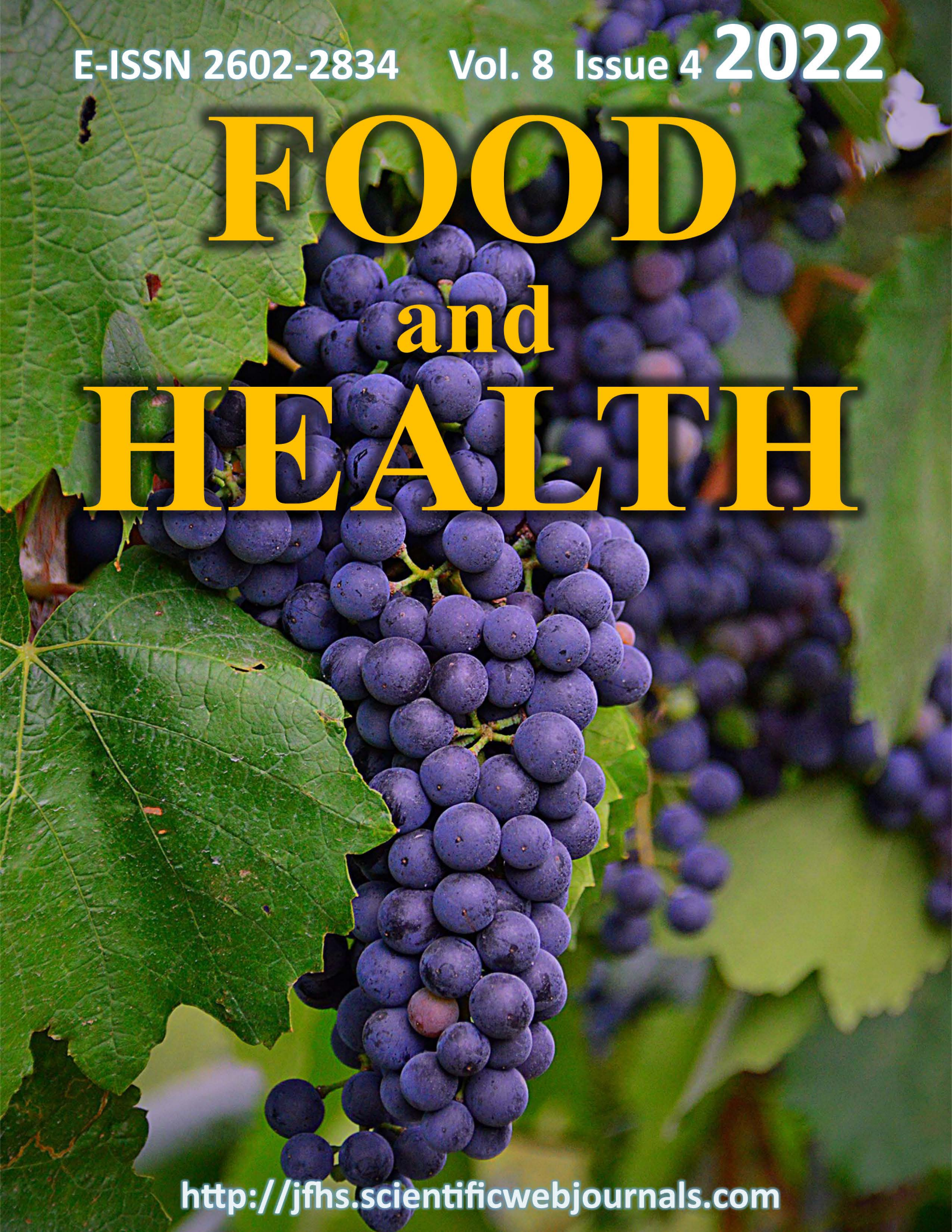
E-ISSN 2602-2834 Vol. 8 Issue 4 2022

FOOD and HEALTH



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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Publisher Özkan Özden

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

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Aims and Scope

FOOD and HEALTH

Abbreviation: **FOOD HEALTH**

e-ISSN: 2602-2834

Journal published in one volume of four issues per year by

http://jfhs.scientificwebjournals.com web page

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FOOD and HEALTH

FOOD and HEALTH

E-ISSN 2602-2834

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 8(4), 273-283 (2022) • https://doi.org/10.3153/FH22025 Research Article

Investigation of cyto-genotoxic effects of a food sweetener Acesulfame potassium

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Cite this article as:
Mamur, S., Yüzbaşıoğlu, D., Bülbül, S.N., Ünal, F. (2022). Investigation of cyto-genotoxic effects of a food sweetener Acesulfame potassium. *Food and Health,* 8(4), 273-283. https://doi.org/10.3153/FH22025

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Submitted: 07.06.2022
Revision requested: 05.07.2022
Last revision received: 19.07.2022
Accepted: 19.07.2022
Published online: 16.08.2022

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

ABSTRACT

Acesulfame potassium (ACE-K) is an artificial sweetener widely used in many foods. This investigation assessed the cytotoxic effect of ACE-K using MTT assay in human hepatocellular carcinoma (HepG2) cell line and the genotoxic effect using chromosomal aberrations (CAs), micronucleus (MN), and comet assays in human lymphocytes. 7.5-240 µg/mL concentrations of ACE-K were applied to cells. ACE-K notably decreased the cell viability on HepG2 cells, especially at 120 and 240 µg/mL at 24 and 48 h. It also significantly reduced the mitotic index (MI) at 60, 120, and 240 µg/mL at both treatments (24 and 48 h) in human lymphocytes. The frequency of the CAs significantly increased at 60, 120, and 240 µg/mL for 48 h treatment compared to control. However, no difference was observed in the frequency of MN and nuclear division index (NDI) at all the treatments. ACE-K also induced comet tail length, tail intensity, and moment at 15 µg/mL in isolated human lymphocytes. Therefore, ACE-K showed a cytotoxic effect in HepG2 cells as well as human lymphocytes at higher concentrations. It also exhibits a mild genotoxic effect by increasing the frequency of CAs at long-term treatment and DNA damaging effect only at 15 µg/mL.

Keywords: Acesulfame potassium, Food sweetener, MTT assay, Chromosomal aberrations, Micronucleus, Comet assay

Introduction

Sweeteners are used as food additives in all kinds of foods and beverages to make foods tastier (Cruz-Rojas et al., 2019; Schiano et al., 2021). They entered the food industry around the 1800s and are the basis of foodstuffs today (Carocho et al., 2017). Due to their low calorie, cost, and having a higher sweetness than natural table sugar, they are increasingly being included in foods and beverages. Sweeteners are added to foods and beverages as sugar substitutes such as artificial sweeteners, sugar-free sweets, and sugar-free sodas (Cao et al., 2020). They can be grouped into artificial sweeteners, modified sugars, sugars, and sugar alcohols, natural caloric sweeteners, and zero-calorie sweeteners (Hernández-Pérez et al., 2020). Artificial sweeteners are obtained by chemical synthesis and are primarily used in food, beverages, pharmaceuticals, and animal feeds with high sweetening power without extra energy (Li et al., 2020; Schiano et al., 2021). There are more than 3000 additives approved for use around the world (Whitehouse et al., 2008; Cao et al., 2020). These sweeteners are aspartame, acesulfame potassium, neotame, saccharin, cyclamate, sucralose, and neohespiridin dihydrochalcone (Kokotou et al., 2012; Heredia-García et al., 2019; Li et al., 2020).

Acesulfame potassium (ACE-K) (also known as Sweet One, and Sunett) is a widely-used artificial sweetener worldwide (Cruz-Rojas, 2019; Belton et al., 2020). It is one of several low- and no-calorie sweeteners used as an alternative to sugar. In 1967, ACE-K was discovered by chemist Karl Clauss. In 1988, the Food and Drug Administration (FDA) was approved as a food/drink sweetener (Whitehouse et al., 2008; Belton et al., 2020). Additionally, the acceptable daily intake (ADI) value for ACE-K has been recommended by FDA is 15 mg/kg body-weight in the same year. The ADI value has been determined by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) to be 0–15 mg/kg body-weight in 1991, and by the European Scientific Committee on Food to be 0-9 mg/kg body-weight in 2000 (WHO 1980; JECFA 1991; Belton et al., 2020; Chappell et al., 2020). ACE-K, whose sweetness is nearly 200 times higher than that of sucrose, is known as a potential sweetener due to its superior sweet taste and high water solubility (Magnuson et al., 2016; Ibi et al., 2018). Additionally, ACE-K is often mixed with other sweeteners (aspartame or sucralose) and exhibits a synergistic effect that makes the mixture sweeter than its components (Chattopadhyay et al., 2014). It can be included in some foods like baked goods, frozen desserts, candies, chewing gum, desserts, non-alcoholic beverages, and breath mints (Whitehouse et al., 2008; Fındıklı and Türkoglu, 2014). It is not metabolized in the body and is excreted unaltered in the urinary (Whitehouse et al., 2008; van Eyk, 2015; Najam et al., 2017). ACE-K contains the chemical methylene chloride, which has a known carcinogenic effect (Fındıklı and Türkoglu 2014).

Some *in vitro* and *in vivo* studies revealed that ACE-K exhibited cytotoxic and genotoxic effects (Mukherjee and Chakrabarti 1997; Bandyopadhyay et al., 2008; van Eyk, 2015). Cytotoxicity studies on cancer cell lines related to ACE-K have been observed to be very limited. van Eyk (2015) determined that ACE-K had a cytotoxic effect at concentrations >10 mM in colon HT-29 and Caco-2 cells and kidney HEK-293 cells using MTT assay. However, it had no significant effect on DNA damage using comet assay at all treatments. Mukherjee and Chakrabarti (1997) demonstrated that ACE-K (60, 450, 1500, and 2250 mg/kg) caused an increase in the frequency of chromosomal aberrations in *vivo* Swiss albino male mice. However, Mukhopadhyay et al. (2000) determined that the combination of ACE-K (1.5, 15, and 150 mg/kg) and Aspartame (3.5, 35, and 350 mg/kg) did not show any genotoxic effect using chromosomal aberration assay in Swiss albino male mice. Bandyopadhyay et al., (2008) demonstrated that ACE-K induced DNA damage in bone marrow cells of mice at 150, 300, and 600 mg/kg concentrations with the comet assay. This result is consistent with other results obtained in isolated human lymphocytes at 2.5 and 5 ppm using comet assay (Fındıklı and Türkoglu, 2014). Additionally, Silva et al. (2008) reported that ACE-K and Aspartame (ACE-K+AS) mixture increased the DNA tail length in human lymphocytes at 0.5% and 5% concentrations using the comet assay.

In literature, limited studies have been conducted on the genotoxic effects of ACE-K despite its frequently used in foods. Moreover, there are not enough studies about the cytotoxic/antiproliferative effects of ACE-K on human hepatocellular carcinoma (HepG2) cells. Therefore, this study purposed to investigate the cytotoxic effect of ACE-K in HepG2 cells using MTT assay as well as genotoxic effects in human lymphocytes using chromosomal aberrations (CAs), micronucleus (MN), and comet (SCGE) assays in human lymphocytes.

Materials and Methods

Chemicals

The test substance Acesulfame Potassium (ACE-K) (Cas. No: 55589-62-3) was obtained from Merck. The molecular formula of ACE-K is $C_4H_4KNO_4S$ and the molecular weight is

201.24. It was dissolved in distilled water. LymphoPlus Medium was obtained from Cegrogen Biotech. Dulbecco's Modified Eagle Medium with phenol red and without phenol red (DMEM, Cas. No: F0445, Cas. No: F0475, respectively), fetal bovine serum (FBS, Cas. No: S0613), PBS (Cas. No: L1825), trypsin (Cas. No: L2163), L-glutamine (Cas. No: K0283) and penicillin/streptomycin (Cas. No: A2213) were obtained from Biochrome. *In vitro* toxicology assay kit (MTT based, Tox-1), mitomycin-C (MMC, Cas. No: 50-07-7), cytochalasin-B (Cyt-B, Cas. No: 14930-96-2), NaCl (Cas. No: 7647-14-5), colchicine (Cas. No: 64-86-8) were obtained from Sigma. Low Melting Agarose (Cas. No: 9012-36-6), Normal Melting Agarose (Cas. No: 9012-36-6), NaOH (Cas. No: 1310-73-2), Tris (Cas. No: 77-86-1), Triton X-100 (Cas. No: 9002-93-1), EtBr (Cas. No: 1239-45-8), DMSO (Cas. No: 67-68-5), EDTA (Cas. No: 6381-92-6) and H_2O_2 (Cas.No: 7722-84-1) were obtained from Applichem.

MTT Assay

Human hepatocellular carcinoma (HepG2) cells were cultured according to ATCC protocols (https://www.atcc.org/products/hb-8065). The cytotoxicity of ACE-K was examined using an MTT assay with *In Vitro* Toxicology Assay kit. The experiment was carried out according to the method of Mossman (1983) with some modifications (Mamur et al. 2018). The grown cells were seeded in 96-multi-well plates, including $5x10^3$ cells per well, and cultured in a CO_2 incubator for 24 h. The cells were treated with 7.5, 15, 30, 60, 120, and 240 µg/mL concentrations of ACE-K for 24 and 48 h. A negative control was also maintained.

The absorbance (ABS) values of the wells were read at 570 nm wavelength in the ELISA microplate reading device (Molecular Devices Spectramax, M5). Then, the cell viability (%) values and also IC_{50} values that killed half of the cell population were determined. All experiments were repeated three times independently.

Lymphocyte Cultures

In this study, human peripheral lymphocyte cells from three healthy volunteers (a male and two female, non-alcoholic, nonsmokers, aged 24–27 years) were used. This study was confirmed by the Ethical Committee of the Faculty of Medicine, Gazi University (11.11.2019/144). Cells were treated with between 7.5 and 240 µg/mL concentrations of ACE-K. A positive control (Mitomycin-C; MMC, 0.20 µg/mL for CAs and MN; Hydrogen peroxide; H_2O_2, 100 µM for comet assay) and negative control (distilled water) were also maintained.

Chromosomal Aberrations (CAs) Assay

For the CAs assay, Evans's (1984) method was applied with some modifications according to Yuzbasıoglu et al. (2022). A blood sample (with heparin added to prevent clotting) was added to 2.5 mL of LymphoPlus medium. The cells were treated with 7.5, 15, 30, 60, 120 and 240 µg/mL concentrations of ACE-K for 24 and 48 h.

In CA analysis, a total of 300 (100 metaphases for each donor) metaphases per concentration were examined. In addition, the mitotic index (MI) was determined by scoring a total of 3000 cells per concentration (1000 cells from each donor).

Micronucleus (MN) Assay

The methods of Fenech (2000) and Palus et al. (2003) were followed for the preparation of MN with some modifications (Yuzbasioglu et al. 2022). Whole blood (0.2 mL) was added to 2.5 mL the LymphoPlus medium and incubated for 72 h at 37°C. Human lymphocytes were treated with 7.5, 15, 30, 60, 120 and 240 µg/mL of ACE-K at 48 h treatment period. Both of a negative control (distilled water) and positive control (Mitomycin C) were also maintained.

MN was scored from 1000 binucleated cells (BN) from each donor (a total of 3000 BN per concentration). The nuclear division index (NDI) was determined via analyzing 500 cells from each donor (a total of 1500 cells per concentration) according to the methods of Surrales et al (1995).

Comet (Single cell gel electrophoresis, SCGE) Assay

The alkaline comet assay was applied according to the methods of Singh et al. (1988) with some modifications (Erikel et. al. 2020). Lymphocytes were treated with six different concentrations of ACE-K (7.5, 15, 30, 60, 120, and 240 µg/mL) for 1 h at 37°C. In addition, negative and positive (100 µM H_2O_2) controls were also maintained.

Finally, the slides were evaluated using a fluorescence microscope (Olympus) equipped with an excitation filter (546 nm) and a barrier filter (590 nm), at 400× magnification. For each ACE-K concentration, the tail length (µm), intensity (%) and moment of the randomly selected 300 (100 cells from each donor) comets were determined using a specialized image analysis system (Comet Assay IV, Perceptive Instruments Ltd., Haverhill, UK).

Statistical Analysis

MTT assay was analysed by using One Way ANOVA followed by Dunnet's multiple comparison test and a p value less than 0.05 was considered as statistically significant. z-test was performed for statistical analysis of the percentage of abnormal cells, CAs/cell number, MN frequency, MI and

NDI results. For the analysis of Comet assay results, t-test was used. Besides, correlation and regression analysis were performed in order to designate the concentration-response relationship for the experiment groups.

Results and Discussion

Recently, artificial sweeteners are widely used by millions of people worldwide in various substances including pharmaceuticals, diet drinks and foods, and in consumer products such as toothpaste (Oldfield et al., 2020). Food consumers often select those foods with sweeteners because they want the taste of sweetness without added calories (Chattopadhyay et al., 2014). Artificial sweeteners supply the sweetness of sugar without the calories (Whitehouse et al. 2008). However, the safety of these sweeteners has been contradictory issue and there is some concern about their health effects (Cao et al., 2020). Several previous studies reported that artificial sweetener consumption can be related to psychotic conditions (Lindseth et al., 2014), oxidative stress (Ashok et al., 2017), type 2 diabetes mellitus (De Koning et al., 2011), weight gain (Fowler et al., 2008), obesity (Fowler, 2016), metabolic syndrome (Lutsey et al., 2008), coronary heart disease (Fung et al., 2009) and even cancer (Soffritti et al., 2006). It has been determined that the frequent use of these additives is also a risk factor for higher-grade tumors (Sturgeon et al., 1994). Another study reported that there is a positive association between the consumption of artificial sweeteners and well-differentiated thyroid carcinoma (Singh et al., 2020). In addition, artificial sweeteners are also known as environmental pollutants that occur permanently in aquatic environments (Dong et al., 2020; Yang et al., 2021). ACE-K has been identified as a new type of environmental pollutant because it is ubiquitous in the ecosystem and is extremely persistent (Cruz-Rojas et al., 2019; Dong et al., 2020). Considering the human health and food safety point, determining the potential cytotoxicity and genotoxicity of ACE-K have the utmost necessary. However, no other cytotoxicity study of ACE-K with HepG2 cells was available. Moreover, limited studies conducted on the genotoxic potential of ACE-K in human lymphocytes. Therefore, the main goal of the present study is to investigate the potential cytotoxic and genotoxic effects of ACE-K *in vitro*.

The potential cytotoxic effect of ACE-K was analyzed in HepG2 cell line using MTT assay. The cell viability was significantly and concentration-dependently decreased at 15, 60, 120, and 240 µg/mL for 24 h and at 30, 120 and 240 µg/mL for 48 h compared to negative control (24 h r= -0.68; 48 h r=-0.67, respectively) (Table 1). IC_{50} value was detected as 120 µg/mL for 24 h and >240 µg/mL for 48 h (Figure 1). The MTT assay is noted as the "gold standard" for cytotoxicity testing (van Tonder et al., 2015; Pintor et al., 2020). The results of the MTT assay demonstrated that ACE-K exhibited the cytotoxic effect in HepG2 cells, especially at 120 and 240 µg/mL at both treatments. HepG2 cells are frequently used in *in vitro* models (Choi et al. 2015). There is only one study evaluated the cytotoxic effect of ACE-K on the different cell lines. Van Eyk (2015) has investigated the cytotoxic effect of ACE-K on Caco-2, HT-29 (colon), and HEK-293 (kidney) cells at 1, 4, 10, 20, and 50 mM concentrations for 24, 48, and 72 h by using the MTT assay. The cell viability decreased at concentrations >10 mM for both concentrations- and treatment-dependently in all cell lines. The results obtained from this study are consistent with previous results. This study also evaluated the potential cytotoxic effect of ACE-K in human peripheral lymphocytes using MI and NDI which are other techniques for evaluating cellular proliferation and its kinetics (Eroglu et al. 2007). Our study revealed that ACE-K significantly decreased the MI at 60, 120, and 240 µg/mL for 24 h and at 30, 60, 120, and 240 µg/mL for 48 h versus control (Table 2). It has been determined that these decreases were in a concentration-dependent manner in both treatments (24 h r = -0.90; 48 h r= -0.88). The decrease in the rate of MI may be due to a blockage in G2, preventing the cell from entering mitosis, or a decrease in ATP level and pressure from the energy production center (Jain and Sorbhoy, 1988). Contrary to the results regarding MI in this study, NDI values did not verify other cytotoxicity results.

Genotoxicity is related to serious health effects and contains different types of DNA lesions, gene mutations, structural, and numerical chromosomal abnormalities comprising breakage and/or rearrangement of chromosomes (Dusinska et al., 2019). Considering the close relationship between genotoxicity and carcinogenesis, various assays have been developed to detect genetic damage (Souza et al., 2019). The CAs, MN, and Comet assays are commonly used measurement techniques for detecting the genotoxic, mutagenic, and carcinogenic effects (Stice et al., 2019). Clastogenicity and aneugenicity can be assessed by a CA test that identifies agents that cause chromosomal or chromatid breaks, dicentrics, and other chromosomal abnormalities (Dusinska et al., 2019).

In the present study, the effects of ACE-K on the frequency of CAs and CAs/cells were shown in Table 2. ACE-K significantly and concentration-dependently increased the CAs and CAs/cell frequency for 48 h treatment at 60, 120 and 240 µg/mL (r=0.78; r=0.79, respectively) (Table 2). However, ACE-K did not affect the frequency of CAs in 24 h treatment compared to the negative control (Table 2). Six types of structural abnormalities were observed such as chromatid breaks (31.95%), chromosome break (7.73%),

sister chromatid union (35.05%), dicentric chromosomes (5.15%), fragment (2.06%), chromatid exchange (9.27%) and one type of numerical abnormality polyploidy (8.76%) in cultured human lymphocytes. The commonly observed structural aberrations were sister chromatid unions (35.05%) and chromatid breaks (31.95%). The formation of structural chromosomal aberrations (CAs) can be caused by unrepaired or insufficiently repaired DNA double-strand breaks (Vodicka et al., 2018). Chromosomal aberrations and chromosomal instability are often associated with human cancers (Bach et al., 2019). In *in vivo* study, Mukherjee and Chakrabarti (1997) investigated the potential genotoxic and clastogenic effects of ACE-K. Swiss albino male mice were exposed to ACE-K by gavage using concentrations of 15, 30, 60, 450, 1500, and 2250 mg/kg (bw). ACE-K caused a significant raise in the frequency of chromosomal aberrations at the concentrations of 60, 450, 1500, and 2250 mg/kg (bw). Authors reported that it exhibited clastogenic and genotoxic effects. However, Mukhopadhyay et al. (2000) reported that the combination of ACE-K (1.5, 15, and 150 mg/kg) and Aspartame (3.5, 35, and 350 mg/kg) was not genotoxic in Swiss albino male mice using CAs assay.

Table 1. Cytotoxic effect of ACE-K on HepG2 cells

Test Substance			24 hour	48 hour
	Concentration (µg/mL)	N	Mean±SD	Mean±SD
Control	0.00	3	2.833±0.054	3.423±0.187
ACE-K	7.5	3	2.657±0.715	3.212± 0.091
	15	3	1.693±0.616 *	2.674± 0.234
	30	3	2.093±0.138	2.068± 0.560 *
	60	3	1.794±0.407 *	3.029± 0.000
	120	3	1.408±0.184 *	2.536± 0.454 *
	240	3	1.514±0.101 *	1.925±0.286 *

ACE-K: Acesulfame potassium, HepG2: Human hepatocellular carcinoma cell line, SD: Standard deviation
N: The number of repetitions
* Significantly different from the control P < 0.05 (One-way ANOVA-Dunnet Test).

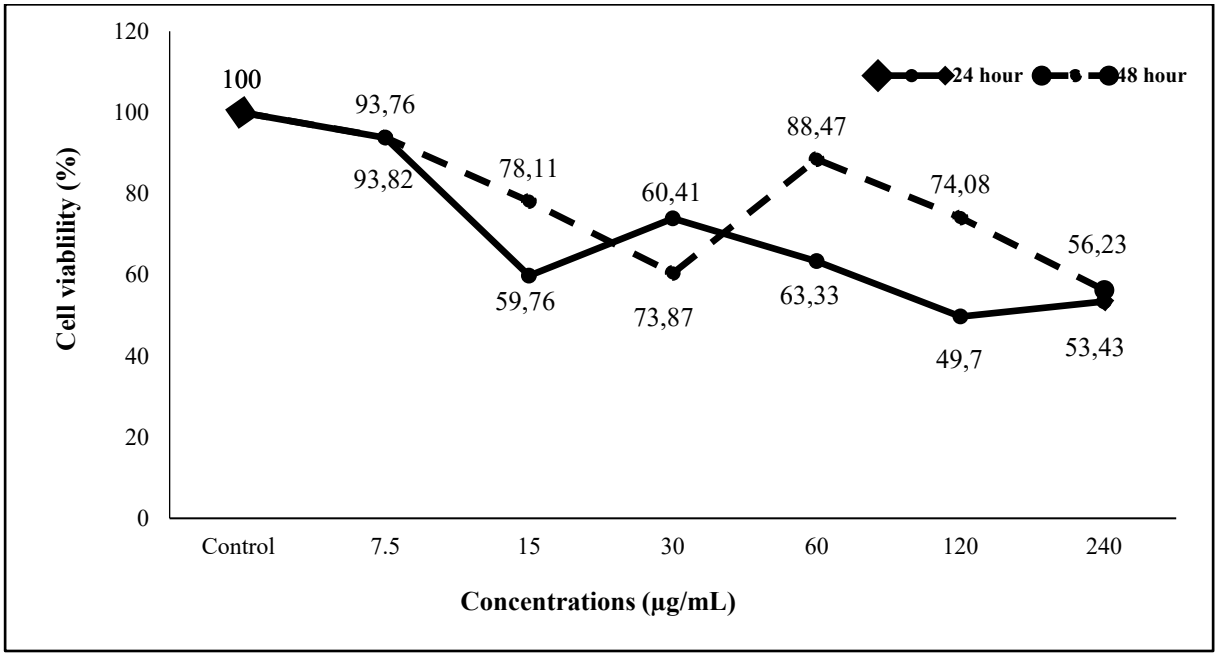

Figure 1. Cell viability of ACE-K on HepG2 cells

Table 2. The effect of ACE-K on the frequencies of chromosomal abnormalities and mitotic index in cultured human lymphocytes

Test substance	Treatment		Abnormalities							Abnormal cell ± SE (%)	CA/Cell ± SE	MI ±SE
	Time (hour)	Concent. (µg/mL)	ctb	csb	scu	cte	dic	f	p			
Control	24	0	-	1	2	2	-	-	-	1.66±0.73	0.016±0.007	7.23±0.47
PC (MMC)	24	0.20	15	2	7	2	2	-	2	9.66±1.70	0.100±0.017	4.00±0.35
ACE-K	24	7.5	2	-	3	-	-	-	2	2.33±0.87	0.023±0.008	6.80±0.45
		15	1	-	6	1	-	-	-	2.66±0.92	0.026±0.009	6.23±0.44
		30	-	-	6	1	-	-	1	2.66±0.92	0.026±0.009	6.10±0.43
		60		1	2	1	3	-	2	3.00±0.98	0.030±0.010	5.73±0.42 *
		120	1	-	5	1	1	1	2	3.66±1.08	0.036±0.010	5.73±0.42 *
		240	2	-	4	2	-	1	2	3.33±1.08	0.036±0.010	4.66±0.38 ***
Control	48	0	1	-	-	-	1	1	-	1.00±0.57	0.010±0.005	7.26±0.46
PC (MMC)	48	0.20	18	2	4	6	-	-	-	10.0±1.73	0.100±0.017	4.00±0.35
ACE-K	48	7.5	4	1	2	-	-	-	1	2.66±0.92	0.026±0.009	6.70±0.45
		15	4	-	6	-	-	-	-	3.33±1.03	0.033±0.010	6.30±0.44
		30	2	-	3	1	-	1	1	2.66±0.92	0.026±0.009	5.96±0.43 *
		60	6	2	4	-	1	-	1	4.66±1.21*	0.046±0.011*	5.73±0.42 *
		120	3	4	6	-	2	-	1	5.33±1.29**	0.053±0.012**	5.83±0.42 *
		240	3	2	8	1	-	-	2	5.33±1.29**	0.053±0.012**	4.70±0.38 ***
The percentage of chromosomal abnormalities (%)			31.95	7.73	35.05	9.27	5.15	2.06	8.76			

PC (MMC): Positive control- Mytomycin-C, ACE-K: Acesulfame potassium , SE: Standard error, MI: Mitotic index,
ctb: chromatid break, csb: chromosome break, scu: sister chromatid union, cte: chromatid exchange, dic: dicentric chromosome, f: fragment, p: polyploidy
*Significantly different from the control $p < 0.05$ (z test)
**Significantly different from the control $p < 0.01$ (z test)
***Significantly different from the control $p < 0.001$ (z test)

Micronuclei are one of the most studied biomarkers of DNA damage and chromosomal instability in humans. The most commonly used cells are lymphocytes due to a better understanding and easy attainment of MN formation (Fenech et al. 2020). It has been documented that MN formed in these cells is a reliable biomarker for the prediction of cancer risk in human (Setayesh et al., 2020). In this study, ACE-K did not caused significant difference on the frequency of MN at all concentrations. ACE-K slightly increased the MN frequency in all treatments versus negative control. However, it was not significant. Therefore, it did not exhibit clastogenic and aneugenic effects in human lymphocytes *in vitro*. The nuclear division index (NDI) was not induced at all concentrations of ACE-K (Table 3).

The Comet assay is a simple method commonly used to determine DNA breaks *in vitro* as well as *in vivo* (Dusinska et al., 2019). In comet assay, ACE-K significantly and concentration-dependently increased the comet tail intensity at 7.5 µg/mL and 15 µg/mL versus control ($r = 0.43$) in isolated human lymphocytes. Besides, a statistically significant increase in comet tail length and tail moment were observed only at 15 µg/mL concentration. However, this induction was not concentration-dependent manner (Table 4).

These current results were consistent with previous reports. Fındıklı and Türkoglu (2014) showed that ACE-K caused DNA damage by increasing tail DNA and tail moment parameters at 2.5 and 5 ppm concentrations in human lymphocytes treated for three hours by the comet assay. In another study, van Eyk (2015) investigated the genotoxic effect of ACE-K on human colon carcinoma (Caco-2 and HT-29) cells and human embryonic kidney HEK-293 cells by the treatment with 1, 4, 10, 20, and 50 mM concentrations for 24, 48 and 72 h using the comet assay. The author indicated that the cells treated with ACE-K had no or little DNA fragmentation at all treatment times and all the cells tested. In *in vivo* study, Bandyopadhyay et al. (2008) assessed the genotoxic effect of ACE-K (150, 300, and 600 mg/kg body weight) in mouse bone marrow cells using comet assay and the mutagenic effect using Ames assay. They found that ACE-K increased the tail DNA and tail extent values in mice bone marrow cells at all treatments. However, it did not cause any mutagenic effect using the Ames assay. Additionally, Jeffrey and William (2000) determined that ACE-K caused DNA damage in hepatocytes of F-344 and Sprague-Dawley rats using a hepatocyte/DNA repair assay. However, ACE-K did not affect DNA damage in rat hepatocytes. Silva et al.

(2008) found that a significant increase was observed in DNA damage of the alone ACE-K (5% concentration) and the combination of ACE-K+Aspartame at 0.5% and 5% concentrations in human peripheral lymphocytes using the comet assay. It was concluded that alone ACE-K did not exert genotoxic activity at low concentrations. Najam et al. (2017) have evaluated the *in vitro* genotoxic effect of alone ACE-K (100, 200, 400, 800, and 1600 µg/mL in concentrations) and combination with Sitagliptin (100+190, 200+380, 400+760, 800+1520, and 1600+3040 µg/mL in concentrations) on lymphocytes by Comet assay and mutagenic effect by Ames assay. ACE-K induced a significant and concentration-dependent DNA damage in lymphocytes compared to the negative control. It also showed the mutagenic effect at the concentrations of 800 µg/plate and 1600 µg /plate. The combination of ACE-K + Sitagliptin showed a mutagenic potential at the combined concentrations of 760+400 µg/plate and 1520+800 µg/plate.

Table 3. Frequencies of the micronucleus and nuclear division index in cultured human lymphocytes treated with ACE-K

Test sub-stance	Treatment		The number of counted BN cell	Distribution of BN cells according to the No. of MN			MN ± SE (%)	NDI±SE
	Time (hour)	Concentration (µg/mL)		(1)	(2)	(3)		
Control	48	0	3000	8	1	-	0.33±0.10	1.54±0.31
PC (MMC)	48	0.20	3000	40	2	-	1.40±0.21	1.40±0.30
ACE-K	48	7.5	3000	8	-	1	0.36±0.11	1.70±0.33
		15	3000	9	1	-	0.36±0.11	1.55±0.31
		30	3000	12	1	-	0.46±0.12	1.57±0.32
		60	3000	17	-	-	0.56±0.13	1.51±0.31
		120	3000	14	1	-	0.53±0.13	1.53±0.31
		240	3000	15	1	-	0.56±0.13	1.40±0.30

PC (MMC): Positive Control-Mytomycin C, ACE-K: Acesulfame Potassium, SE: Standard error, BN: Binucleat, MN: Micronucleus, NDI: Nuclear division index.

Table 4. DNA damage caused by ACE-K in isolated human lymphocytes

Test sub-stance	Treatment		Tail intensity (%)	Tail Length (µm)	Tail Moment
	Time (hour)	Concent. (µg/mL)			
Control	1	0.00	3.92±0.29	37.80±0.52	0.77±0.06
PC (H_2O_2)	1	100 (µM)	17.16±1.16	64.82±1.10	4.55±0.37
ACE-K	1	7.5	4.88±0.34 *	38.40±0.46	0.92±0.07
		15	5.17±0.46 *	39.44±0.47 *	1.01±0.09 *
		30	3.93±0.31	37.67±0.40	0.76±0.06
		60	4.52±0.36	38.38±0.42	0.88±0.07
		120	4.23±0.32	37.11±0.45	0.80±0.06
		240	3.90±0.31	38.19±0.58	0.78±0.08

PC (H_2O_2): Positive Control (Hydrogen peroxide)

* Significantly different from the control $p < 0.05$ (t- test)

Conclusion

Considering all the results, ACE-K showed a cytotoxic effect in HepG2 cells as well as human lymphocytes, especially at higher concentrations. This sweetener exhibited a mild genotoxic effect by increasing the frequency of CAs at 60-240 µg/mL in long-term treatment and DNA damaging effect especially at 15 µg/mL using the comet assay. Since the health effects of artificial sweeteners are not yet fully known, foods containing these substances should not be consumed excessively. In this regard, more *in vivo* studies would be needed.

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 confirmed by the Ethical Committee of the Faculty of Medicine, Gazi University (11.11.2019/144).

Funding disclosure: This study was financially supported (except for comet assay) by Gazi University Scientific Research Projects Coordination Unit under grant number No. 64/2020-01.

Acknowledgments: -

Disclosure: -

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 8(4), 284-289 (2022) • https://doi.org/10.3153/FH22026 *Research Article*

The effect of ultrasound times and amplitudes on the solubility and turbidity of whey protein concentrate

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Cite this article as:
Bulut, M. (2022). The effects of ultrasound times and amplitudes on the solubility and turbidity of whey protein concentrate. *Food and Health,* 8(4), 284-289. https://doi.org/10.3153/FH22026

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Submitted: 14.11.2021
Revision requested: 31.03.2022
Last revision received: 04.05.2022
Accepted: 05.05.2022
Published online: 19.08.2022

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

ABSTRACT

The current work was conducted to explore the influence of ultrasound times and amplitudes on the solubility and turbidity of whey protein concentrate (WPC). Ultrasound (US) application was employed using VC-750 ultrasonic power equipment with the frequency of 20 kHz at various times (10, 20, and 30 minutes at 50% amplitude) and amplitudes (60%, 80%, and 100% for 5 min). The outcomes exhibited that the US process have a significant impact on both solubility and turbidity ($p<0.05$). The highest protein recovery was obtained for the samples exposed to 30 min the US at 100% amplitude (65.56%). WPC samples treated at 100% amplitude showed higher solubility compared to the other samples at 60% and 80% amplitudes. While the solubility of WPC samples treated with 10 min showed the lowest solubility (9.13%), samples treated with 30 min showed the highest solubility (38.14%). There is a negative relationship between solubility and turbidity. All US-treated samples showed less turbidity and higher solubility where the control WPC samples showed the most turbid structure (0.88 NTU) with the lowest solubility (4.15%). Overall, US treatment with 30-minutes at 100 % amplitude showed the highest solubility (65.56%) and least turbidity (0.26 NTU) compared to the other sonication times and amplitudes.

Keywords: Ultrasound, Amplitude, Protein solubility, Turbidity, Whey protein concentrate

Introduction

Whey protein is a crucial material of functional protein components for several conventional and novel food materials (Kumar et al., 2018). Whey proteins are recognized as complete proteins since they include all 9 essential amino acids. The lactose content is low in whey products. When the liquid whey is obtained as a by-product of cheese or yoghurt fabrication, it is subjected to different processes in order to make the protein content higher (Liu et al., 2014). After enough protein concentration is obtained, the liquid could be dried to develop whey protein concentrate (WPC) including nearly 80% protein. The major proteins found in whey can be listed as b-lactoglobulin, a-lactalbumin, and bovine serum albümin (BSA), and these proteins are composed of almost seventy percent of all whey proteins (Arzeni, 2012). These proteins are in charge of the functional features of WPC, such as solubility in water, and propose various nutritional benefits to functionalized products (Krešić et al., 2008).

Various methodologies have been promoted to alter the native protein structure for the purpose of improvement of functionality. Modified whey proteins exhibit a very high level of functional capacity. Through molecular and physical alterations, it is achievable to reorganize protein compounds so that they develop into more practical and useful forms. Ultrasound (US) technology is a cost-effective and fast application that has been employed to alter both the structure and functional properties of protein molecules (Masonn et al., 1996; Jambrak et al., 2008; Yıldız et al., 2018). The impact of US treatment is accomplished by the chemical, molecular, and physical consequences of acoustic cavitation. Cavitation is mostly defined as the creation, development, and powerful breakdown of tiny droplets in solution. The cavitation could be the reason for protein structure modification thanks to hydrogen bonds and hydrophobic cooperation, and the falling part of the protein molecules (Yildiz et al., 2017). By taking into account the benefits of the US application such as being a cost-effective, non-toxic, fast, and efficient process, it is anticipated to reach a goal of advanced WPC functionality by using the US application. For this reason, the purpose of the present work is to analyze the impact of US application on the protein recovery and turbidity features of whey protein.

Materials and Methods

Whey Protein Concentrate (WPC)

Whey protein concentrates (WPCs) were supplied from Bulk-Supplements (Henderson, NV, USA). The WPC consists of 80% protein on a dry base. All chemicals were bought from Sigma-Aldrich (St. Louis, MO, USA), and Fisher Scientific (Pittsburgh, PA, USA).

WPC Samples and Ultrasound Application

US application was progressed using a VC-7500 US power equipment along with the frequency of 20 kHz (Sonic & Materials, Inc., USA) at three different times (10, 20, and 30 minutes at 50% amplitude) and amplitudes (60%, 80, and 100% for 5 min). Insoluble WPC (3 g) was blended with a 100 mL distilled H_2O and stirred for about 60 min at room temperature (RT) conditions with the help of a magnetic stirrer. The beaker stayed in a cup filled with ice cubes at the time of US treatment for the prevention of temperature rise. The protein solution following the US application were centrifuged (1200 g and 20 ºC) for 15 min. Soluble WPC was collected right after centrifugation. For the control WPC samples, no US treatment was conducted; 3 g WPC in 100 mL water was agitated at 25 ºC for 30 min. While table 1 exhibits the description of the WPC samples and treatments, table 2 shows the processing steps applied for each treatment.

Table 1. The description of the WPC samples and treatments

Sample names	Treatments
Control	Untreated WPC, no ultrasound
US1	Ultrasound treatment with 10 min (50% amp.)
US2	Ultrasound treatment with 20 min (50% amp.)
US3	Ultrasound treatment with 30 min (50% amp.)
US6	Ultrasound treatment at 60% amp. (5 min)
US8	Ultrasound treatment at 80% amp. (5 min)
US10	US treatment at 100% amplitude (5 min)
US16	US treatment with 10 min at 60% amp.
US18	US treatment with 10 min at 80% amp.
US110	US treatment with 10 min at 100% amp.
US26	US treatment with 20 min at 60% amp.
US28	US treatment with 20 min at 80% amp.
US210	US treatment with 20 min at 100% amp.
US36	US treatment with 30 min at 60% amp.
US38	US treatment with 30 min at 80% amp.
US310	US treatment with 30 min at 100% amp.

Table 2. The processing steps applied for each treatment

Treatments	Stirring	US (10 min)	US (20 min)	US (30 min)	US (60% amp.)	US (80% amp.)	US (100% amp.)	Centrifuge
Control	A	NA	NA	NA	NA	NA	NA	A
US1	A	A	NA	NA	NA	NA	NA	A
US2	A	NA	A	NA	NA	NA	NA	A
US3	A	NA	NA	A	NA	NA	NA	A
US6	A	NA	NA	NA	A	NA	NA	A
US8	A	NA	NA	NA	NA	A	NA	A
US10	A	NA	NA	NA	NA	NA	A	A
US16	A	A	NA	NA	A	NA	NA	A
US18	A	A	NA	NA	NA	A	NA	A
US110	A	A	NA	NA	NA	NA	A	A
US26	A	NA	A	NA	A	NA	NA	A
US28	A	NA	A	NA	NA	A	NA	A
US210	A	NA	A	NA	NA	NA	A	A
US36	A	NA	NA	A	A	NA	NA	A
US38	A	NA	NA	A	NA	A	NA	A
US310	A	NA	NA	A	NA	NA	A	A

(A: displays the stages applied; and NA: displays the stages that were not applied)

Protein Solubility

Solubility of the WPC samples was determined by a Bio-Rad Protein Assay based on the technique described by Bradford (1976). Bovine serum albumin (BSA) was utilized as the standard assay. Dye reagent was arranged by diluting 1 part of dye reagent concentrate into 4 parts of DI water, and filtered through a filter paper. The prepared solution was blended with soluble WPC. The protein concentration of soluble WPC was measured by spectrophotometer at the wavelength of 595 nm. Protein solubility was calculated as below and represented as "%":

$$Recovery\ of\ soluble\ protein = \frac{Protein\ concentration\ in\ soluble\ WPI}{Initial\ protein\ concentration} \times 100 \quad (1)$$

Turbidity (NTU)

The turbidity of the WPC dispersions was figured out by a spectrophotometer according to the methodology proposed by Yildiz et al. (2017). DI water was used as the blank, and the absorbance at 600 nm was read.

Statistical Analysis

The differences were achieved with the General Linear Model process in SAS (version 9.3, SAS Insttute, Inc., Cary, North Carolin, USA). Significant differences between the mean values were identified by Fisher's least significant difference (LSD) test at alpha = 0.05.

Results and Discussion

Solubility

Table 3 displays the findings related to protein solubility values of the WPC samples exposed to different US treatments. All US-treated WPC samples displayed significantly higher solubility in comparison with the control whey protein concentrates. Moreover, the highest protein solubility was obtained for the WPC samples exposed to 30 min the US at 100% amplitude (65.56%). WPC samples treated at 100% amplitude showed higher solubility compared to the other samples at 60% and 80% amplitudes. A positive relationship between the solubility and ultrasound amplitude was determined. The higher the amplitude, the higher the solubility. While the amplitude was the lowest (60%), the solubility was the lowest (8.65%). On the other hand, while the amplitude was highest (100%), the solubility was highest (21.38%). It was clearly seen that increasing US advances the solubility of whey protein concentrates. WPC samples treated for 30 min showed the highest solubility (38.14%) compared to the WPC samples treated with 10 and 20 min (Table 3). Similar to the amplitude, ultrasound times have also positive relationships with solubility. Increasing ultrasound time from 10 to 30 min leads to higher solubility. While the solubility of WPC samples treated with 10 min showed the lowest solubility (9.13%), samples treated with 30 min showed the highest solubility (38.14%). Solubility is a main functional property for

whey protein (Hussain et al., 2012; Feng et al., 2022). Solubility is related to several functional features such as molecular weight, not the primary but the secondary and tertiary structure, hydrophobic, and electrostatic charges (Lee et al., 2016; Chang et al., 2021). Processing treatments used to manufacture whey protein may result in heat-induced protein denaturation, which then reduces whey protein solubility. Native whey proteins remain soluble at around pH 7; however, heat-induced denaturation renders whey proteins less soluble than native whey proteins (Jambrak et al., 2014). Thus, the protein solubility of whey protein is helpful to estimate protein denaturation (Morr and Ha, 1993). The development of protein solubility increase following a US application has been figured out in different works (Le et al., 2016; Jiang et al., 2017; Yildiz et al., 2017). The physical forces developed by US cavitation such as shear forces could alter the protein structure which comes out with developed protein solubility. Also, sonication can be the reason for the breakage of both non-covalent and covalent bonds which lead to protein solubility increase (Hue et al., 2003). Jambak et al. (2008) examined the influence of US (20 kHz probe & 40 kHz bath), on solubility, emulsifying and foaming attributes of different whey protein types consisting of whey protein isolate, whey protein concentrate, and whey protein hydrolysate. It was figured out whey protein solubility increased significantly for all whey samples for the treatment of 20 kHz probe and 40 kHz baths.

Table 3. Protein solubility (% recovery) & turbidity of WPC samples

Treatments	Solubility (%)	Turbidity (NTU)
Control	4.15 ±0.43^{j}	0.88 ±0.7^{a}
US1	9.13 ±0.58^{i}	0.79 ±0.2^{b}
US2	16.65 ±0.35^{h}	0.74 ±0.3^{bc}
US3	38.14 ±0.22^{f}	0.66 ±0.1^{c}
US6	8.65 ±0.11^{i}	0.79 ±0.6^{b}
US8	15.14 ±0.19^{h}	0.75 ±0.5^{bc}
US10	21.38 ±0.11^{g}	0.74 ±0.7^{bc}
US16	38.06 ±0.01^{f}	0.65 ±0.4^{c}
US18	44.14 ±0.81^{e}	0.63 ±0.3^{c}
US110	53.66 ±0.74^{c}	0.44 ±0.9^{e}
US26	48.73 ±0.62^{d}	0.55 ±0.7^{d}
US28	51.82 ±0.14^{cd}	0.51 ±0.2^{de}
US210	58.85 ±0.53^{b}	0.37 ±0.5^{f}
US36	53.19 ±1.17^{c}	0.45 ±0.1^{e}
US38	59.23 ±0.89^{b}	0.38 ±0.3^{f}
US310	65.56 ±0.46^{a}	0.26 ±0.8^{g}

$^{a-j}$ Mean ± standard deviation (n=3) of properties with the same letter are not significantly different ($p<0.05$)

* All the statistics were done separately for each parameter (solubility, turbidity)

Turbidity

The turbidity findings of WPC samples are demonstrated in Table 3. Both the solubility and particle size of soluble protein aggregates determines the turbidity of a protein dispersion (Lee et al., 2016). Martin et al. (2010) investigated the optimization of the use of power ultrasound to reduce the turbidity of whey solutions. It was concluded that around a 90% decrease was observed in the turbidity of samples treated with ultrasound processing. The highest decline in turbidity values was determined for the samples treated with 30 min at 100% amplitude (US310 samples). While the highest turbidity was obtained for the untreated WPC (0.88 NTU), the lowest turbidity was observed for the US310 samples (0.26 NTU). There is a negative relationship between the variables of solubility and turbidity. All US-treated samples showed less turbidity and higher solubility where the control WPC samples exhibited the most turbid appearance and lowest solubility (Table 3). Overall, US treatment with 30-minutes at 100 % amplitude showed the highest solubility (65.56%) and least turbidity (0.26 NTU) compared to the other sonication times and amplitudes. Both the number of soluble protein components in the dispersion figured out by solubility and the sizes of the soluble protein components determine the turbidity of a whey protein dispersion (Yildiz et al., 2017). Employing the US at 20 kHz raised the clearness and transparency of whey protein suspensions mostly because of the decrease in the size of the suspended insoluble protein components (Zisu et al., 2011; Ghasemi et al., 2018).

Conclusion

Ultrasound treatment was examined for the purpose of modification and enhancement of the whey protein functionality. Compared with other US treatments, a significant improvement in the solubility and turbidity properties of WPC samples was achieved with a US310 treatment. Overall, US310 is a promising treatment to strengthen the physicochemical characteristics of WPCs as indicated within the present study by its ability to higher solubility and less turbidity right after ultrasonication. The results of the current study showed the potential of the US310 treatment as an effective method for protein modification. The functionalized WPC produced by the US310 treatment can be used in a liquid food with high solubility and less precipitation.

Compliance with Ethical Standard

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

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

Funding disclosure: -

Acknowledgments: -

Disclosure: -

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 8(4), 290-301 (2022) • https://doi.org/10.3153/FH22027 Research Article

Horoz Karası üzüm çeşidinde bazı kalite parametrelerinin belirlenmesi

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Cite this article as:
Balbaba, N., Bağcı, S. (2022). Horoz karası üzüm çeşidinde bazı kalite parametrelerinin belirlenmesi. *Food and Health,* 8(4), 290-301. https://doi.org/10.3153/FH22027

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Submitted: 13.05.2022
Revision requested: 23.06.2022
Last revision received: 21.07.2022
Accepted: 28.07.2022
Published online: 22.08.2022

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

ÖZ

Bu çalışma ile, Horoz Karası üzüm çeşidinin bazı kalite unsurları incelenmiştir. Bu amaçla Kahramanmaraş ilinde bulunan üretici bağlarından örnek alınan Horoz Karası üzüm çeşidi salkımlarında salkım, tane, suda çözünebilir kuru madde (SÇKM), titrasyon asitliği, pH düzeyi, tane rengi, toplam fenol ve antioksidan aktivite düzeyi belirlenmiştir. Horoz Karası üzüm çeşidinde SÇKM düzeyi %16.6-25.35, titre edilebilir asitlik % 0.189-0.517, pH düzeyi 3.62 ile 3.94 değerleri arasında kaydedilmiştir. Horoz Karası üzüm örneklerinde parlaklığı ifade eden L*, 25.64 ile 29.97, a* değeri 0.32-1.33, b* değeri -0.91 ile -0.23 değerleri arasında belirlenmiştir. Chroma değeri 0.43-1.85, CIRG (Colour Index of Red Grapes) indeksi 7.02 ile 8.25 değerleri arasında kaydedilmiştir. Hue açısı CIE (Commission Internationale de L'éclairage) renk koordinatında -40.88- (-33.31) değerleri arasında kaydedilmiştir. Hue açısı değerlerine göre Horoz Karası çeşidi tane kabuk rengi mor olarak kaydedilmiştir. Toplam fenol değerleri 360.5 mg GAE 100 g^{-1} ile 484.7 mg GAE 100 g^{-1}, antioksidan aktivite düzeyi % 87-98 arasında değişmektedir. Horoz Karası üzüm çeşidinde toplam fenol düzeyi (484.7 mg GAE) ve antioksidan aktivite bakımından en yüksek olan örnek (% 98) Çobanlı 2 olarak kaydedilmiştir.

Anahtar Kelimeler: Horoz Karası, Antioksidan aktivite, Kalite özellikleri, Tane rengi

ABSTRACT

Determination of some quality parameters in Horoz Karası grape variety

In this study, some quality elements of the Horoz Karası grape variety were investigated. For this purpose, cluster, berry, total soluble solids (TSS), titration acidity, pH level, berry color, total phenol, and antioxidant activity were determined in sample clusters of the Horoz Karası grape variety taken from the producer vineyards in Kahramanmaraş. In the Horoz Karası grape cultivar, total soluble solids (TSS) level was recorded between 16.6-25.35%, titratable acidity between 0.189-0.517, pH level 3.62 and 3.94. In Horoz Karası grape samples, L*, which expresses brightness, was determined between 25.64 and 29.97, a* value between 0.32-1.33, b* value between -0.91 and -0.23. Chroma value was recorded between 0.43-1.85 and CIRG (Color Index of Red Grapes) values were between 7.02 and 8.25. Hue angle was recorded between -40.88 - (-33.31) values in CIE (Commission Internationale de L'éclairage) color coordinate. According to the Hue angle values, the color of the berry of the Horoz Karası variety was recorded as purple.

Keywords: Horoz Karası, Antioxidant activity, Quality characteristics, Berry color

Giriş

Kültür asmasının (*Vitis vinifera* L.) anavatanı olan Anadolu'da bağcılığın tarihi M.Ö. 3500 yıllarına kadar dayanmaktadır. Ülkemizin gerek Dünya üzerindeki coğrafi konumu ve gerekse ekolojik faktörlerin elverişli olması nedeniyle bağcılık, yurdumuzda en uygun koşullara sahip olan tarımsal uğraşılardan birini oluşturmaktadır. Ülkemizin pek çok bölgesine yayılan bağcılık içinde zamanla farklı çeşit zenginliği oluşmuştur. Ayrıca, iklime göre yetiştirme teknikleri gelişerek tüketim ve değerlendirme artış göstermiştir (Çelik, 1998).

Dünya üzüm üretimi 6.950.930 hektar alanda 78.034.332 ton olarak gerçekleşmektedir (FAO, 2022).

Son yıllara ait veriler incelendiğinde Türkiye'de 3.902.211 dekar alanda 1.856.929 ton sofralık üzüm, 1.430.160 ton kurutmalık üzüm, 382.911 ton şaraplık üzüm olmak kaydıyla toplam 3.670.000 ton üzüm üretilmiştir. (TÜİK, 2022).

Kahramanmaraş ilinde 125.127 dekar alanda yapılan toplam üzüm üretiminin ülke genelindeki payı % 1.63 şeklindedir. Sofralık üzüm üretiminde % 2.35 oranındaki payı ile il düzeyinde 11. sırada yer alırken; kurutmalık üzüm üretim miktarı bakımından % 1.15 ile 10. sırada yer almaktadır (TÜİK, 2022).

Sultana ve ark. (2012), meyve ve sebze tüketiminin hastalıklara karşı vücut dayanımını artırdığını belirtmektedirler. Böylece polifenolik maddeler ile vitaminleri içeren antioksidan besin elementleri sağlık açısından son derece önemli hale gelmektedir. Tosun ve Yüksel (2003), daha önce yapılan çalışmalarda kırmızı erik, üzüm, çilek, böğürtlen, ahududu, bektaşi üzümü ve yaban mersini meyvelerinde antioksidan aktivite düzeyinin oldukça yüksek olduğunu ifade etmişlerdir.

Gündüz ve Özdemir (2014), pek çok çalışmaya göre, üzümsü meyveler veya nar gibi kırmızı renkli meyve suyunun kalp hastalıkları, yaşlanma ve kanserin etkilerini azalttığını ifade etmişlerdir. Meyve türleri arasında üzümsü meyvelerin antioksidan etkisi oldukça önemlidir.

Üzümler fenolikler, flavonoidler, antosiyaninler ve resveratrol gibi sağlığa yararlı pek çok fitokimyasal içerir. Üzümlerde serbest radikallerin etkisini azaltan antioksidan aktivite yüksek düzeydedir (Du ve ark., 2012; Yang ve Xiao, 2013). Fenolik bileşikler (sekonder metabolitler) sağlık için son derece faydalı olmalarından dolayı son zamanlarda büyük ilgi görmektedir. Fenolik bileşikler antibakteriyel, antiviral, antikanserojenik, antiinflamatuar etkiye sahiptir (Topalovic ve ark., 2012).

Üzümlerde bulunan fenolik bileşiklerin antioksidan etkisi (antioksidan kapasite) bu bileşiklerin konsantrasyonu ile bağlantılıdır. Kırmızı üzümlerin antioksidan aktivitesi hem fenol bileşiklerinin hem de flavonoid kapsamıyla doğru orantılıdır (Yang ve Xiao, 2013).

Antioksidan aktivite kanser, kardiyovasküler hastalıklar, diyabet gibi hastalıkları önleyebilen ve vücuttaki zararlı etkileri önemli derecede azaltabilen temel unsurlardandır (Farhadi, 2016; Genova, 2012). Günümüzde bitkisel kaynaklardan elde edilebilen doğal antioksidanların kullanımı ve etkinliği önemlidir (Farhadi, 2016).

Üzümlerde yüksek düzeyde bulunan polifenoller tane kabuğu, meyve eti ve çekirdek kısmında bulunur (Genova, 2012). Kabuk, çekirdek ve üzüm suyu gibi ekstraktlardan elde edilen fitokimyasallar; karetenoid, melatonin ve fenolik bileşikler (stilbenler, fenolik asitler ve flavonoidler) olarak tanımlanır. Bu fitokimyasallar sadece antioksidan değil aynı zamanda antikanser, antiinflamatuar, LDL- kolesterol oksidasyon, antiplatelet ve antimikrobiyal etkiye sahiptir (Yang ve Xiao, 2013). Üzümlerin kimyasal bileşimi olgunluk, genotip ve büyüme koşulları gibi bazı faktörlerden etkilenir (Munoz-Robredo, 2011; Cagnasso ve ark., 2011).

Üzüm ve şaraplarda bulunan fenolik bileşikler farklı sınıflarda ve konsantrasyonlarda bulunan önemli kalite özellikleridir. Üzüm ve şarapta renk ve tat oluşumunda önemli bir rol üstlenirler. Renk pigmenti olan antosiyaninler üzümde kırmızı ve mor rengin oluşumundan sorumludur (Dharmadhikari, 1994).

Pek çok araştırıcının bildirdiği gibi; üzüm tane kabuklarında antosiyanin birikimini çevre koşulları ve tarımsal uygulamalar etkilemektedir (Esteban ve ark., 2001; Ojeda ve ark., 2002; De La Hera Orts ve ark., 2005; Ortega-Regules ve ark., 2006).

Bu çalışma ile, Kahramanmaraş ilinin farklı havzalarında yetiştirilen Horoz Karası üzüm çeşidinin pomolojik özellikleri (salkım ağırlığı, salkım boyu, salkım eni, tane boyu, tane eni, tane ağırlığı, tanede bulunan çekirdek sayısı, SÇKM (suda çözünebilir kuru madde miktarı), pH, titrasyon asitliği), tane kabuk rengi, toplam fenol ile antioksidan aktivite düzeyi belirlenmiştir.

Materyal ve Metot

Materyal

Horoz Karası: İri ve kanatlı salkım yapısı olan, tane kabuk rengi mavi siyah, uzun-eliptik iri taneli, çekirdekli yapıda ve orta mevsimde olgunlaşan bir üzüm çeşididir (Çelik, 2006).

Bu araştırma 2018 yılında Kahramanmaraş ilindeki Bertiz Havzası, Pazarcık (Yumaklıcerit-Taşdemir-Kizirli-Akçalar) Havzası ile Şahinkayası-Kürtül Havzasında yürütülmüştür. Bölgede 2018 yılında hasat zamanında ortalama sıcaklık 35 °C civarında olup yağış gerçekleşmemiştir. Çalışmanın yürütüldüğü her üç havzada benzer ekolojik özelliklere sahiptir. Araziler engebeli ve küçük parçalı olup, üzüm bağları güneye meyilli arazilerde tesis edilmiş olup, genellikle % 10-15 meyile sahiptir. Üretim alanlarını temsil edecek şekilde Havzalarda bulunan üretici bağlarındaki Horoz Karası üzüm çeşidi omcalarında 20 Ağustos 2018 ile 30 Ağustos 2018 tarihleri arasında derim yapılmıştır. Hasat zamanı SÇKM (suda çözünebilir kuru madde) düzeyine göre belirlenmiştir. 15-20 yaşındaki bağlarda bulunan omcalar yerde serbest uzanan ve yöresel olarak serpene olarak tabir edilen terbiye şekillerinden oluşmaktadır. Dikim sıklığı 3*3 m olan bağlar, yerli fidanla kurulmuştur.

Üretim alanlarını temsil edecek şekilde farklı köylere ait üzüm bağlarında bulunan omcalardan Horoz Karası üzüm örnekleri alınmıştır. Bağlardan alınan örnekler, örnek kodu 1'den başlamak üzere sıralandırılmıştır. Örnek kodu 1-2 numaralı olanlar Çobanlı köyü, 3-6 numaralı olan Kizirli Köyü, 7-10 numaralı olanlar Taşdemir köyü, 11-14 numaralı olanlar Şahinkayası ve 15-18 numaralı olanlar Yumaklıcerit Köyünden elde edilmiştir. 3 tekerrürlü ve her tekerrürde 10'ar adet salkım örneği alınmıştır. Analizler için, asmalardan seçilmeden alınan üzüm örnekleri polietilen torbalara konarak analiz zamanına kadar -80 ^{0}C sıcaklıkta muhafaza edilmiştir.

Metot

Örnek olarak alınan üzümlerde incelenen kalite özellikleri:

Salkım ağırlığı (g), Salkım boyu (cm), Salkım eni (cm), Tane boyu (mm) ve Tane eni (mm), Tane ağırlığı (g), Tanede bulunan çekirdek sayısı (n), SÇKM miktarı (%), Titrasyon asitliği (%), pH, Tane kabuk rengi, Toplam fenol (mg GAE 100 g^{-1}) ve Antioksidan aktivite (%) düzeyi.

Salkım, tane ve şıra değerleri için üretici bağlarında bulunan farklı omcalardan salkım örnekleri alınmıştır. Bu örnekler omcalardan tesadüfen, 3 tekerrürlü ve her tekerrürde 10 adet salkım bulunacak şekilde elde edilmiştir. Örnek salkımlarda, salkım ağırlığı (g), boyu (cm) ile salkım eni (cm) ölçülmüştür. Salkımların orta kısmından alınan 20 adet tanede; dijital kumpas ile tane eni (mm) ve boyu (mm) belirlenmiştir. Tane ağırlığı (g) değerleri hassas terazi ile kaydedilmiştir. Tanede bulunan çekirdek sayısı (n), tane örneklerinde sayılarak kaydedilmiştir.

Tanelerden elde edilen şırada; SÇKM (suda çözünür kuru madde) dijital bir refraktometre ile, titre edilebilir asitlik (TA) şıra örneklerinin 0.1 N'lik NaOH ile titre edilerek dijital büret ile % olarak, pH değeri bir pH metre ile ölçülmüştür.

Tane Kabuk Rengi Analizi

Commission Internationale de L'éclairage (CIE)'ye göre renk koordinatları (L*, a*, b*) bir renk ölçer cihazıyla belirlenmektedir. (Carreno ve ark., 1995). Buna göre; CIE (International Commission on Illumination) Lab renk sistemine göre L*, a* ve b*, C (Chroma) ve h^0 (hue açısı) değerleri esas alınarak renk ölçer cihaz yardımıyla üzüm örneklerinde tane kabuk rengi belirlenmiştir. L* değeri 0 ile 100 arasında bir değer olarak siyah renkten beyaza parlaklığı, +a* değeri kırmızı ve mor rengi -a* değeri yeşil rengi, +b* değeri sarı rengi ve -b* değeri mavi renk aralığını belirler. Chroma renk yoğunluğunu, Hue renk tonu açısını belirtmektedir. Buna göre 0^o kırmızı-mor rengi, 90^o sarı, 180^o mavi- yeşil, 270^o mavi rengi ifade etmektedir (McGuire,1992).

Eşitlik 1: h^o= arctan(b*/a*) (McGuire, 1992).

Eşitlik 2: Chroma = $(a^{*2}+b^{*2})$ 1/2 (McGuire,1992; Belafi-Bako ve Nemestothy, 2017).

Kırmızı üzümlerde renk indeksi olan CIRG (Colour Index of Red Grapes) aşağıda yer alan eşitlikte olduğu gibi hesaplanır (Carreno ve ark., 1996).

Eşitlik 3: CIRG= 180-hue^o/ (L*+C*)

Toplam Fenol Bileşiklerinin Analizi

Folin Ciocalteu kolorimetrik metodu kullanılarak Singleton ve Rossi (1965)'ye göre yapılmıştır. Folin Ciocalteu reagent, Merck; Gallic asit ise Sigma-Aldrich firmasından elde edilmiştir. Tanelerdeki toplam fenolik bileşik miktarı spektrofotometrede gallik asit cinsinden mg 100 g^{-1} olarak hesaplanmıştır.

Antioksidan Aktivite Analizi

Her örneğin serbest radikalleri indirgeme kapasitesi aşağıda belirtilen formül aracılığıyla antioksidan aktivite olarak belirlenmiştir.

DPPH inhibisyonu (%)= [(Ac – As)/ Ac *100].

Ac: Kontrol absorbansı, As: Örneklerin absorbansı

Üzüm ektraktlarında antioksidan aktivitenin belirlenmesi DPPH (2,2-diphenyl-1-picrylhydrazyl) serbest radikali kullanılarak yapılmıştır (Özden ve Vardin, 2009; Kelebek ve ark., 2009). DPPH Sigma-Aldrich firmasından elde edilmiştir.

İstatistiki Analiz

Deneme 3 tekerrürlü olarak Tesadüf Parselleri Deneme düzenine göre düzenlenmiş verilere JMP 8.0 istatistik programı uygulanarak, standart sapma değerleri kaydedilmiştir. Tablolarda ortalama ve standart sapma değerleri verilmiştir.

Bulgular ve Tartışma

Horoz Karası üzüm çeşidinin salkım özellikleri ile ilgili olarak elde edilen değerler Tablo 1'de görülmektedir. Horoz Karası üzüm çeşidinde salkım ağırlığı 123.28 g ile 509.43 g, salkım uzunluğu 7.77 cm ile 21.37 cm arasında, salkım genişliği 8.13 cm ile 15.94 cm, salkım büyüklüğü değerleri ise 71.35 cm^2 ve 272.48 cm^2 arasında değişmektedir (Tablo 1).

Kök ve Bal (2017), Horoz Karası üzüm çeşidinde salkım ağırlığını 846.65 g, salkım uzunluğunu 17.85 cm, salkım genişliğini 16.20 cm olarak bildirmişlerdir. Kılıç ve ark., (2018), Horoz Karası üzüm çeşidinde salkım ağırlığını denemenin ilk yılı 586.9 g, 2. yıl 645.2 g olarak belirlemişlerdir. Aslan ve ark., (2018), Horoz Karası üzüm çeşidi salkım ağırlığının 402.7 g ile 565 g arasında değiştiğini kaydetmişlerdir.

Bu çalışmada; Horoz Karası üzüm çeşidi salkım ağırlığının anılan literatürlere göre daha düşük olduğu gözlenmiştir. Bu farklılığın, terbiye şekli, gübreleme veya sulama nedeniyle olduğu düşünülmektedir.

Tablo 2'de görüldüğü üzere Horoz Karası çeşidinde tane ağırlığı 3.85 g ile 7.98 g, tane uzunluğu 18.84 mm ile 32.28 mm, tane genişliği değerleri ise 15.12 mm ile 20.00 mm arasında kaydedilmiştir.

Kök ve Bal (2017), Horoz Karası üzüm çeşidinde tane ağırlığını 9.29 g, tane uzunluğunu 32.34 mm, tane genişliğini 21.35 mm olarak ifade etmişlerdir. Kılıç ve ark., (2018), Horoz Karası üzüm çeşidinde tane ağırlığını denemenin ilk yılı 6.1 g, 2. yıl 8.7 g olarak bildirmişlerdir. Aslan ve ark., (2018)'nın çalışmasına göre, Horoz Karası üzüm çeşidinin tane ağırlığı 4.65 g ile 5.18 g arasında değişmektedir.

Araştırmada; Horoz Karası üzüm çeşidi tane ağırlığı değerleri ortalama 5.92 g elde edilmiş olup Aslan ve ark. (2018)'nın bulguları ile benzerlik göstermektedir.

Tablo 1. Horoz Karası üzüm çeşidinin salkım özellikleri

Table 1. Cluster characteristics of Horoz Karası grape variety

Örnek Kodu	Salkım ağırlığı (g)	Salkım uzunluğu (cm)	Salkım genişliği (cm)	Salkım büyüklüğü (cm^2)
1	386.63	18.14	14.18	262.10
2	399.39	17.16	15.94	272.48
3	429.02	19.40	13.29	257.24
4	509.43	15.65	13.35	211.13
5	393.21	15.31	12.78	195.32
6	419.72	17.15	12.41	213.22
7	173.28	12.61	10.43	141.81
8	138.17	7.77	9.64	74.42
9	220.81	17.73	9.63	172.29
10	181.35	13.3	9.87	131.71
11	252.55	18.23	11.37	207.31
12	328.98	17.57	13.66	239.70
13	213.96	14.83	9.87	146.21
14	251.30	21.37	10.77	230.13
15	123.28	8.77	8.13	71.35
16	355.58	15.57	14.61	227.70
17	218.61	12.47	8.33	104.23
18	232.12	12.27	10.35	134.43
Min.	123.28	7.77	8.13	71.35
Max.	509.43	21.37	15.94	272.48
Ort.	316.36	14.57	12.04	171.92
Standart sapma	58.548	1.130	0.781	22.724

Tablo 1 ve Tablo 2 incelendiğinde; Kizirli'den alınan salkım örneklerinde salkım ağırlığı ile tane ağırlığı ortalamasının diğer bölgelere göre daha üst sırada yer aldığı görülmektedir. Bunun nedeni, örnek alınan bağlarda gerçekleştirilen kültürel uygulamalardan kaynaklanmış olabilir.

Tanede olgunluğu ve tadı belirleyen kriterlerden biri Brix değeri, diğeri ise en önemlileri tartarik ve malik asit olmak üzere titrasyon asitliğidir. Pek çok parametre Brix değerinin değişimi üzerine etkilidir. İklimsel faktörlerden sıcaklık ve yağış Brix değeri için önemlidir. Üzümde bulunan kalite faktörlerinden biri de üzüm suyunda çözünmüş halde bulunan madde miktarının hesaplanmasıyla elde edilen şeker konsantrasyonudur. Brix değeri solüsyonda bulunan yaklaşık % şeker miktarını ifade eder (Creasy ve Creasy, 2009).

Horoz Karası üzüm çeşidi şıra özellikleri incelendiğinde kalite parametrelerinden biri olan SÇKM düzeyi % 16.6-25.35, titre edilebilir asitlik % 0.189-0.517, pH 3.62 ile 3.94 değerleri arasında belirlenmiştir (Tablo 3). Taze üzüm suyunda % 70-80 düzeyinde su ve içinde pek çok çözünmüş maddeler (şeker, organik asit, fenolik bileşikler, azotlu bileşikler, aroma maddeleri, mineraller ve pektik maddeler) bulunur. Meyve suyundaki şeker kapsamı ^{o}Brix olarak isimlendirilir. Briks derecesi 100 g üzüm suyunda bulunan şeker miktarıdır (Dharmadhikari, 1994). Olgunlaşma başlangıcında SÇKM oranında artış başlar ve olgunluğa kadar devam eder. Mevsim koşulları, özellikle sıcaklık bu düzeye etki eder. Yüksek sıcaklık nedeniyle tanede olgunlaşma hızlı şekilde ilerler (Winkler ve ark.,1974).

Tablo 2. Horoz Karası üzüm çeşidinde incelenen tane özellikleri

Table 2. Berry characteristics of Horoz Karası grape variety

Örnek Kodu	Tane ağırlığı (g)	Tane uzunluğu (mm)	Tane genişliği (mm)	Çekirdek sayısı (n)
1	6.45	25.10	17.79	3
2	4.42	24.13	16.12	2
3	7.98	31.28	20.01	2
4	6.96	30.45	19.92	3
5	5.23	27.02	18.83	2
6	5.56	26.25	19.29	3
7	5.70	25.89	15.35	2
8	5.02	22.55	16.91	2
9	6.51	27.93	19.38	2
10	4.67	23.03	15.29	1
11	6.84	26.05	19.01	2
12	5.96	23.65	18.70	3
13	5.56	24.52	18.41	2
14	4.68	25.79	17.73	2
15	3.85	18.84	15.12	2
16	6.75	28.05	19.34	2
17	4.31	23.06	17.77	3
18	4.97	23.32	17.41	2
Min.	3.85	18.84	15.12	1
Max.	7.98	32.28	20.00	3
Ort.	5.92	25.56	17.56	2
Standart sapma	0.253	0.790	0.525	0.192

Kök ve Bal (2017), Horoz Karası üzüm çeşidinde SÇKM miktarını % 16.62, toplam asitliği 7.10 g L^{-1}, pH düzeyini 3.43 olarak bildirmişlerdir. Kılıç ve ark., (2018), Horoz Karası üzüm çeşidinde SÇKM oranını % 17.1, toplam asitlik düzeyini 5.30 g L^{-1} ile 4.90 g L^{-1} arasında belirlemişlerdir. Çağındı (2016), Red Globe üzüm çeşidi üzüm suyunda toplam kuru madde miktarını % 14.22, titrasyon asitliğini % 0.39, pH düzeyini 3.90 olarak bildirmiştir.

Çalışmada; Horoz Karası üzüm çeşidi salkım örneklerinde SÇKM düzeyi ortalama olarak % 20.98, titrasyon asitliği % 0.360 olarak elde edilmiştir.

Üzümlerde tadı etkileyen titrasyon asitliğinin önemine rağmen üreticiler olgunlaşmayı belirleyen faktör olarak SÇKM (tatlılık) düzeyini kullanırlar. Ticari çeşitlerde SÇKM düzeyi % 15-18 olduğunda olgunluk kabul edilir (Munoz- Robredo, 2011).

Tablo 3 incelendiğinde; Taşdemir'den alınan salkım örneklerinde ortalama SÇKM düzeyi % 22.32, pH düzeyi ise 3.91 olarak diğer bölgelere daha yüksek değerde kaydedilmiştir. Bu farklılığın ekolojik koşullar sebebiyle olduğu düşünülmektedir.

0.1 normal sodyum hidroksid (NaOH) ile yapılan titrasyon asitliği veya üzümlerde baskın organik asit olan tartarik asit, 1 litre solusyonda çözünen organik asit miktarıdır (Creasy ve Creasy, 2009).

Olivares ve ark., (2017), Crimson Seedless üzüm çeşidi kontrol grubu omcalarında titrasyon asitliğini % 0.4-0.6 arasında değişmekte olduğunu bildirmiştir.

Özden ve Vardin (2009), Merlot, Cabernet Sauvignon ve Şiraz üzüm çeşitlerinde Briks kapsamı sırasıyla; 24.50, 22.70, 23.50; Toplam asitlik düzeyi 4.40 g tartarik L^{-1}, 6.73 g tartarik L^{-1}, 6.51 g tartarik L^{-1}, pH düzeyi 3.82, 3.55,3.17 olarak belirlemişlerdir.

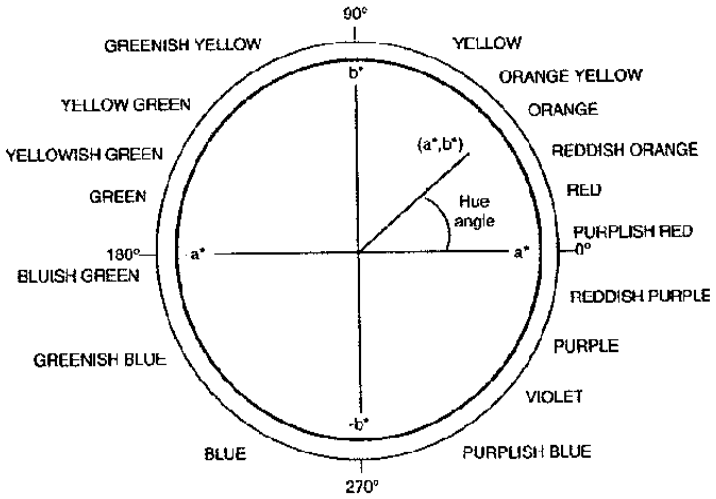
Mulero ve ark., (2010), Monastrell üzüm çeşidinde titrasyon asitliğini 8.16 g L^{-1}, pH kapsamını 3.60 olarak kaydetmişlerdir.

Farklı araştırıcıların bulgularına göre; CIELAB (Commission International De L'éclairage) parametreleri çiçeklerin, meyvelerin ve sebzelerin rengini değerlendirmek ve tanımlamak için kullanılmıştır (Rolle and Guidoni, 2007).

Tablo 4'de Horoz karası üzüm çeşidi renk parametreleri incelenmiştir. Horoz Karası çeşidine ait tane kabuk rengi bulgularına göre; L* değeri 25.64 ile 29.97 değerleri arasında, a* değeri 0.32-1.33, b* değeri -0.91 ile -0.23 arasında belirlenmiştir. Horoz Karası üzüm örneklerinde Chroma değeri 0.43-1.85, Hue açısı ise -40.88 ile -33.31 arasında değişmiştir. Hue açısı değerlerine göre Horoz Karası çeşidi tane kabuk rengi mor olarak kaydedilmiştir (Şekil 1).

Çağındı (2016), Red Globe üzüm suyu örneklerinde L* değerini 16.09, a değerini 9.57, b* değerini 2.39, Chroma 10.24 ve Hue açısını 13.09 olarak bildirmiştir.

Peppi and Fidelibus (2008), Flame Seedless üzüm çeşidinde iki farklı hasat tarihinde tane kabuğu rengiyle ilgili olarak L* değerinin 40.79 ve 41.78 arasında, Chroma değerinin 14.84 ve 15.25, hue açısının 59.5 ile 61.8 arasında elde edildiğini saptamışlardır.



Şekil 1. CIE renk koordinatları (a*, b* ve hue açısı). (McGuire,1992)

Figure 1. CIE color coordinates (a*,b* and hue angle). (McGuire,1992)

Olivares ve ark., (2017), Crimson Seedless üzüm çeşidi kontrol grubu örneklerinde L*değerini 32.7-38.8, a* değerini 0.5-8.1, b* değerini 7.8-4.7, Chroma 8.69-9.61 değerleri arasında bildirmişlerdir.

Rolle and Guidoni (2007), Cabernet Sauvignon üzüm çeşidinde L* değerini ortalama 29.3, a*, b* ve Chroma, hue açısı, CIRG değerlerini sırasıyla; 0.48, -0.33, 0.62, -0.56, 12.41 olarak kaydetmişlerdir.

CIRG indeksine göre üzümler farklı gruplara ayrılmaktadır. Sarı-yeşil renkli üzümlerde CIRG<2, pembe üzümlerde 2< CIRG<4, kırmızı üzümlerde 4<CIRG<5, koyu kırmızı üzümlerde 5< CIRG<6, mavi-siyah üzümlerde CIRG>6 şeklinde kaydedilmiştir (Carreno ve ark., 1996). Tablo 4 incelendiğinde; Horoz Karası üzüm çeşidinde CIRG indeksi 7.02 ile 8.25 değerleri arasında belirlenmiştir.

Orak (2007), kırmızı üzüm çeşitlerinde renk parametrelerini inceleyerek, L* değerinin 7.89 ile 34.78 değerleri arasında, a* renk değerinin 6.31 ile 13.29, b* değerinin 0.61 ile 15.12, hue açısının 0.43 ile 13.84 ve Chroma değerinin 7.74 ile 16.38 değerleri arasında olduğunu bildirmiştir. Ayrıca Öküzgözü, Muscat Hamburg, Cabernet Sauvignon, Tekirdağ Çekirdeksizi gibi mavi-siyah renkli üzüm çeşitlerinde CIRG indeksini sırasıyla; 6.60,6.29,9.32, 6.34 olarak kaydetmiştir.

Bu çalışmada, Horoz Karası üzüm örneklerinde L* değeri ortalama 27.81, a*, b* ve Chroma ortalama değerleri sırasıyla; 0.8, -0.5, 1.14 olarak belirlenmiştir. Bu değerler Rolle and Guidoni (2007)'nin Cabernet Sauvignon üzüm çeşidinde elde etmiş olduğu L*, a*, b*, Chroma değerleri ile benzerlik göstermektedir.

Tablo 5'de Horoz Karası üzüm örneklerinin toplam fenol ve antioksidan aktivite kapsamı görülmektedir. Toplam fenol değerleri 360.5 mg GAE 100 g^{-1} ile 484.7 mg GAE 100 g^{-1} arasında değişmektedir. Antioksidan aktivite düzeyi ise % 87-98 arasında belirlenmiştir. Fenolik bileşikler, üzüm ve şarapta bulunan önemli unsurlardır. Bu bileşikler farklı yapılarda ve miktarlarda bulunabilir. Fenolik bileşikler özellikle tane kabuğu ve çekirdeklerde yer alır. Daha az bir miktarı ise üzüm suyunda bulunur. Fenol miktarı gallik asit eşdeğeri olarak (GAE) ifade edilir (Dharmadhikari, 1994).

Özden ve Özden (2014) yaptıkları çalışmada toplam fenolik madde miktarını Wonderful nar çeşidinde 1136.54 mg GAE kg^{-1} taze ağırlık, siyah dutlarda 2153.51 mg GAE kg^{-1} taze ağırlık, Şiraz kırmızı üzüm çeşidinde 1465.64 mg GAE kg^{-1} taze ağırlık olarak kaydetmişlerdir. Antioksidan aktivite düzeyini ise aynı çeşitlerde sırasıyla % 89.82, % 73.79, % 60.42 olarak bildirmişlerdir.

Tablo 5'de; toplam fenol düzeyi ortalama değer bakımından yüksek rakımda yer alan Çobanlı'dan alınan salkım örneklerinin diğer bölgelere göre daha üst sırada yer aldığı görülmektedir. Üzümde toplam fenol düzeyi düşük rakımlı bölgelere göre yüksek rakımlı bölgelerde daha yüksek değerlere ulaşmaktadır (Hess, 2007; Aslantaş ve Karakuş, 2007).

Bunea ve ark., (2012), Napoca ve Muscat Hamburg üzüm çeşitlerinde toplam fenol düzeyi ile antioksidan aktivite kapsamı sırasıyla; 1231.38 mg GAE kg- 935.04 mg GAE kg; 25.07 mg Trolox g^{-1} ile 22.77 mg Trolox g^{-1} olarak kaydetmişerdir. Üzümdeki antioksidan aktivite düzeyi flavonoid, fenolik asit, antosiyanin ve karetenoidler gibi antioksidan unsurlardan dolayıdır.

Tablo 3. Horoz Karası üzüm çeşidi şıra özellikleri

Table 3. Must characteristics of the Horoz Karası grape variety

Örnek Kodu	SÇKM (%)	Asitlik (%)	pH
1	20.20	0.205	3.94
2	22.37	0.221	3.93
3	21.49	0.287	3.88
4	22.26	0.318	3.76
5	17.91	0.206	3.62
6	22.25	0.372	3.75
7	24.55	0.240	3.89
8	21.40	0.517	3.93
9	21.31	0.222	3.92
10	22.05	0.269	3.93
11	19.35	0.324	3.83
12	19.15	0.422	3.72
13	17.72	0.343	3.87
14	25.01	0.294	3.82
15	22.75	0.189	3.70
16	25.35	0.201	3.89
17	16.64	0.420	3.36
18	21.57	0.310	3.63
Min.	16.64	0.189	3.62
Max.	25.35	0.517	3.94
Ort.	20.98	0.360	3.78
Standart sapma	0.309	0.057	0.085

Tablo 4. Horoz Karası üzüm çeşidinde incelenen renk özellikleri

Table 4. Color characteristics of Horoz Karası grape variety

Örnek Kodu	L*	a*	b*	Chroma	Hue açısı	CIRG
1	27.15	0.32	-0.26	0.66	-39.01	7.87
2	28.64	0.35	-0.23	0.72	-33.31	7.26
3	29.58	0.41	-0.28	0.59	-34.33	7.21
4	29.97	0.68	-0.53	1.05	-37.93	7.02
5	25.64	0.48	-0.34	0.43	-35. 31	8.25
6	28.91	0.51	-0.91	1.12	-37.27	7.23
7	29.13	0.58	-0.47	1.25	-39.61	7.29
8	26.12	0.69	-0.58	1.30	-40.04	8.02
9	29.46	0.67	-0.58	1.36	-40.88	7.17
10	28.76	1.33	-0.90	1.61	-34.08	7.04
11	27.29	0.68	-0.57	1.56	-39.97	7.62
12	28.56	0.92	-0.75	1.52	-39.18	7.29
13	26.47	0.66	-0.50	1.09	-37.14	7.88
14	25.65	0.62	-0.45	1.15	-35.97	8.06
15	26.87	1.18	-0.90	1.85	-37.33	7.56
16	28.94	0.44	-0.30	0.58	-34.28	7.26
17	27.59	1.05	-0.70	1.56	-33.69	7.33
18	28.47	0.99	-0.82	1.33	-39.63	7.37
Min.	25.64	0.32	-0.91	0.43	-40.88	7.02
Max.	29.97	1.33	-0.23	1.85	-33.31	8.25
Ort.	27.81	0.83	-0.57	1.14	-37.10	7.64
Standart sapma	1.018	0.161	0.193	0.195	1.512	0.103

Tablo 5. Horoz Karası üzüm örneklerinin toplam fenol ve antioksidan aktivite düzeyi

Table 5. Total phenol and antioxidant activity levels of Horoz Karası grape samples

Örnek Kodu	Toplam fenol (mg GAE 100 g^{-1})	Antioksidan aktivite (%)
1	456.8	91
2	484.7	98
3	429.9	96
4	445.2	92
5	440.8	97
6	386.4	90
7	412.3	92
8	447.6	98
9	416.1	95
10	457.7	96
11	366.1	87
12	462.6	93
13	427.7	89
14	438.2	92
15	453.9	96
16	360.5	92
17	393.6	95
18	402.7	94
Min.	360.5	87
Max.	484.7	98
Ort.	409.1	92
Standart sapma	22.48	2.113

Dani ve ark. (2007); beyaz ve kırmızı üzüm çeşitlerine ait üzüm şıraları ile ilgili yaptıkları çalışmada, toplam fenolik içerik (Folin-Ciocalteu) ile antioksidan aktivite (DPPH) arasında pozitif bir ilişki olduğunu belirlemiştir.

Çağındı (2016), Red globe çeşidi üzüm suyunda toplam fenol ile antioksidan aktivite düzeyini sırasıyla; 304.42 mg l^{-1}, % 63.83 olarak kaydetmiştir.

Du ve ark., (2012), Cabernet Sauvignon, Cabernet Franc ve Merlot üzüm çeşitlerinde toplam fenol miktarını 219.5 mg 100 g^{-1}, 128.3 mg 100 g^{-1}, 179.1 mg 100 g^{-1}; antioksidan aktivite düzeyini ise %92.77, % 89.30, % 89.91 olarak bildirmiştir.

Orak (2007), çalışmasında Alfonse Lavallee, Boğazkere, Adakarası üzüm çeşitlerinde toplam fenol miktarını 1728, 2649, 2695 µg mL^{-1} GAE olarak belirlemiştir. Genova (2012), Sangiovese üzüm çeşidinde toplam fenol kapsamını 419.9 mg GAE 100 g^{-1} olarak kaydetmiştir.

Chorti ve ark., (2016), Agiorgitiko kırmızı üzüm çeşidinde toplam fenol miktarının 400 mg l^{-1} ile 560 mg L^{-1} arasında değiştiğini ifade etmişlerdir. Kök ve Bal (2017), Horoz Karası üzüm örneklerinde toplam fenol miktarını 886.44 mg GAE kg^{-1} olarak bildirmiştir.

Serrano ve ark., (2006), Crimsonn Seedless üzüm çeşidinde toplam fenol miktarını 53.6 mg GA 100 g^{-1}, toplam antioksidan aktiviteyi 396.8 mg GA 100 g^{-1} şeklinde kaydetmişlerdir. Paun ve ark., (2017), siyah üzüm kabuğunda toplam fenol düzeyini 21.32 mg g^{-1} olarak belirlemişlerdir. Farhadi (2016), Black Pearl ile Purple grape üzüm çeşitlerinde çekirdek ve kabukta bulunan toplam fenol düzeyini sırasıyla; 18.34 mg g^{-1} ile 40.20 mg g^{-1}; 15.79 mg g^{-1} ile 27.36 mg g^{-1} olarak kaydetmiştir. Aynı çalışmada antioksidan aktivite kapsamı Muscat ve Hosseini üzüm çeşidinde meyve pulpunda % 93.14 ile % 94.58 olarak kaydedilmiştir. Mulero ve ark., (2010), Monastrel üzüm çeşidinde toplam fenol miktarını 447.7 mg kg^{-1}, antioksidan aktivite düzeyini 4.40 Trolox g^{-1} olarak elde etmişlerdir.

Çalışmada, toplam fenol miktarı ortalama olarak 409.1 mg GAE 100 g^{-1} belirlenmiştir. Bu düzey, Mulero ve ark., (2010), Genova (2012), Chorti ve ark., (2016), Yang ve Xiao (2013)'nın farklı kırmızı üzüm çeşitleri ile yürüttükleri çalışmalarda elde ettiği toplam fenol miktarı ile benzerlik göstermektedir. Antioksidan aktivite düzeyi Horoz Karası üzüm çeşidinde ortalama olarak % 92 olarak belirlenmiştir. Bu değer, Du ve ark., (2012), Farhadi (2016)'nin araştırmalarında farklı kırmızı üzüm çeşitlerinde belirlenen antioksidan aktivite bulgularına yakın değerler olarak görülmektedir.

Topalovic ve ark., (2012), üzüm tanesinde bulunan fenolik bileşiklerin miktarlarının genetik, iklimsel, coğrafik etmenler ile bitkinin vejetatif kuvveti, tarımsal uygulamalar ve olgunlaşma aşamalarına bağlı olarak değişkenlik gösterdiği ile ilgili sayısız çalışma yapıldığını ifade etmişlerdir.

Üzüm ve özellikle kırmızı üzüm ürünleri fenolik bileşikler açısından zengindir (Fuleki ve Ricardo-da-Silva, 2003). Dani ve ark. (2007); yürüttükleri çalışmada kırmızı üzüm suyunun beyaz üzüm suyundan daha fazla fenolik içeriğe sahip olduğunu belirlemişlerdir. Horoz Karası çeşidinin kırmızı renkte olması nedeniyle toplam fenol ve antioksidan aktivite kapsamının yüksek düzeyde olduğu bu çalışmada belirlenmiştir.

Yang ve Xiao, (2013), üzüm suyunda toplam fenol kapsamını Cabernet Franc ve Pinot Noir üzüm çeşitlerinin çekirdeklerinde 424.6 mg 100 g^{-1} GAE ile 396.8 mg 100 g^{-1} olarak bildirmiştir.

Üzümde bulunan fenolik bileşiklerin konsantrasyonu çeşit, vejetasyon dönemi, kültürel koşullar ve çevresel koşullara bağlı olarak farklılık gösterebilmektedir (Bunea, 2012; Farhadi, 2016).

Horasan Sağbasan (2015), toplam fenol miktarını siyah üzümde 634.3 mg GAE/100g, mor erikte 416.5 mg GAE/100g, kızılcıkta 1081.9 mg GAE/100g, yaban mersininde 313.9 mg GAE/100g olarak belirlemiştir. Antioksidan kapasite düzeyi ise aynı çeşitlerde sırasıyla; 133.3 ±3.7 µ mol Trolox/100 g, 127 ±1.9 µ mol Trolox/100 g, 144.4 ±1.9 µ mol Trolox/100 g ve 133.3 ±1.5 µ mol Trolox/100 g örnek olarak belirlenmiştir.

Sonuç

Bu çalışma ile Kahramanmaraş ilinin önemli havzalarında yetiştirilen Horoz Karası üzüm çeşidinin salkım, tane ve şıra özellikleri ile tane kabuk rengi, toplam fenol ve antioksidan aktivite değerleri ortaya konulmuştur. Horoz Karası üzüm çeşidine ait veriler incelendiğinde; salkım ağırlığı açısından Kizirli öne çıkmaktadır. Salkım iriliğinin omcaların bulunduğu toprak özellikleri ve budama uygulamaları ile doğrudan etkilendiği düşünülmektedir.

Tane ağırlığı ortalama değer bakımından Kizirli'den alınan salkım örnekleri diğer bölgelere göre daha üst sırada yer almaktadır.

Salkım ve tane özellikleri bakımından; Kizirli'nin yer aldığı Pazarcık Havzasının öne çıkmasının nedeni; omcalara goble terbiye şekli verilmiş olması ve bununda kalite özelliklerine olumlu etki ettiği düşünülmektedir.

Şıra özelliklerine ait veriler incelendiğinde; Horoz Karası üzüm çeşidinde Taşdemir'den alınan salkım örneklerinde ortalama SÇKM düzeyi ile pH düzeyi diğer bölgelere daha yüksek değerde kaydedilmiştir. Çalışmanın yürütüldüğü havzalarda çeşide ait tane rengi, şıra miktarı, iriliği, antioksidan içeriği, şeker oranı gibi parametrelerde farklı değerlerin belirlenmesi ekolojik koşulların etkisi nedeniyle olduğu düşünülmektedir.

CIE (Commission Internationale de L'éclairage) renk koordinat sisteminde hue açısı değerlerinin mor renge karşılık geldiği, CIRG indeksinin 7.02 ile 8.25 değerleri arasında değiştiği belirlenmiştir. Mor renkli Horoz Karası üzüm çeşidinde toplam fenol düzeyi (484.7 mg GAE) ve antioksidan aktivite bakımından en yüksek olan örnek (% 98) 2 numaralı örneğin (Çobanlı 2) alındığı Bertiz Havzası olarak kaydedilmiştir.

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: Bu çalışma, Kahramanmaraş Sütçü İmam Üniversitesi BAP Koordinasyon Birimi tarafından desteklenmiştir. Proje numarası: 2018/3-32 M.

Teşekkür: -

Açıklama: -

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 8(4), 302-311 (2022) • https://doi.org/10.3153/FH22028 *Research Article*

COVID-19 salgınında tüketicilerin gıda satın alma, gıda hijyeni ve beslenme davranışları

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Cite this article as:
Coşansu, S., Başyazıcı, E., Atasoy, E., Mazreku, G., Çetin, S., Toupal, S. (2022). COVID-19 salgınında tüketicilerin gıda satın alma, gıda hijyeni ve beslenme davranışları. *Food and Health,* 8(4), 302-311. https://doi.org/10.3153/FH22028

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Submitted: 13.05.2022
Revision requested: 30.06.2022
Last revision received: 07.07.2022
Accepted: 28.07.2022
Published online: 01.09.2022

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

ÖZ

COVID-19 salgını sürecinde bireylerin gıda satın alma, gıda hijyeni ve beslenme davranışlarını belirlemek amacıyla 24 sorudan oluşan bir anket uygulanmıştır. Gönüllülük esasına dayalı olarak çevrimiçi gerçekleştirilen anket çalışmasına toplam 658 kişi katılmış olup, %67'si kadın, %33'ü erkek, %60'ı 18-24 ve %25'i 25-31 yaş aralığındadır. Anket sorularına verilen cevaplara göre salgın döneminde gıda temini için en fazla market alışverişi (%73.9) tercih edilmiş olup, en fazla bakliyat (%59.1) stoklanmıştır. Satın alma sırasında ürünlerin besin değerine salgın öncesine göre daha fazla dikkat edilmiş ve öncelikli olarak ambalajlı gıdalar tercih edilmiştir (%79.3). Taze meyve ve sebzelerin temizlenmesinde en fazla tercih edilen yöntemler akan su altında yıkama (%59.7) ve sirkeli suda bekletmedir (%43.2). Genellikle bireylerin salgın döneminde daha sağlıklı beslenme eğiliminde oldukları, ancak öğün sayısında bir miktar artış olduğu görülmüştür. Bağışıklık sisteminin güçlendirilmesi amacıyla başta C vitamini (%73) ve D vitamini (%40.5) olmak üzere besin takviyesi kullanıldığı anlaşılmaktadır. Ayrıca sosyal medya (%31.9) ve televizyon programları (%20.3) bireylerin beslenme davranışlarına yön vermede doktor tavsiyesinden (%32) sonra en fazla etkili olan kaynaklıdır.

Anahtar Kelimeler: COVID-19, Salgın, Satın alma, Gıda hijyeni, Beslenme

ABSTRACT

Food purchasing, food hygiene, and nutritional behaviors of consumers during the COVID-19 pandemic

An online questionnaire, consisting of 24 questions was applied to determine individuals' food purchasing, food hygiene, and nutritional behaviors during the COVID-19 pandemic. Of 658 respondents, 67% were female, 33% were male, 60% were 18-24 years old, and 25% were 25-31 years old. Supermarket shopping (73.9%) was the most preferred way to supply food, while the primary stocked up food was dried legumes (59.1%). The nutritional values and contents of foods were more paid attention to than before the COVID-19 pandemic, and the packaged foods (79.3%) were primarily preferred. The most preferred methods for cleaning fresh fruits and vegetables were washing under running water (59.7%) and soaking in water with vinegar (43.2%). The participants intended to eat healthy foods during the COVID-19 pandemic. However, the daily meal counts increased slightly. Food supplements, mainly vitamin C (73%) and vitamin D (40.5%) were consumed to strengthen the immune system. Additionally, social media (31.9%) and TV programs (20.3%) were very effective after medical doctor recommendations (32%) for orienting the nutritional behaviors of participants.

Keywords: COVID-19, Pandemic, Food purchasing, Food hygiene, Nutrition

Giriş

COVID-19, 2019 yılının son aylarında Çin'in Wuhan şehrinde başlayıp kısa sürede tüm dünyayı etkisi altına almış olan ve günümüzde devam eden küresel bir salgındır. Bu hastalıkta etken SARS-CoV-2 olup, yeni bir koronavirüs suşudur. COVID-19 enfeksiyonu damlacık yoluyla insandan insana bulaşmakta, akut solunum sıkıntısı sendromuna benzer şekilde yüksek ateş, kuru öksürük, genel yorgunluk ve boğaz ağrısına neden olmaktadır. Diğer belirtiler arasında ishal, kas ve baş ağısı bulunmaktadır (Tsai ve ark., 2021). COVID-19, 11 Mart 2020 tarihinde Dünya Sağlık Örgütü (DSÖ) tarafından küresel pandemi ilan edilmiştir (Çöl ve Güneş, 2020). Salgının başlangıcından Nisan 2022 tarihine kadar dünyada doğrulanmış toplam vaka sayısı 490 milyonu, ölüm sayısı ise 6 milyonu aşmıştır. DSÖ verilerine göre Türkiye'de Nisan 2022 itibariyle doğrulanmış vaka sayısı yaklaşık 15 milyon, ölüm sayısı ise 98 binin üzerindedir (WHO, 2022).

DSÖ tarafından pandemi olarak ilan edildiği tarihte, Türkiye'de de ilk pozitif vakanın görülmesi ile birlikte COVID-19 önlemleri alınmaya başlanmıştır. Bu önlemler kapsamında okullarda yüz yüze eğitime ara verilmiş, kafe, restoran, alışveriş merkezi vb. pek çok hizmet sektörünün faaliyetleri durdurulmuş, ayrıca belirli günlerde uygulanan sokağa çıkma yasakları getirilmiştir. Diğer ülkelerde de buna benzer uygulamalara gidilmiştir. Sokağa çıkma yasakları ve sosyal mesafenin tüketici alışkanlıklarını ve davranışlarını önemli düzeyde etkilemiştir (Sheth, 2020). COVID-19 salgını süresince yaşanan sokağa çıkma yasakları, uzaktan eğitim, evden çalışma vb. nedenler her yaştan bireyler için alışılmış yaşam tarzının dışına çıkılması anlamına gelmektedir. Bu koşullarda bireyler yaşanılan kaygı ve stresin etkisi ile sağlıksız yiyeceklere yönelebilmektedirler. Fiziksel aktivitenin de azalması ile birlikte obezite, diyabet, kardiyovasküler hastalıklar ve kanser dahil pek çok hastalık riski ortaya çıkmaktadır (Sánchez-Sánchez ve ark., 2020).

Pandemi başlangıcından itibaren yapılan çalışmalar bireylerin kaygı düzeylerinin arttığını ve yaşam tarzlarındaki değişiklikler nedeniyle fiziksel sağlık yanında ruhsal sağlığın da olumsuz etkilendiğini göstermektedir (Altun, 2020; Göksü ve Kumcağız, 2020). Bireylerin pandemi döneminde beslenme, uyku ve fiziksel aktivite düzeyleri değişmiştir (Macit, 2020). Ayrıca sosyal izolasyon döneminde karbonhidrat ve yağ içeriği yüksek besinlerin daha fazla tüketildiği, diyetlere devamlılığın azaldığı ve buna bağlı olarak vücut ağırlığının arttığı diğer tespitler arasındadır (Garipoğlu ve Bozar, 2020).

Bu çalışmada, COVID-19 salgınının toplumda hemen her yaştan bireyin yaşam tarzının değişmesine neden olduğundan yola çıkılarak, bireylerin gıda ürünlerini satın alma, gıda hijyeni uygulamaları ve beslenme tercihlerinin ne yönde etkilendiğinin belirlenmesi hedeflenmiştir.

Materyal ve Metot

Kitlesel tipte bir çalışma olan bu araştırmada, COVID-19 salgını sürecinde bireylerin beslenme, gıda takviyesi kullanımı, gıda temini ve gıda hijyeni uygulamaları ile ilgili tercihlerinin belirlenmesi hedeflenmiştir. Bu amaçla iki bölüm ve toplam 24 sorudan oluşan bir anket çalışması yapılmış olup, bu ankete verilen cevaplar çalışmanın verilerini oluşturmuştur. Çalışmada kullanılan anket Google Forms ile hazırlanmış (https://docs.google.com/forms) ve çevrimiçi olarak gerçekleştirilmiştir. Toplam katılımcı sayısı 658 olup, anketlerin tamamı değerlendirmeye alınmıştır. İlk bölümde katılımcıların demografik özelliklerini belirlemek amacıyla cinsiyet, yaş, öğrenim durumu, aylık gelir ve yaşanılan yer sorulmuştur. İkinci bölümde ise gıda satın alma, gıda hijyeni ve beslenme tercihleri ile ilgili 19 adet soru sorulmuştur (Garipoğlu ve Bozar, 2020; Macit, 2020). Anket cevaplarının değerlendirilmesinde Microsoft Excel programı ve Google Forms'un ilgili özellikler kullanılarak grafik ve tablolar oluşturulmuş, veriler frekans ve % dağılım olarak sunulmuştur.

Etik İlkeler

Sakarya Üniversitesi Fen ve Mühendislik Bilimleri Etik Kurulu Başkanlığı'nın 13.05.2022 tarihli ve 25 sayılı toplantısında alınan 04 nolu karar ile "COVID-19 Salgının Tüketicilerin Gıda Satın Alma, Gıda Hijyeni ve Beslenme Davranışlarının Araştırılması " başlıklı çalışmanın Etik açıdan uygun olduğuna oy birliği ile karar verilmiştir.

Bulgular ve Tartışma

Katılımcıların Demografik Özellikleri

Katılımcılara ait demografik özellikler Şekil 1'de sunulmuştur. Buna göre ankete katılan bireylerin %33'ü (n=216) erkek, %67'si (n=442) kadındır. Bireylerin yaşa göre dağılımına bakıldığında %60'ının (n=395) 18-24 yaş, %25'inin (n=168) ise 25-31 yaş aralığında olduğu görülmektedir. Ankete katılan bireylerin önemli bir kısmı (%79.3; n=522) üniversite öğrencisi veya mezunudur. Katılımcıların %48'inin (n=302) aylık geliri 2000 TL veya altında olup, önemli bir kısmı (%61; n=404) büyük şehir veya il merkezinde yaşamaktadır. Buna göre ankete katılan bireylerin önemli bir kısmı 18-31 yaş aralığında, üniversite öğrencisi veya mezunu, 2000 TL veya altında aylık geliri olan, büyük şehir veya il merkezinde yaşayan bireylerden oluşmaktadır. Anketin gönüllülük esasına da-

yalı olarak çevrimiçi gerçekleştirildiği göz önünde bulundurulduğunda, ortaya çıkan genel katılımcı profilinin beklenen bir durum olduğu düşünülmektedir.

COVID-19 *Salgını Sürecinde Bireylerin Gıda Temini ve Satın Alma İle İlgili Tercihleri*

Çalışmada bireylerin COVID-19 salgını sürecinde gıda temini ve satın alma tercihleri ile ilgili dört adet soru sorulmuş olup, cevapların frekans ve % dağılımları Tablo 1'de sunulmuştur. Verilen cevaplara göre, süpermarket alışverişi (%73.9) gıda temininde birinci sırayı almakta, onu bakkal-yerel market (%49.8) ve pazar (%43) izlemektedir. Salgın sürecinde özellikle sokağa çıkma yasakları ve izolasyon nedeniyle daha da önemli hale gelen online market ve paket servisin tercih edilme oranları sırasıyla %24.6 ve %18.7'dir. Salgın sürecinde kafe, restoran, lokanta vb. işletmeler kapanması veya sınırlı süre ve kapsamda hizmet vermeleri nedeniyle gıda temini marketlere kaymıştır. Gıda temininde dağılım salgın öncesi %50 market ve %50 servis sektörü şeklinde iken, salgın döneminde neredeyse %100 market olacak şekilde değişmiştir. Gıda alışverişi için markete gidiş sayısı azalmakla birlikte, her bir gidişte harcanan para miktarı artmıştır (Aday ve Aday, 2020). Ayrıca kontaminasyon riski nedeniyle temastan kaçınma ve satış noktalarına erişimin güçleşmesi nedeniyle online alışverişe olan talebin arttığı bildirilmektedir (Baltacı ve Akaydın, 2020).

Salgın sürecinde, ihtiyaç duyulduğunda erişememe endişesi nedeniyle normal koşullara göre daha fazla ürün satın alma eğilimi sıklıkla karşılaşılan bir durumdur (Aday ve Aday, 2020). Katılımcıların %54'ü salgın döneminde gıda stokladığını belirtmiştir (Tablo 1). En çok stoklanan ürün grubu bakliyattır (%59.1). Salgın sürecinde bağışıklık sistemini güçlendirmek için sağlıklı beslenme çabası satın alma davranışlarına da yansıyabilmektedir. Katılımcıların %40.1'i satın alma sırasında gıdaların besin değerine ve içeriğine salgın öncesine göre daha fazla dikkat ettiğini belirtmiştir (Tablo 1). Pandemi sürecinde tüketicilerin özellikle gıda ve hijyen ürünleri ile ilgili stok yapma eğiliminde oldukları ve uzun ömürlü gıdaları taze gıdalara tercih ettikleri pek çok ülkede yapılan araştırmalarla ortaya konmuştur (Baltacı ve Akaydın, 2020). İtalya'da Bracale ve Vaccaro (2020) tarafından yapılan bir çalışmada kapanma döneminde bir önceki yılın aynı dönemine göre satışı en fazla olan gıdaların başında makarna, ton konservesi, dondurulmuş sebze ve meyve, UHT süt, yumurta, dondurulmuş balık ve portakal geldiğini bildirmişlerdir. Ayrıca ekmek mayası, un, hazır un karışımları ve bakliyat ve konserve gıdalar satışında en fazla değişim olan ürünlerdir.

Diğer yandan, "Salgın döneminde satın aldığınız gıda ürünlerinin besin değerine ve içeriğine salgın öncesine göre daha fazla dikkat ediyor musunuz?" sorusuna katılımcıların %40.1'i "evet", %25.4'ü ise "bazen" cevabını vermiştir (Tablo 1). Bu durum salgın döneminde bireylerin bağışıklık sistemlerini güçlendirme ve kilo alımından kaçınma eğiliminin bir sonucu olarak değerlendirilebilir.

Tablo 1. Katılımcıların gıda temini ve satın alma ile ilgili sorulara verdikleri cevapların frekans ve % dağılımı

Table 1. Frequency and % distribution of the responses given by the participants to food supply and purchasing questions

		n	%
Salgın sürecinde gıda ürünlerini ne şekilde temin ediyorsunuz? (Birden fazla seçenek işaretlenebilir)	Süpermarket	486	73.9
	Bakkal ve yerel market	328	49.8
	Pazar	283	43
	Online market	162	24.6
	Paket servis	123	18.7
	Kafe/restoran	53	8.1
Salgın sırasında tedarikinden duyduğunuz endişe nedeniyle gıda ürünlerini stoklama yaptınız mı?	Evet	303	46
	Hayır	355	54
Salgın sürecinde hangi gıda ürününü stoklama yaptınız? (Birden fazla seçenek işaretlenebilir)	Bakliyat ürünleri	389	59.1
	Meyve ve sebze ürünleri	136	20.7
	Et ve et ürünleri	115	17.5
	Konserve ürünleri	113	17.2
	Süt ve süt ürünleri	111	16.9
	Fırıncılık ürünleri	82	12.5
	Kuruyemiş ürünleri	80	12.2
	Diğer	13	1.9
Salgın döneminde aldığınız gıda ürünlerinin besin değerine ve içeriğine salgın öncesine göre daha çok dikkat ediyor musunuz?	Evet	264	40.1
	Hayır	227	34.5
	Bazen	167	25.4

COVID-19 Salgını Sürecinde Bireylerin Gıda Hijyeni Uygulamaları

Salgının başlangıcında henüz koronavirüsün nasıl bulaştığı, gıdalar aracılığı ile bulaşıp bulaşmadığı vb. konularda yeterli bilgi bulunmamaktaydı. Bu nedenle meyve ve sebzelerden virüs bulaşabileceği endişesi ile satın alma sonrası temizlemek ve dezenfekte etmek için çeşitli işlemler uygulanabilmektedir. Katılımcıların bu amaçla tercih ettiklerin işlemlerin başında akan su altında yıkama (%59.7), sirkeli suda bekletme (%43.2) ve dışarıda/balkonda bekletme (%34.3) gelmektedir (Tablo 2). COVID-19'dan korunmak için öneriler maske kullanılması, ellerin sık sık yıkanması yüzeylerin dezenfekte edilmesi, kalabalık ortamlarda bulunulmaması, bulunulması haline diğer insanlarla en az 2 m mesafe olacak şekilde sosyal mesafeye dikkate edilmesi şeklinde sıralanmaktadır. Ayrıca çiğ ve pişmiş gıdaların ayrılması, sebze ve meyvelerin tüketilmeden önce iyice yıkanması ve gıdaların iç sıcaklığı en az 70°C olacak şekilde pişirildikten sonra tüketilmesi de diğer öneriler arasındadır (FDA, 2020).

Katılımcıların %79.3'ü salgın sürecinde gıda satın alırken ambalajlı olmasına dikkat ettiğini belirtmiştir. Bracale ve Vaccaro (2020) İtalya'da salgının ilk altı haftasında 10769 marketin satışlarını inceledikleri çalışmada özellikle paketlenmiş gıdaların satışında artış olduğunu bildirmişlerdir. Paketlenmiş gıdalar hem hijyenik açıdan tercih edilmekte, hem de mağazada geçirilen süreyi kısaltmaktadır.

Satın alınan gıdaların belirli bir süre dışarıda, balkonda vs. bekletildikten sonra ev içine alınması da yapılan uygulamalardan biridir. Katılımcıların %39.8'i ürünleri eve almak için bekletmediğini, diğerleri ise bir saatten azdan 5 güne kadar değişen sürelerde karantinada beklettiklerini ifade etmişlerdir. SARS-CoV-2 virüsünün enfekte kişi ile temas, aerosol veya damlacık yoluyla olmak üzere üç şekilde bulaştığı bildirilmektedir. COVID-19'un, virüsle kontamine olmuş gıdanın tüketilmesi ile yani sindirim yoluyla bulaştığına dair yeterli kanıt bulunmamaktadır (Zhang ve ark., 2021). Ancak gıdaların virüsün insanlara taşınmasında aracı olabileceği düşünülmektedir. Örneğin enfekte bir kişi direkt olarak gıda üzerine hapşırır veya öksürürse gıdayı virüs ile kontamine edebilir. Singapur'da fiziksel temas ve gıda tüketiminin olduğu bir bilimsel toplantı sonrası katılımcıların çoğuna COVID-19 bulaşmıştır (Rizou ve ark., 2020). SARS-CoV-2 gıda ile temas eden yüzeylerde aktif kalabilmektedir. Örneğin, somon dilimlenmiş kesme tahtasından SARS-CoV-2 virüsü izole edilebilmiştir (Zhang ve ark., 2021). SARS-CoV-2 plastik ve paslanmaz çelik yüzeylerde bakır ve mukavvaya göre daha uzun süre aktif kalabilmekte, bulaştıktan 72 saat sonra bu yüzeylerde tespit edilebilmektedir (Doremalen ve ark., 2020). Bu nedenle gıda tedarik zinciri ile yayılma potansiyeli olduğu düşünülmektedir. Ayrıca SARS-CoV-2'nin 4°C'de muhafaza eden gıdalarda canlılığı araştırılmış olup, tavuk derisi, somon ve karideste 24 saatte virüs konsantrasyonunda önemli bir azalma olmadığı ancak elma ve mantar yüzeyinde önemli azalma meydana geldiği belirlenmiştir (Dhakal ve ark., 2021).

COVID-19 Salgını Sürecinde Bireylerin Beslenme Alışkanlıklarındaki Değişim

Katılımcıların beslenme alışkanlıklarına ilişkin sorulara verdikleri cevapların frekans ve % dağılımı Tablo 3'te verilmiştir. Ankete katılan her üç bireyden biri (%31) pandemi sürecinde beslenme alışkanlığının değişmediğini ifade etmiş, diğerleri ise "evet" (%41) veya "kısmen" cevabını vermiştir. Salgın öncesi ve salgın sürecinde tüketilen öğün sayıları ile ilgili sorulara verilen cevapların dağılımına göre, iki ya da üç öğün tüketenlerin oranının azaldığı, buna karşın dört ya da daha fazla öğün tüketenlerin oranın arttığı görülmektedir. Katılımcılara hangi gıdaların tüketimini artırdıklarının sorulduğu soruda birden fazla seçeneği işaretleyebilecekleri belirtilmiştir. Katılımcıların %23.1'i hiçbir ürün grubunun tüketimi artırmadığı cevabını vermiştir. Diğer yandan tüketimi en fazla artan ürün grubu meyve (%42.6), onu sebze ve sebze yemekleri (%38.9) ve hamur işleri (%31.3) izlemektedir. Diğer yanıtını veren bireyler ise sirke, limon, pekmez, bal, bitki çayları ve turşu tüketimini artırdıklarını ifade etmişlerdir. Verilen cevapların dağılımından genel olarak bireylerin sağlıklı yiyeceklere yöneldiği sonucuna varılabilir. Ancak katılımcıların üçte biri hamur işleri tüketimini artırdığı cevabını vermiştir. Bu durum salgın döneminde hissedilen stres, kaygı ve endişe nedeniyle karbonhidrat içeriği yüksek gıdalara daha fazla yönelme olduğu şeklinde yorumlanabilir. Pandemi öncesine göre evde daha uzun süre geçirilmesi aşırı yeme eğilimine yol açabilmektedir (Muscogiuri ve ark., 2020). Sánchez-Sánchez ve ark. (2020) kapanma süresince ev yapımı tatlı, hamur işi tüketiminin %4.6 arttığını bildirmişlerdir.

Günlük su tüketim miktarı bireylerin önemli bir kısmında (%57.4) 1-2 litre aralığındadır. Pandemi öncesi yapılan çalışmalarda bireylerin günlük ortalama su tüketimlerinin 2 litreden az olduğu görülmektedir (Karagöz ve ark., 2004; Eroğlu, 2018). Buna göre günlük su tüketiminde pandemiye bağlı olarak bir artış olmadığı söylenebilir. Diğer yandan, dışarıda yemek yeme alışkanlığının önemli oranda (%63.5) azaldığı anlaşılmaktadır. Bunda hem restoran ve kafelerin özellikle salgının ilk yılında uzun süre kapalı kalmasının hem de bireylerin bulaşı endişesi nedeniyle kalabalık ortamlardan kaçınmalarının etkili olduğu düşünülmektedir. Katılımcıların %66.4'ü salgın sürecinde tüketiminden vazgeçtikleri herhangi bir ürün olmadığını ifade etmişlerdir. Tüketiminden en fazla vazgeçilen ürünler sırasıyla abur cubur (%20.4) ve hamur işleridir (%13.4).

Tablo 2. Katılımcıların gıda hijyeni uygulamalarına ilişkin sorulara verdikleri cevapların frekans ve % dağılımı

Table 2. Frequency and % distribution of the responses given by the participants to food hygiene practices

		n	%
Salgın sürecinde aldığınız meyve ve sebzeleri tüketim öncesi temizlemek / dezenfekte etmek için ne yapıyorsunuz? (Birden fazla seçenek işaretlenebilir)	Akan su ile yıkama	393	59.7
	Sirkeli suda bekletme	284	43.2
	Dışarıda/balkonda bekletme	226	34.3
	Hiçbir işlem uygulamıyorum	72	10.9
	Deterjanla yıkayıp durulama	60	9.1
	Karbonatlı suda bekletme	40	6.1
Salgın sürecinde gıda satın alırken ambalajlı olup olmamasına dikkat ediyor musunuz?	Evet	522	79.3
	Hayır	136	20.7
Salgın sürecinde gıda ürünlerini tüketmeden önce (karantinada) ne kadar bekletiyorsunuz?	Bekletmiyorum	262	39.8
	Yaklaşık 1 gün	217	33
	1 saatten az	156	23.7
	2-5 gün	23	3.5

Tablo 3. Katılımcıların beslenme alışkanlıklarına ilişkin sorulara verdikleri cevapların frekans ve % dağılımı

Table 3. Frequency and % distribution of the responses given by the participants to eating habits questions

		n	%
Salgın beslenme alışkanlığınızı etkiledi mi?	Evet	264	41
	Hayır	203	31
	Kısmen	191	29
Salgın öncesi bir günde kaç öğün yemek yerdiniz?	1 öğün	6	0.9
	2 öğün	278	42.2
	3 öğün	330	50.2
	4 öğün ve fazlası	44	6.7
Salgın sürecinde bir günde kaç öğün yemek yediniz?	1 öğün	16	2.4
	2 öğün	223	33.9
	3 öğün	302	45.9
	4 öğün ve fazlası	117	17.8
Salgın sürecinde beslenmenizde hangi gıdaların tüketimini artırdınız? (Birden fazla seçenek işaretlenebilir)	Tüketimimi artırmadım	152	23.1
	Meyve	280	42.6
	Sebze ve sebze yemekleri	256	38.9
	Hamur işleri	206	31.3
	Abur cubur ve çeşitleri	190	28.9
	Et ve et ürünleri	187	28,4
	Tatlı ve tatlı türleri	178	27,1
	Süt ve süt ürünleri	155	23,6
	Baharat çeşitleri	109	16.6
	Diğer	8	1.2
Salgın sürecinde günlük tüketilen su miktarınız ne kadar?	<1 litre	131	19.9
	1-2 litre	381	57.9
	>2 litre	146	22.2
Salgın sürecinde dışarıda yeme alışkanlığınız nasıl değişti?	Azaldı	418	63.5
	Değişmedi	120	18.2
	Dışarıda yemek yeme alışkanlığım yok	77	11.7
	Arttı	43	6.5
Salgın sürecinde tüketmekten vazgeçtiğiniz ürün grubu var mı? (Birden fazla seçenek işaretlenebilir)	Vazgeçtiğim herhangi bir ürün yok	437	66.4
	Abur cubur çeşitleri	134	20.4
	Hamur işleri	88	13.4
	Tatlı ve tatlı ürünleri	48	7.3
	Meyve	24	3.6
	Et ve et ürünleri	15	2.3
	Baharat ve çeşitleri	15	2.3
	Sebze ve sebze yemekleri	13	2

Katılımcıların %29.6'sı koronavirüsten koruyan herhangi bir besin veya besin takviyesi olduğuna inanmamaktadır. Yaklaşık her üç bireyden ikisi (%64.1) C vitamininin koronavirüse karşı koruma sağladığını düşünmektedir (Tablo 4). Koronavirüse karşı koruyucu etkisi olduğu düşünülen diğer besin veya besin takviyelerinin başında sırasıyla sarımsak (%40.9), ilik suyu (%29.6), bal (%29), sirke (%28.9), zencefil (%24.2) ve pekmez (%21.7) gelmektedir. Katılımcıların yaklaşık %70'i en az bir besin veya besin takviyesinin koruma sağladığını belirtmekle birlikte, %49.4'ü salgın sürecinde herhangi bir besin takviyesi kullanmadığını ifade etmiştir. Diğer yandan besin takviyesi kullananların özellikle C vitamini (%73) ve D vitamini (%40.5) tercih ettikleri görülmektedir.

Dünya genelinde COVID-19 pandemisi süresince bağışıklık sistemini destekleyici gıda takviyesi pazarı da diğer gıda sistemleri (gıda güvenliği, gıda güvencesi ve sürdürülebilirlik) gibi krizden etkilenmiştir. Tüketiciler özellikle C vitamini ve bitkisel takviyelere yönelmiş, bu durum besin takviyelerinin piyasada hızla tükenmesine yol açmıştır (Galanakis ve ark., 2020). Çalışmada takviye olarak en çok C ve D vitamininin tercih edildiği görülmektedir (Tablo 4). A, C, E ve D vitaminlerinin bağışıklık sistemini desteklediği, soğuk algınlığı ve ayrıca virüsün hücreye girişini sağlayan ACE2 aktivitesini sınırlayarak SARS-COV-2'ye karşı koruyucu etki sağladıkları bilinmektedir (Muscogiuri ve ark., 2020; Galanakis ve ark., 2020; Carr ve Maggini, 2017). Klinik çalışmalarda, D vitamininin COVID-19 hastalarında ortaya çıkan sitokin fırtınasını baskılayarak hastalığın daha hafif seyretmesini sağladığı belirlenmiştir (Daneshkhah ve ark., 2020).

Vitaminlerden sonra en fazla kullanılan besin takviyesi probiyotik ve prebiyotik ürünleridir (%20). Probiyotik metabolitlerinin COVID-19 üzerine antiviral etkisi konusunda çalışmalar bulunmaktadır (Anwar ve ark., 2021). Probiyotik alımının COVID-19'un hastalık ve ölüm oranlarını düşürebileceği ve hastalığın şiddetini azaltabileceği bildirilmektedir (Kurian ve ark., 2021).

Çalışmada COVID-19'a karşı koruma sağladığına inanılan ve tüketilen besin ve besin takviyeleri arasında zencefil, zerdeçal, sarımsak, kara mürver ekstresi, kantaron yağı, kekik yağı vb. yer almaktadır (Tablo 4). Yapılan çalışmalar karabiber, zencefil, sarımsak, soğan gibi bitkisel gıdalarda bulunan biyoaktif bileşenlerin COVID-19'a karşı koruma sağlayabileceğini ortaya koymuştur (Rajagopal ve ark., 2020; Donma ve Donma, 2020; Williamson ve Kerimi, 2020). Ayrıca pek çok bitkisel gıdada bulunan polifenollerin viral replikasyonu önlediği, viral spike proteinini yıkıma uğrattığı ve SARS-COV-2 proteazı inhibe ettiği bildirilmektedir (Galanakis ve ark., 2020). Örneğin kara mürverin grip ve soğuk algınlığına bağlı semptomların giderilmesinde faydalı olduğu ve üst solunum yolu hastalıklarına karşı koruyucu etki gösterdiği bilinmektedir (Alıç ve ark., 2021). Kara mürverin COVID-19'a karşı etkin olduğunu gösteren bir çalışma bulunmamaktadır, ancak diğer virüslere karşı etkili olduğundan COVID-19'a da etkili olabileceği düşüncesiyle salgın döneminde kara mürver ekstresi satışının arttığı bildirilmektedir (Macit, 2020).

Katılımcıların %14.1'i salgın süresinde besin takviyesi olarak çinko kullandığını belirtmiştir. Çinko bağışıklık sistemi fonksiyonları için gerekli bir iz elementtir. Çinkonun, SARS (Severe Acute Respiratory Syndrome) koronavirüsün RNA polimeraz aktivitesini ve virüsün hücre kültüründe çoğalmasını engellediği bildirilmiştir (Muscogiuri ve ark., 2020).

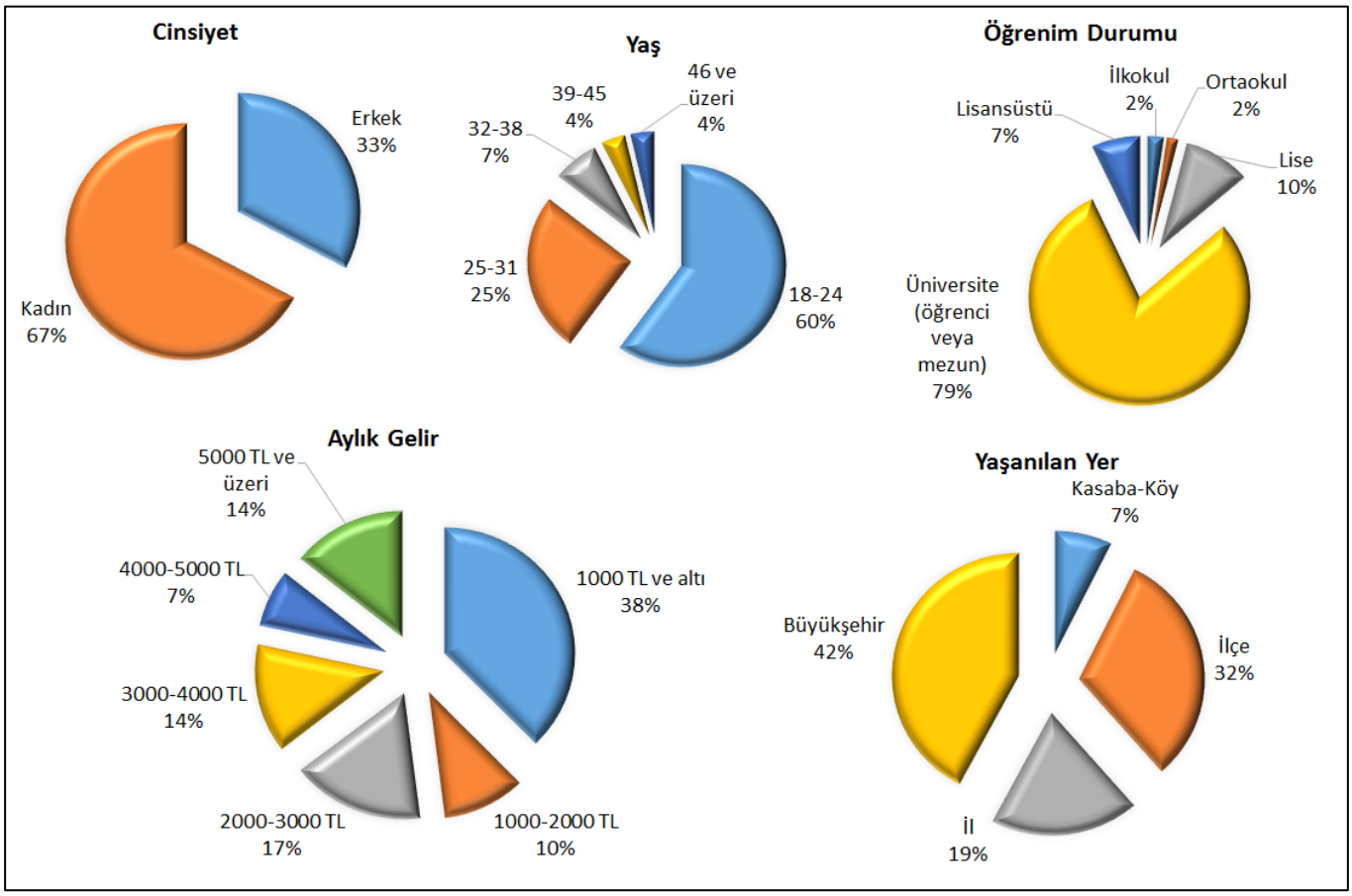
Katılımcıların %86.1'i bağışıklık sistemini güçlendirmek amacıyla besin takviyesi kullandığını belirtmiştir. Besin takviyeleri bağışıklık sisteminin güçlendirilmesine, COVID-19 veya diğer viral enfeksiyonların tedavisine katkıda bulunabilir. Ancak, bilinçsiz veya aşırı miktarlarda kullanımları halinde toksisite oluşabileceği veya sağlığa zararlı yan etkiler ortaya çıkabileceği unutulmamalıdır (Çağındı ve ark., 2022).

Katılımcıların %39.5'i salgın sürecinde herhangi bir kaynaktan gelen beslenme tavsiyelerinden etkilenmediklerini ifade etmişlerdir. Diğer yandan sosyal medyanın (%31.9) da en az doktor tavsiyesi (%32) kadar beslenme davranışlarına yön verme açısından etkili olduğu anlaşılmaktadır. Sosyal medyadan sonra üçüncü sırada televizyon programları yer almaktadır (%20.3). İngiltere'de Goodyear ve ark. (2021) tarafından yapılan bir çalışmada, özellikle kapanma süresince olmak üzere salgın döneminde sosyal medya kullanımının arttığı, sosyal medyanın bireylerin egzersiz, diyet ve yaşam kalitesini artırmaya yönelik bilgilere erişimini kolaylaştırdığı, arkadaş, aile bireyleri ve sosyal gruplar içinde bu konularla ilgili etkileşime fırsat yarattığı bildirilmektedir.

Tablo 4. Katılımcıların besin takviyesi kullanımına ilişkin sorulara verdikleri cevapların frekans ve % dağılımı

Table 4. Frequency and % distribution of the responses given by the participants to the utilization of nutritional supplements questions

		n	%
Koronavirüsten koruduğuna inandığınız besin/takviye hangisidir? (Birden fazla seçenek işaretlenebilir)	Koronavirüsten koruyan bir besin/takviye olduğuna inanmıyorum	195	29.6
	C vitamini	422	64.1
	Sarımsak	269	40.9
	İlik suyu	99	29.6
	Bal	191	29
	Sirke	190	28.9
	Zencefil	159	24.2
	Pekmez	143	21.7
	Zerdeçal	112	17
	Kefir	104	15.8
	Soğan suyu	61	9.3
	Kekik yağı	43	6.5
Salgın sürecinde takviye kullandınız mı?	Evet	333	50.6
	Hayır	325	49.4
Salgın sürecinde hangi besin takviyesini kullandınız? (Birden fazla seçenek işaretlenebilir)	C vitamini	243	73
	D vitamini	135	40.5
	Multivitamin	85	25.5
	Probiyotik-prebiyotik	70	21
	Balık yağı	55	16.5
	Sarımsak ekstratı	54	16.2
	Çinko	47	14.1
	Zencefil ekstresi	31	9.3
	Kara mürver ekstresi	21	6.3
	Beta glukan	15	4.5
	Kantaron yağı	9	2.7
Salgın sürecinde kullandığınız takviyelerini hangi amaçla kullandınız? (Birden fazla seçenek işaretlenebilir)	Bağışıklığımı sistemimi güçlendirmek için	273	86.1
	Sağlıklı olduğunu düşündüğüm için	115	36.3
	Mevcut hastalığım için kullanıyorum	93	29.3
	Diğer	47	14.8
Salgın sürecinde hangi kaynaklardan aldığınız beslenme tavsiyeleri sizin beslenme alışkanlıklarınıza yön verdi? (Birden fazla seçenek işaretlenebilir)	Etkilenmedim	259	39.5
	Doktor takviyesi	210	32
	Sosyal medya	209	31.9
	Televizyon programları	133	20.3
	Aile/Akraba	130	19.8
	Arkadaş	73	11.1



Şekil 1. Katılımcılara ait demografik özellikler

Figure 1. Demographic characteristics of the participants

Sonuç

Bu çalışmada COVID-19 salgınında bireylerin gıda temini, gıda hijyeni ve beslenme davranışlarını belirlemek amacıyla 24 sorudan oluşan bir anket uygulanmıştır. Ankete verilen cevaplara göre salgın döneminde gıda temini için en fazla market alışverişi tercih edilmiş ve en fazla bakliyat stoklanmıştır. Satın alma sırasında ürünlerin besin değerine salgın öncesine göre daha fazla dikkat edilmiş ve öncelikli olarak ambalajlı gıdalar tercih edilmiştir. Taze meyve ve sebzelerin temizlenmesinde en fazla kullanılan yöntem akan su altında yıkama ve sirkeli suda bekletmedir. Genellikle bireylerin salgın döneminde daha sağlıklı beslenme eğiliminde oldukları, bağışıklık sistemini güçlendirmek için başta C ve D vitaminleri olmak üzere gıda takviyesi kullandıkları anlaşılmaktadır. Ayrıca sosyal medya ve televizyon programları bireylerin beslenme davranışlarına yön verme açısından etkili olmuştur.

Sonuç olarak 2020 yılının Mart ayında başlayan ve tüm dünyayı etkisi altına alan COVID-19 salgını aradan geçen iki yılı aşkın sürede, alınan tedbirler ve yapılan aşılama çalışmaları sayesinde dünya genelinde şiddetinin önemli ölçüde azaltmakla birlikte günümüzde devam etmektedir. Bu çalışmada elde edilen sonuçlara göre, COVID-19 veya olası başka salgınlarda toplumun beslenme, besin takviyesi kullanımı ve gıda hijyeni konularında doğru bilgilendirilmesine, özellikle kısa sürede çok kişiye ulaşma potansiyelinden dolayı sosyal medya ve televizyon programlarında bilimsel olarak doğrulanmış bilgilerin paylaşılmasına dikkat edilmesi önerilmektedir.

Etik Standart ile Uyumluluk

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

Etik izin: Bu çalışma, Sakarya Üniversitesi Fen ve Mühendislik Bilimleri Etik Kurulu Başkanlığı'nın 13.05.2022 tarihli ve 25 sayılı toplantısında alınan 04 no'lu karar ile etik açıdan onaylanmıştır.

Finansal destek: -

Teşekkür: -

Açıklama: -

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 8(4), 312-320 (2022) • https://doi.org/10.3153/FH22029 Research Article

The effect of heat treatment on the nutritional and antioxidant content of different milk types

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Cite this article as:
Gürel Gökmen, B., Taslak, H., Özcan, O, Sivas, G.G., Yılmaz Karaoğlu, S., Tunalı Akbay, T. (2022). The effects of heat treatment on the nutritional and antioxidant content of different milk types. *Food and Health,* 8(4), 312-320. https://doi.org/10.3153/FH22029

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Submitted: 21.04.2022
Revision requested: 20.06.2022
Last revision received: 07.07.2022
Accepted: 13.08.2022
Published online: 14.09.2022

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

ABSTRACT

Heat treatments may cause some chemical and physicochemical changes in milk, although milk is a heat-stable system. Heat treatments can cause different changes in different types of milk. This study aimed to compare the effects of pasteurization and boiling on goat and cow milk's macro-molecular contents, glutathione levels, and superoxide dismutase activities. The protein level of both types of milk decreased with the pasteurization process, and boiling also reduced the protein level of goat milk. Both heat treatments reduced superoxide dismutase activity and glutathione levels in both types of milk. While the boiling process did not change the cow's milk lactose level, it increased the goat milk lactose level. It was determined that pasteurization reduced the lactose level in both types of milk. Pasteurization did not change the fat level in cow milk but decreased the fat level in goat milk. In conclusion, cow milk was less affected by these heat treatments, which can be attributed to having large fat globules, high lactose concentration, and high heat resistance protein content compared to goat milk.

Keywords: Milk, Boiling, Pasteurization, Antioxidants, The Nutritional Content of Milk

Introduction

Milk and dairy products are the major sources of protein, fat, carbohydrate, mineral, and vitamin in the human diet (Kliem et al. 2013). The macronutrients of milk are protein, fat, and carbohydrate and micronutrients are minerals and vitamins. The milk macronutrient content varies depending on the animal's diet, the season the milk is collected, and the type of animal. When the nutrient content of goat and cow milk is compared, there is a structural difference between them. Goat milk is richer in protein and fat; on the other hand, cow milk is rich in lactose content. Goat and cow milk have different biochemical properties because of these differences in nutritional content. Goat milk is less allergenic compared to cow milk, due to the low α-s1 casein and β-lactalbumin protein levels in goat milk. The goat milk fat globules are small in size (<3.5 μm), have a homogeneous structure and are easier to digest compared to other animal-based milk. Goat milk also has a higher oligosaccharide (25-30 mg/100 mL) concentration when compared to cow milk oligosaccharide concentration (2-3 mg/100 mL). These oligosaccharides stimulate the release of interleukin-2, increase the growth of bifidobacteria and help the nervous system to develop by elevating the ability to make long-chain polyunsaturated fatty acids (Altun and Sarici 2017).

Milk has antioxidant effects in addition to nutritional benefits. The antioxidant properties of milk are due to its high-quality protein content (Korycka-Dahl, Richardson, and Hicks 1979; Pocius, Clark, and Baumrucker 1981). It has been suggested that glutathione (GSH) and superoxide dismutase (SOD) are the antioxidant parameters for the oxidative stability of milk (Granelli, Björck, and Appelqvist 1995; Talukder et al. 2015). Glutathione is the non-protein sulfhydryl compound in mammalian cells and is generally considered a good indicator of the scavenging of reactive oxygen species (Talukder et al. 2015). Dismutation of superoxide anion by SOD may be of importance in preventing lipid peroxidation (Granelli et al. 1995). It has been observed that the GSH and SOD are excreted into the milk from mammary secretory cells. SOD is only found in skim milk fractions of cow milk, with concentrations ranging from 0.15 mg to 2.4 mg/L. (Khan et al. 2019). Öner et al. reported that goat milk contains higher free radical scavengers than cow milk. They also stated that dry matter, protein, and fat levels did not correspond to the antioxidant capacity (Öner, Sanlıdere-Aloglu, and Dedebaş 2011).

Heat treatment of raw milk reduces the microbial population, inactivates enzymes, and minimizes chemical reactions and physical changes during storage. Pasteurization, sterilization, ultra-high temperature, and high-temperature processing are accepted methods for extending the shelf life of dairy products in the dairy industry (Stojanovska et al. 2017). Pasteurization is one of the most commonly used techniques for the processing of fluid milk. During pasteurization, milk is exposed to a certain heat treatment for a specific period (Khan et al. 2017). It has been demonstrated that industrial pasteurization may cause remarkable modifications in milk structure, like enzyme inactivation, protein denaturation, modification, masking or unmasking cross-linking between proteins and other food components, such as lactosylation, lipid oxidation products, and the generation of Maillard reaction products (Lamberti et al. 2017). During pasteurization, approximately 5 - 15% of milk whey protein is denatured by the pasteurization process. Pasteurization does not cause dephosphorylation and reduction in pH and ionic calcium and causes a negligible effect on the heat-sensitive water-soluble vitamins (Deeth and Lewis 2017). In addition, SOD activity and GSH level are also affected by the heat treatments (Hicks, Bucy, and Stofer 1979; Li et al. 2018; Martysiak-Żurowska, Puta, and Kiełbratowska 2019). Much is known about the industrial thermal processing of food whereas there is a lack of information about the impact of domestic heat treatments (boiling) on the biochemical quality of milk (Lamberti et al. 2017). Boiling is the mildest heat treatment given to milk. Boiling aims to reduce the growth of psychrotrophic bacteria that may release heat-resistant proteases and lipases into the milk if allowed to reach high levels (Deeth and Lewis 2017). Boiling does not denature the milk whey proteins, does not affect the heat stability of milk as measured by the heat coagulation time at 130°C (Coghill, Mutzelburg, and Birch 1982), and reduces lipase activity by about 50% (Humbert et al. 1985).

Although heat treatments are effective in removing germs from milk, it is known that they change the milk's biochemical composition. However, no research has been conducted to determine which types of milk are more influenced by these processes. Therefore, this study aimed to see how different heat treatments affected the nutritional content of cow and goat milk, as well as glutathione levels and superoxide dismutase activity for antioxidant characteristics.

Materials and Methods

Milk Samples

Raw and pasteurized milk were obtained from the same local brand (Berk Süt, Kocaeli, Turkey). Each milk sample was purchased as one liter and used as a pool. The study was carried out by taking 8 different samples from cow and goat milk. Raw milk was boiled at 100°C for 1 minute, and pasteurized milk of the same brand was purchased. Milk samples were divided into six groups designated as follows; RC: raw

cow milk, BC: boiled cow milk, PC: pasteurized cow milk, RG: raw goat milk, BG: boiled goat milk, and PG: pasteurized goat milk.

Total Protein Determination

Cow and goat milk total protein levels were determined according to the method of Bradford (Bradford 1976). The fat (cream) layer was removed before the milk protein determination. Subsequently, the skim-milk samples were incubated with Coomassie Brillant Blue dye solution. The absorbance of the blue colour formed at the end of the incubation was recorded at 595 nm.

Lactose Determination

The milk lactose level was detected by the colorimetric picric acid method (Khramov, Kolomeĭtseva, and Papichev 2008). The milk samples were mixed with an aqueous and saturated solution of picric acid (Sigma-Aldrich, 197378) and then incubated in a boiling water bath. At the end of incubation, the color of the solution changes from yellow to red. The quantity of lactose in milk is directly proportional to the reddish color intensity in the solution.

Fat Determination (Lucas et al. 1978)

The milk samples were drawn into a capillary tube (ISOLAB, length of 75 mm, wall thickness of 0.2 mm) and sealed with a lighter flame for fat content analysis. Each milk sample performed a double determination. Sealed tubes were centrifuged by a hematocrit centrifuge for 40 minutes. The percentage of the fat (cream) layer was calculated by measuring the fat (cream) layer and the full milk length with a ruler. The calculated percentage of the fat (cream) layer is linearly related to the fat content in goat and cow milk.

Determination of GSH Level

GSH level was determined by the method of Beutler (Beutler 1975). The colored product resulting from the reaction of the sulfhydryl groups with Ellmann's reagent, 5-5 'dithiobis 1-2 nitrobenzoic acid (Merck, D8130) was evaluated spectrophotometrically. Results were presented as % mg glutathione using an extinction coefficient of 13600 $M^{-1}cm^{-1}$.

Determination of SOD Activity

SOD activity was measured by the method of Mylorie et al. (Mylroie et al. 1986). This method measures the ability of SOD to increase the effect of riboflavin (Sigma-Aldrich, R9504) sensitized photo-oxidation of o-dianisidine (Sigma-Aldrich, D3252). The activity of SOD is generated by illuminating the reaction mixture that contains o-dianisidine dihydrochloride and riboflavin with light from a fluorescent lamp. The oxidation of o-dianisidine, which is sensitized by riboflavin, is enhanced by superoxide dismutase, and the increase in the absorbance is linearly dependent on superoxide dismutase concentration. The absorbance of the resulting colored product is evaluated spectrophotometrically at 460 nm. Results were presented as kU/mL.

pH Measurement and Milk Energy Level

Cow and goat milk pH levels were determined by a benchtop pH meter (Mettler Toledo FE20-Basic Five Easy). The milk's macro components are multiplied by the suitable conversion factor to determine the total level of energy contained in the milk samples. Conversion factors are 4 kcal/dL for protein values, 9 kcal/dl for fat values, and 4 kcal/dl for lactose values, respectively. The total energy content was calculated as follows:

Total energy (kcal/dl)= (Fat x 9)+(Protein x 4)+(Lactose x4) (García-Lara et al. 2012).

Statistical Analysis

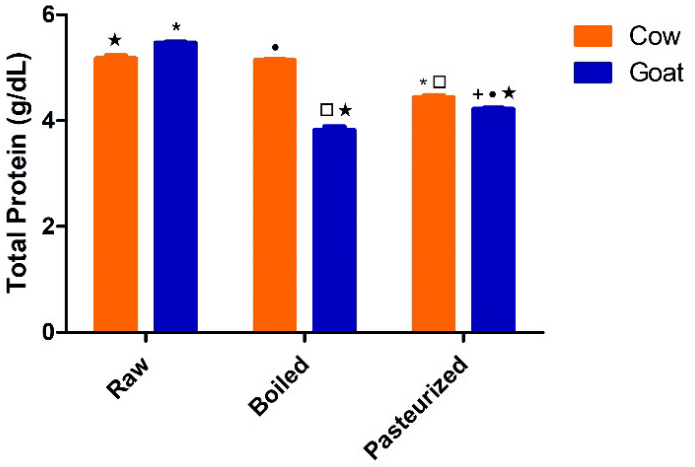
Graph Pad Prism 5.0 (Graph Pad Software, San Diego, CA, USA) statistical package program was used for the statistical analysis. Groups of data were analyzed by using ANOVA followed by Tukey's multiple comparison tests. Values of $p<0.05$ were regarded as significant. All data were given as mean and standard deviation.

Results and Discussion

The effect of heat treatments on milk macronutrients and antioxidant capacity has been the subject of many researchers over the years (Hicks et al. 1979; Coghill et al. 1982; Villamiel and de Jong 2000; García-Lara et al. 2012; Deeth and Lewis 2017; Khan et al. 2017). Milk heat treatments are used to eliminate microorganisms that might spoil the milk and to maintain its safety for daily consumption. Heat treatments, on the other hand, may change the nutritional characteristics of milk. In this study, the nutritional composition, GSH level, and SOD activity changes of cow and goat milk were investigated to determine the most affected milk type.

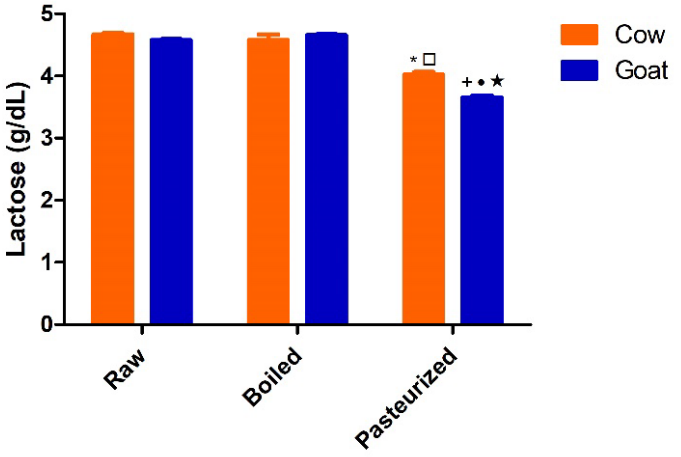
The total protein level of raw goat milk was significantly higher than raw cow milk ($p<0.05$, Figure 1). Raw cow milk's total protein level was not significantly changed with boiling, while pasteurization reduced the total protein level by 14%. However, after boiling and pasteurization, the total protein level of raw goat milk significantly decreased ($p<0.05$, Figure 1). Raw cow and goat milk lactose levels were not significantly changed by boiling (Figure2). Pasteurization significantly reduced the lactose level of both cow and goat milk ($p<0.05$). For goat milk, the decline was 20%, while for cow milk, it was 13%. Heat treatment had no significant effect on

the fat level of raw cow milk. While the fat level of goat milk did not change significantly after boiling, it decreased significantly after pasteurization ($p<0.05$, Figure 3).



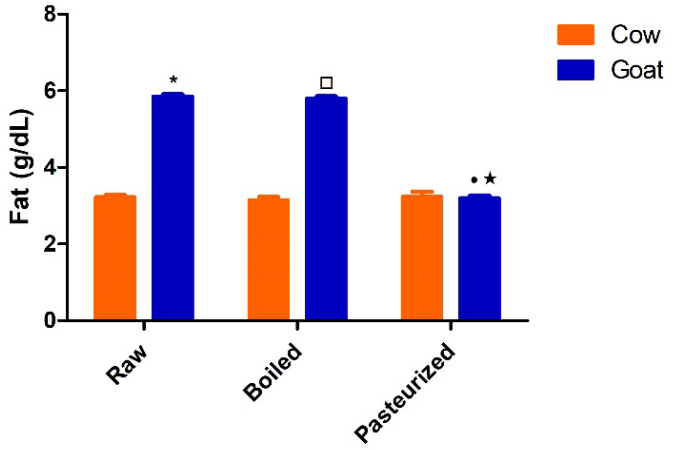
*: $p<0.05$ compared to Raw Cow Milk, □: $p<0.05$ compared to Boiled Cow Milk, +: $p<0.05$ compared to Pasteurized Cow Milk, ★: $p<0.05$ compared to Raw Goat Milk, •: $p<0.05$ compared to Boiled Goat Milk, n= 8

Figure 1. Total protein levels of cow and goat milk after the thermal processes



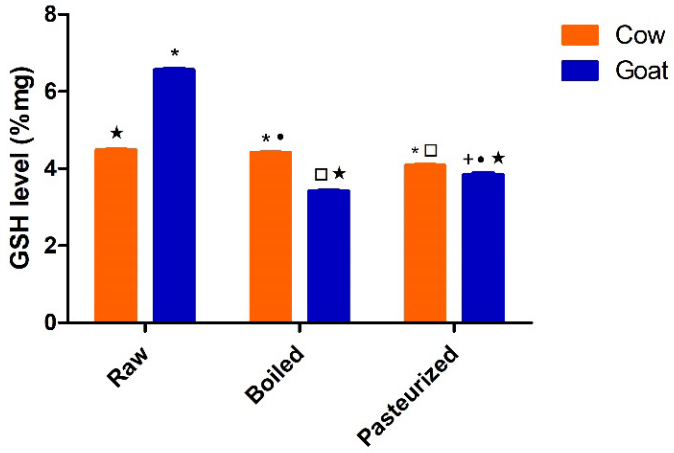
*: $p<0.05$ compared to Raw Cow Milk, □: $p<0.05$ compared to Boiled Cow Milk, +: $p<0.05$ compared to Pasteurized Cow Milk, ★: $p<0.05$ compared to Raw Goat Milk, •: $p<0.05$ compared to Boiled Goat Milk, n= 8

Figure 2. Lactose levels of cow and goat milk after the thermal processes



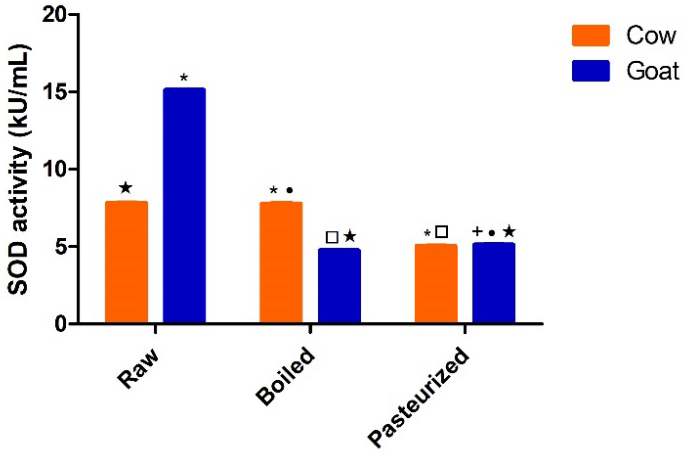
*: $p<0.05$ compared to Raw Cow Milk, □: $p<0.05$ compared to Boiled Cow Milk, ★: $p<0.05$ compared to Raw Goat Milk, •: $p<0.05$ compared to Boiled Goat Milk, n= 8

Figure 3. Fat levels of cow and goat milk after the thermal processes



*: $p<0.05$ compared to Raw Cow Milk, □: $p<0.05$ compared to Boiled Cow Milk, +: $p<0.05$ compared to Pasteurized Cow Milk, ★: $p<0.05$ compared to Raw Goat Milk, •: $p<0.05$ compared to Boiled Goat Milk, n= 8

Figure 4. GSH levels of cow and goat milk after the thermal processes.



*: p<0.05 compared to Raw Cow Milk, □: p<0.05 compared to Boiled Cow Milk, +: p<0.05 compared to Pasteurized Cow Milk, ★: p<0.05 compared to Raw Goat Milk, •: p<0.05 compared to Boiled Goat Milk, n= 8

Figure 5. SOD activities of cow and goat milk after the thermal processes

Raw cow and raw goat milk consists of basic parts (fat, protein and lactose) but goat milk has higher protein and fat level compared to cow milk (Posati & Orr, 1976; Jenness, 1980; Park & Haenlein, 2016). In the study by Khan et al., it was found that protein levels of cow's milk increased after 30 minutes of boiling, while the total protein level decreased with pasteurization. The reason for the increased total protein level was attributed to the decrease in water loss during boiling (Khan et al. 2017). Farrell et al. suggested that boiling milk induces structural changes in protein structure by disrupting intramolecular or intermolecular interactions and disulfide bonds, causing the loss of secondary structure and protein aggregation (Farrell et al. 2004).

Boiling raw milk for more than 5-10 minutes at home causes the milk to lose its structural and content properties. Laszlo et al (2017) advised shorter milk boiling times as the antibiotic residues that can be found in milk of animal origin may be more harmful than microorganisms (László, Lányi, and Laczay 2017). In the literature, there are also many milk-boiling procedures according to the purposes of the study. Condas et al (2012) boiled milk for 1 and two minutes at 100 ºC (Condas et al. 2012). Kilango et al. boiled milk for 5 minute at 95ºC (Kilango et al. 2012). Khan et al. boiled milk for 1 minute at 100ºC (Khan et al. 2017). Joishy et al. boiled milk for 10 minutes at 100ºC (Joishy, Dehingia, and Khan 2019). In this study, raw cow and goat milk samples were boiled for 1 minute at 100ºC. There was no significant change in the total protein level of cow milk at the end of controlled boiling, a decrease was found in the total protein level of goat milk. The heat stability of lactoferrin is such that typical pasteurization processes have little effect on structure (Farrell et al. 2004). The fact that cow's milk contains more lactoferrin than goat's milk causes cow's milk to be less affected by the pasteurization process (Rachman, Maheswari, and Bachroem 2015). The five major proteins of goat milk, α-lactalbumin, β-lactoglobulin, κ-casein, β-casein, and αs2-casein are very similar to their homologs in cow's milk. Goat milk lacks bovine αs1-casein, the most abundant protein in cow's milk. Caseinate micelles of goat milk contain more calcium and inorganic phosphorus, are less soluble and less heat resistant, and lose β-casein more easily than bovine micelles. For this reason, goat's milk proteins are less heat resistant (Jenness, 1980; Montilla & Calvo, 1997; Farrell et al., 2004) .

Khan et al. found that the lactose level of cow's milk increased with boiling and decreased with pasteurization (Khan et al. 2017). In this study, the lactose level was not affected by boiling in raw cow and goat milk, and it was decreased with pasteurization. As heat treatment causes several chemical modifications in milk nutrients, the lactose level of milk can change depending on the severity of heating. Heat treatment causes the degradation of lactose to acids (with a concomitant decrease in pH), isomerization of lactose (e.g., to lactulose), production of compounds such as furfural, and interactions with amino groups of proteins (by Maillard reaction) (Lamberti et al. 2017). Lactose is a reducing sugar that reacts with the amino groups of proteins. Since the proteins in goat milk are less heat resistant, lactose reacts more easily with the proteins in goat milk. With the pasteurization of goat milk, the lactose level decreases more than in cow's milk.

Heat treatments have been found to reduce the diameters of fat globules of cow milk (Villamiel and De Jong 2000). The decrease in the diameter of fat globules does not indicate a change in fat levels. The core of all milk fat globules contains triacylglycerols, polyunsaturated fatty acids, and monounsaturated fatty acids. The small fat globules, which are not heat resistant, are more affected by the boiling and pasteurization process (Jenness 1980). In light of this information, it can be concluded that the small-sized fat globules of goat milk were disrupted as a result of the pasteurization process.

Figures 4 and 5 show the effects of heat treatments on GSH levels and SOD activities in cow and goat milk, respectively. Raw goat milk's GSH level and SOD activity were significantly higher than raw cow's milk ($p<0.05$). The raw cow milk GSH level decreased after boiling and pasteurization. On the other hand, the GSH level decrease of raw goat milk was higher than the cow milk after boiling and pasteurization. Both heat treatments were significantly reduced the SOD ac-

tivity of cow milk ($p<0.05$). On the other hand, the SOD activity of raw goat milk decreased by 68% and 66% by boiling and pasteurization processes, respectively.

One of the most often used methods for processing fluid milk is pasteurization, but another widely used method is boiling the milk at home for use in the home. In this study, the effect of pasteurization and boiling on antioxidant characteristics of cow and goat milk in terms of GSH level and SOD activity were investigated for improved consumption patterns. GSH is an antioxidant that protects cells from the toxic effects of reactive oxygen species such as free radicals, peroxides, and heavy metals, in a tripeptide structure consisting of the amino acids glutamate, cysteine, and glycine. When the amino acid profiles of cow and goat milk were examined, it was found that the amount of glutamate, cysteine, and glycine amino acids in goat milk was higher than the cow milk (Kamal et al., 2007; Barlowska et al., 2011; Medhammar et al., 2012). In this study, the GSH level was higher in raw goat milk compared to raw cow milk. Pasteurization and boiling significantly decreased the high raw goat milk GSH level ($p<0.05$).

SOD is the only antioxidant enzyme that scavenges the superoxide anion by converting this free radical to oxygen and hydrogen peroxide (Wang and Zhang 2015). SOD enzyme is rich in amino acids alanine, glycine, leucine, arginine, serine, and valine amino acids (Folz and Crapo 1994). In the amino acid profiles of cow and goat milk, the amount of alanine, arginine, serine, and valine amino acids were found to be higher in cow milk (Kamal et al., 2007; Barlowska et al., 2011; Medhammar et al., 2012). This situation can be thought of as if the SOD enzyme activity in cow's milk should be more than in goat's milk, but in this study, SOD enzyme activity was found to be higher in goat milk compared to cow milk. This finding is compatible with the studies that show the high SOD activity of goat milk compared to cow milk (Granelli et al. 1995; Li et al. 2018). The antioxidant content of the milk also changes as the macro components of milk decrease as a result of heat treatment. In the study of Li et al., it was determined that heat treatments higher than 75ºC inactivated more than 20% of SOD activity in goat milk. SOD activity is easily affected by temperature, pH, rotation speed, calcium ion concentration, and fermentation time. SOD activity gradually decreased as the storage time increased, the heating temperature and the Ca^{2+} concentration decreased (Li et al. 2018). In the study by Hicks et al. was found that cow milk SOD activity decreased depending on the increasing heat intensity (Hicks et al. 1979). Another study with breast milk revealed that convective heating at temperatures above 66ºC caused significant changes in the activity of antioxidant enzymes in human milk when applied for more than 20 minutes (Martysiak-Żurowska et al. 2019). In this study, heat treatments decreased both raw cow and raw goat milk SOD activity.

Both boiling and pasteurization did not significantly change the pH of cow and goat milk but decreased the cow milk energy level. This energy decrease was higher in the pasteurization process compared to the boiling process (Table 1). In goat milk, pasteurization significantly decreased the energy level ($p<0.05$, Table 2). When the energy levels of the cow and goat milk were compared, the energy level of goat milk was found to be higher than cow milk in raw and boiled samples ($p<0.05$). This difference can be associated with the high fat content of goat milk.

Table 1. Cow milk pH□ and energy level changes (means) after the heat treatments

	Raw Cow Milk	Boiled Cow Milk	Pasteurized Cow Milk
pH	6.76 ±0.16	6.71 ±0.18	6.75 ±0.13
Energy (kcal/mol)	87.64 ±2.7	80.69 ±2.8*	75.98 ±1.6□ Δ.

* $p<0.05$ compared to raw cow milk, □: $p<0.05$ compared to boiled cow milk, n= 8

Table 2. Goat milk pH• and energy level changes (means) after the heat treatments

	Raw Goat Milk	Boiled Goat Milk	Pasteurized Goat Milk
pH	6.75 ±0.17	6.75 ±0.17	6.70 ±0.13
Energy (kcal/mol)	111.8 ±5.6	114.1 ±5.8	101.7 ±1.5• Δ.

• $p<0.05$ Compared to raw goat milk, Δ: $p<0.05$ Compared to boiled goat milk, n=8

Conclusion

Goat milk was more affected by boiling and pasteurization than cow's milk. The reason that cow milk was less affected by these heat treatments can be attributed to having large fat globules, high lactose concentration, and high heat resistance protein content compared to goat milk.

Compliance with Ethical Standard

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

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

Funding disclosure: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgments: -

Disclosure: -

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 8(4), 321-333 (2022) • https://doi.org/10.3153/FH22030 *Research Article*

Evaluation of university students' knowledge, attitudes, and practices on food safety in Bolu, Türkiye

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Cite this article as:
Gökçeel, E., Akoğlu, A. (2022). Evaluation of university students' knowledge, attitudes, and practices on food safety in Bolu, Türkiye. *Food and Health,* 8(4), 321-333. https://doi.org/10.3153/FH22030

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Submitted: 02.06.2022
Revision requested: 29.07.2022
Last revision received: 29.08.2022
Accepted: 05.09.2022
Published online: 20.09.2022

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

ABSTRACT

This study aims to assess the food safety knowledge, attitudes, and practices of university students in Türkiye. A cross-sectional study was conducted with the participation of 630 university students from the departments of Gastronomy and Culinary Arts (GCA), Food Engineering (FE), Nursing (NUR), and Physical Therapy and Rehabilitation (PTR) in Bolu, Türkiye. The GCA students scored the highest (25.46 ±2.85) and the NUR students scored the lowest (21.81 ±3.27) on food safety knowledge. The GCA students scored the highest (51.42 ±5.58) and the NUR students scored the lowest (49.00 ±6.07) on food safety attitudes. The FE students scored the highest (10.12 ±1.69) and the NUR students scored the lowest (9.69 ±1.77) on food safety practices. The students who took a course on food safety had significantly higher scores on food safety knowledge, attitudes, and practices than those who did not. Although the students' food safety scores were above average, they had a lack of knowledge and concerns in many areas. It was emphasized the importance of food safety education to fill the knowledge gaps and thus to prevent foodborne illnesses.

Keywords: University students, Food safety, Knowledge, Attitude, Practice

Introduction

Foodborne diseases, which are generally caused by the consumption of foods contaminated with pathogenic microorganisms or microbial toxins, have clinical manifestations such as life-threatening neurological, hepatic, and renal syndromes and mostly progress with gastrointestinal symptoms (CDC, 2007). Unsafe foods containing harmful bacteria, viruses, parasites, or chemical substances cause more than 200 diseases ranging from diarrhea to cancers. An estimated 600 million – almost 1 in 10 people in the world – fall ill after eating contaminated food and 420,000 die every year, resulting in the loss of 33 million healthy life years (WHO, 2020). Foodborne diseases put a huge burden on the public healthcare system and significantly increase healthcare expenditures. If not controlled, they create significant gaps in the budgets of countries (Egan et al., 2007). Unsafe foods cost low- and middle-income countries US$ 110 billion each year in lost productivity and medical expenses (WHO, 2020). In Türkiye, it is not obligatory to report foodborne illnesses to a specified agency; therefore, data on foodborne infections and intoxications do not reflect the real situation (Şanlıer, 2009).

Food safety is an important issue that needs to be examined in detail in a broad perspective with all stakeholders of the food industry including governments, standing government authority, farmers, food manufacturers, processors, wholesalers, dealers, retail outlets, and consumers. Systematic controls carried out by the food industry to ensure a more conscious service are an assurance in terms of the continuity of food safety. In parallel with this, the continuity of food safety practices can be sustained with the awareness of consumers (Hobbs and Roberts, 2001; McMichael and Schneider, 2011). Consumers are the last element of the food chain. Most food poisoning outbreaks are known to be caused by human handling errors. Reported foodborne illness data show that a significant proportion of foodborne illnesses is attributed to inappropriate food handling in households (Clayton et al., 2002; Greig et al., 2007; Medeiros et al. 2001). A significant portion of food preparation, processing, and storage takes place in the home environment; so, understanding consumer behaviors and educating consumers about the risk of unsafe food processing practices is important to prevent foodborne illnesses (Ergönül, 2013).

Young adults, an important consumer group, can be at an increased risk for foodborne illnesses due to their risky food practices and low level of knowledge on food safety (Chuang et al. 2021). University students, who can be both consumers and food producers in their future professional business lives, play a key role in ensuring food safety. Many studies have reported that university students put their health at risk due to insufficient knowledge, attitudes, and practices about food safety (Ferk et al., 2016; Giritlioglu et al., 2011; Hassan and Dimassi, 2014; Lazou et al., 2012; Luo et al., 2019; Strateva et al., 2017; Şanlier and Konaklioglu, 2012). There is a limited number of studies on food safety for university students in Türkiye (Açıkalın, 2019; Avşar, 2019; İncedal-Sonkaya, 2018). This study, it is aimed to evaluate the food safety knowledge, attitudes, and practice of university students in Türkiye and to reveal the differences between their departments and socio-demographic variables. This study is different from previous studies in that it shows the effect of the food safety course given at the university and the difference between the departments of the students on food safety knowledge, attitudes, and practice.

Materials and Methods

Study Design and Participants

A cross-sectional study was conducted among 630 university students from the departments of Gastronomy and Culinary Arts (GCA), Food Engineering (FE), Nursing (NUR), and Physical Therapy and Rehabilitation (PTR), Bolu Abant Izzet Baysal University, Bolu, Türkiye. The departments both offering and not offering a compulsory food safety course and a career in the field of food were included in the study in order to make a comparison in this regard. This study was carried out between February and June 2019. The research data was collected through a questionnaire and face-to-face interviews. The students were informed about the study, and they participated in the research on a voluntary basis. The time devoted to administering the questionnaire ranged between 10 and 15 minutes per classroom. The Ethics Committee of Human Studies in Social Sciences of Abant Izzet Baysal University approved the study (no: 2018/329).

Questionnaire Design

The questionnaire was developed based on the knowledge, attitudes, and practices (KAP) model. The food safety KAP questionnaire was designed based on the related literature (Al-Shabib et al., 2017; Luo et al., 2019; Osaili et al., 2011). The questionnaire was finalized after a pilot study and repeated discussions with experts. Cronbach's alpha value was 0.73 for the knowledge scale, 0.83 for the attitudes scale, and 0.73 for the scale of the practice. When the scale reliability was analyzed based on the scale sections, it was found that the internal consistency of the KAP questionnaire was acceptable.

The questionnaire was divided into four sections: (a) socio-demographic characteristics, (b) food safety knowledge, (c)

food safety attitudes, and (d) food safety practices. Socio-demographic data consisted of gender, age, department, grade, history of foodborne illness, food safety training, and frequency of eating out. Food safety knowledge was assessed using 32 items. Each item was scored 1 if the answer was right and scored 0 if the answer was wrong or the answer choice "I do not know" was selected. The total score ranged from 0 to 32, and the higher the score the higher the level of knowledge on food safety. Food safety attitudes were assessed using 12 items. Each item consisted of five levels with a score ranging from 1 (strongly disagree) to 5 (strongly agree). The total score for this part ranged from 12 to 60, and the higher the score the higher the concern about food safety. Food safety practices were assessed using 14 items. The correct answers given to the practice statements were coded as 1, while the other options were coded as 0. The correct scale response differs for each statement. The total score for these items ranged from 0 to 14 and the higher the score the better the food safety practices.

Data Analysis

The data were analyzed using SPSS software (Statistical Package for the Social Sciences, version 11.0, SPSS Inc, III, USA). The food safety scores were expressed as mean and standard deviation (SD). Independent sample t-test and one-way analysis of variance (ANOVA) were carried out to identify the relationship between the participants' socio-demographic characteristics and their KAP scores. The frequency and percentages of the responses in each category were calculated and tabulated. In all analyses, the statistical significance was set at $p \leq 0.05$.

Results and Discussion

Socio-Demographic Characteristics of the Participants

Table 1 presents the university students' socio-demographic characteristics. The majority of the respondents were female (80 %). This can be explained by the structure and types of the departments. Most of the students were studying at the department of Nursing (38.9 %) followed by Gastronomy and Culinary Arts (26.0 %), Food Engineering (22.4 %), and Physical Therapy and Rehabilitation (12.7 %). 14.6 % of them were freshmen, 30.5% were sophomores, 30.2 % were juniors, and 24.8 % were seniors. 21.7 % of the participants stated that they had a history of foodborne illness, and 61.7 % of them had taken food safety training. About half of the participants (55.6 %) reported the frequency of eating out as once or twice a week.

Table 1. Socio-demographic characteristics of the participants (n=630)

Demographic Variables		Total (n=630)	Departments			
			Food Engineering	Gastronomy and Culinary Arts	Nursing	Physical Therapy and Rehabilitation
Gender	Male	126 (%20.0)	19 (%13.5)	62 (%37.8)	30 (%12.2)	15 (%18.8)
	Female	504 (%80.0)	122(%86.5)	102 (%62.2)	215 (%87.8)	65 (%81.2)
Age	18 years old	16 (%2.5)	0 (%0)	8 (%4.9)	8 (%3.3)	0 (%0.0)
	19 years old	68 (%10.8)	10 (%7.1)	21 (%12.8)	32 (13.1)	5 (%6.3)
	20 years old	165 (%26.2)	30 (%21.3)	39 (%23.8)	64 (%26.1)	32 (%40.0)
	21 years and older	381 (%60.5)	101 (%71.6)	96 (%58.5)	141 (%57.6)	43 (%53.8)
Grade	Freshman	92 (%14.6)	9 (%6.4)	36 (%22.0)	46 (%18.8)	1 (%1.3)
	Sophomore	192 (%30.5)	41 (%29.1)	38 (%23.2)	74 (%30.2)	39 (%48.8)
	Junior	190 (%30.2)	42 (%29.8)	63 (%38.4)	47 (%19.2)	38 (%47.5)
	Senior	156 (%24.8)	49 (%34.8)	27 (%16.5)	78 (%31.8)	2 (%2.5)
History of foodborne illness	Yes	137 (%21.7)	30 (%21.3)	52 (%31.7)	35 (%14.3)	20 (%25.0)
	No	493 (%78.3)	111 (%78.7)	112 (%68.3)	210 (%85.7)	60 (%75.0)
Having food safety training	Yes	389 (%61.7)	88 (%62.4)	163 (%99.4)	137 (%55.9)	1 (%1.3)
	No	241 (%38.3)	53 (%37.6)	1 (%0.6)	108 (%44.1)	79 (%98.8)
Frequency of eating out	Everyday	158 (%25.1)	46 (%32.6)	44 (%26.8)	49 (%20.0)	19 (%23.8)
	1-2 days per a week	350 (%55.6)	76 (%53.9)	102 (%62.2)	130 (%53.1)	42 (%52.5)
	1-2 days per a month	122 (%19.4)	19 (%13.5)	18 (%11.0)	66 (%26.9)	19 (%23.8)

Association Between The Participants' Scores and Socio-Demographic Characteristics

The students' mean scores for knowledge, attitude, and practices were found to be 23.63 ±3.61 (72%), 49.73 ±6.33 (79%), and 9.86 ±1.70 (70%), respectively (Table 2). Based on these results, it can be asserted that the student's knowledge, attitude, and practice scores were above average. Previous studies also reported similar results in this regard (Byrd-Bredbenner et al., 2007, Garayoa et al., 2005, Hassan and Dimassi, 2014, Lazou et al., 2012; Sharif and Al-Malki, 2010). For instance, Lazou et al. (2012) reported the mean score for food safety knowledge as 60% among Greek university students, Byrd-Bredbenner et al. (2007) as approximately 60% among American university students, and Hassan and Dimassi (2014) as 53.6% among Lebanese university students. On the other hand, the university students in Türkiye were found to have higher scores for food safety attitudes and practices compared to those reported in many previous studies (Hassan and Dimassi, 2014; Lazou et al., 2012; Unklesbay et al., 1998).

When the students were compared in terms of their departments, it was found that the GCA students had the highest score in food safety (25.46 ±2.85) and the FTR students the lowest (21.81 ±3.27). The differences among the four departments were statistically significant in terms of food safety knowledge ($p=0.001$. $p<0.05$). As for the food safety attitudes, the GCA students scored the highest (51.42 ±5.58) and the FE students scored the lowest (49.00 ±6.07). The differences among the four departments were statistically significant in terms of food safety attitudes ($p=0.001$. $p<0.05$). However, the differences among the four departments were not statistically significant in terms of food safety practices ($p=0.122$. $p>0.05$). The FE students were found to have the highest score for food safety practices (10.12 ±1.69) and the NUR students the lowest (9.69 ±1.77). The knowledge and attitude scores of the GCA students were significantly higher than those of the others ($p=0.000$, $p=0.001$. $p<0.05$). This can be attributed to the fact that the GCA students have greater access to knowledge of nutrition and food hygiene. Students studying in health sciences (NUR and FTR), on the other hand, got the lowest score in food safety knowledge and practice. Luo et al. (2019) also compared the food safety knowledge and practice scores of the students studying at the departments of education, medicine, and nursing in China and reported that the nursing students had the lowest scores, which is in agreement with the results of the present study. Contrary to this study, Lazou et al. (2012) in their study on Greek university students reported that students from health-related faculties, in general, had the highest average food safety practices and knowledge scores. They stated that this result could be attributed to the presence of modules relevant to food hygiene in health-related curricula. In our country, a food safety course is not a compulsory course in health-related departments. Additionally, Istanbullugil and Gürbüz (2019), in their study on university students in Bishkek, reported that there was no statistically significant difference between the departments in terms of the level of food safety awareness among students.

The female students were found to have a statistically significantly better food safety attitude than the males ($p=0.002$. $p<0.05$). This could be due to the fact that females tend to behave more responsibly in cooking, food handling, and kitchen hygiene (Chuang et al., 2021). This is even more evident in developing countries such as Türkiye. This result was in line with those reported in previous studies in which female university students outperformed males (Hassan and Dimassi, 2014; Lazou et al., 2012). On the other hand, gender did not have a significant effect on the student's scores for food safety knowledge and practices.

The food safety knowledge and practice scores of the senior students were significantly higher than the other participants ($p=0.000$ and $p=0.004$, respectively. $p<0.05$). This may be due to the fact that senior students have a long experience in their department, have already taken food safety courses, and do internships. As Chuang et al. (2021) asserted, consumers' food safety knowledge could increase over time as they are exposed to more learning opportunities and practice the recommended behaviors more. Previous studies also reported higher scores among seniors (Hassan and Dimassi, 2014; Lazou et al., 2012; Osaili et al., 2011).

Food safety knowledge, attitude, and practice mean scores of the students who had had food safety training were significantly higher than those of the students who had not ($p=0.000$, $p=0.002$, and $p=0.020$, respectively. $p<0.05$). This finding confirms the previous studies surveying university students in various countries (Chuang et al., 2021; Courtney et al., 2016; Hassan and Dimassi, 2014; Lazou et al., 2012). An important reason that the students in previous studies had low food safety knowledge was the lack of food safety training or courses on food handling (Chuang et al., 2021). Several studies highlighted the importance of food safety education and the need for teaching children food safety at school (Jevsnik et al., 2008; Lange et al., 2018; Marklinder et al., 2020). In Türkiye, the FE and GCA departments offer hygiene and food safety courses on a compulsory basis, while other departments usually offer them as elective courses. Such courses generally aim to provide students with knowledge about hygiene and sanitation rules, possible risk factors, and precautions to be taken in the preparation and service of foods. Offering food safety education as part of the school

curriculum could enable the development of safe habitual food handling practices and the dissemination of food safety information (Rössvoll et al., 2013). Since there is no food safety education at the primary and high school levels in Türkiye, it is of great importance to offer food safety education to university students.

Food safety attitudes of the students who had a history of foodborne illness were found to be higher than those who did not ($p=0.029$. $p<0.05$). On the other hand, the frequency of eating out was found to have no significant effect on food safety knowledge, attitude, and practice scores ($p > 0.05$).

Table 2. Total food safety knowledge, attitude and practice scores of participants by soci-demographic characteristics

Demographic Variables			Food safety knowledge		Food safety attitude		Food safety practice	
			Mean scores ± SD	P	Mean scores ± SD	P	Mean scores ± SD	P
		Total	**23.63 ± 3.61 (Range 0-32)**		**49.73 ± 6.33 (Range 12-60)**		**9.86 ± 1.70 (Range 0-14)**	
Gender	Male	126	23.26 ±4.24	0.261	47.73 ±8.34	0.002*	9.61 ±1.81	0.076
	Female	504	23.72 ±3.43		50.23 ±5.62		9.92 ±1.67	
Age	18 years old	16	22.12 ±4.36	0.006*	46.31 ±10.87	0.166	8.93 ±2.20	0.054
	19 years old	68	22.66 ±3.98		50.04 ±6.70		9.60 ±1.72	
	20 years old	165	23.35 ±3.77		49.61 ±6.36		9.82 ±1.57	
	21 years and older	381	23.98 ±3.38		49.87 ±5.99		9.96 ±1.72	
Departments	Food Engineering	141	24.06 ±3.41	0.000*	49.00 ±6.07	0.001*	10.12 ±1.69	0.122
	Gastronomy and	164	25.46 ±2.85		51.42 ±5.58		9.87 ±1.66	
	Culinary Arts	245	22.74 ±3.70		49.17 ±6.78		9.69 ±1.77	
	Nursing	80	21.81 ±3.27		49.30 ±6.31		9.87 ±1.56	
	Physical Therapy and Rehabilitation							
Grade	Freshman	92	22.60 ±3.70	0.000*	49.17 ±7.62	0.693	9.42 ±1.88	0.004*
	Sophomore	192	23.13 ±4.01		49.82 ±6.22		9.90 ±1.65	
	Junior	190	23.94 ±3.29		49.59 ±5.90		9.74 ±1.71	
	Senior	156	24.45 ±3.16		50.12 ±6.18		10.20 ±1.60	
History of foodborne illness	Yes	137	23.86 ±3.61	0.383	50.78 ±5.74	0.029*	9.86 ±1.69	0.994
	No	493	23.56 ±3.61		49.44 ±6.46		9.86 ±1.71	
Having food safety training	Yes	389	24.93 ±2.96	0.000*	50.36 ±5.57	0.002*	9.98 ±1.63	0.020*
	No	241	21.52 ±3.57		48.72 ±7.30		9.65 ±1.80	
Frequency of eating out	Everyday	158	23.42 ±3.42	0.116	49.68 ±5.49	0.368	9.78 ±1.78	0.274
	1-2 Days A Week	350	23.88 ±3.60		49.51 ±6.40		9.81 ±1.73	
	1-2 Days Per Month	122	23.16 ±3.80		50.45 ±7.13		10.08 ±1.49	

SD: standard deviation

Food Safety Knowledge

Table 3 shows the university students' food safety knowledge. There was a significant difference between the departments in terms of the student's answers to many statements ($p<0.05$), and the FE and GCA students were found to have more correct answers than the others. The students were found to be more knowledgeable (90% and above) about storage conditions (the statement: "purchased perishable food should be stored at refrigerator", ratio of correct answers: 90.8%), cross contamination ("cooked and uncooked food can be kept in the same container of the refrigerator", 94%), personal hygiene ("washing hands before handling food reduces the risk of contamination", 91%), food packaging ("damage in food packaging can cause food spoilage", 94.9%), and foodborne disease symptoms and results ("diarrhea, vomiting, abdominal pain are symptoms of foodborne illness", 95.2%). The students were less knowledgeable (50-80%) about thawing frozen foods ("frozen foods should be thawed in the refrigerator", 64.6%), safe storage temperatures for foods ("temperature danger zone for foods is 5°C-60°C", 61.6%; "The correct temperature for a refrigerator is 1-4°C",73.2%), HACCP food safety system ("HACCP is an international food safety system", 67%), and the differences between "recommended consumption date" and "expiration date" (64.9%). These findings are rather similar to those of the prior studies which reported that university students has limited knowledge about food safety (Al-Shabib et al., 2017; Luo et al., 2019; Madaki and Bavorova, 2019; Marklinder et al., 2020). On the other hand, it was found that the participants had insufficient knowledge about the following topics: reheating of foods, storage time of leftover food in the refrigerator, and safe time for keeping foods at room temperature. For instance, only 32.7% of the participants stated that "the internal temperature should be at least 74 °C when the cooked food is reheated", only 40.3% stated that "foods should not be consumed if kept at room temperature for more than 4 hours", and only 37.9% stated that "leftover food can be stored in the refrigerator for up to 3-4 days". The rules in these three statements are very important for ensuring food safety; however, most of the students answered these questions incorrectly. It takes time for the food poisoning bacteria to grow to unsafe levels. If the food has been out of temperature control for 4 hours or more, it must be disposed of. Moreover, when the leftover food is reheated, it should be ensured that it reaches 74 °C (USDA, 2021). Furthermore, Al-Shabib et al. (2017) asserted that 45.6% of foodborne disease outbreaks were due to temperature abuse during food processing and inappropriate storage temperatures of leftover or recently cooked meals. Therefore, the participant's lack of knowledge of these subjects is a remarkable result.

Food Safety Attitudes

Table 4 shows the university students' food safety attitudes. There was a significant difference between the departments in terms of the student's answers to many statements ($p<0.05$). The majority of the participants stated that they were concerned about pesticides, veterinary drug residues, and metallic contamination in food and that they thought that foodborne diseases had serious health and economic effects on society. Similarly, a previous study carried out in China reported that about 80% of university students were concerned about pesticide residues in vegetables, veterinary drug residues in meat, and the heavy metal pollution of foods (Luo et al., 2019). More than half of the participants stated that they were concerned about the food safety incidents in recent years, plastic packaging, food additives, and food safety behaviors of those working in the canteens and restaurants around schools and that they thought that foodborne diseases are more common in developing countries. It is thought that the participants' concerns about many issues may be related to the food problems experienced in Türkiye in recent years such as animal diseases, the inadequacy of inspections, and legal regulations (Food Safety News, 2015; Tosun and Demirtaş, 2012). In his study, Erden (2012) asserted that, in the field of food safety, Türkiye's weakness lay in the inadequacy of its legislation and the lack of in-service training of inspection personnel and laboratory personnel. The government is in charge of addressing the concerns of students and indeed all consumers. In this context, legal regulations should be developed for food safety, and control mechanisms should be implemented more effectively. The control mechanisms should eliminate the experts' deficiencies in in-service food safety training.

Food Safety Practices

Table 5 shows the university students' food safety practices. There was no significant difference between the departments in terms of the student's answers to many statements. The majority of the participants answered correctly the questions about food storage, cross-contamination, cleaning, and personnel hygiene. The most frequently reported food safety practice was hand washing; almost 98% of the participants stated that they wash their hands with soap and water before handling food. This percentage is the highest compared to those reported in previous studies on university students' hand-washing practices (Hassan and Dimassi, 2014; Luo et al., 2019; Marklinder et al., 2020). 69.4% of the participants stated that they do not use unwashed chopping boards or knives, 62.5% of them stated that they do not use foods with damaged packaging, and 70.3% of them stated that they decline the use of expired foods. Most of the participants stated that they store raw foods separately from cooked foods, wash

their hands before cooking, and do not purchase expired food and products with damaged packaging. These practices indicate a good implementation of food safety knowledge, which plays a key role in the control of an outbreak of foodborne diseases. On the other hand, about 23 % of the participants stated that they always prepare food when they have wounds, bruises, or injuries on their hands; whereas 26.7 % of them stated that, they never do this. This result indicates a poor implementation of the knowledge about the preparation of food with hands having wounds, bruises, or injuries. This finding is similar to that reported by Al-Shabib et al. (2017) for university students in Saudi Arabia. About 62% of the participants stated that they always guide their parents about food safety practices. This percentage is higher than those reported by Al-Shabib et al. (2017) for Saudi Arabia, Haapala and Probart (2004) for the USA, and Ovca et al. (2014) for Slovenian. The students' guiding their parents can ensure the dissemination of correct information and thus reduce the risk of foodborne diseases. Therefore, this high percentage is promising in terms of preventing foodborne diseases.

Table 3. Correct responses on food safety knowledge of students by four departments (n=630)

Statements	Departments					X^2	P
	FE (%)	GCA (%)	N (%)	PTR (%)	Total (%)		
Purchased perishable food should be stored at refrigerated.	93.6	92.1	88.6	90.0	90.8	7.811	0.252
Leftover food can be stored in the refrigerator for up to 3-4 days.	44.0	37.8	29.8	52.5	37.9	20.758	0.002*
Food should not be consumed if it has been left at room temperature for more than 4 hours.	51.1	40.9	38.4	26.3	40.3	16.516	0.011*
The Temperature danger zone for foods is 5°C-60°C	69.5	79.9	48.2	53.8	61.9	50.396	0.000*
The correct temperature for a refrigerator is 1-4°C.	80.1	90.9	60.8	62.5	73.2	62.275	0.000*
Cooked and uncooked food can be kept in the same container of the refrigerator.*	94.3	98.8	93.5	85.0	94.0	20.549	0.002*
Vegetables and meat can be chopped up with the same chopping board or knife.	85.8	91.5	80.4	61.3	82.1	41.074	0.000*
Damage in food packaging can cause food spoilage.	95.0	97.6	92.2	97.5	94.9	11.989	0.062
When reheating cooked food, it should be heated to a minimum internal temperature of 74°C.	43.3	61.0	13.9	13.8	32.7	145.060	0.000*
Inadequate cooking of raw foods can cause foodborne illness.	94.3	96.3	95.1	91.3	94.8	10.187	0.117
Frozen foods should be thawed in the refrigerator.	72.3	86.6	51.0	47.5	64.6	73.142	0.000*
Frozen foods should be thawed on the counter.*	65.2	83.5	43.7	42.5	58.7	78.502	0.000*
Shelf life is defined as the length of time a product may be stored without becoming unsuitable for use or consumption.	95.7	97.0	91.4	91.3	93.8	10.020	0.124
'Recommended consumption date' and 'Expiration date' have the same meaning.*	58.2	89.6	54.3	58.8	64.9	60.567	0.000*
Washing hands before handling food reduces the risk of contamination.	90.1	92.7	91.4	87.5	91.0	4.517	0.607
Washing hands after using toilets reduces the risk of foodborne illness.	86.5	88.4	91.0	86.3	88.7	14.054	0.029*
Washing hands time should be at least 20 seconds.	76.6	75.0	85.7	82.5	80.5	21.884	0.001*
There is no harm in using watches, earrings, rings during food preparation.*	74.5	90.9	86.1	78.8	83.8	22.479	0.001*
Food should not be touched with injured hands.	97.2	97.6	91.0	96.3	94.8	12.231	0.057
Using of gloves during food preparation reduces the risk of contamination.	92.2	89.0	91.4	88.8	90.6	3.420	0.755
Diarrhea, vomiting, abdominal pain are symptoms of foodborne illness.	92.9	97.0	95.5	95.0	95.2	3.322	0.767
High blood pressure is a symptom of foodborne illness.*	36.2	35.4	31.8	23.8	32.7	20.110	0.003*

Foodborne illnesses can result in death.	92.9	98.2	93.9	87.5	94.0	14.002	0.030*
Children, pregnant women and the elderly are more at risk of food poisoning.	78.0	71.3	86.5	73.8	79.0	20.428	0.002*
Milk is one of the most common food allergens.	73.0	89.0	72.2	68.8	76.3	24.288	0.000*
Food allergy can result in death.	82.3	82.9	93.9	91.3	88.1	20.054	0.003*
Food additives can cause food allergies.	85.8	73.8	90.6	93.8	85.6	29.288	0.000*
Shortness of breath is a symptom of a food allergy.	48.2	37.2	75.5	60.0	57.5	67.813	0.000*
Allergens should be stated on the label as different from other ingredients.	91.5	97.6	91.8	93.8	93.5	7.595	0.269
HACCP is an international food safety system.	89.4	97.6	49.0	20.0	67.0	230.141	0.000*
HACCP is a mandatory system in Türkiye.	77.3	73.2	36.7	17.5	52.9	193.401	0.000*
Consumer is responsible for food safety after purchase.	73.0	76.8	73.1	80.0	74.9	10.993	0.089

* Incorrect statements.
FE: Food Engineering; GCA: Gastronomy and Culinary Arts; N: Nursing; PTR: Physical Therapy and Rehabilitation

Table 4. Responses on food safety attitude of students by four departments (n=630)

Expressions	Departments				Total (%)	X^2	P
	FE (%)	GCA (%)	N (%)	PHR (%)			
You are concerned about food safety incidents in recent years in our country	1.4	1.8	3.3	2.5	2.4	16.282	0.179
Strongly disagree	4.3	3.7	4.5	2.5	4.0		
Disagree	9.9	12.8	19.2	22.5	15.9		
Neutral	49.6	42.1	45.3	38.8	44.6		
Agree	34.8	39.6	27.8	33.8	33.2		
Strongly agree							
You are concerned about pesticide residues in vegetables.							
Strongly disagree	2.8	1.8	2.0	5.0	2.5	41.776	0.000*
Disagree	3.5	0.0	1.2	0.0	1.3		
Neutral	7.8	0.6	7.3	7.5	5.7		
Agree	44.7	28.7	38.8	26.3	35.9		
Strongly agree	41.1	68.9	50.6	61.3	54.6		
You are concerned about the veterinary drug residue of meat.							
Strongly disagree	1.4	1.8	1.6	2.5	1.7	26.677	0.009*
Disagree	3.5	1.2	1.2	2.5	1.9		
Neutral	12.8	3.0	9.8	10.0	8.7		
Agree	46.1	32.9	41.2	35.0	39.4		
Strongly agree	36.2	61.0	46.1	50.0	48.3		
You are concerned about the heavy metal pollution of food.							
Strongly disagree	2.8	1.2	1.2	2.5	1.7	29.799	0.003*
Disagree	0.7	1.2	2.9	2.5	1.9		
Neutral	9.9	4.3	7.8	1.3	6.5		
Agree	42.6	31.7	44.5	28.8	38.7		
Strongly agree	44.0	61.6	43.7	65.0	51.1		
You are concerned about the transfer of plasticizers in food containers and packaging materials.	4.3	1.8	0.8	2.5	2.1	40.571	0.000*
Strongly disagree	14.9	3.0	3.7	6.3	6.3		
Disagree	19.9	14.0	15.1	6.3	14.8		
Neutral	30.5	38.4	41.2	38.8	37.8		
Agree	30.5	42.7	39.2	46.3	39.0		
Strongly agree							
Eating too much monosodium glutamate is bad for your health.							
Strongly disagree	1.4	1.8	0.4	0.0	1.0	16.423	0.173

Disagree	2.8	2.4	2.0	3.8	2.5		
Neutral	22.0	31.1	35.9	41.3	32.2		
Agree	34.8	26.2	25.7	21.3	27.3		
Strongly agree	39.0	38.4	35.9	33.8	37.0		
You are concerned about the current situation of food safety in the school canteen.	3.5	0.0	2.4	2.5	2.1	16.434	0.172
Strongly disagree	14.9	6.7	9.0	13.8	10.3		
Disagree	26.2	30.5	22.4	21.3	25.2		
Neutral	31.9	34.1	36.3	33.8	34.4		
Agree	23.4	28.7	29.8	28.8	27.9		
Strongly agree							
You are concerned about the safety of food in restaurants around the school.	2.8	1.2	2.4	0.0	1.9	21.849	0.039*
Strongly disagree	9.2	4.3	6.5	7.5	6.7		
Disagree	24.1	14.0	22.4	26.3	21.1		
Neutral	34.8	40.9	43.3	43.8	40.8		
Agree	29.1	39.6	25.3	22.5	29.5		
Strongly agree							
You are willing to improve your knowledge of food safety.							
Strongly disagree	0.7	1.2	0.8	1.3	1.0	81.618	0.000*
Disagree	1.4	0.0	3.3	2.5	1.9		
Neutral	4.3	4.9	11.4	8.8	7.8		
Agree	24.1	28.0	51.4	56.3	39.8		
Strongly agree	69.5	65.9	33.1	31.3	49.5		
You are willing to change your inappropriate food safety practices.							
Strongly disagree	1.4	0.6	1.6	1.3	1.3	53.766	0.000*
Disagree	1.4	0.6	1.2	1.3	1.1		
Neutral	4.3	4.9	13.1	7.5	8.3		
Agree	29.8	34.1	50.2	56.3	42.2		
Strongly agree	63.1	59.8	33.9	33.8	47.1		
You think that foodborne diseases have serious health and economic effects on society.	0.7	0.6	0.8	1.3	0.8	10.082	0.609
Strongly disagree	1.4	1.8	2.0	3.8	2.1		
Disagree	2.8	2.4	5.3	1.3	3.5		
Neutral	34.8	32.3	39.2	41.3	36.7		
Agree	60.3	62.8	52.7	52.5	57.0		
Strongly agree							
You think that foodborne diseases are more common in developing countries.	3.5	2.4	2.9	3.8	3.0	19.317	0.081
Strongly disagree	14.2	7.3	6.1	11.3	8.9		
Disagree	28.4	23.2	19.2	15.0	21.7		
Neutral	26.2	34.1	35.1	40.0	33.5		
Agree	27.7	32.9	36.7	30.0	32.9		
Strongly agree							

FE: Food Engineering; GCA: Gastronomy and Culinary Arts; N: Nursing; PTR: Physical Therapy and Rehabilitation

Table 5. Responses on food safety practice of students by four departments (n=630)

Expressions	Departments				Total(%)	X^2	P
	FE (%)	GCA (%)	N (%)	PTR (%)			
Do you use food with damaged packing?							
Always	19.9	13.4	20.0	16.3	17.8	5.861	0.439
Never	63.8	64.6	61.6	58.8	62.5		
Occasionally	16.3	22.0	18.4	25.0	19.7		
Do you use unwashed chopping board/knife?							
Always	22.0	13.4	19.2	13.8	17.6	11.324	0.079
Never	71.6	70.1	66.9	71.3	69.4		
Occasionally	6.4	16.5	13.9	15.0	13.0		
Do you check the temperature of the refrigerator before opening it?							
Always	27.0	20.1	21.6	17.5	21.9	16.049	0.013*
Never	15.6	17.7	29.4	26.3	22.9		
Occasionally	57.4	62.2	49.0	56.3	55.2		
Do you prepare food when you have wounds, bruises or injuries on hands?							
Always	24.1	18.9	27.3	17.5	23.2	12.897	0.045*
Never	26.2	24.4	24.1	40.0	26.7		
Occasionally	49.6	56.7	48.6	42.5	50.2		
Do you save the leftovers in the refrigerator?							
Always	59.6	56.1	58.8	58.8	58.3	1.265	0.974
Never	2.1	3.0	3.7	2.5	3.0		
Occasionally	38.3	40.9	37.6	38.8	38.7		
Do you wash dishes with warm water?							
Always	89.4	75.6	82.0	81.3	81.9	18.414	0.005*
Never	1.4	0.0	2.0	0.0	1.1		
Occasionally	9.2	24.4	15.9	18.8	17.0		
Do you clean the kitchen counter and utensils after food preparation?							
Always	94.3	97.0	94.7	96.3	95.4	2.843	0.828
Never	1.4	0.6	1.6	0.0	1.1		
Occasionally	4.3	2.4	3.7	3.8	3.5		
Do you check the cleanliness of the utensils before cooking?							
Always	94.3	94.5	95.1	95.0	94.8	4.443	0.617
Never	2.1	0.6	2.0	0.0	1.4		
Occasionally	3.5	4.9	2.9	5.0	3.8		
Do you use soap when washing hands?						6.892	0.331
Always	95.7	97.0	95.1	100.0	96.3		
Never	0.7	0.0	1.6	0.0	0.8		
Occasionally	3.5	3.0	3.3	0.0	2.9		
Do you remove watches, rings and jewelry before cooking?							
Always	68.1	79.9	73.1	72.5	73.7	9.614	0.142
Never	2.1	0.6	3.7	1.3	2.2		
Occasionally	29.8	19.5	23.3	26.3	24.1		
Do you use expired food items?							
Always	21.3	17.1	21.6	15.0	19.5	6.840	0.336
Never	66.0	70.7	70.6	76.3	70.3		
Occasionally	12.8	12.2	7.8	8.8	10.2		
Do you wash hands before cooking?							
Always	95.7	95.7	94.3	92.5	94.8	5.092	0.532
Never	1.4	0.6	3.3	3.8	2.2		

Occasionally	2.8	3.7	2.4	3.8	3.0		
Do you guide your parents regarding food safety practices?							
Always	80.1	60.4	57.1	51.3	62.4	27.908	0.000*
Never	2.1	3.0	2.4	1.3	2.4		
Occasionally	17.7	36.6	40.4	47.5	35.2		
Do you store raw food separately from cooked food?							
Always	80.9	81.1	70.6	76.3	76.3	11.725	0.068
Never	6.4	3.0	6.5	2.5	5.1		
Occasionally	12.8	15.9	22.9	21.3	18.6		

FE: Food Engineering; GCA: Gastronomy and Culinary Arts; N: Nursing; PTR: Physical Therapy and Rehabilitation

Conclusion

University students are an important target group as they are most likely to engage in risky eating behaviors and food handling practices making them susceptible to foodborne illnesses. Additionally, they also have the potential to work in the food industry. Our study presents insights into food safety knowledge, attitudes, and practices among university students in Türkiye. Although the university students' food safety knowledge, attitudes, and practices were found to be above average, they had some knowledge deficiencies and concerns in many areas. The students who had received a food safety education had higher scores for food safety knowledge, attitudes, and practices than the others. This once again emphasizes the importance of education. Especially in developing countries such as Türkiye, food safety education is insufficient both at primary and secondary school levels and at the university level; therefore, more attention should be paid to this education. In fact, this subject could be included in the curriculum of basic education before university. The increase in the number of responsible and conscious consumers will urge companies in the food industry to be more careful in food production. It should be known that education is the most effective solution for ensuring effective food safety in the long term and that the objectives of information and protection will only yield more positive results with education. Although this study is limited with respect to the number of respondents and the place where the research was conducted, it gives insight and direction for further studies on food safety knowledge and education.

Compliance with Ethical Standard

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

Ethics committee approval: The Ethics Committee of Human Studies in Social Sciences of Abant Izzet Baysal University approved the study (no: 2018/329).

Funding disclosure: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Acknowledgments: Authors are also thankful for the students who participated in this research study.

Disclosure: This study was a part of the Master Thesis of Emrah Gökçeel (Bolu Abant Izzet Baysal University, Department of Gastronomy and Culinary Arts).

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 8(4), 334-343 (2022) • https://doi.org/10.3153/FH22031 *Review Article*

Çimlendirilmiş besinler ve sağlık üzerine etkileri

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Cite this article as:
Kalaycı, Z. Şahin Kaya, A. (2022). Çimlendirilmiş besinler ve sağlık üzerine etkileri. *Food and Health,* 8(4), 334-343. https://doi.org/10.3153/FH22031

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Submitted: 04.03.2022
Revision requested: 07.06.2022
Last revision received: 22.06.2022
Accepted: 22.06.2022
Published online: 20.09.2022

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

ÖZ

Bitki filizleri birçok ülkede tüketime hazır sağlıklı gıdalar olarak dikkatleri üzerine çekmektedir. Nem ve sıcaklık başta olmak üzere, ortama besin ilavesi gibi farklı işlemler de optimum çimlenmenin sağlanması için gereklidir. Çimlenme, bitkilerin besin kalitesini iyileştirmek için ucuz ve etkili bir yöntemdir. Geçmişte buğday ve arpa gibi bazı tahıllar başta olmak üzere, baklagillerin çimlendirilmesi de yaygın bir uygulama iken günümüzde yonca, brokoli, soya fasulyesi ve diğer bazı tahıl taneleri çimlendirilerek filiz halinde tüketilmesi söz konusudur. Çimlenmeyle antibesinsel faktörlerinin miktarı azalmakta; fenolik bileşikler, fitosteroller, folat, gama aminobütirik asit gibi biyoaktif bileşiklerin miktarı, antioksidan aktivite ve sindirilebilirlik de artabilmektedir. Ayrıca Ɣ-orizanol ve aminoasitler gibi yeni bileşiklerin sentezi de çimlenme sürecinde artmaktadır. Çimlendirilmiş besinlerin birçok fizyolojik etkisinin bulunmasının yanı sıra; bu besinlerin kanser, diyabet, kalp-damar hastalıkları ve nörolojik hastalık gibi hastalık riskinde azalmaya sahip olabileceği bildirilmiştir. Bu nedenle çimlendirilmiş besinler fonksiyonel besin olarak kabul edilmektedir. Çimlendirilmiş besinlerin çeşitli biyolojik aktiviteleri halen kesin olarak kanıtlanmamıştır. Bu nedenle çimlendirilmiş besinlerin çeşitli biyoaktif bileşen içerikleri ve insan sağlığı üzerindeki etkileriyle ilgili daha fazla sayıda çalışmaya ihtiyaç vardır.

Anahtar Kelimeler: Çimlendirme, Fonksiyonel besin, Sağlık

ABSTRACT

Germinated foods and their effects on health

Plant sprouts attract attention as ready-to-eat healthy foods in many countries. Different processes such as adding nutrients to the environment, especially humidity and temperature, are also necessary to ensure optimum germination. Germination is an inexpensive and effective method for improving the nutritional quality of plants. In the past, germination of legumes, especially some grains such as wheat and barley, was a common practice, but today, alfalfa, broccoli, soybean and some other cereal grains are germinated and consumed as sprouts. The amount of antinutritional factors decreases with germination; The amount of bioactive compounds such as phenolic compounds, phytosterols, folate, gamma aminobutyric acid, antioxidant activity and digestibility can also increase. In addition, the synthesis of new compounds such as Ɣ-oryzanol and amino acids increases during the germination process. In addition to the many physiological effects of germinated nutrients; It has been reported that these foods may have a decrease in the risk of diseases such as cancer, diabetes, cardiovascular diseases and neurological diseases. Therefore, germinated foods are considered as functional foods. Various biological activities of germinated nutrients have still not been conclusively proven. Therefore, there is a need for more studies on the various bioactive component contents of germinated foods and their effects on human health.

Keywords: Germination, Functional food, Health

Giriş

Bitki filizleri birçok ülkede tüketime hazır sağlıklı gıdalar olarak dikkatleri üzerine çekmektedir. Filizlenme olarak da adlandırılan çimlenme, tohumların, kabuklu yemişlerin, baklagillerin veya tahılların birkaç saat ıslatma sonrasında 24-48-72 saat gibi sürelerde nemli tutulması gerekliliği olan bir durumdur. Tohum veya tanenin çimlenebilmesi için belli ortam koşullarının uygun olması öncelikli kuraldır. Nem ve sıcaklık başta olmak üzere, ortama besin ilavesi gibi farklı işlemler de optimum çimlenmenin sağlanması için gereklidir. Çimlenme, bitkilerin besin kalitesini iyileştirmek için ucuz ve etkili bir yöntemdir ve bitkisel besinlerin besin değerini büyük ölçüde artırmaktadır. Ultraviyole ışık, filiz büyümesi ve gelişimi üzerinde çevresel bir faktör olarak belirgin bir etkiye sahiptir (Mewis ve ark., 2012).

Geçmişte buğday ve arpa gibi bazı tahıllar başta olmak üzere, baklagillerin çimlendirilmesi yaygın bir uygulama iken günümüzde yonca, brokoli, soya fasulyesi ve diğer bazı tahıl taneleri çimlendirilerek filiz halinde tüketilmesi söz konusudur (Yetim ve ark., 2010). Son zamanlarda bitki filizli gıdalar fonksiyonel gıda olarak kabul edilmekte ve çok fazla ilgi görmektedir. Bununla birlikte, biyolojik aktiviteleri ve sağlık yararları ile ilgili yapılan çalışmalar yetersizdir. Bu derlemenin amacı, çimlendirme işlemi uygulanan besinlerin sağlık üzerine olan yararlarını incelemektir.

Filizlendirmeyle Besinlerde Oluşan Değişiklikler

Çimlenme sırasında tohumda, enzimatik aktivite ve biyoaktif bileşikler artmaktadır. Çünkü metabolik aktivite ile; depo karbonhidratlar, proteinler ve lipitlerden çimlenme için gerekli enerji üretimi sağlanmaktadır. Ayrıca Ɣ-aminobütirik asit (GABA), Ɣ-orizanol ve kullanışlı aminoasitler gibi yeni bileşiklerin sentezi de çimlenme sürecinde artmaktadır (Xu ve ark., 2020).

Fenolik bileşikler, bitkisel besinlerde bulunan ikincil metabolitlerdir. Genellikle serbest radikallerden, reaktif oksijen türlerinden ve prooksidanlardan türetilen oksidatif stresle mücadele ederek, onları oksidasyondan korumak için antioksidan görevi görmektedirler. Diyetimizdeki doğal fenolik bileşiklerin antioksidan özellikleri, insan sağlığını geliştirmedeki çok yönlü işlevleriyle bağlantılıdır (Xu ve ark., 2020).

Tahıl ve bakliyat ürünleri, insan diyetine en büyük kalori ve protein kaynağını sağlayan ilk iki bitkisel besindir. İnsanlar ve hayvanlar üzerinde yapılan son müdahale araştırmaları, tahıl ve bakliyat mahsullerinin biyolojik olarak aktif, ancak nispeten düşük miktarlarda fenolik bileşikleri içerdiğini ve fenolik bileşiklerin kronik hastalık riskinin azalmasıyla ilişkili olduğunu göstermektedir (Jayathilake ve ark., 2018). Tahıl ve bakliyatlardaki fenolik bileşiklerin miktarını ve antioksidan aktivitesini geliştirmek amacıyla birtakım yöntemler kullanılmıştır. Yüzyıllardır tohum çekirdeklerini yumuşatmak için kullanılan süreç olan çimlendirme, besin değerini iyileştirme ve özellikle bakliyatlarda antibesinsel faktörleri azaltma başarısı sayesinde son zamanlarda büyük ilgi görmektedir (Mäkinen ve Arendt, 2015; Yeo ve Shahidi, 2015). Çimlendirme yöntemi insan sağlığını iyileştirmek için insan diyetine dahil edilmesinin yanı sıra, aynı zamanda lipit oksidasyonunu etkili bir şekilde önlemek için gıda antioksidanları olarak da kullanılabilmektedir (Xu ve ark., 2020).

Çok sayıda araştırma (Yeo ve Shahidi, 2015; Guzmán-Ortiz ve ark., 2017), çimlenmenin tahıl ve bakliyatlardaki fenolik bileşiklerin miktarını artırmak için umut verici bir yol olduğunu gösterse de, fenolik bileşiklerin dinamik değişimleri nedeniyle bu konu hala tartışmalıdır. Örneğin López-Amorós ve ark. (2006) ve Xu ve ark. (2018), çimlenme sürecinde mercimekten ekstrakte edilebilen fenolik bileşik içeriğinin azaldığını ifade ederken; diğer araştırmacılar (Yeo ve Shahidi, 2015) çimlenme sürecinde mercimekteki ekstrakte edilebilen fenolik bileşik içeriğinin arttığını rapor etmişlerdir. Tohumun çimlenmesi sürecinde, fenolik bileşiklerin metabolizmasıyla ilgili genel olarak üç olay vardır. Bunlardan ilki, çekirdek çimlenmesi sürecinde glukoz veya aromatik aminoasitlerden, doğal fenolik bileşiklerin sentezinin başlamasıdır. Sitozolde, oksidatif pentoz fosfat, glikolitik ve şikimat yolu (folat ve aromatik aminoasitlerin biyosentezi için bitkiler tarafından da kullanılabilen bir metabolik yoldur) ile birlikte fenilalanin gibi aromatik aminoasitler üretilebilmekte ve fenolik asitlere dönüşebilmektedir (Stalker ve ark., 1985). İkincisi, makromoleküler besinler, enzimler tarafından ayrıştırılmakta ve bunun sonucunda bağlı formlarından fenolik bileşikler salınmaktadır. (Paucar-Menacho ve ark., 2017). Üçüncüsü, fenolik bileşikler, serbest radikalleri temizlemek için veya sinyal bileşiklerinin ara ürünleri olarak tüketilirler. Tohumun türü ve çimlenme ortamı, çimlenme sürecinde fenolik bileşiklerin bu üç olaydaki transformasyonunun belirlenmesindeki iki önemli faktördür. Bu sebeplerle kompleks çimlenme prosedürü sonrasında, fenolik bileşiklerin toplam miktarı ve antioksidan aktivitesinin azalması veya artması şaşırtıcı değildir (Xu ve ark., 2020).

Avenantramitler, esas olarak yulafta bulunan ve yulaf ürünlerinin taze tadına katkıda bulunan fenolik bileşiklerdir. Çimlenmeyle, bu bileşiklerin yaklaşık %20 oranında arttığı bildirilmiştir. Çimlendirmenin besinlerde sterol içeriğini artırdığına dair kanıtlar da mevcuttur (Skoglund ve ark., 2008).

Çimlenmenin ayrıca buğdayın E vitamini içeriğinde artışa neden olduğu bildirilmiştir. Lee ve ark. (2007), Kore'ye özgü

olan sert pirinçte çimlenme sonrasında; ham protein, yağ, toplam diyet posası ve şeker içeriğinin önemli ölçüde arttığını belirtmişlerdir. Sert pirincin özellikle esansiyel protein içeriğinde artış meydana geldiği rapor edilmiştir. Ayrıca GABA, Ɣ-orizanol, α-tokoferol, α-tokotrienol, Ɣ-tokotrienol içeriklerinin de arttığı bildirilmiştir (Lee ve ark., 2007). GABA, doğada yaygın olarak bulunan, anti-hipertansif ve antidepresan aktiviteler gibi sayısız ve önemli fizyolojik fonksiyonlara sahip güçlü bir biyoaktif bileşik olarak kabul edilmektedir (Cui ve ark., 2020). Ɣ-orizanol ise, temel kaynağı pirinç kepeği olan önemli bir biyoaktif maddedir. Ɣ-orizanolün kolesterol düşürücü, antioksidan, trombosit agregasyonunu azaltıcı, sinirsel dengesizlikleri ve menopoz sıkıntılarını azaltıcı, kas kütlesini artırıcı, fekal safra asidi atımını artırıcı ve tümör büyümesini engelleyici etkileri rapor edilmiştir (Tuncel, 2016). Ayrıca çimlendirilen buğday, arpa, karabuğday ve kinoada işlem sonrası belli oranlarda C vitamini bulunduğu bildirilmiştir (Hübner ve Arendt, 2013).

Frias ve ark. (1998) Hindistan'da yetişen 10 farklı tür pirincin, çimlenme sonrasında, amiloz içeriklerinin önemli ölçüde azaldığını bildirmişlerdir. Ayernor ve Ocloo (2007) tarafından Gana'da yapılan bir çalışmada, sert pirinçte çimlenme boyunca, nişasta içeriğinde ciddi düşüş gözlenmiştir. Sert pirincin şeker içeriğinin, önemli ölçüde arttığı rapor edilmiştir (Ayernor ve Ocloo, 2007). Cornejo ve ark. (2015), farklı çimlendirme sürelerinde (0 saat, 12 saat, 24 saat ve 48 saat) çimlendirilmiş pirinçten elde edilen ekmeklerin beslenme faydalarını araştırmışlardır. Farklı çimlendirme süreleriyle karşılaştırıldığında, 48 saat çimlendirilen pirinçten elde edilen undan yapılan ekmek; daha yüksek protein, lipit ve biyoaktif bileşik (GABA ve polifenoller) içeriğine sahip olması, antioksidan aktivitesinin artması, fitik asit içeriğinin (anti-besinsel öge) azalması ve glisemik indeksinin azalması nedeniyle, çalışmadaki diğer ekmek türlerine göre besleyici olarak daha üstün kalitede olduğu bildirilmiştir. Çalışmada ayrıca proteinlerin *in vitro* sindirilebilirliğinde hafif bir azalma gözlenmiştir (Cornejo ve ark., 2015). Başka bir çalışmada (Ijarotimi ve Keshinro, 2012), çimlenme ve fermantasyonun Afrika locust fasulyesi ununun besin kalitesi üzerindeki etkileri araştırılmıştır. Çalışmada fermantasyon tekniğinin anti-besin konsantrasyonunu önemli ölçüde düşürdüğü ve ayrıca besin bileşimi kompozisyonunu, özellikle filizlendirilmiş fasulye ununun amino asit profilini geliştirdiği bulunmuştur (Ijarotimi ve Keshinro, 2012).

Pal ve ark. (2016) yaptıkları çalışmada, esmer pirinçte çimlenmeyle protein içeriğinin arttığını; yağ ve amiloz içeriğinin azaldığını bulmuşlardır. GABA, histidin, arjinin, prolin, metiyonin ve asidik aminoasitlerin çimlenmeyle önemli derece arttığı ve bu artışın glutelin ve prolamin birikimindeki değişimle ilişkili olduğu bildirilmiştir. Glutelin ve prolamin birikiminin, çimlenmeyle birlikte azaldığı rapor edilmiştir (Pal ve ark., 2016).

Genel olarak çimlenme, tam tahılların biyokimyasal kompozisyonunda önemli değişikliklere yol açmaktadır. Çimlenmeyle birlikte;

- Nişasta rezervleri, α-amilazın etkisiyle mobilize olur,
- Nitrojen içeren fraksiyonlar, oligopeptidlere ve serbest amino asitlere doğru kayar ve amino asitlerin bileşimi değişir,
- Triaçilgliseroller hidrolize olmaya başlar ve doymuş/doymamış yağ asitleri oranı yükselir,
- Anti-besinsel faktörlerinin (örneğin fitat, tripsin inhibitörü, tanen) miktarı önemli ölçüde azalır,
- Fenolik bileşikler, fitosteroller, folatlar ve GABA gibi biyoaktif bileşikler artar (Benincasa ve ark., 2019).

Çimlendirilmiş Besinlerin Sağlık Üzerine Etkileri

Tohumların çimlendirilmesi, mutfak tarihinin önemli bir bileşeni olarak Doğu ülkeleri tarafından çok uzun zamandır bilinmektedir. 1980'lerden itibaren tüketicilerin diyetetik ve egzotik sağlıklı gıdalara olan talebi nedeniyle, çimlendirilmiş tohumların tüketimi Batı ülkelerinde de popülerlik kazanmıştır (Geng ve ark., 2021).

Çimlendirme sürecinde meydana gelen modifikasyonlar, sindirim sistemi gibi işlev görmektedir ve çeşitli *in vitro* ve *in vivo* çalışmalarla onaylandığı gibi, makro ve mikro besinlerin ulaşılabilirliğini artırmaktadır. Çimlendirilmiş besinler, çeşitli antioksidan ve biyoaktif bileşikler açısından zengin, sağlığı teşvik edici gıdalar arasında sayılmaktadır (Geng ve ark., 2021).

Antioksidan Etki

Antioksidanlar, insan vücuduna çeşitli faydaları olan önemli biyokatif bileşiklerdir. Gıdalardaki antioksidan kapasiteden sorumlu olan başlıca bileşikler, L-askorbik asit ve fenolik bileşiklerdir (Huang ve ark., 2014).

Soya fasulyesinin çimlenmesi, biyokimyasal özelliklerinde önemli değişikliklere sebep olur. Bu değişiklikler soya fasulyesinin besinsel özelliklerini ve sağlığı geliştirici özelliğe sahiptir. Soya fasulyesi filizlerinin antioksidan kapasitesinin araştırıldığı bir çalışmada; çimlendirmenin antioksidan aktiviteyi artırıcı etkilerinin olduğu sonucuna varılmıştır (Huang ve ark., 2014). Gawlik-Dziki ve ark. (2014), brokoli filizi ek-

lenen ekmeğin antioksidan aktivitesini araştırmışlardır. Brokoli filizi takviyesinin, non enzimatik ve enzimatik aktivitelerde, antioksidan aktiviteyi artırdığı gösterilmiştir (Gawlik-Dziki ve ark., 2014). Świeca ve Dziki (2015) koşulları optimize ederek, buğday filizinin antioksidatif kapasitesini araştırmışlardır. En yüksek antioksidan aktivite değeri ve toplam fenolik bileşik içeriğine, 20 °C'de 4 gün süren filizlendirme işlemi sonucunda ulaşılmıştır (Świeca ve Dziki, 2015).

Pişirmenin soya fasulyesi filizlerinin antioksidan özelliği üzerine etkilerinin incelendiği bir çalışmada, pişirmenin fenolik bileşikler ve antioksidan aktivitede önemli düşüşlere yol açtığı gösterilmiştir (Kumari ve Chang, 2016). Ultraviyole-B radyasyonunun soya fasulyesinin antioksidan sistemi üzerinde etkisinin araştırıldığı bir çalışmada, ultraviyole-B radyasyondan kaynaklanan nitrik oksit üretiminin; antioksidanları, antioksidan enzim aktivitelerini ve onların gen ekpresyonlarını modüle ederek, antioksidan aktiviteyi artırdığı bulunmuştur (Añón ve ark., 2014).

Soya fasulyesi filizi ekstrelerinin antioksidan aktivitesinin araştırıldığı bir çalışmada, isoflavonların antioksidan aktivite sonuçlarıyla yüksek korelasyon gösterdiği bulunmuştur (Guzmánortiz ve ark., 2017). Dongyan ve ark. (2014) maş fasulyesinin antioksidan kapasitesini gözlemlemişlerdir. Farklı sürgün uzunluklarının şelasyon kapasitesi %85'in üzerinde iken; ekstraktların toplam antioksidan kapasitesinin 4.0 olduğu bulunmuştur. İlk gün filizlerinin ekstraktlarının şelasyon kapasitesinin yaklaşık %80 olduğu ve antioksidan aktivite sergilediği saptanmıştır (Dongyan ve ark., 2014).

Melatonin, mercimek filizi gibi bitkisel gıdalarda bulunan çok işlevli bir antioksidan nörohormondur. Rebollo-Hernanz ve ark. (2020) yaptıkları çalışmada, çimlenmeyle mercimeğin melatonin içeriğinin arttığını bildirmişlerdir. Ancak fenolik asit ve flavan-3-ollerin kaybı nedeniyle fenolik bileşik içeriğinin azaldığı rapor edilmiştir. Flavonol içeriğinin değişmeden kaldığı, mercimek filizlerindeki ana fenolik bileşiğin, esas olarak kaempferol glikozitlerden oluştuğu belirtilmiştir. Çalışmada sıçanlara mercimek filizi ekstresi oral yoldan verilmiş ve ratların melatonin seviyeleri, uygulamadan 90 dakika sonra maksimum konsantrasyona ulaşmıştır. Plazma antioksidan statüsü de mercimek filizi uygulamasından sonra arttığı gözlenmiştir (Rebollo-Hernanz ve ark., 2020).

Çözünmeyen bağlı fenoliklerin (IBP) çözünür fenoliklere (SP) oranı, çimlenme sırasında mercimeklerin ve diğer besinlerin antioksidan aktivitesindeki değişiklikleri izlemek için etkin bir yöntem olarak önerilmektedir. Yeo ve Shaidi (2015) çimlendirilmiş Richlea mercimek çeşidinin 4 gün boyunca, SP, IBP ve toplam fenoliklerin miktarını ve bu bileşiklerin antioksidan aktivitesini incelemişlerdir. Çimlenme süresince; IBP'lerin SP'lere oranının arttığı ve bu durumun muhtemelen çimlenme işlemi sırasında fenolik bileşik oluşumunun çözünürden çözünmez bağlı forma dönüşmesinden kaynaklanabileceği bildirilmiştir (Yeo ve Shahidi, 2015).

Yapılan çalışmalarda genel olarak, çimlenmeyle birlikte besinlerdeki antioksidan özellik gösteren bileşiklerin ve antioksidan etkinin arttığı gösterilmiştir (Huang ve ark., 2014).

Antikanser Etki

Kanser, dünya çapında en önemli ölüm nedenlerinden biri olmaya devam etmektedir. Flavonoidler ve gallik asit, klorojenik asit, ferulik asit, benzoik ve salisilik asitler, kuarsetin, kaempferol gibi fenolik bileşikler, C vitamini, glukosinolatlar, antikanser aktivite gösteren çeşitli biyokatif bileşiklerdir. Antikanser özellik gösteren diyetteki koruyucu unsurlar; selenyum, folik asit, B_{12} vitamini, D vitamini, klorofil ve antioksidanlardır (Wu ve ark., 2013).

Çimlendirilmiş esmer pirincin, kanser hücrelerinde proliferasyon ve apoptoz üzerine etkilerinin incelendiği bir çalışmada (Oh ve Oh, 2004), çimlendirilmiş esmer pirinç ekstresinin, GABA seviyelerini artırarak, lösemi hücre proliferasyonunu inhibe ettiği ve kanser hücrelerinin apoptozunu stimüle ettiği bulunmuştur.

Amici ve ark. (2008) buğday filizi ekstresinin kanserde antioksidan aktivitesini araştırmışlardır. Buğday filizi ekstresiyle tedavi edilen hücrelerden, stimüle edilemeyen hücrelerde proteazom baskılanmasının meydana geldiği ve TPA (12-o-tetradekanoilforbol-13-asetat)'nın aracılık ettiği etkilerin hafiflediği bildirilmiştir (Amici ve ark., 2008).

Alumkal ve ark. (2015) sülforafanın insanlarda karsiyogenezi engelleme yeteneğinin mekanizmasını aydınlatmak için, erkek prostat kanseri vakalarında sülforafanla brokoli filizi ekstresinin etkisini inceleyen bir klinik çalışma yürütmüşlerdir. İlginç olarak, sülforafan bakımından zengin brokoli filizi ekstreleri, prostat kanseri hücrelerinde androjen reseptör sinyalini inhibe ettiği bulunmuştur (Alumkal ve ark., 2015). Gawlik-Dziki ve ark. (2014) brokoli filizinin, mide kanseri hücrelerine karşı antikanser aktivitesini değerlendirmişlerdir. Bulgular, brokoli filizi takviyesinin mide kanseri hücreleri için kimyasal önleyici etkisinin olduğunu göstermiştir (Gawlik-Dziki ve ark., 2014).

Haghparast ve ark. (2017) yaptıkları çalışmada, mercimek filizi ekstresinin, lenfosit hücresi üzerinde bir miktar radyo koruyucu etki gösterdiğini bulmuşlardır. Ayrıca toplam mercimek filizi ekstresinin, kök kısmına göre daha fazla antioksidan aktiviteye sahip olduğu bildirilmiştir (Haghparast ve ark., 2017). Ling ve Chang (2017) soya fasulyesi filizinden elde edilen kumestrolün, prostat kanserini baskılayabileceği ve

bazı hücre sinyal yolakları aracılığıyla, prostat kanserinin tedavisinde yeni bir bileşik olabileceği belirtilmiştir.

Çimlendirilmiş besinlerin, çimlendirilmemiş olanlara göre biyoaktif bileşik içeriği daha yüksektir. Bu nedenle çimlendirilmiş besinlerin kanserin önlenmesinde etkili olabilecek bir diyet için iyi bir seçim olabileceği düşünülmektedir (Ling ve Chang, 2017).

Antihipertansif Etki

Kalp-damar hastalıklarının bir belirtisi olan yüksek kan basıncı veya hipertansiyon, önemli bir morbidite ve ölüm nedenidir. Yüksek kan basıncının diyet yoluyla kontrolü, önemli bir halk sağlığı stratejisidir (Wu ve ark., 2013).

Wu ve ark. (2013) tarafından, çimlendirilmiş esmer pirinç diyetinin hipertansif ratlarda ve hastalarda kan basıncı artışının önlenmesi üzerindeki etkilerini araştıran çalışmalar derlenmiş ve çimlendirilmiş esmer pirincin, hipertansiyonun terapötik diyetinin bir parçası olarak kullanılabileceği önerilmiştir. Ebizuka ve ark. (2009), diyetlerinin %40'ı çimlendirilmiş esmer pirinçten oluşan spontan hipertansif ratlarda, çimlendirilmiş esmer pirincin hipertansiyon ve kan biyokimyası üzerindeki etkilerini incelemişlerdir. Çalışma sonunda çimlendirilmiş esmer pirincin, spontan hipertansif ratlarda, önemli ve güçlü bir antihipertansif etkisinin olduğu bildirilmiştir (Ebizuka ve ark., 2009). Esmer pirinçte çimlendirmeyle miktarı önemli ölçüde artan ferulik asitin, streptozotosin kaynaklı diyabetik ve hipertansif ratlarda kan basıncını düşürmede etkili olduğu belirtilmiştir (Ohsaki ve ark., 2008).

Meschini ve ark. (2015), hipertansif hayvan modellerinde, tartar karabuğday filizinin anti-genotoksik etkilerini araştırmışlardır. Kontrol grubuyla kıyaslandığında; tartar karabuğday filiziyle beslenen ratlarda, DNA hasarında ciddi bir azalma ve daha etkin bir DNA onarımı gösterilmiştir (Meschini ve ark., 2015).

Nakamura ve ark. (2016) karabuğday ve maş fasulyesi filizlerinin, fruktoz kaynaklı spontan hipertansif ratlarda antidiyabetik etkisini incelemişlerdir. Bulgular, karabuğday filizi tozunun, kalp atışında ve serum trigliseritlerinde önemli düşüşlere neden olduğunu göstermiştir. Maş fasulyesi filizi tozunun, kalp atımı ve serum trigliseritlerinde belirgin bir azalmaya neden olduğu bildirilmiştir. Dahası, maş fasulyesi filizi tozunun, serum total kolesterolünü önemli ölçüde azalttığı rapor edilmiştir (Nakamura ve ark., 2016).

Çimlendirilmiş besinlerin antihipertansif etkisinin, GABA, diyet posası, ϒ orizanol ve ferulik asit gibi çeşitli biyoaktif bileşenlerin karmaşık aktivitelerinden kaynaklandığı belirtilmektedir (Wu ve ark., 2013).

Antidiyabetik Etki

Diyabet insidansı, dünya çapında gelişmiş ve gelişmekte olan ülkelerde artış göstermektedir. Yüksek postprandiyal kan glukoz ve insülin seviyeleri, diyabet ve kardiyovasküler hastalıklar için risk faktörüdür (Wu ve ark., 2013).

Pirinç tüketen toplumlarda diyabet hastalığının etkin yönetimi için çimlendirilmiş esmer pirincin, tip 2 diyabetin seyrinde önemli bir değişken olduğu bildirilmiştir. *İn vitro* veriler ve hayvan deneyleri, çimlendirilmiş esmer pirincin, bu hastalığı yönetmek için fonksiyonel bir diyet potansiyeline sahip olduğunu göstermektedir. GABA, ϒ-orizanol, diyet lifi, fenolikler, vitaminler gibi biyoaktif bileşikleri içermesi nedeniyle esmer pirincin; düşük insülin indeksi, kan glukozunu düşürücü, antioksidatif, antitromboz, antihipertansif, hipokolesterolomik ve nöroprotektif etkilere sahip olduğu bildirilmektedir. Beyaz pirincin, esmer pirinçle yer değiştirmesi durumunda, pirinç tüketen popülasyonlarda diyabet hastaları için çok büyük fayda sağlanabileceği düşünülmektedir (Imam ve ark., 2012a). Hem diyabetik olmayan, hem de kontrolsüz diyabetli hiperglisemili bireylerden, esmer pirinçle takviye edilen diyet alanlarda, beyaz pirinç tüketenlere göre postprandiyal kan glukozunun daha düşük olduğu bildirilmiştir (Ito ve ark., 2005).

Ren ve ark. (2020) otoklavlama işleminin, çimlendirilmiş esmer pirincin (ÇEP) beslenme ve sağlık fonksiyonları üzerine etkilerini incelemişlerdir. 115°C'de 20 dakika otoklavlama işleminin, çimlendirilmiş esmer pirincin GABA ve ferulik asit seviyelerini artırdığı bulunmuştur. Buna ek olarak, 1 ay otoklavlanmış çimlendirilmiş esmer pirinç tüketiminin metabolik sendromlu hastalarda; açlık glukozu, 0.5, 1 ve 2 saatlik postprandiyal plazma glukozu, trigliserit, toplam kolesterol, yüksek yoğunluklu lipoprotein kolesterol ve düşük yoğunluklu lipoprotein kolesterolü önemli derecede azalttığı bildirilmiştir. Çalışmada, otoklavlamanın umut verici bir işleme stratejisi olduğunun altı çizilmiştir (Ren ve ark., 2020).

Imam ve ark. (2012b), ÇEP'in, streptozotosin enjeksiyonu ve yüksek yağlı diyetle indüklenen tip 2 diyabetli ratların antioksidan durumu üzerine etkisini incelemişlerdir. Ayrıca beyaz pirinç, esmer pirinç ve çimlendirilmiş esmer pirincin, katalaz ve süperoksit dismutaz genleri üzerindeki etkilerini değerlendirmişlerdir. Diyet bileşenleri olarak, esmer pirinç ve ÇEP'in, glisemiyi ve böbrek hidroksil radikalini süpürme aktivitesini iyileştirdiği ve tip 2 diyabetli ratlarda antioksidan durumun kötüye gitmesini önlediği bulunmuştur. Benzer olarak ÇEP'in, serum kreatininin yanısıra, karaciğer enzimlerini de koruyucu özellik gösterdiği bildirilmiştir. Süperoksit dismutaz geninin upregülasyonunun, esmer pirinç ve ÇEP'in antioksidan etkilerinin altında yatan mekanizma olabileceği belirtilmiştir (Imam ve ark., 2012b).

Çimlendirilmiş besinlerin, açlık kan glukoz seviyeleri, postprandiyal kan glukoz seviyeleri ve insülin cevabına olumlu etkileri, birçok araştırmacı tarafından gösterilmiştir (Imam ve ark., 2012a, Ito ve ark., 2005).

Diğer Sağlık Etkileri

Yapılan çalışmalarda çimlendirilmiş esmer pirincin; anti-obezite (Golzarand ve ark., 2021), anti-depresyon (Mamiya ve ark., 2007), hipokolesterolomik (Roohinejad ve ark., 2010) etkilerinin de olduğu gösterilmiştir (Wu ve ark., 2013).

Lee ve ark. (2013) tarafından yürütülen, karabuğday filizinin anti-adipogenez aktivitesinin olduğunu iddia ettikleri çalışmanın sonucunda; kontrol grubuyla karşılaştırıldığında, karabuğday filizinin adipozit farklılaşmasını ve adipoz hücrelerinde reaktif oksijen türlerinin sekresyonunu önemli derecede baskıladığı bulunmuştur.

Golzarand ve ark. (2021), beyaz pirinçle karşılaştırıldığında esmer pirincin obeziteyi önleyici etkisinin olduğunu; ancak lipit profili ve glisemik göstergeler üzerinde herhangi bir etkisinin olmadığını bildirmişlerdir. Esmer pirincin aksine, önceden çimlendirilmiş esmer pirincin, beyaz pirince göre; vücut ağırlığında azalmaya neden olduğu, lipit profilini ve açlık kan glukozu seviyelerini azalttığı bulunmuştur (Golzerand ve ark., 2021).

Chang ve ark. (2015), *Helicobacter pylori* enfeksiyonunda brokoli filizi ekstraktının gastrik mukozal hasar üzerinde antioksidan aktivite sergilediğini bildirmişlerdir. Sonuçlar, brokoli filizi ekstraktı tedavisinin, mukozal malondialdehit konsantrasyonunu önemli ölçüde zayıflattığını gösterirken; gastrik mukozal glutatyon konsantrasyonunun tedavi altında değişmediği rapor edilmiştir. Brokoli filizi ekstraktının, gastrik mukozada lipit peroksidasyonunu önlediği ve *H. pylori* ile indüklenen gastritte sitoprotektif etkisinin olabileceği bildirilmiştir (Chang ve ark., 2015). Ek olarak, Müller ve ark. (2016), sülforafanla brokoli filizi tüketiminin, bir enfeksiyon modelinde viral yük göstergelerini azalttığı gözlemlemiştir. Bu model, zayıflatılmış canlı influenza virüsü ile inoküle edilmiştir (Müller ve ark., 2016).

Feruzza ve ark. (2016) yürüttükleri bir çalışmada, brokoli filizi sularının ince bağırsak hücrelerinin *in vitro* inflamasyon modellerindeki biyolojik aktivitesini araştırmışlardır. Bulgular brokoli filizi sularının, marjinal çinko eksikliği altında, tümör nekrozis faktör α'ya maruz kalmış insan intestinal epitel hücrelerinde, intestinal bariyer bütünlüğünü koruduğunu göstermiştir (Feruzza ve ark., 2016). Kujawska ve ark. (2016), soya fasulyesi filizinin, erkek ve dişi Wistar ratlarında, demir eksikliğini önleyici ve anti-inflamatuar aktivitesini incelemişlerdir. Çalışma sonucunda soya fasulyesi filizlerinin; süperoksit dismutaz, glutatyon peroksidaz ve glutatyon S-transferaz özelliklerini artırdığı bulunmuştur. Soya fasulyesi tohumlarına ferroz sülfat eklenmesinin, inflamatuar bağırsak hastalıklarında ve demir eksikliği anemisi yaşayan bireylerde besin takviyesi olarak düşünülebileceği bildirilmiştir (Kujawska ve ark., 2016).

Hafidh ve ark. (2015) sitotoksisite ve virüs verim azaltma, virüsidal aktivite ve profilaktik aktivite testlerini kullanarak, maş fasulyesi metanol ekstraktlarının, solunum sinsityal virüsü (RSV) ve Herpes Simplex virüs-1 (HSV-1) üzerindeki antiviral atkilerini incelemişlerdir. Maş fasulyesi ekstraktının, RSV ve HSV-1 için antiviral aktivite sergilediği bulunmuştur (Hafidh ve ark., 2015).

Okada ve Okada (2016), karabuğday, brokoli, kırmızı lahana ve brüksel lahanası filizlerinin nöroprotektif özelliğini, β-amiloid seviyelerini ölçerek değerlendirmişlerdir. Çalışma sonucunda bitki filizlerinin, β-amiloid seviyelerini önemli ölçüde azalttığı bulunmuştur (Okada ve Okada., 2016).

Demeekul ve ark. (2021b) yaptıkları çalışmada, çimlendirilmiş esmer pirincin kardiyomyositleri mitokondriyal fonksiyon yoluyla iskemik/reperfüzyon hasarına karşı koruduğunu bulmuşlardır. Bu etkinin, çimlendirilmiş esmer pirincin biyofonksiyonel bileşik içeriğine bağlı olabileceği bildirilmiştir (Demeekul ve ark., 2021b). Başka bir çalışmada (Demeekul ve ark., 2021a), kardiyoplejik solüsyonla birlikte ÇEP tedavisinin, domuz kardiyomyositlerinde hücre canlılığını iyileştirdiği gösterilmiştir. Kardiyak cerrahiden elde edilen bulgulara göre, ÇEP grubu ile kombine kardiyoplejik solüsyonda ortalama arter basıncı ve kalp hızının sürekli stabil olduğu, ÇEP uygulanan hayvanlarda potasyum ve laktaz konsantrasyonunun eğiliminin azaldığı bildirilmiştir. Bu nedenle, ÇEP'in, antiinflamatuar yanıt oluşturması nedeniyle, iskemik reperfüzyon hasarına karşı kalp sağlığını koruyucu etkiler gösterebileceği vurgulanmıştır (Demeekul ve ark., 2021a).

Sonuç

Çimlenme, besinlerin biyokimyasal bileşiminde önemli değişikliklere yol açmaktadır. Çimlenmeyle antibesinsel faktörlerin (fitat, tripsin inhibitörü, tanen) miktarında önemli ölçüde azalmaktadır. Fenolik bileşikler, fitosteroller, folat, GABA gibi biyoktif bileşiklerin miktarı artmaktadır. Ayrıca GABA, Ɣ-orizanol ve aminoasitler gibi yeni bileşiklerin sentezi de çimlenme sürecinde yükselmektedir. Besinlerin antioksidan özellikleri ve bazı vitamin içeriklerinde de artış meydana gelebilmektedir. Bu değişiklikler çimlendirilmiş besinlere "fonksiyonel besin" özelliği kazandırmaktadır. Yapılan çalışmalarda farklı çimlendirilmiş besinlerin, antioksidan, antidiyabetik, antiobezite, antikanser, nöroprotektif vb. özellikleri gösterilmiştir. Çimlendirilmiş besinlerin çeşitli biyolojik

aktiviteleri *in vitro* ve *in vivo* çalışmalarda halen kesin olarak kanıtlanmamıştır. Bu nedenle çimlendirilmiş besinlerin çeşitli biyoaktif bileşen içerikleri ve insan sağlığı üzerindeki etkileriyle ilgili daha fazla sayıda çalışma yapılmalıdır.

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: -

Açıklama: -

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 8(4), 344-358 (2022) • https://doi.org/10.3153/FH22032 Review Article

Coffee: Health effects and various disease treatments

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Cite this article as:
Vo, T.S., Vo, T.T.B.C. Vo, T.T.T.N. (2022). Coffee: Health effects and various disease treatments. *Food and Health,* 8(4), 344-358. https://doi.org/10.3153/FH22032

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Submitted: 12.03.2022
Revision requested: 27.04.2022
Last revision received: 25.06.2021
Accepted: 06.07.2022
Published online: 22.09.2022

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

ABSTRACT

To respond the growing demands for consuming natural foods, biochemical compounds originated from natural sources can be one of significant purposes for numerous researchers. In this review, we summarize the literatures regarding to the health effects of coffee consumption toward various human disease treatments, i.e., diabetes, cancer, liver diseases, and neurodegenerative diseases. The consumption of natural products is being common and considered significantly, for example, the potentially functional features of biochemical compounds contained in coffee have significantly contributed to clinical treatments of different human diseases. The experimental and epidemiologic evidences are indicated in this review to probably contribute to elucidate the protective effects of coffee consumption on several human diseases; besides, it is not still certain whether the consumption of coffee should be recommended to patients in some cases. Moreover, the chemical features and health benefits of coffee are introduced shortly, which can support readers understanding in detail to the benefits and the roles of coffee compounds.

Keywords: Coffee, Disease treatment, Diabetes, Liver diseases, Parkinson's disease, Alzheimer's disease

Introduction

Coffee drink (Figure 1) is one of the most widely used beverages in the world, which contains several biochemical compounds that may influence the uptake and the metabolism of glucose (Ejaz et al., 2004). So far, numerous researches have been conducted to demonstrate the beneficial health effects of coffee on various diseases (Ngueta, 2020). Indeed, coffee showed its beneficial effects in irregular/regular coffee drinkers by changing mood, enhancing cognitive performance and endurance with exercises (Campbell et al., 2013). However, the extraction of coffee soluble from the roasted and ground coffee bean is a complex operation and brewing/cooking method plays an important role on the extraction and amount of the key compounds in the coffee drink. For instance, Ilkay et al. provided in detail how the roasting level and brewing techniques affect the key compounds, physicochemical attributes, and health of coffee beverage (Gök, 2021). More notably, coffee is utilized to support in sports that is useful for a significantly improved performance of athletes after the consumption of coffee (Mc Naughton et al., 2008). Coffee, as is known, is one of the most essential sources of caffeine, although soft and energy drinks also contain a significant part of caffeine. Its consumption probably increases alertness and enhances the performance of manual works, i.e., driving and encoding of new information (Gök, 2021).

Coffee contains over a thousand compounds including caffeine, sugars, polysaccharides, chlorogenic acids, aromatics, phenols, organic acids, and etc. (Table 1); nonetheless, the exact content of bioactive compounds containing in coffee can differently depend to the species, farming processes and preparation of final product, i.e., blend, roast, or brew (Yesil and Yilmaz, 2013). The use of coffee was first reported that it could provide a beneficial health effect against the cirrhosis development, similarly for an inverse relationship with total and non-cancer related mortality, as well as uncertainty over caffeine content and the information of coffee preparation techniques (Gök, 2021).

According to the epidemiological studies, the use of coffee could reduce the risk of liver enzyme disorder and decrease death rate and hospitalisations in all cirrhotic patients (Ruhl and Everhart, 2005). Besides liver diseases, the consumption of coffee also effectively resulted in treating other diseases, i.e., diabetes, cancer, neurodegenerative diseases, and so on. Hence, it is truly important to clearly understand the biochemical compounds and bioactive functions containing in coffee, aiming to apply it in bioactive and pharmacological applications after its reliability has been demonstrated. Thereby, we hope that it is an effective approach for various disease treatments, and its chemical components and beneficial health effects will be briefly summarized in several disease treatments.

Figure 1. Schematic illustration of the coffee drink.

Table 1. Background of chemical compositions containing in coffee.

Chemical compositions	Background
Lipids	Coffee oil (triglycerides, unsaponifiables and sterols/tocopherols) and diterpenes (cafestol and kahweol).
Minerals	Phosphorus and potassium. Magnesium, sodium, calcium, and sulfur.
Proteins	Peptides and free amino acids, i.e., regard to the coffee flavor.
Caffeine	Alkaloid, using as a psychoactive stimulant of the central nervous system.
Chlorogenic acids	Using as an ester compound of the caffeic acid. They are assumed to cause gastrointestinal discomfort at some people with higher coffee consumption, and can cause a slight reduction in blood pressure and have been investigated concerning an anti-inflammatory effect, and an antioxidant effect.
Trignonelline	Alkaloid. When roasting it can partly metabolize to niacin.
Carbohydrates	Common carbohydrates: fructose, glucose, mannose, arabinose, and rhamnose and oligosaccharides, raffinose and stachyose. Other ones: • *Sucrose*: Important for coffee flavor and quality. • *Polysaccharides*: The main polysaccharides in coffee are galactomannan and arabinogalactan (soluble compounds). • *Lignin*: A class of complex organic polymers, structural material in the support tissue of plants, important for the formation of cell walls.
Productions of caramelizations and condensation	Substances, influencing the color, and aroma of coffee.

Chemistry and General Properties of Coffee

So far, coffee is one of the most common beverages in the world, as well as its beneficial effects have been demonstrated to human health. Besides, coffee contains lots of chemicals, for example, vitamins, lipids, carbohydrates, nitrogenous compounds, minerals, alkaloids and micronutrients, which significantly depends on the variety and processes. Table 1 listed in detail for information of availably chemical compositions containing in coffee. Chemical structure of principal compounds contained in coffee is shown in Figure 2. Among these chemicals, the main components containing in coffee are caffeine, cafestol and kahweol, chlorogenic acid, ferulic acid, and micronutrients, at same time that the major polyphenols in coffee contributes to making coffee as a real functional food. Besides, it reveals promising anti-oxidative properties owing to their beneficial activities, especially for ferulic acid and chlorogenic acid (Higdon and Frei, 2006). More specifically, chlorogenic acid containing in roasted coffee is considered as a major antioxidant in the diet (Yanagimoto et al., 2004), whereas diterpenes cafestol and kahweol are contained much in boiled or unfiltered coffee.

Chlorogenic acid, as is known, is a phenolic compound (a family of polyphenols), which is reached from the combination of (L)-quinic acid and caffeic acid to be concerned as anti-oxidative agent. Meanwhile, ferulic acid is also a phenolic acid (a derivative of trans-cinnamic acid) and available in coffee, which can intervene in the expression and cytotoxic enzymes' activity (i.e., caspases, nitric oxide synthase, and cyclooxygenase-2), aiming to apply it in treating cardiovascular, neurodegenerative and diabetic disorders (Perumpail et al., 2018).

For chlorogenic acids containing in green coffee, there are some major subclasses including caffeoylquinic acids, dicaffeoylquinic acids, *p*-coumaroylquinic acids, feruloylquinic acids, and caffeoylferuloylquinic acids (Chu, 2012). Among them, 5-caffeoylquinic acid reaches ~60.0% of the total chlorogenic acids content, and thus is named as chlorogenic acid due to a commercial standard, at same time that dicaffeoylquinic acids are known as potent inhibitors contributing against various viruses (Chu, 2012). Concomitantly, caffeine-contained coffee (1,3,7-trimethylxanthine) favors to increase peroxiredoxin-1, which has positive effects on reactive oxygen species and lowering oxidative stress at hepatocytes level (Perumpail et al., 2018). Thereby, the coupling influences of

caffeine- and polyphenols-contained coffee on hepatocytes probably reduce insulin resistance, which is revealed as an anti-fibrotic effect on the liver through the effective investigations on obese rats (Watanabe et al., 2017).

Additionally, chlorogenic acids can be completely degraded with severely roasting conditions that is mainly due to its thermal instability, leading to forming bioactive lactones in medium roasting process and degrading then. Concomitantly, several volatile compounds are formed, and these acids are partially incorporated into melanoidines' backbones during coffee roasting process (Chu, 2012). Meanwhile, caffeine is not affected much during coffee roasting process, as well as this methylxanthine is heat stable. Trigonelline is considered as an alkaloid and obtained from enzymatic methylation of nicotinic acid, which is also degraded during this roasting process, yielding several compounds regarding nicotinic acid (3%) (Hirakawa et al., 2005). Overall, the various chemical composition-contained coffee is truly modified significantly from combinations of the coffee roasting techniques, regard with the species and other factors, i.e., agricultural processes, degree of fruit ripeness, and storage conditions (Chu, 2012).

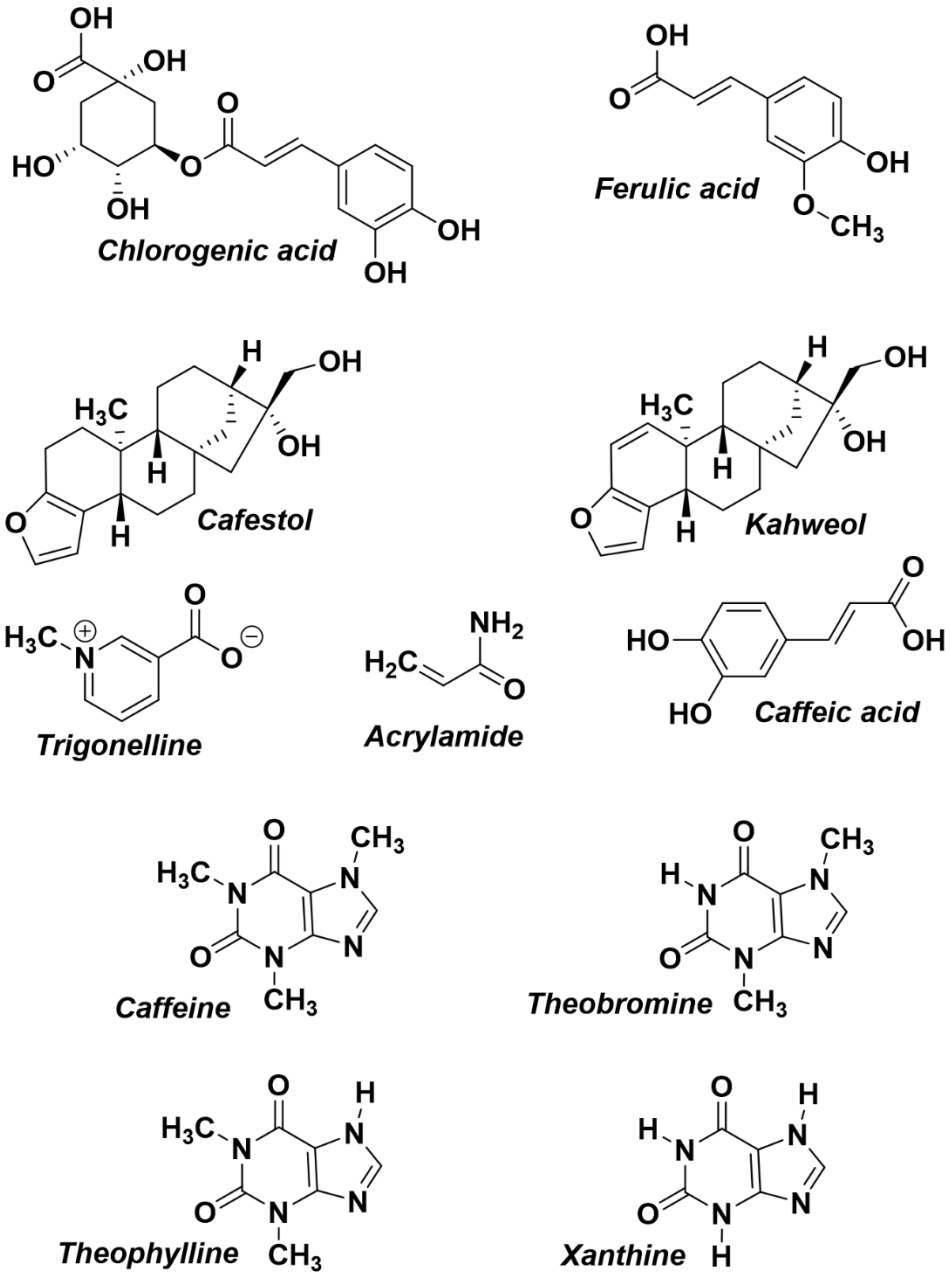

Figure 2. Chemical structure of the principal compounds contained in coffee.

More notably, caffeine is able to be the most well-known bioactive compound contained in coffee. Caffeine is an alkaloid and can be well absorbed in the stomach, which is metabolized in the liver (after 45-60 min of absorption period) to be converted into dimethylxanthines, i.e., xanthine, theobromine, theophylline, leading to enhancing its beneficial health effects. In other words, caffeine is quickly absorbed from the gastrointestinal way and well distributes in all tissues consisting of brain (Higdon and Frei, 2006); similarly, the caffeine consumption could lower a risk of raised aminotransferases, as well as its obviously hepatoprotective effect is to against liver disease (Ruhl and Everhart, 2005). Caffeine is concerned to be the purines, which acts as a psychostimulant in the central nervous system. It means that its stimulant ability can reduce the adenosine transmission in the different regions of brain (Fisone et al., 2004), as well as it probably lowers the sleeps and stimulate the heart muscle (Farah et al., 2006). Caffeine also induce to a bronchodilation and a peripheral vasodilation that can be due to its positive inotropic effect leading to increasing the contractility and efficiency of the heart. Moreover, it well stimulates digestion through promoting peristalsis, and which attains the diuretic effects on kidney (Maughan and Griffin, 2003). It could synergistically combine with phenylpropanolamine to be associated with severe hypertension, stroke and myocardial infarction. Additionally, the anti-fibrotic influences of coffee is controlled by lowering growth factor and connective tissue one (Chen et al., 2014), whereas tocopherols and chlorogenic acid in coffee showed anti-oxidative activities.

Moreover, cafestol and kahweol in coffee are pentacyclic diterpene alcohols, increasing cholesterol level, but they can be served as an anti-carcinogenic effect. Their amount containing in filtered coffee can be reduced but still maintain an amount of chlorogenic acid and caffeine to probably provide the maximum health benefit (Torres and Harrison, 2013). Their bioactive derivatives can be salts or esters of saturated and un-saturated fatty acids (~20.0%) (Chu, 2012); in particular, cafestol (0.2-0.6%, wt.) is a primary compound of unsaponifiable fraction of coffee oil, while kahweol (less abundant) is unstable to light, heat, oxygen, and acids . For acrylamide, it is found in coffee beans during the roasting process, and its content in coffee has a significant difference, besides, dietary acrylamide exposure in animal is higher than that in human studies. These coffee diterpenes revealed as hepatoprotective and anti-carcinogenic properties in vitro; however, these compounds are highly consumed, leading to elevating levels of homocysteine and low-density lipoprotein in human plasma that can indirectly induced to the cardiovascular diseases' risk (Chu, 2012). Therefore, coffee is served as non-toxic and highly promising compounds to apply for beneficial health effects and various disease treatments based on an appropriately utilized dose and each studying aim.

Health Effects and Various Disease Treatments of Coffee

As mentioned above, coffee is one of the most widely used beverages in the world. Roasted coffee accommodates chlorogenic acids that is known as a major antioxidant in the diet (Yanagimoto et al., 2004), whereas the diterpenes cafestol and kahweol can obtain after boiling or un-filtering coffee . With the concomitant existence of these components and other bioactive compounds containing in the coffee, the beneficial health effects and various disease treatments of coffee have been investigated as well (Grosso et al., 2017, Poole et al., 2017), especially for cancer, diabetes, liver diseases, Alzheimer's disease, and Parkinson's disease. Among these diseases, an inverse relationship between the use of coffee and liver cancer has long been recognized (Bravi et al., 2013, Godos et al., 2017); however, its mechanisms regarding these anti-carcinogenic influences have yet to be explained in detail. They are induced from liver enzymes to probably lead to cirrhosis and hepatocellular carcinoma; besides, the γ-glutamyltransferase of 2,494 male self-defense officials was ~30.0% lower in who drank coffee (≥ five cups/day) compared to nondrinkers , at same time that the inverse relationship between coffee and γ-glutamyltransferase was limited and stronger in alcohol drinkers. Also, chlorogenic acids and caffeic acids showed anti-oxidative effects in vitro (Iwai et al., 2004), while caffeine while caffeine has been reported regarding to its psychostimulant and positive short-term effects on attention and mental condition (i.e., cognition and memory) (Hameleers et al., 2000). From several researches, 3,5-icaffeoylquinic acid (family of chlorogenic acids) was utilized to be a potent inhibitor of the human immunodeficiency virus integrase , at same time that dicaffeoylquinic acids could be used to investigate against influenza and herpes virus, as well as regarded to other anti-bacterial properties (Antonio et al., 2010). Besides, chlorogenic acids probably impacted to mobility and replication of murine macrophages, anti-mutagenic properties, inhibition of glucose-6-phosphatase in lowering blood glucose and other mechanisms. Also, trigonelline has recently been considered an anti-bacterial agent to be against a cariogenic bacterium, i.e., Streptococcus mutants (Antonio et al., 2010).

Liver Cancer and Liver Diseases

Liver cancer, as is known, is a common malignancy worldwide, which can cause cancer deaths (Ferlay, 2004), especially for chronic infection with hepatitis B (HBV) or C (HCV) viruses and alcohol consumption (Franceschi et al.,

2006, Llovet et al., 2021). In general, coffee contains lots of biologically active components, especially for anti-tumor effects, at same time that trigonelline has well impeded the cancer cells' invasiveness in vitro (Hirakawa et al., 2005). Several investigations were conducted on animal models indicating that the direct use of coffee had the barring influences against the chemical carcinogenesis in liver tissue; concomitantly, several epidemiological investigations indicated on the inverse relation between the effects of coffee and liver cancer regarding serum liver enzyme activity, as well as the inconsistent relation with the incidence of liver cirrhosis.

To further clarify this approach, Sang et al. conducted an investigation of prospective–cohort and case–control studies, resulting that the use of coffee could relate to a reduced risk of liver cancer; nevertheless, this should be treated with caution more (Sang et al., 2013). Whereas, Shimazu et al. carried out an available data analysis of 2 cohort studies, including 22,404 people with the frequency consumption of coffee (10,588 men and 11,816 women) and 38,703 people with other health habits (18,869 men and 19,834 women), who were ≥40 years old and no previous history of cancer (Shimazu et al., 2005). It manifested that the consumption of coffee significantly contributed to a decreased incidence of liver cancer, and which is truly need more investigations to elucidate the role of coffee in prevention of liver cancer. Additionally, Loftfield et al. also demonstrated that coffee has been consistently involved to reducing risk of liver cancer and chronic liver disease, which based on serum metabolites in case-control studies of liver cancer (221 people) and fatal liver disease (242 people) (Loftfield et al., 2020).

Moreover, hepatocellular carcinoma can be also added into other digestive tract cancers regarding to beneficial health effects of coffee drinking has been suggested (i.e., oral-pharyngeal and oesophageal-colorectal cancers) (Tavani and La Vecchia, 2004). The use of coffee has been suggested to reduce the risk for hepatocellular carcinoma; nonetheless, controversy exists about the exactly used dose. Bhurwal et al. (Bhurwal et al., 2020) investigated the association of coffee utilization and risk of hepatocellular carcinoma and/or liver cancer (20 people, one cup/day), evaluating that the relationship between the use of coffee with hepatocellular carcinoma or liver cancer development along with the suitably used amount of coffee to probably prevent hepatocellular carcinoma or liver cancer. Also, more highly used doses of coffee have better benefits in terms of risk reduction, but further biological and epidemiological investigations are truly necessary to be required to demonstrate the exact mechanism and determine specific subgroups (i.e., HBV- or HCV-related hepatocellular carcinoma). Concomitantly, Freedman et al. also examined the relationship between the use of coffee and liver disease progression in individuals with advanced HCV–related liver disease (766 people), suggesting that the regular utilization of coffee related to lowering the disease progression (Freedman et al., 2009). As such, coffee consumption probably reduced the fibrosis progression's risk in HCV and improved the interferon based anti-HCV therapy (Freedman et al., 2011, Freedman et al., 2009), at same time that it also protected against the hepatocellular carcinoma development (Johnson et al., 2011). In other studies, 59 people with alcohol-related cirrhosis drank the coffee (≥ four cups/day), manifesting that it reached about five fold lower the risk of non-coffee drinkers , as well as the use of coffee was also inversely involved to the risk of cirrhosis death (Komorita et al., 2020, Teramoto et al., 2021). Besides, a case-control study (115 people) indicated an inverse relation between the use of coffee and the risk of cirrhosis, especially for a favorable health effect of coffee on alcohol-related cirrhosis risk. This was similar with another bigger study (274 people and 458 controls), which revealed a strong inverse relation between coffee drinking and cirrhosis, meaning a relative risk of 0.16 for coffee drinkers (≥ four cups/day) comparing to non-coffee drinkers.

In general, coffee contains lots of bioactive compounds that probably reach beneficial health effects on the liver, which probably based on their antioxidant and anti-inflammatory properties, defense mechanisms, and angiogenesis inhibition to favor the tumors' growth with oxygen and nutrients (Bohn et al., 2014). Also, the use of decaffeinated coffee in an animal model was able to decrease liver fibrosis, steatosis, and inflammation (Vitaglione et al., 2010).

Non-Alcoholic Fatty Liver Diseases and Diabetes

For non-alcoholic fatty liver disease, it is a highly common condition and signalized in the initial stages by hepatic steatosis. In another word, this disease is originated from hepatic fat accumulation (>5.0%), which is not due to excess alcohol consumption or other established liver diseases (Friedman et al., 2018). This disease is a phenomenon of alterations scaling from simple hepatic steatosis to non-alcoholic steatohepatitis, cirrhosis and hepatocellular carcinoma; besides, the liver biopsy non-alcoholic steatohepatitis can be classified with the degree of fibrosis, i.e., mild fibrosis, significant fibrosis, advanced fibrosis and cirrhosis. Some clinical meta-analysis indicated that potentially bioactive compounds (i.e., vitamin, silymarin, resveratrol, curcumin, etc.) exert positive influences on this disease that was probably attributed to their anti-inflammatory or antioxidant properties (Vo et al., 2021a, b, Vo et al.); nonetheless, it could be also significantly involved to the differently used doses, formulation issues, or tested duration.

Among them, the consumption of coffee containing caffeine could reduce lipid infiltration in the liver via anti-inflammatory, anti-oxidation, and fatty acid metabolism-related mechanisms. Also, the utilization of coffee can be beneficial in treatment of non-alcoholic fatty liver disease based on a direct effect on the liver and beneficial systemic metabolic influences. Needed, coffee has plenty of potentially bioactive properties, i.e., anti-inflammatory, antioxidant, and anti-fibrotic properties, which reported a reverse relationship between the coffee utilization and non-alcoholic steatohepatitis on base of a dose-dependent manner (Chen et al., 2014). A daily used coffee drink (n=177, > two cups/day, ~6 months) was associated with significantly lower odds of liver fibrosis (Modi et al., 2010), similarly with a non-alcoholic steatohepatitis specific cohort (n=306, > two cups/day, 2 years) (Molloy et al., 2012). At the same time, it revealed that non-alcoholic fatty liver-associated patients (n=5,147) drank the coffee liquid (> three cups/day, 7 years) resulting a lower fibrosis score (Zelber-Sagi et al., 2015), and the coffee consumption (≥ three cups/day) could also decrease the growing hepatocellular carcinoma's risk in 63,257 people (~44.0%) (Johnson et al., 2011). In other studies, it manifested that caffeine decreased intrahepatic fat accumulation in rat models, but they did not definite clearly its used dose based on animal weight (Fang et al., 2019, Helal et al., 2018).

In addition to caffeine, coffee contains the other biochemical compounds, and which are especially rich in polyphenols, i.e., chlorogenic acids, was responsible for its beneficial health effects. Thereby, Velázquez et al. conducted an investigation of green coffee extract and caffeine effects (5.00 mg/day) on hepatic lipids in female rat models with steatosis (Velázquez et al., 2020). As a result, a low dose of caffeine did not decrease hepatic steatosis in these rat models, but the same used dose of green coffee extract led to lowering liver triglyceride levels. Trovato et al. surveyed the drinking espresso coffee in 161 obese women and 34 men after bariatric surgery, which explained clearly in its beneficial health effect (Trovato et al., 2013).

Theoretically, coffee is used to can protect the liver on base of increased PPAR-α mediated fatty acid oxidation and protective antioxidants, and reduced collagen deposition (Carvalhana et al., 2012), so the use of caffeine-contained coffee significantly related to lower the hepatic fibrosis' risk in patients with non-alcoholic steatohepatitis. Molloy et al. also surveyed 400 non-alcoholic fatty liver-associated patients, suggesting that greater use of coffee significantly reduced risk of advanced fibrosis (Molloy et al., 2012). However, the exactly used dose of coffee regarding the greatest risk reduction was not still clear. The case of 782 non-alcoholic fatty liver-associated adults also showed a lowered risk for advanced non-alcoholic steatohepatitis through regularly drank coffee, especially in patients with low levels of insulin resistance (Bambha et al., 2014). Besides, the use of coffee could protect against the development of metabolic syndrome and non-alcoholic fatty liver disease conducted in experimental and clinical models (Yesil and Yilmaz, 2013) (i.e., 3 animal studies and 11 epidemiological and clinical studies). Thereby, the health effects of coffee on liver disease are multiple factors, as well as which is truly necessary to elucidate further, especially for the use of filtered unsweetened coffee was be able to be a rational extension to diet and exercise in the fatty liver-associated patients (Chen et al., 2014, Yesil and Yilmaz, 2013).

More interestingly, caffeine could lower the gene expression of the transcription factors Sterol regulatory element-binding protein 1c and 2 in HepG2 cells regarding to the combination of cholesterol and triglycerides in the liver (Quan et al., 2013), which was able to cause the reduction of 3-hydroxy 3-methylglutaryl CoA reductase and low-density lipoprotein receptor in an appropriately used dose. In another study, the utilization of caffeine could improve liver damage induced by a high-fat diet in animal models (Helal et al., 2018), which was evaluated on base of alanine/aspartate aminotransferase, albumin, bilirubin, triglycerides and cholesterol. As result, it showed that the use of caffeine lowered elevated serum levels of alanine/aspartate aminotransferase, bilirubin/hepatic mRNA expression of fatty acid synthase and acetyl CoA carboxylase. Furthermore, the use of coffee also impacted to an improvement of insulin sensitivity (Bohn et al., 2014) and lowered risks of metabolic syndrome and type 2 diabetes (Tunnicliffe and Shearer, 2008). Diabetes is considered as an auto-inflammatory syndrome with plenty of possible disorders, i.e., insulin resistance, hyperglycemia, dyslipidemia, impaired β-cell functioning and insulin secretion (Bosun-Arije et al., 2020, Hussain and Chowdhury, 2019), which also accelerates liver fibrosis and inflammation. Generally, the traditional anti-diabetic agents probably induce several potentially adverse occurrences (Bosun-Arije et al., 2020, Hussain and Chowdhury, 2019), at same time that there are also some natural anti-inflammatory agents with anti-diabetic effects to be able to against type 2 diabetes (Akash et al., 2013), and their limitations in short biological half-life (Akash et al., 2012). Besides, coffee can be well used to protect liver cancer through improving the insulin sensitivity as well as lowering the risk of diabetes (Tunnicliffe and Shearer, 2008). Recently, several researches have indicated a significantly reduced risk of cardiovascular disease and type 2 diabetes in coffee drinkers (Ding et al., 2014). Specifically, some biochemical components of coffee are able to ameliorate type 2 diabetes symptoms through impacting glucose regulation,

i.e., the effects of chlorogenic acid on glucose-6-phosphatase, the beneficial influences of caffeine on insulin secretion, and the antioxidant activity of polyphenols on α-glucosidase (Tuomilehto et al., 2004).

Neurodegenerative Diseases

Mitochondrial dysfunction and oxidative stress are considered as prior occurrences in neurodegenerative diseases (NDDs), i.e., Alzheimer's disease (AD), Parkinson's disease (PD), and so on. In fact, mitochondria are essential factors in cellular function grounded on their energy produced and their major role in cell physiology. Also, neurons significantly relate to this energy production, so the mitochondrial dysfunction can induce deadly influences regarding to neuronal function and survival that are due to their high energy demand and reduced glycolytic capacity. Especially, the use of coffee has protectively affected in these NDDs through epidemiological and clinical studies (Beghi et al., 2011), for example, the protective effects of aqueous coffee extract investigated effectively on amyloid-beta (Aβ) peptide toxicity in the AD (Dostal et al., 2010).

More specifically, AD is known as the most typical dementia on base of a gradual descent of cognitive functions and memory deficiencies (Reitz and Mayeux, 2014), meaning that it involves to extra-neuronal deposition of Aβ protein in the formation of plaques and intra-neuronal aggregation of the hyper-phosphorylated microtubule-associated protein tau in the cortex, hippocampus and amygdala (Lloret et al., 2015). Moreover, a neuro-inflammatory constituent regarded strongly to the AD (Ikonomovic et al., 2008). In other words, the presence of Aβ oligomers probably leads to microglia-mediated neuro-inflammatory response, which can induce the neuronal-loss and -toxicity (Pan et al., 2011). As such, microglia reactivity is occurred not only in the brain, but also in the retinas of AD animal models (Ning et al., 2008), at same time that activation is also considered as a consequence or a cause of the AD. Thereby, interventions are suitably selected to control the microglia reactivity that probably delay the AD progression.

Interestingly, the use of caffeine can favor for reducing the cognitive decline in AD patients and healthy subjects with advanced age (Ritchie et al., 2007). This approach was also investigated in AD animal models, which showed that the use of caffeine could effectively reach in amelioration of cognitive impairments and dementia (Arendash et al., 2009, Eskelinen and Kivipelto, 2010), at same time that increased caffeine amounts in the plasma could reduce levels of inflammatory cytokine in the hippocampus (Cao et al., 2009). Notably, caffeine was also used in a mouse model with AD-like tau pathology reducing some oxidative stress and pro-inflammatory markers in the hippocampus, as well as which interfered the spatial memory deficits' development (Laurent et al., 2014). Concomitantly, caffeine could be applied to well protect against AD-associated blood-brain barrier dysfunction (Chen et al., 2010) and probably control an increase in AD-associated inflammatory mediators (Farkas et al., 2003).

More notably, the use of caffeine significantly improved memory deficits and reduced the expression of reactive oxygen species, pro-inflammatory cytokines TNF, IL-1β, and further granted anti-apoptotic effects (Ullah et al., 2015) in an animal model with age-related central nervous system alterations; at the same time, the caffeine actions were mediated through A2aR blockade, indicating that this blockade based on pharmacological features and genetic inactivation well provided neuroprotection against Aβ toxicity (Canas et al., 2009). As such, these demonstrated the use of caffeine reached desired and potential properties to apply for the AD treatment.

For the cased of PD, it is considered as the second most common NDD based on a progressive loss of dopaminergic neurons with the Lewy bodies' occurrence, which leads to appearing resting tremor, muscular rigidity, bradykinesia and postural instability (Klockgether, 2004). Concomitantly, this PD has been also coupled with oxidative stress and chronic neuro-inflammation inducing the blood-brain barrier disruption, meaning that the brain can be susceptible to cause oxidative stress because of the high oxygen consumption (Hald and Lotharius, 2005). Notably, the oxidative stress could be a main cause inducing the neuronal damage in the PD based on postmortem human samples (Jenner and Olanow, 2006). Moreover, plenty of currently epidemiological studies have been also significantly investigated the use of caffeine to favor reducing risk of developing PD (Rodrigues et al., 2015). For instance, the daily use of caffeine could attenuate microglia reactivity and prevent the blood-brain barrier dysregulation in the MPTP mouse model, which led to reducing dopaminergic neuronal loss (Chen et al., 2008). The caffeine also probably depress the inflammatory procedure and microglial cell expression in the later neurodegenerative processes suggesting its ability to delay or arrest neuro-inflammation and neurodegeneration (Chen et al., 2008); besides, the lowly used doses of caffeine can invert functional motor deficiencies in the PD animal models probably involving to the A2AR antagonism (Bata-Garcia et al., 2010). Furthermore, the long- and short-term use of caffeine also impacted to acetylcholine and its receptors in the brain, at same time that the acetylcholine response displayed no trend to tolerance (Acquas et al., 2002). In case of long-term use of caffeine to mice, it indica-

ted that it could increase the number of muscarinic and nicotinic receptors in the brain, as well as probably increased their cholinergic function.

So far, the potential benefits and roles of coffee are attracting interests in treatment of age-related NDDs. In particular, Fisicaro et al. conducted the evaluation between various quantities of used mocha coffee (two cups/day) and performance of cognitive-mood in 300 non-demented patients with subcortical ischemic vascular disease (Fisicaro et al., 2021), indicating that daily use of mocha coffee involved to higher cognitive-mood performance, similarly with a dose-response association to probably identify the factors regarding vascular dementia and geriatric depression. However, there are some limitations occurred during this investigation, i.e., the drinking habit can become an effect of cognitive performance rather than causally regarded (Arab et al., 2013). The caffeine could significantly contribute to the evaluation, but the other potential compounds contained in the coffee, i.e., flavonoids, were missed and need further investigation. Besides, trigonelline could also recreate axons and dendrites in animal models, which led to probably improving memory (Tohda et al., 2005).

Coronavirus Disease 2019

As known, the first cases of coronavirus disease 2019 (COVID-19) were reported in Wuhan (China) at the end of 2019, which is as an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Especially, handwashing (i.e., soap and water/warm water-based handwashing, or alcohol-based hand sanitizers) and wearing masks (i.e., cloth masks, medical masks, N95 respirators, and surgical masks) are considered as one of the ways that can prevent the influenza virus infection (Vo et al., 2020a, b). People in the world are now self-isolating at their homes, the use of supplement products probably support enhancing the immune system and prevent SARS-CoV-2 infection through the daily diet, that may reduce the COVID-19 infection risk and a recovery in SARS-CoV-2 infection cases (Vo et al., 2021a, b, Vo et al.). Moreover, 1,3,7-trimethylxanthine is known as the most common psychoactive drug worldwide, which is used effectively on the tolerance to central hypovolemia (Zores and Rebeaud, 2020) and the prevention of hypotension-related syncope (Pizzey et al., 2020), at same time that the exposure to lower body negative pressure led to decreasing significantly blood pressure was conducted in coffee-used 13 patients, which contained caffeine and 1,3,7-trimethylxanthine. It means that syncope is caused by a decrease in blood flow, especially from low blood pressure, so its control can allow a better perfusion of the brain and a reduced risk of syncope. Interestingly, Belaroussi et al. performed a pedagogic comparative investigation aiming to evaluate the effect of active part (1,3,7-trimethylxanthine) contained in the coffee for the treatment of 93 COVID-19 infected patients (Belaroussi et al., 2020), meaning that this pedagogic investigation aimed to highlight potential biases in research on COVID-19 treatment. However, this study cannot be still concluded for any association between coffee or 1,3,7-trimethylxanthine and COVID-19.

Conclusion

In brief, coffee is one of the widely used beverages in the world, which was also applied effectively for treating various human diseases to assess correspondingly its beneficial health effects. For liver diseases, the health effect of coffee is almost undoubtable on liver cancer risk, chronic liver diseases, and etc., indicating that it is not only one specific benefit to liver cancer, but also a favorable effect on a whole of liver functions. Nonetheless, prospective mechanisms have not still been determined in detail and need to be resolved further in this approach. Coffee could also directly affect to the pathogenesis of type 2 diabetes and non-alcoholic fatty liver disease, revealing that the use of coffee imparted the advantageous effects to stop the pathogenesis of these diseases. Besides, this approach also needs to be concerned further to be an alternate supplement along with other anti-diabetic agents, while the effects of caffeine to neuro-inflammation is truly important to be elucidated more. Interestingly, the biochemical compounds of coffee could also be known as a preventive and therapeutic role in the neurodegenerative diseases, i.e., Alzheimer's and Parkinson's diseases. It means that these potentially functional features have contributed to antioxidant, anti-apoptotic, and etc.; concomitantly, the clinical studies with long-term approach should be recommended more in the future.

Compliance with Ethical Standard

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

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

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

Acknowledgments: -

Disclosure: -

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Abstract

Keywords (indexing terms), normally 3-6 items

Introduction

Material and Methods

Results and Discussion

Conclusion

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Compliance with Ethical Standard

- **Conflict of Interest:** When you (or your employer or sponsor) have a financial, commercial, legal, or professional relationship with other organizations or people working with them, a conflict of interest may arise that may affect your research. A full description is required when you submit your article to a journal.
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Manuscript Types

Original Articles: This is the most important type of article since it provides new information based on original research. **The main text should contain "Introduction", "Materials and Methods", "Results and Discussion", "Conclusion", "Compliance with Ethical Standard**" and **"References" sections.**

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Table 1. Limitations for each manuscript type

Type of manuscript	Page	Abstract word limit	Reference limit
Original Article	≤25	180	40
Review Article	no limits	180	60
Short Communication	≤5	150	20

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Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text).

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References

Reference System is APA 6th Edition (with minor change's)

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....(Erkan, 2011).

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

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A book in print

Harrigan, W.F. (1998). Laboratory Methods in Food Microbiology. Academic Press, pp. 308. ISBN: 9780123260437

A book chapter

Craddock, N. (1997). Practical management in the food industry A case study. In Food Allergy Issues for the Food Industry; Lessof, M., Ed.; Leatherhead Food RA: Leatherhead, U.K., pp 25-38. ISBN: 4546465465

A webpages

CDC (2020). Rift Valley Fever | CDC. https://www.cdc.gov/vhf/rvf/index.html (accessed 20.08.2020)

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