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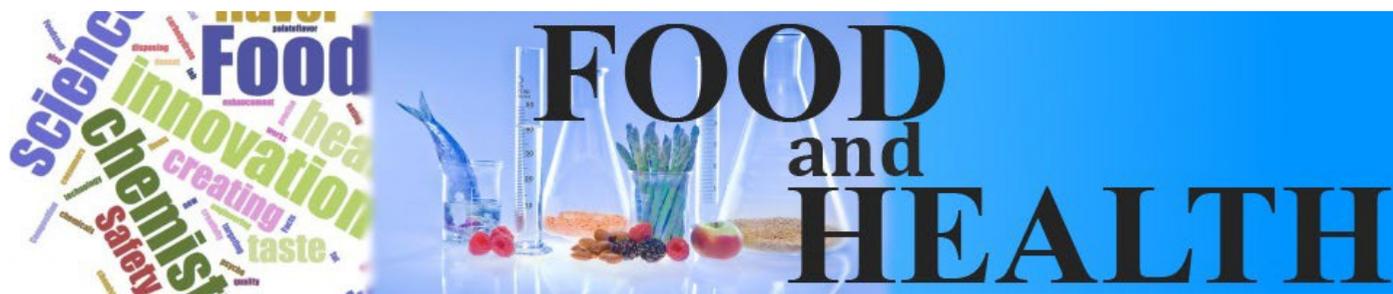
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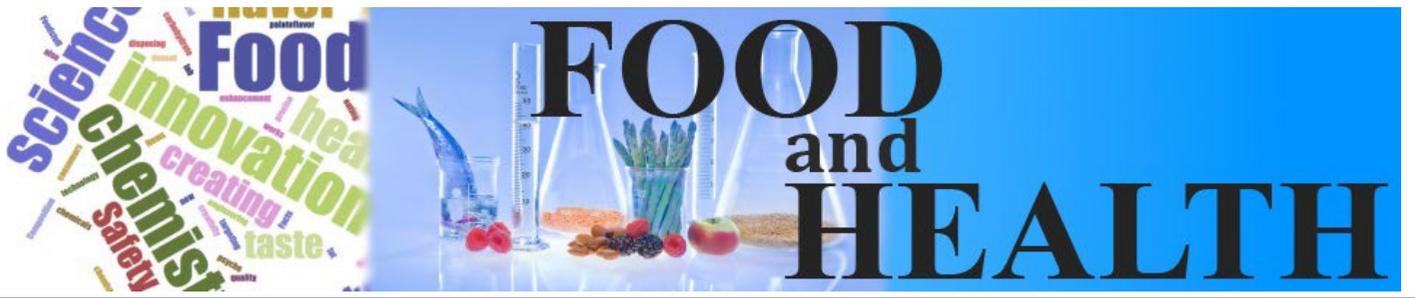
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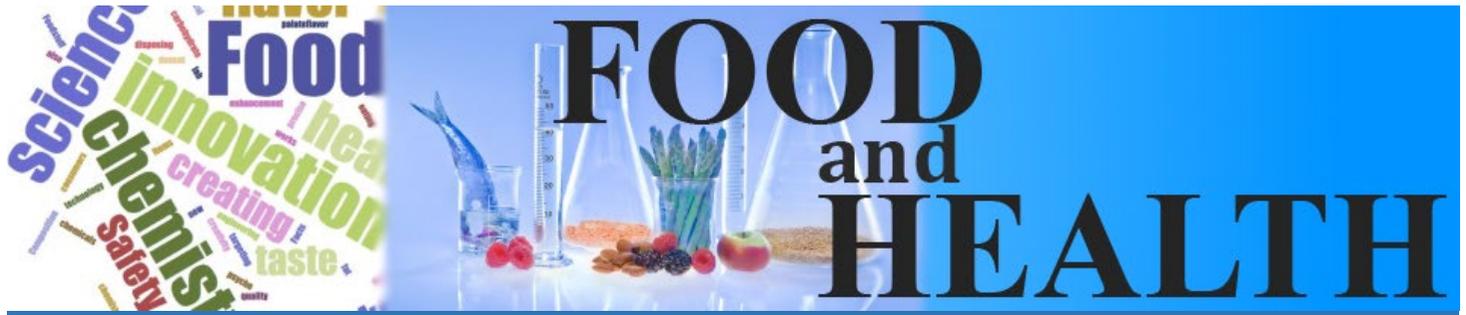
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**Research Article**

## **EVALUATING THE PERFORMANCE OF ATP BIOLUMINESCENCE METHOD BY COMPARISON WITH CLASSICAL CULTURAL METHOD**

**Pınar Öz , Özge Özgen Arun **

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### **ABSTRACT**

Verification of the performance of the sanitation procedure is as important as the actual application. The methods that are going to be used for such verification needs to be user friendly and rapid resulting to apply the corrective actions promptly. Additionally, these methods should also be reliable besides their fast resulting. ATP bioluminescence method is recommended to be a solution for such procedures for many years. In this present study, our aim was to evaluate the performance of ATP bioluminescence method by comparison with golden standard classical cultural method. For this we performed parallel testing on surfaces which were experimentally contaminated with different types of microorganisms. The results showed that ATP bioluminescence gives more reliable results in surfaces which do not have any physical dirt and are contaminated with high level of microorganisms. The most reliable results were obtained from *Salmonella* contaminated surfaces.

**Keywords:** ATP bioluminescence, Food hygiene, Hygiene monitoring, Poultry hygiene, Rapid hygiene monitoring

## Introduction

The microbiological safety of food products is strongly related to the hygienic properties of the processing environment. In such conditions, applying convenient sanitation methods becomes mandatory for final product safety. Evaluation of the efficiency of these methods is necessary for verification of such processes. As a matter of fact, these verification practises are required in all food safety standards (BRC 2014; ISO 2009; TSE 2006). Therefore, researchers put significant effort on development of rapid and reliable methods to meet the requirement of routine hygiene monitoring in food production.

Although classical cultural methods are the most reliable golden standards, the time consuming process necessary for these methods are not very practical for routine use. The main expectation from such methods are practicability, rapidity and cost efficiency while their reliability is still a major expectation. For this purpose, several alternative rapid methods have been developed (Rosmini et al., 2004). Most well-known samples of these alternative methods are; 3MTM Petrifilm™, CompactDry, Contact Plate, Dipslide, DEFT, ELISA, PCR ve ATP bioluminescence hygiene monitoring system (Jasson et al., 2010).

ATP bioluminescence is one of these alternative methods and being recommended for online monitoring of the microbiological activity in biological processes since 1960s (Chu et al., 2001). The method is based on the reaction between luciferin-luciferase and ATP which transforms the chemical energy to light energy and the measurement of this energy with a luminometer (Griffith et al., 1997). Because, this method can be performed with a small and practical portable device it has gained a significant application area in food industry for monitoring of production surfaces. With this method it is possible to monitor the level of hygiene in minutes and thus, it gives the possibility of promptly starting the corrective actions if a nonconformity is detected (Chen and Godwin 2006; Chollet et al., 2008; Siragusa et al., 1995).

Several studies have been done so far to evaluate the effectiveness of the method in hygiene monitoring (Ayçiçek et al., 2006; Boyce et al., 2009; Carroscosa et al., 2012; Chen and Godwin 2006; Costa et al., 2006; Davidson et al., 1999; Larson et al., 2003; Lehto et al., 2011; Moore and Griffith 2002; Moore et al., 2010; Sala et al., 2008; Valat et al., 2003). Lehto et al., (2011) used ATP bioluminescence method in a vegetable processing environment in Finland and determined that ATP bioluminescence method can even be used for monitoring of production surfaces with a low

contamination level. Similarly, Ayçiçek et al., (2006) compared the results of hygiene monitoring with classical cultural method and ATP at hospital kitchen.

In these studies the evaluation is studied on naturally contaminated surfaces. In contrast, the main purpose of our study was to evaluate the performance of ATP bioluminescence method by comparison with the classical cultural (swab) method on surfaces artificially contaminated with different microorganisms (*Salmonella*, *E. coli*, *Staphylococcus aureus*, *Saccharomyces cerevisiae*). Additionally, we evaluated the performance of the method on naturally contaminated environment. For this, we made parallel sampling with these two methods from a poultry processing plant.

## Materials and Methods

**Sampling surfaces and Reference strains:** 30x40 cm stainless steel surfaces which are most commonly used materials in food production were used as model sampling surfaces. Before the artificial contamination, the surfaces were cleaned and disinfected in accordance with a sanitation procedure used by a poultry processing plant in Turkey. For this, the surfaces were sprayed with 1/250 diluted Antec DSC 1000 (Refarm, Istanbul) and rinsed with water for 2-3 min. Following cleaning, the surfaces were sprayed with 1/200 diluted Antec Ambicide (Refarm, Istanbul) for disinfection. Different than the practical approaches, the surfaces were soaked with alcohol and flamed. The residual microorganisms on the surfaces were checked with swab sampling and enumerated on Plate Count Agar (PCA, Oxoid, UK).

For the artificial contamination of the surfaces; lyophilised reference strains of *E. coli* ATCC 25922, *Salmonella enterica subsp. enterica serovar Typhi*. ATCC 14028, *S. aureus subsp. aureus* ATCC 11632, *S. cerevisiae* ATCC 9763 were used. The contamination was performed with stock cultures prepared in compliance with the manufacturer's instructions.

**Contamination of the surfaces:** a single colony from each reference strain was inoculated in Brain Heart Infusion Broth (BHIB, Oxoid, UK) and incubated under the appropriate condition and time (at 35°C for 24 h.; 44°C for 24 h.; 35°C for 48 h.; 25°C for 5-7 days; for *Salmonella*, *E. coli*, *S. aureus*, *S. cerevisiae* respectively). The presumptive colony count of these inoculation solutions were determined with McFarland Optical density Equipment (Biosan, 050102-1108-0341). The inoculation solution was then 10 fold diluted to prepare the necessary concentration for target contamination level.

Each of the two parallel surfaces (each for swab and ATP bioluminescence method) were streaked with 0.1 mL from the appropriate dilution of inoculation solution to give the high ( $10^5$ - $10^6$  cfu/cm<sup>2</sup>), medium ( $10^2$ - $10^4$  cfu/cm<sup>2</sup>) and low ( $10^1$ - $10^2$  cfu/cm<sup>2</sup>) contamination levels. To determine the accurate bacterial count of the inoculation solution, it was plated on PCA (e.g. Plate Count Agar) in duplicate in parallel with the artificial contamination and incubated 48 h at 35°C. The obtained enumeration results were used for the determination of the contamination level. This application was repeated for each trial and each reference strain.

**ATP measurements:** In this study ATP bioluminescence Hygiene (SystemSURE Plus, INS0047) was used according to the instructions manual. Samples were taken from one of the parallel contaminated surface. Sampling were made from 100 cm<sup>2</sup> surface. The RLU values read from the instrument was recorded.

**Classical cultural method:** Swab sampling was performed from the second parallel contaminated surface and the swab stick was left in sterile physiological saline water. Following 10 fold dilutions with sterile physiological saline water, petri dishes containing adequate media were inoculated with appropriate dilution fluid (FDA, 2003; ISO, 2004). For *E.coli* enumeration TBX agar (Oxoid, UK), for *S.aureus* Baird Parker Agar (Oxoid, UK), for Salmonella XLD agar (Oxoid, UK) and for total mesophilic aerobic count PCA (Oxoid, UK) were used in accordance with the reference standard methods (FDA, 2001a; FDA 2001b; FDA 2001c; FDA, 2007, ISO 2001).

The whole experimental design was repeated three times.

The correlation between log<sub>10</sub> cfu and RLU values obtained from the artificially contaminated surfaces were calculated with Spearman Correlation method.

## Results and Discussion

The main objective of this study was to evaluate the performance of ATP bioluminescence hygiene monitoring method by comparison with golden standard classical cultural method. For this, sampling from the experimentally contaminated surfaces were analysed with both methods and the results were compared. Additionally, the results of parallel sampling from naturally contaminated processing environment was performed.

The swab sampling taken from the disinfected surfaces did not show any microbiological growth on PCA. This negative total aerobic count showed that the surfaces were successfully decontaminated and the microbial flora we detected after artificial contamination belongs to target microorganisms.

The mean (mean of three repeated experiments) cfu and RLU values for the four contaminated microorganism and all contamination levels are summarized in Table 1.

According to the user's manual of the ATP Bioluminescence equipment (SystemSURE Plus, INS0047) the RLU values <10, 11-29 and >30 reflects clean, conditionally clean and dirty surfaces (Whitehead et al. 2008). The evaluation at the last column of the table were made according to these limits.

The mean RLU results of artificially contaminated surfaces with *Salmonella* was 189 for high contamination level (6.11 log<sub>10</sub> cfu/cm<sup>2</sup>), while 31 and 0 respectively for medium (4.53 log<sub>10</sub> cfu/cm<sup>2</sup>) and low (2.29 log<sub>10</sub> kob/cm<sup>2</sup>) contamination levels (Table 1). The results for *E.coli* were 265, 8 and 1 for high (5.57 log<sub>10</sub>cfu/cm<sup>2</sup>), medium (3.25 log<sub>10</sub>cfu/cm<sup>2</sup>) and low (1.51 log<sub>10</sub> cfu/cm<sup>2</sup>) respectively, for *S.aureus* 136, 19 and 1.6 for high (5.55 log<sub>10</sub> cfu/cm<sup>2</sup>), medium (4.46 log<sub>10</sub> cfu/cm<sup>2</sup>) and low (2.95 log<sub>10</sub> cfu/cm<sup>2</sup>) respectively and for *S. cerevisiae* 135, 0 and 3 for high (6.14 log<sub>10</sub> cfu/cm<sup>2</sup>), medium (2.76 log<sub>10</sub>cfu/cm<sup>2</sup>) and low (1.32 log<sub>10</sub> cfu/cm<sup>2</sup>) respectively. According to these results, ATP bioluminescence evaluated the highly contaminated surfaces which carried 5-6 log<sub>10</sub> cfu/cm<sup>2</sup> microorganisms as "dirty". The detection for medium level contaminated surfaces (3-4 log<sub>10</sub> cfu/cm<sup>2</sup>) differed for microorganism and resulted as dirty and doubtful for *Salmonella* and *S.aureus* respectively while it resulted as clean for *E.coli*. The medium level contamination was 2 log<sub>10</sub> cfu/cm<sup>2</sup> for *S.cerevisiae* and ATP again resulted as clean. The results for low level contamination of *S.cerevisiae* which is 1-2 log<sub>10</sub> cfu/cm<sup>2</sup> ATP failed to detect or detected as very low. Similarly, Chen and Godwin (2006) also informed that ATP results were lower than 20 RLU for surfaces contaminated with total aerobic bacteria below 3 log<sub>10</sub>/cfu. Likewise, some other researchers calculated the detection limit of ATP 3-4 log<sub>10</sub> cfu/cm<sup>2</sup> (Siragusa et al., 1995; Davidson et al., 1999; Corbit et al., 2000; Larson et al., 2003; Leon ve Albrech 2007; Sala et al., 2008; Jasson et al., 2010; Carroscosa et al., 2012).

**Table 1.** The mean cfu and RLU values for the four contaminated microorganism and all contamination levels

Microorganism	Contamination level	Log <sub>10</sub> cfu/cm <sup>2</sup>	RLU	Clean/Dirty
<i>Salmonella</i>	High	6.11	189	Dirty
	Medium	4.53	31	Dirty
	Low	2.29	0	Clean
<i>E.coli</i>	High	5.57	265	Dirty
	Medium	3.25	8	Clean
	Low	1.51	1	Clean
<i>S.aureus</i>	High	5.55	136	Dirty
	Medium	4.46	19	Conditionally clean
	Low	2.95	1.6	Clean
<i>S. cerevisiae</i>	High	6.14	135	Dirty
	Medium	2.76	0	Clean
	Low	1.32	3	Clean

The correlation between the results of classical cultural methods and ATP bioluminescence method was calculated with Spearman correlation test. According to this calculation, correlation coefficient (r) was 0.911, 0.724, 0.644 and 0.623 for *Salmonella*, *E. coli*, *S. aureus*, *S. cerevisiae* respectively. Nevertheless, the correlation coefficient (r) between the classical cultural and ATP bioluminescence method calculated from all results irrespective from the microorganism was 0.761 (P<0.001), which indicated that there is a significant correlation between the results. Similar to our results, some other researchers also determined that the correlation between cfu and RLU values differs for different microorganisms and was between 0.68 and 0.90 (Siragusa et al., 1995; Cutter et al., 1996; Chen and Godwin 2006; Sala et al., 2008). In our study, the highest correlation was determined for *Salmonella* (r=0.911) while it was as low as r=0.623 for *S. cerevisiae*. The p values were calculated as p=0.073 and p=0.061 for *S. cerevisiae* and *S. aureus* respectively. Chen and Godwin (2006), also informed that the values determined for different bacteria (total mesophyll aerobic count and psychophilic count) were different and best results were obtained from mean results of pooled data. This also explains the variations of (r) values obtained from different studies. The results of Moore et al., (2010) on *S. aureus* were in compliance with our results. In their study, the efficiency of the cleaning procedure was inspected in a hospital intensive care service by performing measurements with classical cultural method and ATP bioluminescence method. The results of the study indicated that ATP method resulted as clean while presence of *S. aureus* was confirmed with classical cultural method.

Our results, particularly the results of *Salmonella* and *E.coli* is significantly differed from similar studies. Most probably,

because we used artificially contaminated surfaces which did not contain any physical dirt. However, in other studies most commonly naturally contaminated surfaces were used and the dirt on these surfaces probably interfered with the results. Similarly, in some other studies the researchers also studied on naturally contaminated surfaces and determined low correlation and finally recommended to perform study with previously disinfected surfaces (Costa et al., 2006; Carroscosa et al., 2012; Vilar et al., 2008). Additionally, Sokolinska ve Pikul (2008) also informed that the surface material is effective on the results and thus this might be another effective factor on the difference of the results.

Our results and results of several other researchers proved that ATP bioluminescence method has a low reliability on naturally contaminated surfaces and does not give specific information about the microorganism and thus it could only be an alternative for monitoring of the sanitation performance on clean surfaces (Davidson et al., 1999; Griffith et al., 2000; Aytaç et al., 2001; Ayçiçek et al., 2006; Chen and Godwin 2006; Costa et al., 2006; Boyce et al., 2009; Jasson et al., 2010; Moore et al., 2010).

## Conclusions

Our results showed that there are several factors effective on the correlation of the results of ATP bioluminescence method and golden standard classical cultural method. However, the meaningful correlation of pooled data obtained from ATP irrespective from the microorganism type showed that the method can successfully be used for general verification purposes of the clean surfaces. Additionally because method gave the best correlation with *Salmonella* and taking it rapidly in to consideration, the method could be recommended for poultry industry as a practical solution.

## Compliance with Ethical Standard

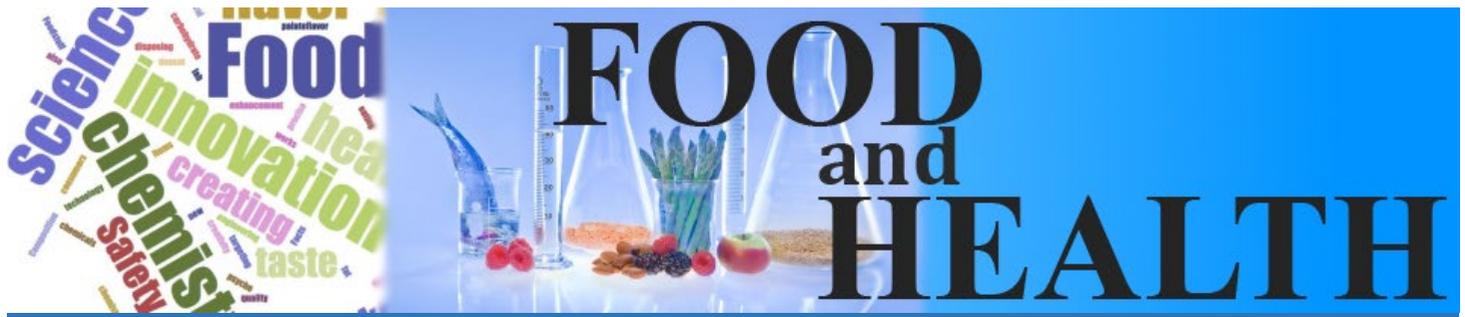
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*Research Article*

## THE ACQUISITION OF HEALTHY FOOD CHOICES, BREAKFAST AND EATING HABITS TO THE PRE-EDUCATION AND AFTER EDUCATION IN ADOLESCENTS

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### ABSTRACT

Adolescence is a period when the fastest growing happens in individuals after babyhood. The study was conducted with a total of 540 students from the 5<sup>th</sup>, 6<sup>th</sup>, 7<sup>th</sup>, and 8<sup>th</sup> Grade students (284M and 256F) attending secondary school. In the first stage, 540 questionnaires that had 39 questions were applied to the students who were trained; and the children were given nutrition training; and after 20 days, another 540 questionnaires were applied again. The data of a total of 540 pre-training and 540 post-training students were recorded in terms of their eating habits, regular breakfast and healthy food selection before and after the study. The body parameters according to gender (height, weight, BMI) show normality with the percentile values formed in Turkish children. The percentages of the answers given by the students to the questions before and after the training are given under each question category. A significant difference was detected between the habits of not having breakfast according to gender ( $P=0.021$ ) ( $P<0.05$ ). As a conclusion, these data show that food selection may be developed with training.

**Keywords:** Adolescence, Nutrition training, Eating habits, BMI, Body parameters

## Introduction

Nutrition in adolescents is important for healthy growth and development. One of the periods in which sufficient and balanced nutrition is very important is the adolescence period, which is between childhood and maturity, where growth and development are accelerated. Adolescence is a period when the fastest growing happens in individuals after babyhood. Adolescence means growth and reaching maturity (Spear, 2002; Demirezen and Cosansu, 2005).

In this period, nutrition habits generally differ from children and adults. Adolescents have the tendency to skip meals and the tendency to eat most of the meals out of their homes, to consume fizzy drinks, candy, dietary foods and readymade meals and drinks is more (Stea et al., 2012). In the adolescence period, energy and nutrition items should be taken in adequate and balanced amounts; otherwise, the growth of the body may slow down, success at school may decrease; and important diseases such as cardiovascular, diabetes, hypertension may be seen in further ages (Malik et al., 2013).

There is no single nutrient element that contains all the necessary nutrients to meet the requirements of the human body (Aksoy and Selen, 2018). For this reason, the human body can only stay healthy if all nutrients are taken properly, balanced and correctly from all foods groups (Aksoy et al., 2017). Foods have been divided into different groups; and the amount of from food groups be consumed every day has been determined by national food guides over the world (Willett and McCullough, 2008; Aktas, 2011; TUBER, 2015).

Scientists in the food guides generally have grouped the nutrients under five groups (TUBER, 2015), the amounts of the nutrients included in these groups have been determined, and daily nutrition plans have been made. In this way, people have been guided to learn the calories and their varieties, which should be taken according to the needs of the body, the nutrient groups that are to be taken from outside when they consume nutrients (Cooke and Wardle, 2005; Neumark-Sztainer et al., 1999; Auestad et al., 2015).

If healthy eating habits, which will continue lifelong, are acquired during the adolescence period, possible future risks will be reduced. Regular breakfast habit is important for the health and development in adolescence and breakfast is as the most essential meal of the day, but it is the most skipped meal (Cebirbay et al, 2011). The energy and nutrients needed between 10-12 hours between dinner and breakfast are provided by the body storages. Breakfast time should not be missed after this time period. When breakfast is skipped,

weakness, headache, attention and perception problems may appear during the day (Wouters et al., 2010).

In reinforcing healthy eating habits, which are acquired during childhood and adolescence, the school environment has a great importance. Schools offer many opportunities to promote healthy dietary and physical activity patterns for children, and are also preventing child malnutrition in all its forms (i.e. undernutrition, micronutrient deficiencies, and obesity and other nutrition related chronic diseases) (Aktas, 2017). Keeping foods like fruit, milk, and fruit-milk at school may be important in shaping the food variety and portion control at lunchtime. Non-alcoholic drinks that contain sugar that is consumed in schools and low-nutritional foods can cause that unhealthy eating habits to emerge (Wordell et al., 2012; Cebirbay et al., 2011). The present health conditions are important for adolescents; possible future health problems are not perceived as a worrying situation by them; and different priorities push health and nutrition issues into the background (Wouters et al., 2010).

In this study, the aim was to show how the training on nutrition at schools affect the level of nutrition habits of adolescents; and to determine the levels of the changes that were detected in the pre-training and post-training nutrition habits. In parallel to this; education in adolescents is aimed to show how effective they are in changes in eating habits

## Materials and Methods

### *Material*

The study was conducted with a total of 540 students from the 5<sup>th</sup>, 6<sup>th</sup>, 7<sup>th</sup>, and 8<sup>th</sup> Grade students (284 males and 256 females) attending Alparslan Secondary School of Malazgirt district, National Education Directorate of Mus Turkey.

### *Methods*

The study was conducted in two stages. In the first stage, 540 questionnaires that had 39 questions were applied to the students who were trained; and the children were given nutrition training; the training was taught by an expert dietician throughout a one-week, healthy and correct nutrition, healthy breakfast habits, healthy nutritional items, face and visual materials. Nutrition education provided by using modern education tools with the active participation of students. The training were given by the researchers. And after 20 days, another 540 questionnaires were applied again. The data of a total of 540 pre-training and 540 post-training students were recorded in terms of their eating habits, regular breakfast and healthy food selection before and after the

training study. Training on nutrition habits had not been provided before in the study school. The experimental method was used in the study. The experimental design of the study is a single group pre-test, final-test model was applied in the study. The Questionnaire Model was used in the present study. The Questionnaire Form, which was developed as a data collection tool, was preferred because it is an appropriate tool in data collection. The questionnaire consisted of three parts. In the first part, there were questions on determining the information about the students and their families; in the second part, there were questions on determining the eating habits of the students; and in the third part, there were questions on determining the frequency of food consumption. The height measurements of the children were made by using the Harpenden Stadiometer (ADE/Hamburg, MZ10020) ultrasonic height measurement unit with an accuracy of 0.1 cm sensitivity, as the individual was standing parallel to the ground with nothing to affect the measurement, with light clothing; and shoes removed; the weight of the individuals were measured with the InBody230 (MW160) Bioelectrical Impedance Body Analyzer device. While the height and weight of the students were measured; body weight and height measurement procedures were applied to 12 and older age groups (TBSA, 2010). The data to determine the nutrient consumption frequency consisted of a 5-point Likert Scale as “Every day”; “5-6 times a week”, “at least 3-4 times a week”, “1-2 times a week”, “Never”. The Questionnaire Form was applied to the students who participated in the study under the surveillance of the author

of the study. The necessary explanations about the questionnaire were made to the students; it was accepted that the students gave accurate and unbiased answers to the questions.

*Statistical Analysis*

The questionnaire data were entered into Microsoft Excel; and the statistical evaluation of the data was made with the IBM SPSS Statistics 17® Package Program; and the results were evaluated by performing T-test, ANOVA and correlation tests. A value of P<0.05 was considered to be statistically significant.

*Ethical Principles*

The study protocol was approved by the Secretary General’s Office of Bitlis Eren University on 22.12.2016 and with the Ethical Board Approval with the number E.3624 on 13.12.2016 with 2016/16-VIII decision.

**Results and Discussion**

*Demographical Characteristic*

No specific selections were made for the gender distributions of the students who were included in the present study. Participation was formed a class-based manner, and the age range was distributed according to the numbers of the students in the classes. The number of students who were 10 years old due to the early start to school, and the ones who were 10 years of age due to the late start to school was also included in the table.

**Table 1.** The gender, age and grade distributions of the students

<b>Gender distribution of students</b>						
Gender	n	%			Total	
Boys (Male)	284	52.6			540	
Girls (Female)	256	47.4			100.0	
<b>Distribution of students according to classes</b>						
Classes	5 <sup>th</sup> Class	6 <sup>th</sup> Class	7 <sup>th</sup> Class	8 <sup>th</sup> Class	Total	
n	151	122	138	129	540	
%	27.9	22.6	25.6	23.9	100.0	
<b>Body Mass Index (BMI) values by age groups comparisons</b>						
Age Range	n	Mean ± SD	Minimum	Maximum	F	P
10 years old	37	17.50 ± 3.01	12.81	25.57	5.497	0.000
11 years old	107	16.97 ± 2.31	10.06	22.26		
12 years old	123	17.88 ± 3.07	13.13	26.84		
13 years old	134	18.44 ± 3.14	11.11	32.47		
14 years old	115	18.88 ± 3.01	12.50	29.14		
15 years old	21	19.36 ± 1.85	16.98	23.15		
16 years old	3	19.48 ± 4.67	14.87	24.22		

SD: Standard Deviation

When the BMI values of the students were examined, they were ranked according to their ages and the BMI averages were given. The expected difference that was based on age was found to be significant ( $P < 0.05$ ).

When the educational status of the parents was examined it was determined that literacy levels in mothers were found to be 30.2% (163), in fathers 5.2% (28); again 3.3% (18) of the

mothers and 14.4% (78) of the fathers were university graduates. There was a significant relation between fathers' educational levels and the having breakfast habits of the children ( $P = 0.015$ ); the income levels of the families and what the children brought to school to eat ( $P = 0.021$ ); and where the children ate lunch ( $P = 0.028$ ) ( $P < 0.05$ ).

The average BMI, height and weight values based on gender and age of the students are given in Table 3.

**Table 2.** Educational status of the parents

	Father education		Mother education	
	n	%	n	%
<b>Reader-Writer Not</b>	163	30.2	28	5.2
<b>Primary education</b>	298	55.2	221	40.9
<b>High school</b>	61	11.3	213	39.4
<b>University</b>	18	3.3	78	14.4
<b>Total</b>	540	100.0	540	100.0

**Table 3.** Body measurement analysis values according to gender and age

	Age	Boys (M)		Girls (F)		P
		n	Mean ± SD	n	Mean ± SD	
<b>BMI</b>	10.00	20	17.37 ± 2.61	17	17.64 ± 3.50	0.043
	11.00	59	17.56 ± 2.23	48	16.26 ± 2.23	
	12.00	69	18.29 ± 3.08	54	17.37 ± 3.00	
	13.00	64	18.71 ± 3.29	70	18.20 ± 3.01	
	14.00	60	18.81 ± 2.65	55	18.96 ± 3.38	
	15.00	11	19.40 ± 2.05	10	19.32 ± 1.72	
	16.00	1	24.22	2	17.11 ± 3.17	
<b>Height</b>	10.00	20	1.37 ± 0.05	17	1.39 ± 0.07	0.664
	11.00	59	1.40 ± 0.09	48	1.37 ± 0.07	
	12.00	69	1.45 ± 0.09	54	1.46 ± 0.09	
	13.00	64	1.52 ± 0.07	70	1.53 ± 0.07	
	14.00	60	1.58 ± 0.10	55	1.56 ± 0.08	
	15.00	11	1.62 ± 0.10	10	1.54 ± 0.09	
	16.00	1	1.60	2	1.65 ± 0.02	
<b>Weight</b>	10.00	20	32.85 ± 5.65	17	34.11 ± 1.67	0.830
	11.00	59	35.05 ± 7.30	48	30.87 ± 6.17	
	12.00	69	38.84 ± 7.19	54	37.50 ± 8.42	
	13.00	64	43.84 ± 8.76	70	42.91 ± 7.12	
	14.00	60	47.55 ± 9.17	55	46.03 ± 7.86	
	15.00	11	51.72 ± 9.26	10	46.20 ± 6.81	
	16.00	1	62.00	2	47.00 ± 9.89	

**Table 4.** The nutrition habits of the students before and after the training

Pre-and Post-Training Nutrition Habits in Adolescents								
					Correlation		T-Test	
Habits	Answers given	TS	n	Mean ± SD	r	P	t	P
Having breakfast habit in the morning	A-Daily, B-3-4 times a week, C-Weekly 1 day D-do not	Pre-T	540	1.44 ± 0.80	0.189	0.000	8.301	0.000
		Post-T	540	1.15 ± 0.37				
Pre-T: A %57.3(310), B %11.8(64), C %16.1(87), D%14.6(79) Post-T: A %73.8(399), B%12.6(68), C %10.0(54), D %3.5(19)								
Where do you eat lunch?	A- At home B-At school, C-Outside	Pre-T	540	1.07 ± 0.32	0.030	0.485	-2.222	0.027
		Post-T	540	1.13 ± 0.44				
Pre-T: A %90.9(491) B %5.0(27) C %4.1(22) Post-T : A %93.5(506), B %4.6(25), C %1.7(9)								
What do you bring with the highest frequency from home to eat during breaks?	A-Fresh Fruit, B-Dried Fruit, C-Dried Fruit, D-Milk-Ayran, E-Ready Fruit Juice, F-Ready Cake Biscuits, G-Home Made Cake, H-none	Pre-T	386	7.09 ± 7.95	0.022	0.660	-1.071	0.285
		Post-T	386	9.00 ± 8.19				
Pre-T; A-%15.3(83), B-%0.9(5), C-%2.1(11), D-%3.5(19), E-%2.1(11), F- %5(27), G-%5.5(30), H-%65.7(355), Post-T: A-%7.4(40), B-%0.3(2), C-%0.3(2), D-%2.1(11), E-%5.1(28), F- %9.6(52), G-%4.6(25), H%70.5(381),								
Do you think you receive adequate and balanced nutrition?	A-Yes B-No	Pre-T	540	1.06 ± 0.25	0.051	0.239	-8.606	0.000
		Post-T	540	1.26 ± 0.47				
Pre-T: A-%74.5(403), B-25.4(137), Post-T: A-%93.0(503), B-%6.8(37)								
How often do you consume milk, yogurt and ayran?	A- Daily, B- 5-6 times a week, C- 3-4 times a week D- 1-2 times a week E-None	Pre-T	540	2.79 ± 1.23	0.169	0.000	1.001	0.317
		Post-T	540	2.72 ± 1.22				
Pre-T :A-%19.4(105), B- %21.5(115), C-%29.2(158), D-%20.5(111), E-%9.4(51) Post-T:A- %23.1(125), B- %18.3(98), C-%26.4(143), D-%27(146), E-%5.2(28)								
How often do you consume cheese?	A- Daily, B- 5-6 times a week, C- 3-4 times a week D- 1-2 times a week E-None	Pre-T	540	2.13 ± 1.07	0.126	0.003	-0.464	0.643
		Post-T	540	2.16 ± 1.42				
Pre-T: A-%51,2(277), B- %14(76). C-%11,3(60), D-%13.9(75), E-%9.6(52) Post-T:A- %37.9(205), B- %23.8(129), C-%25.9(140), D-%11.7(62), E-%0.7(4)								
How often do you consume red meat, chicken and fish?	A- Daily, B- 5-6 times a week, C- 3-4 times a week D- 1-2 times a week E-None	Pre-T	540	1.75 ± 1.10	0.037	0.385	-17.203	0.000
		Post-T	540	2.93 ± 1.17				

<p><b>Pre-T:</b> A-%59.1(320), B- %19(103). C-%11,8(63). D-%6.5(35), E-%3.5(19)  <b>Post-T:</b>A- %15.2(82), B- %19.6(105), C-%29.8(161), D-%27.9(151), E-%7.6(41)</p>								
How often do you consume egg?	<p><b>A-</b> Daily, <b>B-</b> 5-6 times a week, <b>C-</b> 3-4 times a week  <b>D-</b> 1-2 times a week  <b>E-</b>None</p>	Pre-T	540	2.49 ± 1.06	0.144	<b>0.001</b>	-6.510	<b>0.000</b>
		Post-T	540	2.92 ± 1.29				
<p><b>Pre-T:</b> A-%21.1(114), B- %29.4(159), C-%30.7(166), D-%16.5(88), E-%2.4(13)  <b>Post-T:</b>A- %20.7(112), B- %15(81). C-%24.6(132), D-%30.1(163), E-%9.6(52)</p>								
How often do you consume legumes?	<p><b>A-</b> Daily, <b>B-</b> 5-6 times a week, <b>C-</b> 3-4 times a week  <b>D-</b> 1-2 times a week  <b>E-</b>None</p>	Pre-T	540	2.47 ± 1.20	0.226	<b>0.000</b>	-14.187	<b>0.000</b>
		Post-T	540	3.37 ± 1.14				
<p><b>Pre-T:</b> A-%29(157), B- %21.5(115), C-%26.6(144), D-%18.7(101), E-%4.3(23)  <b>Post-T:</b>A- %10.4(56), B- %9.8(53), C-%24.8(134), D-%42.3(228), E-%12.8(69)</p>								
How often do you consume green-leaf vegetables?	<p><b>A-</b> Daily, <b>B-</b> 5-6 times a week, <b>C-</b> 3-4 times a week  <b>D-</b> 1-2 times a week  <b>E-</b>None</p>	Pre-T	540	2.87 ± 1.18	0.164	<b>0.000</b>	-4.402	<b>0.000</b>
		Post-T	540	3.17 ± 1.24				
<p><b>Pre-T:</b> A-%16.3(88), B- %21.4(116), C-%26.8(144), D-%29(157). E-%6,5(35)  <b>Post-T:</b>A- %12.8(69), B- %17.6(94), C-%23.7(128), D-%31.4(170), E-%14.6(79)</p>								
How often do you consume Potatoes?	<p><b>A-</b> Daily, <b>B-</b> 5-6 times a week, <b>C-</b> 3-4 times a week  <b>D-</b> 1-2 times a week  <b>E-</b>None</p>	Pre-T	540	2.77 ± 1.28	0.164	<b>0.000</b>	-1.206	0.228
		Post-T	540	2.86 ± 1.13				
<p><b>Pre-T:</b> A-%21.8(118), B- %20.1(109), C-%26.3(141), D-%21.8(118), E-%10(54)  <b>Post-T:</b>A- %15.9(86), B- %21.5(115), C-%27(146), D-%32.2(174), E-%3.5(19)</p>								
How often do you consume fresh fruits?	<p><b>A-</b> Daily, <b>B-</b> 5-6 times a week, <b>C-</b> 3-4 times a week  <b>D-</b> 1-2 times a week  <b>E-</b>None</p>	Pre-T	540	2.49 ± 1.11	0.130	<b>0.002</b>	3.458	<b>0.001</b>
		Post-T	540	2.27 ± 1.16				
<p><b>Pre-T:</b> A-%24.4(132), B- %24.5(132), C-%29.9(161), D-%19.4(105), E-%1.8(10)  <b>Post-T:</b>A- %35.3(191), B- %22.4(120), C-%24.4(132), D-%15.5(84), E-%2.4(13)</p>								
How often do you consume bread?	<p><b>A-</b> Daily, <b>B-</b> 5-6 times a week, <b>C-</b> 3-4 times a week  <b>D-</b> 1-2 times a week  <b>E-</b>None</p>	Pre-T	540	1.92 ± 1.06	0.085	<b>0.048</b>	7.719	<b>0.000</b>
		Post-T	540	1.47 ± 0.97				
<p><b>Pre-T:</b> A-%47.5(257), B- %23.7(127), C-%18.3(99), D-%9.6(52), E-%0.9(5)  <b>Post-T:</b>A- %76.5(414), B- %8.7(47), C-%7.6(41), D-%5(27), E-%2(11)</p>								
		Pre-T	540	1.43 ± 0.89	0.035	0.421	-19.035	<b>0.000</b>

How often do you consume bulgur, rice and pasta?	A- Daily, B- 5-6 times a week, C- 3-4 times a week D- 1-2 times a week E-None	Post-T	540	2.59 ± 1.13				
<p><b>Pre-T:</b> A-%76.9(415), B- %9.4(51), C-%8.7(47), D-%3.7(20), E-%1.3(7)  <b>Post-T:</b>A- %22.2(120), B- %22.8(123), C-%31.6(171), D-%20.2(109), E-%3.1(17)</p>								
How often do you consume olives?	A- Daily, B- 5-6 times a week, C- 3-4 times a week D- 1-2 times a week E-None	Pre-T	540	2.17 ± 1.13	0.102	<b>0.018</b>	-5.888	<b>0.000</b>
		Post-T	540	2.63 ± 1.57				
<p><b>Pre-T:</b> A-%37.8(204), B- %24.1(130), C-%23.7(128), D-%12.2(66), E-%2.2(12)  <b>Post-T:</b>A- %38.7(209), B- %12.8(69), C-%14(76), D-%15(81), E-%19.4(105)</p>								
How often do you consume butter?	A- Daily, B- 5-6 times a week, C- 3-4 times a week D- 1-2 times a week E-None	Pre-T	540	2.66 ± 1.55	0.098	<b>0.022</b>	-6.536	<b>0.000</b>
		Post-T	540	3.25 ± 1.54				
<p><b>Pre-T:</b> A-%36.3(196), B- %14.6(79), C-%14.4(78), D-%15.3(83), E-%19.2(104)  <b>Post-T:</b>A- %22.6(122), B- %10.7(58), C-%16.6(90), D-%18.9(102), E-%31.1(168)</p>								
How often do you consume margarine?	A- Daily, B- 5-6 times a week, C- 3-4 times a week D- 1-2 times a week E-None	Pre-T	540	3.77 ± 1.31	0.169	<b>0.000</b>	-0.397	0.692
		Post-T	540	3.80 ± 1.37				
<p><b>Pre-T:</b> A-%10.6(57), B- %7.4(40), C-%14.1(76), D-%29.6(160), E-%38.3(207)  <b>Post-T :</b>A- %11.9(64), B- %6.9(37), C-%14.6(79), D-%22(119), E-%44.6(241)</p>								
What is your frequency of consuming drinks with sugar?	A- Daily, B- 5-6 times a week, C- 3-4 times a week D- 1-2 times a week E-None	Pre-T	540	4.10 ± 1.17	0.103	<b>0.017</b>	19.305	<b>0.000</b>
		Post-T	540	2.59 ± 1.51				
<p><b>Pre-T:</b> A-%6.7(36), B- %4.1(22), C-%11.6(63), D-%27.7(150), E-%49.7(269)  <b>Post-T:</b>A- %39.4(213), B- %11.3(61), C-%13.7(74), D-%21.9(118), E-%13.7(74)</p>								
How often do you consume jam and cream chocolate?	A- Daily, B- 5-6 times a week, C- 3-4 times a week D- 1-2 times a week E-None	Pre-T	540	3.05 ± 1.50	0.104	<b>0.015</b>	3.439	<b>0.001</b>
		Post-T	540	2.75 ± 1.48				
<p><b>Pre-T:</b> A-%25.6(138), B- %12(65), C-%16.3(88), D-%23.7(128), E-%22.4(121)  <b>Post-T :</b> A- %31.3(269), B- %15(81), C-%16.3(88), D-%21.5(116), E-%15.9(86)</p>								
How often do you consume honey, boiled grape juice, or sesame oil with boiled grape juice?	A- Daily, B- 5-6 times a week, C- 3-4 times a week D- 1-2 times a week E-None	Pre-T	540	2.80 ± 1.42	0.212	<b>0.000</b>	-3.377	<b>0.001</b>
		Post-T	540	3.07 ± 1.55				

<p><b>Pre-T:</b> A-%28(151), B- %15.4(83), C-%19.6(106), D-%22.8(123), E-%14.3(77)  <b>Post-T:</b>A- %26.3(142), B- %12.6(68), C-%14.3(77), D-%21.3(115), E-%26.6(138)</p>								
How often do you consume desserts with milk and syrup?	<p><b>A-</b> Daily, <b>B-</b> 5-6 times a week, <b>C-</b> 3-4 times a week  <b>D-</b> 1-2 times a week  <b>E-</b>None</p>	Pre-T	540	3.13 ± 1.48	0.131	<b>0.002</b>	-4.478	<b>0.000</b>
		Post-T	540	3.48 ± 1.19				
<p><b>Pre-T:</b> A-%20.4(110), B- %17(92), C-%16.3(88), D-%20.9(113), E-%25.4(137)  <b>Post-T:</b>A- %9.1(49), B- %12(65), C-%20.2(109), D-%39.1(211), E-%19.6(106)</p>								
How often do you consume coke, fizzy drinks, ready-made fruit juice?	<p><b>A-</b> Daily, <b>B-</b> 5-6 times a week, <b>C-</b> 3-4 times a week  <b>D-</b> 1-2 times a week  <b>E-</b>None</p>	Pre-T	540	3.69 ± 1.11	0.056	0.192	6.171	<b>0.000</b>
		Post-T	540	3.23 ± 1.38				
<p><b>Pre-T:</b> A-%6.3(34), B- %8.1(44), C-%19.6(106), D-%41.5(224), E-%24.4(132)  <b>Post-T:</b>A- %18(97), B- %12.8(69), C-%17(92), D-%32(173), E-%20.2(109)</p>								
How often do you consume tea?	<p><b>A-</b> Daily, <b>B-</b> 5-6 times a week, <b>C-</b> 3-4 times a week  <b>D-</b> 1-2 times a week  <b>E-</b>None</p>	Pre-T	540	3.67 ± 1.21	-	0.981	24.461	<b>0.000</b>
		Post-T	540	1.83 ± 1.26				
<p><b>Pre-T:</b> A-%63(340), B- %12(65), C-%9.3(50), D-%10.2(55), E-%5.6(30)  <b>Post-T:</b>A- %56.3(304), B- %16.5(89), C-%11.3(61), D-%10.6(57), E-%5.4(29)</p>								
How often do you consume chips, biscuits, and cereals?	<p><b>A-</b> Daily, <b>B-</b> 5-6 times a week, <b>C-</b> 3-4 times a week  <b>D-</b> 1-2 times a week  <b>E-</b>None</p>	Pre-T	540	1.92 ± 1.25	-	0.753	-12.946	<b>0.000</b>
		Post-T	540	2.96 ± 1.36				
<p><b>Pre-T:</b> A-%18(97), B- %12.8(69), C-%17(92), D-%32(173), E-%20.3(109)  <b>Post-T:</b>A- %19.8(107), B- %18.3(99), C-%24.8(134), D-%19.8(107), E-%17.2(93)</p>								
How often do you consume salami and sausages?	<p><b>A-</b> Daily, <b>B-</b> 5-6 times a week, <b>C-</b> 3-4 times a week  <b>D-</b> 1-2 times a week  <b>E-</b>None</p>	Pre-T	540	3.49 ± 1.23	0.160	<b>0.000</b>	0.153	0.878
		Post-T	540	3.48 ± 1.36				
<p><b>Pre-T:</b> A-%9.1(49), B- %11.5(62), C-%25(135), D-%30(162), E-%24.4(132)  <b>Post-T:</b> A- %11.3(61), B- %15.7(85), C-%17.8(96), D-%23.9(129), E-%31.3(169)</p>								
How often do you consume fermented sausages?	<p><b>A-</b> Daily, <b>B-</b> 5-6 times a week, <b>C-</b> 3-4 times a week  <b>D-</b> 1-2 times a week  <b>E-</b>None</p>	Pre-T	540	4.14 ± 0.98	0.261	<b>0.000</b>	13.828	<b>0.000</b>
		Post-T	540	3.28 ± 1.33				
<p><b>Pre-T:</b> A-%19.8(107), B- %18.3(99), C-%24.8(134), D-%19.8(107), E-%17.2(93)  <b>Post-T:</b>A- %13.7(74), B- %14.8(80), C-%22.8(123), D-%26.3(142), E-%22.4(121)</p>								

TS: Training Status; Pre-T: Pre-Training; Post-T: Post-Training; SD: Standard Deviation

When the BMI values of the boys were compared with those of the girls according to age, the difference between them were found to be significant ( $P=0.043$ ). No significant differences were detected between the height and weight values according to the gender variable ( $P>0.05$ ). When the percentile values were compared in children according to body weights, it was determined that the weights of the males were between 25-50% percentile; height values were between 10-25 percentile; and the percentile values of the BMI averages were 25%. It was also determined that the weights of the girls were between 25-50% percentile; their heights were between 10-25% percentile; and the percentile value of the BMI average value was 15-25%.

To perform an obesity evaluation in children and adolescents, the age and gender-specific BMI values were established. According to these evaluation criteria, BMI-percentile the value being below 5 shows malnutrition; its being between 5-85 shows normal weight; being between 86-95 shows being overweight (slightly obese), being over 95 shows obesity (Bundak et al., 2006; Neyzi et al., 1978; Neyzi et al., 2008). The body parameters according to gender (height, weight, BMI) show normality with the percentile values formed in Turkish children.

The nutrition habits of the students before and after the training are given in Table 4.

The percentages of the answers given by the students to the questions before and after the training are given under each question category. Significant differences were determined before and after the training between having breakfast in the morning ( $r=0.186$ ); What is brought from home to eat during breaks ( $r=0.022$ ); Thinking that s/he has adequate and balanced nutrition ( $r=0.051$ ); Consuming habits of milk, yogurt and ayran ( $r=0.169$ ); Red meat, chicken and fish ( $r=0.037$ ); Eggs ( $r=0.144$ ); Legumes ( $r=0.226$ ); Green-leaf vegetables ( $r=0.164$ ); Fresh fruit ( $r=0.130$ ); Bread ( $r=0.085$ ); Bulgur, rice and pasta ( $r=0.035$ ); Olives ( $r=0.102$ ); Butter ( $r=0.098$ ); Drinks with sugar ( $r=0.103$ ); Jam, cream chocolate ( $r=0.104$ ); Honey, boiled grape juice or boiled grape juice with sesame oil ( $r=0.212$ ); Dessert with milk and syrup ( $r=0.131$ ); Coke, fizzy drinks, ready-made fruit juice ( $r=0.056$ ); Tea ( $r=-0.001$ ); Chips, biscuits, cereals ( $r=-0.014$ ) and Sausage ( $r=0.261$ ) ( $P<0.05$ ); and a very weak a correlation was detected between them. There was a very weak and negative relationship between consumption habits of tea, chips, biscuit, and cereals. No significant differences were determined between the variables Where lunch is eaten ( $r=0,030$ ); consuming Cheese ( $r=0.126$ ); Potatoes ( $r=0.164$ ); Margarine ( $r=0.169$ ); Salami and sausage ( $r=0.160$ ) before and after the training ( $P>0.05$ ); however, a

very weak and positive correlation was determined between them.

There are reports that children in school age are more likely to change their ways of doing than their right behavior. It is very common in children and adolescents to skip breakfast. It was determined that skipping breakfast was more in adolescent girls than in males (Steyn, 2010). This a situation has been explained with by fact that skipping breakfast was a weight control method in adolescent girls (Keshi-Rahkonen et al., 2003). Pulp, vitamins A, C and E, B<sub>6</sub>, B<sub>12</sub> and folate, iron, calcium, phosphorus, magnesium and potassium values of the individuals who skipped breakfast were found to be lower than in those who did not skip breakfast (Deshmukh-Taskar et al., 2010). In another study, it was reported that skipping breakfast could affect concentration, learning, and school performance (Story and Stang, 2005). In our study, a significant difference was detected between the habits of not having breakfast according to gender ( $P=0.021$ ) ( $P<0.05$ ). Again, according to gender, there was a significant difference between healthy and balanced nutrition habits ( $P=0.001$ ) ( $P<0.05$ ). It was found in a study conducted in the Mardin that overweight children between the ages of 7-15 consumed toast, coke, chips and chocolate more (Battaloglu, 2014); and in another study, it was reported those children ate bagels, chocolate, biscuits at the highest level, and preferred drinks like water, cola, fizzy drinks and fruit juice (Orhan and Celik, 2014). In our study, the rate of those who consumed coke, fizzy drinks and ready-made drinks 3-4 times a week was found to be 41.5% before the training; however, after the training, this rate decreased significantly. As a result of the training, when the benefits of other foods were learned, the diversity in food consumption increased; and after the training, the students consuming meat, poultry and fish showed a significant difference ( $P<0.05$ ); however, a weak correlation ( $r=0.037$ ) was also found between them. No significant differences were detected in terms of the gender variable ( $P>0.05$ ). In another the study, a significant difference was reported based on the gender variable (Cebirbay et al., 2011). In a study conducted with children aged between 4 and 14 years and adolescents, it was determined that girls loved fruits and vegetables more than boys; and boys loved fatty and foods with sugar, meat products, processed meats and eggs more than girls (Cooke et al., 2005). In our study, a significant difference was detected after the training in the egg consumption of the students who consumed eggs every day before the training ( $P<0.05$ ); and the relation between them was found to be very weak. According to the results of the research conducted before and after the training, sausages, tea, milk, fresh fruits and egg consumption (Sabbag and

Surucuoglu, 2012). The relationship between pre-training and post-training data in our study was significant ( $P < 0.05$ ). In another a study, it was determined that most of the students who had very good, moderate and low nutrition knowledge drank tea; drinking coffee was very common among the subjects (60.2%); and 26.1% of those who had very good nutrition knowledge consumed milk and ayran; and 20% of them consumed fruit juice (Birer and Ersoy, 1987). In our study, although a significant difference was detected in terms of consuming tea before and after the training, there was a very weak negative relationship between them. In a study that was conducted for the purpose of determining the nutrition knowledge and habits of senior high school students, it was determined that the students preferred coke at the intermediary meals were cola with 27.9%; and the lowest consumed ones were yogurt and milk with 14.9% (Sagun, 1987). No significant differences were detected between the consumption frequencies for this product group ( $P > 0.05$ ).

In a study, it was determined in the weight and height measurements in boys and girls aged 12 years, girls were found to be overweight and taller than their boys (Neyzi et al., 1978). In the present study of ours, when we examined the 12-year age range participants, we found that their heights of the males and females were nearly the same; and there was a slight difference in the weight averages in favor of the males; however, the difference between males and females was not found to be significant ( $P > 0.05$ ). In another study, it was found that the sugar-chocolate consumption of the students was found to be 88% as once or twice or more per week; chips-potato consumption was like 71.7%; and coke consumption were as 40.4% (Kutlu and Civi, 2009). In another a study, it was shown that the children consumed sweets and chocolate (32.8%), chips, cracker etc. (15.4%) and fizzy drinks (59%) (Rakicioglu et al., 2000). In our study, after the training, those who said that they consumed coke and fizzy drinks 1-2 times a week were found to be 32%, and those who said they consumed chips-biscuit-cereals were found to be 19.8%. In a survey, results revealed 83.3% change in students' nutrition habits following education program (Sebbag, 2017). In our study this rate was found as 76.92%.

## Conclusions

As a conclusion, these data show that food selection may be developed with learning. As a result of the nutrition training provided by the dietitian in the adolescent children, there were significant differences despite the positive relation compared to the pre-training period. This study showed that

it is important to provide nutrition trainings at schools in order to acquire healthy eating habits. Classes on healthy food selection and nutrition should be included in the curricula of schools and appropriate awareness about healthy lifestyle and its effects must be provided. The trainings should focus on healthy eating habits and lifestyles and should be given to all children in early staged of their developments.

## Compliance with Ethical Standard

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

**Ethics committee approval:** The study protocol was approved by the Secretary General's Office of Bitlis Eren University on 22.12.2016 and with the Ethical Board Approval with the number E.3624 on 13.12.2016 with 2016/16-VIII decision.

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Research Article

## ZEYTİN KARASUYU FENOLİKLERİNDEN 3,4-DİHİDROKSİFENİL ETANOLÜN FARKLI PROSTAT KANSERİ HÜCRE HATLARINDAKİ SİTOTOKSİK ETKİLERİ

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### ÖZ

Zeytinyağı üretimi sırasında oluşan yan ürünlerden zeytin karasuyunun zengin fenolik içeriği ve buna bağlı olarak antioksidan, antitrombotik, antiinflamasyon, hipokolesterolemik, antimikrobiyal, antiviral ve antikanserijenik aktiviteleri bilimsel çalışmalarla ortaya konmuştur. Zeytin karasuyunun temel bileşenlerinden biri 3,4-dihidroksifenil etanol (3,4-DHPEA)'dır. 3,4-DHPEA'nın birçok kanser tipi üzerinde antikanserijenik etkileri araştırılmasına rağmen prostat kanseri üzerindeki etkilerini irdeleyen çalışmalar literatürde sınırlı sayıdadır. Bu amaçla çalışmamızda, 3,4-DHPEA'nın orijin, genetik yapı ve karakteristik özellikler bakımından farklı 5 insan prostat kanseri hücre hatları üzerindeki benzer ve farklı sitotoksik etkileri araştırılmıştır. 3,4-DHPEA farklı konsantrasyonlarda prostat kanseri hücre hatları (LNCaP, C4-2, 22Rv1, PC3 ve DU-145) üzerinde 72 saate kadar uygulandı ve hücre canlılıkları Cell Titer-Glo luminesan testi uygulanarak belirlendi. Tüm deneyler 3 paralel yapıldı ve 3 kere tekrarlandı. Sonuçlar varyans analizi (ANOVA) ile karşılaştırıldı ve %5'den düşük olasılıklar ( $P<0.05$ ) anlamlı olarak değerlendirildi. 3,4-DHPEA, kullanılan tüm hücre hatları üzerinde zamana ve doza bağlı sitotoksikite göstermiştir. En hassas hücre hattının 72 saatlik  $IC_{50}=25.64 \mu\text{g/mL}$  değeriyle LNCaP ve en az hassas hücre hattının ise 72 saatlik  $IC_{50}=58.81 \mu\text{g/mL}$  değeriyle PC3 olduğu tespit edildi. LNCaP popülasyonunun 48 saat 75  $\mu\text{g/mL}$  3,4-DHPEA muamelesi sonunda tamamına yakınının öldüğü görülmüştür. Prostat dokusundan gelişen farklı kanser hücre hatlarının 3,4-DHPEA'ya karşı farklı derecelerde hassasiyet göstermeleri hücrelerin farklı orijin, farklı genetik yapı (bazı hücrelerin fosfataz ve tensin homolog (PTEN) gen mutasyonuna sahip olması gibi) ve farklı karakteristik özelliklere (kullanılan hücre hatlarının bazılarının androjene bağımlı bazılarının androjenden bağımsız olması gibi) sahip olması sebebiyle olduğu düşünülmüştür.

**Anahtar Kelimeler:** Prostat kanseri, 3,4-DHPEA, Sitotoksikite, Zeytin karasuyu

### ABSTRACT

#### CYTOTOXIC EFFECTS OF 3,4-DIHYDROXYPHENYL ETHANOL, AN OLIVE MILL WASTE WATER PHENOLIC, ON DIFFERENT PROSTATE CANCER CELL LINES

The rich phenolic content of olive mill waste water obtained as by-product during olive oil production and its antioxidant, antithrombotic, anti-inflammatory, hypocholesterolemic, antimicrobial, antiviral, and anticancerogenic activities have been revealed by scientific studies. One of the main components of olive oil is 3,4-dihydroxyphenyl ethanol (3,4-DHPEA). Although the effect of 3,4-DHPEA was studied on many types of cancer, studies on prostate cancer are limited in the literature. For this purpose, similar and different cytotoxic effects of 3,4-DHPEA on 5 human prostate cancer cell lines differing in origin, genetic structure, and characteristic features were investigated. The 3,4-DHPEA was applied on prostate cancer cell lines (LNCaP, C4-2, 22Rv1, PC3, and DU-145) at different concentrations for up to 72 hours and cell viabilities were measured by the Cell Titer-Glo luminescence assay. All experiments were performed in 3 parallel and repeated 3 times. Results were compared with analysis of variance (ANOVA) and the probabilities less than 5% ( $P<0.05$ ) were evaluated as significant. The 3,4-DHPEA showed time- and dose-dependent cytotoxicity on all cell lines used. The most sensitive cell line was LNCaP with 72 hr  $IC_{50}=25.64 \mu\text{g/mL}$  value and the least sensitive cell line was PC3 with 72 hr  $IC_{50}=58.81 \mu\text{g/mL}$  value. Nearly entire LNCaP population was found to be dead after 75  $\mu\text{g/mL}$  of 3,4-DHPEA treatment for 48 h. The fact that different cancer cell lines developed from prostate tissue show different degrees of sensitivity to 3,4-DHPEA could be due to the different origins, different genetic structures (e.g. some cells have phosphatase and tensin homolog (PTEN) gene mutation), and different characteristic features (e.g. some of the cell lines used were androgen-dependent and the others are androgen-independent).

**Keywords:** Prostate cancer, 3,4-DHPEA, Cytotoxicity, Olive mill waste water

## Giriş

Prostat kanseri, erkek üreme sisteminin önemli bir üyesi olan prostatta görülen malign değişikliklerdendir ve ileri yaş erkeklerde en sık rastlanılan kanserlerden birisidir. Prostat kanseri, erkeklerde en fazla tanı konulan neoplazm olup ABD’de kansere bağlı mortalitede ikinci sırada yer almaktadır. Avrupa’da her yıl tahmini 2,6 milyon yeni olguya kanser tanısı konmaktadır. Erkeklerde görülen kanserlerin %11’ini prostat kanseri oluşturmaktadır ve erkekler arasındaki tüm kanser ölümlerinin %9’unun sebebi olmaktadır (Bono, 2004; Siegel vd., 2016). Kemoterapi ve radyoterapi, prostat kanser tedavisinde uygulanan en yaygın ve etkili yöntemlerdir (Cansino Alcaide ve Martínez-Piñero, 2006; Zhou vd., 2016). Ancak bu tedavilerin yüksek maliyetli oluşu, hastayı ve ülke ekonomisini olumsuz etkilemeleri sebebiyle yeni ulusal kaynaklı sitotoksik etkisi yüksek bileşiklerin araştırılmasına ihtiyaç duyulmaktadır.

Zeytinin, yağa işlenmesi sonucunda elde edilen zeytin karasuyunun 30’dan fazla fenolik bileşik içerdiği bildirilmekle birlikte bu bileşikler genel olarak fenolik asitler (3,4-DHPEA, 3,4-Dihidroksifenil asetik asit, *p*-Dihidroksifenil asetik asit, kafeik asit, *p*-kumarik asit, ferulik asit) fenolik alkoller, flavonoidler, sekoiridoidler ve lignanlar olarak gruplandırılır. Zeytin karasuyunun temel bileşeni 3,4-DHPEA’dır (Artajo vd., 2006).

Zeytin karasuyu ve zeytin yaprağının yapısında bulunan fenolik bileşenlerin antibakteriyel, antiviral, antifungal ve sitotoksik etkileri yapılan *in vitro* çalışmalarla ortaya konulmuştur (Capasso vd., 1995; Soler-Rivas vd., 2000; Sousa vd., 2006). Birçok araştırmacı tarafından antimikrobiyal aktivite gösteren bileşikler 3,4-DHPEA, oleuropein, 4-hidroksibenzoik asit, vanilik asit ve *p*-kumarik asit olarak bildirilmiştir. Nitekim 3,4-DHPEA’nın solunum ve bağırsak enfeksiyonlarına neden olan Gram pozitif ve Gram negatif bakterilere karşı etkili olduğu yapılan çalışmalarla ortaya konmuştur (Ibarra ve Sniderman, 2008). Bouaziz ve ark. tarafından Tunus bölgesinde yetişen zeytin türlerinde bulunan saf flavonoidler ve düşük molekül ağırlıklı fenoller serbest radikal süpürme aktiviteleri bakımından kendi aralarında ve bütilhidroksitoluen (BHT) ile karşılaştırılmıştır. Elde edilen sonuçlara göre, BHT’ye göre en yüksek antioksidan aktiviteyi 3,4-DHPEA’nın daha sonra kafeik asitin ve kersetinin gösterdiği bildirilmiştir (Bouaziz vd., 2005). Bir başka çalışmada 3,4-DHPEA’nın ve oleuropein’in oksidasyona karşı BHT ve E vitamininden çok daha etkili olduğu saptanmıştır (Aruoma vd., 1998). 3,4-DHPEA ve kafeik asitin *ortho* grubunda iki hidroksil grubunun bulunması yüksek antioksidan aktivite göstermelerine, tirozol, *p*-kumarik asit ve *p*-hidroksifenil asetik asitin ise tek hidroksil

grubu içermeleri nedeni ile düşük antioksidan aktivite göstermelerine neden olduğu şeklinde bir açıklama yapılmıştır (Fki vd., 2005). Yapılan çalışmalarda 3,4-DHPEA’nın hem apoptoz aktivitesini hem de süperoksit üretimini arttırarak anti kanserojenik etkileri olabileceği, yine 3,4-DHPEA’nın H<sub>2</sub>O<sub>2</sub> ile oluşturulan DNA hasarı deneylerinde DNA hasarını düşürerek hücreleri koruyucu etkisi olduğu saptanmıştır (Luo vd., 2013; Rosigmoli vd., 2016; Fabiani vd., 2012).

3,4-DHPEA’nın kolon, meme ve hepatoselüler karsinomalarda sitotoksik etkilerinin saptanmasına rağmen prostat kanserindeki etkileri çok bilinmemektedir. İnsan prostat, meme, kıkırdak hücreleri ve monositler üzerinde yapılan çalışmalarda 3,4-DHPEA’nın DNA oksidasyonunu önleyici etkileri olduğu bildirilmiştir (Facchini vd., 2014; Quiles vd., 2002; Warleta vd., 2011). 2017 yılında 3,4-DHPEA’nın LNCaP ve C4-2 prostat kanser hücrelerine karşı antiproliferatif etki göstermesine karşın normal prostat epitel hücre hatlarının (RWPE1 ve PWLE2) büyümelerine karşı herhangi bir negatif etki göstermediği rapor edilmiştir (Zubair vd., 2017). PC3, DU-145, LNCaP insan prostat kanseri hücre hatlarının 3,4-DHPEA ile muamele edildiği kısıtlı çalışmalarda, 3,4-DHPEA’nın hem apoptoz aktivitesini hem de süperoksit üretimini arttırarak anti kanserojenik etkileri olabileceği üzerinde durmuşlardır (Luo vd., 2013; Rosignoli vd., 2016). Orenay-Boyacioglu oksidatif strese karşı 3,4-DHPEA’nın prostat hücre hatlarında PTEN statüsüne göre sitotoksik etkilerini araştırdığı çalışmada wild tip PTEN içeren 22Rv1’in 3,4-DHPEA’ya karşı en büyük duyarlılığı gösterdiğini rapor etmiştir (Orenay Boyacioglu, 2018). Kısıtlı çalışmalarla sitotoksik etkisi gösterilen zeytin karasuyu bileşiği 3,4-DHPEA’nın farklı prostat kanseri hücre hatlarında sitotoksik etkilerinin araştırılması amaçlanarak, hem literatürdeki kısıtlı çalışmalara katkıda bulunmak hem de zeytin karasuyu fenoliklerinin sağlık alanında yararlı kullanımlarının arttırılması hedeflenmiştir.

## Materyal ve Metot

### *Prostat Hücre Hatlarının Üretilmesi*

Prostat kanseri hücre hatları Prof. Dr. William H. Gmeiner’den (Wake Forest Üni., ABD) tedarik edilmiştir. Beş farklı insan prostat kanseri hücre hattı (LNCaP, C4-2, 22Rv1, PC3 ve DU-145) %10 fetal sıgır serumu (FBS) (Lonza, Allendale, NJ, ABD) içeren RPMI-1640 (Lonza) besiyerinde 37°C’de, %5 CO<sub>2</sub> ve %89 nemli inkübatörde kültüre edilerek, %80-85 yoğunluğa ulaştıklarında pasajlandı. Klonal seleksiyonu en az seviyede tutmak için çalışmalarda 5-20. pasajlar arasındaki hücreler kullanıldı.

### 3,4-DHPEA Muamelesi

3,4-DHPEA'nın (Cayman Chemical, Ann Arbor, MI, ABD) son seyreltmesi test kuyucuklarında %1 etanol içerecek şekilde RPMI-1640 içinde hazırlandı. Toplam  $10^4$  hücre/kuyu 96 kuyucuklu plakaya inoküle edildi. Ertesi gün 0, 10, 25, 50 ve 75  $\mu\text{g}/\text{mL}$  konsantrasyonlarda filtre ile sterilize edilmiş 3,4-DHPEA eklendi ve 24, 48 ve 72 saat inkübe edildi.

### Cell Titer-Glo Luminesan Testi

Muameleden sonra hücre canlılığı Cell Titre-Glo® Luminesan testi (Promega, Madison, WI, ABD) kullanılarak üretici firmanın talimatlarına uygun olarak yapıldı. Canlı hücre sayısının dolaylı belirteci olan luminesan, LMAXII 384 mikropilok okuyucu (Molecular Devices, Toronto, ON, Kanada) ile ölçüldü.

### IC50 Değerlerinin Hesaplanması

Test edilen 3,4-DHPEA dozları ile 72 saat sonunda gözlemlenen ölüm oranları kullanılarak her hücre hattı için oluşturulan dağılım (scatter plot) grafikleri üzerinde polinom regresyon eğri denklemleri hesaplanmıştır. Denklem üzerinden hücrelerin %50'sinin proliferasyonunu inhibe eden 3,4-DHPEA IC50 dozu hesaplanmıştır. 24 ve 48 saat muamele sonunda PC3 ve DU-145 hücre hatlarında minimum %50 büyüme inhibisyonu gözlemlenmediğinden ve en yüksek doz olan 75  $\mu\text{g}/\text{mL}$ 'nin üzerinde bir değer bildirmekten kaçınıldığı için 24 ve 48 saat muameleler için IC50 değerleri hesaplanmamıştır.

### İstatistiksel Analizler

Bu çalışmada tüm deneyler 3 paralel yapıldı ve 3 kere tekrarlandı. Sonuçlar varyans analizi (ANOVA) ile karşılaştırıldı ve %5'den düşük olasılıklar ( $P<0.05$ ) anlamlı olarak değerlendirildi.

## Bulgular ve Tartışma

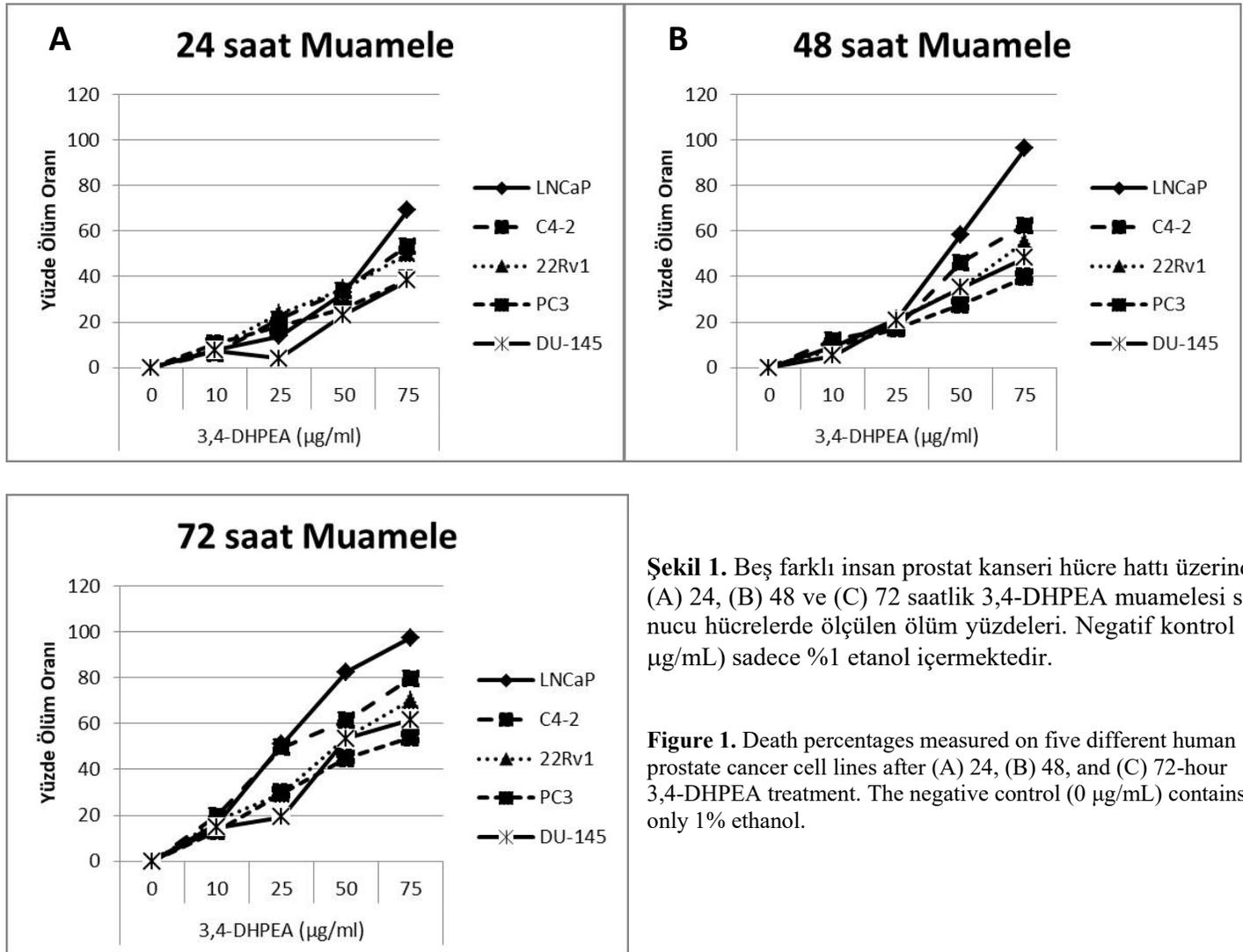
3,4-DHPEA, test edilen tüm prostat kanser hücre hatları üzerinde zamana ve doza bağlı olarak sitotoksik etki göstermiştir (Şekil 1). Test edilen tüm uygulama sürelerinde 3,4-DHPEA'nın en fazla sitotoksik etkisi LNCaP hücre hattında ve en az sitotoksik etkisinin PC3 hücre hattında olduğu gözlemlenmiştir (Şekil 1). 72 saat 3,4-DHPEA ile muamele sonuçlarına göre hücre hatlarının IC50 değerleri şu şekilde hesaplanmıştır: LNCaP 25.64  $\mu\text{g}/\text{mL}$ , C4-2 30.53  $\mu\text{g}/\text{mL}$ , 22Rv1 44.39  $\mu\text{g}/\text{mL}$ , PC3 58.81  $\mu\text{g}/\text{mL}$  ve DU-145 52.80  $\mu\text{g}/\text{mL}$ . LNCaP popülasyonunun 48 ve 72 saatlik 75  $\mu\text{g}/\text{mL}$  3,4-DHPEA muamelesi sonunda tamamına yakınının öldüğü görülmüştür (Şekil 1B, 1C). Tüm hücre hatlarında 3,4-DHPEA'nın uygulama süresi ve elde edilen ölüm oranları

arasındaki ilişki istatistiksel olarak anlamlı bulunmuştur ( $P<0.05$ ).

Bu çalışmada, beş farklı insan prostat kanseri hücre hattında zeytin karasuyu ana fenolik bileşiği olan 3,4-DHPEA'nın sitotoksik etkisi araştırıldı. Hücre hatları üzerinde gözlemlenen farklı seviyedeki sitotoksik etkilerin, hücrelerin farklı orijin, farklı genetik yapı (bazı hücrelerin *PTEN* (*fosfataz ve tensin homolog*) gen mutasyonuna sahip olması gibi) ve farklı karakteristik özelliklere (kullanılan hücre hatlarının bazılarının androjene duyarlı bazılarının androjene duyarsız olması gibi) sahip olması sebebiyle olduğu düşünülmüştür. 3,4-DHPEA'nın kolon, meme ve hepatoselüler karsinomalarda anti kanserojenik etkileri saptanmasına rağmen prostat kanserindeki etkileri sınırlı miktarda bilinmektedir. Fabiani vd. (2008) yaptıkları bir çalışmada insan periferik kan mononükleer hücrelerinde (PBMC) ve promiyelositik lösemi hücrelerinde (HL60) hidrojen peroksit ( $\text{H}_2\text{O}_2$ ) ile oluşturulan DNA hasarında 3,4-DHPEA'nın etkisini ölçmüşlerdir. 3,4-DHPEA'nın ortamda  $\text{H}_2\text{O}_2$  (40  $\mu\text{M}$ ) ile birlikte uygulandığında 1  $\mu\text{M}$  gibi düşük konsantrasyonlarda DNA hasarını düşürdüğü, 10  $\mu\text{M}$  da ise HL60'da %93 ve PBMC'de %89 koruma sağladığı bulunmuştur. Araştırmacılar deneylerinde diğer pürifiye bileşikler de aynı hücre hatlarında denediklerinde *p*-HPEA'nın oksidatif DNA hasarı önlemede 3,4-DHPEA'ya göre daha etkin olduğunu göstermişlerdir (Fabiani vd., 2008). Sepporta vd. (2013) 3,4-DHPEA'dan derive tioasetat ve disülfid bileşiklerini HL60 ve multi-drug resistant HL60R hücre hatlarında denemişler ve disülfid bileşiklerinin HL60 ve HL60R hücrelerinde pro-apoptotik ve anti-proliferatif etkilerinin en fazla olduğunu göstermişlerdir (Sepporta vd., 2013). Fabiani vd.'nin bir diğer çalışmasında 3,4-DHPEA'nın HL60, HT-29 ve HT-29 kolon 19 hücreleri üzerinde hücre döngüsünün G1 fazında duraklatarak ve apoptoza sevk ederek etkisi olduğunu ileri sürmüşlerdir (Fabiani vd., 2002). Prostat kanseri hücre hatları ile yapılan çalışmalarda, Zubair vd. 3,4-DHPEA'nın bizim çalışmamıza benzer şekilde LNCaP ve C4-2 prostat kanser hücrelerine karşı antiproliferatif etki gösterdiğini bildirmişlerdir. Ancak normal prostat epitel hücre hatlarının (RWPE1 ve PWLE2) büyümelerine karşı herhangi bir negatif etki göstermediğini rapor etmişlerdir (Zubair vd., 2017). PC3, DU-145, LNCaP insan prostat kanseri hücre hatlarının 3,4-DHPEA ile muamele edildiği kısıtlı çalışmalarda, 3,4-DHPEA'nın anti kanserojenik etkileri olabileceği üzerinde durulmuştur (Luo vd., 2013; Rosignoli vd., 2016). Luo vd., PC3, DU-145 hücre hatları ve kontrol olarak malign olmayan prostat epitel hücrelerini 3,4-DHPEA ile muamele ederek, 2 grubun karşılaştırılması sonucu 3,4-DHPEA'nın hem apoptotik aktivitesini hem de süperoksit üretimini arttırarak anti kanserojenik etkileri olabileceğini bildirmişlerdir (Luo

vd., 2013). Rosignoli vd., PBMC, meme (MDA ve MCF-7), prostat (LNCaP ve PC3) ve kolon (SW480 ve HCT116) hücre hatları üzerinde yaptıkları 3,4-DHPEA muamelesi sonucu 3,4-DHPEA'nın  $H_2O_2$ 'nin indüklediği oksidatif DNA hasarını kaldırdığı, kanserin başlangıç ve ilerlemesi aşamalarında önleyici bir kimyasal ajan olabileceği sonucuna varmışlardır (Rosignoli vd., 2016). Fabiani vd., 3,4-DHPEA'nın meme (MDA ve MCF-7), prostat (LNCaP ve PC3) ve kolon (SW480 ve HCT116) kanser hücre hatları üzerindeki kimyasal önleyici etkinliklerinde  $H_2O_2$ 'nin oynadığı rolü açıklığa kavuşturmak için yaptıkları araştırmada, 3,4-DHPEA'nın  $H_2O_2$ 'yi indükleme kabiliyetinin piruvat tarafından tamamen engellendiği ve hücrelerin  $H_2O_2$  birikimini (katalaz veya piruvatın kültür ortamına ilavesi) desteklemeyen koşullara maruz bırakılmasının anti-proliferatif etkiyi inhibe ettiğini göstermişlerdir (Fabiani vd., 2012). Bizim çalışmamızda da Luo vd., Rosignoli vd. Ve Fabiani

vd.'nin sonuçlarını destekler şekilde, 3,4-DHPEA'nın PC3, DU-145 ve LNCaP hücre hatları üzerindeki sitotoksik etkisi saptanmıştır. Ancak çalışmamızda  $H_2O_2$ 'nin indüklediği bir DNA hasarı oluşturulmamıştır ve 3,-DHPEA'nın antiapoptotik etkisi araştırılmamıştır. Örenay-Boyacıoğlu oksidatif strese karşı 3,4-DHPEA'nın prostat hücre hatlarında *PTEN* mutasyonu statüsüne göre sitotoksik etkilerini araştırdığı çalışmada, wild tip *PTEN* içeren 22Rv1'in 3,4-DHPEA'ya karşı en büyük duyarlılığı gösterdiğini ve bizim çalışmamıza benzer şekilde PC3'ün en az duyarlılık gösterdiği rapor etmiştir (Örenay-Boyacıoğlu, 2018). Bizim çalışmamızda en fazla duyarlılık bu çalışmanın tersine LNCaP ve C4-2 hücre hatlarında saptanmıştır. Bu farklılık Örenay-Boyacıoğlu'nun çalışmasında oksidatif stres oluşturan bir ajanın kullanılması ve *PTEN* mutasyonlarının göz önünde bulundurulmasından kaynaklandığı düşünülmektedir.



**Şekil 1.** Beş farklı insan prostat kanseri hücre hattı üzerinde (A) 24, (B) 48 ve (C) 72 saatlik 3,4-DHPEA muamelesi sonucu hücrelerde ölçülen ölüm yüzdeleri. Negatif kontrol (0 µg/mL) sadece %1 etanol içermektedir.

**Figure 1.** Death percentages measured on five different human prostate cancer cell lines after (A) 24, (B) 48, and (C) 72-hour 3,4-DHPEA treatment. The negative control (0 µg/mL) contains only 1% ethanol.

## Sonuç

Bu çalışmada 3,4-DHPEA, kullanılan tüm prostat hücre hatları üzerinde zamana ve doza bağlı sitotoksikite göstermiştir. Çalışmamızda 3,4-DHPEA'nın LNCaP ve C4-2 hücreleri üzerinde diğer prostat kanser hücre hatlarına göre daha fazla sitotoksikite gösterdiği dikkat çekmiştir. PC3 hücre hattının 3,4-DHPEA'ya karşı en az hassas olduğu tespit edilmiştir. LNCaP popülasyonunun 48 saat 75 µg/mL 3,4-DHPEA muamelesi sonunda tamamına yakınının öldüğü görülürken PC3 hücrelerinin aynı süre ve doz karşısında sadece %40'ı ölmüştür. Prostat dokusundan gelişen farklı kanser hücre hatlarının 3,4-DHPEA'ya karşı farklı derecelerde hassasiyet göstermeleri hücrelerin farklı karakteristik özelliklere sahip olduklarını düşündürmektedir.

## Etik Standart ile Uyumluluk

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

**Teşekkür:** Yazar katkılarından dolayı Prof. Dr. William H. Gmeiner'e teşekkür eder.

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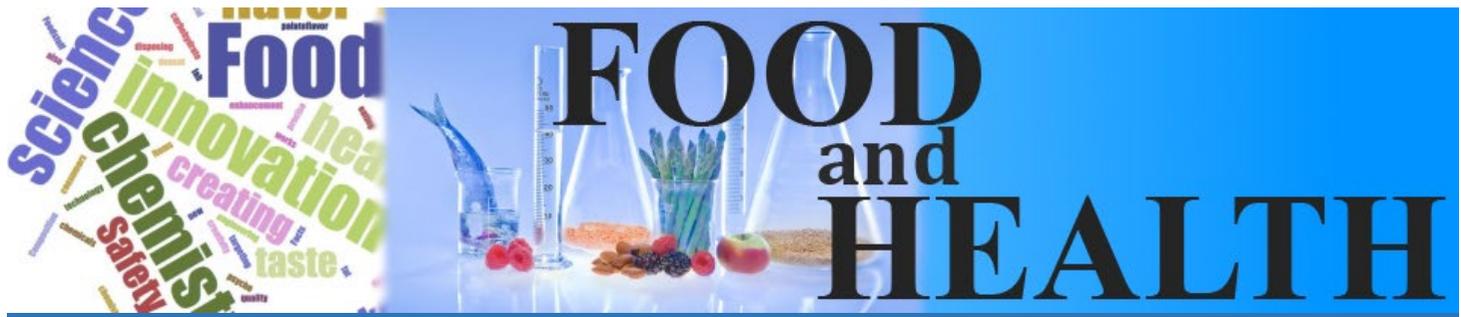
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*Research Article*

## NUTRITION-SOCIO-ECONOMIC STATUS RELATIONS EFFECT ON THE PHYSICAL DEVELOPMENT OF PRESCHOOL CHILDREN

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### ABSTRACT

This study was conducted to question the socio-economic and educational status of preschool 4-6-years old children and their families living in Bitlis and its counties; and also, to examine the effects of these variables on the nutrition and physical development status of children. A total of 960 students, 479 boys and 481 girls, aged between 4-6, who were attending preschools in Bitlis and its counties were included in the study. There was a significant difference ( $P<0.05$ ) between the BMI values of the children and the educational status of their parents; however, there was a very weak correlation between these values (mother ( $r=0.163$ ), father ( $r=0.121$ ). When the total daily sleep durations and the BMIs of the children were compared, it was determined that there was a significant difference ( $P<0.05$ ) and a relation at a moderate severity ( $r=0.54$ ). It was determined that the nutrition and physical development status of the children was related with the socio-economic and educational levels of the parents with a weak correlation and with a positive and very high correlation with the consumption of some food groups.

**Keywords:** Preschool feeding, Physical development, Socio-Economic status, Nutrition, BMI

## Introduction

Healthy nutrition in childhood means covering all the energy and nutritional requirements to provide and sustain a healthy life for children in terms of growth and development. Healthy eating habits in childhood ensure that children have a body structure that is appropriate for the genetic burden of a human in adulthood period (Shloim et al., 2015; Yalvaç et al., 2008). The consumption of healthy foods by young children is essential to provide normal growth and development with children and to prevent a variety of nutrition-related health problems (Aktaş and Angin, 2011). Today, almost all of the parents try hard to raise their children in the best possible way. The inadequacy of financial aids, the social family structure, the educational status, the financial acquisitions of the families, the elementary family structure, regional and local developments, social disasters (Acharya et al., 2015) (i.e. fires, wars, terrorism and migrations), the position of the residential area in relation to intensive settlement areas, poverty, underdevelopment, rapid population growth, rapid and uncontrolled and unplanned structures in dangerous areas, destruction or misuse of the environment, destruction of green spaces, ignorance and lack of education and all similar factors affect life itself and, of course, mostly children in the first place.

According to the analyses in the 2002 Health Report of the World Health Organization, it was reported that the poverty rates in all regions was increasing as the rate of low-weight births increased (Hashemian-Nejad et al., 2014; Özmert et al., 2005) and the weight of 27% of the world's under-five-year-old children was low compared to their age and that most of them lived in developing countries. Children who experience nutritional deficiencies in such countries are at a serious risk due to problems like malnutrition, vitamin-mineral deficiency (Hwalla et al., 2017), childhood deaths, fever diseases, diarrhea, frequent infectious diseases, decreased learning ability in children, stopping of the mental development, growth and physical development deficiencies and similar health problems (Prado and Dewey, 2014; Uauy et al., 2008).

On the one hand, children with nutritional deficiencies try to adapt to hunger and slow growth; and on the other hand, the risk of getting sick due to bodily wasting also increases.

There are nutrient groups which are necessary to be provided with healthy nutrition, and the nutrient groups to be consumed by preschool children, who are also the study group, are as follows (Auestad et al., 2015; Ekinçi, 2018). Scientists have classified in five groups of nutrients (TUBER, 2015). Quantities of nutrients in these groups were determined and daily nutrition plans were made. Food groups which are necessary to be provided with healthy nutrition and food groups to be consumed by preschool children also, food groups and their peculiarities are the key concepts for nutrition education activities in preschool education (Angin et al., 2015). Nutrition shows itself in physical development as well as by affecting all kinds of developmental and behavioral stages. The direct relationship with BMI, height and weight is the most important indicator of the physical development (Aksoy et al., 2017). In general, physical development refers to the physical development of an individual as age grows from the prenatal period time of birth. Physical development is described as the development of organs that form the body such as increase in height, weight gain, development of bones and teeth, muscles, brain, nerves, digestion, circulation, respiration and excretion systems and the development of sensory organs (Garipagaoglu and Özgüneş, 2008). Especially in this study, the subject that is emphasized in physical development will be the BMI values, which are calculated together with height and weight according to nutrition status. In recent years, there has been an increase in the prevalence of obesity that is associated with increases in BMI values, which is also the case in the whole world, and the prevalence is also increasing in children (Tam and Çakır, 2012). In general terms, obesity is the desired level of body weight being over the height of the body as a result of the excessive increase in the rate of lean mass to body fat mass when daily received energy is more than the consumed energy (WHO, 1995). In our country, obesity was also more common in family members with moderate and high-income levels (Kondolot et al., 2011)

*Physical Development and Body Mass Index (BMI) Percentile Values in Children*

One of the most reliable criteria to evaluate the health status of both children and adults and to measure physical development is the age-related BMI (Aksoy and Selen, 2018). The BMI is obtained by dividing the body weight (kg) of the individual by the square of the height (m<sup>2</sup>) (kg/m<sup>2</sup>) (WHO, 1995; WHO, 2000). BMI values corresponding to the percent of a group percentile by the age values of the children created with information compiled by Turkish researchers for Turkey in 2008 are also given in Table 1.

According to the above chart, percentage intervals that correspond to the weight classification category according to BMI percentiles created for the children by the World Health Organization are as in Table 2 below.

Children with a percentile range below 5% are classified as wasting, those between 5% and 85% are normal or healthy, those between 85% and 95% are fat, and those above 95% are classified as obese (Kondolot et al., 2011).

The city of Bitlis has borders with Van, Siirt, Batman, Muş and Ağrı, and is located in the Eastern Anatolian Region

(Demirtaş, 2008). According to SEGE-2011 data (SEGE, 2013) while Turkey’s most developed provinces are located in the Marmara Region, and in western and coastal areas; the poorly- developed cities are located in the Eastern and Southeastern Anatolian Regions; and according to the report, Bitlis ranks the 76<sup>th</sup> in terms of socio-economic development among the 81 provinces. Socioeconomic wasting gives rise to differences in terms of quality of life, education, social life, lifestyle, and social status; and brings deficiencies or differences in terms of nutrition, which is a permanent part of our lives.

The purpose of this study is to determine the nutritional status of children between 4-6 years old who go to preschool classes in Bitlis city center and 6 counties (Tatvan, Ahlat, Adilcevaz, Hizan, Güroymak, Mutki), which differ from each other in terms of education and development levels depending on the socio-economic situation; and also, to reveal the effects of these variables on the physical development of children. Similar studies have not been done on children before in the province and districts of Bitlis

**Table 1.** BMI values corresponding to the percentile by age for Turkish children between 4-6 years of age (TBSA, 2010)

Smoothed age specific BMI for Turkish Boys and Girls (4-6 years of age)														
Percentile Values for Boys							Age	Percentile Values for Girls						
5	15	25	50	75	85	95		5	15	25	50	75	85	95
13.7	14.4	14.8	15.7	16.7	17.3	18.4	4	13.6	14.2	14.6	15.4	16.4	17.0	18.1
13.6	14.2	14.7	15.6	16.6	17.2	18.4	4.5	13.5	14.2	14.6	15.4	16.5	17.1	18.2
13.5	14.2	14.6	15.5	16.5	17.1	18.3	5	13.4	14.1	14.5	15.4	16.5	17.2	18.5
13.4	14.1	14.5	15.4	16.5	17.1	18.4	5.5	13.4	14.0	14.5	15.5	16.6	17.3	18.8
13.4	14.1	14.5	15.4	16.5	17.2	18.5	6	13.3	14.0	14.5	15.5	16.7	17.5	19.1

**Table 2.** Classification of children’s percentile values by weight status category according to the WHO (WHO, 2006)

Weight Status Category	Percentage Interval
Wasting	< 5
Normal or Healthy	5 ≤ BMI < 85
Overweight	85 ≤ BMI < 95
Obese	95 ≤ BMI

## Materials and Methods

### Materials

This study is a descriptive and cross-sectional qualitative study. The study was carried out with a total of 960 children (479 Girls (Female) and 481 Boys (Male) who attended pre-schools in Bitlis/Center and 6 counties (Tatvan, Ahlat, Adilcevaz, Hizan, Güroymak, Mutki) in 2018. The Questionnaire Model was used in the present study. A questionnaire consisting of 40 questions was applied. The questionnaire prepared by examining the literature was composed of three parts (TBSA, 2010), the questionnaire consisted of three parts. The data to determine the nutrient consumption frequency consisted of a 5-Point Likert Scale as "Always"; "Often", "Sometimes", "Rarely", "None". In the first part, there were questions on determining the information about the students and their families; in the second part, there were questions on determining the eating habits of the students; and in the third part, there were questions on determining the frequency of food consumption. Data were collected by face to face interviews with parents of children in school

### Methods

The height measurements of the children were made by using the Harpenden Stadiometer (ADE/Hamburg, MZ10020) ultrasonic height measurement unit with an accuracy of 0.1 cm sensitivity, as the individual was standing parallel to the ground with nothing to affect the measurement, with light clothing; and shoes removed; the weight of the individuals was measured with the InBody230 (MW160) Bioelectrical Impedance Body Analyzer device. After the measurements of height and weight were made, questionnaires were answered by interviewing the parents of the students in a face-to-face manner. All ethical principles, which were included in the Helsinki European Human Rights Declaration, were obeyed on a basis of voluntariness.

The Independent Variables were; gender, age, place of residence, consumed food, demographic information of the parents, monthly expenditures of the children, and monthly income of the families, education and working status of the parents, and social security status of the family.

The Dependent Variables were; children's physical development.

### Statistical Analysis

Using the SPSS Statistics 20.0 Package Program; the Chi-Square (single sample chi-square test), Mann-Whitney U, Test was employed to determine whether the observed values corresponding to the expected values; Non-Parametric and the Spearman Rank Correlation Coefficient Test Analysis was used to determine the direction of the relation between the variables. A level of  $P < 0.05$  was considered to be statistically significant in evaluating the obtained results.

### Ethical Principles

The study protocol was approved by Bitlis Eren University, Ethical Principles and Ethics Committee decision dated 13.12.2017 and with the number 2017/12-V.

## Results and Discussion

### Findings on Children's Age, Gender and Counties Resided

A total of 49.9% (479) of the children who participated in the study were girls; and 50.1% (481) of them were male. 106 of the participants (54F, 52M) were 4 years old, 163 (91F, 72M) were 4.5 years, 248 (138F, 110M) were 5 years old, 328 (152F, 176M) were 5.5 years old and 115 (44F, 71M) were 6 years old. The distribution of the children according to the counties was as follows; Bitlis/Center 168 (90F+78M), Hizan 143 (75F+68M), Güroymak 133 (61F+72M), Mutki 134 (72F+62M), Tatvan 140 (62F+78M), Ahlat 121 (61F+60M), Adilcevaz 121 (60F+61M) children (Table 3).

### Findings on the BMI, Gender and Age of the Children

It was determined that a total of 12.9% (62) of the girls were wasting; 71.0% (340) were normal, 9.0% (43) were fat, 7.1% (34) were obese; 13.5% (65) of the boys were wasting, 67.6% (325) were normal, 13.1% (63) were fat, 5.8% (28) were obese; and the BMI values of the girls showed a distribution that was similar to each other.

When we look at the children's BMI distributions according to age, the distribution is similar in proportion to the participation rate. In the light of the present data, a statistical analysis was made which revealed that there was no significant difference ( $P > 0.05$ ) between age and gender and the BMI of the children (Table 3).

**Table 3.** The BMI percentile distribution of the participating children according to the counties they live

Child BMI	District Center of Lived							Total
	Bitlis	Hizan	Guroymak	Mutki	Tatvan	Ahlat	Adilcevaz	
<b>Wasting</b>	12	16	24	36	15	19	5	127
<b>Normal</b>	124	106	85	88	94	82	86	665
<b>Overweight</b>	22	12	12	5	23	11	21	106
<b>Obese</b>	10	10	14	3	7	9	9	62
<b>Total</b>	168	144	135	132	139	121	121	960

**Table 4.** The BMI percentile distribution of the participating children according to age

Child BMI	Age					Total
	4	4.5	5	5.5	6	
<b>Wasting</b>	12	24	34	41	16	127
<b>Normal</b>	69	122	178	221	75	665
<b>Overweight</b>	13	11	23	42	17	106
<b>Obese</b>	12	6	13	24	7	62
<b>Total</b>	106	163	248	328	115	960

#### *Findings on the BMI of the Children and the BMI of the Parents of the Children*

A total of 960 mothers and 960 fathers participated in the study, and the BMI values of a total of 1920 participants were measured. According to the Obesity Classification of the BMU value that was determined by the WHO for adults (Forno et al., 2018), the rate of the 1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> degree fat participants was 13.6% (262) (this percentage may be accepted as low when the nearly 20% obesity average in our country is considered); the rate of those who were slightly fat was 43.1% (828), normal 38,6% (742), acceptable 3.5% (67), wasting 1.1% (21).

When the BMI of the children and that of the parents were compared, it was determined that there was a parallel increase in the number of obese children depending on the obesity status of the mothers, the BMI of the children and the BMI of the mothers. It was also observed that there was a significant difference at a statistically significant level ( $P < 0.05$ ). No statistically significant differences were determined between the percentile values of the BMI values of the fathers and the BMI values of the children ( $P > 0.05$ ).

#### *Findings on the Children's BMI and the Total Monthly Income of Their Families, Monthly Expenditure of the Children and on the Inhabited Counties Resided*

When the monthly income levels of the families of the children were compared, it was determined that 42.3% (406) of

them were between 500 and 1609 TL; 15.9% (153) were between 1610 and 2499 TL; 15.9% (153) were between 2500 and 3499 TL; 10.2% (98) were between 3500 and 4449 TL; and 15.7% (150) were over 4500 TL monthly incomes. The income levels of most of the families were at or below the Official Minimum Wage.

When we look at the data that children marked as monthly spending, it was determined that 40.4% (389) of the children spend less than 100 TL, which is a very high rate; while 20.5% (196) spend 400 TL or more. 39.1% (375) this increase in the monthly expenditure on children suggests that they do not comply with the socio-economic indicators of the province. A statistically significant difference ( $P < 0.05$ ) was found between the monthly expenditures of the children and the percentile values of BMI, but the relationship between them was found to be very wasting ( $r = 0.160$ ). There was a significant difference ( $P < 0.05$ ) in the statistical analysis between the monthly incomes of the children and the percentile values of the BMIs and the percentile values based on BMIs of the children according to the BMI category classification.

Families with low income levels were mostly found in Mutki and Hizan, while families with higher incomes were found in the counties of Bitlis/Center and Tatvan. The increase in the number of fat and obese children were found to be significant ( $P < 0.05$ ) but showed a poor correlation

( $r=0.166$ ) in the regions where the income increased and the parents were in the upper level status.

#### *Findings on How the Children Travel to Schools and on Their BMI Values*

In the data related to how the children traveled to schools, the students stated that 67% (643) walked to the school; 33% (317) traveled to school with service buses or private vehicles. When these data and the travel styles of the children to schools and their BMI values were associated with each other, it was determined that there were no significant differences between these variables and obesity in children ( $P>0.05$ ).

#### *Findings on the BMI and Educational Status of Their Parents*

When the educational status of the mothers was examined, it was determined that 15% (144) of the participants were literate; 49,7% (478) were primary school graduates; 18,6% (178) were high school graduates; 16,7% (160) were university graduates; and 4,6% (42) of the fathers were literate; 26,4% (254) were primary school graduates, 32,1% (309) were high school graduates, 37,9% (355) were university graduates. When the BMI values of the children and the educational status of the parents were compared, it was determined that there was a significant difference ( $P<0.05$ ); and as the educational status of the parents increased, the number of the fat and obese children also increased. However, the correlation of these values (mothers' educational status ( $r=0,163$ ), fathers' educational status ( $r=1,121$ ) was found to be very wasting.

#### *Findings on the BMI values of the Children and the Social Security of Their Families*

When the BMI of the children and the social security status of the families were compared, it was determined that there was a significant difference ( $P<0.05$ ). However, the relation between them was found to be very wasting ( $r=0,096$ ). It is considered that this situation is related to the income status of the families and the educational levels of the parents.

#### *Findings on the BMI of the Children and the Professions of the Parents*

When the BMI of the children and the professions of the parents were compared, it was determined that there was a significant difference ( $P<0.05$ ); and the children of families who had high-level income (2.500.00 TL and over) were more inclined to be fat and obese. However, the relation between them was found to be very little and might be ignored ( $r=0.001$ ).

#### *Findings on the BMI of the Children, the Working Status of the Parents and the Number of Children in Families*

When the number of siblings was questioned in the families who were included in the study, it was determined that 8.9% (86) had one, 35.7% (343) had two, 29.3% (282) had three, 15.2% (146) had four, 5.5% (53) had five, 5.2% (50) had six siblings. When the findings on the total number of children in families and their BMI values were examined, no significant difference was found when the BMI values were compared with those of the siblings ( $P>0.05$ ). The number of children may have varied according to the age of the parents and the duration of their marriages.

When the working status of the mothers was examined, it was determined that 10.6% (101) did not work, and 89.4% (859) worked. When the working status of the fathers were questioned, it was determined that 0.8% (7) did not work; and 99.2% (859) worked. There was no significant difference ( $P>0.05$ ) when children's BMI and mothers' and fathers' working status were compared.

#### *Findings on the BMI of the Children and the Daily Sleep Hours*

It was observed that there was a significant difference ( $P<0.05$ ) when the BMI and total sleeping hours of the children were compared; and fatness and obesity increased in children who had increased sleep durations. A moderately severe relation was detected between the BMI and sleep durations of the children ( $r=0.54$ ) (Table 5).

#### *Findings on Meal Intake of the Children*

There was no significant difference ( $P>0.05$ ) between the 3 main meals (breakfast, lunch, dinner) and 3 intermediate meals (brunch, afternoon, night) consumption frequency ( $P>0.05$ ) according to the income levels of the families and the BMI values of the children (Table 6).

#### *Findings on the Consumption of Some Nutrient Groups by Children*

While no significant differences were detected between the total monthly incomes of the families and the BMI of the children and consumption of chocolate, pastries, biscuits, pudding, ice cream, nuts, crisps, popcorn, cokes, drink powders, sweets and candies, sandwich, milk, ayran, yogurt, cheese, fruit and vegetables, fruit yogurt, grissini, melba toast, oats, cornflakes, which are prepared at home, sweets, melba toast, oat, cornflakes, herbal teas, coffee, tea, ready-made juice, meat, fish, chicken, turkey, egg and legumes ( $P>0.05$ ); a significant difference was determined between the consumption of fruit yogurt, pizza, hamburger, sausage, salami, etc. ( $P<0.05$ ) (Table 7).

**Table 5.** The BMI percentile distribution of the preschool children according to their daily sleeping durations

Child BMI	Daily Sleep Time (Hours)								Total
	6	7	8	9	10	11	12	13	
Wasting	0	2	28	34	47	6	10	28	127
Normal	2	18	86	171	292	47	47	86	665
Overweight	0	1	15	24	36	17	13	15	106
Obese	0	2	12	12	35	1	0	12	62
<b>Total</b>	2	23	141	241	410	71	70	141	960

**Table 6.** The frequency of daily meal intake of the participating children

The food menu	Always		Often		Sometimes		Rarely		None	
	n	%	n	%	n	%	n	%	n	%
Breakfast	792	82.50	53	5.52	89	9.27	19	1.97	7	0.72
Mid-Morning Meal	81	8.43	53	5.52	244	25.41	120	12.50	462	48.12
Lunch	780	81.25	82	8.54	80	8.33	12	1.25	6	0.62
Early Dinner	121	12.60	73	7.60	294	30.62	140	14.58	332	34.58
Dinner	862	89.79	45	4.68	39	4.06	11	1.14	3	0.31
Night Meal	45	4.68	45	4.68	206	21.45	166	17.29	498	51.87

**Table 7.** The consumption frequency of some food groups by the preschool children

Foods	Always		Often		Sometimes		Rarely		None	
	n	%	n	%	n	%	n	%	n	%
Milk, Yogurt, Ayran, Cheese	623	64.89	190	19.79	102	10.62	30	3.12	15	1.56
Fruit yogurt	107	11.14	86	8.95	245	25.52	182	18.95	340	35.41
Meat, Chicken, Fish, Turkey	438	45.62	232	24.16	227	23.64	38	3.95	25	2.60
Egg	595	61.97	159	16.56	157	16.35	24	2.50	25	2.60
Pizza, Hamburger	30	3.12	44	4.58	263	27.39	215	22.39	408	42.50
Sausage, Salami, Sausage etc.,	27	2.81	46	4.79	262	27.29	220	22.91	405	42.18
Homemade sandwich	152	15.83	158	16.45	300	31.25	141	14.68	209	21.77
Fruit and vegetable	616	64.16	195	20.31	120	12.5	14	1.45	15	1.56
Prepared fruit juice	304	31.66	217	22.60	308	32.08	86	8.95	45	4.68
Herbal teas	22	2.29	25	2.60	114	11.87	121	12.60	678	70.62
Coffee, Tea	94	9.79	70	7.29	278	28.95	242	25.20	276	28.75
Nuts	187	19.47	216	22.5	362	37.70	163	16.97	32	3.33
Legumes	305	31.77	178	18.54	273	28.43	96	10	108	11.25
Pastry work, Biscuit etc.,	164	17.08	192	20	499	51.97	93	9.68	12	1.25
Oats, cornflakes, etc.,	31	3.22	34	3.54	135	14.06	157	16.35	603	62.81
Chips, Popcorn	125	13.02	114	11.87	367	38.22	280	29.16	74	7.70
Breadsticks, Melba toast	5	0.52	12	1.25	57	5.93	116	12.08	770	80.20
Chocolate	158	16.45	142	14.79	545	56.77	93	9.68	22	2.29
Pudding, Ice Cream, Desserts etc.,	79	8.22	99	10.31	489	50.93	242	25.20	51	5.31
Cola drinks, Powder drinks etc.,	47	4.89	39	4.06	241	25.10	313	32.60	320	33.33
Sugar, Confectionery	116	12.08	143	14.89	382	39.79	242	25.20	77	8.02

The physical development of the body shows variations according to age and affects the developmental stages of height as well as the weight of individuals, and the development of the organs that make up the body (Erkan and Akyol, 2018). In order to have a healthy life, it is necessary to keep a balance between the energy consumed and the energy taken in (Essah et al., 2014). Today, the greatest problem of undeveloped countries is hunger and famine. In this study, the nutritional status and physical development of children aged 4-6 years are discussed.

There were no statistically significant differences in terms of the relation between the BMI and gender of the children ( $P>0.05$ ). In a study in which the obesity prevalence among children living in the United States and in our country was reported, it was determined that there was a similar distribution between girls and boys; and there was no significant difference between the BMI (Kondolot et al., 2011; Ogden et al., 2014).

Although the BMIs of the children were not related to fathers' BMI, there was a correlation between mothers' BMI at  $r=0.158$ . Although this relation was wasting, it is possible to claim that in the case where maternal BMI was increased, there is also an increase in the BMIs of the children. Similar results were reported in a study investigating the factors that affect obesity formation (Ulutaş et al., 2014).

When the correlation parameters were examined, a wasting effect was determined ( $r=0.163$ ) of fathers' education status ( $r=0.121$ ), occupations ( $r = 0.001$ ), family social security status ( $r = 0.096$ ), the total monthly income of the family ( $r=0.166$ ), the monthly expenditures of the children ( $r=0.160$ ), and the residential areas ( $r = 0.018$ ) on the BMI of the children; and it was also observed that the education, parents' professions and the income level of families affected the children's BMI values as much as the opportunities offered to the children. Previous studies also reported that maternal education has positive effects on children's education and development (Rowe et al., 2016). In this respect, it was also determined that especially in the provinces where the income of the families was higher (Bitlis/Center and Tatvan) there were increases in the number of fat and obese children; and it increased significantly compared to other counties ( $P<0.05$ ).

Although there was no significant difference between the BMIs of the children and how they traveled to schools (service buses provided by the Ministry of National education/private vehicles), there are data showing that facilitated transportation improves obesity (Kimm et al., 1996).

Although there was no statistically significant difference between the BMIs of the children and the total number of siblings in families and the working status of the parents, this situation might have stemmed from the fact that very few of the mothers who participated in our study worked 10.6% (101); and very few of the fathers 0.8% (7) did not work. This can be examined in a more comprehensive study.

There was a moderate correlation between the BMI values and the sleep durations of the children ( $r=0.54$ ). There are similar studies in which fatness and obesity were reported to increase as well as the increase in sleep durations (Sancak et al., 1999). The fact that children spend a certain amount of time by sleeping which may be spent with other activities and that the metabolism works in a lower level during sleep might have been influential in this outcome.

It was determined that there was no difference in the meals of the children and their BMI according to the income level of the families;

- The answers given in general to the question on the frequency of consumption of the three main meals of the day (breakfast, lunch, dinner) were "always" or "often".
- While the consumption of brunch was answered as "sometimes" and "never"; there were answers showing that the afternoon and night snacks were consumed "frequently" or "sometimes".

Snacks like brunch are the mostly skipped snack. It is known that afternoon snacks and night snacks are preferred relatively more (Al-Bashtawy, 2017). It should be noted that although these data on meals seem interesting, it must be kept in mind that the amount of food consumed during meals will affect the BMI rather than the frequency of meals.

The total monthly incomes of the families and the BMI of the children did not differ significantly;

- The consumption frequency of foods such as milk, ayran, yogurt, cheese, fruit, vegetables, meat, fish, chicken, turkey and eggs were "always" and "often",
- The consumption frequency of legumes, ready-made fruit juice, and chocolate consumption frequency was "always" and "sometimes",
- The frequency of consuming pastries, biscuits, and nuts was "often" and "sometimes",
- The frequency of consuming pudding, ice cream, sweets and candies, crisps and popcorn was "sometimes" and "rarely",

- The frequency of consumption of home-made sandwiches was “sometimes” and “none”,
- The frequency of consumption of cokes, drink powders, grissini, melba toast, herbal teas, coffee, tea, oats, cornflakes etc. was distributed between “rarely” and “none”, as shown in Table 7.

It was determined that there was a significant difference ( $P<0.05$ ) between the total monthly incomes of the families and the BMIs of the children; and in terms of correlation relations, it was determined that there was a very high relation between the use of some food types such as fruit yogurt ( $r=0.87$ ), pizza, hamburger ( $r=0.90$ ), sausage, salami, etc. ( $r=0.89$ ). In this respect, while the consumption frequency of these foods was “high” and “occasionally” in families with high socio-economic status, the frequency of this consumption in children with families that had lower socio-economic status was “rarely” and “never”. Similar results were found in a study on socioeconomic status and obesity (Sisson et al., 2017) and in another study on the consumption of such foods (Savaşhan et al., 2015).

In the food groups that are necessary for children, the inclusion of foods such as milk, ayran, yogurt, cheese, fruit, vegetables, meat, fish, chicken, turkey, egg, legumes, and nuts in meals, and children from almost any background consuming them is a good sign. However, ready-made fruit juice, chocolate, pastries, biscuits, pudding, ice cream, sweets and candies, crisps and popcorn, cokes, drink powder, etc. are nutrients that are easily accessible today, and in long-term use, they may give rise to all kinds of health problems and diseases, especially obesity not only in children but also in young people and in adults (TUBER, 2015; Kırılan et al., 2005; Işık et al., 2013).

The amount and the diversity of nutrients that are available for children are increasing in direct proportion with the high income levels (Kazak et al., 2015). It may be claimed that depending on the increase in income levels, the BMI of children increase and cause the consumption of high-calorie but unhealthy fruits such as yogurt, pizza, hamburger, sausage, salami, etc., the foods in this group are prepared especially in industrial environments, and are processed, and contain too many simple sugars or trans fats. Similarly, it is evident that such foods increase the BMI values, fatness and obesity as well as several health problems.

## Conclusion

Our findings showed that nutrition and physical development of children was associated positively with a weak correlation with the socio-economic and educational levels of

the parents; and was associated positively with a very high correlation with the consumption of certain food groups.

## Compliance with Ethical Standard

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

**Ethics committee approval:** The study protocol was approved by Bitlis Eren University, Ethical Principles and Ethics Committee decision dated 13.12.2017 and with the number 2017/12-V.

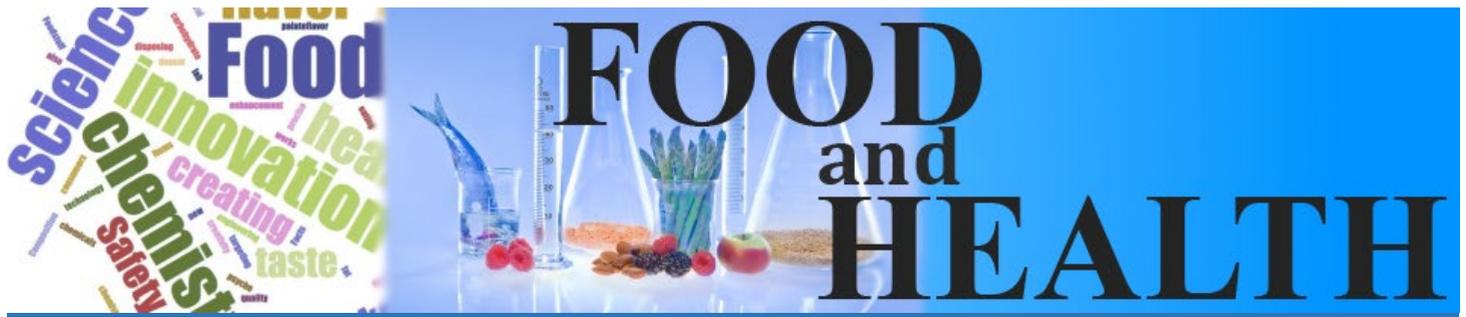
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*Research Article*

## DETERMINING THE LEVEL OF FOOD SAFETY AWARENESS AMONG FOOD PROFESSIONALS: A CASE STUDY OF TURKEY AND NIGERIA

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### ABSTRACT

In this study, 284 food professionals including chefs and students of food science-related courses were surveyed to determine their level of business regarding allergens in foods and food safety in general were determined through questionnaire survey. A questionnaire comprising of 19 questions covering specific food safety issues and allergens in foods were used to collect information for the study through face to face interviews. Interviews were conducted for 284 people in Nigeria and Turkey. One hundred and ninety-five (68.7 %) of the respondents were from Turkey whereas 89 (31.3 %) were from Nigeria. Out of the 68.7% respondents from Turkey; 68.7% were students and 31.3% were chefs. The 31.3 % respondents from Nigeria were 33.7% students and 66.3% chefs. The result of the study indicated that the findings of scientific studies on food safety have not been directly applied by the people working in the food sector. Additionally, the knowledge level of the gastronomy and culinary arts students on food safety and allergens are higher than the restaurant chefs that prepare foods for the consumers. The restaurant as a significant part of the food chain industry must be well-prepared to face an increasing number of clients with food allergies.

**Keywords:** Allergens, Food safety, Consumer awareness, Questionnaire survey

## Introduction

The single most vital task of food scientists and the food industry as a whole is to ensure the safety of foods supplied to consumers. The recent trends in global food production, distribution and preparation necessitated the need for an increased emphasis on hygienic practices at all levels and for increased research in food safety in order to ensure a safer global food supply (Anna and Richard, 2007; Gong et al., 2016). Prokopov and Tanchev (2007) opined that it is specifically an important role of food scientists to make foods as safe as possible regardless of whether they are used fresh or being processed. The judicious utilization of application of food processing, storage, and preservation techniques will help to prevent foodborne illness outbreaks thereby ensuring adequate protection of the health of the consumers.

In this 21st century that we are, food safety issues have had as high a priority and significance as they did over 100 years ago. There have been rising public concerns regarding high-profile food-borne illness outbreaks due to contamination of food with certain pathogens like *Salmonella*, *Escherichia coli* 0157:H7, *Listeria monocytogenes*, and others which have serious acute impact and potential chronic long-term complications especially in the ever-increasing high-risk population segment which include elderly, children and immuno-compromised (Ronald and Gary, 2002).

In addition to the foodborne related diseases, allergens in foods are also a growing food safety and public health concern globally and affect up to 2-8% of the world population and in the United States alone, 4%–6% of children are affected according to a report by the Centre for Disease Control and Prevention (CDC) of the United States. Further, allergic reactions happen when there is a specific and reproducible immune response by the human body systems that can be severe and life-threatening, particularly anaphylaxis. In people with food allergy, the immune system that supposedly protect them from germs will mistakenly respond to food as if it were harmful (Branum, et al., 2008; Zhang, et al., 2016). There are eight foods that are said to contain allergenic materials accounting for 90% of serious allergic reactions in the United States and Europe. These include; milk, eggs, fish, crustacean shellfish, wheat, soy, peanuts, and tree nuts (FSA, 2013). The allergen rules came into effect in the UK and the EU on 13 December 2014. According to this regulation, food businesses must emphasize the major allergens on the product label within the ingredients list (FSA, 2013).

Implementation of the studies in food safety, identified standards, legal regulations issued in this regard by the con-

scious people is extremely important since they should provide solid outcomes for the public health (Hassan and Dimassi, 2014). In order to realize this aim, gastronomy, and food science departments were established in many universities in the world to train qualified personnel in the food sector (Santich, 2004; Aduriz, 2012). As obtained in other parts of the world, there exist regulations on food allergens in Nigeria and Turkey where this research will be undertaken. However, there is no evidence to suggest that consumers are made to understand the risks associated with allergens and whether these regulations are fully enforced and effective on food businesses particularly in Nigeria and this makes it difficult to have any data on allergen prevalence, incidences, and measures taken so far.

This study is therefore aimed at determining the awareness level of Chefs and Students of Food related areas on food safety in general and allergens, in particular, giving the recommendations on the way forward.

## Materials and Methods

A questionnaire comprising of 19 questions covering many food safety issues and allergens in foods were used to collect information for the study through face to face interviews. Questions were developed using samples of questions being used by the Food Standards Agency of the United Kingdom for its annual and biannual surveys on consumer attitudes to foods and food safety and other food safety agencies in the World. The demographic information of the respondents was also collected to determine whether it influences their responses to questions.

About 284 respondents from the age of 18 years and above participated in the survey conducted in Kano state, Nigeria and Ankara city in Turkey. Responses were collected specifically from students in Gastronomy and Culinary Arts, Nutrition, Food Science, and Chefs. Questions were translated to the respondents in the local language where necessary for easy interpretation.

Study 1 Survey on the consumer level of awareness on food safety issues

Respondents were asked six questions regarding their level of concern and awareness regarding general food safety issues. These include 1) Whether food safety is a major issue of concern facing them today 2) Whether they are concerned about food safety at all. 3) Respondents level of concern on food safety issues such as amounts of fats and salt in foods, use of additives, use of pesticides and food poisoning. Respondents were also asked whether they were getting any information on food safety and through which ways and

who should be held responsible for providing information on food safety. The survey items were measure as using scale [1 as Yes, 2 as No, Concerned (1), Neutral (2) and not concerned (3)].

#### Study Survey on respondents' knowledge about allergens in foods and food labeling

Respondents were asked nine questions here to determine their level of awareness and concerns about food allergens and food labeling. The aim was to understand if there is a connection between knowledge on food allergens on one hand and food labeling on the other. Questions asked include 1) whether the respondents have any food allergy. 2) The level of concerns about food allergens and the perspective from which food allergy is important. 3) Respondents ability to identify allergenic foods. 4) Whether respondents have ever come across allergen warning on food packaging. Respondents were also asked on the specific information they look at when buying packaged foods, how easy they are finding food labels to understand, how important is food label in their decision of which food to buy and if they understand the meaning of "use by date" found in some food labels.

#### *Statistical Analyses*

The gathered data was analyzed using The Statistical Package for Social Sciences for Windows (SPSS Inc., version 17.0 Chicago, IL, 2007). Cross-tabulation tests were performed to determine the respondents' awareness level and concerns about food safety issues and food allergens on a comparative basis between Turkey and Nigeria. Statistical significance of  $p < 0.05$  was used for all tests.

## **Results and Discussion**

### *Demographics*

Of the 284 respondents, 68.7% were from Turkey and 31.3% from Nigeria. The vast majority of respondents from Turkey were students (68.7%) and 31.3% were chefs. On the other hand, the majority of respondents from Nigeria were chefs (66.3%) and 31.3% were students. In total, 57.7% students and 42.3% of chefs participated in this research. The percentage of male (49.7%) in Turkey was almost the same to female (50.3%), despite the fact that their counterpart from Nigeria was 56.2% male and 43.8% of them were female. The demographic information will be discussed were it has an influence on the responses to questions.

The research question 1 from the food safety section asked today's top major concern. The result as shown in figure 1 indicated that the majority of respondents in both Turkey

and Nigeria mentioned that their top major concern is terrorism. This result was expected because nowadays terrorism has increased rapidly in all over the world. On the other hand, the interesting results are that respondents from Turkey indicated that the second major problem is standards in education (27.8%), and food safety issue becomes a third major problem (27.8%). However, respondents from Nigeria mentioned the second major problem today is food safety issues (31.5%). According to research, FSA and WHO indicate that cases of foodborne related diseases are growing every day in all over the world, for example in 2010, 582 million incidents and 351,000 deaths associated with 22 different foodborne diseases have been reported (Chapman et al., 2016). That is why it was expected from respondents (chefs & culinary students) that at least food safety issues become a second major problem today.

#### Study 2 Survey on respondents' knowledge about allergens in foods and food labeling.

Respondents were asked to identify food allergens from the listed items. These are approved list of allergens as identified in the EU food standards. Hattersley et al., (2014) mentioned that allergenic foods are a major concern all over the world and all food handlers must know how to handle them. The result as presented in Table 1 indicated that 16.7% of chefs do not know anything about food allergens. This is very dangerous because they deal with food on daily basis and they serve people with these foods. Chefs must be able to understand allergens in foods and guide consumers to avoid negative effects of allergens. Furthermore, significant number of allergic reactions happens in the restaurants resulting from cross contamination, miscommunication between the waiters and cooks and undeclared ingredients (Lee & Xu, 2015; Pratten & Towers, 2004). It is interesting to know that only a few percentages of students (8.5%) in food science, gastronomy, and culinary arts are not aware of food allergens. They are given the needed knowledge on food allergens. Kabacık (2013) in his study to Determine the Food Safety Knowledge Level of the Kitchen Staff Working For 4 And 5 Star Hotel Kitchens reported that students' knowledge level on food safety is significantly higher than that of the chefs.

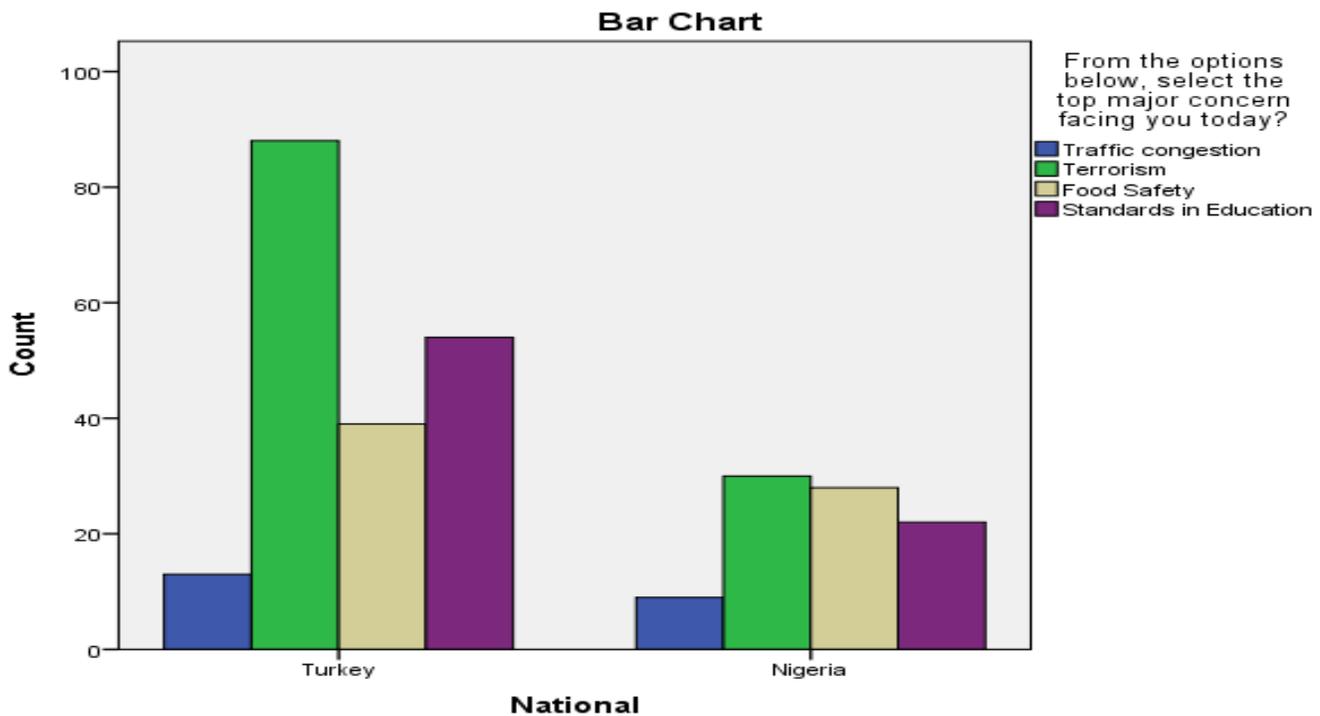
Further, there is a significant difference between the respondents from Nigeria and that of Turkey with respect to their knowledge on food allergens. As indicated in Table 2, a quarter of the respondents from Nigeria (28%) mentioned that they do not know allergenic foods but a majority (51.7%) claimed they have allergy while only fewer percentage (20%) of them correctly identify allergens from the list. In Turkey, on the other hand, the vast majority (70.8%)

of the respondents correctly identify allergens from the list with just a few (4.6%) who mentioned that they don't know anything about allergenic foods. Additionally, fewer percentage (3.1%) of the respondents indicated that they have an allergy. A graphical presentation of this distribution is presented in Figure 2.

**Importance of food allergen from different points of view**

Respondents were asked about the importance of food allergens from different points of view. The result in Table 3, indicated that the vast majority of the respondents in both countries are concerned about food allergens from a health point of view. Allergens in foods can cause a health risk to

the consumers who are allergic to its components or ingredients added. It is to be noted that allergens can also be important from the food safety point of view meaning that the food will not cause any harm when prepared or consumed based on its intended use. Surprisingly, just a few percentages of the respondents were able to indicate that in their responses. Other authors have indicated a general lack of knowledge of food handlers on scientific information on food safety (Kunadu et al., 2016). These points clearly show that the scientific information and finding must be implemented by the food handlers otherwise foodborne related problems will continue. The study of Burke et al., (2016) indicated that food servers and producers have low knowledge of foodborne pathogens.



**Figure 1.** Major Concerns Facing Respondents Today

**Table 1.** Knowledge amongst respondents about foods that contain allergens.

<b>Which of the following foods contains allergens?</b>									
	Wheat	Shell fish	Milk	Egg	Fish	Soy	Peanuts and its derivatives	All of them	I don't know anything about allergic foods
Chefs	8.3%	6.7%	5.8%	13.3%	3.3%	3.3%	3.3%	39.2%	16.7%
Students	3.0%	6.1%	6.1%	3.0%	0.6%	1.8%	4.3%	54.9%	12.0%

**Table 2.** Which of the following foods contains allergens?

<b>Which of the following foods contains allergens (count within Nationalities)?</b>										
		Wheat	Shell fish	Milk	Egg	Fish	Soy	Peanuts and its derivatives	All of them	I don't know anything about allergic foods
Nationality	Turkey	3.6%	7.7%	1.0%	6.2%	1.5%	1.0%	3.6%	70.8%	4.6%
	Nigeria	9.0%	3.4%	16.9%	10.1%	2.2%	5.6%	4.5%	20.2%	28.1%

**Table 3.** The importance of allergenic foods from different points of view.

<b>Allergens are important from which point of view?</b>					
		Food safety	Health point	Food quality	Not important at all
Nationality	Turkey	11.9%	79.4%	3.6%	1.5%
	Nigeria	23.6%	57.3%	4.5%	13.5%

*Consumer Awareness on Food Labelling*

On food labelling, there is a significant difference between the respondents from Nigeria and that of Turkey with respect to ‘how easy do you personally find foods label to understand’. The results were presented in Figure 3. The majority of the respondents from Nigeria (56.8%) mentioned they understand food labels easily while a few percentages of them indicated that it is very hard to understand (8%). On the other hand, only 39.6% of the respondents from Turkey indicated that they understand food labels easily while a quarter of them (25.6%) indicated that it is hard to understand. Accordingly, it looks like Nigeria’s food labels are more understandable than their Turkish counterparts.

Respondents were asked about the information they look up when buying packaged foods. As shown in Figure 3, majority of the respondents in both Turkey (52.8%) and Nigeria (51.1%) lookup for all the mentioned information on the package. However, only a quarter (although the largest percentage) of the respondents in all the countries look up for best before/use by date despite its importance from the food safety point of view. Furthermore, despite the importance of nutritional information especially on allergens, very few of

the respondents look for it when buying packaged foods. The 2007 survey conducted by the FSA in the UK on Consumer Attitudes to Food Standards revealed that respondents were more likely to look at the nutritional value and ingredient information than the “use by date”. The change in attitude is likely to be attributed to the rising concern about food safety in recent years and the importance of the “use by date” as an indicator of food safety.

On the other hand, the results indicated only 40% of the chefs look at all the information on the food package. It is expected that the chefs should be more concerned about the information on the package especially in regards to their role in food preparation. However, 59% of students mentioned that they look up all information on the package. This suggests a good understanding of the importance of food labels. A similar work was carried out by the Kunadu et al., (2016) indicated that food handlers never check the integrity of food package before use. Webb and Morancie (2016) also indicated food handlers do not have significant knowledge of food safety.

The respondents were asked if they ever come across allergen warning in the packaged foods or menu list at restaurants. The results show on Table 4, shows that, there is a

significant difference between Nigerians and Turkish participants. The vast majority of Turkish respondents (66.2%) indicated that they have not come across any warning in the packaged foods and menu at restaurants. The EU food regulation stipulated that major allergens must be emphasized on the product label (Smigic et al., 2016) and many restau-

rants in Europe mention allergens alert in their menu. Answers from Nigeria’s participants are so close (yes 32.6%, no 34.8%, I don’t know 32.6%) and if we look at those results, it clearly indicated that respondents from Nigeria are more concerned about this situation and that explains why respondents from Nigeria claimed to have more food allergy than their Turkish counterparts.

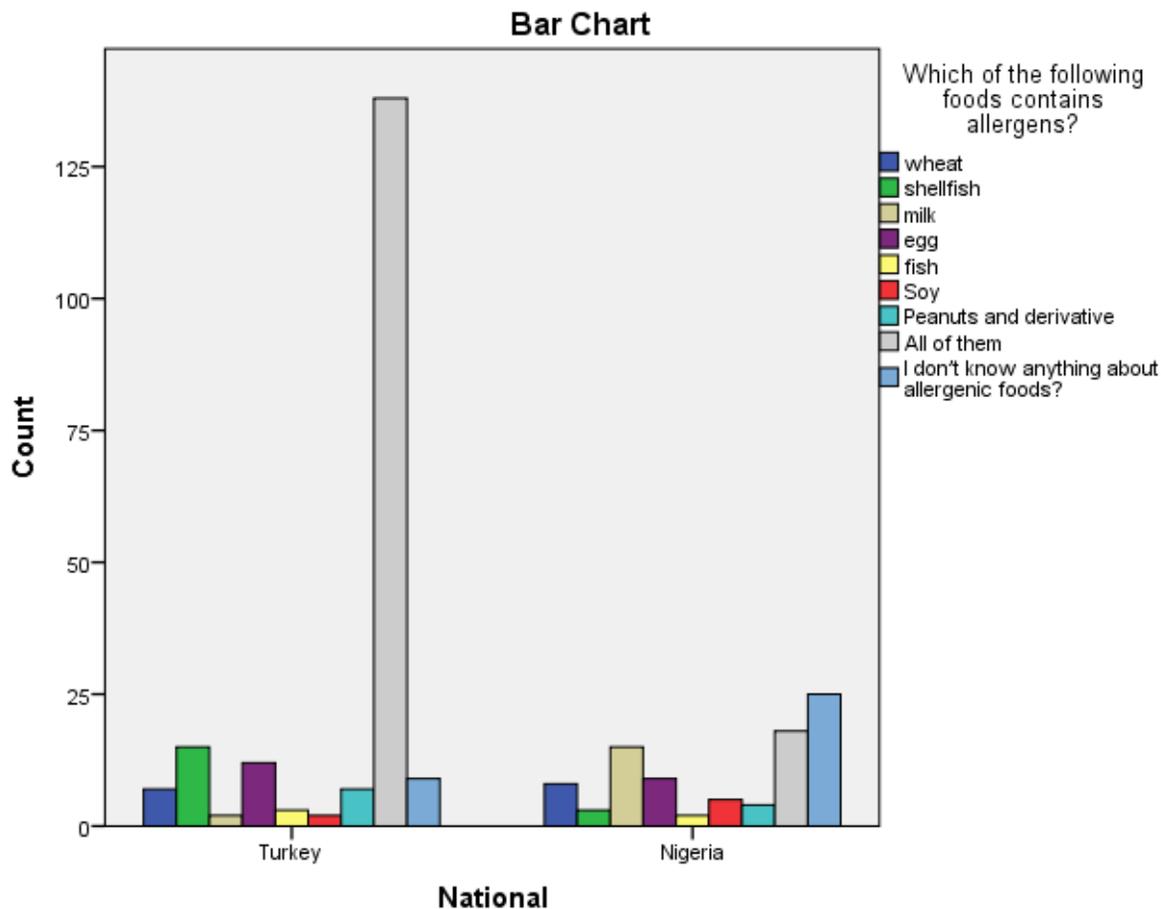
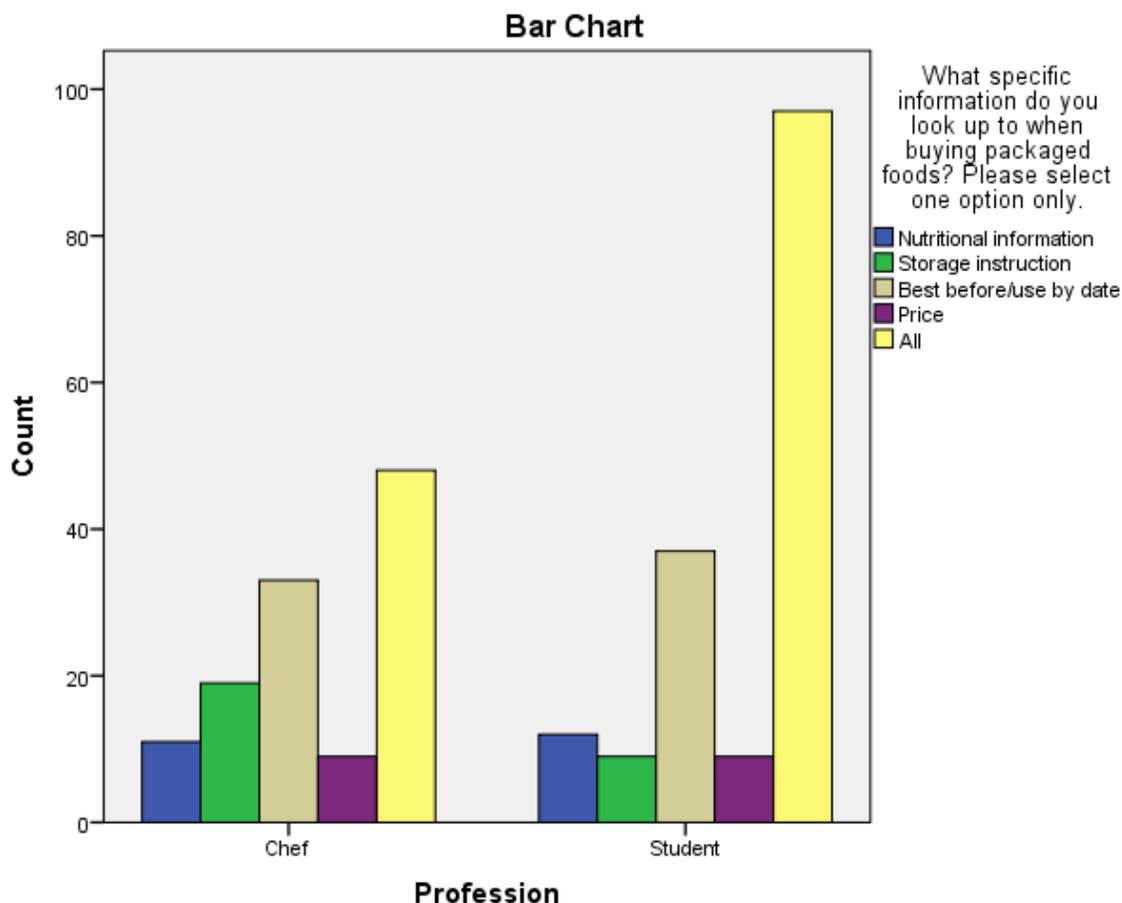


Figure 2. Knowledge of Allergens across Turkey and Nigeria.

Table 4. Have you ever come across allergen warning in the packaged foods that you are buying?

Nationality	Yes	No	I do not know
	Turkey	13.3%	66.2%
Nigeria	32.6%	34.8%	32.6%



**Figure 3.** Information looked up when buying foods.

## Conclusion

This study has examined the awareness level and attitude of chefs and students of food-related areas on food safety in general and allergens in particular. All over the world, there is an abundance of works dealing with the knowledge level of the people on food safety. What makes this study different is that the target participants are the chefs currently working in the food industry sector and the gastronomy and culinary arts students who will be qualified staff in the food sector. As a result of this study, it has been concluded that the findings of scientific studies on food safety have not been directly applied by the people working in the food sector. Additionally, another finding of this study is that the knowledge level of the gastronomy and culinary arts students on food safety and allergens are higher than the restaurant chefs that prepare foods for the public. It has been observed that there is a significant difference between the chefs and the students from both countries about allergens and food labels. In the official list of the most well-known

allergens, 66.5 % of the students know all of them correctly whereas only 39% of the chefs correctly identify these allergens. Moreover, 16.7% of the chefs have never heard anything about allergens which means a great problem for the consumers.

Despite this rapid development of food technology, food-related problems still continue. This problem can be handled by the support of the institutions offering gastronomy and culinary art through training qualified people for the sector. In addition, Government must be sure that measures to reduce food safety cases are fully applied in the food sector.

### Compliance with Ethical Standard

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

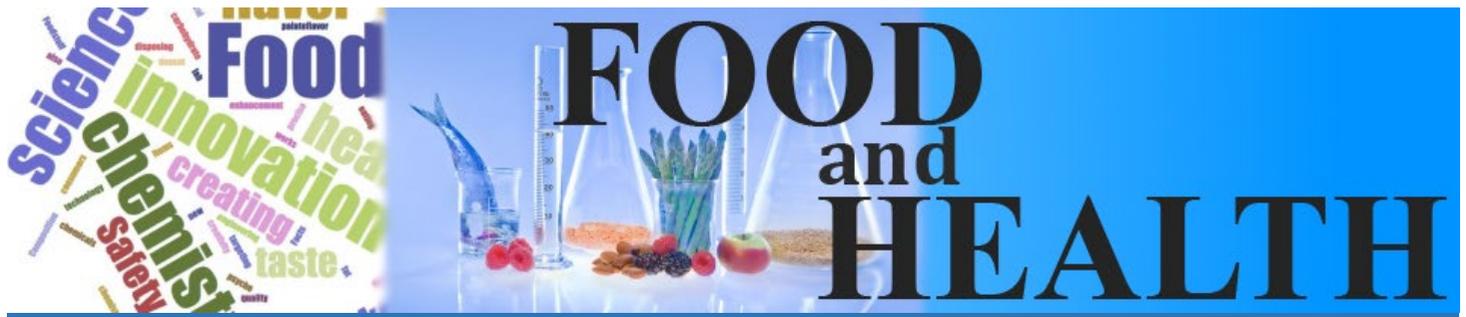
**Ethics committee approval:** Informed consent was obtained from all participants prior to their participation in the study.

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*Research Article*

## CONTROL OF ENZYMATIC BROWNING IN POTATO WITH CALCIUM CHLORIDE AND ASCORBIC ACID COATINGS

Gülçin Yıldız 

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### ABSTRACT

This study deals with the inactivation of browning enzymes in potato by using various chemical agents. Potato purchased from a supermarket was treated by ascorbic acid and calcium chloride solutions alone and in combination. The effects of different chemical treatments on the surface appearance and selected quality indexes of potato slices during 2-weeks of storage at refrigeration conditions (0-4°C) were evaluated. Potato slices were immersed directly to the distilled water at room temperature for the Control samples to evaluate the browning process. The combined chemical treatments showed less browning in the potato slices compared to the applied solutions alone. Beside the inactivation of the enzyme, the combined treatment also showed a better success to prevent color degradation of potatoes. This study showed that the combining chemicals rather than using them alone is important in terms of processing of cut potato slices with a less browning and acceptable colors.

**Keywords:** Ascorbic acid, Color, Calcium chloride, Potato, Polyphenol oxidase enzyme

## Introduction

Enzymatic browning is a process which occurs in several plants and is in charge of important loss of food product. Because of enzymatic browning almost half of tropical fruits are discarded because of the quality defects (Whitaker *et al.*, 1995). The browning is mainly catalyzed by the enzyme called as polyphenol oxidase (PPO) (Marshall *et al.*, 2000). When plant tissues are damaged by physical effects such as cutting, peeling or slicing, PPO is activated by releasing into the cytosol. In the presence of O<sub>2</sub> and PPO, monophenol is hydroxylated to *o*-diphenol and diphenol can be oxidized to *o*-quinones, which then undergoes polymerization to produce dark brown polymers (Caodi, 2007).

Potatoes are one of the most famous product which is consumed in many countries, and are exposed to enzymatic browning and microbial spoilage. The browning reaction could be reduced or prevented by enzyme inactivation with some parameters such as reducing the pH (i.e. addition of lemon juice or other acids), reducing the amount of available oxygen (i.e. vacuum packing of cut food), and adding several preservative chemicals (such as sulfur dioxide) (Annese *et al.*, 1997; Sapers *et al.*, 1990). Among these chemical, citric, acetic, malic, and ascorbic acids are used frequently in order to prevent or reduce enzymatic browning (Whitaker *et al.*, 1995). It was concluded that the differences in browning reduction efficiency could be arise from the food products used in the research and/or the concentrations of the chemicals (Sapers *et al.*, 1987).

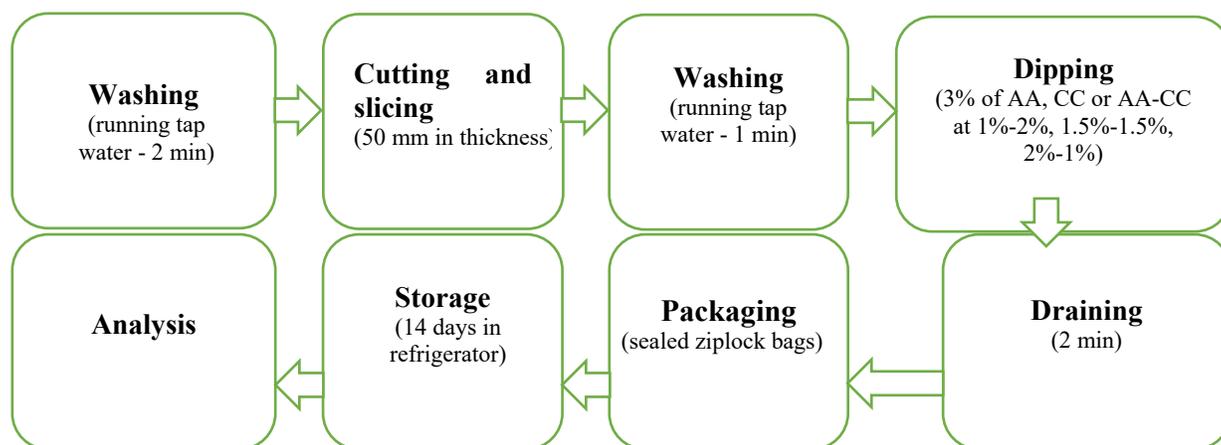
Ascorbic acid is an isomer which have been used commonly in the food industry due to its antioxidant properties. The role of this acid in browning is to reduce the *o*-quinones to diphenols (Golan-Goldhirsh *et al.*, 1984), and its effectiveness is determined by redox potential, rate of oxidation, and stability during processing. According to Tortoe *et al.*

(2007), ascorbic acid (AA) was found to be the most effective treatment, whereas calcium chloride (CC) was most efficient in limiting the decay of fruits. By taking care of this statement, the combination of those two could be the best compounds for minimally processed foods because of their minimized browning. Therefore, the objective of this study was investigate the effects of different chemical treatments alone or in combination on the surface appearance and selected quality parameters of potato slices during 2-week of storage at refrigeration conditions (0- 4°C). This will provide a significant information to prevent enzymatic browning in potato with the most effective and efficient chemical treatment with the best concentration.

## Materials and Methods

### Potato Samples and Chemical Treatments

Russet potatoes were supplied from a supermarket were stored at 4°C. Potatoes were washed with running tap water during 2 min, and followed by cutting with a sharp stainless steel knife for the production of potato slices (50 mm thickness). And then, these potato slices were washed by running tap water during 1 min and instantly dipped into 3.0% (w/w) chemicals during 3 min (Figure 1-Flow chart). On the other hand, control potato samples were prepared by dipping them in distilled water with no chemical treatments. After dipping, slices were drained with a paper towel for 2 min and immediately packed in polyethylene bags, labeled and stored at 4°C for during 2 weeks for the shelf life study. All dipping solutions (ascorbic acid and calcium chloride) were prepared at 3 % (w/w) concentration using distilled water, in addition to the combination of AA and CC in various rates (i.e. 1% AA-2% CC; 1.5% AA-1.5% CC; and 2% AA-1% CC). Experiments were done in triplicate. Analysis were done at days 0, 7, and 14.



**Figure 1.** Processing diagram for fresh cut potato slices

### Color Analysis

Colors of the potatoes were analyzed by a Minolta Chroma Meter CR-300 from the surface of the samples (Yildiz *et al.*, 2016). White standard plate was used for the calibration of the machine. For each samples color values (L, a, and b) were read 5 times at different locations on the sample surface (one reading was from the center, one from the left-top side, one from the right-top side, one from the left-bottom, and one from right-bottom of the sample) at room temperature (RT). And finally, just one number for averaged L, a, and b values was reported. 3 replications were used for the color measurement, and the analysis was done at 0, seventh and fourteenth days.

### PPO Activity Analysis

According to the method stated by Montgomery *et al.* (1975) was followed for the PPO activity measurement in the potato samples using a spectrophotometer. Thirty grams of tissue were homogenized using a blender in 0.2 g polyvinylpyrrolidone and 70 mL of 0.5 M phosphate buffer for a minute. The clear supernatant after centrifugation (1200 g, 15 min, 20°C) was obtained as enzyme extracts.

2 mL of phosphate buffer (0.05 M) stored in a refrigerator at 4°C and 0.5 mL enzyme extract were added to a glass tube. Before the reading, fresh 0.5 mL catechol was put into the mix. The mixture was poured into cuvettes, and the increase in absorbance was read over 3 min in every 15 s at 420 nm. The PPO activity analysis was done at the 0, 7<sup>th</sup> and 14<sup>th</sup> days.

The absorbance of the assay solution was plotted against the reaction time to show the enzyme kinetics. Slope of the absorbance vs. time curve was calculated, and the result was expressed as a “U/g FW”. The data was divided by 0.001 and PPO activity was described as “U/mL” in agreement with Cemeroglu (2007).

$$\text{Activity} \left( \frac{\text{Unit}}{\text{mL}} \text{ enzyme extract} \right) = \frac{E}{0.001 H_e} (H_{rk})(S_f)$$

where E: the slope of the absorbance vs. time curve,

0.001: a constant value,

$H_e$ : the volume of the enzyme extract

$H_{rk}$ : the total volume of the reaction mixture (mL), and

$S_f$ : a dilution factor.

### Statistics

The differences were determined by using the General Linear Models procedure in SAS program. Significant differences among the means were identified with Fisher's least significant difference (LSD) test at  $\alpha = 0.05$ .

### Results and Discussion

Color is an important quality parameter which is very effective on consumer decision and inform us related to many other properties of food products, such as ripeness degree in fruit and vegetables (Dorante-Alvarez *et al.*, 2000). Fresh-cut potatoes are expected to have a bright surface color and be free of decay. The color readings of control and treated potato samples over storage at 4°C are shown in Tables 1. The L (lightness) values of all treated potatoes samples significantly higher than the control at all storage times (Table 1). The highest L values were observed for the samples treated with combined ascorbic acid (AA, 2%)-calcium chloride (CC, 1%) application. The L values decreased significantly with the storage time in all treated samples except AA (1.5 %) + CC (1.5 %) and AA (2 %) + CC (1 %) samples. The similar results were observed in the work of Yildiz (2018). It was observed that the L values of the banana slices treated with different chemical agents decreased with the storage time. The a (redness) values of the potatoes significantly increased during storage (Table 1). While the lowest a values were found at day 0 (for all the treatments), the highest a values were observed on day 14. Similarly, the b (yellowness) values of the potatoes significantly increased with the storage time in all samples (Table 1). An increase in redness (a) and yellowness (b) values, and a decrease in lightness (L) values indicated an increased enzymatic browning in all cut potatoes during 2 weeks of storage. Pérez-Gago *et al.* (2005) also stated the findings where color changes of apples were announced with a decrease in L (lightness) values, and increase in a (redness) and b (yellowness) values during the storage. It was also reported that sweet potatoes samples treated with different chemical preservatives showed lower L values with the storage (Sgroppo *et al.*, 2010). Moreover, browning measured by a (yellowness) value showed a significant increase mostly from seventh to fourteenth day of storage in apple slices treated with different chemicals including ascorbic acid and calcium chloride (Tortoe *et al.*, 2007).

The processing of products such as cutting caused removal of skins in the case of fruits and this leads to water loss and deterioration (Altekruse *et al.*, 1997; Agar *et al.*, 1999). It also causes increase in respiration rate and ethylene production (Saltveit, 1997). In addition, reaction between phenols and PPO causes browning discoloration (Martinez *et al.*,

1995; Heaton *et al.*, 1996). In this study, different chemical agents including ascorbic acid and calcium chloride alone and in combination were used to reduce the browning of potato slices. The PPO activity changes of potato slices during a two-week period at 4°C are listed in Table 2. The PPO activities of potatoes treated with AA (2%) + CC (1%) were in the range of 78 to 110 U/mL during 2 weeks of storage, and that for the Control 185 to 292 U/mL. The PPO activities of all treatments including control significantly increased during storage, namely in untreated (control) potatoes samples. It has been frequently reported that the most effective browning in fresh-cut products is achieved by using a combination of treatments (Ahvenainen, 2000). In this study, even though all chemical treatments decreased the PPO activity significantly compared to the control samples, the combination of chemicals rather than applying them alone was resulted with less browning. In addition, especially AA (2%) + CC (1%) treated potato samples showed lowest PPO activity compared to the other treatments, which is the indication of less browning. This is supported by the color values (Table 1). AA (2%) + CC (1%) treated samples showed less browning by having a higher L value, and lower

a and b values compared to the other treated samples. In overall, the AA (2%) + CC (1%) treated potato samples yielded the best result showing less browning and optimum quality of fresh-cut potatoes. In the study of Tortoe *et al.* (2007) the moderate browning was observed in Golden Delicious apples treated with combined ascorbic acid-sodium chloride solution after 14 days while the other chemicals were resulted with severe browning. It was reported that as the concentration of ascorbic acid increased with different samples, the inhibitory effect on the initial rate of reaction improved (Dziedzak, 1986). This statement is in a good agreement with the findings of this study. The less browning activity was observed when the concentration of ascorbic acid changed from 1% to 2% (Table 2). For example, the PPO activity was found as 98 U/mL for the first day samples treated with ascorbic acid at 1% concentration with calcium chloride. On the other hand, significantly less browning was achieved with ascorbic acid at 2% concentration (78 U/mL). Finally, Lee *et al.* (1999) found that the combining the ascorbic acid, erythorbic acid, citric acid and acetic acid is more effective compared to when applying them alone in pre-cut lotus roots.

**Table 1.** Color changes in control and treated potato samples over storage at 4°C

Treatments	Storage (days)	L	a	b
<b>Control</b>	0	68.3 ± 0.8 <sup>c</sup>	-0.5 ± 0.5 <sup>e</sup>	12.3 ± 0.4 <sup>c</sup>
	7	63.3 ± 0.6 <sup>d</sup>	1.2 ± 0.3 <sup>b</sup>	15.5 ± 0.4 <sup>b</sup>
	14	54.2 ± 0.8 <sup>e</sup>	1.8 ± 0.2 <sup>a</sup>	19.2 ± 0.6 <sup>a</sup>
<b>Ascorbic acid</b>	0	70.4 ± 0.3 <sup>b</sup>	-0.3 ± 0.4 <sup>e</sup>	9.6 ± 0.3 <sup>d</sup>
	7	67.3 ± 0.5 <sup>c</sup>	0.1 ± 0.2 <sup>d</sup>	12.3 ± 0.2 <sup>c</sup>
	14	64.2 ± 0.6 <sup>d</sup>	1.3 ± 0.7 <sup>b</sup>	12.8 ± 0.4 <sup>c</sup>
<b>Calcium chloride</b>	0	73.8 ± 0.7 <sup>a</sup>	-0.4 ± 0.1 <sup>e</sup>	9.3 ± 0.3 <sup>d</sup>
	7	71.2 ± 0.2 <sup>b</sup>	-0.1 ± 0.3 <sup>d</sup>	12.5 ± 0.5 <sup>c</sup>
	14	67.7 ± 0.1 <sup>c</sup>	1.2 ± 0.5 <sup>b</sup>	12.8 ± 0.5 <sup>c</sup>
<b>Ascorbic Acid (1%) -Calcium chloride (2%)</b>	0	75.1 ± 0.1 <sup>a</sup>	-0.6 ± 0.2 <sup>e</sup>	9.9 ± 0.1 <sup>d</sup>
	7	74.2 ± 0.3 <sup>a</sup>	0.6 ± 0.7 <sup>c</sup>	12.4 ± 0.2 <sup>c</sup>
	14	71.9 ± 0.3 <sup>b</sup>	1.3 ± 0.2 <sup>b</sup>	12.9 ± 0.3 <sup>c</sup>
<b>Ascorbic Acid (1.5%) -Calcium chloride (1.5%)</b>	0	72.3 ± 0.2 <sup>b</sup>	-0.6 ± 0.3 <sup>e</sup>	9.1 ± 0.1 <sup>d</sup>
	7	71.7 ± 0.5 <sup>b</sup>	0.7 ± 0.3 <sup>c</sup>	12.3 ± 0.3 <sup>c</sup>
	14	71.2 ± 0.6 <sup>b</sup>	1.3 ± 0.2 <sup>b</sup>	12.5 ± 0.5 <sup>c</sup>
<b>Ascorbic Acid (2%) -Calcium chloride (1%)</b>	0	75.4 ± 0.3 <sup>a</sup>	-0.7 ± 0.3 <sup>e</sup>	9.8 ± 0.5 <sup>d</sup>
	7	74.8 ± 0.5 <sup>a</sup>	0.8 ± 0.1 <sup>c</sup>	10.1 ± 0.3 <sup>d</sup>
	14	74.3 ± 0.5 <sup>a</sup>	1.1 ± 0.7 <sup>b</sup>	10.6 ± 0.1 <sup>d</sup>

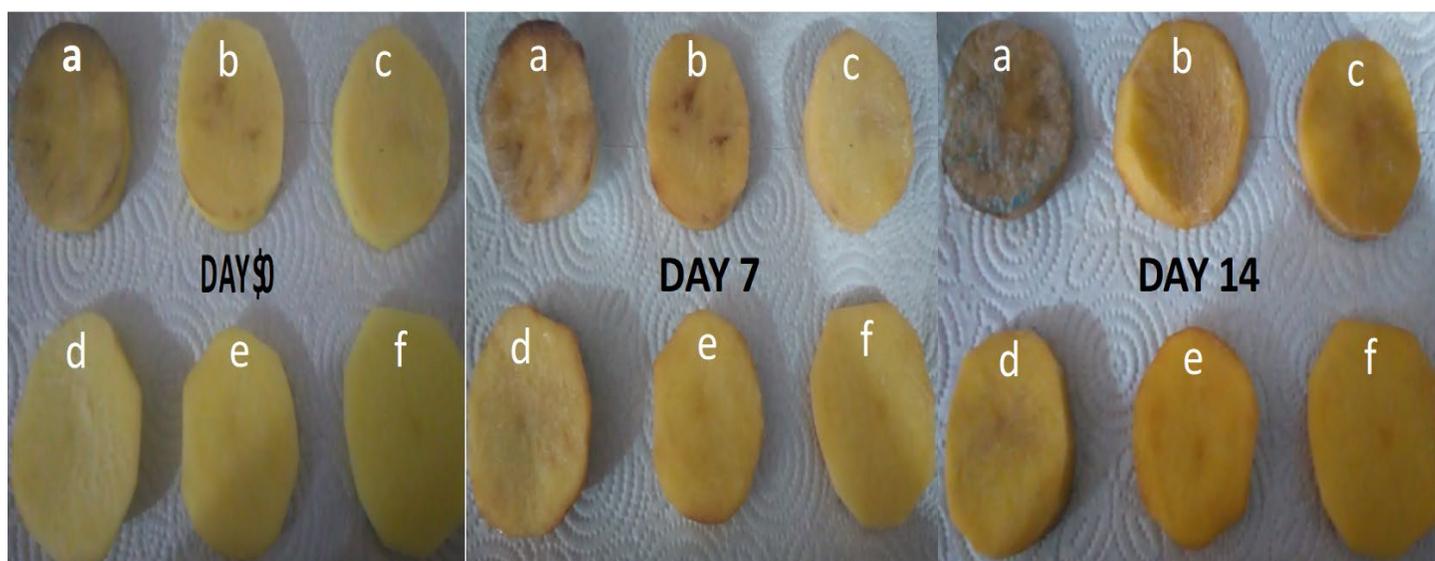
<sup>a-c</sup> Treatment means within storage times (columns) with the same letter in each sample are not significantly different ( $p < 0.05$ ).

The surface pictures of potatoes sliced with different chemical treatments are shown in Figure 2. All treated and control samples showed darker colors compared to the combined treatments (Figure 2–d, e, f), but especially for AA (2%) + CC (1%) treated potatoes (Fig.2-h). This is in agreement with the Hunter L (lightness) values where AA (2%) + CC (1%) treated potato slices had significantly higher L values compared to the control. All samples showed a color degradation starting from first day to last day. Specifically, 14<sup>th</sup> days-samples showed a severe color degradation, namely in control samples. Appearance plays an important role which influence the consumer’s perception and final acceptance of a food product (Lund *et al.*, 2000). It is possible to predict that people will be favor of purchasing potatoes treated by AA (2%) + CC (1%) since they have less browning and preferable color.

**Table 2.** Polyphenol oxidase (PPO) activity changes of potato slices during a two-week period at 4°C

Treatments	Storage (days)	PPO activity (U/mL) at 4°C
Control	0	185 ± 1.1 <sup>c</sup>
	7	235 ± 1.7 <sup>b</sup>
	14	292 ± 1.5 <sup>a</sup>
Ascorbic acid	0	104 ± 1.8 <sup>g</sup>
	7	143 ± 2.1 <sup>e</sup>
	14	167 ± 1.5 <sup>d</sup>
Calcium chloride	0	102 ± 1.7 <sup>g</sup>
	7	141 ± 1.2 <sup>e</sup>
	14	165 ± 1.3 <sup>d</sup>
Ascorbic Acid (1%) - Calcium chloride (2%)	0	98 ± 2.5 <sup>g</sup>
	7	123 ± 3.7 <sup>f</sup>
	14	138 ± 3.8 <sup>e</sup>
Ascorbic Acid (1.5%) - Calcium chloride (1.5%)	0	95 ± 3.2 <sup>g</sup>
	7	120 ± 2.2 <sup>f</sup>
	14	136 ± 1.7 <sup>e</sup>
Ascorbic Acid (2%) - Calcium chloride (1%)	0	78 ± 1.7 <sup>h</sup>
	7	96 ± 1.3 <sup>g</sup>
	14	110 ± 2.1 <sup>f</sup>

<sup>a-h</sup> Treatment means within storage times (columns) with the same letter in each sample are not significantly different (p<0.05).



(a: no treatment; b: calcium chloride c: ascorbic acid d: ascorbic acid (1%)-calcium chloride (2%); e: ascorbic acid (1.5 %)-calcium chloride (1.5%); f: ascorbic acid (2%)-calcium chloride (1%)).

**Figure 2.** Appearance of potato slices with different treatments during a two-week period at 4°C

## Conclusion

The quality of potato samples treated with different chemical agents was evaluated for a period of two weeks. A significant difference in color and PPO activity was observed among the different treatments. It was observed that the inactivation of polyphenol oxidase (PPO) enzyme was achieved for the combined ascorbic acid (2%)-calcium chloride (1%) application at most. Browning measured by yellowness (a) value showed a significant increase from first to last day of storage, especially in fresh potato samples. The ascorbic acid (2%)-calcium chloride (1%) treated potato samples showed significantly higher L (lightness) values than that control and other treated samples. In addition, this combination showed relatively less browning activity. Overall, potato samples treated with ascorbic acid-calcium chloride combination showed a better properties during two weeks of storage compared to the control and other treatments as evidenced by the measurements with instruments, and the appearance of the samples. This study is important in terms of applying chemical treatments with the best and effective concentration to inhibit browning activity in fresh-cut potato samples. This piece of work will be beneficial to produce processed raw potato products with a longer shelf life and higher consumer acceptability by preserving the color and inhibiting the enzymatic browning. A practical implication is that applying combined chemical agents with a best ratio can be used to get a high-quality browning-free products.

## Compliance with Ethical Standard

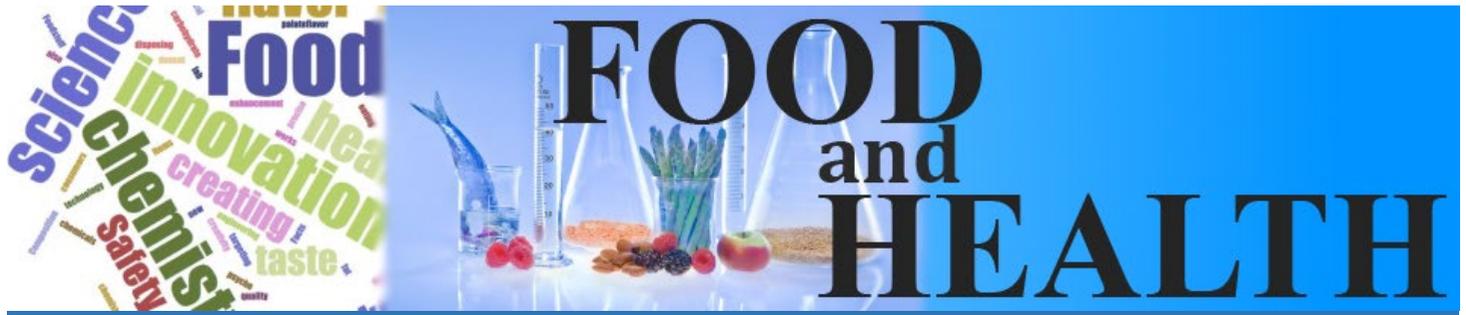
**Conflict of interests:** The authors declare that for this article they have no actual, potential or perceived the conflict of interests.

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Review Article

## GEBELERDE FOLİK ASİT DESTEĞİ VE GÜNCEL YAKLAŞIMLAR

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### ÖZ

Suda çözünen B grubu vitaminler içerisinde yer alan folat besinler içerisinde doğal olarak bulunmaktadır. Folik asit vitaminin sentetik formudur, besin desteklerinde ve zenginleştirilmiş besinler içerisinde yer almaktadır. Gebe ve fetus fizyolojisinde önemli bir role sahip olan folat gereksiniminin gebe kadınlarda tek başına diyet ile karşılanması mümkün değildir. Folat ile başta nöral tüp defekti ve megaloblastik anemi olmak üzere, postpartum depresyon, doğum ağırlığı, çocukluk çağı alerjik hastalıkları, konjenital kalp hastalıkları, otizm vb. sağlık sorunları yakından ilişkilidir. Bu nedenle tüm dünyada nöral tüp defekti, anemi vb. sağlık sorunlarını önlemek amacıyla planlı bir gebelik ile gebelik öncesinde başlayan ve gebelik süresince devam edilen folik asit desteği (400 µg/gün) yaygın olarak önerilmektedir.

**Anahtar Kelimeler:** Folat, Folik asit, Gebelik, Besin desteği

### ABSTRACT

#### CURRENT APPROACHES: FOLIC ACID SUPPLEMENTATION FOR PREGNANT WOMEN

Folate belongs to B group of vitamins. It exists naturally in foods and it is a water soluble vitamin. Folic acid is a synthetic form of folate and it exists in food supplements and fortified foods. Folate has an important role in pregnant women and fetus physiology. Only diet is not enough to supply required amount of folate. There is an important relationship between folate and neural tube defects, megaloblastic anemia, postpartum depression, birth weight, childhood allergic disorders, congenital heart disorders, autism etc. So with a planned pregnancy folic acid supplementation (400 µg/day) beginning before pregnancy and continued during pregnancy is recommended to prevent neural tube defects, anemia and other health problems.

**Keywords:** Folate, Folic acid, Pregnancy, Food supplement

## Giriş

Geberelerde mikrobesein öğeleri yetersizliğinin tüm ülkelerde ve bölgelerde yaygın görülen bir sorun olduğu bilinmektedir. Dünya Sağlık Örgütü (DSÖ) tarafından dünyada 32 milyon gebe kadının anemik olduğu, 19 milyon gebede A vitamini ve milyonlarca gebede ise folat, çinko ve iyot eksikliğinin görüldüğü rapor edilmiştir. Vitamin ve mineral yetersizliklerinin gebe ve fetus sağlığı üzerinde olumsuz etkilere sahip olduğu aynı zamanda çocukluk döneminde morbidite ve mortalite riskini de arttırdığı belirtilmiştir (World Health Organization - WHO, United Nations International Children's Emergency Fund - UNICEF ve Micronutrient Initiative - MI, 2015). Bu derleme yazıda gebelerde folat yetersizliği ve folik asit desteği konusunda yaklaşımlar güncel yayınlar doğrultusunda irdelenmiştir.

### Folat ve Folik Asit

Suda çözünen B grubu vitaminler arasında yer alan Folat, 1941 yılında ıspanaktan izole edilmiştir ve latince 'folium' (Ebera, 2017; Hoffbrand ve ark., 2001). Yetersizliği durumunda görülen başlıca sorunlar megaloblastik anemi ve fetüste nöral tüp defektidir (NTD). Ek olarak kardiyovasküler hastalıklar, kanser ve bilişsel zeka üzerinde de etkili olduğu bilinmektedir (Ebera, 2017). Folik asit ve folat terimleri birbirinden farklıdır. Folat besinlerde ve dokularda doğal olarak yer almaktayken, folik asit vitaminin oksidasyonu ile meydana gelen, besin zenginleştirme ve besin destekleri içerisinde kullanılan formudur (Gropper ve Smith, 2013). Besin destekleri içerisinde tek başına veya diğer B grubu vitaminler ile birlikte yer alır. Besin destekleri megaloblastik anemi, kardiyovasküler hastalıklar, NTD vb. sağlık sorunlarını önlemek amacı ile kullanılmaktadır (Hamishehkar ve ark., 2016). Folatın özellikle gebe kadın ve fetus fizyolojisinde deoksiribo nükleik asit (DNA) sentezi, metilasyon reaksiyonları ve hücre bölünmesi üzerinde etkili olduğu bilinmektedir. Gebe kadınlarda artan folat gereksinmesi tek başına diyet ile karşılanamamakta ve bu nedenle folik asit desteği önerilmektedir (Yan ve ark., 2017). Aynı zamanda MTHFR 677C → T (metilen-tetrahidrofolat redüktaz T allel) polimorfizmi olan bireylerde de folik asit desteği önerilmektedir. MTHFR 677TT polimorfizm prevelansı Kuzey ve Güney Avrupa ülkelerinde sırasıyla %12 ve %24 olarak bilinmektedir. Polimorfizm görülen bireylerde folat düzeyi düşüktür ve istenmeyen sağlık sorunları görülmektedir. Bu nedenle toplumda polimorfizmin sıklığına bağlı olarak günlük gereksinimin düzenlenmesi gerekmektedir. Bu doğrultuda %15 varyasyon katsayısı düşünülerek gereksinimler elde edilir. Serum folat düzeyinin  $\geq 10$  nmol ve kırmızı kan hücrelerinde folat

düzeinin  $\geq 340$  nmol/L olması kesişim noktaları olarak önerilmektedir (European Food Safety Authority - EFSA, 2014).

### Folat Gereksinmesi

Günlük karşılanması önerilen folat alım miktarı farklı formların biyoyararlılığı esas alınarak 'diyet folatı eşdeğeri' olarak hesaplanmaktadır. Besinlerde yer alan folat %50, besin destekleri ve zenginleştirilmiş besinler içerisinde yer alan folik asit ise %85 biyoyararlılık değerine sahiptir (Saini ve ark., 2016).

Türk toplumunda yer alan gebeler için önerilen folat vitamini güvenilir alım düzeyi 600 mcg/gün, üst alım düzeyi ise 1 mg/gün olarak bilinmektedir (Türkiye Beslenme Rehberi - TÜBER, 2015). 1 mcg diyet folat eşdeğeri (DFE) besinler içerisinde yer alan 1 mcg folat vitaminine, yemekle birlikte alınan 0.6 mcg folik asit desteğine ve aç karnına alınan 0.5 mcg folik asit desteğine eş değer olarak bilinmektedir (National Institutes of Health - NIH, 2016). Besin folatı ile folik asit birlikte alındığında DFE, 'DFE (mcg) = [besin folatı (mcg) + (folik asit (mcg) x 1.7)]' olarak hesaplanmaktadır. Gebe kadınlar için Avrupa Gıda Güvenliği Otoritesi (EFSA) tarafından önerilen yeterli folat alım düzeyi 600 mcg/gün'dür. Doğal besin folatı alımı güvenlidir ve yüksek miktar alımın yan etkisi bulunmamaktadır. EFSA tolere edilebilir üst alım düzeyini folik asit (sentetik form) alımına göre belirlemiştir. Gebe kadınlar için üst alım düzeyi 1000 mcg/gün olarak bildirilmektedir (EFSA, 2014).

### Folat Kaynakları

Doğal olarak folat birçok besin çeşidinde bulunmasına karşın, sınırlı sayıda besin zengin folat kaynağıdır. Zengin kaynaklar koyu yeşil yapraklı sebzeler (brokoli, ıspanak vb.), kurubaklagiller (nohut, mercimek, fasulye vb.), portakal, greyfurt, yer fıstığı, badem ve karaciğerdir. Ancak karaciğer depo organı olması nedeni ile gebelerde tüketimi önerilmemektedir (EFSA, 2014).

### Folik Asit ile Zenginleştirilmiş Besinler

Vitamin ve mineral eksikliklerinin önlenmesinde besin zenginleştirme temel yaklaşımlar arasında yer alan bir uygulamadır. Dünya'da birçok ülkede unlar folik asitle zenginleştirilmektedir. Ülke uygulamalarında sıklıkla 100 gram una 140-150 mcg folik asit eklenmesi yapılmaktadır (Zimmermann, 2011). DSÖ ülkenin tüketim örüntüsüne göre 100 gram una,  $\geq 300$  g/gün tüketimde 100 mcg ve  $\leq 75$  gramdan az tüketimde 500 mcg folik asit eklenmesini önermektedir (WHO, 2009). Ülke deneyimlerine göre folik asit ile zenginleştirme sonrası NTD insidansında %30-70 azalma

olduğu bilinmektedir (Zimmermann, 2011). Öte yandan 100 g ekmeğe 70 mcg folik asit eklenmesi ile 7000 sağlıklı yaşam yılı kaybında (DALY) yarar sağlandığı gösterilmiştir (Verhagen ve ark., 2012). Belirtildiği üzere folik asit folat vitamininin sentetik formudur ve besin destekleri içerisinde ve tam tahıl taneleri başta olmak üzere beyaz un, makarna ve mısır gevrekleri vb. bazı besinlerin zenginleştirilmesinde kullanılmaktadır (Fischer ve ark., 2017; Dietitians of Canada, 2016).

### **Gebelik ve Folik Asit**

Her kadın için oldukça doğal bir süreç olan gebelik, insan yaşamında beslenme ve besin öğelerinin en önemli olduğu dönemlerden biri olarak bilinmektedir (Köksal ve Gökmen, 2013). Bu dönemde annenin beslenmesi ve yaşam tarzı kendi sağlığının yanı sıra fetüs sağlığı için de oldukça önemlidir (Uzdil ve Özenoğlu, 2015). Gebe kadınlarda bu süreçte fetüsün artan besin ögesi gereksinmelerini karşılamak gerekmektedir (Salcedo-Bellido ve ark., 2017).

Gebelikte folat gereksinmesinin artış nedeni hem fetüs hem de gebe kadınlarda doku sentezi vd. fizyolojik değişiklikler olarak bilinmektedir (Greenberg ve ark., 2011). Artan folat gereksinmesinin tek başına diyet ile karşılanması mümkün olmadığından gebelerde folik asit desteği tüm dünyada önerilmektedir (Wang ve ark., 2016; Yan ve ark., 2017). Gebelik boyunca, mümkünse öncesinde NTD, anemi vd. sağlık sorunlarını önlemek amacı ile DSÖ her kadına 400 mcg/gün veya haftada bir sefer 2800 mcg folik asit desteği ve folik asitten zengin besin tüketimini önermektedir (WHO, 2012a ve 2012b). Önceki gebeliklerinde NTD sorunu ile karşı karşıya kalmış olan kadınlarda sonraki gebeliklerde bu riski azaltmak adına ilaç dozu olarak nitelendirilen 4-5 mg/gün folik asit desteği öneriler arasında yer almaktadır (Czeizel ve ark., 2013). Planlı bir gebelik ile gebelik öncesinde folik asit desteğine başlanması (400 mcg/gün) ve birinci trimester boyunca mutlaka kullanılmaya devam edilmesi gerekmektedir (EFSA, 2014). Gebeliğin 2.5-3.5 ay öncesinde desteğe başlanmasının gerektiği bilinmektedir. Folat yetersizliğinde erkeklerde de sperm sayısında azalma ve kalitesinin düşük olduğu bildirilmiştir (Stegers-Theunissen ve ark., 2013).

### **Folik Asit ve Nöral Tüp Defekti**

Beyin ve omuriliğin konjenital bir anormalliği sonucunda gelişmekte olan ve mortalite ve morbidite riski yüksek olan Nöral Tüp Defekti intauterin gelişim sürecindeki folat yetersizliği sonucu ortaya çıkmaktadır (Moussa ve ark., 2016). Spina bifida, anensefali ve sinir sistemini etkileyen diğer birkaç konjenital bozuklukları da içerisinde barındıran NTD gebelik öncesi ve gebelik sırasındaki serum folat düzeyleri

ile yakından ilişkilidir. Çünkü folat nöral tüpün kapanmasından sorumlu olan nükleotid sentezi ve metilasyon reaksiyonları gibi bazı biyosentetik süreçlerin gerçekleşmesini sağlayan tek karbon metabolizmasında etkili bir vitamindir (Greene ve Copp, 2014). Nöral tüpün konsepsiyonu takip eden ilk 21-28 gün içerisinde kapandığı bilinmektedir (EFSA, 2014).

Çin’de doğurganlık çağındaki kadınlar ile 24 ay boyunca yapılan bir araştırmada kontrol grubunda yer alan bireylere (n:7037) hiç folik asit ile zenginleştirilmiş un verilmezken, müdahale grubunda yer alan bireylere (n: 16,648) yaklaşık olarak 800 mcg/gün folik asit sağlayan zenginleştirilmiş un (200 mcg/100 g zenginleştirilmiş un) ve ürünleri verilmiştir. Araştırma sonucunda kontrol grubunda yer alan kadınların gerçekleştirdiği 2139 doğumun 49’unda, müdahale grubunda meydana gelen 5898 doğumun ise 43’ünde NTD gözlemlenmiştir. Özetle folik asit zenginleştirilmiş besin tüketimi NTD görülme sıklığını 229.1/10,000’den 72.9/10,000’e düşürmüştür (Wang ve ark., 2016). Türkiye’de 2010-2012 yılları arasında takip edilen gebelikler sonucunda dünyaya gelen veya operasyon için söz konusu hastanelere başvuran Türk ve Suriye uyruklu 40 NTD’li bebeği dünyaya getiren kadınlar ile retrospektif bir çalışma yürütülmüştür. Sonuç olarak bireylerin hiçbirinin gebelik öncesi dönemde ve gebelik süresi boyunca düzenli olarak folik asit desteği almadığı saptanmıştır (Altaş ve ark., 2012). Çin’in kuzeyinde ilk gebeliklerinde NTD gözlemlenmiş 851 kadın ile yapılan retrospektif bir araştırmada bu kadınların 578’inin yeniden gebe kaldığı ve ikinci gebeliklerinde FA desteği almayanların %2.6’sında, destek alanların ise %1.5’inde yeniden NTD gözlemlendiği saptanmıştır. Kadınların büyük bir çoğunluğu (%73) ilk gebeliklerinde hamile kaldıklarını öğrendikten sonra folik asit desteği kullandıklarını bildirmişlerdir (Liu ve ark., 2017).

Folat yetersizliği tanısında serum folat ve kırmızı kan hücrelerinde folat parametreleri sıklıkla kullanılmaktadır (Bailey ve Hausman, 2017). Dünya Sağlık Örgütü (DSÖ) gebelerde kırmızı kan hücrelerinde folat düzeyinin > 400 ng/mL (906 nmol/L) olması gerektiğini bildirmektedir (WHO, 2015). Diyetle folat alımında her %10’luk artış ile kırmızı kan hücrelerinde folat düzeyinde %6 ve serum folat düzeyinde %7 artış olduğu bulunmuştur. Her gün 450 mcg veya üzerinde diyet folat eşdeğeri doğal besinlerin tüketimi ile yeterli kırmızı kan hücreleri folat düzeyinin (~1050 nmol/L) sağlandığı ve NTD riskinin azaldığı belirlenmiştir (~6 NTD/10 000 canlı doğum) (WHO, 2012a ve 2012b).

**Tablo 1.** Bazı besinlerin yenebilen 100 gramlarının içerdiği ortalama folat miktarları (Türkiye Ulusal Gıda Kompozisyonu Veri Tabanı, 2018)**Table 1.** Folate amounts of some foods (National Food Comity of Turkey, 2018)

Besin	Folat miktarı (mcg/100 g)	Besin	Folat miktarı (mcg/100 g)
Kırmızı mercimek	328	Karalahana	89
Yeşil mercimek	286	Marul (iceberg)	81
Ispanak	264	Kuşkonmaz	80
Buğday ruşeymi	212	Buğday kepeği	66
Pazı	211	Marul (uzun yapraklı)	66
Yer fıstığı	189	Roka	60
Dondurulmuş ıspanak	184	Pırasa	59
Isırgan otu	183	Marul (kıvrıkcık)	57
Maydanoz	172	Kepekli ekmek	56
Brokoli	155	Mandarin	49
Ebegümeçi	136	Taze fasulye	48
Dereotu	125	Nohut	46
Turp (siyah)	118	Bezelye	45
Turp (kırmızı)	117	Portakal (Valensiya)	43
Yumurta sarısı (tavuk)	114	Beyaz ekmek	40
Tere	114	Tam buğday ekmeği	38
Nane	102	Mısır ekmeği	34

**Tablo 2.** Gebelerde folik asit desteği kullanım önerileri (Kanada Kadın Doğum Uzmanları ve Jinekologlar Birliği, 2007)**Table 2.** Recommendation of folic acid supplementation in pregnancy (The Society of Obstetricians and Gynaecologists of Canada, 2007)

Öneri	Öykü	Folik asit dozu	Süre
<b>A</b>	Sağlık sorunu yok. Planlı gebelik durumunda.	Folat içeriği yüksek bir diyetle ek olarak <b>400-1000 mcg/gün</b>	Konsepsiyondan <b>en az 2-3 ay önce</b> başlanmalı, tüm gebelik boyunca ve postpartum süreçte devam edilmelidir. (4-6 hafta boyunca ve emzirme devam ettiği müddetçe)
<b>B</b>	Sağlık sorunu var. (Epilepsi, insülin bağımlı diyabet) Beden Kütle İndeksi >35 kg/m <sup>2</sup> Ailede NTD öyküsü mevcut. Yüksek riskli etnik kökene sahip.	Folat içeriği yüksek bir diyetle ek olarak <b>5000 mcg/gün</b> <b>400-1000 mcg/gün*</b>	Konsepsiyondan 3 ay önce başlanmalı, ilk 12 hafta devam edilmelidir.  *12. haftadan sonra tüm gebelik boyunca ve postpartum süreçte devam edilmelidir.
<b>C</b>	Yoksul yaşam öyküsü, dengesiz ve değişken diyet, düzensiz doğum kontrolü, olası teratojenik madde kullanımı (alkol, tütün vb.)	Folat içeriği yüksek bir diyetle ek olarak <b>5000 mcg</b>	Daha uygun bir RBC folat düzeyi elde etmek için kullanılmalıdır.

Pakistan'da jinekoloji servisi tarafından takip edilen 209 kadın hasta ile bir araştırma yürütülmüştür. Kontrol grubunda yer alan kadınlar (n: 100) sağlıklı bebek dünyaya getirmiş, çalışma grubu ise (n: 209) NTD tanısı alan bebeği olan kadınlardır. Araştırma kapsamında bu bireylerin serum folat ve kırmızı kan hücreesindeki folat düzeylerine bakıldığı zaman kontrol grubunda yer alan kadınların her iki parametre düzeylerinin de çalışma grubunda yer alan kadınlara göre daha yüksek olduğu rapor edilmiştir (Nauman ve ark., 2018). Başka bir çalışmada gebelik öncesi 400 mcg/gün folik asit desteği kullanan kadınlara kırmızı kan hücreesindeki folat düzeyinin daha yüksek olduğu ve bu durumun NTD riskini azalttığı sonucuna ulaşılmıştır (Crider ve ark., 2014).

### ***Folik Asit ve Megaloblastik Anemi***

Megaloblastik anemi folat ve B<sub>12</sub> vitamin eksikliğinde gelişen, kemik iliğinde megaloblastik eritropoez olarak adlandırılan, morfolojik anormallikler ile karakterize makrositik anemi grubunda yer alan bir kansızlık türüdür (Wickramasinghe, 2006; Castellanos-Sinco ve ark., 2015). Tek karbon metabolizmasında enzim kofaktörü olarak gerekli olan folat, tek karbon ünitelerinden Ribo Nükleik Asit (RNA) ve DNA sentezi için gerekli olan nükleotidlerin oluşmasından sorumludur. Aynı zamanda folat homosisteinden metionin oluşumunda da görevlidir. Bu dönüşümün sonucunda meydana gelen S-adenosilmetionin'in (SAM) metil grupları DNA, hormonlar, proteinler, nörotransmitterler ve membran fosfolipdleri vb. pek çok fizyolojik süreçte yer alan bileşiklerin oluşumu için kullanılır. Dolayısıyla folat yetersizliği durumunda DNA replikasyonu, hücre bölünmesi olumsuz yönde etkilenmekte bu durum da kemik iliği vb. hızlı çoğalan dokuları etkileyerek normal olmayan çekirdeğe sahip, makrositik hücre üretilmesine neden olmaktadır. Bunun sonucunda megaloblastik anemi adı verilen önemli bir sağlık sorunu gelişmektedir (EFSA, 2017).

Dolayısıyla gebelerde anemi ve benzer diğer sağlık sorunlarına neden olabileceğinden çok uzun süredir folik asit desteği uygulamaları yapılmaktadır (Chandra, 2010).

### ***Folik Asit ve Postpartum Depresyon***

Postpartum depresyon, her 100 doğumdan 10-15'inin ardından gözlemlenen, gebelik sürecindeki depresif ruh hali ve yoğun stres, prenatal nörotisizm, düşük sosyoekonomik durum, sosyal açıdan yetersiz destek, doğuma bağlı komplikasyonlar ve preeklamsi gibi gebeliğe bağlı sağlık sorunları nedeni ile gelişen önemli bir psikolojik sorundur (Anderson ve Maes, 2013). Doğumu takip eden ilk birkaç yıl içerisinde gelişebilen, bebeğe zarar verme ve intihar ile

sonuçlanabilen postpartum depresyon tanısı en kısa süre içerisinde konulmalı ve tedavi sağlanmalıdır (Tezel, 2006). Folat, homosisteinden SAM üretilmesinden sorumlu olan bir vitamindir. SAM ise serotonin, dopamin ve nörepinefrin adı verilen nörotransmitterlerin sentezinden sorumludur. Folat yetersizliği durumunda plazma homosistein düzeyi yükselmektedir. Araştırmalar yüksek seyreden homosistein düzeyi ile postpartum depresyon arasında önemli bir ilişki olduğunu göstermektedir (Yan ve ark., 2017).

Toplam 6809 gebe kadının katıldığı prospektif bir çalışmada bireylerin tamamı folik asit desteği kullandıklarını bildirmişlerdir. Sonuç olarak folik asit desteğinin gebelik boyunca ve doğumdan sonraki ilk 8 ay içerisinde depresyon riski üzerinde etkili olmadığını, ancak doğumu takip eden 21. ayda depresyon skorunda azalma sağladığı rapor edilmiştir (Lewis ve ark., 2012). Singapur'da gebelik sürecinde bakıma gelen kadınlar üzerinde yapılan bir çalışmada (n: 1000) serum folat düzeyi ile postpartum depresyon arasında bir ilişki olmadığı sonucuna erişilmiştir (Chong ve ark., 2014). Öte yandan Çin'de doğumdan sonra ilk 6-12 hafta içerisinde yer alan ve gebelik boyunca folik asit desteği kullandığı bilinen 1592 kadın ile yürütülen çalışmada bireylerin %24.9'unda postpartum depresyon görüldüğü, postpartum depresyon prevalansının 6 aydan daha fazla folik asit desteği kullanan bireylerde, 6 ay ve daha az kullanan bireylere göre anlamlı derecede düşük bulunduğu bildirilmiştir (Yan ve ark., 2017).

### ***Folik Asit ve Doğum Ağırlığı***

Yeni doğan bebekler AGA (Gestasyonel yaşa uygun - Appropriate for gestational age), SGA (Gestasyonel yaşa göre küçük - Small for gestational age) ve LGA (Gestasyonel yaşa göre büyük - Large for gestational age) olmak üzere 3 grupta incelenmektedir. AGA, gebelik süresine göre gelişimi normal olan bebekleri, SGA, gebelik süresine göre düşük doğum ağırlıklı bebekleri ve LGA ise gebelik süresine göre yüksek doğum ağırlığına sahip olan bebekleri ifade etmektedir (Köksal ve Gökmen, 2013). Gebelik dönemi ve öncesi ile ilgili bazı risk faktörleri bebeğin düşük doğum ağırlıklı doğma olasılığını arttırmaktadır (Salcedo-Bellido ve ark., 2017).

Gebe kadında kan şekeri regülasyonu sağlanamayan tip I-II diyabet veya gestastasyonel diyabet, obezite veya kontrolsüz ağırlık artışı olması LGA riskini arttırmaktadır (Morrens ve ark., 2016).

**Tablo 3.** Düşük doğum ağırlığı ile ilişkili bazı risk faktörleri (Salcedo-Bellido ve ark., 2017)**Table 3.** Some of risk factors for low birth weight (Salcedo-Bellido et al., 2017)

Dönem	Risk faktörleri
<b>Prekonsepsiyon</b>	1. Sosyoekonomik durum 2. Kronik hastalık varlığı
<b>Gebelik</b>	1. Sigara kullanımı 2. Alkol kullanımı 3. Benzer diğer bağımlılıklar 4. Yetersiz besin ögesi alımı

Gebeliğin erken dönemlerinde kontrol amaçlı hastaneye başvuran 2644 kadın ile folik asit desteğinin doğum ağırlığı üzerine etkileri araştırılmıştır. Bireyler 4 grup halinde incelenmiştir. 1. Grup (n: 2188) 2. ve 3. trimesterler boyunca hiç folik asit desteği almayan, 2. Grup (n: 109) 2. trimesterde folik asit desteğine devam eden ancak 3. trimesterde destek kullanmayan, 3. Grup (n: 223) 2. trimesterde folik asit desteği almayan ancak 3. trimesterde yeniden başlayan ve 4. Grup (n: 131) tüm trimesterler boyunca folik asit desteği alan katılımcılardan meydana gelmektedir. Araştırma sonucunda düzenli olarak folik asit kullanan kadınların meydana getirdiği doğumlarda LGA prevalansı daha yüksek çıkmıştır. Öte yandan SGA prevalansını azaltıcı bir etki gözlemlenmemiştir (Wang ve ark., 2016). Meta-analizler sonucunda incelenen 9 çalışmada yer alan 707 katılımcı 12-22 hafta arasında 250-5000 mcg/gün folik asit desteği kullanmıştır. Birinci trimesterden sonra folik asit desteği kullanımının doğum ağırlığı üzerindeki etkileri incelendiği zaman çalışma gruplarının kontrol gruplarına kıyasla daha yüksek doğum ağırlıklı bebekler dünyaya getirdikleri ve folat alımındaki yaklaşık iki katlık bir artışın doğum ağırlığını %2 oranında arttırdığı saptanmıştır (Fekete ve ark., 2012).

#### **Folik Asit ve Çocukluk Çağı Alerjik Hastalıkları**

Çocukluk çağı alerjik hastalıklarının etiolojisinde genetik yatkınlıklar, çevresel koşullar ve bazı diyetel faktörler yer almaktadır. Folik asit DNA metilasyonu üzerinde etkili olan

metil grubu verici olarak görev almaktadır. Hayvanlar üzerinde yapılan birçok araştırmada folik asitin alerjik hastalıklarla ilişkili gen ekspresyonunu değiştirdiği gözlemlenmiştir (McStay ve ark., 2017).

Koreli 917 gebe kadın ve bebekleri ile yürütülen araştırmada gebeliğin 12-28 haftalarında serum folat düzeyi medyan değer üzerinde olan (>9.5 ng/mL) kadınların bebeklerinde 6. ayda solunum yolu enfeksiyon riski ve 12. ayda ise atopik dermatit gelişme riskinin daha düşük olduğu sonuçları elde edilmiştir. Öte yandan gebeliğin ileri dönemlerinde kullanılan folik asit desteği ile bebeklerde meydana gelen alerjik bulgular arasında bir ilişki bulunamamıştır (Ja Hye-ong ve ark., 2015). Roy ve ark. (2017) 858 anne ve bebeği ile meydana getirdikleri araştırmada bebeklerden alınan geri bildirimlerin 2. ve 3. trimesterde annenin serum folat düzeyleri ile ilişkili olmadığını bildirmişlerdir. Avustralya'da alerjik öyküsü olan 628 gebe kadının 3. trimesterdaki kan bulguları ve besin tüketimi üzerinden elde edilen sonuçlar 12 aylık bebeklerinin alerjik geri bildirimleri ile ilişkilendirilmiştir. Kadınların %25'inden daha azı önerilen folik asit gereksinmesini (600 mcg/gün) karşılayabilmektedir. Folik asit alımı <200 mcg/gün, 200-500 mcg/gün ve >500 mcg/gün olarak sınıflandırıldığı zaman yapılan analizler >500 mcg/gün folik asit alımına maruz kalan bebeklerde egzema gelişme olasılığının <200 mcg/gün alıma maruz kalanlara göre daha düşük olduğunu göstermektedir (Dunstan ve ark., 2012). Wang ve ark. (2015) tarafından rapor edilen, içerisinde 16 kohort, 7 vaka kontrol ve 3 kesitsel çalışmayı barındıran meta analiz sonuçlarına göre gebelikte folik asit desteği kullanımı astım, atopik dermatit ve egzema gelişimi ile ilişkili değilken, hışırtılı solunum ile ilişkilidir.

#### **Folik Asit ve Konjenital Kalp Hastalıkları**

Amerikan Kalp Birliği [American Heart Association (AHA)] en yaygın konjenital kalp defektlerini şu şekilde sıralamaktadır (*American Heart Association [AHA]*, 2018):

1. Aortik kapak darlığı,
2. Atrial septal defekt,
3. Aortun koarktasyonu,
4. Tam atrioventriküler kanal defekti,
5. Büyük arterlerin d-transpozisyonu,
6. Fallot tetralojisi,
7. Ebstein anomalisi,
8. Hipoplastik sol kalp sendromu,
9. Pulmoner atrezi,

10. Pulmoner kapakçık darlığı,
11. Tek ventrikül defekti,
12. Triküspid atrezi,
13. Truncus arteriosus ve
14. Ventriküler septal defekt vb.

Konjenital kalp hastalıkları (KKH) gebelik sırasında alkol kullanımı, yüksek lityum ve homosistein düzeyi ile ilişkilidir (Huhta ve Linask, 2015). Folat homosisteinden metionin oluşumundan ve dolayısıyla homosistein düzeyinin azalmasından sorumludur (EFSA, 2017). Yapılan birçok araştırma sonucunda yüksek doz folik asit kullanımının KKH riskini azaltıcı etkisi olduğunu göstermektedir (Huhta ve Linask, 2015).

Çin’de bir Kadın Doğum ve Çocuk Hastanesi’nde 2010-2012 yılları arasında 10.087 gebe kadının dahil edildiği bir araştırmanın sonucunda 94 kadının gerçekleştirmiş olduğu canlı doğumlar sonucunda konjenital kalp hastası bebek dünyaya getirdikleri saptanmıştır. Araştırma sonucunda uzun dönem folik asit desteği kullanımının konjenital kalp hastalıkları riskini azaltıcı bir etkisi olduğu bildirilmiştir (Baohong ve ark., 2017). Norveç’te Kardiyovasküler Hastalıklar Projesi kapsamında 1999-2009 doğum kayıtları ile konjenital kalp hastalıkları tanısı alan bireyler değerlendirilerek bir araştırma yürütülmüştür. Araştırmaya 517.784 katılımcı dahil edilmiştir. 6200 çocukta her hangi bir konjenital kalp hastalığı var iken, 1153 çocukta şiddetli konjenital kalp hastalığı bulunmaktadır. Bebekleri dünyaya getiren kadınların çok azı (%18,4) gebe kalmadan önce folik asit desteği kullanmaktayken, %31,6’sı gebe kaldığını öğrendiği zaman, %8,4’ü sadece konsepsiyon döneminde folik asit desteği kullanmıştır. %41,6’sı ise hiçbir zaman folik asit desteği kullanmamıştır. Araştırma sonucunda Norveç halkında gebelik sırasında ve öncesinde folik asit desteği kullanımını ile şiddetli konjenital kalp hastalıkları arasında önemli bir ilişki olmadığı saptanmıştır (Leirgul ve ark., 2015). Çin’de 2010-2011 yılları arasında vaka (n: 358) ve kontrol (n: 422) grubu olmak üzere toplam 780 katılımcı ile bir araştırma yürütülmüştür. Vaka grubunda yer alan kadınların %68,72’sinin gebelikte folik asit desteği kullandığı ve hiç folik asit desteği kullanmayan kadınların dünyaya getirdikleri bebeklerde konjenital kalp hastalıkları görülme riskinin arttığı sonucuna erişilmiştir. Aynı zamanda gebe kalmadan 3 ay ve 1 aydan daha kısa bir süre folik asit desteği kullanan kadınlar değerlendirilmiş ve KKH ile pre-konsepsiyonel folik asit kullanımı arasında anlamlı bir ilişki olmadığı görülmüştür. Özetle konjenital kalp hastalıklarının önlenmesinde folik asit desteğinin önemli bir role sahip

olduğu saptanmış ancak konsepsiyondan önceki kullanım süresinin etkili olmadığı görülmüştür (Li ve ark., 2013). Bir vaka kontrol çalışmasının sonuçlarına göre yüksek doz folik asit desteği (6 mg/gün) atrial septal defekt görülme riskini azaltmaktadır. Aynı zamanda 3 mg/gün folik asit desteğinin diğer KKH riskini azalttığı da bildirilmiştir (Czeizel ve ark., 2015).

### **Folik Asit ve Otizm**

Otizm bedensel bazı engellerin varlığı ile tanımlanan, bilişsel gelişim bozukluğuna neden olan bir hastalıktır. Üç yaşına kadar belirgin hale gelen iletişim kuramama, tekrarlayan davranışlar vb. semptomlar ile kendini göstermektedir (Schmidt ve ark., 2011). Folat metabolizmasında meydana gelen birkaç anormallik otizm ile yakından ilişkilidir. Metabolizmada yer alan metilentetrahidrofolat redüktaz ve dihidrofolat redüktaz enzimlerinde meydana gelen polimorfizmler sonucunda folat taşınması azalır. Dolayısıyla otizm tanısı alan çocuklarda çoğunlukla folat yetersizliği gözlemlenmektedir (Frye, 2014).

Folik asit ve otizm arasındaki ilişkiyi belirlemeye yönelik yapılan araştırmaların bir kısmı gebelik öncesinde ve süresince folik asit desteği kullanımının otizm riskini azalttığını vurgulamaktayken bir kısmı da yüksek dozda FA desteği kullanımının otizm riskini arttırdığını ve bilişsel gelişimi olumsuz yönde etkilediğini göstermektedir (Wiens ve ark., 2017).

İçlerinde 86 tanesinin otistik olduğu toplam 1257 anne ve çocuğu ile yürütülen bir araştırmada haftada 3-5 kez FA desteği kullanımının otizm riskini azalttığı,  $\leq 2$  ve  $> 5$  kez kullanımın ise otizm riskini arttırdığı saptanmıştır. Öte yandan gebelikte plazma folat düzeyinin  $\geq 60.3$  nmol/L olmasının otizm riskini 2.5 kat arttırdığı sonucuna ulaşılmıştır (Raghavan ve ark., 2017). Katılımcılar içerisindeki 114 çocuğun otistik olduğu bir diğer çalışmada annesi folik asit desteği kullanan çocukların %0,10’unda otizm görüldüğü, konsepsiyondan önceki 4-8 hafta boyunca FA desteği kullanımının otizm riskini azalttığı vurgulanmıştır. Prekonsepsiyon döneminde düşük plazma folat düzeyinin önemli bir otizm nedeni olduğu saptanmıştır (Suren ve ark., 2013). Otistik (n: 429), gelişim geriliği (n: 130) ve normal gelişim (n: 278) gösteren çocukların ve annelerinin dahil edildikleri araştırmanın sonucunda  $\geq 600$  mcg/gün folik asit alımının otizm gelişme riskini azalttığı ayrıca konsepsiyon öncesi folik asit desteği kullanımının otizm üzerinde olumlu etki gösterdiği sonucuna ulaşılmıştır (Schmidt, 2012). Geniş popülasyonlarda yapılan vaka kontrol çalışmaları sonucunda konsepsiyona yakın bir zaman dilimi içerisinde folik asit desteğinin otizm riskini %40 oranında azalttığı sonucuna varılmıştır (Lyll, 2014).

## Sonuç

Gebelikte hem maternal hem de fetal sağlığın korunması ve geliştirilmesi için artan besin ögesi gereksinmelerinin karşılanması gerekmektedir (Salcedo-Bellido ve ark., 2017). Bu nedenle DSÖ tarafından mümkünse gebelik öncesinde ve gebelik boyunca 400 mcg/gün folik asit desteği önerilmektedir (WHO, 2012).

Folik asit desteği kullanımının nöral tüp defekti ve megaloblastik anemi üzerinde olumlu etki gösterdiği yönünde azımsanmayacak sayıda çalışma mevcuttur. Öte yandan uzun dönem yüksek doz folik asit desteği kullanımının doğum ağırlığını arttırabildiği varsayılmaktadır. Folik asit desteği ve postpartum depresyon, konjenital kalp hastalıkları, alerjik hastalıklar ve otizm üzerinde yapılan araştırmalar ise çift yönlüdür.

Sonuç olarak; folat yetersizliğinde görülen sağlık sorunları toplumda büyük önem taşımaktadır. Folat yetersizliğinin önlenmesi için folattan zengin besinlerin tüketilmesi, besin hazırlama ve pişirme yöntemlerine dikkat edilmesi, ekmelek unun folik asitle zenginleştirilmesi, planlı gebeliklerin sağlanması ve gebelere gebelik öncesinden başlayarak folik asit desteğinin verilmesi (400 mcg/gün), halkın beslenme konusunda eğitilmesi ve bilinç düzeyinin artırılması gerekmektedir. Güncel yaklaşım olarak demir ve folik asit desteğinin multivitamin ve mineral desteği içerisinde sağlanması üzerinde durulmaktadır (Silva Lopes ve ark., 2017; WHO, UNICEF ve MI, 2015). Bu durumun da hekimler tarafından değerlendirilmesi gerekmektedir.

## Etik Standart ile Uyumluluk

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

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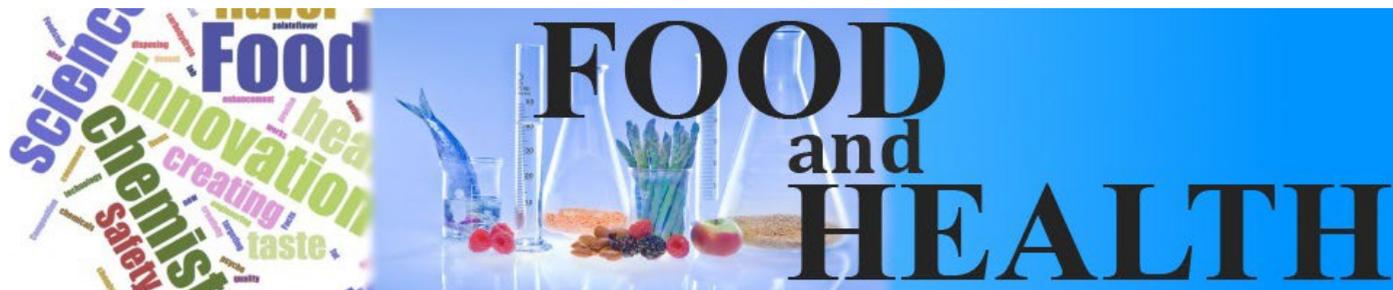
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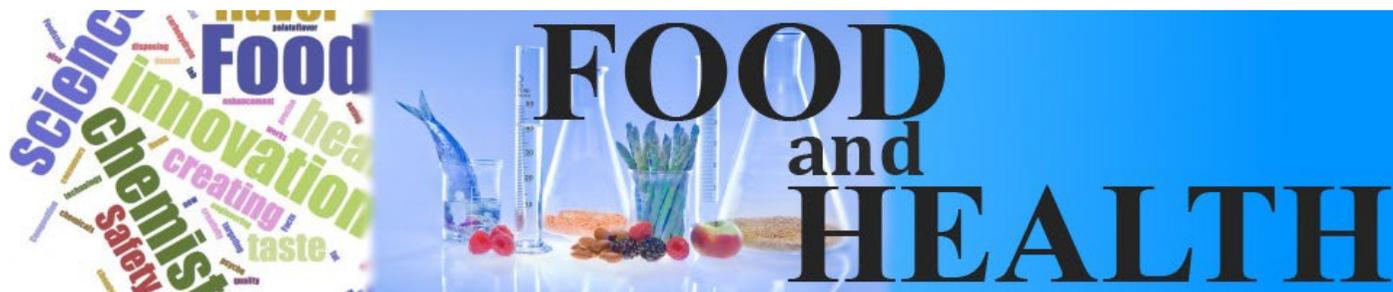
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**ORCID ID for all author (s)** (<http://orcid.org/>)

**Address (es) of affiliations and e-mail (s)**

**Complete correspondence address and e-mail**

**Abstract**

**Key words (indexing terms), normally 3-6 items**

**Introduction**

**Material and Methods**

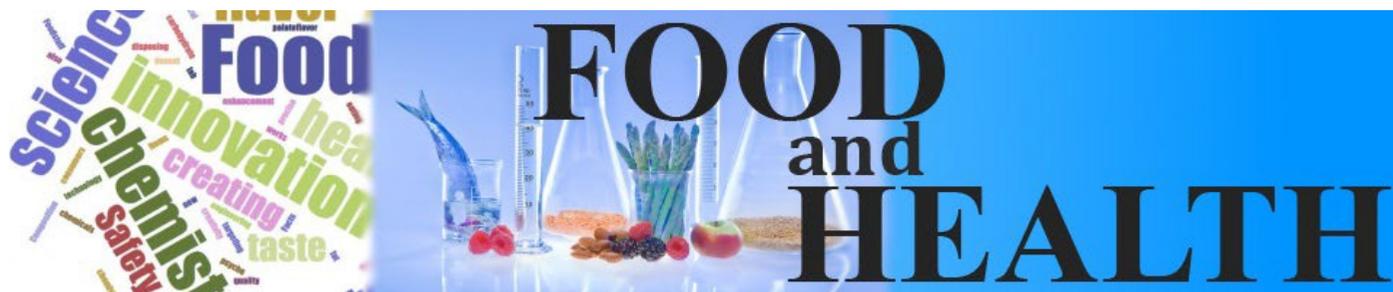
**Results and Discussion**

**Conclusion**

**Compliance with Ethical Standard**

**Conflict of interests:** 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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#### References

#### Tables

#### Figures

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**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", "Result and Discussion" and Conclusion sections.

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**Short Communication:** This type of manuscript discusses important parts, overlooked aspects, or lacking parts of a previously published article. Articles on subjects within the scope of the journal that might attract the readers' attention, particularly educative cases, may also be submitted in the form of a "Short Communication" Readers can also present their comments on the published manuscripts in the form of a "Short Communication". The main text should contain Introduction, "Materials and Methods", "Result and Discussion" and Conclusion sections.

**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

### Tables

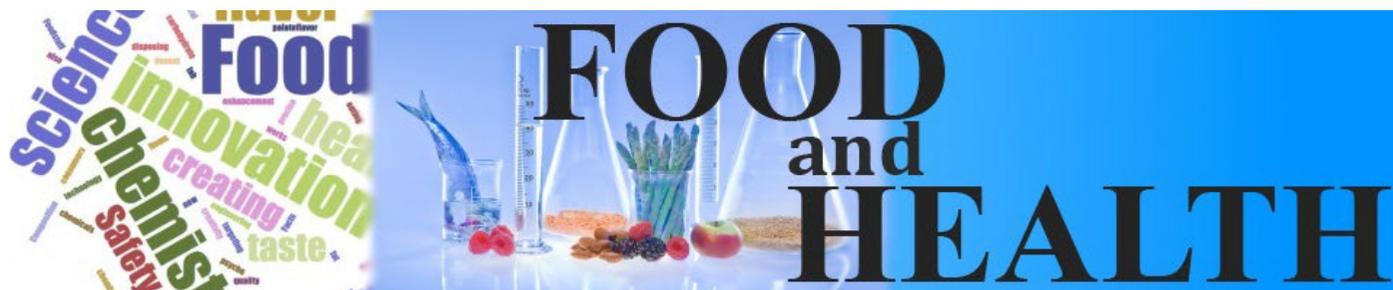
Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

### Figures and Figure Legends

Figures, graphics, and photographs should be submitted as separate files (in TIFF or JPEG format) through the submission system. The files should not be embedded in a Word document or the main document. When there are figure subunits, the subunits should not be merged to form a single image. Each subunit should be submitted separately through the submission system. Images should not be labelled (a, b, c, etc.) to indicate figure subunits. Thick and thin arrows, arrowheads, stars, asterisks, and similar marks can be used on the images to support figure legends. Like the rest of the submission, the figures too should be blind. Any information within the images that may indicate an individual or institution should be blinded. The minimum resolution of each submitted figure should be 300 DPI. To prevent delays in the evaluation process, all submitted figures should be clear in resolution and large (minimum dimensions: 100 × 100 mm). Figure legends should be listed at the end of the main document.

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

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



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

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

## References

### Reference System is APA 6<sup>th</sup> Edition

#### In-text Citation with APA

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

....(Crockatt, 1995).

#### Direct quote from the text

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

#### Major Citations for a Reference List in Table 2.

Table 2.

Material Type	Reference List/Bibliography
<b>A book in print</b>	Baxter, C. (1997). <i>Race equality in health care and education</i> . Philadelphia: Ballière Tindall, p. 110-115, ISBN 4546465465
<b>A book chapter, print version</b>	Haybron, D.M. (2008). Philosophy and the science of subjective well-being. In M. Eid & R. J. Larsen (Eds.), <i>The science of subjective well-being</i> (p. 17-43). New York, NY: Guilford Press. ISBN 4546469999
<b>An eBook</b>	Millbower, L. (2003). <i>Show biz training: Fun and effective business training techniques from the worlds of stage, screen, and song</i> . p. 92-90. Retrieved from <a href="http://www.amacombooks.org/">http://www.amacombooks.org/</a> (accessed 10.10.15)
<b>An article in a print journal</b>	Carter, S. & Dunbar-Odom, D. (2009). The converging literacies center: An integrated model for writing programs. <i>Kairos: A Journal of Rhetoric, Technology, and Pedagogy</i> , 14(1), 38-48.
<b>Preview article in a journal with DOI</b>	Gaudio, J.L. & Snowdon, C. T. (2008). Spatial cues more salient than color cues in cotton-top tamarins ( <i>Saguinus oedipus</i> ) reversal learning. <i>Journal of Comparative Psychology</i> , doi: 10.1037/0735-7036.122.4.441
<b>Websites - professional or personal sites</b>	<i>The World Famous Hot Dog Site</i> . (1999, July 7). Retrieved January 5, 2008, from <a href="http://www.xroads.com/~tcs/hotdog/hotdog.html">http://www.xroads.com/~tcs/hotdog/hotdog.html</a> (accessed 10.10.15)
<b>Websites - online government publications</b>	U.S. Department of Justice. (2006, September 10). Trends in violent victimization by age, 1973-2005. Retrieved from <a href="http://www.ojp.usdoj.gov/bjs/glance/vage.htm">http://www.ojp.usdoj.gov/bjs/glance/vage.htm</a> (accessed 10.10.15)
<b>Photograph (from book, magazine or webpage)</b>	Close, C. (2002). <i>Ronald</i> . [photograph]. Museum of Modern Art, New York, NY. Retrieved from <a href="http://www.moma.org/collection/object.php?object_id=108890">http://www.moma.org/collection/object.php?object_id=108890</a> (accessed 10.10.15)
<b>Artwork - from library database</b>	Clark, L. (c.a. 1960's). <i>Man with Baby</i> . [photograph]. George Eastman House, Rochester, NY. Retrieved from ARTstor
<b>Artwork - from website</b>	Close, C. (2002). <i>Ronald</i> . [photograph]. Museum of Modern Art, New York. Retrieved from <a href="http://www.moma.org/collection/browse_results.php?object_id=108890">http://www.moma.org/collection/browse_results.php?object_id=108890</a> (accessed 10.10.15)

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

## REVISIONS

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

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