides, according to Turkish legislation, hygiene certification must be obtained by food workers to work in food establishments. Moreover, food workers from catering restaurants tend to be more disciplined than food workers that worked in kebap restaurants. And, this result may be dependant on various meals that are prepared–precooked/cooked and different procedures of cooling and reheating meals- in catering restaurants as opposed to kebap restaurants. Our results are different from Taha et al.'s (2020) study; they found a significant association between education, age, and experience of food workers in their research. However, we did not find a significant association between working experience and education level, monthly income, and frequency of hygiene education of food workers. This may be because of food workers who all have similar education levels, working experience, and similar monthly incomes in the present study. Considering the hygiene training frequencies of food workers; all of them took hygiene education, and the higher scores were found in the "one-time hygiene trained group''. And other scores show some similarities in Table 5. This result may be depending on the effectiveness of the hygiene education taken by the participants that affected several aspects. These are education strategies, training areas, voluntary participation in education, a culture of work that is influenced by education, and food processors' beliefs, norms, and perceptions of their peers (Griffith et al., 2010; Taha et al., 2020). The possible reasons for these distinct results may be due to the efficiency of the training/practices. To ensure correct behaviors of food workers about their practices to prevent the occurrence of foodborne diseases and forging a positive food safety culture; the training programs should be designed to be comprehensive and according to necessities of the working conditions as well as suggestions of food workers.

Considering the students; as seen in Table 6; age, graduated high school, working in any food business had no significant association with total food safety knowledge (p>0.05). Similarly in a recent study conducted in Turkey has reported no significant association between age and food safety knowledge of food workers (Akoğlu and Tuncer, 2017). However, they found a significant association between the graduated high school, working in any food business in their study (p<0.005). The probable causes of our results might be most of the students have similar age groups and most of them graduated from an Anatolia high school in which they were not given occupational education. Therefore, the students were not educated for a job in the food sector. Besides no correlation was found between students' experiences with and without doing part-time jobs. On the other hand, gender, hygiene certification, frequency of receiving hygiene education, and their class (education status) had a significant association with total food safety scores of the students (p<0.05). Our results have shown some similarities and differences with some recent studies conducted in Turkey by Akoğlu and Tuncer (2017) and Ertopcu et al. (2019). They had found an association between education level(class) and food safety training with total food safety scores of the students (p<0.05). This finding is in agreement with our results. However, they had

no association with gender and food safety scores of the students (p>0.05). Besides, Ertopcu et al. (2019) had found an association between gender and total food safety scores of the students (p<0.05). Hygiene trained students have more scores than not trained students about food hygiene and safety in our study. This result is parallel to findings reported recently by Osaili et al. (2018) in Jordan and Taha et al. (2020) in Saudi Arabia. It is well known that hygiene certification and effective hygiene education are mandatory factors to maintain food safety for gastronomy and culinary arts students as well as food workers.

Table 5. Comparison of total food safety scores by characteristics of food workers (*n=75*)

		Total Score			
Group 1(Food workers)		**N**	**Mean±Sd**	**Median (Min, Max)**	**p value**
Gender	Female	12	86±8.78	90(72,96)	0.284^{MW}
	Male	63	89.97±4.24	92(80,100)	
Age	<30 years	19	88.0±5.66	88(72,96)	0.348^{A}
	30-40 years	35	89.37±6.21	88(72,100)	
	>40 years	21	90.48±2.96	92(84,96)	
Education Level	Primary School	24	87.83±5.84	88(72,96)	0.325^{KW}
	Middle School	29	90.07±4.61	88(80,96)	
	High Scool	15	88.33±5.98	92(72,96)	
	University	7	91.43±4.86	92(84,100)	
Hygiene Certification	Yes	68	93.71±3.15	92(92,100)	**0.013*^{MW}**
	No	7	88.88±5.35	88(72,100)	
Frequency of Receiving Hygiene Education	Once	47	89.79±5.77	92(72,100)	0.642^{KW}
	2 or 3 times	21	88.57±4.78	88(80,100)	
	4 andovertimes	7	88.57±4.28	88(84,96)	
Occupation	Chefcook	34	90.82±4.24	92(80,100)	**0.044*^{t}**
	Waiter	12	90.33±2.67	92(84,92)	
	Assistantcook	16	87.5±4.35	88(80,96)	
	Other (manager, dishwasher, etc.)	13	86.77±8.85	92(72,96)	
Working Experience	0-6 years	17	87.06±7.28	92(72,96)	0.154^{KW}
	6-11 years	31	89.68±5.04	88(80,100)	
	12-20 years	18	91.11±4.24	92(80,100)	
	>20 years	9	88.89±2.67	88(84,92)	
Monthly Income (Türkish Liras)	2500-3000	49	88.98±5.80	92(72,100)	0.635^{KW}
	3000-3500	21	89.71±4.66	92(80,96)	
	3500-4000	5	91.20±3.35	92(88,96)	
Marital Status	Single	13	89.54±4.18	92(80,96)	0.994^{MW}
	Married	62	89.29±5.60	92(72,100)	
Working Establishments	Catering	55	88.51±5.76	88(72,100)	**0.005*^{t}**
	Kebap Restaurant	20	91.60±3.15	92(72,100)	

*p-value **<0.05** is significantly different

p-value used in the tables; Mann Whitney test result is 0.284^{MW}, T-test is 0.005^{t}, for Anova, it is 0.348^{A} and Kruskal-Wallis is 0.325^{KW}

Table 6. Comparison of total food safety scores by characteristics of the students(*n=80*)

		Total Score			
Group 2(Students)		**N**	**Mean±Sd**	**Median (Min, Max)**	**p value**
Gender	Female	54	77.19 ±11.93	76 (48,100)	**0.026*^{t}**
	Male	26	82.42 ±14.98	84.5 (41,100)	
Age	18	11	74.10 ±7.67	71 (63,88)	
	19	19	77.84 ±15.91	80 (18,100)	0.338^{KW}
	≥20	50	80.34 ±13.14	79.5 (41,100)	
Graduated High School	Occupation high school	21	78.52 ±14.66	80 (41,99)	
	Anadolu high school	42	80.02 ±12.54	79 (51,100)	
	Basic-Open highs chool	6	74.67 ±9.60	71.50 (66,91)	
	Religious high school	11	77.55 ±14.99	78 (48,99)	0.792^{KW}
Hygiene Certification	Yes	14	87.07 ±9.45	87.50 (71,100)	
	No	66	77.15 ±13.20	77 (41,100)	**0.008*^{MW}**
Frequency of Receiving Hygiene Education	0	51	74.33 ±12.73	75 (41-100)	
	Once	15	87.80 ±8.95	88 (64,99)	**<0.001*^{KW}**
	2 or 3 times	14	85.93 ±10.64	86 (70,100)	
Working in any Food Business	Yes	32	80.47 ±14.90	82 (41,100)	
	No	48	77.83 ±11.87	79 (48,99)	0.293^{t}
Education Status	1. Class	44	70.68 ±9.77	71 (41,88)	
	2. Class	36	88.92 ±9.17	90 (58,100)	**<0.001*^{t}**

*p-value **<0.05** is significantly different
p-value used in the tables; Mann Whitney test result is 0.284^{MW}, T-test is 0.005^{t}, for Anova it is 0.348^{A} and Kruskal-Wallis is 0.325^{KW}

Conclusions

In this study, we compared knowledge levels in safe food production and hygiene practices between gastronomy and culinary arts students and food workers. Our results indicated that students had less information in some important food safety practices such as time/temperature control, storage/holding temperatures of the meals, cooling, freezing, and thawing methods of the food, important foodborne pathogens, preventive methods for cross-contamination, and the importance of handwashing than food workers. However, the food workers had less knowledge in some aspects including time and temperature control, holding/storage temperature of the meals especially cross-contamination. Besides, two groups highly cared about the importance of personal hygiene applications in the food production line. Food workers' safe food knowledge and practices can be crucial in public health and the economic standing of businesses in the catering industry. Also, the students will be food production workers or managers/chefs in the future. Their food safety knowledge level and practices also will affect either positively or negatively on the economy and public health Therefore, hygiene education and practices and HACCP education should be given to both groups, routinely. The most proactive method for guaranteeing safe food production in the future may be an effective HACCP and ISO 22000 Quality Management Systems implementation in food establishments and especially the catering sector.

Compliance with Ethical Standard

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

Ethics committee approval: This study was approved by the Scientific Research, and Publication Ethics Committee of Osmaniye Korkut Ata University was received on 11.05.2020 with the document number 2020 /18 /3.

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Tarihin gelişim sürecinde Türk yemek kültürü ve beslenme alışkanlıklarının değişimi

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ÖZ

İnsanın yaradılışından bu yana yaşamsal faaliyetlerini sürdürebilmesi için gereken unsurlardan biri beslenmedir. Fizyolojik bir zorunluluk olan beslenme, insanlığın yaşam şartlarının gelişimi ile paralel ilerlemiştir. İçgüdüsel olarak gerçekleşen bu durum, doğada önce toplayıcılıkla başlamıştır. İnsanlar doğada buldukları zararlı bitkileri ayırt ederek yenebilir yiyecekleri bulmuşlardır. Ateşin bulunmasıyla pişirmeyi öğrenen insanoğlu, zamanla hayvanlardan da elde edebileceği gıda maddelerini keşfetmiştir. Bu keşifle farklı lezzetleri denemiş ve vücudun gerekli olan protein, vitamin ve yağ gibi ihtiyaçlarını karşılamıştır. Beslenme çeşitliliği damak tadı olgusunu da beraberinde getirmiştir. Tüketilen yiyeceklerin farklılık göstermesinin başlıca sebepleri; o dönemdeki toplulukların yaşadıkları bölgenin iklimi, doğa koşulları ve bu bölgelerde yetişen sebze, meyve ve tahıllardır. Yine bölgede yaşayan hayvanların çeşitliliği sebebiyle tüketilen yiyeceklerin farklılık göstermesi kaçınılmazdır. Ekolojik dengenin sonucu olan bu durum, zaman içerisinde toplumlar arası kültür farklılıklarının başlıca sebeplerinden biri olmuştur. Yemek kültürü, coğrafi bölgelere göre ayrılmış ve "yöresel yemekler" diye adlandırdığımız kültür oluşmuştur. Günümüze kadar olan süreçte insanoğlu birçok gıda hazırlama ve pişirme tekniğini deneyerek yeni lezzetler geliştirmiştir. Bu çalışmada Türk yemek kültürü ve beslenme alışkanlıklarının tarih boyunca gelişim süreçleri derlenmiştir.

Anahtar Kelimeler: Beslenme alışkanlıkları, Yemek kültürü, Türk yemek kültürü

ABSTRACT

Turkish food culture and nutrition habits in the development of history

Since the existence of mankind, nutrition is one of the necessities to maintain their vital activities. Nutritional habit, a physiological movement, has progressed in parallel with the development of living conditions of mankind. This instinctive behaviour has started with gathering in the nature originally. People have found edible foods by distinguishing the harmful plants in the nature. Mankind, who had learned cooking with the invention of fire, has discovered foodstuffs that can be obtained from animals in time. Due to this discovery, they had an opportunity to try different flavours and supply a greater variety of needed macro components of their body such as proteins, vitamins and essential oils etc. This nutrition diversity has brought with the taste phenomenon. The major reasons of consumed food variety are the climate of the region they live in, the condition of nature and the kinds of vegetables, fruits, grains that grow in these regions. Furthermore, it is inevitable that the diversity of animals living in the region causes food diversity. This situation, which is the result of ecological balance, has been one of the main causes of cultural differences between societies over time. The culinary culture has been seperated by geographical regions over time and a sub-culture called "regional dishes" has formed. Until today, mankind have developed new tastes by experimenting with many food preparation and cooking techniques. In this study, the development of Turkish food culture and nutrition habits throughout the history have been reviewed.

Keywords: Nutrition diversity, Food culture, Turkish food culture

Giriş

İnsanlık tarihi Paleolitik, Mezolitik, Neolitik, Tunç ve Demir Çağları gibi çeşitli dönemlere ayrılmaktadır. İnsanoğlunun Anadolu'da en eski görülme tarihi ise Eski Taş Çağı ya da Yontma Taş Çağı adı verilen Paleolitik Çağ'ın başlarına kadar uzanmaktadır. Paleolitik Çağ'da insanoğlu, çevre ve iklim koşullarındaki değişikliklerin beraberinde biyolojik ihtiyaçlarını karşılamak için, beslenebilecekleri kaynak bulmak ve avlanmak amacıyla küçük topluluklar halinde sürekli yer değiştirerek, konar-göçer bir yaşam sürdürmüşlerdir (Aksoy ve Çetin, 2018). Biyolojik ihtiyaçlar doğrultusunda karşımıza çıkan yemek yeme, tarihsel süreçte ihtiyacın da ötesine geçerek toplumda dinsel, toplumsal ve kültürel kimliği belirtmenin farklı bir yöntemi olarak kullanılmaya başlanmıştır (Ay ve Şahin, 2014).

Bir toplumun kültürel değerleri, diğer toplumların yaşam biçimlerini ne kadar etkilerse yeme-içme alışkanlıklarını da bir o kadar etkilemektedir. Bu nedenle toplumun yeme-içme alışkanlıkları, yaşadığı yörenin tarımsal, sosyo-kültürel ve coğrafik özellikleri ile diğer toplumlarla olan ilişkilerinden de etkilenmektedir (Güler, 2008). Her kültürün etkilenmeler konusunda da yaklaşımı farklılık gösterebilmektedir. Örneğin bir toplumda beğenilmeyen bir yiyecek diğer bir toplumda önde gelen lezzetlerden sayılabilmektedir (Talas, 2005).

İnsanın yeryüzünde görülmesinden başlayarak yerleşik hayata geçişine kadar olan süreç Neolitik Çağ adını almaktadır (Akın ve ark., 2015). Bu çağda fizyolojik ve toplumsal adaptasyon kaynaklı olarak alet ve silah kullanmada erkeğin doğal yeteneği sebebiyle avcı, kadının da koruma ve çocuk yetiştirme yeteneği sebebiyle sadece toplayıcı rolü üstlendiği görülmektedir (Beardsworth ve Keil, 2011). Avcı-toplayıcı dönemin ardından yoğun olarak Anadolu'ya göç ederek yerleşen toplumlar bu topraklarda uzun yıllar tarım ve hayvancılıkla beslenme ihtiyaçlarını karşılamışlardır (Akın ve ark., 2015). Yerleşik hayat düzenine alıştıktan sonra insanlar, biyolojik ihtiyaçlarını gidermek için beslenmekten ziyade, yemek sofrası kurma ve bu sofrada farklı yemekleri birlikte denemeyi bir kültür haline getirmeye başlamışlardır (Düzgün ve Durlu Özkaya, 2015). Önceleri kilden çanak-çömlek yapmış ve besinlerini saklama kuyularında depolamışlardır. Neolitik Çağ'ın sonlarına doğru Kalkolitik Çağ ile bakır madeninin işlenmesi ardından Tunç Çağı ve Demir Çağı ile daha fazla kesici alet, mutfak eşyası geliştirmişlerdir (Akın ve ark., 2015). Özellikle Demir Çağı'nda mutfak gereçleri olarak altın, gümüş, bakır ve tunç gibi madenlerin sıklıkla kullanıldığı bilinmektedir. İlk Çağ'da Anadolu Uygarlıklarında daha sonrasında devam eden Orta Çağ'da ise Roma ve Bizans İmparatorluklarında farklı beslenme kültürlerinin şekillendiği ifade edilmektedir. Türk beslenme kültürü ise Orta Asya Dönemi, Selçuklu ve Beylikler Dönemi, Osmanlı Dönemi ve Cumhuriyet Dönemi olmak üzere çeşitli evrelerden geçmiştir (Düzgün ve Durlu Özkaya, 2015). Orta Asya'dan sosyal ve biyolojik pek çok sebeple Anadolu'ya göçen Türkler, burada karşılaştıkları Anadolu insanının sahip olduğu beslenme alışkanlıkları ile kendi bilgilerini sentezleyerek yeni ve zengin bir mutfak kültürüne ulaşmıştır. Osmanlı İmparatorluğu'ndan Cumhuriyet Dönemi'ne kadar olan 600 yılı aşan süreçte, padişahlara ve saraya verilen önem sebebiyle gösterişli mutfaklar ön plana çıkmaktadır. Cumhuriyet'in ilanıyla başlayarak günümüzde hızla devam eden ekonomik ve teknolojik gelişmelerin beslenmeye etkileri ve gıda sektörünün oluşmasıyla yemek kültürü artık bambaşka bir noktaya gelmiştir (Akın ve ark., 2015).

Türk toplumu köklü ve zengin bir tarihe ve kültürel yapıya sahiptir. Kültürel zenginlik ile beslenme kültürünün de zenginleştiği görülmektedir (Kılıç ve Albayrak, 2012). Türk yemek kültürünün, Orta Asya'dan günümüze kadar uzanan tarihsel bir gelişim süreci bulunmaktadır. Türk yemeklerinin farklılığını ve zenginliğini sağlayan unsurlar; Asya ve Anadolu topraklarında yetişen farklı gıda ürünleri, Selçuklu ve Osmanlı saraylarında keşfedilen yeni lezzetler ve Anadolu'da kurulmuş tüm uygarlıklara ait izlerdir. Türk yemek kültürü göçebe hayattan Anadolu Selçuklu ve Osmanlı zamanına kadar olan süreçten günümüze çok değişim göstermiş ve olgunlaşmıştır (Güler, 2008). Bu çalışmada, insanlığın varoluşundan günümüze kadar olan süreçteki beslenme alışkanlıkları ile bu alışkanlıkların süreç içerisindeki değişimi ve bu değişime kültürün etkisi derlenmiştir.

Tarih Öncesi Çağlarda Yemek Kültürü ve Beslenme Alışkanlıkları

21. yüzyılın sosyal ve ekonomik düzeyinin temelini oluşturan Neolitik Çağ, insanlığın kültürel evrimi ve gelişimindeki en önemli evre olarak bilinmektedir (Akın ve ark., 2015). 9000 yıldan öncesine dayanan Neolitik Çağ'da evlerin, Orta Anadolu'da ırmak kenarlarına art arda ve kıyı boyunca inşa edildiği ifade edilmektedir (Redman, 1978). Bu dönemde evlerin içindeki çeşitli ocaklar, fırınlar, saklama çukurları, mimari bölümler ve dekorasyonlar yaklaşık 1000 yıl aynı şekilde yapılarak kullanılmıştır (Atalay ve Hastorf, 2006).

Neolitik Çağ'da ilk yerleşimlerde yalnızca avcılık ve toplayıcılıkla geçinilirken, Çatalhöyük'teki höyük diziliminin altıncı katından sonra tarım ile geçinildiği görülmektedir. Kaplıca (einkorn) ve germik (emmer) buğdaylarıyla ekmeklik buğday, yalın arpa ve bezelye yetiştirilmiştir (Richards ve ark., 2003). Böylece üretime başlanmış ve insanlar ektikleri ürünlerin yetişmesini beklemek için günümüzdeki şehir yaşantısının temelini oluşturan yerleşik yaşam düzenine geçmişlerdir.

O dönem insanları yalnız et ile beslenmeyi bırakıp farklı bitki, baharat ve hayvansal ürünleri birlikte pişirerek yemek yapma sanatını geliştirmişlerdir (Düzgün ve Durlu Özkaya, 2015). Aynı dönemde tarımsal faaliyetlerle birlikte avcılığı da yoğun olarak sürdürmüşlerdir (Richards ve ark., 2003).

Yemek pişirme işlemleri önceden fırın içinde sıcak taş üzerinde yapılırken, İ.Ö. 6000-5000 tarihlerinde kaplarda yapılmaya başlanmıştır. Bu dönemle ilgili yapılan araştırmalarda, kullanılan kapların sürüngen kabukları, hayvanın kürek kemiği ve midesinden olduğu tespit edilmiştir (Sürücüoğlu ve Özçelik, 2008). Pişirmeye dayanıklı kil toplarından üretilen kapların kullanılmasıyla beraber fırınların boyutlarının da büyüdüğü anlaşılmaktadır (Aksoy ve Çetin, 2018). Şekil 1'de görülen, Anadolu Medeniyetler Müzesinde sergilenmekte olan toprak pişirme kabı Çatalhöyük'te bulunmuştur (Işın, 2018). Üçayaklı kapların tarihlendiği zamana bakılırsa yemeği doğrudan ateş üstünde pişirme işleminin ateşin bulunmasından çok sonra öğrenildiği anlaşılmaktadır (Önçel, 2015).

Şekil 1. Toprak pişirme kabı ve üçayak (Anadolu Medeniyetler Müzesi) (Işın, 2018)

Figure 1. Earthenware pot and tripod (Anatolian Civilizations Museum) (Işın, 2018)

Yapılan kazı çalışmalarında botanik kalıntılardan, kuş kemiklerinden, kuş yumurtalarından ve organik moleküllerden dönemin yemek kültürüne ait çıkarımlar yapılabilmektedir. Nekropol kazılarında bulunan iskeletlerin kemiklerinde yapılan izotop analizi, insanların günlük işleri ve beslenme şekilleri hakkında bilgiler sunmaktadır (Le Bras-Goude, 2016). Literatürde Neolitik Çağ'da balık ve kuş eti az tüketilirken genellikle koyun ve keçi etinin tercih edildiği, domuz ve sığır etinin de özel şölenlerde yenildiği bilgisine ulaşılmaktadır (Delemen, 2001).

Arkeolojik kazılarda elde edilen buluntulardan, insanların anatomik özelliklerinden sosyal yaşamlarına kadar birçok sonuca ulaşılmaktadır. Toplumların beslenme alışkanlıklarının ve beslenme çeşitliliğinin gelişimi açısından da ipuçları elde edilmektedir. Bu anlamda, insan vücudunun en kalıcı yapılarından olan diş ve kemikler ile insan dışkısı arkeolojik buluntu olarak büyük önem taşımaktadır. Buluntularda elde edilen dişlerde görülen çürükler ne tür besinlerin, hangi sıklıkla tüketildiğini göstermektedir. Taş Devri'ne ait olduğu düşünülen buluntularda diş çürüğüne rastlanmazken, Neolitik Çağ'da diş çürüklerinde ciddi bir artış olduğu gözlenmiştir. Bu bulgular o dönem insanlarının karbonhidrat ağırlıklı beslendiğini göstermektedir (Özüşen, 2013). Bu dönemde kurutulmuş meyve, kurutulmuş et, tam tahıllar ve kabuklu yemişlerin tüketildiğine de yine diş analizlerinden ulaşılmaktadır (Atalay ve Hastorf, 2006). Bu konuda derinlemesine sonuç alınabilen yerlerden biri olan Çatalhöyük'te, insanların protein ve karbonhidrat ağırlıklı beslendikleri tespit edilmiştir (Yurdakök, 2013). Dışkı örneklerine bakıldığında ise yabani fıstık, palamut ve badem kabuğu bulunduğu saptanmıştır. Bu veriler ışığında genelde yiyeceklerin kabukları ile tüketildiği kanısına varılmıştır (Hastorf, 2012).

Çatalhöyük araştırmaları sonucunda ortaya çıkan diğer bulgularda mayalama, öğütme, kesme, süzme, tütsüleme, doğrama, kurutma, dövme, tuzlama, kızartma gibi çeşitli yemek hazırlama teknikleri görülmektedir (Şensoy ve Tiritoğlu, 2018). Ayrıca, üretim, işleme, yemek pişirme, yemek yeme gibi gıda faaliyetlerinin uygulandığı tespit edilmiştir. Meyvelerin ve kabuklu yiyeceklerin kümeler halinde bulunması da depolandıklarını göstermektedir (Atalay ve Hastorf, 2006).

Gün yüzüne çıkan arkeolojik kalıntılar, Erken Bronz Çağı'nda zeytinin yetiştirilmesinin ve işlenmesinin, Neolitik Çağ'daki gıda faaliyetlerinin geliştirilmesiyle yapıldığını göstermektedir. Girit Adası'nda bulunan yazıtlardan, yabani zeytinin gıda olarak tüketildiği ve ayrıca kozmetik ürün, merhem ve parfüm yapımında kullanıldığı anlaşılmaktadır (Kaplan ve Arıhan, 2017).

Neolitik Çağ'da Çatalhöyük'te bulunan arkeolojik kalıntıların yanı sıra bu döneme dair en eski buluntuların bugünkü

Şanlıurfa'da yer alan Göbeklitepe yerleşim yerinde mevcut olduğu bilinmektedir. Bu nedenle sonraki dönemlere ait keşiflerin kökenlerinin Anadolu ve Mezopotamya olduğu ifade edilmektedir (Düzgün ve Durlu Özkaya, 2015).

Orta Asya Türklerinde Yemek Kültürü ve Beslenme Alışkanlıkları

Tarihsel süreçte, Türklerin diğer toplumlarla karşılaştırıldığında, yeme ve içmeye daha fazla önem verdiği görülmektedir. Yaşam biçimleri, yaşadıkları bölgeler ve etkileşim halinde oldukları topluluklar, beslenme şekillerini ve yemek kültürlerini etkilemiştir. Türklerin kültürel zenginliği Türk mutfak kültürüne de yansımıştır. Tarihte yer almaya başladıklarından itibaren yeme-içme sosyal yaşamlarında önemli bir yer tutmuştur (Kızıldemir ve ark., 2014).

Türkler Cilalı Taş Devri'nde Orta Asya'ya gelerek buraya yerleşmiş, sonrasında Ural ve Altay dağları arasında bulunan geniş bozkırları yurt edinmişlerdir (Koşay, 1982). İslamiyet öncesinde Türkler yalnızca Orta Asya ile sınırlı kalmayarak Kafkaslar, Karadeniz'in kuzeyi ve Macaristan ovalarına kadar uzanan bölgelere yerleşmişlerdir. Dolayısıyla atlı göçebe kültürü burada doğmuş ve gelişmiştir. Geçim kaynakları tarım ve hayvancılık olmuştur (Seyitoğlu ve Çalışkan, 2014). At, koyun, keçi gibi hayvan etlerini mutfaklarında sıklıkla kullandıkları görülmektedir (Kaplan ve Arıhan, 2017). Yaşadıkları coğrafya sebebiyle beslenmelerini çoğunlukla hayvansal gıdalardan sağladıkları ancak aynı dönemde buğday ve buğday unuyla yapılan hamur işlerinin de yemeklerde yer almaya başladığı kaynaklarda ifade edilmektedir (Kızıldemir ve ark., 2014). Böylece hayvancılık ilk ve çoğu zaman tek geçim kaynağı iken buğdayı da temel besin kaynağı olarak kabul ettikleri ve ekmeklerini buğdaydan yaptıkları görülmektedir (Sürücüoğlu ve Özçelik, 2008) (Ögel,1982).

Zamanla yerleşik hayata geçen Türkler; buğdayın yanında çavdar, arpa ve darı yetiştirmeye başlamıştır (Demirgül, 2018). Orta Asya Türkleri Çin'in de etkisiyle pirinci yetiştirmeyi öğrenmişlerdir (Sürücüoğlu ve Özçelik, 2008). Aynı dönemde dağ ıspanağı, şalgam, havuç, soğan, turp, patlıcan, kabak, pancar, sarımsak gibi sebzelerin o dönem tüketim tercihinde yer aldığı görülmektedir (Kaplan ve Arıhan, 2017). Sıklıkla tüketilen meyveler ise; elma, dut, kayısı, armut, üzüm, karpuz, kavun, şeftali, iğde, fıstık gibi ürünlerdir (Ergüzel, 2006). Üzümden pekmez, şarap ve sirke yapıldığı da kaynaklarda yer almaktadır (Akın ve ark., 2015).

Orta Asya'da Türkler sürekli göç ettiklerinden ve geçim kaynakları hayvancılık olduğundan etin beslenmedeki yeri büyük önem arz etmektedir (Güldemir, 2014). Yapılan kazılardaki buluntulardan İ.S. 6. ve 8. yüzyıllarda koyun ve at eti ile sığır ve geyik etlerinin de tüketildiği tespit edilmiştir. İ.S. 8. yüzyıl sonrasındaki buluntularda kuş ve balık tüketildiğine dair sonuçlara ulaşılmıştır. Elde edilen bu bulgular zaman içerisinde yemek kültürünün değiştiğini göstermektedir (Ögel, 1982). Orta Asya Türkleri eti tuzlayarak güneş altında kurutmuşlar ve bu yiyeceğe "kak et" adını vermişlerdir. Güneşte kurutma, etin korunmasında bilinen en eski yöntemdir (Alçay ve ark. 2015). Günümüzde benzer şekilde üretilen sucuk ve pastırma göçebe hayatının doğal bir ihtiyacı olarak üretilmiştir. Eti uzun süre koruma yöntemini keşfeden Türklerin, bu ürünleri sefer ve göç esnasında tükettikleri düşünülmektedir (Özkan, 2013).

Orta Asya Türklerinin at eti ve kısrak sütünden elde edilen kımız tükettikleri görülmektedir (Beşirli, 2010). Kımızın taze kısrak sütü ve bu sütün üçte biri oranında olan mayalık kımızla birlikte bir deri torbaya doldurularak ılık bir ortamda saklanması ve torbanın ağzından bir sopa ile sık sık dövülerek, içilecek kıvama gelinceye kadar 12 veya 24 saat bekletilmesiyle hazırlandığı ifade edilmiştir (Ögel, 1991). Kımızın yanı sıra içecek olarak hububat çeşitlerinin alkole çevrilip tüketildiği, bunların dışında ritüel olarak at kanı içildiği de belirtilmektedir (Kızıldemir ve ark., 2014).

Orta Asya Dönemi'nde Türk yemek kültürünü yansıtan en eski yazılı kaynaklar; Kül Tigin'in yeğeni olan Yolluğ Tigin tarafından yazılan Kül Tigin ve Bilge Kağan anıtlarından oluşan Orhun Yazıtları'dır. Bu anıtlarda o döneme ait yemek kültürü ile ilgili bilgilere rastlanmıştır (Tekin, 2014). Yapılan kazılarda elde edilen yine bu döneme ait düz tabanlı ağzı geniş kulpsuz kaplar ve içki kadehleri Türklerin yemek kültürünün o zamanlarda bile gelişmiş olduğunu kanıtlamaktadır. Kazılarda bunların dışında bulunan fincan, kaşık, küçük bıçak, kulplu güveç ve farklı formdaki mutfak eşyaları da gelişmiş yemek kültürünün bir başka göstergesidir (Abalı, 2017). New York'ta bulunan Metropolitan Sanat Müzesi'nde sergilenen Şekil 2'deki eser, Orta Asya'da tüketilen yemek çeşitliliğini ve yapılış şeklini görsel olarak yansıtmaktadır (Işın, 2018).

Selçuklu Döneminde Yemek Kültürü ve Beslenme Alışkanlıkları

Ziraat ve tarıma uygun Anadolu topraklarına yerleşen göçebe Selçuklu, zaman içerisinde yerleşik hayata geçmişler ve bölgede yaşayan mevcut toplumların etkisinde kalarak tarım ile daha fazla ilgilenmişlerdir (Düzgün ve Durlu Özkaya, 2015). Bu dönemde tüketilen yiyeceklerin tarımsal gıdalar başta olmak üzere küçükbaş ve büyükbaş hayvan eti ile bu hayvanların sütleri ve kümes hayvanları olduğu anlaşılmaktadır (Kızıldemir ve ark., 2014).

Şekil 2. Ziyafet Hazırlığı 15. Yüzyıl Sonu. (The Metropolitan Museum of Art) (Işın, 2018)

Figure 2. The Banquet Preparation End of the 15th Century. (Metropolitan Museum of Art) (Işın, 2018)

Divanü Lügat-it Türk, dönemin Türk yemek kültürünü yansıtan en eski kaynakların başında gelmektedir (Güldemir, 2014). Divanü Lügat-it Türk'te çorbalar, unlu mamüller, süt ve süt ürünleri, et, sakatat, balık, otlar ve tahıllar, meyveler, sebzeler, yemişler, tatlılar ve içecekler ile ilgili bilgiler bulunmaktadır (Atalay, 1998). Ayrıca Dede Korkut Hikayeleri'nde de aynı dönemdeki et kızartmaları, kavurmalar ve yahniler, süt ve süt ürünleri, unlu mamüller, yemişler, meyveler ve içecekler gibi pek çok yiyecek ve yemek çeşidi yer almaktadır (Güldemir, 2014).

Selçuklu Dönemi'nde diğer dönemlere nazaran özgün bir yemek kültürü ortaya çıkmıştır (Sürücüoğlu ve Özçelik, 2005). Yemek çeşitlerinin yanı sıra yemek pişirme ve muhafaza yöntemlerinin de farklılık gösterdiği tespit edilmiştir (Serçeoğlu, 2014). Bu dönemde tüketilen birçok yiyecek ve yemek çeşidi, adıyla birlikte değişmeden günümüze kadar gelmiştir (Sürücüoğlu ve Özçelik, 2005). Günümüzde de isimleri aynı olan süt, peynir, yoğurt, yağ, kaymak gibi yiyecekler Selçuklu Dönemi'nde sıklıkla tüketilen yiyecekler arasında yer almıştır (Sürücüoğlu ve Özçelik, 2008). Özellikle yoğurt Selçuklu yemek ve ikramlarında ayrı bir önem taşımaktadır (Ünver, 1982). Et, un ve yağ yeme alışkanlığı bu dönemin simgesi olarak görülmüştür. En çok yenen yemeklerin kuzu, erkeç, keçi, at eti ve sakatatlar olduğu ifade edilmiştir. Bunların yanında kuş türleri de tüketilmiştir (Karaca ve ark., 2015).

Türklerin Anadolu'ya gelmeden önce de yetiştirdiği buğday, Bizans, Selçuklu ve Beylikler dönemlerinde en çok yetiştirilen tahıl ürünü olmuştur. Selçuklu Dönemi'nde Anadolu topraklarında sıklıkla üretilen buğday zaman içinde önemli bir ihracat ürünü haline gelmiş, hatta devletlerarası ilişkilerde kimi zaman "ekonomik koz" olarak kullanılmıştır (Özgüdenli ve Uzunağaç, 2014).

Selçuklu Dönemi'ne ait sofra kültürü ile ilgili olarak günümüzdeki gibi yere "sofra yaygısı" serildiği bilinmekte olup (Genç, 1982), yemek servisinde sahan, bakır veya çiniden kaseler, sini ve testi kullanıldığı, tek kaptan kaşıkla yemek yendiği ifade edilmektedir (Sürücüoğlu ve Özçelik, 2008). Ayrıca Kutadgu Bilig adlı eserde bu dönemde yapılan şölen, tören ve sofra düzeni ile ilgili bilgiler verilmektedir (Akın ve ark., 2015). Öğünlerin kuşluk ve zevale yemeği olarak ikiye ayrıldığı bilinmektedir. Bu öğünlerin ev ahalisinin uyanma ve evden ayrıldıkları zamanlara göre değişiklik gösterdiği (Ünver, 1982), kuşluk yemeğinde yağlı ve hamur işleri, zevale yemeğinde ise bol çeşitte yemeğin hava kararmadan yendiği ifade edilmektedir (Serçeoğlu, 2014).

Günümüzde sofraların ayrılmaz bir parçası olan ekmek, Selçuklu yemek kültüründe sadece ana yemeği destekleyici bir öge olarak değil, tek başına yemek olarak da tüketilmiştir. Bu dönemde Anadolu'da ekmeğin belirli aralıklarla ve ihtiyaca göre pişirildiği İbn Battuta Seyahatnamesi'nde yer almaktadır. İbn Battuta, 1333 senesinde seyahat ettiği Alanya şehrinden söz ederken, burada ekmeğin haftada bir kez pişirildiğini söylemektedir (Aykut, 2019). Selçuklu Anadolu'sunda ekmek yapımını tasvir eden Şekil 3'teki minyatür 18. yüzyıla tarihlenirken, günümüzde tandır ekmeği hala aynı şekilde yapılmaktadır (Özgüdenli ve Uzunağaç, 2014).

Şekil 3. Selçuklu Dönemi'nde Ekmek Yapımını Tasvirleyen Minyatür (Topkapı Sarayı Müzesi Kütüphanesi) (Özgüdenli ve Uzunağaç, 2014)

Figure 3. Miniature Depicting Bread Making in the Seljuk Period (Topkapı Palace Museum Library) (Özgüdenli and Uzunağaç, 2014)

Osmanlı Döneminde Yemek Kültürü ve Beslenme Alışkanlıkları

Türk yemek kültürünün hem çok eskiye dayanması hem de en zengin yemek kültürlerinden biri olmasının en önemli sebebi, Osmanlı İmparatorluğu'nun çok uluslu bir yapıya sahip olmasıdır (Alabacak, 2018). Orta Asya kökenli beslenme alışkanlığı, göç esnasındaki Arap ve Fars kültürlerinin etkileri, Anadolu'nun zenginliği ve Rumların etkisi Osmanlı İmparatorluğu'nun yemek kültüründeki çeşitliliğini sağlayan temel unsurlardır (Üner, 2014).

İmparatorluğun geniş bir coğrafyada, farklı etnik grupları içermesinin etkisiyle saray mutfağında birden fazla kültüre özgü yemek çeşidi bulunmaktadır. Özellikle Çerkez tavuğu, Tatar böreği, Arnavut ciğeri ve Laz böreği gibi sıklıkla tüketildiği bilinen yemekler, isimlerini ait olduğu etnik gruplardan almıştır (Hatipoğlu ve Batman, 2014). Ayrıca çeşitli kaynaklarda kabak yemeği, ıspanak kavurması, kavurmalı pırasa, lapa, mantı, yoğurtlu bazu (pazı), patlıcan yahnisi, herise, nohutlu yahni, hünkârbeğendi, imam bayıldı, kebap, medfune, kızartma, köfte ve külbastı gibi yemeklerin de tüketildiği ifade edilmektedir (Samancı, 2008; Solmaz, 2018; Ünver, 1948; Hatipoğlu ve Batman, 2014; Akkoyunlu, 2012).

Türk yemek kültürü Osmanlı İmparatorluğu zamanında daha da gelişmiş ve en görkemli çağını yaşamıştır (Kızıldemir ve ark., 2014). Osmanlı Dönemi'nde saraylarda kullanılan gümüş siniler ve lüks mutfak eşyalarından yemek kültürünün ne kadar gösterişli olduğu anlaşılmaktadır (Abalı, 2017). Yabancı devlet adamlarının Osmanlı'ya yaptıkları ziyaretlerde yedikleri Türk yemeklerinden aşırı derecede etkilendikleri ve kendi aşçılarını yetişmeleri için Türk aşçıların yanına gönderdikleri bilinmektedir (Kızıldemir ve ark., 2014).

Orta Asya döneminde yoğun et tüketimi Osmanlı yemek kültürüne de yansımıştır (Solmaz, 2018). Özellikle sarayda yapılan ziyafetlerde şaşırtıcı ölçüde et tüketildiği ifade edilmektedir (Yerasimos, 2014). Balık etinin diğer et çeşitlerine göre daha az tercih edildiği yabancı seyyahlar tarafından belirtilse de yazılı kaynaklarda deniz mahsullerinin ve yemeklerinin sofra kültüründe önemli bir yere sahip olduğu ifade edilmektedir (Yiğit ve Ay, 2016; Güldemir, 2020). Saray mutfağında yapılan balık biryan, istiridye-i hassa, karidye, müferreke, piyazlı balık, Vardar balığı kapaması, havyarlı levrek balığı, ıstakoz, midyeli salça, Papaz yahnisi, uskumru balığı yahnisi ve Yaka yahnisi gibi yemek çeşitleri dikkat çekmektedir (Güldemir, 2020; Yerasimos, 2007).

Saray sofralarında çeşitli pilavlar ve çorbalar ön planda tutulan yemeklerdir (Solmaz, 2018). Çorba çeşitlerinden buğday çorbası, lahana çorbası, maydanozlu çorba, tutmaç, balık çorbası, işkembe çorbası, terbiyeli çorba, mercimek çorbası ve tarhana çorbasının halk ve saray sofralarında yer aldığı bilinmektedir (Alabacak, 2018; Yerasimos, 2014). Evliya Çelebi Seyahatnamesi incelendiğinde Amber pilavı, avşıla pilavı, çilav pilavı, dud pilavı, firik pilavı, rumman pilavı ve yoğurtlu pilav gibi sayısız pilav çeşidi karşımıza çıkmaktadır (Yerasimos, 2014). Bunların yanında yaş ve kuru meyve hoşafı, şerbet gibi içecekler sofralarda yer almıştır (Solmaz, 2018). Özellikle bu dönemde hem sarayda hem de halk içinde önemli bir yer tutan şerbet günümüze kadar ulaşmıştır. Söz ve nişan törenleri, mevlitler, düğünler ve iftar yemeklerinde tüketilen şerbet, Türk geleneklerinde yer almaktadır (Ceyhun Sezgin ve Durmaz, 2019). Düğünlerde şerbetin yanı sıra baklava, güllaç, kadayıf, helva ve reçel gibi pek çok çeşit tatlı sunulduğu bilinmektedir (Solmaz, 2018; Hatipoğlu ve Batman, 2014). Yapılış biçimlerine göre adlandırılan baklava çeşitleri için hala aynı isimler kullanılmaktadır. Ayrıca tahıllı tatlıların bu dönemde sıklıkla tüketildiği ifade edilmektedir. Özellikle günümüzde Ramazan ayında yapılan aşure ve Konya'da geleneksel olarak düğünlerde yapılan zerde, geçmişten günümüze değişmeden gelen yiyecekler arasında yer almaktadır (Hatipoğlu ve Batman, 2014).

Osmanlı mutfağında günümüz sofralarında olduğu gibi ekmeğin temel gıda olarak tüketildiği bilinmektedir (Alabacak, 2018). Çeşitli kaynaklarda gündelik ekmeğin nan-ı aziz, orta kaliteli has ekmeğin nan-ı has orta, vasat ekmeğin nan-ı adi, yassı beyaz ekmeğin fodula ve uzun iyi beyaz ekmeğin fırancala olarak un kalitesine göre adlandırıldığı ifade edilmiştir. Diğer temel gıdalar arasında süt ve süt ürünleri dikkat çekmektedir. Özellikle mutfaklara alınan peynir türlerinin kaşar, kaşkaval, Felemenk, parmezan, Arnavut, beyaz ve tulum peynirleri olduğu belirtilmiştir (Samancı,2008).

Geleneklerimizde önemli bir yer tutan ve kültürümüzle özdeşleşmiş Türk kahvesi ve kahvehane kültürü Osmanlı'yı ziyaret eden bürokratlar tarafından diğer devletlere ve kültürlere yayılmıştır (Yerasimos, 2007). Şekil 4'te yer alan 1747 yılına ait portre Türk kahvesinin diğer toplumlarda da tüketildiğini göstermektedir (Işın, 2014). Dünyada ilk kahvehane İstanbul'da günümüzde Tahtakale olarak bilinen Tahtü'l-Kale'de 1554-1555 yıllarında açılmıştır (Hatipoğlu ve Batman, 2014). Osmanlı'nın ihtişamlı, keyfe düşkün ve göz kamaştıran alışkanlıkları büyük ilgi görmüştür (Bilgin, 2008).

Osmanlı'da 17. yüzyıldan itibaren muz, domates, mandalina, ananas, portakal gibi sebze ve meyvelerin tüketilmeye başlandığı görülmektedir. O dönemde Avrupa ile olan ticari ilişkilerin artmasının ve güçlenmesinin bu tüketim değişikliğinin sebebi olduğu düşünülmektedir (Kızıldemir ve ark., 2014). Böylece bu görkemli mutfağa domates ve patates gibi ürünler de katılmıştır (Yerasimos, 2007).

Şekil 4. Türk kıyafetiyle kahve içen Madame de Pompadour (Işın, 2014)

Figure 4. Madame de Pompadour drinking coffee in Turkish dress (Işın, 2014)

18. yüzyıl boyunca kültürel katkılar ve ekonominin gelişmesiyle daha da yenilenen mutfak kültürü Yeni Dünya'dan gelen ürünlerin etkisinde kalmıştır (Sağır, 2012). Osmanlı yemek kültürü, imparatorluğun ilerleyen zamanlarında zenginleşerek bu yüzyılda en parlak dönemine ulaşmıştır (Sürücüoğlu ve Özçelik, 2008). Bu son parlak dönem günümüzdeki zengin Türk yemek kültürünün temellerini oluşturmuştur (Bilgin, 2008).

Günümüzde Yemek Kültürü ve Beslenme Alışkanlıkları

Türkiye'nin Avrupa, Asya ve Afrika'nın kavşak bölgesinde olması sebebiyle Türk yemek kültürü Cumhuriyet ile daha da zenginleşmiş ve genişlemiştir (Seyitoğlu ve Çalışkan, 2014). Gıda biliminin katkıları ile şekillenen gıda endüstrisi, her toplumda olduğu gibi Türk toplumunda da yemek kültürü ve beslenme alışkanlıklarını etkilemiştir (Sürücüoğlu ve Özçelik, 2008). Ticaret ağının gelişmesiyle birlikte çeşitli gıdalar ve mevsiminde olmayan meyve-sebzeler pazarlarda ve büyük marketlerde rahatlıkla bulunabilir hale gelmiştir (Közleme, 2012). Önceki dönemlerde iki öğün yenen yemekler üç öğüne yükselmiştir (Önçel, 2015). Geçmiş zamanlarda sıklıkla hayvansal yağ tercih edilirken margarin ve bitkisel yağlar da kullanılmaya başlanmış, dana eti küçümsenirken kuzu ve koyun eti kadar tercih edilir hale gelmiş, zenginliğin göstergesi beyaz ekmek tüketilmeye devam ederken son zamanlarda sağlıklı yaşam üzerine tüketicinin eğiliminin artması ve toplumda sağlıklı yaşam trendinin başlaması esmer ekmek ve doğal köy ekmeği tüketimini arttırmıştır (Sürücüoğlu ve Özçelik, 2008). Bunun yanı sıra yoğurt tüketiminde de değişiklikler olmuştur. Kaymaksız endüstri yoğurdu geleneksel yoğurt kadar sevilerek tüketilmeye başlanmıştır (Közleme, 2012).

Sofra adabı değişerek günümüzde çoğunlukla yer sofrası yerine masa ve sandalye kullanımı başlamıştır. Geçmiş dönemlerde büyük ve tek kaptan yemek yenirken günümüzde küçük ve kişiye özel tabaklardan yemek yenmektedir. Yemeğin sonunda ikram edilen gülsuyu ve güzel kokular, günümüzde yerini ıslak mendil ya da kolonyaya bırakmıştır. Bu gelişmeler sonucunda evlerde mutfak ve salon dışında sadece yemek yenilen "yemek odaları"nın tasarlandığı görülmektedir (Hatipoğlu ve Batman, 2014).

Cumhuriyet Dönemi ile sanayi ve teknolojinin gelişmesi, ekonomik gereklilik gibi nedenlerle kadının çalışma hayatına katılması, yemek yeme alışkanlıklarının ev dışına taşması ve daha kısa sürede yemek yeme ihtiyacını giderme zorunluluğu hazır yemek kültürünün ortaya çıkmasına sebep olmuştur (Önçel, 2015). Televizyon, gazete, radyo gibi görsel ve yazılı basındaki reklamlar ve bu reklamlarda yer alan sloganlar önce çocuklar daha sonra çok kişinin beslenme alışkanlıklarını değiştirmiştir (Mengü, 2006).

Gıda endüstrinin gelişmesi, hızlı kentleşmenin sonucunda ev dışında yemek yeme zorunda olan kişilerin tercihleri, kadının iş hayatına atılması sebebiyle yemek hazırlama zamanının daralması, toplumda yalnız yaşayanların sayısındaki artış, kitle iletişim araçlarının etkisi gibi çeşitli nedenlerden dolayı, hazır ve yarı hazır hale getirilen yemeklerin tüketiminde artış olduğu görülmüştür (Önçel, 2015). Bugünkü Türk yemek kültürünün geniş yelpazesinin oluşmasında özellikle Selçuklu ve Osmanlı dönemlerindeki lezzetler başta olmak üzere tüm Anadolu lezzetleri büyük rol oynamıştır (Sürücüoğlu ve Özçelik, 2008). Örnek olarak tandır ekmeği, Fırancala ekmeği, süt, kaşar peyniri, beyaz peynir, tulum peyniri, parmesan peyniri, yoğurt, kaymak, sucuk, pastırma (kak et), hoşaf, baklava, güllaç, kadayıf, helva, reçel, aşure, zerde, Türk kahvesi, imambayıldı, mantı, hünkârbeğendi, külbastı, kebap ve yahni gibi lezzetlerin bir kısmı hazırlanış biçimlerine göre değişikliğe uğrasa da bir kısmı değişmeden günümüze kadar ulaşmıştır (Hatipoğlu ve Batman, 2014; Samancı, 2008; Özkan, 2013; Sürücüoğlu ve Özçelik, 2008; Özgüdenli ve Uzunağaç, 2014; Alabacak, 2018; Solmaz, 2018; Yaresimos, 2007). Tüm bu yemek çeşitleri geçmişte sadece saray mutfağı ile sınırlı kalırken günümüzde restoranlar, hazır yemek işletmeleri ve fast food firmaları konseptine ulaşmıştır.

Günümüzde en çok tüketilen gıdaların %19.4 oranında et, balık ve deniz ürünleri olduğu bunu %17.8 ile ekmek ve tahıllar; %16.6 ile sebzeler; %13.9 ile süt, peynir ve yumurta; %11,5

ile meyveler; %6.0 ile şeker, bal ve çikolata; %5.4 ile sıvı ve katı yağların takip ettiği rapor edilmiştir. En az tüketilen gıdaların ise, %3.2 ile kahve, çay ve kakao; %3.8 ile alkolsüz içecekler; %2.3 ile diğer gıda ürünleri olduğu belirtilmiştir (TÜİK, 2019).

Genel olarak tarihin gelişim sürecinde beslenme alışkanlıkları ele alındığında et, ekmek gibi sıklıkla tüketilen temel gıda maddelerinin aynı olduğu görülmektedir. Diğer yandan günümüzde gıda endüstrisinin gelişimi ve ticaret olanakları da dikkate alındığında, ürün çeşitliliğinden dolayı tüketim tercihlerinin değişim göstermesi kaçınılmazdır.

Sonuç

Yaşadığı çevreye uyum sağlama güdüsüne sahip olan insanoğlu ilk çağlardan bu yana gelişim sürecini sürdürmektedir. Araştırmaların ışığında, insanoğlunun varoluşundan günümüze kadar yemek yeme alışkanlığında birçok etkiyle değişim ve gelişim yaşandığı ortaya çıkmaktadır. Toplumlarda yaşanan savaşlar, göçler, teknolojik gelişmeler ve ticaret gibi olaylar yeni yiyecek çeşitlerinin yayılmasında ve tanınmasında etkili olmuştur. Hala gelişmekte ve değişmekte olan yemek kültürünün, kültürler ve milletler arası etkileşimi ile daha da ilerlemesi kaçınılmazdır. Gıda endüstrisi, turizm sektörü, iklim ve doğa koşullarının devamlı değişmesi ve iletişimin artması ile yeme-içme alışkanlıklarının eskiye oranla daha da hızlı değişeceği öngörülmektedir. Tüm bunların yanı sıra günümüzdeki yemek çeşitliliğinin temeli, bu topraklarda daha önce yaşamış olan toplumların miras bıraktığı beslenme kültürlerinin senteziyle oluşmaktadır. Bu derleme ile tarihsel süreç içerisinde Türk yemek kültüründeki benzerlikler, değişimler ve günümüze yansımalarından kesitler sunulmaya çalışılmıştır.

Etik Standart ile Uyumluluk

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

Etik izin: Araştırma niteliği bakımından etik izne tabii değildir.

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Gıda ve su kaynaklı önemli viral enfeksiyonların güncel durumu ve korunma stratejileri

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ÖZ

Günümüzde enfeksiyöz etkenlerin tanı tekniklerindeki ilerlemeler ve kullanımlarının yaygınlaşması ile viral etkenlerin gıda ve su kaynaklı hastalıklardaki prevalansları dünya genelinde daha da önem kazanmıştır. Gıda ve su kaynaklı virüslerin, gastroenterit ve hepatit yanı sıra nörolojik bozukluklar, solunum yolu hastalıkları, myokardit, glomerulonefrit ve hemorajik ateşe neden olabildiği, özellikle bebek/çocuk ve immun yetmezliği olan bireylerde ölüm oranının yüksek olduğu belirtilmektedir. Ayrıca etkenlerin, çevresel şartlara ve gıda proseslerine karşı dirençli olması nedeni ile sıcaklık ve yüksek basınç uygulamaları, doğal antiviral bileşikler, UV uygulamaları ve konvansiyonel temizlik-dezenfeksiyon ile eliminasyonları diğer mikroorganizmalara göre daha zor olmakta, hatta yetersiz kalmaktadır. Viral enfeksiyonlardan korunmada, aşı uygulamaları ile birlikte, üretimde GMP, GHP ve HACCP sistem yaklaşımları, etkenlerin gıda ortamında ve toplumda minimize edilmesini sağlayan en etkin yol olarak görülmektedir. Bu derleme makalesinde, gıda ve su kaynaklı viral etkenlerden dünya çapında özellikle yüksek prevalans ile seyrettiği rapor edilen, enterotropik virüslerden NoV, AstV, RoV ve AdV ile hepatotropik virüsler olan HAV ve HEV'in genel özellikleri, oluşturdukları hastalık tabloları ve epidemiyolojileri ile uygulanan korunma ve kontrol tedbirlerine dair güncel bilgilere yer verilmiştir.

Anahtar Kelimeler: Gıda, Su, Virüs, Enfeksiyon

ABSTRACT

Current status of major foodborne and waterborne viral infections and their prevention strategies

Advances in diagnostic techniques and their widespread use for infectious agents revealed the considerably high current prevalence of viral agents in foodborne and waterborne diseases. Foodborne and waterborne viruses are indicated to cause not only gastroenteritis and hepatitis but also neurological disorders, respiratory tract diseases, myocarditis, glomerulonephritis and hemorrhagic fever, with a particularly high mortality rate in infants/children and in individuals with immune deficiency. Additionally, due to their resistance to environmental conditions and food processes compared to other microorganisms, elimination of these viruses by heat and high pressure applications, natural antiviral compounds, UV applications and conventional cleaning-disinfection remains difficult even inadequate. In protection from viral infections, vaccine applications together with GMP, GHP and HACCP system approaches in production seem to be the most effective approaches to ensure the minimization of viruses in food environment and in public. In this review article, up-to-date information is presented on the general characteristics and the diseases caused by enterotropic viruses; NoV, AstV, RoV, AdV and hepatotropic viruses; HAV and HEV, with a particularly high worldwide prevalence, as well as their epidemiology, prevention and their control measures.

Keywords: Food, Water, Virus, Infection

Giriş

Dünya Sağlık Örgütü (WHO)'nün 2015 tarihli raporunda, gıda ve su kaynaklı hastalık etkenlerinin, güvenli gıda üretim ve tüketimi yönünden hayati önem taşıdığı bildirilmiştir. Gıda güvenliği ile ilgili olarak fiziksel, kimyasal ve biyolojik potansiyel tehlikeler içerisinde en önemlisini oluşturan biyolojik tehlikelerden yılda ortalama 600 milyon bireyin etkilendiği ve bunların 420.000'inin öldüğü rapor edilmiştir. Ayrıca, bu tehlikelerin 5 yaş altı çocukların %40'ında gıda kaynaklı hastalığa neden olarak, yıllık 125.000 çocuğun ölümüne sebep olduğu belirtilmiştir. Yaşanan ölümlerin yanı sıra, gıda kaynaklı hastalıkların düşük ve orta gelirli ülkelerde, yılda ortalama 110 milyar dolarlık bir ekonomik kayba da neden olduğu bildirilmiştir.

2017 WHO raporuna göre, Avrupa'da 23 milyondan fazla insan kontamine gıda tüketimi sonrasında hastalanmış, vakaların ortalama 5.000'i ölüm ile sonuçlanmıştır. Aynı raporda; gıda kaynaklı hastalıklarda en sık gözlenen semptomun ishal ve en yaygın saptanan mikrobiyal etkenin de norovirüs (NoV) olduğu belirtilmiştir. Benzer şekilde, dünya genelinde gıda kaynaklı gastroenterit ile seyreden vakaların ortalama %20'sinin NoV'ler tarafından oluşturulduğu tahmin edilmektedir.

Son yıllarda gerçekleştirilen risk değerlendirmeleri ve ileri tanı metotlarının kullanımı ile virüslerin, akut gastroenterit (AGE) vakalarında en sık karşılaşılan etken olduğu saptanmıştır. Gıda ve su kaynaklı viral enfeksiyonlar; ishal, kusma, bulantı, abdominal ağrı ve daha nadir olarak ateş, baş ağrısı ile seyreden viral gastroenteritler ile ishal, kusma, ateş ve sarılık gibi semptomlarla seyreden viral hepatitler olarak ikiye ayrılmaktadır (İncili ve Çalıcıoğlu, 2016; Bosch ve ark, 2018). Gıda ve su ile taşınabilen virüslerle kontaminasyon sonucunda daha nadir de olsa şiddetli sinirsel semptomlar, flasid paralizi, myokardit, solunum yolu hastalıkları ve hemorajik ateş gibi semptomlar da görülebilmektedir. Gıda ve su aracılığıyla insanlara bulaşabilecek viral enfeksiyon etkenleri arasında en önemlilerini; NoV, rotavirüs (RoV), aichi virüs (AiV), astrovirüs (AstV), adenovirüs (AdV), poliovirüs (PoV), sapovirüs (SaV), reovirüs, parvovirüs, nipah virüsü, ebola virüsü, avian influenza virüsü (H5N1), coronavirüs (SARS-CoV, MERS-CoV), tick-borne encephalitis virüsü (TBE), hantavirüs ve enterovirüs (EV) oluşturmaktadır. Gıda aracılı viral hepatit etkenleri ise, hepatit A virüsü (HAV) ve hepatit E virüsü (HEV)'dür. Bu virüslerin enterotropik, hepatotropik, nörotropik, pnömotropik ve multitropik yatkınlıkları olmakla birlikte, gastroenteritten felce ve menenjite varan farklı semptomlarla seyrettiği görülmektedir (Bosch ve ark., 2016).

Bu derleme makalesinde, gıda ve su kaynaklı viral etkenlerden insanlarda dünya çapında özellikle yüksek prevalans ile seyrettiği rapor edilen, enterotropik virüslerden NoV, AstV, RoV ve AdV ile hepatotropik virüsler olan HAV ve HEV ile ilgili genel özellikleri, oluşturdukları hastalık tabloları ve epidemiyolojileri ile uygulanan korunma ve kontrol tedbirlerine dair güncel bilgilere yer verilmesi amaçlanmıştır.

Gıda ve Su Kaynaklı Virüsler

Enterotropik Virüsler

Norovirüs. *Caliciviridae* ailesi içerisinde yer alan, zarfsız, küçük (28-35 nm), tek sarmallı (ss) bir Ribonükleik asit (RNA) virüsüdür. Hızlı moleküler evrim kabiliyetine sahip olan bu virüse ait yeni genotip ve varyantlar rapor edilmektedir. Günümüzde virüsün yedi genotipinden (GI-GVII) GI, GII ve GIV genotiplerinin (Human NoV - HNoV) insanlarda enfeksiyon oluşturduğu belirlenmiştir (Erol, 2007; Demirci ve ark., 2018). İnsan norovirüslerinin (HNoV) üretilmesinde karşılaşılan güçlükler nedeni ile bilimsel çalışmalarda aynı aile içerisinde yer alan, HNoV ile benzer inaktivasyon özellikleri gösteren ve üretilmesi daha kolay olan murine norovirüs (MNV-1), feline calicivirüs (FCV-F9), tulane virüs (TV) ve domuz sapovirüsü gibi virüsler kullanılmaktadır (Patwardhan ve ark., 2020).

NoV, tek başına dünyadaki gıda ve su kaynaklı diyare ile seyreden hastalıkların %20'sini oluşturarak, yılda ortalama 125 milyon insanı etkilemektedir. Virüs ile enfekte olduktan 12-48 saat sonra mide bulantısı, kusma, ishal, yorgunluk, kas ve karın ağrısı gibi semptomlar ile seyreden hastalık genellikle 1-3 gün sürmekte, iyileşme sonrasında ise iki haftaya kadar dışkı ile virüs saçılımı devam etmektedir. Bazı bireylerde, hastalık asemptomatik seyretmekte ve bu kişiler virüsün yayılımında etkili olmaktadır. Virüse karşı ticari bir aşı henüz bulunmamaktadır (WHO, 2017; O'Shea ve ark., 2019).

Gastroenterit vakalarının sistematik olarak NoV yönünden test edildiği iki ülkeden biri olan Hollanda'da, NoV insidansının her 10.000 kişide 380, Birleşik Krallık'da ise 10.000'de 450 olduğu belirlenmiştir. Aynı değerin ABD'de 10.000'de 650, Kanada'da ise 10.000'de 1040 olduğu tahmin edilmektedir. Ayrıca NoV'ler sebebiyle, organ nakli sonrasında veya onkoloji hastalarında immunsupresyona bağlı olarak aylarca süren kalıcı gastroenterit vakaları bildirilmiştir (Belliot ve

ark., 2014). Dünyada bu virüsün prevalansının kontamine sular, bu tür suların kirlettiği sularda yaşayan deniz kabukluları (midye, kum midyesi, istiridye vb), yine bu tip sular ile kontamine olan ve çiğ tüketilen sebze ve meyvelerde çok yüksek olduğunu gösteren güncel çalışmalar bulunmaktadır. Bunlardan Güney Kore'de Kim ve ark. (2016) tarafından gerçekleştirilen bir araştırmada, Mart 2014 - Şubat 2015 arasında örnekledikleri 504 adet suyun 104'ünün (%20.6) HNoV yönünden pozitif olduğu, HNoV prevalansının kış-ilkbahar aylarında, yaz-sonbahar aylarına göre daha yüksek olduğu belirlenmiştir. Bir diğer çalışmada, ülkemizde Ocak 2017 - Ocak 2018 tarihleri arasında Mardin, Şanlıurfa, Gaziantep ve Kahramanmaraş il ve ilçelerinde bulunan kuyu ve derelerden alınan 60 adet su örneğinin 10'unda (%16.67) HNoV GI, GII ve GIV bulunmuş, pozitiflik tespit edilen örneklerin 3'ünün lokal kuyulardan, 7'sinin ise derelerden elde edildiği bildirilmiştir. Ayrıca, HNoV tespit edilen 6 örneğin GII (%10), 3 örneğin GI (%5) ve 1 örneğin (%1.67) ise GIV genotipine ait olduğu belirlenen çalışma sonuçları, HNoV salgınlarında kontamine suların önemli bir kaynak oluşturabileceğini göstermiştir (Demirci ve ark., 2018). Kıyı sularında avlanan ya da yetiştirilen deniz kabuklularında NoV prevalansının yüksek olduğuna dair yapılan çalışmalardan birinde, İspanya'da 168 deniz kabuklusu (vahşi ve kültür midye, kum midyesi, deniz tarağı) örneği üzerinde, NoV GI, GII ve HAV prevalansı araştırılmış, örneklerin %55.4'ünün en az bir virüs ile, %11,3'ünün de iki veya daha fazla virüs ile kontamine olduğu belirlenmiştir. NoV GI, en prevalan virüs olarak bulunmuş (%32.1), bunu NoV GII (%25.6) ve HAV (%10.1) takip etmiştir. Kontamine deniz kabukluları içerisinde en yüksek pozitifliğe kültür midyesinde (%61.4) rastlanırken, bunu kum midyesi (%59.4), vahşi midye (%54.3) ve deniz tarağı (%38.7) izlemiştir (Polo ve ark., 2015). Benzer şekilde Tan ve ark. (2018) tarafından Çin'de faaliyet gösteren çiftlik, market ve restoranlardan toplanan istiridyelerin analiz edildiği çalışmada, 463 örneğin 96'sında (%20.7) NoV tespit edilmiş, bunlardan 94'ünün (%20.3) NoV GII, 2'sinin (%0,4) NoV GI olduğu rapor edilmiştir. İngiltere'de Cook ve ark. (2019)'nın gerçekleştirdiği çalışmada ise, 568 marul ile 310 taze ve 274 donmuş ahududu örneği NoV prevelansı yönünden incelenmiş, örneklerin sırasıyla %5.3, %2.3 ve %3.6 oranında kontamine olduğu bulunmuştur.

Astrovirüs. *Astroviridae* ailesi, *Mamastrovirus* cinsi içerisinde yer alan, zarfsız, 28-40 nm büyüklüğünde, tek sarmallı (ss) bir RNA virüsüdür. Tespit edilen 8 adet insan astrovirüs (HAstV) serotipi (HAstV 1-8)'nden, HAstV-1'in en prevalan serotip olduğu, ayrıca bu cinste yer alan virüslerin sığır, kedi, köpek, koyun, domuz, kemirgenler, yarasa ve deniz memelilerinde de bulunduğu bildirilmiştir (Greening ve Cannon, 2016; Vu ve ark., 2017). HAstV'lerin dünya genelinde bakteriyel olmayan sporadik gastroenteritlerin %20'sini ve epidemik gastroenteritlerin %0.5-15'ini oluşturduğu tahmin edilmektedir (Greening ve Cannon, 2016). Virüs bulaşı, fekal-oral yolla gerçekleşmekle birlikte, özellikle kontamine su kaynaklı enfeksiyonlar sıkça görülmektedir. Ayrıca, kontamine sularda yetişen deniz kabukluları ve bu tip sularla sulanan ya da yıkanan sebze ve meyveler bulaşta önemlidir. Semptomları 2-4 gün süren sulu ishal, kusma, ateş, abdominal ağrı, anoreksi ve baş ağrısı olan ve çoğunlukla asemptomatik olarak seyreden hastalığın özellikle bağışıklık sistemi zayıf bireylerde gastrointestinal bulgular yanında, merkezi sinir sistemi (MSS) (menenjit ve ensefalit) hastalıklarına da yol açabildiği, MSS tutulumlarında ölüm oranının yüksek olduğu bilinmektedir (O'Shea ve ark., 2019). Oldukça fazla genetik çeşitlilik gösteren AstV'lerin zoonotik yolla bulaşı henüz net olarak ortaya konulmamış olsa da, insan ve hayvan AstV'leri arasındaki genetik benzerlik bu ihtimalin göz ardı edilmemesi gerektiğini düşündürmektedir (Vu ve ark., 2017).

Rotavirüs. *Reoviridae* ailesi içerisinde yer alan, zarfsız, 70-75 nm büyüklüğünde, çift sarmallı (ds) RNA virüsüdür. Sekiz grubu (A-H) olan RoV'ler arasında en çok A olmak üzere, B, C ve H grubundaki RoV'ler insanlarla birlikte diğer memelileri de enfekte etmektedir. Çoğunlukla gelişmekte olan ülkelerde olmak üzere, 5 yaş altı çocuklarda yıllık RoV kaynaklı 138 milyon vakanın ortalama 527.000'i ölümle sonuçlanmaktadır (Martella ve ark., 2010; O'Shea ve ark., 2019). Minimum enfektif dozunun oldukça düşük olması nedeniyle, yaygın olarak görülen (Erol, 2007) enfeksiyonun, özellikle evcil hayvanlarla yakın temas halinde yaşayan bireylerde, zoonotik yolla da bulaşabildiğine dair kanıtlar bulunmaktadır (Greening ve Cannon, 2016). Fekal-oral yolla, insandan insana doğrudan temas ve dışkı ile kontamine gıdalar aracılığıyla bulaşan RoV enfeksiyonlarında, kontamine suların en önemli paya sahip olduğu bildirilmiştir. Enfeksiyonda, oldukça sulu ishal, kusma, ateş, solunum yolu hastalıkları ve dehidratasyon görülmektedir (Kiulia ve ark., 2015).

Adenovirüs. *Adenoviridae* ailesine bağlı, 70-110 nm büyüklüğünde, zarfsız, çift sarmallı (ds) DNA virüsünün beş cinsi mevcuttur. Bunlardan *Mastadenovirüs* cinsi içerisinde yer alan virüsler, insan dahil memelileri enfekte etmektedir. Yedi farklı türü (A-G) olan insan adenovirüslerinin (HAdV) pri-

mer kontaminasyon kaynaklarını yüzme havuzu, içme ve deniz suları oluşturmakta, etkenin vücuda alındığı yol ve tropizmine bağlı olarak gastrointestinal ya da solunum yolu enfeksiyonlarının yanı sıra konjonktivit, hemorajik sistit, nefrit, hepatit ve ensefalit gibi farklı hastalık tabloları da şekillendirebilmektedirler (Greening ve Cannon, 2016; O'Shea ve ark., 2019).

Hepatotropik Virüsler

Hepatit A virus. *Picornaviridae* ailesi içerisinde yer alan *Hepatovirus* cinsine ait zarfsız, tek sarmallı (ss) bir RNA virüsüdür. HAV'ın tek serotipi, üç genotipi (I, II ve III) ve yedi alt genotipi (IA, IB, IC, IIA, IIB, IIIA, IIIB) bulunmaktadır. Dünya genelinde en sık rastlanan alt genotipler IA, IB ve IIIA olarak rapor edilmektedir (Bosch ve ark., 2016; O'Shea ve ark., 2019). Bununla birlikte, Güney Kore'de en yaygın alt genotip IA olsa da son zamanlarda alt genotip IIIA'nın prevalansının hızla yükseldiği, ayrıca her 100.000'de 1.7 olan 2013 insidansının 2016'da 8.2 olduğu bildirilmiştir (Shin ve ark., 2017). HAV, zarfsız bir virüs olması nedeni ile klor, ozon, asit, sıcaklık, kurutma, gamma ve UV ışın uygulamalarına direnç göstermektedir. Dolayısı ile patojen bakterileri elimine edebilen gıda proseslerinde canlı kalabilen HAV, sütteki yağ içeriğinin virüs üzerine koruyucu etkisinden de yararlanarak, 62.8°C'de 30 dakikada sütten tamamen elimine edilememektedir (Erol, 2007; Wu ve ark., 2019).

HAV enfeksiyonları, iyi hijyen ve sanitasyon uygulamaları sayesinde gelişmiş ülkelerde az görülmekle birlikte, gelişmekte olan ülkelerde endemik seyretmektedir. Çocuklar enfeksiyonu genellikle asemptomatik geçirebilirken, özellikle 60 yaş ve üzerindeki yaş gruplarında çok şiddetli hastalık tabloları meydana gelmekte ve yüksek morbidite ve mortalite ile seyretmektedir. Endemik bölgelerde, çocuklar hastalığı küçük yaşlarda geçirdiği için bağışıklığa bağlı olarak ileri yaşlarda şiddetli HAV enfeksiyonları nadiren görülmektedir. Bunun aksine, hastalığın endemik olarak görülmediği gelişmiş ülkelerde ise, hastalık çoğunlukla yetişkinlik döneminde meydana gelmekte ve şiddetli seyretmektedir (Bosch ve ark, 2016).

Ateş, baş ağrısı, iştahsızlık, koyu renkli idrar, açık renkli dışkı, mide bulantısı, kusma ve bazen ishal gibi semptomlar ile başlayan hastalıkta, 1-2 hafta sonra meydana gelen viremi ile sarılık tablosu oluşmaktadır. Genel olarak enfeksiyonun 4.-7. haftalarına rastlayan bu sarılık döneminde etkenin dışkı ile saçılımı söz konusu olup (Greening ve Cannon, 2016) bazı vakalar fulminan hepatit, hepatik ensefalopati ve ölümle sonuçlanmaktadır (Maki ve ark., 2020).

Hepatit E virüsü. *Hepeviridae* ailesine bağlı *Orthohepevirus* cinsi içerisinde yer alan, küçük (27-34 nm), zarfsız, tek sarmallı (ss) bir RNA virüsüdür. *Orthohepevirus* cinsi içerisinde dört (A-D) tür olmakla birlikte insanları sadece *Orthohepevirus* A türü enfekte etmektedir. *Orthohepevirus* A türünde HEV-1 ve HEV-2 genotiplerinin sadece insanları enfekte ettiği, HEV-3 ve HEV-4 genotiplerinin ise zoonotik karakterde olup hem insanlarda hem de domuz, geyik, tavşan ve yaban domuzu gibi hayvanlarda enfeksiyona neden olduğu belirtilmektedir (Pavio ve ark., 2015).

HEV enfeksiyonlarında, 2-8 haftalık inkübasyon periyodunun ardından mide bulantısı, ateş, abdominal ağrı, artralji, koyu renkli idrar, hepatomegali, genel iştahsızlık, halsizlik durumu ve en sonunda sarılık tablosu oluşmaktadır. Enfeksiyonda Guillain-Barre sendromu, sinir sistemi bozuklukları, ensefalit, akut pankreatit, glomerulonefrit, trombositopeni, hemolitik anemi gibi semptomlar da görülmektedir. Hastalık 2 yaş altı çocuklarda, hamilelerde ve bağışıklık sistemi baskılanmış bireylerde daha ciddi seyretmekte, hamilelerde görülen %10'luk mortalite daha çok gebeliğin son 3 aylık döneminde gerçekleşmektedir (Greening ve Cannon, 2016; O'Shea ve ark., 2019).

HEV enfeksiyonları, gelişmekte olan ülkelerde yetersiz hijyenik koşullara bağlı olarak genellikle insan veya domuz dışkısıyla kontamine su yoluyla HEV-1 ve HEV-2 genotipleri tarafından, gelişmiş ülkelerde ise daha çok zoonotik yolla, HEV-3 ve HEV-4 genotipleri tarafından oluşturulmaktadır. Domuz eti ve karaciğeri, av hayvanlarının eti, kontamine sularda yetişen deniz kabukluları ve kontamine sular ile sulanan sebze ve meyveler bulaşta önemli kaynaklar olup bunun yanında, kan ve organ naklinden sonra da insandan insana bulaş rapor edilmiştir (van der Poel, 2014; Adlhoch ve ark., 2019). Yetersiz hijyenik koşullara bir örnek olarak, HEV enfeksiyonlarının özellikle hamilelerde daha ciddi bir tablo ile seyrettiği ve daha yüksek mortaliteye neden olduğunun belirtildiği, Hindistan'da gerçekleştirilen bir çalışmada, 2005-2010 yılları arasında, akut viral hepatit (AVH) ve akut karaciğer yetmezliği (AKY) tanısı konulan 550'si hamile toplam 1088 hasta serolojik yönden incelenmiş, hamile olanların 411 ve 139'unda, hamile olmayanların ise 357 ve 181'inde sırasıyla AVH ve AKY tespit edilmiştir. AVH'li hamile ve hamile olmayan hastalarda HEV oranı sırasıyla %82.72 (340/411) ve

%43.41 (155/357) olarak belirlenmiştir. AKY bulunan hamile ve hamile olmayan hastalarda ise HEV prevalansı sırasıyla %73.38 (102/139) ve %61.32 (111/181) olarak saptanmıştır. Hamile hastaların %80,36'sında (442/550) enfeksiyon etkeni HEV olarak belirlenmiş ve bu kadınlardan 129'u (%75.96) hayatını kaybetmiştir. (Karna ve ark., 2020). HEV'in özellikle gelişmiş ülkelerde zoonotik yolla bulaştığının kanıtı olarak rapor edilen bir vakada ise, Fransa'da 3 günlük bir halsizlik şikâyetiyle doktora başvuran, ardından akut hepatit ve sarılık tablosu şekillenen ve serolojisi HEV pozitif olan hastada, etkenin bulaş kaynağının domuz karaciğeri ve etinden üretilen kontamine bir sosis olduğu tespit edilmiştir (Renou ve ark., 2014). Benzer şekilde, İtalya'da HEV varlığı yönünden inceleme amacı ile üretim işletmelerinden alınan 384 deniz kabuklusu ve 39 su örneğinin sırasıyla %2.6 ve %12.8'inin etkeni taşıdığı, kanalizasyon deşarj bölgeleri yakınlarından alınan 29 su örneğinde ise HEV bulunmadığı, karakterize edilen HEV'lerin hepsinin HEV-3 olduğu belirtilmiştir (La Rosa ve ark., 2018).

Gıda ve Su Kaynaklı Virüslerin Epidemiyolojileri

Uluslararası gıda ticaretinin artması, küresel iklim değişiklikleri, hızlı nüfus artışı ve kentleşme, ülke içi ve dışı turizmin artması, çiğ ya da yetersiz pişmiş gıda tüketimi gibi toplumdaki beslenme tarzının değişmesi, immun sistemi baskılanmış bireylerin sayısındaki artış, antimikrobiyal direnç, temiz su kaynaklarının azalması gibi faktörler gıda kaynaklı patojenlerin epidemiyolojisinde de önemli değişiklikler meydana gelmesine neden olmuştur (Erol, 2016; Miranda ve Schaffner, 2019). Küresel iklim değişiklikleri sonucunda artan yüzey sıcaklıkları, sel ve kuraklık gibi anormal iklim olayları sonucunda gıda, su, parazit ve vektör kaynaklı hastalıkların artması beklenmektedir. Özellikle arbovirüs enfeksiyonları başta olmak üzere, viral gastroenteritler ve viral hepatitlerin yanında diğer birçok viral etkenin prevalans ve insidansında artış olacağı düşünülmektedir (Akman ve Gümüşova, 2016).

Virüsler, fekal-oral ya da kusma yoluyla enfekte insandan sağlıklı insana, gıdaya, suya ve diğer yüzeylere yayılabilmektedir (Yeargin ve ark., 2016). Enfekte bir bireyin dışkısında 10^{11}/g, kusmuğunda ise 10^{7}/30 mL virüs partikülü bulunduğu (Kotwal ve Cannon, 2014), şiddetli kusma olgularında hava yolu ile de bulaş gerçekleşebildiği bildirilmiştir (Sökel ve ark., 2018). Ayrıca, viral hepatit geçiren asemptomatik ve anikterik bireylerin de dışkılarında uzun süre virüs saçmaları nedeni ile bulaşta önemli rol oynadıkları rapor edilmiştir (İncili ve Çalıcıoğlu, 2016). Özellikle zarfsız virüsler çevresel koşullara oldukça dirençli olmaları nedeni ile gıda üzerinde, ellerde, dışkıda ve gıda ile temas eden yüzeylerde uzun süre canlılıklarını koruyabilmekte, bu durum da virüslerin yayılımını ve enfeksiyon oluşumunu kolaylaştırmaktadır (Miranda ve Schaffner, 2019). Bununla birlikte, HAV ve HEV gibi virüslerin parenteral yolla da bulaşabileceği belirtilmiştir (Bosch ve ark., 2016).

Gıda ve su kaynaklı viral enfeksiyonlar otel, okul, hastane, huzurevi, gündüz bakımevi, kreş, büyük yolcu gemisi, yaz kampı, catering hizmeti, restoran gibi toplu yemek tüketilen yerlerde daha sık görülmektedir (Hall ve ark., 2014; O'Shea ve ark., 2019). Viral enfeksiyonlar, gelişmekte olan ülkelerde büyük çoğunlukla hijyenik yetersizlikler ve kontamine su ile, gelişmiş ülkelerde ise daha çok hastalığın endemik olarak görüldüğü düşük gelirli ülkelere seyahat ile veya çiğ ya da az pişmiş et ve karaciğer tüketimi sonucunda zoonotik kökenli olarak meydana gelmektedir (Di Bartolo ve ark., 2015; Hennechart-Collette ve ark., 2019; Garcia ve ark., 2020). Kanalizasyon sularında sıklıkla rastlanan HAV, HEV, RoV, NoV, AdV, AstV, parvovirüs, CoV, PoV gibi enterovirüslerle mücadelede, konvansiyonel arıtmanın viral yükün büyük ölçüde azaltılmasında etkin bir tedbir olmasına rağmen, özellikle PoV ve AdV'lere karşı daha az etkili olduğu rapor edilmektedir. Kanalizasyon suyunun konvansiyonel arıtma ile birlikte ozon ile muamele edilmesinin viral yükü daha da azalttığı, hatta bazı virüsleri tespit edilemeyecek seviyelere indirdiği, buna rağmen iki yöntemin de kanalizasyondaki patojen virüsleri tamamen yok edemediği bildirilmektedir (Wang ve ark., 2018).

Gıda ve su kaynaklı viral enfeksiyonlarda öne çıkan gıdaların, hasat öncesi ve/veya sonrasında etken ile kontamine olabilen deniz kabukluları ve taze sebze ve meyveler olduğu bilinmektedir. Deniz kabuklularının viral yükü, beslenmeleri sırasında filtre ettikleri deniz suyunun kanalizasyon ile kontamine olması durumunda artmaktadır. Sebze ve meyvelerin yetiştirilmesinde kontamine su ile sulama, hasat öncesi bulaş, yetersiz hijyenik koşullar altında yapılan hasat, proses, hazırlama, paketleme ve dağıtımda kontamine su kullanımı ve enfekte işçiler, hasat sonrası bulaş nedenlerinden en önemlilerini oluşturmaktadır (Seo ve ark., 2014; Bosch ve ark., 2016; Miranda ve Schaffner, 2019).

İnsanlarda, dünya çapında yüksek prevalans ile seyrettiği rapor edilen enterotropik virüslerden NoV, AstV, RoV ve AdV

ile hepatotropik virüsler olan HAV ve HEV varlığının deniz kabukluları, sebze ve meyveler ile bunların üretimleri ile ilgili olan suların öncelikli örnek olarak test edildiği araştırmalar incelendiğinde, güncel birçok veri ile karşılaşılmaktadır. Bu çalışmalar içerisinde, deniz kabukluları ve su ile ilgili olanlardan, 2015-2017 yılları arasında, İtalya'nın güneybatı bölgesine ait 289 deniz kabuklusunda, %62 viral patojen prevalansının bulunduğu bildirilen bir çalışmada, 26'şar örnekte HAV ve RoV (%8.9), 31 örnekte NoV GI (%10.8), 114 örnekte NoV GII (%39.7), 60 örnekte AstV (%20.8), 16 örnekte AdV (%5.6) varlığı tespit edilmiştir (Fusco ve ark, 2019). Aynı ülkede gerçekleştirilen bir diğer çalışmada, 108 donmuş ve taze deniz kabuklusu, 70 sebze, 23 su, 17 yabanmersiniböğürtlen-ahududu karışımı ile 28 yüzey svap örneği bazı enterotropik ve hepatotropik virüsler yönünden incelenmiştir. Deniz kabuklularında %18.5 oranında tespit edilen NoV'un genotip dağılımı %10.2 GI, %5.6 GII ve %2.8 GIV iken, RoV ise örneklerin hiçbirinde saptanamamıştır. İncelenen 23 adet su örneğinin %21.7'sinin NoV GII ile, %4.3'ünün RoV ile kontamine olduğu belirlenmiştir. Yetmiş sebze örneğinde %2.9 oranında NoV G1 kontaminasyonu bulunurken, HEV varlığı sadece su örneklerinin %4.3'ünde bulunmuştur. Meyvelerde ve yüzey svaplarında ise enterik virüs varlığına rastlanmamıştır (Purpari ve ark., 2019). Güney Kore'de yapılan ve 51 istiridye, 51 deniz tarağı ve 50 midye olmak üzere toplam 152 deniz kabuklusunda NoV, HAV, HEV ve RoV varlığının araştırıldığı bir prevalans çalışmasında, NoV GII'nin %21.7 (33/152), NoV GI'in %5.9 (9/152), HAV'ın %0.7 (1/152) oranında bulunduğu, HEV ve RoV varlığı saptanmadığı rapor edilmiştir (Seo ve ark., 2014). İspanya'da yedi üretim sahasından 18 aylık bir zaman periyodu içerisinde toplanan 81 midye örneğinin 12'sinde (%14.81) HEV-3 varlığı belirlenirken (Mesquita ve ark., 2016), Vietnam'da iki deniz ürünü satış yeri ile iki süpermarketten elde edilen 121 deniz kabuklusu örneğinin 99'unun (%81.8) NoV, 15'inin (%12.4) AstV, 14'ünün (%11.6) HEV ve 2'sinin (%1.7) HAV taşıdığı bildirilmiştir (Suffredini ve ark., 2020).

Çevresel suların incelendiği çalışmalardan Almanya'da gerçekleştirilen ve bir nehirin üç farklı bölgesinden, farklı zamanlarda alınan yirmi dörder su, biyofilm ve sediment örneklerinin birlikte değerlendirildiği araştırmada, enterik virüs prevalansları yüksekten düşüğe olmak üzere HAdV>EV>RoV>NoV GII olarak gösterilmiştir. Su örneklerinin %87.5'inin HAdV, %20.8'inin EV, %16.7'sinin RoV ve %8.3'ünün NoV GII yönünden pozitif olduğu tespit edilirken, biyofilm ve sediment örneklerinde HAdV varlığı %54.2 olarak bulunmuş, RoV ve NoV GII varlığına ise rastlanmamıştır (Mackowiak ve ark., 2018). Arjantin'de yapılan başka bir çalışmada, 2012 yılında bir barajdan alınan 48 adet, 2013 ve 2015 yılları arasında ise baraj kenarındaki turistik plajların bulunduğu alandan alınan 66 adet su örneği RoV, HAstV, NoV, HAV ve HEV varlığı yönünden incelenmiştir. İlk periyotta alınan 48 örnekte, RoV genogrup A %52.1 (25/48), HAstV %50 (24/48), NoV %60.4 (29/48), HAV %22.9 (11/48), HEV %2,1 (1/48) olup toplam enterovirus varlığı %64.6 (31/48) olarak belirlenmiştir. İkinci periyotta ise, 66 örnekte RoV ve HAstV %18.2 (12/66), NoV %31.8 (21/66), HEV %7.57 (5/66) olarak bulunmuş, HAV varlığına rastlanmamış, toplamda örneklerin %66,7'sinin (44/66) enterovirüsler yönünden pozitif olduğu saptanmıştır (Masachessi ve ark., 2018).

Sebze ve meyve örneklerinde enterotropik ve hepatotropik virüs prevalansı belirlenmesine yönelik olarak Güney Kore'de 2016-2017 yılları arasında yapılan bir çalışmada, 80 adet çiftlikten toplanan 541 taze sebze ve meyve, tarım alanlarından alınan 191 toprak, 14 sulama suyu ve 27 çalışan eldiveni olmak üzere toplam 773 örnekten 2 salatalık ve 2 sulama suyunda NoV GI (%0.51, 4/773), 1 salatalık ve 2 sulama suyunda NoV GII (%0.38, 3/773), 1 çilek ve 1 eldiven örneğinde ise HAV (%0.25, 2/773) bulunmuştur (Shin ve ark., 2019). İtalya'da faaliyet gösteren marketlerde, ambalajlı şekilde, tüketime hazır olarak satışa sunulan toplam 911 sebze örneğinden 18'inde HAV (%1.9), 6'sında HEV (%0.6) varlığı tespit edilirken NoV varlığına rastlanmamıştır (Terio ve ark., 2017). İspanya'da, uluslararası bir havaalanında, ülkeye gelen yolcular tarafından yasal olmayan yollarla ülkeye sokulmaya çalışılırken tespit edilip alıkonulan ve NoV GI, NoV GII, HEV ve HAV yönünden test edilen 122 et ve et ürününün 65'i (%53.3) HEV, 3'ü (%2.5) NoV GI, 1'i ise (%0.8) NoV GII yönünden pozitif bulunmuş, HAV varlığına ise rastlanmamıştır (Rodriguez-Lazaro ve ark., 2015).

Korunma ve Kontrol

İnsanlarda enfeksiyon yayılımının azaltılması/önlenmesinde birincil ve en etkin yöntem aşılamadır. Bu derleme kapsamında yer alan bazı viral etkenlerin de insanlarda enfeksiyon oluşturmasının önlenmesinde aşı uygulamaları bulunmaktadır. Bu nedenle, öncelikle korunma ve kontrol kapsamında, enfeksiyonun insanlarda yayılımının önlenmesi amacı ile gerçekleştirilen aşılamalara yer verilecektir. Önemli bir enterotropik virüs olan RoV'e karşı aşılama, ilk olarak 1998 yı-

lında ABD'de kullanılmaya başlanılmıştır. Yeni patojen suşları da kapsayacak şekilde farklı firmalar tarafından geliştirilen yeni tip aşılar günümüzde de kullanılmaktadır (Jain ve ark., 2014). En yaygın akut viral hepatit etkeni olan HAV'a karşı da aşılanma ile korunma sağlamak mümkündür. Dünya'da, 1995 yılından itibaren HAV aşısı kullanılmaya başlanılmış ve ülkemizde de 2012 yılından itibaren, bebeklik döneminde 18. ve 24. ayların sonlarında olmak üzere iki doz olarak uygulanmaktadır. Aşının koruyuculuğunun ortalama olarak 20 yıl olduğu öngörülmekle birlikte, sonrasında bir hatırlatma dozu önerilmektedir (Afyon ve ark., 2018). Bir diğer önemli hepatit etkeni olan HEV'e karşı Çin'de geliştirilen aşı 2011 yılından itibaren 16-65 yaş arasındaki bireylerde kullanılmaktadır. Aşının 16-65 yaş arasındaki yetişkinlerde güvenilirliği kanıtlanmış olup yapılan çalışmalarda 65 yaş üzeri kişilerde de güvenli olduğu belirtilmiştir (Yu ve ark., 2019; Yin ve ark., 2020).

Günümüz koşullarında, gıda ve su patojenlerinden korunmada en yeni yaklaşım risk temelli değerlendirmelerdir. Bir gıdada bulunma olasılığı olan biyolojik, kimyasal ve fiziksel tehlikelerin belirlenmesi ve derecelendirilmesi, alınacak kontrol önlemlerinin tanımlanması ve geçerliliğinin oluşturulması yönünden büyük önem taşımaktadır. Özellikle biyolojik risk değerlendirmelerinde, sadece bakteriyel gıda patojenlerinin göz önünde bulundurularak, prevalansları göz ardı edilemeyecek kadar yüksek olan enterotropik ve hepatotropik virüslerin bu değerlendirmenin dışında bırakılması, önemli bir tehlikenin atlanmasına neden olmaktadır (Miranda ve Schaffner, 2019). Ancak, gıda kaynaklı hastalıklarda virüslerin de oldukça büyük bir yer tuttuğunun kabul edilmesi sonrasında, işletmelerin gıda güvenliği stratejileri içerisinde viral kontaminasyonlardan korunma ve kontrol tedbirleri de yer almaya başlamıştır.

Gıda ve su kaynaklı viral kontaminasyonlar, güvenli hammadde, üretim sırasında hijyen prosedürlerine uyulması, güvenli su kullanımı, işleme öncesi, sırası ve sonrasında çapraz kontaminasyonların önlenmesi, çevre hijyeni, personel eğitimi, iyi üretim uygulamaları (GMP), iyi hijyen uygulamaları (GHP) ve Kritik Kontrol Noktalarında Tehlike Analizi (HACCP) prosedürlerine uyulması ile etkin şekilde önlenebilmektedir. Bunun yanı sıra, gıdaların işlenmesi sırasında uygulanan soğutma, dondurma, asidifikasyon, su aktivitesinin düşürülmesi, modifiye atmosfer paketleme, pastörizasyon, yüksek hidrostatik basınç, gıda ışınlama gibi birçok teknolojik uygulamanın, gıda kaynaklı virüslerin eliminasyonunda yetersiz kalabileceği de bildirilmiştir (Keyvan ve ark., 2018). Bu konu ile ilgili olarak, bazı meyve ve sebzelerde enterotropik ve hepatotropik virüslerin eliminasyonuna yönelik uygulamaları içeren güncel çalışmalara dezenfektan uygulamaları, ışınlama, pişirme ve hidrostatik basınç uygulaması başlıkları altında yer verilmiştir.

***Dezenfektan uygulamaları*:** Bu uygulamalardan ilkinde, deneysel olarak HuNoV G1 ve G2 ile kontamine edilmiş tüketime hazır karışık salatanın, 3 farklı dezenfektan (100 ppm sodyum hipoklorür, 80 ppm perasetik asit ve 20 ppm klor dioksit) içeren su ile yıkanma sonrasında antiviral etkilerinin incelendiği çalışmada, en yüksek antiviral etkinin perasetik asitte, en düşük etkinin ise klor dioksitte görüldüğü tespit edilmiştir. Çalışma sonucunda, sodyum hipoklorür ve özellikle perasetik asitin, HACCP ilkeleriyle belirlenen hijyen ve kontrol kurallarıyla birlikte, taze sebze/meyve endüstrisinde bu virüsün eliminasyonunda etkin bir dezenfektan olarak kullanılabileceği belirtilmiştir (Anfruns-Estrada ve ark., 2019). Bir diğer çalışmada, FCV, HAV ve MNV-1 ile kontamine edilen taze marul örneklerinin, aktif klor (15 ppm) ve peroksiasetik asit bazlı (100 ppm) dezenfektan içeren suda yıkanması sonrasında, iki bileşiğin de test edilen virüsler üzerindeki etkisi benzer olarak saptanmıştır. En yüksek antiviral etkinin FCV'de peroksiasetik asit kullanımı (3.2 log azalma) ile olduğu, bunu aktif klor kullanımının izlediği (2.9 log azalma), en düşük etkinin ise HAV'de aktif klor kullanımında (0.7 log azalma) görüldüğü belirlenmiştir (Fraisse ve ark., 2011). Bu durum, farklı konsantrasyon ve etken maddelerin yanı sıra, farklı virüslerin de etken maddelere karşı duyarlılıklarında fark olabileceğini göstermesi açısından önem taşımaktadır. Ayrıca, aktif klor ve peroksiasetik asitin önemli bir enterotropik virüs olan NoV üzerinde çok düşük bir inhibitör etki göstermesi, bu etkenin taze meyve ve sebzelerde eliminasyonunda farklı yaklaşımların gerekli olabileceğini düşündürmektedir. Bir diğer çalışmada, MNV ve HAV ile kontamine edilen taze ve donmuş çilek, ahududu ve yaban mersininde güvenli dozlarda Ultraviyole-C (UV-C) uygulaması gerçekleştirilmiş, sonuçta uygulamanın yeterli bir antiviral etki sağlamadığı, dolayısı ile gıda kaynaklı virüslerin inaktivasyonunda, UV-C'nin tek başına yeterli bir yöntem olmadığı ortaya konulmuştur (Butot ve ark., 2018). Bir diğer etkili dezenfektan olan ozon gazının, MNV-1 ve HAV ile kontamine edilen taze ahududu örneklerinde virüsid etkisinin incelendiği bir araştırmada, bu gazın sadece HNoV inaktivasyonunda ve kontrolünde iyi bir aday olduğu rapor edilmiştir (Brie ve ark., 2018).

***Işınlama*:** Gıdalara uygulanan koruyucu yöntemlerden bir tanesi de gıda ışınlama teknolojisidir. Gıda ışınlama yöntemi, patates, soğan, sebze, meyve, baharat, beyaz et gibi ürünlerin belirli dozlarda ışınlanarak bozulmasını önlemek, raf ömrünü uzatmak ve patojen mikroorganizmaları elimine etmek için kullanılmaktadır (Erol, 2007). Türk Gıda Kodeksi (TGK) Gıda Işınlama Yönetmeliği (2019) Ek-2'de, çeşitli gıda gruplarına uygulanabilecek ışınlama dozları kilo Grey (kGy) birimi üzerinden verilmiş olup, buna göre uygulanabilecek en yüksek doz kurutulmuş sebzeler, baharatlar, kuru aromatik bitkiler, otlar, çeşniler ve bitkisel çaylar gıda grubuna olmak üzere, 10 kGy olarak belirtilmiştir. Bununla birlikte, yapılan çalışmalarda 20-25 kGy'lik gamma radyasyon dozunun bile virüsleri tam olarak elimine etmediği ortaya konulmuştur (Akakçe ve Çam, 2019). Ayrıca, gıdaya uygulanan yüksek dozlardaki ışının, gıdanın yapısında bozulmalar meydana getirdiği, besin değeri kaybı ve istenilmeyen tat ve koku oluşturduğu bildirilmiştir (Erol, 2007).

***Pişirme*:** Geleneksel pişirme yönteminin HAV üzerinde inaktive edici etkisinin araştırıldığı bir çalışmada, deneysel olarak HAV ile kontamine edildikten sonra kabukları açılarak 100°C sıcaklıkta pişirilen deniz taraklarında etkenin tamamen inaktive olması, bu tip ürünleri kabukları açılmış olarak en az 2 dakikası 100°C'de olmak üzere 12 dakika boyunca pişirmenin, gıda kaynaklı hastalıklardan korunmak için yeterli olduğu belirtilmiştir (Pascoli ve ark., 2016). İspanya'da deniz taraklarında pişirme esnasında iç sıcaklığın 5 dakika boyunca 90°C'de tutulmasının HAV, HuNoV GI ve GII üzerine etkisinin araştırıldığı bir başka çalışmada ise, yapılan uygulama sonucunda HAV miktarında 3.89 log10, HuNoV GI miktarında 2.96 log10 ve HuNoV GII miktarında da 2.56 log10 azalma meydana geldiği saptanmıştır (Fuentes ve ark., 2021). 2016 yılında Ettayebi ve ark., (2016) HuNoV içeren dışkı süspansiyonlarına 15 dakika boyunca 60°C sıcaklık uygulamasından sonra virüsün enfektivitesini kaybettiğini belirlemiştir. Yine EV, HuNoV, HAV ve HEV gibi virüslerin, kaynayan suda 1 dakika sonunda viral yüklerinde 4 $\log_{10}$'dan fazla azalma meydana geldiği bildirilmiştir (CDC, 2009). Benzer şekilde 2 dakika boyunca 70°C sıcaklık uygulamasının ardından, MNV tespit limitlerinin altına düşmüş (Hirneisen ve Kniel, 2013), ayrıca pişirme sırasında domuz etinde merkez sıcaklığın 20 dakika boyunca en az 71°C'de tutulmasının, HEV inaktivasyonu için gerekli olduğu belirtilmiştir (Barnaud ve ark., 2012)

***Hidrostatik basınç uygulaması*:** Yüksek hidrostatik basınç yöntemi de viral patojenlerin inaktivasyonunda kullanılan bir yöntemdir. 22°C'de, 5 dakika boyunca, 450 MPa basınç uygulaması ile HAV ve RoV miktarında 7-8 $\log_{10}$ azalma meydana gelmiştir (Shukla ve ark., 2018). Avrupa ülkelerinde sık görülen ve zoonotik yolla da bulaşabilen HEV-3 genotipinin yüksek basınç uygulaması karşısındaki stabilitesi üzerine yapılan bir çalışmada, 20°C'de 200 MPa basınç uygulaması sonrasında viral yükte 0,5 $\log_{10}$, 400 MPa basınç uygulaması sonrasında 1 $\log_{10}$, 4°C 'de 200 MPa basınç uygulamasından sonra 1 $\log_{10}$ ve 400 MPa basınç uygulamasından sonra ise 2 $\log_{10}$ azalma meydana gelmiştir. İki sıcaklık derecesinde de 600 MPa basınç uygulamasının ardından virüs büyük ölçüde inaktive olmuştur (>3,5 $\log_{10}$) (Johne ve ark., 2021). Gıda matriksi üzerinde, farklı virüslerin farklı sıcaklık, basınç uygulaması ve pH değerindeki durumlarının incelendiği bir başka çalışmada ise, pH 7.0 değerinde, 4°C'de, 2 dakika boyunca uygulanan 300 MPa yüksek basınç sonrasında, RoV miktarında 4.1 $\log_{10}$, aynı parametrelerde, pH 4.0 değerinde ise 1.9 $\log_{10}$ azalma meydana gelmiştir. pH 7.0 değerinde, 4°C'de, 2 dakika boyunca 350 MPa basınç uygulaması sonrasında HuNoV miktarında 8,1 $\log_{10}$ azalma meydana gelirken, aynı parametrelerde 20°C'de yapılan uygulama sonrasında ise 4.1 $\log_{10}$ azalma meydana gelmiştir. Yine pH 4.0 değerinde, 4°C'de, 2 dakika boyunca uygulanan 350 MPa basınç sonrasında, MNV-1 miktarında 6.0 $\log_{10}$ azalma meydana gelirken, pH 6.0 değerinde, 20°C'de, 1 dakika boyunca uygulanan 250 MPa basınç sonrasında FCV miktarında 4.1 $\log_{10}$ azalma oluşmuştur. Bununla birlikte, AiV ve PoV gibi virüsler yüksek basınç uygulamalarına dirençli olup, 600 MPa basınç uygulaması sonrasında dahi virüs miktarında bir indirgenme meydana gelmemiştir (Lou ve ark., 2015).

Yukarıda bahsedilen yaklaşımlar dışında ticari olarak erişilebilen antiviral ilaçlar, virüslerde gelişen çoklu ilaç direnci sebebiyle eliminasyonda her zaman etkili olamayabilmektedirler. Bu nedenle, birtakım alternatif uygulamalara ait birçok güncel yaklaşım bulunmaktadır. Bunlardan ilki, doğal kaynaklardan elde edilen, bazı antiviral etki potansiyeli bulunan bileşik ve ekstraktların (polifenolik bileşikler, saponin, sitrik asit, yaban mersinindeki proanthocyanidin, nar suyu, üzüm çekirdeği ekstraktı, kitosan, siyah ahududu suyu ve dut suyu, kızılcık, limon otu yağı, yeşil çay ekstraktı, *Hibiscus sabdariffa* ekstraktı vb) ya da gıdanın kendi içerisinde doğal olarak bulunabilen antimikrobiyal etkili maddelerin kullanımına yönelik çalışmalardır (Lee ve ark., 2014). Farklı 16 bitkisel ekstraktın HAV üzerine inhibitör etkisinin incelendiği bir çalışmada, *Alnus japonica* (Japon kızılağacı), *Artemisia annua* (Peygamber süpürgesi), *Allium sativum* (Sarımsak), *Allium*

fistulosum (Yeşil soğan), *Agrimonia pilosa* (Kasık otu), *Pleuropterus multiflorus* (Hasuo bitkisi), *Eleutherococcus senticosus* (Sibirya gingsengi), *Coriandrum sativum* (Kişniş), *Ginko biloba* (Mabet ağacı) ve *Torilis japonica* (Japon çit maydonozu) ekstraktları olmak üzere 10 ekstraktın HAV'e karşı etkili olduğu, en kuvvetli antiviral etkiyi *Alnus japonica* ekstraktının gösterdiği rapor edilmiştir (Seo ve ark., 2017). Resveratrolün NoV kaynaklı gıda enfeksiyonlarından korunmada önemli rol oynayabileceği belirtilmiştir (Oh ve ark., 2015). Kekik uçucu yağı ve primer bileşiği olan karvakrolün MNV üzerine antiviral etkisinin araştırıldığı bir başka çalışmada, karvakrolün virüs sayısında 1 saatte 3.84 $\log_{10}$ düşüş sağlayabilmesi, bu maddelerin NoV kontrolünde potansiyel bir gıda ve yüzey dezenfektanı olarak kullanılabileceğini göstermektedir (Gilling ve ark., 2014). Falco ve ark. (2019b)'nın çalışmasında, portakal suyu, elma suyu, süt ve Meksika'ya özgü bir içecek olan horchata gibi gıdaların HAV ve MNV ile kontamine edilmesi sonrasında, gastrik koşullarda, yeşil çay ekstraktının antiviral etkisi incelenmiş, 5 mg/mL oranındaki yeşil çay ekstraktının elma suyundaki MNV miktarını tespit edilebilir limitlerin altına düşürdüğü, ayrıca süt, horchata ve portakal suyunda da 1.0-1.8 log azalmaya neden olduğu belirlenmiştir. Aynı çalışmada 5 mg/mL oranındaki yeşil çay ekstraktının HAV miktarında portakal suyunda 1.2 log, elma suyunda 2.1 log, horchatada 1.5 log ve sütte 1.7 log düşüş sağladığı tespit edilmiştir. Çalışma sonunda, yeşil çay ekstraktının gıda kaynaklı viral hastalıkların önlenmesinde doğal bir seçenek olarak kullanımının uygun olduğu ortaya konulmuştur. Falco ve ark. (2019a)'nın başka bir çalışmasında ise, yeşil çay ekstraktı içeren aljinat-oleik asit bazlı yenilebilir film kaplamaların iki farklı pH değerinde (5.5-7.0), 10°C ve 25°C sıcaklıklarda, çilek ve ahududu üzerindeki antiviral ve antioksidan özellikleri incelenmiştir. Antioksidan özelliklerin pH değerine bağlı olarak değişmediği, ancak antiviral özelliklerin pH 5.5'te daha fazla olduğu belirlenmiştir. Saf yeşil çay ekstraktına göre yeşil çay içeren filmlerin antioksidan aktivitesi daha düşük bulunmuş, bu da yeşil çaya antioksidan özellikler kazandıran polifenoller gibi bileşiklerin, filmlerden salınımının sınırlı olmasına bağlanmıştır. Antiviral özellikler ise HNoV ile aynı familya içerisinde yer alan MNV ve HAV üzerinde araştırılmış, örnekler deneysel olarak enfekte edilmiştir. Çilek ve ahududu örnekleri, kaplama uygulamasından sonra 10°C'de 4 gün muhafaza edilmiş ve muhafaza sonrasında kontrol gruplarıyla karşılaştırıldığında, viral yüklerde 1.5-2.0 $\log_{10}$ azalma meydana geldiği, 25°C'de 1 gece muhafaza sonrasında ise iki virüsün de tamamen inaktive olduğu belirlenmiştir. Sonuçta, yenilebilir film kaplamaların gıda güvenliğini sağlamada potansiyel antiviral etkilerinin olduğu ortaya konulmuştur. Falco ve ark. (2020)'nın meyve sularındaki enterik virüsler üzerine yeşil çay ekstraktı ve düşük sıcaklıkta pastörizasyon uygulamasının etkisini inceledikleri bir çalışmada, 50°C'de 30 dakika düşük pastörizasyon uygulanan meyve sularında yeşil çay ekstraktı kullanımının, sadece sıcaklık uygulamasına göre MNV-1 miktarını 4 log daha fazla düşürerek, daha yüksek antiviral etki oluşturduğu, kombine şekilde yapılan uygulamanın gıda güvenliğini artırdığı belirlenmiştir. Elma suyu ve sütün FCV-F9, MNV-1 ve HAV ile kontaminasyonu sonrasında, gastrik koşullarda, üzüm çekirdeği ekstraktının antiviral etkisinin incelendiği başka bir araştırmada, en duyarlı virüsün FCV-F9 olduğu, ekstraktın 37°C'de daha yüksek etki gösterdiği, 1 mg/mL ekstrakt ile 37°C'de 15 dakika inkübasyon sonucunda FCV-F9, 2 mg/mL üzüm çekirdeği ekstraktı ile 37°C'de 6 saat inkübasyon sonunda ise HAV ve MNV-1 titrelerinin tespit edilebilir seviyenin altına indiği belirlenerek, ekstraktın düşük maliyeti ile de gıda kaynaklı viral enfeksiyonların önlenmesi, gıda güvenliğinin artırılması ve halk sağlığının korunmasında uygun bir doğal bileşik olduğu belirtilmiştir (Joshi ve ark., 2015). Benzer şekilde, üzüm çekirdeği ekstraktı, gingerol ve kurkumin varlığında HAV'ın sıcaklığa karşı duyarlılığının arttığı tespit edilmiş ve bu virüsün sıcaklık uygulanarak eliminasyonunda, bu tip ekstraktların ısıl işlemler ile kombine olarak kullanılabileceği önerilmiştir (Patwardhan ve ark., 2020). Ayrıca, sütün doğal yapısında bulunan kazein, α-laktalbumin, β-laktoglobulin, laktoferrin, laktoferrisin gibi proteinlerin de antiviral etkilerinin olduğu rapor edilmiştir (Ng ve ark., 2015).

Bunların dışında, veteriner otoritesi öncülüğünde başlatılıp, ilgili diğer disiplinleri de kapsayacak şekilde hayvan, insan ve çevre sağlığını bir bütün olarak ele alan tek sağlık yaklaşımı da korunma ve kontrolde oldukça etkilidir. İnsanları enfekte eden patojenlerin % 61'inin zoonoz kökenli olduğunun anlaşılması ve insan ve hayvan hastalıklarının tedavisinde kullanılan ilaçlara karşı mikroorganizmalarda gelişen antimikrobiyal direncin artması nedeni ile tek sağlık yaklaşımı gün geçtikçe daha da önem kazanmaktadır (Ryu ve ark., 2017). Diğer zoonozlar ile mücadelede olduğu gibi gıda ve su kaynaklı hastalıkların önlenmesinde de tek sağlık yaklaşımı son 10 yıldır gelişmiş ülkelerde başarı ile uygulanmaktadır (Aguirre ve ark., 2019).

Sonuç

Son yıllarda tanıya yönelik teknolojik gelişmelerin ivme kazanması ile viral enfeksiyonların prevalansları hakkında daha güvenilir bilgilere ulaşılmıştır. Bunun sonucunda daha önce bilinenin aksine, viral etkenlerin özellikle gastroenterit ile seyreden hastalıklardaki payının oldukça büyük olduğu anlaşılmıştır. Virüslerle mücadele, çevresel şartlara dayanıklı olmaları, gıda proseslerine bakterilere göre daha direnç göstermeleri, ticari antiviral etkenlerin oldukça kısıtlı olması gibi sebeplerle diğer mikroorganizmalara göre daha zor olmakta, bu nedenle özellikle gıda sektöründe korunma ve kontrol önlemleri büyük önem taşımaktadır.

Gıda ve su kaynaklı viral enfeksiyonlardan korunmada; sıcaklık uygulamaları, yüksek basınç uygulamaları, doğal antiviral bileşikler, UV ışını ve çeşitli dezenfektanların kullanımı gibi yöntemler olsa da, en etkilisi işletmelerin personel ve su hijyeni başta olmak üzere iyi hijyen uygulamaları doğrultusunda faaliyet göstermeleri gerekliliğidir. Bu kapsamda, GMP, GHP ve HACCP sisteminin eksiksiz ve ciddiyetle uygulanması, gıda ve su kaynaklı enfeksiyon ve intoksikasyonlardan korunma sağlayacaktır. Bununla birlikte, RoV, HEV, HAV gibi aşısı mevcut olan etkenlere karşı uygulanan aşılama programları ise korunmada en etkili yoldur. Ayrıca, son yıllarda üzerinde sıklıkla durulan, doğal bileşiklerden elde edilen antiviral ajanların gıdalarda kullanılması ile oluşan antiviral etki birçok çalışmada gösterilmiş, gelişen antimikrobiyal direnç tehlikesine karşı doğal bileşiklerden elde edilen antimikrobiyal ajanların kullanımının önemi vurgulanmıştır.

Etik Standart ile Uyumluluk

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

Etik izin: Araştırma niteliği bakımından etik izne tabii değildir.

Finansal destek: -

Teşekkür: -

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All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition.

When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

All references, tables, and figures should be referred to within the main text, and they should be numbered consecutively in the order they are referred to within the main text.

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

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References

Reference System is APA 6th Edition

In-text Citation with APA

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

....(Crockatt, 1995).

Direct quote from the text

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

Major Citations for a Reference List in Table 2.

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

REVISIONS

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

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

Table 2.

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