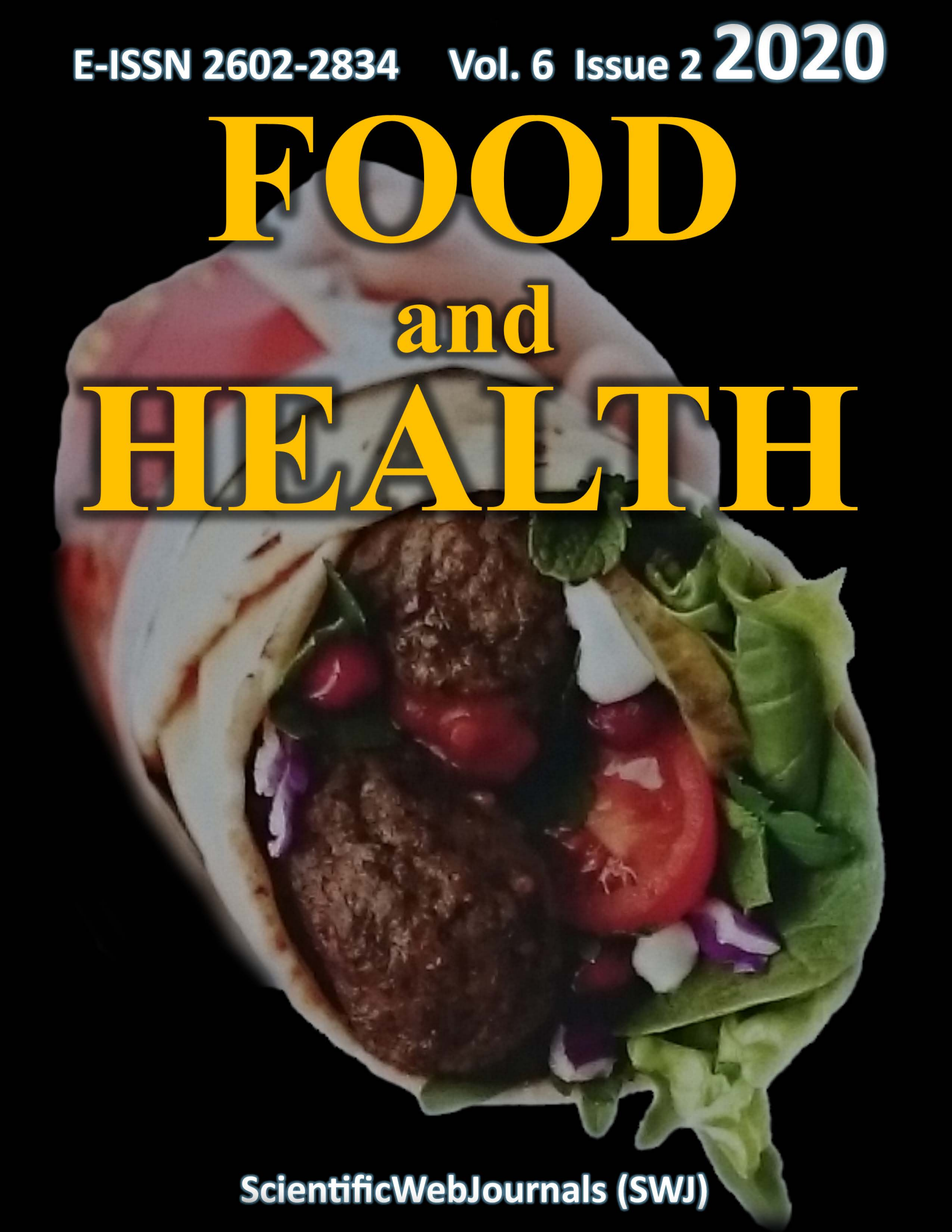
E-ISSN 2602-2834 Vol. 6 Issue 2 2020

FOOD and HEALTH



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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Publisher

Adress: Abdi Bey Sok. Kentplus Kadıköy Sitesi No:24B D. 435 Kadıköy/Istanbul, Türkiye
E-Mail: swj@scientificwebjournals.com

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Aims and Scope

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Abbreviation: **FOOD HEALTH**

e-ISSN: 2602-2834

Journal published in one volume of four issues per year by

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

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REVIEW ARTICLE

FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 6(1), 67-76 (2020) • https://doi.org/10.3153/FH20008 Research Article

Influence of heating on chemical composition, antioxidant activity and protein quality of an advanced line *Amaranthus cruentus* L. seed flour

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Cite this article as:
Razzeto, G.S., Rojas Moreno, J.E., Aguilar, E.G., Peiretti, E.G., Lucero López, V.R., Albarracín G. de J., Escudero, N.L. (2020). Influence of heating on chemical composition, antioxidant activity and protein quality of an advanced line *Amaranthus cruentus* L. Seed flour. *Food and Health,* 6(2), 67-76. https://doi.org/10.3153/FH20008

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Submitted: 16.09.2019
Revision requested: 24.10.2019
Last revision received: 10.11.2019
Accepted: 17.11.2019
Published online: 04.01.2020

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ABSTRACT

Amaranth is a pseudocereal of Andean origin, and compared to other crops, its seeds have a higher content of proteins, lipids and bioactive compounds of nutraceutical relevance.

The goal of the present work is to study the chemical composition, antioxidant activity and biological value of the protein of an advance line *Amaranthus cruentus* L. seed flour (ACRU), compared with the same flour subjected to thermal treatment (90 ºC, 1 h). Regarding the proximal chemical composition, the protein and lipid contents stand out, reaching values of 19.59 g % and 7.47 g %, reflecting an increase of 17% and 50% in the treated sample, respectively. A significant increase ($p<0.05$) is observed in the ash content, as well as in the content of the main elements of nutritional interest, of the treated samples. The anti-nutrients values are within the acceptable limits in all samples, and present an adequate content of total phenols, with an antioxidant activity highlighted by its free-radical scavenging capacity. In the biological tests, the Net Protein Utilization (NPU) presents lower values for the treated samples, the True Digestibility (tD) does not show significant differences, and the Biological Value (BV) turns out to be lower in the treated sample ($p<0.05$). A significant hypotriglyceridemic effect is observed. The applied thermal treatment, even though increases the nutrients concentration and the total phenols, according to the biological tests, it decreases the protein quality. These are aspects that should be contemplated in the food technology to optimize the nutritional quality of this amaranth.

Keywords: Amaranth, Antioxidant capacity, Biological value, Chemical composition, Thermal treatment

Introduction

Amaranth is an indigenous plant from America that has been used for more than 4000 years. In the last decades, it has regained popularity due to beneficial nutritional aspects, its agronomic advantages, such as its wide adaptability, and the possibility of its use as horticultural, graniferous and fodder, as well as for being one of the most promising resources to contribute towards mitigating food deficit (Dodok, 1997).

The amaranth nutritional composition is distinguished by the protein and lipid contribution. The protein content is between 12 to 22% (Tosi et al. 2001; Escudero et al. 2004; Barba de la Rosa et al. 2009). The proteins quality depends on the composition of essential amino acids and digestibility. Protein digestibility, lysine availability and net protein utilization of the amaranth proteins are higher than of cereals, and similar to those of casein (Salcedo-Chávez et al. 2002). The constituent amino acids of food proteins are not always fully available, due to that the protein digestibility and amino acid adsorption can be incomplete. Thus, the thermal treatment and milling applied to the seeds for the production of flour can raise the foods nutritional quality by the denaturalization of its proteins and the digestibility increase (Giami et al. 2001; Sun et al. 2014).

In order to expand the biodiversity and increase the agronomical efficiency of crops in the Central - West region of Argentina, new improved varieties have been obtained, such as the Acru-G10/13II, the advanced line belonging to the *Amaranthus cruentus* L. species. The latter is characterized for expressing good yields and excellent adaptation; it presents dark green foliage, semi-compact to compact dark red panicle, and a height between 1.40 and 1.70 m. It has a total cycle of 115 days and an acceptable behavior against "stem borer" (*Conotrachelus spp.*). The goal of this study is to evaluate the effect of thermal treatment on the chemical composition, antioxidant activity and biological quality of a new amaranth variety seed flour.

Materials and Methods

Sample

Seeds of a new variety of *Amaranthus* (Acru-G10/13 II) were supplied by the Faculty of Agronomy and Veterinary, National University of Río Cuarto, Cordoba, Argentina (experimental crop from 2016 vintage).

Sample Treatment

Dried seeds were ground in a grain mill and sieved through a 200 µm diameter mesh. A flour portion was used untreated (ACRU) and another was exposed to 90ºC for 1 hour in an air-current oven (Dalvo HER/F/I, Argentina) (ACRU treated). The flours obtained were kept in closed containers protected from light, in a cool and dry environment until analysis.

Reagents

All reagents used were of analytical grade and acquired from Sigma (St. Louis, MO). All standard solutions were prepared using reagents of spectroscopic grade supplied by Merck (Darmstadt, Germany). Ultrapure water (18.2 MΩ cm) was used to prepare all standard and sample solutions.

Chemical Composition

The determination of moisture, ash, protein, total lipids and crude fiber was performed according to the methodology proposed by the AOAC (2012). Carbohydrates are determined by the following calculation:

Carbohydrates = 100 – (% Ash) – (% Total Fat) – (% Moisture) – (% Protein)

The quantification of mineral elements was performed by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-OES). The procedure was performed following the methodology used by Aguilar et al. (2011).

The antinutrients determined were: nitrates (Cataldo et al. 1975), hemagglutinin activity (lectins) (Das Gupta and Boroff 1968; Do Prado et al. 1980), saponins (WHO/PHARM/92559 1992; Duarte Correa and Carlsson, 1986), antitrypsin activity (Kakade et al. 1974), oxalic acid (AOAC 1995) and phytic acid (Rucci and Bertoni, 1974).

The extraction of total phenols was performed from a defatted sample with 1.2 M HCl in 50% methanol/water. The sample was heated at 90 °C for 3 h, cooled, and then diluted with methanol. The supernatant was used for the determination of total phenols and antioxidant activity (Vinson et al. 2001). The concentration of the obtained extract was 5 mg/mL. The determination of total phenols was performed using Folin Ciocalteu reagent with gallic acid as a standard. The absorbance was measured at 750 nm (UV–vis BeckmanDK-2ª). Results were expressed as mg/100 g of dry weight of gallic acid equivalent (Emmons et al. 2001).

Antioxidant Activity

DPPH Free Radical-Scavenging Assay. This spectrophotometric assay uses the stable radical DPPH (1,1-diphenyl-2-picrylhydrazyl) as a reagent (Burits and Bucar, 2000). Various concentrations of the extract in methanol were added to a 40 mg/L methanol solution of DPPH. The absorbance was read at 517 nm (UV-Vis Beckman DK-2a). Results were expressed as percentage (%) of radical scavenging activity (RSA).

Scavenging Activity against Nitric Oxide (NO Test). Nitric oxide (NO) was generated from sodium nitroprusside and measured by Griess reaction (Marcocci et al. 1994; Saija et al. 1999). Nitrite concentration was calculated by referring to the absorbance of standard solutions of sodium nitrite. Absorbance was measured in a spectrophotometer (UV-Vis Beckman DK-2a) at 542 nm. Results were expressed as percentage (%) of RSA with respect to blank.

β-Carotene–Linoleic Acid Assay

This assay involves measuring *β*-carotene bleaching, at 470 nm, resulting from the *β*-carotene oxidation by linoleic acid degradation products at 50 ºC (Koleva et al., 2002). The absorbance at 470 nm was taken at time zero ($t = 0$), and measured every 15 min until the color of β-carotene disappeared in the control tubes ($t = 60$ min). A mixture prepared as above but without β-carotene served as blank. BHT (butylated hydroxytoluene) was included in the experiments as a positive control. Results were expressed as percentage (%) of RSA.

All determinations above were performed in triplicate.

Biological Assay

The Pprotein quality of the amaranths flour was measured by three different indices: Net Protein Utilization (NPU), true digestibility (tD), and Biological Value (BV) (Miller and Bender, 1955; Pellet and Young, 1980). Four groups of 30-day-old *Wistar* rats weighing 30–40 g (± 2 g weight difference) were used (six animals per group). One group received a protein-free diet, another received a control diet (casein), and the remaining groups received a diet with protein provided by the material under study. The preparation and composition of the diets were carried out according to AIN 93G (Table 1) and the NPU method at 10% of proteins and 7% of lipids (Reeves et al. 1993). The amount of flour incorporated in the diets to achieve 10 % of protein, was defined taking into account the protein content of the sources, ACRU: 19.59 % and ACRU treated: 23.54 %. Casein: 80 % purity. The amount of oil added to achieve 7 % of lipids, was defined taking into account the fat content obtained for the sources, ACRU: 7.47 % and ACRU treated: 14.70 %.

The animals were kept in individual suspended cages with screen bottoms. Temperature and relative humidity were held at 21 ±1 ºC and 60%, respectively. Lighting was controlled by alternating 12-h periods of light and darkness. All animals received potable water and food *ad libitum* for 14 days. Ingestion was recorded on days 3, 6, and 10; weight gain was recorded at the end of the experiment. Feces were collected and weighed. After the experiment, the euthanasia of the animals was performed through a carbon dioxide chamber. Subsequently, a thoracic and abdominal incision was performed, and the rats were weighed and placed in a forced air oven at 100 – 105 ºC for 48 hours to determine the body water by weight difference. We followed the general guidelines for the care and use of laboratory animals recommended by the Animal Care Committee of the National University of San Luis.

The NPU is defined as the portion of nitrogen intake that is retained. The formula used was

$$\text{NPU} = \frac{B - (B\text{K} - I\text{K})}{I} \times 100$$

where B is the corporal nitrogen of the experimental group; BK is the corporal nitrogen of the group on the protein-free diet; IK is the nitrogen intake of the group on the protein-free diet; and I is the nitrogen intake in the experimental group. Corporal nitrogen (N) was calculated by using the following equation:

$$Y = 2.92 + 0.02X \quad (1)$$

where X is the rats age in days, and Y is calculated as

$$Y = \frac{\text{N (g)} \times 100}{\text{H}_2\text{O (g)}} \quad (2)$$

By equating Eqs. (1) and (2), N is calculated as

$$\text{N (g)} = \frac{\text{H}_2\text{O}\,(2.92 + 0.02X)}{100}$$

tD was determined along with NPU, and was considered as the absorbed nitrogen with respect to the N intake. Unabsorbed nitrogen was calculated by quantification of the fecal nitrogen in the group fed with the protein-free diet. The formula used was

$$\text{tD} = \frac{I - (F - FK)}{I} \times 100$$

where I is the ingested nitrogen; F is the fecal nitrogen in the group that received the experimental diet; and FK is the fecal nitrogen of the group consuming the protein-free diet.

The biological value (BV) was calculated as the NPU/tD ratio.

Table 1. Composition of diets AIN 93-G

Nutrients (g/kg)	Protein free	Casein	ACRU	ACRU Treated
Cornstarch	397.48	397.48	85.16	164.16
Protein[a]	0	125.00	510.50	424.71
Soybean oil[b]	70.00	70.00	31.86	7.56
Fiber	50.00	50.00	20.08	29.23
Sucrose	100.00	100.00	100.00	100.00
Mineral mix	35.00	35.00	35.00	35.00
Vitamin mix	10.00	10.00	10.00	10.00
Choline bitartrate	2.50	2.50	2.50	2.50
L-Cystine	0	3.00	0	0
Dextrinized cornstarch	335.00	207.00	284.87	226.80
Tert-butylhydroquinone	0.014	0.014	0.014	0.014

[a] The amount of flour incorporated in the diets to achieve 10 % of protein was defined taking into account the protein content of the sources, ACRU: 19.59 % and ACRU treated: 23.54 %. Casein: 80 % purity.

[b] The amount of oil added to achieve 7 % of lipids, was defined taking into account the fat content obtained for the sources, ACRU: 7.47 % and ACRU treated: 14.70 %.

Blood Analysis

Glucose, total cholesterol and triglycerides, were determined by enzymatic methods using commercial kits.

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Statistical Analysis

Results are expressed as mean ± standard deviation. Statistical differences were tested by the Student's *t*-test and ANOVA. Probabilities of 0.05 or less indicate significant difference (Snedecor and Cochran 1991).

Results and Discussion

The present work studied the effect of thermal treatment on the proximal chemical composition, elemental profile, antioxidant activity and biological value of the protein of a new *Amaranthus cruentus* L. variety seed flour, as well as the effect of its intake on some serum parameters.

Table 2 shows the proximal chemical composition, where the protein and lipid contents stand out, reaching values of 19.59 and 7.47 g/100 g, respectively, in ACRU. These values are higher than the informed by Bressani (2003) for proteins, and similar in the case of lipids. In ACRU treated, these results increase significantly in 17% and 50%, respectively.

Regarding the elemental profile (Table 3), in general, the results obtained are increased in the treated sample, in accordance with the ash value informed. Among the main elements of nutritional interest (expressed in µg/g of flour), it is highlighted the presence of Ca: 1592.15 and 1974.25, Fe: 85.50 and 106.02, Na: 49.00 and 60.76, S: 7.71 and 9.56, and Cu: 6.49 and 8.03, for ACRU and ACRU treated, respectively, and a high content of P and K in both samples. The concentrations of these minerals is similar to the informed by Nascimento et al. (2014), and do not exceed the maximum tolerable levels. The Ca contribution of the studied flours could participate in the prevention of osteopenia and osteoporosis, which frequently affect coeliacs patients. No presence of toxic mineral elements, such as As and Cd, is detected, while Pb and Cr slightly exceed the limit allowed by the FAO/WHO (2015) (0.20 ppm for Pb and 0.10 ppm for Cr).

Form the investigated anti-nutrients (Table 4), it is observed that nitrates increase significantly (35%) in the treated sample; however, both values obtained (206.44 and 316.27 mg/100 g) are within the acceptable range. The Joint FAO/WHO Expert Committee has determined as Acceptable Daily Intake (ADI) of nitrates a value of 0-3.7 mg/kg of body weight (FAO/WHO 2002). Regarding the hemagglutinin activity, it is observed that they are concordant with the obtained by Escudero et al. (2004) for grain amaranths, and do not affect health. No presence of saponins is observed. A significant decrease is observed in the antitryptsin activity in

ACRU treated, due to that these proteases inhibitors are thermolabile. The results obtained for ACRU and ACRU treated, 3.49 and 2.86 TIU/mg, respectively, are similar to the reported for seeds of other amaranth species (Bressani, 1994). The values informed are close to the levels considered as safe (5 TIU/mg of sample). The concentration of oxalic acid in ACRU is 156.00 mg/100 g, decreasing in 8% for ACRU treated, value that is similar to regular consumption cereals. Considering that the Hendek Ertop and Bektaş (2015) recommends patients with kidney stones an oxalate-restricted diet with values below 40 to 50 mg per day, the daily intake of ACRU should be reduced. The phytic acid content was of 0.36 and 1.38 mg P/100g in ACRU and ACRU treated, respectively, lower than the informed for amaranth (82 mg/100 g) by Ferreira and Arêas (2010).

The amaranth grain contributes with natural antioxidants that play an import role in the inhibition of free radicals, preventing oxidative deterioration. The values obtained for

total phenols were 16.23-39.23 mg gallic acid / 100 g for ACRU and ACRU treated, respectively (Table 5), similar to the informed by Repo de Carrasco and Encina Zelada (2008) for six varieties of *Amaranthus caudatus*. The significant increase of phenols in the treated sample would be a consequence of the release of these compounds by the action of the thermal treatment. The evaluated antioxidant activity does not present significant differences between both samples, with varying ranges for % Inhibition for DPPH: 88.84-89.51; NO: 65.73-72.21; and β-carotene: 25.03-26.65. It is noted the capacity of free radical scavenging given by the DPPH inhibition percentage. It is interesting the high percentage obtained for the NO Test, considering that NO produced in excess interacts with oxygen forming nitrites that transform into harmful peroxides; Czerwinski et al. (2004) inform lower values that vary between 23.00 and 25.10 % NO inhibition. The bleaching percentage of β-carotene, as a measurement of the lipid peroxidation inhibition, is relatively low and in agreement with the informed for *Amaranthus hypochondriacus* by the same author.

Regarding the evaluation of the biological quality of the studied samples (Table 6), it is observed that the diets consumption does no present significant differences between the experimental diets, and neither with respect to casein taken as reference. However, the weight gain decreases significantly (87%) with respect to the animal protein; these results can be attributed to the characteristics that distinguish a vegetable protein. The feces weight presents a significant increase with respect to casein, probably due to the fiber present in the vegetable diet. In the biological tests, NPU presents lower values for the studied samples with respect to casein, being lower for the treated sample. The tD does not show significant differences between the experimental samples, so it can be inferred that it is not affected by the thermal treatment. The BV results are lower for the treated sample, indicating that this treatment decreases the protein quality, and consequently, its utilization for protein synthesis.

Table 2. Chemical composition of the new variety in the dry weight of Acru-G10/13 II and Acru-G10/13 II treated seed flours

Determination (g/100g)	**ACRU**	**ACRU treated**
Moisture	7.28 ± 0.15^{a}	$1.13 \pm 0,01^{b}$
Ash	$4.46 \pm 0,16^{a}$	$5.53 \pm 0,04^{b}$
Protein (N x 6.25)	$19.59 \pm 0,13^{a}$	$23.54 \pm 0,15^{b}$
Total lipids	7.47 ± 0.18^{a}	14.70 ± 0.12^{b}
Carbohydrates*	61.20 ± 0.43^{a}	55.10 ± 0.30^{b}
Crude fiber	5.86 ± 0.14^{a}	5.91 ± 0.06^{a}

Values are mean ± standard deviation of three measurements. Different letters indicate significant differences ($p<0.05$).
*Carbohydrates are determined by the following calculation:
Carbohydrates = 100 – (% Ash) – (% Total Fat) – (% Moisture) – (% Protein)

Table 3. Concentration of 21 elements analyzed in the dry weight of Acru-G10/13 II and Acru-G10/13 II treated seed flours (µg/g)

Element	ACRU	ACRU treated
As	ND	ND
Ca	1592.15 ± 75[a]	1974.25 ± 83[b]
Cd	ND	ND
Co	0.11 ± 0.005[a]	0.14 ± 0.008[b]
Cu	6.49 ± 0.03[a]	8.03 ± 0.06[b]
Cr	0.16 ± 0.007[a]	0.18 ± 0.009[b]
Fe	85.50 ± 5.70[a]	106.02 ± 7.50[b]
K*	>10	>10
Hg	ND	ND
Li	0.98 ± 0.06[a]	1.21 ± 0.04[b]
Mg*	>10	>10
Mn	37.47 ± 2.40[a]	46.46 ± 3.35[b]
Mo	0.53 ± 0.06[a]	0.64 ± 0,07[a]
Na	49.00 ± 2.78[a]	60.76 ± 4.25[b]
Ni	0.37 ± 0.001[a]	0.46 ± 0.002[b]
P*	>10000	>10000
S	7.71 ± 0.50[a]	9.56 ± 0.45[b]
I	ND	ND
Pb	1.15 ± 0.009[a]	1.43± 0.01[b]
Se	ND	ND
Zn	32.33 ± 1.87[a]	40.09 ± 2.25[b]

Values are mean ± standard deviation of three measurements. Different letters indicate significant differences ($p<0.05$).
*Exceeds the upper quantification limit. ND: not detected

Table 4. Anti-nutrient factors in the dry weight of Acru-G10/13 II y Acru-G10/13 II treated seed flour

Anti-nutrient factors	ACRU	ACRU treated
Nitrates (mg/100 g)	206.44 ± 17.67[a]	316.27 ± 32.19[b]
Hemagglutinin activity	1/64	1/16
Hemolytic activity	ND	ND
Foam index*	<100	<100
Antitrypsin activity (TIU/mg sample)#	3.49 ± 0.30[a]	2.86 ± 0.10[b]
Oxalic acid (mg/100 g)	156.00 ± 4.04[a]	144.32 ± 3.90[b]
Phytic acid (mg P/100 g)	0.36 ± 0.01[a]	1.38 ± 0.09[b]

Values are mean ± standard deviation of three measurements. Different letters indicate significant differences ($p<0.05$)
ND: Not detected.
*1000/a; a = mL of filtrate in the tube that reached, when no tube exhibited 1 cm of foam, foam index <100.
#Trypsin inhibited units per mg of flour.

Table 5. Total phenols content and antioxidant activity in the dry weight of Acru-G10/13 II and Acru-G10/13 II treated seed flours

	ACRU	ACRU treated
Total phenols (mg gallic acid /100 g)	16.23 ± 0.10[a]	39.23 ± 0.99[b]
DPPH inhibition (%)	89.51 ± 1,66[a]	88.84 ± 2.14[a]
NO inhibition (%)	65.73 ± 1.63[a]	72.21 ± 2.58[a]
β-carotene inhibition (%)	25.03 ± 1.89[a]	26.65 ± 0.97[a]

Values are mean ± standard deviation of three measurements.
Different letters indicate significant differences ($p<0.05$)

Table 6. Biological quality from seed flour of Acru-G10/13 II y Acru-G10/13 II treated

	Casein	ACRU	ACRU treated
Intake (g)	74.08±10.94[a]	60.70±7.17[a]	66.90±7.37[a]
Weight gain (g)	17.70±2.38[a]	2.75±0.75[b]	1.83±0.15[b]
Feces weight (g)	5.51±0.46[a]	8.44±1.96[b]	7.44±0.85[b]
NPU	63.21±6.85[a]	45.39±5.23[b]	33.99±3.22[c]
tD	96.23±2.19[a]	83.90±4.32[b]	86.33±4.60[b]
BV	65.69±5.69[a]	54.10±4.53[a]	39.37±3.20[b]

The results are expressed as mean ± standard deviation
Different letters indicate significant differences ($p<0.05$)
NPU: Net protein utilization, tD: true digestibility, BV: Biological value

Table 7. Effect of ACRU-G10/13 II and ACRU-G10/13 II treated seed flours on biochemical variables

Biochemical variables (mg/dL)	Casein	ACRU	ACRU treated
Glucose	53.25 ± 11.70[a]	62.40 ± 8.88[a]	77.66 ± 21.08[a]
Total Cholesterol	53.40 ± 11.33[a]	39.60 ± 12.34[a]	38.00 ± 5.05[a]
Triglycerides	131.40 ± 33.28[a]	89.40 ± 6.58[b]	70.20 ± 18.40[b]

The results are expressed as mean ± standard deviation
Different letters indicate significant differences ($p<0.05$)

The evaluated serum parameters (Table 7) indicate that the triglycerides level decreases significantly in the experimental samples, with a tendency in the decrease of total cholesterol with respect to casein, values similar to the reported by Escudero et al. (2006). This could be explained by the presence of dietary fiber and the prevalence of unsaturated fatty acids in the vegetal diet.

Conclusions

The obtained results allow to conclude that the thermal treatment used (90°C for 1h) affects the content of nutrients. It improves the protein and lipid contents, however, the biological study indicates that the thermal treatment decreases the protein quality when its biological value and net protein utilization are affected. The content of minerals of nutritional importance increases in the treated sample, highlighting the presence of Ca, Fe and P, without observing toxic elements. The antinutrients studied are within ranges that do not affect health, suggesting the potential consumption of this variety without apparent toxicity risk. The concentration of total phenols indicates that the amaranth grain contributes with antioxidants that play an important role in free-radical scavenging, preventing oxidative deterioration; the sample subjected to thermal treatment increases its phenol content. On the other hand, a beneficial effect of the amaranths grains is observed by producing a hypotriglyceridemic effect. However, and despite that, in general, the thermal treatment improves some of the evaluated parameters, it decreases the protein quality. This is an aspect that should be contemplated for performing future studies, subjecting that sample to different types of thermal treatments that do not affect the biological quality of the protein. This also becomes a challenge for the food technology, with the goal of optimizing the nutritional quality of this amaranth.

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.

Acknowledgements: The authors thank Dr. Eloy Salinas for his contribution in the serum determinations.

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 6(2), 77-89 (2020) • https://doi.org/10.3153/FH20009 Research Article

Food safety knowledge of food handlers working in hotel kitchens in Turkey

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Cite this article as:
Tuncer, A., Akoğlu, A. (2020). Food safety knowledge of food handlers working in hotel kitchens in Turkey. *Food and Health,* 6(2),77-89.
https://doi.org/10.3153/FH20009

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ABSTRACT

This study was aimed to examine the food safety knowledge of food handlers in hotels' kitchen, to determine existing knowledge gaps in food safety, and to examine relationship between food safety knowledge and some sample characteristics such as gender, age, education level, professional experience, and past attendance to food safety training course. A total of 378 food handlers working in hotel kitchens, located at six different cities in Turkey, participated in the cross-sectional study. The food safety knowledge score of participants was average with 53.70%. Knowledge scores related the different food safety aspects including personal hygiene (53.60%), food hygiene (53.91%), cross contamination (61.13%), health problems that would affect food safety (52.14%), symptoms of foodborne illnesses (52.00%), HACCP (51.00%) and food allergy (50.89%) were found average level. The most striking result of this study is that although the number of employees receiving food safety training is considerably high (82.3%), the food safety knowledge score was found less than expected. When viewed from this aspect, this work is remarkable about examining into content and adequacy of food safety training in Turkey.

Keywords: Food safety, Hotels kitchen, Food handlers, Knowledge, Turkey

Submitted: 06.09.2019
Revision requested: 10.11.2019
Last revision received: 15.11.2019
Accepted: 17.11.2019
Published online: 08.01.2020

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Introduction

Recent studies show that foodborne illnesses affect more than one-third of the total population in developing countries (Sani and Siow, 2014). Several foodborne illness outbreaks are associated with various factors, with the most common being food personnel's poor hygiene (Pichler et al., 2014). Presence of pathogenic microorganisms on food handlers' hands contributes to the existence of those illnesses (Egan et al., 2007; Rebouças et al., 2017). The Centre for Disease Control and Prevention (CDC) has reported that food handlers cause as much as 20% of food-related infections (Assefa et al., 2015). Furthermore, the mishandling of food seems the source of 97% of all foodborne illnesses spread through catering outlets (Egan et al., 2007). Inconvenient practices which are responsible for foodborne microbial illnesses are cross-contamination of raw and cooked foodstuffs, inadequate cooking or reheating of foods, usage of unsafe ingredients, storing food at incorrect temperatures, and cooling food inappropriately (Egan et al., 2007; Webb and Morancie, 2015). All of these factors are generally associated with a low level of knowledge and practices (Webb and Morancie, 2015). Lack of knowledge about food safety of food handlers contributes to the spread of those pathogens during food processing (Pichler et al., 2014). Thus, increase the comprehensive knowledge of food handlers about food safety and the efficient application of current information in food processing are crucial to maintaining safe food production (Bolton et al., 2008). Many studies have been conducted about the food safety knowledge of food handlers in different countries so far, for instance Brazil (Rebouças et al., 2017; Soares et al., 2012), Malaysia (Sani and Siow, 2014), Vietnam (Samapundo et al., 2016), Ghana (Kunadu et al., 2016), Jordan (Osaili et al., 2017; 2018) and Turkey (Baş et al., 2006; Tokuç et al., 2009). In many of these studies' results show that participants had limited knowledge about food safety.

Food that consumed at food and beverage establishments have been continuing to be a significant source of foodborne illnesses. The eating habit of many people have changed especially in large cities, therefore the safety of food is extremely important for trade success of food businesses. As individuals continue to become busier, the number of people eating outside is expected to continue to increase (Choi and Rajagopal, 2013). According to the report of the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control, about twenty-five percent of the foodborne epidemy, occurred in European Union countries, had been found associated with restaurants, cafes, pubs, and hotels (ECDC - European Food Safety Authority and European Centre for Disease Prevention and Control, 2017). Similarly, catering services has also a critical role in the spread of those infections. In the USA, Europe, and Ireland those had been partially traced to catering establishments (approximately 45%, 22%, and 50% respectively) (Giritlioglu et al., 2011).

Hotels, as an important part of the tourism industry, are one of the most common food production places. Hence, food safety, hygiene, and sanitation are the most critical issues need to be considered by hotel management. If the importance that required is not given to those issues during the preparation and service of the food, it might cause to the health threat for both personnel and customers (Baser et al., 2017). Bolton et al. (2008) stated that the food preparation personnel, and also customers may seriously be affected by the improper hygienic conditions in the hotel kitchens. In Turkey, there were some regional studies had been made for the evaluation of the food safety knowledge of food handlers working in hotel kitchens (Baş et al., 2006; Sanlier et al., 2010; Baser et al., 2017). However, there is no comprehensive study that includes the whole country. The current study aims to examine the food safety knowledge of food handlers working in hotels' kitchen in different cities of Turkey.

Materials and Methods

Research Design and Participants

A cross-sectional study had been made between May 2017 and September 2017 and involved 378 food handlers working in 22 different food establishments, as a participant. Assessments are comprised of four- and five-star hotels' kitchens in the following cities of Turkey: Ankara, İstanbul, Muğla, Hatay, Diyarbakır, and Nevşehir. The reason why the provinces mentioned in the research are selected as sample was related to the high number of city hotels in these provinces. Since the universe size could not be calculated exactly, the sample size scale table (Yazıcıoğlu and Erdoğan, 2004) was used for determining the sample size. In cases where the universe is 1.000.000 and above, the sample size was considered as min 323, so the 378 sample size reached in this study was sufficient. In sampling, simple random sampling was used in which each element of the universe had equal chance of entering the sample (Arıkan, 2004). After the implementation of questionnaires, face-to-face interviews were performed to guarantee the accuracy of responses which had given to survey questions. Participants were given an ample amount of time (~30 min) to answer the questionnaire.

Questionnaire Design

The questionnaire was prepared from previously performed studies Baş et al., 2006; Giritlioglu et al., 2011; Osaili et al., 2011; Panchal et al., 2014; Shafie and Azman, 2015; Smigic et al., 2016). The questionnaire's reliability was tested via

Cronbach alpha test, with a reliability coefficient of 0.913 (Santos, 1999). In the first part of the questionnaire, some characteristics of subjects were collected such as gender, age, education level, professional experience, past attendance to food safety training courses, the habit of updating knowledge and self-confidence situation about food safety, and the kind of and location of their workplace (hotel). Second part included 65 questions that examined respondents' knowledge of personal hygiene (5 items), cross-contamination (8 items), food hygiene (23 items), health problems that would affect food safety (7 items), symptoms of foodborne diseases (7 items), knowledge of HACCP (Hazard Analysis Critical Control Points) (6 items) and food allergy (9 items) issues. Each question consisted of three optional answers of "yes", "no" and "do not know" in order to reduce the probability of respondents in selecting the correct answer by chance.

Data Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences, Version 16.0 (SPSS, Inc., Chicago, IL, USA). Descriptive statistics were calculated for all variables. Food safety knowledge scores were analyzed by using an independent sample t-test for two groups or Analysis of Variance (ANOVA) with post-hoc Duncan test for more than two groups. $p<0.05$ were considered to be statistically significant. The total food safety knowledge score of respondents' was calculated by summation of the correct answers of the 65 questions included in the seven categories (maximum score is 65). Each correct answer was given 1 point but the incorrect or not sure answer was given 0 points (Osaili et al., 2017). Additionally, the score was converted to a percentage by dividing the total score by the maximum score accessible (score/maximum score*100) and a randomized scoring system was used to assess the level of knowledge (Shafie and Azman, 2015). As per that scoring system, the score that lower than 50% was considered as 'poor knowledge', the score between 50% and 75% was considered as 'average knowledge' and the score that higher than 75% was considered as 'excellent knowledge' (Samapundo et al., 2016).

Results and Discussion

Sample Characteristics

Characteristics of the participants were shown in Table 1. The vast majority of the participants were men (72.2%). In the previous studies, it was mentioned that there were many female kitchen employees in Turkey (Baş et al., 2006; Çakıroğlu and Uçar, 2008). This is because of cultural conditions in Turkey where females do not prefer to work in the hotels' kitchen. Almost half of the participants (53.4%) were between the age ranges 21–30. The total of 79.3% of all participants had finished elementary and high school, whereas 14.0% had a bachelor degree. The number of food handlers who have baccalaureate is very low in Turkey and the findings of the current study are consistent with that level of education. Regarding professional experience in the food services, 20.1% of all participants had <1 year, 40.3% of them had between 1 year and 3 years, 18.3% of them had between 4 years and 6 years of and 21.2% of them had more than 7 years' work experience. About 82.3% of the participants reported that they were previously attended to training courses about food safety. About three of four all participants stated that they had a habit of following new information (77.2%) and feel confident (79.4%) about food safety. A lot of participants (43.9%) have also reported using the internet to follow new information about food safety.

Results of Knowledge Scores in Relation to Sample Characteristics

There were a significant association between food handlers' knowledge and the variables such as their gender (p=0.000), age (p=0.000), professional experience (p=0.000), past attendance to food safety training course (p=0.000), the type (p=0.000) and location (p=0.000) of their workplaces, the habit of updating information (p=0.000), and confidence about food safety (p=0.001). Male participants' knowledge score (35.95) was significantly higher than the female's (32.30) ($p<0.05$). Similarly, it was also determined by Çakıroğlu and Uçar (2008) that the different was significant between knowledge scores about kitchen and equipment hygiene from the aspect of gender. Nevertheless, unlike our study, it was also mentioned that knowledge scores of female employees about general hygiene were higher than male employees. On the other hand, Şanlıer et al. (2010) reported that there the different was insignificant ($p>0.05$) between genders in their study, which had investigated food safety knowledge levels of food handlers in the hotels' kitchen. Even though the different was found insignificant ($p>0.05$) amongst participants with regards to education levels, knowledge score of high school graduates (36.49) who are nearly half of the participants (48.9%) was slightly higher than the others. Unlike our study, the different was found significant ($p<0.05$) between general hygiene konowledge and education status in another study (Şanlıer et al., 2010). The participants who both older than 40 years and the occupational experience longer than 7 years were achieved a higher knowledge score by far (43.15 and 44.60, respectively) ($p<0.05$). Likewise, Şanlıer et al. (2010) had also pointed out the higher score of those who have had 16 years or more experience about general hygiene in their study. Additionally,

Martins et al. (2012) reported that the knowledge scores increase through age and experience. Results of those studies are concordant with our study and show a positive influence of age and experience on food safety knowledge. The food preparers' knowledge score (36.36) who had received food safety training course (82.3%) were significantly higher than those who had not (28.32%) (p<0.05). Many studies have been declared that the most important component for ensuring food safety was a knowledge. Hence, education and training about food safety have been emphasized on various other studies as a must for prevention of foodborne diseases (Ajala et al., 2010; Choi and Rajagopal, 2013; Mullan et al., 2013; Shafie and Azman, 2015). In terms of hotel types, the knowledge score of food handlers working in five-star hotels (39.72) was significantly higher than those working in the four-star hotels (27.90) (p<0.05). Moreover, the knowledge score of food preparers working in hotels in different cities had remarkably varied (p<0.05). Those who constantly update their information and declared their self-confidence about food safety have had significantly higher knowledge score than the others (p<0.05).

Table 1. Characteristics of food handlers and differences in the food safety knowledge scores

		n	%	Mean (Knowledge Score)	Score percent	Within p-value
	Total	**378**	**100**	**34.91**	**53.70**	
Gender	Male	273	72.2	35.95	55.31	**0.000**
	Female	105	27.8	32.3	49.69	
Age	15-19	27	7.1	37.14	57.14	**0.000**
	20-24	89	23.5	32.15	49.46	
	25-29	113	29.9	33.18	51.05	
	30-34	82	21.7	35.56	54.71	
	35-39	27	7.1	34.00	52.31	
	≥40	40	10.6	43.15	66.38	
Education Level	Literate	25	6.6	32.04	49.29	0.197
	Elementary school	115	30.4	33.24	51.14	
	High school	185	48.9	36.49	56.14	
	Two-year degree	30	7.9	36.03	55.43	
	Undergraduate	22	5.8	32.77	50.42	
	Post graduate	1	0.3	31.00	47.69	
Professional experience	<1 year	76	20.1	28.42	43.72	**0.000**
	1-3 years	153	40.5	31.11	47.86	
	4-6 years	69	18.3	39.42	60.65	
	≥7 years	80	21.2	44.60	68.62	
Past attendance to food safety training course	Yes	311	82.3	36.36	55.94	**0.000**
	No	67	17.7	28.32	43.57	
Type of hotel	Five-star hotel	153	40.5	39.72	61.11	**0.000**
	Four-star hotel	225	59.5	27.90	42.92	
Location of hotel	Ankara	71	18.8	25.66	39.48	**0.000**
	Diyarbakır	75	19.8	55.05	84.69	
	Hatay	39	10.3	36.87	56.72	
	İstanbul	81	21.4	25.48	39.20	
	Muğla	92	24.3	30.71	47.25	
	Nevşehir	20	5.3	46.45	71.46	
The habit of following new information about food safety	Yes	292	77.2	36.27	55.80	**0.000**
	- Book / Magazine	*29*	*7.7*			
	- Television programs	*88*	*23.3*			
	- Internet	*166*	*43.9*			
	- Others	*9*	*2.4*			
	No	86	22.8	30.40	46.77	
Self-confident in food safety	Yes	300	79.4	36.06	56.15	**0.001**
	No	78	20.6	30.64	45.08	

Total Food Safety Knowledge Score

The knowledge scores related to different food safety areas and the total knowledge status were evaluated according to the percentile score (arbitrary scoring system). The total knowledge score of participants was 53.70% (34.91/65) as shown in Table 2. This result indicated that food handlers working in hotels' kitchen had average knowledge about food safety. The total knowledge score reported in this study was found better than 43.4% as reported in Turkey (Baş et al. 2006) and 46% as reported in small and micro enterprises in South Africa (Cape et al., 2007) for food handlers. Nevertheless, our results on total knowledge of food safety was found quite lower than the studies of Pichler et al. (2014), Osaili et al. (2013), Osaili et al. (2018), Panchal et al. (2014), Gomes-Neves et al. (2007), Martins et al. (2012) and which had been found that score as 76%, 69.4%, 67.1%, 65%, 62.9%, 56%, respectively. We were expected from participants to receive relatively higher scores in food safety knowledge due to the majority of them had reported that they attended a training course about food safety (82.3%). From this point of view, the result has shown that food safety training is not sufficient alone to have knowledge about it. That result might be related to the quality of the training. Food safety educations should be repeated intermittently, their contents should be updated and its competence should be measured by conducting post-training applications and exams.

In general, knowledge score that involved to the different sections of food safety, like personal hygiene (53.60%), cross-contamination (61.13%), food hygiene (53.91%), health problems which may affect food safety (52.14%), symptoms of foodborne diseases (52.00%), HACCP (51.00%) and food allergy (50.89%) was average level. The highest percentage of correct answers belonged to "knowledge of cross-contamination" (61.13%), while the lowest one belonged "knowledge of food allergy" (50.89%). Food allergy is a more recent issue than the other aspects and it is just mentioned as the subject of food safety training. For that reason, it seemed quite normal to get a lower score. As a result of our study, the knowledge score of personal hygiene (53.60%) was found immensely higher than those in the reports of Martins et al. (2012) and Baş et al. (2006), 51.5% and 31.8%, respectively. Similarly, the knowledge score of cross-contamination in our study (61.30%) was extremely higher than the score calculated both for food handlers at the main campus of University Kebangsaan Malaysia, which reported by Sani and Slow (2014) (44.6%), and at Residential Colleges and Canteen in Malaysia, which reported by Nee and Sani (2011) (46.9%).

Food Safety Knowledge Related to Different Food Safety Areas

Food safety knowledge related to different food safety areas was examined individually and determined some food safety gaps in these areas. All data related to this section are shown in Table 3.

Table 2. Knowledge scores (mean and percentage) related to different food safety areas and total knowledge scores for food handlers in a hotel kitchen in Turkey

Food safety areas	Mean	The possible range of scores	Score percent
Personal hygiene	2.68	0-5	53.60
Cross contamination	4.89	0-8	61.13
Health problems that would affect food safety	3.65	0-7	52.14
Symptoms of foodborne illnesses	3.64	0-7	52.00
Food hygiene	12.40	0-23	53.91
HACCP	3.06	0-6	51.00
Food allergy	4.58	0-9	50.89
Total food safety knowledge score	*34.91*	*0-65*	*53.70*

Table 3. Percentage (%) of correct answers for each question

	Correct answer	
Health problems would affect food safety	**n**	**%**
Sneezing would affect food safety	196	51.90
Hypertension would affect food safety	202	53.40
Diarrhea would affect food safety	179	47.40
Flu would affect food safety	212	56.10
Fever would affect food safety	217	57.40
Smoking would affect food safety	205	54.20
Covered wound in the hand with wearing a glove would affect food safety	172	45.50
Symptoms of foodborne illness	**n**	**%**
Hypertension is a symptom of foodborne illnesses	135	35.70
Diarrhea is a symptom of foodborne illnesses	227	60.10
Nausea is a symptom of foodborne illnesses	206	54.50
Vomiting is a symptom of foodborne illnesses	201	53.20
Pain in the bone is a symptom of foodborne illnesses	199	52.60
Bacteria is the only cause of foodborne diseases	190	50.30
Pathogens in food can cause diseases and even death	220	58.20
Personal hygiene	**n**	**%**
Duration of hand washing ≥ 20 s	206	54.50
Washing hands after touching money	215	56.90
Washing hands after handling raw meats or poultry	191	50.50
Washing hands before preparing meals	200	52.90
Food-services staff with abrasion or cuts on fingers or hands should not touch unwrapped foods	202	53.40
Cross contamination	**n**	**%**
Use same cutting board to cut raw meat or poultry and to chop vegetables	231	61.10
Use same knife to cut raw meat or poultry and to chop vegetables	254	67.20
Wash cutting board used to cut raw meat or poultry with cold water before using it to chop vegetables	227	60.10
Store vegetables salad in the lower shelf in refrigerator if raw meat or chicken in the middle shelf	258	31.70
Store vegetables salad in meat or poultry refrigerator	234	61.90
Wash knife used to cut raw meat or poultry with water and soap then apply sanitizer before using it to chop vegetables	201	53.20
Wash knife used to cut raw meat or poultry with cold water before using it to chop vegetables	239	63.20
Use cap, masks, protective gloves, and adequate clothing reduces the risk of food contamination	206	54.50

***Sentences in bold indicate the correct expressions.**

Table 3 continues. Percentage (%) of correct answers for each question

Food hygiene (safe temperatures, purchasing, storage, thawing, cooking and reheating of the foods)	**n**	**%**
5°C is known as minimum temperature danger zone	198	52.40
Refrigerator operating temperature is 1–5 °C	195	51.60
Freezer operating temperature is −18 °C	202	53.40
Reheat food to temperature of 74 °C	202	53.40
Hot food needs to be kept and served at 60 °C or hotter	178	47.10
Cracked, dirty, broken eggs should not be purchased	208	55.00
Damaged and swollen cans should not be purchased	199	52.60
Conserving cooked food and raw food together causes foodborne illness	234	61.90
Store leftover in the refrigerator	201	53.20
Store leftover on the countertop or table in the kitchen	249	65.90
Store leftover in the oven	215	56.90
Frozen foods should be stored in their own packages	220	58.20
The total time in the temperature danger zone must not be longer than 4 hours	225	59.50
Thaw frozen raw meat or poultry on the kitchen counter in an open container	230	60.80
Thaw frozen raw meat or poultry in the refrigerator	206	54.50
Thaw frozen raw meat or poultry in running tap water	205	54.20
Thaw frozen raw meat or poultry in the microwave	110	29.10
Thaw frozen raw meat or poultry on the kitchen counter in a covered container.	185	48.90
It is necessary to check thermometer settings of refrigerators, freezers and store at least twice a day	211	55.80
Improper heating of food causes foodborne illnesses	206	54.50
Check poultry is sufficiently cooked by thermometer	202	53.40
It is perfectly safe to consume food that tastes and smells normal	188	49.70
Food should be served no later than two hours after preparation	221	58.50
HACCP	**n**	**%**
HACCP is an international food safety system	208	55.00
HACCP is a preventive system that ensures food safety in all stages of food production	203	53.70
HACCP is a mandatory system in Turkey's food law	184	48.70
The HACCP system requires staff training in hygiene	191	50.50
Microbiological hazards cannot be included in HACCP	161	42.60
HACCP is not a very effective system to provide food safety	211	55.80
Food allergy	**n**	**%**
A food allergy is an abnormal response of the immune system to an ordinarily harmless food	202	53.40
Food allergy can result in death	214	56.60
Customers with food allergies can safely consume a small amount of that food	157	41.50
Eczema can be a symptom of food allergy	133	35.20
Asthma can be a symptom of food allergy	198	52.40
Food additives may cause an allergic reaction	204	54.00
Peanut is one of the major foods that cause serious allergic reactions	205	54.20
Labels on food give information about allergic content	203	53.70
Food-allergic reactions occur within from 2 min to 12 h after ingestion	219	57.90

***Sentences in bold indicate the correct expressions.**

Health Problems Would Affect Food Safety

Infected food handlers might contaminate foods and surfaces, thus, leading to spreading foodborne diseases (Todd et al., 2008). For that reason, having knowledge about health problems that would affect food safety has become a considerable aspect. The percentages of participants who knew that sneezing, diarrhea, flu, fever, and smoking would affect food safety during food processing were 51.9%, 47.40%, 56.10%, 57.40%, 54.20%, respectively. On the other hand, even though that is not scientifically relevant, almost 53.40% of participants were believed that hypertension would affect food safety. Less than 50% of them thought that wearing a glove with a covered wound on their hand would affect food safety. The data obtained from our study indicated that about half of the participants were not aware of 'health problems would affect food safety'. As higher than ours, Osaili et al. (2017) have reported that more than 70% of the food service staff, working in hospitals in Jordan were known that sneezing, fever, diarrhea, and smoking during working hours had an impact on food safety. However, there are also studies which have lower results than the current one such as the reports of Jianu and Chis (2012) that mentioned only 31% of the food handlers in Western Romania had knowledge about coughing and/or sneezing might have an effect on food contamination.

Symptoms of Foodborne Illness

When people suffer from food poisoning they cannot be able to understand it is related to food unless they have poor or no knowledge about symptoms of foodborne illnesses. This condition increases the contamination risk since infected food handlers can contaminate food, surface, and other workers (Todd et al., 2008). The most common symptoms of foodborne diseases were reported as diarrhea, nausea, vomiting, and stomach cramps (CDC, 2018). In the current study, more than half of the participants knew that diarrhea (60.10%), nausea (54.50%) and vomiting (53.20%) are the typical symptoms of foodborne illnesses. Although this result showed that the relevant knowledge level is at a moderate level, it is unfortunate that it is a very low rate compared to other studies. For instance, in contrast to that result, Osaili et al. (2013, 2017) reported that 90% of foodservice staff working in the hospitals and restaurants had knowledge about the most common symptoms of foodborne diseases such as diarrhea, vomiting, abdominal pain, and nausea. Similarly, Jianu and Chis (2012) were pointed out that most of the food handlers (77%) in Western Romania could explain these symptoms. Diarrhea, in general, is defined as the most common symptom of those infections in many studies [Tokuç et al., 2009; Osaili et al., 2013, 2017); because of being the most emphasized one in the media (Osaili et al., 2017). Hypertension and bone pain are not symptoms of foodborne diseases. Amongst participants, 35.7% of them knew that that statement was not correct for hypertension and 52.60% knew that was not correct for bone pain. Similarly, in the study of Osaili et al. (2017), in which less than 42% of the participants assumed that hypertension and bone pain were symptoms of foodborne diseases.

Personal Hygiene

Hygienic practices in the responsibility of food handlers are considered as the most effective method to reduce food contamination risk in food establishments. Amidst these hygienic practices, hand hygiene is a more effective method for preventing foodborne diseases when compared with the cleaning and disinfection of surfaces that contact food (Todd et al., 2007). As a matter of fact, it is crucial to have sufficient knowledge about handwashing and concerned staff should pay more attention to this issue. In the current study, the knowledge about the handwashing of employees was determined and the correct response rate was around 50% and this result was quite low compared to other studies (Osaili et al., 2013, 2017). Similar to our finding, Tan et al. (2013) and Rebouças et al. (2017) showed that the majority of food handlers had inadequate knowledge about handwashing. On the other hand, "Duration of handwashing must be minimum 20 sec." statement was correctly identified as true by 54.50% of attendees. When it comes to practice, the time of handwashing is usually shorter than required and not correctly performing by the employees. Although, it is a good result that more than half of the attendees responded correctly to this statement. Unlike our results, Debess et al. (2009), Osaili et al. (2017) and Osaili et al. (2018) reported that only 39%, 29.5%, and 31.9% knew the time that should be spent during hand washing, respectively.

Cross Contamination

Cross-contamination is one of the most contributory risk factors related to foodborne diseases. Cross-contamination and contaminated equipment are referred to as risk factors in England and Wales (25%), and in the US (26%) of general epidemics, respectively. In the catering industry, it was well known that cross-contamination of food via receptacles, hands and surfaces were major risk factors (Bolton et al., 2008). Therefore cleaning those surfaces and equipment is essential to prevent cross-contamination. Over 60% of participants answered "Use the same cutting board to cut raw meat or poultry and to chop vegetables" and "Use the same knife to cut raw meat or poultry and to chop vegetables" statements correctly. 53.20% of participants correctly knew the procedures for cleaning and sanitizing containers. Previous studies

have reported better knowledge level about sanitization procedures of equipment and surfaces than reported in the current one (Tokuçet al., 2009; Jianu and Chiş, 2012; Osaili et al., 2013).

The number of those who knew that raw meat and vegetables must not be chopped on the same cutting board (61.1%) and knew to must change the knife to cut raw meat or poultry and to chop vegetables (67.20%) was substantially high. Pieces of equipment, especially cutting boards have a crucial role with respect to cross-contamination. It was found that more than 50% of selected cutting boards used in hotels in Spain had been contaminated with superfluous levels of microorganisms (Doménech-Sánchez et al., 2011). The number of correct responses given to questions about cross-contamination is higher than in the other food safety areas in the current study, even though this rate is still unsatisfactory and there is still a high risk of cross-contamination. Similarly, in another study which made by Bolton et al. (2008) with 200 chefs and managers of restaurants in Ireland, the risk of cross-contamination sourced from the tools and surfaces were determined as high in kitchens of restaurants. Taking necessary precautions by food handlers such as the hygiene of their hands, bodies, and items of clothing, equipment, and workplace will help to lessen the incidence of cross-contamination (Assefa et al., 2015).

Food Hygiene (Safe Temperatures, Purchasing, Storage, Thawing, Cooking and Reheating of the Foods)

Foodborne diseases have been associated with improper storage, thawing, cooking or reheating of the food and those are frequently due to a lack of awareness or applications about food hygiene. In the current study, the rate of correct answers to statements about this food safety area was usually above 50%. When the statements are viewed singly, it was seen that more than half of the participants (61.9%) had correctly known how to separate raw meat from other food during storage. Unluckily, this result was found weaker than the studies of Walker et al. (2003) and Bolton et al. (2008), in which had been reported that results as 84% for the US, as 97% for the UK and as 92% for Ireland, respectively. 60.80% and 48.90% of attendees stated that they thaw frozen raw meat or poultry on the kitchen counter, sequentially the open and closed container. The distribution of participants who knew the correct thawing procedures was: 54.50% use the refrigerator and 29.10% use the microwave and 54.20% use the tap water. In some other studies, such as Osaili et al. (2013, 2017, 2018) responses related to thawing in a microwave (8.1%, 4.1%, 1.1%, respectively) were similarly low. These results showed that thawing in the microwave is not a common process in the hotel kitchens. About 53.40% of participants knew the right process to determine whether poultry is cooked well by using a thermometer. Unlike this study, a lower rate (12.7% and 19.5%) of attendees had been found correctly answered that question in the study of Osaili et al. (2017, 2018). The statements about correct refrigeration and freezing temperature, the reheating temperature of food and minimum danger zone were correctly answered by more than half of participants (51.60%, 53.40%, 53.40%, and 52.40%, respectively). This finding showed that the majority of participants knew the correct temperature about refrigeration, freezing, reheating and the danger zone. Unlike our results, in many studies (Baş et al. (2006); Tokuç et al., (2009); Martins et al., (2012); Osaili et al., (2013); Webb and Morancie (2015); Kunadu et al., (2016); Osaili et al. (2017)), it was reported that a lack of knowledge among food service staff about critical temperatures.

One of the most common causes of foodborne diseases is inaccurate cooling of cooked food. Even if the food is safely cooked, the bacteria can be contaminated that food. For this reason, remainders must be put in shallow containers for quick cooling and refrigerated at 4 °C or below within two hours (USDA, 2017). In our study, 53.20% and 58.50% of participants knew that “store remainder in the fridge” and “food should be served no later than two hours after preparation”, respectively. Osaili et al. (2018) reported that a small part of the participants (15%) had known the correct retention temperature needed to eat. In contrast to Osaili et al. (2018), in our study, almost half of the attendees correctly answered this statement.

HACCP

Large establishments adopt the HACCP system unlike in the small enterprises in the catering sector. There are some obstacles in small businesses to practice the HACCP system, such as financial constraints and attitudes that restrict the process, absence of legal arrangements, lacked expertise (Egan et al., 2007). Additionally, in catering companies, supervision of the management and lack of motivation, resources and awareness about food safety are other factors that prevent the effective sustaining of the HACCP system (Osaili et al., 2018). Less than half of participants (48.70%) knew that the system is mandatory in our country. Similar results were obtained from the study of Ulusoy and Çolakoğlu (2015) which measured the level of HACCP knowledge of food handlers in enterprises in İstanbul. In the current study, approximately half of the participants correctly responded to the statements about HACCP. Unlike our result, Bolton et al. (2008) reported that head chefs and catering managers in Ireland had inadequate knowledge about HACCP. Similarly, Rebouças et al. (2017) in their study which performed with head chefs and managers in hotels' restaurants of Salvador (Brazil), noticed

that nearly 35.0% of the participants knew what the HACCP means.

Food Allergy

Food allergy is a prominent public health problem as well as food infections. Researchers estimate that up to 15 millions of Americans and about 17 million Europeans have food allergies (FARE, 2017; EAACI, 2017). In this point of view, food handlers might have a critical role to reduce the risk of food allergy and adverse reactions (Dupuis et al., 2016). Therefore, the level of knowledge of food handlers and behavior against food allergy reactions are very important. In the current study, it was seen that more than half of the participants responded correctly to statements such as, "food allergy is an abnormal response of the immune system to food that harmless normally" (53.40%), "food allergy may result in death" (56.60%), "asthma might be a symptom of food allergy" (52.40%), "food additives may cause allergic reaction" (54.0%) and "peanut is a significant food may cause serious allergic reactions" (54.20%). These results showed that more than half of the participants responded correctly to expressions related to food allergy. On the contrary, 41.50% of them incorrectly declared that "customers who have a food allergy can safely consume a small amount of that food". Although food allergy is a current issue and is not frequently included in the training, it is quite a good result that attendees had accurately responded to almost 50% of the statements. The levels of food allergy knowledge and practices of food handlers had seen insufficient in many studies (Ajala et al., 2010; Choi and Rajagopal, 2013; Shafie and Azman, 2015). Food handlers are expected to increase their awareness and knowledge about food allergy since the public health authorities' more interest in food allergy over time.

Conclusion

It is provided important information and displayed many features in that study, concerning the food safety knowledge conditions of food handlers who work in kitchens of 22 hotels located at six different cities in Turkey. The results obtained from our study showed that the level of food safety knowledge of food workers in hotels' kitchens is at an average level. However, there are some significant gaps in food safety areas, such as personal hygiene, food allergy, and HACCP. The most striking result of this study is despite the high number of employees received food safety training the level of knowledge about food safety is medium. In such a group that 82.3% had received food safety training, we would expect a better level. This result may be associated with the adequacy status of food safety training in Turkey. There is a general consensus that trained food handlers are needed to prevent and control foodborne diseases. Thus, food handlers should be taken food safety training regularly. However, as shown in the current study, training is not just enough but the quality of those training should also be measured regularly, effective application of training should be provided and inspections should be made routinely. Not only food handlers, but also food manufacturers, consumers, food and beverage industrialists should take responsibility in this regard, besides that governments are primarily responsible for ensuring and maintaining food safety. Therefore, all these stakeholders should be trained about food safety and systematic control should be made by the government. This is especially important in developing countries such as Turkey, where the risk of foodborne diseases is loud. As this study comprises the employees who work in hotels located in various regions of Turkey, the obtained results can be generalized to all of the hotels in Turkey. Hence, this study is a comprehensive survey that measured the food safety knowledge level of the hotel kitchen staff in Turkey. In the future, some further studies might be performed to determine the relationship between the knowledge, attitudes, and practices of food handlers with food safety training.

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.

Funding: No financial support was received for this study.

Ethics committee approval: "Abant İzzet Baysal University Humanities Ethics Committee in Social Sciences" was received on 17.07.2017 with the document number 2017/175.

Acknowledgements: Authors are thankful for the hotel kitchen staff who participated in this research study and also for Buğra Soykut for editing the article. This study was a part of a Master Thesis of Taner Tuncer (Bolu Abant Izzet Baysal University, Department of Gastronomy and Culinary Arts).

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 6(2), 90-97 (2020) • https://doi.org/10.3153/FH20010 Research Article

Health effects of ethanolic extract from seeds of lady finger

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Cite this article as:
Kaewsrichan, J., Wongwitwichot, P., Manee, S. (2020). Health effects of ethanolic extract from seeds of lady finger. *Food and Health,* 6(2), 90-97. https://doi.org/10.3153/FH20010

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Submitted: 25.09.2019
Revision requested: 10.11.2019
Last revision received: 19.11.2019
Accepted: 22.11.2019
Published online: 10.02.2020

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ABSTRACT

Health benefits of lady finger were explored in this study by which the ethanol extract from the seeds was utilized for testing. In high glucose (350 mmole/L) medium, the proliferative activity of C2C12 cells was inferior, but their viability was improved following treated with the extract. The activities of antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT), were also enhanced. In response to such high glucose concentration, glucose uptake ability of these cells was compromised. The extract was demonstrated to increase the ability of glucose uptake by approximately equal to the effect of 200 nmole/L insulin. High intracellular reactive oxygen species (ROS) of RBM-MCS cells grown in high glucose containing medium was elicited. The level of ROS was reduced when the cells were cultured in contact with the extract. L929 cells were made inflammation and oxidative stress by incubating with 37.5 µmole/L DPPH for 30 min. The release of tumor necrosis factor-α (TNF-α) and interleukin 6 (IL-6) from these inflamed cells was inhibited by such treatment. Thus, lady finger might be advantageous in the protection of cells in the body that would be damaged or being susceptible to hyperglycemia and diabetes.

Keywords: Lady finger, Polyphenols, Healthy diet, Nutrient, Obesity, Chronic inflammation, Metabolic disease

Introduction

Consumption of healthy diets throughout the life-course helps prevent malnutrition and a range of non-communicable diseases (NCDs), such as obesity, diabetes, heart disease, stroke, and cancer. Rapid urbanization changes lifestyles and shift dietary patterns. Having more foods high in energy, fats, free sugars, salt, and sodium, but insufficient of fruit, vegetables, and whole grains, are major epidemic factors of NCDs ("Healthy diet," 2018). A healthy dietary set can be varied depending on individuals and is influenced by both social and economic factors. Indeed, the basic principle of what constitutes a healthy diet is the same and requires a variety of different foods, since no single food contains all the essential nutrients the body needs to stay healthy and work properly ("A healthy, balanced diet," 2016). A healthy diet will provide the right amount of energy to maintain energy balance, which is where the calories taken in from the diet are equal to the calories used by the body. For adults, total fat should not exceed 30% of total energy intake with saturated fats of less than 10% and trans-fats of less than 1%, less than 10% of free sugars, less than 5 g per day of salt, and at least five portions of fruit and vegetables per day ("Healthy diet," 2018). Excess calories intake is stored as fat, and **obesity** is the consequence due to chronic energy imbalance. An association between an unhealthy lifestyle and obesity has been obvious. Obesity changes the body physiological responses through cytokines and pro-inflammatory factors produced by adipocytes, which involve low-grade inflammation. When continuously existing, the inflammation becomes chronic and influences other systems by altering their functions to cause different degenerative diseases (Castro et al., 2017). Therefore, nutritional approach is implicated as an effective tool to manage obesity and obese-associated indisposition. In this study, lady finger, which is a plant with the scientific name *Abelmoschus esculentus (L.)* Moench of the family Malvaceae was chosen for evaluation of health beneficial effects. The selection criteria were that the plant is naturalized in the world's tropical and subtropical areas and its edible fruits are popular for consumption. The fruits are rich in vitamins A, C, and K, polyphenols, protein, and fiber. Accordingly, serious diseases such as cancers, diabetes, chronic inflammation, as well as heart and brain malfunction are expected to improve when the fruits of lady finger are regularly consumed (Ware, 2019). In this work, seeds of lady finger were extracted by using ethanol and examined for optimistic activities, including anti-oxidation, anti-inflammation, inductive proliferation, and beneficial properties of increasing glucose uptake and utilization by muscle cells. Results may assist the daily consumption of lady finger for proper health and prevention/delay the onset of NCDs.

Materials and Methods

Materials

Cell lines of L929 mouse fibroblast (CRL-6364™), PC12 rat adrenal gland (CRL-1721™), and C2C12 mouse myoblast (CRL-1772™) were bought from American Type Culture Collection-Cell lines (ATCC). RBM-MSCs (rat bone marrow derived mesenchymal stem cells) were kindly obtained from authors of the previous study (Wongwitwichot and Kaewsrichan, 2017). RPMI 1640 medium, Dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS), penicillin/streptomycin, Dulbecco's phosphate buffered saline (DPBS) and trypsin ethylenediamine tetraacetic acid (EDTA) were purchased from Gibco™. APO-BrdU™ TUNEL Assay Kit was obtained from Molecular Probes, Inc. Superoxide Dismutase Activity Assay Kit (Colorimetric) was from Abcam®. Catalase Activity Colorimetric/Fluorometric Assay Kit was acquired from BioVision, Inc. Mouse TNF-alpha Quantikine ELISA Kit and Mouse IL-6 Quantikine ELISA Kit were purchased from R&D System (MN, USA). Tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was from Hi Media Labs (Mumbai, India). 2-(*N*-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-Deoxyglucose, (2-NBDG), was bought from Thermo Fisher Scientific (MA, USA). Folin-Ciocalteu reagent, gallic acid, ascorbic acid, sodium acetate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA), 2,4,6-tripyridyl-striazine (TPTZ), $FeCl_3$, L-dopa, and mushroom tyrosinase were acquired from Sigma Aldrich (Darmstadt, Germany). Absolute ethanol was purchased from Loba Chemie (Mumbai, India). Other chemicals were of analytical grade and bought from Merck (NJ, USA).

Seed Extract Preparation

Fruits of lady finger were bought from a farm in Nakhonpathom province, Thailand. Seeds of the fruit were collected and completely dried in a hot-air oven at 60°C. Ethanol was used as a solvent for extraction of dried seeds using a solid: liquid ratio of 1:2. The seed sample was soaked in ethanol overnight at room temperature in close container with regularly shaking. The extract was filtered through a bruckner funnel and concentrated on a water bath at 60°C for 2 h. The extracted residue was reconstituted in ethanol and stored at 4°C until analysis.

Cell Culture

The cell lines of L929 and C2C12 and RBM-MSCs were routinely grown in DMEM medium, whereas RPMI 1640 was used for growing PC12 cells. To prepare a complete medium,

each medium was supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL streptomycin. The cells were routinely cultured in a 5% CO_2 incubator at 37°C and sub-cultured every 3 days.

Cytotoxic Test

The extract was tested for cytotoxicity by using MTT assay. Briefly, cells of 80% confluence were incubated with varying concentrations of a sample for 24 h. The supernatant was withdrawn and replaced by excess MTT reagent (5 mg/mL in PBS). The cells were continually incubated for 4 h at 37°C in the dark before the reagent was removed. DMSO of 150 µL was then added to each well for dissolving the formazan product, and the OD_{570} was measured by using a microplate reader (SPECTROstar Nano, BMG LABTECH, Germany).

Total Phenolic Content (TPC) and Antioxidant Activity

The Folin-Ciocalteu method was used for determination of TPC (Azlim Almey et al., 2010). Briefly, fifty micro litter of sample was added in a tube containing 375 µL of ten-fold diluted Folin-Ciocalteu reagent. After thoroughly mixed for 5 min, 375 µL of 6% w/v sodium carbonate solution was added, mixed, and incubated at room temperature for 90 min. Then, the OD_{725} was measured by using a microplate reader. Gallic acid was a standard. Its calibration curve was constructed by using a concentration range of 0.01-0.10 mg/mL. Data were calculated and expressed as mg/mL gallic acid equivalent.

Antioxidant activity of the extract was determined by two methods, e.g., DPPH and FRAP (Ferric Reducing Antioxidant Power), respectively. For DPPH assay, the method of Alama et al. was performed with some modifications (Alam et al., 2017). In brief, forty micro litter of sample was added to 160 µL of 0.1 µmole/L DPPH (2,2-diphenyl-1-picrylhydrazyl) solution in a well of 96-wells plate and incubated in the dark at room temperature for 30 min. Then, the OD515 was measured using a microplate reader. A calibration curve of trolox at concentrations ranging between 0 and 500 µmole/L was plotted. Data were expressed as µmole/L trolox equivalent. In analysis of FRAP activity (Rehakova et al., 2014), three solutions were prepared, e.g., 300 mmole/L acetate buffer pH 3.6; 10 mmole/L of TPTZ in 40 mmole/L HCl; and 20 mmole/L of FeCl3. FRAP reagent was freshly prepared by mixing 25 mL of acetate buffer, 2.5 mL of TPTZ solution and 2.5 mL of $FeCl_3$. The FRAP reagent of 750 µL and a sample of 25 µL were mixed and incubated in the dark for 10 min. After that the OD593 was measured using a microplate reader. In compared to known concentration of $FeCl_2$, results were calculated and expressed as µmole/mL Fe^{2+} equivalent.

Proliferation Assay

Cells of $5x10^4$ cells/mL in complete medium were seeded in a 6-wells plate and cultured to nearly 100% confluent in a 5% CO_2 incubator at 37 ^{O}C. Then, the growing cells were starved overnight in serum-free medium, and incubated in medium supplemented with 37.5 µmole/L DPPH for 30 min by which cellular oxidative stress was established. A linear scratch was created on a well by using a sterile pipette tip. Any debris was removed by gentle washing with phosphate buffer saline (PBS) solution, followed by incubation in medium supplemented with the extract for 2 days. Photography was carried out on each day and the image J software was used for measuring the scratch distance closure. The percentage (%) of distance closure was calculated by using an equation below.

$$\% \text{ Distance closure} = \frac{\text{Distance of the scratch on day 0} - \text{Distance of the scratch on day 1 or 2}}{\text{Distance of the scratch on day 0}}$$

Apoptotic Activity Assay

The terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labelling (TUNEL) assay was utilized for detection of apoptotic cells as induced by test compounds by using APO-BrdU™ TUNEL Assay Kit according to the manufacturer's recommendations. Cells of 60-70% confluence were challenged with 350 mmole/L glucose with or without the addition of the extract for 72 h. After that the cells were collected and apoptotic determination was performed by using flow cytometer (CytoFLEX S, Beckman Coulter, CA, USA).

Activity of Antioxidant Enzymes

The previously challenged cells were collected by centrifugation (1500xg, 15 min), and lysed by sonication in a specified buffer. The supernatant was collected by centrifugation at 12000xg at 4°C for 10 min and measured for enzyme activity. Controls were cells grown in serum free medium without any challenges.

1. Superoxide dismutase (SOD) activity

SOD activity was determined by using Superoxide Dismutase Activity Assay Kit. Results were calculated by comparing with that of controls and represented as fold increase.

2. Catalase (CAT) activity

CAT activity was measured using Catalase Activity Colorimetric/ Fluorometric Assay Kit. The enzyme activity was reported as mU/mL. One unit of catalase is the amount of CAT able to decompose 1.0 µmole of H_2O_2 per min at pH 4.5 at 25°C.

Tumor Necrosis Factor-α (TNF-α) and Interleukin 6 (IL-6) Inhibition

The activities of TNF-α and IL-6 in culture supernatant were determined based on ELISA technique. Briefly, a monoclonal antibody specific for either mouse TNF-α or mouse IL-6 was pre-coated onto a microplate. Standards, control, and samples were separately pipetted into the wells, and either TNF-α or IL-6 present was bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse TNF-α or IL-6 was added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution was added to the wells. The enzyme reaction yielded a blue product that turns yellow when the Stop Solution was added. The intensity of the formed colour was proportional to the amount of TNF-α or IL-6 bound in the initial step. The sample values were read off by comparing to the standard curve, calculated, and reported as the percentage (%) of inhibition.

Glucose Uptake

C2C12 cells were determined for glucose uptake activity. The cells of 80% confluence were starved in serum free medium for 24 h and incubated with the extract for 24 h. A fluorescent glucose analogue, 2-NBDG, was inoculated into culture medium to a final concentration of 180 µmole/L, which was used to replace the old medium. The treated cells were incubated in a CO_2 incubator for 30 min following such replacement, collected by centrifugation (1500xg, 15 min), and subjected to flow cytometric analysis. Control cells were incubated in medium supplemented with 200 nmole/L insulin for 30 min before the addition of the fluorescent dye.

Intracellular Reactive Oxygen Species (ROS)

RBM-MSCs were cultured in medium supplemented with the extract for 24 h before the old medium was replaced by a new medium that contained 10 µmole/L DCFH-DA. These cells were incubated further for 1 h, washed with PBS, incubated in medium supplemented with 150 µmole/L H_2O_2 for 4 h, and then observed under a fluorescence microscope (OLYMPUS BX61).

Statistical Analysis

All experiments were done in triplicate. Data were presented as mean ± standard deviation (S.D.). One-way ANOVA was used, and $p<0.05$ was considered significant of the results.

Results and Discussion

Previously, the extract from seeds of lady finger was demonstrated to contain polyphenols, tannin, flavonoids, terpenoids, saponins, long chain fatty acids, and glutathione (Manee and Kaewsrichan, 2017). In the present work, data of TPC, and antioxidant activity regarding DPPH and FRAP assays were additionally reported. The TPC was equivalent to 9.2 mg gallic acid/mL. The DPPH radical scavenging activity and FRAP were of 700 µmole/mL trolox equivalent and 340 µmole/mL Fe2+ equivalent, respectively. It is interesting in this study to investigate health benefits of lady finger when regularly consumed as a vegetable.

Testing for cytotoxicity of the extract was priory performed by using MTT assay, and the cell viability of not less than 80% would be indicated as safe. Data corresponding to this cytotoxic test on L929 fibroblastic cells should be referred to those of our previous study (Manee and Kaewsrichan, 2017). Three different cell types, including C2C12 muscle cells, PC12 adrenal cells, and RBM-MSCs bone stem cells that associated to this work were recently tested. Results showed that C2C12 and PC12 cells could tolerate to a concentration range of 1.25-2.5 mg/mL extract, while the viability of RBM-MSCs cells was observed at a lower concentration range of 0.5-1.25 mg/mL extract. In accordance, each maximal concentration level will be used for treating the respective cells in next experiments, i.e., 2.5 mg/mL for C2C12 and PC12 cells, and 1.25 mg/mL for RBM-MSCs cells.

C2C12 cells were cultured in medium supplemented with 350 mmole/L glucose to mimic the main pathological condition of diabetes mellitus (Newsholme et al., 2007). Such hyperosmotic medium induced cell death by approximately 36.92 ±0.90%, as determined by TUNEL assay. After treating with the extract of 2.5 mg/mL, the number of dead cells was significantly decreased to 24.55 ±0.48%. Thus, those injured cells were possibly rescued by the extract, becoming healthy cells which can grow and proliferate. Commonly, diabetes patients may suffer from muscle weakness of the lower limbs, which increases the risk of falling (Woodfield, 2016). Therefore, sufficient consumption of lady finger may be a way for increasing healthy muscle cells, which leads to improved muscle mass and strength. However, deep investigation on mechanisms underlining such muscular effect is awaited to discover. In contrast to C2C12 cells, the proliferative capacity of glucose-treated PC12 cells was not improved by such treatment. These cells seemed to be more vulnerable to glucose toxicity than the muscle cells described above. Regarding the physiology, adrenal cells function in synthesis and secretion of glucocorticoids, mineralocorticoids, and androgenic steroids, which are strictly controlled by corticotrophin-releasing hormone of hypothalamus and adrenocorticotrophic hormone of anterior pituitary gland, respectively. Cortisol is the major glucocorticoid in human. To our knowledge, alteration of circulating cortisol has dramatic effects on the body by increasing appetite, promoting triglyceride accumulation,

and obesity (Lucassen and Cizza, 2012). Therefore, plant extracts capable in protection of adrenal cells death are requested to research.

Free radicals and reactive species, especially super oxide anion (O_2^-), are constantly generated by the normal body metabolisms through mitochondrial energy production pathway. Cellular oxidative stress may occur when the level of oxidant species exceeds that of antioxidants and be implicated in the incidence and progression of several health problems and chronic diseases (Giustarini et al., 2009). SOD, CAT, and glutathione peroxidase (GPX) act promptly against free radicals to protect vital macromolecules and body tissues (Ighodaro and Akinloye, 2018). Determination of SOD and CAT activities before and after treating with 2.5 mg/mL extract was consequently carried out. The SOD activity of C2C12 cells cultured in 350 mmole/L glucose supplemented medium was 1.59-fold compared to the control, and was slightly increased to 1.80-fold after treatment. For PC12 cells, the SOD activity in the present of such high glucose concentration was 11.34-fold compared to the control, but decreased to 2.24-fold by such challenge. Results indicated that the circumstance of high glucose was very toxic to PC12 cells compared to C2C12 cells. It also seemed that PC12 cells were more sensitive to the extract treatment by increasing SOD production than C2C12 cells. The CAT activity of C2C12 cells cultured in glucose containing medium was accountable to be 23.79 mU/mL and was significantly decreased to 17.04 mU/mL after treatment. For non-challenging, non-treating C2C12 cells (control), the enzyme activity was 3.42 mU/mL. In contrast, the CAT activity was not detected for PC12 cells. To limit the oxidative stress of high glucose, C2C12 cells were thus simply challenged by the extract for increased production of the CAT enzyme. Instead, PC12 cells trended to die when in contact with the non-favorable environment due to the scant of CAT activity.

L929 cells that made oxidative stress by culturing in medium containing 37.5 µmole/L DPPH for 30 min were investigated for proliferative activity after treatment with the extract for 2 days. Results showed that the distance closure of the scratched lines was improved by approximately 55% and 84% on day 1 and day 2 of the treatment, respectively. In addition, the extract demonstrated inhibitory activity on the released TNF-α and IL-6 by about 40.5% and 86.3%, after 2 days of incubation. Therefore, the extract might help recovery of the injured cells to become healthier, which could begin proliferation and growth. Inhibition of TNF-α and IL-6 activity by the extract might be suggested as a role for mending the distorted cells. There is an agreement in that prolonged inflammation and increased oxidative stress impair healing in diabetes (Kant et al., 2014). Focusing on beneficial effects by increasing SOD and CAT activity and decreasing TNF-α and IL-6 activity, the extract might be implicated for ameliorating damaged cells, tissues, and organs affected by diabetes.

Hyperglycaemic condition can induce skeletal muscle to be resistant to insulin, resulting in lowered glucose uptake (Shannon et al., 2018). Then, C2C12 cells were determined for glucose uptake ability in response to the elevated glucose concentration in culture medium and the present of the extract. 2-NBDG is a fluorescent glucose analogue, which is used for monitoring glucose uptake in live cells. In this study, the percentage (%) of cells being fluorescent as 2-NBDG was taken up was analysed by flow cytometry. Results showed that glucose uptake activity of C2C12 cells was promoted following treated with the extract in compared to the untreated control. The acquired fluorescent signal was equivalent to those challenged by 200 nmole/L insulin. Consequently, the extract seemed to be advantageous for improving hyperglycemic condition by increasing glucose uptake and reducing glucose tolerance. Several mechanisms regarding glucose uptake amelioration have been reported, such as activation of Peroxisome Proliferator-Activated Receptors (PPARs) (Dubois et al., 2017), and upregulation of adiponectin receptor (Kim and Park, 2019). To this context, mechanisms underlining the improvement of glucose uptake by the extract in C2C12 cells are being investigated.

High blood glucose concentrations can cause injury of a large number of tissues and organs. Although most cells can adapt the rate of glucose influx under hyperglycaemic condition to protect their intracellular milieu, some cells such as beta cells, neuronal cells, and endothelial cells, do not have such adaptive mechanism. These cells are thus more susceptible to oxidative stress caused by hyperglycaemia than others (Marcovecchio, 2017). RBM-MSCs were chosen for measuring intracellular oxidative stress in response to high glucose concentrations, because this cell type is of mesenchymal origin and responsible in forming muscle, and lymphatic and blood vessels, and would be used in estimating hyperglycaemic effect on endothelial cells (Bianco et al., 2008). DCFH-DA is a nonpolar dye. Following taken up into cytoplasm, it is converted into DCFH by cellular esterase, which is nonfluorescent. DCFH is switched to highly fluorescent DCF when oxidized by intracellular ROS and other peroxides. Therefore, if intracellular ROS is generated, the images of green fluorescent cells will be obtained from a fluorescence microscope using 295, 320 or 395 nm cut-off filters (Rastogi et al., 2010). Results indicated that the green fluorescent intensity of the treated cells was lower than that of the untreated cells (Figure 1), suggesting that the extract presented antioxidant activity.

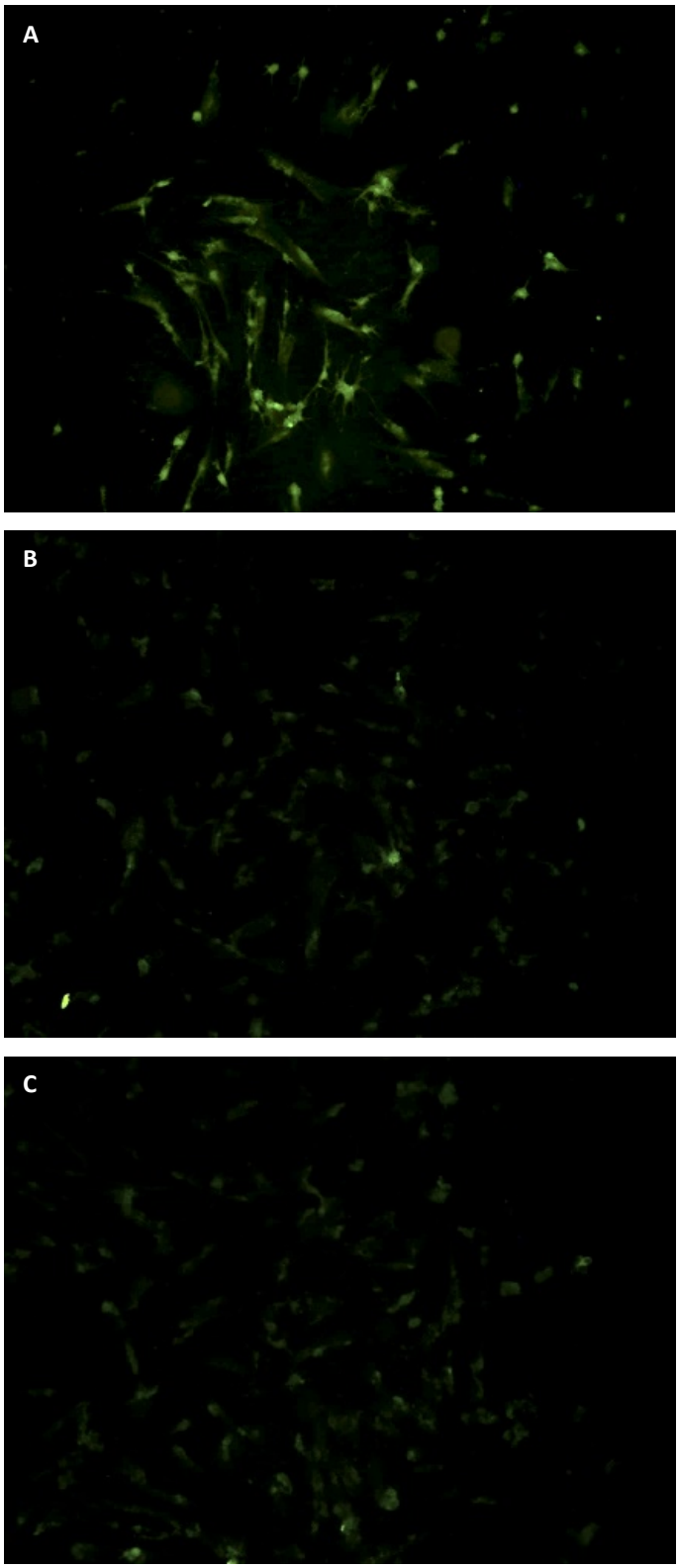

Figure 1. The fluorescence signal of DCFH-DA up taken by RBM-MSC cells. The cells were induced by 150 μmole/L H_2O_2 for 4 h. a, control (without treatment); b, treated with 1.25 mg/mL extract; c, treated with 2.5 mg/mL extract

Conclusion

It was concluded that the extract from seeds of lady finger displayed antioxidant and anti-inflammatory activities, as well as properties for fitting cells, promoting proliferation, and improving glucose uptake and insulin resistance. Thus, when regularly consumed as a vegetable, lady finger would be advantageous in management hyperglycaemia and diabetes.

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.

Funding: The financial support of this project was granted by Thailand national budget for the fiscal year 2018 under integrative research and innovative plan (Project No.: PHA610372S).

Ethics committee approval: No animals are used in this study

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 6(2), 98-109 (2020) • https://doi.org/10.3153/FH20011 *Research Article*

Probiyotikli devam formülünde probiyotiğin canlılık çalışması

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Cite this article as:
Bulca, S., Ağalday, R. (2020). Probiyotikli devam formülünde probiyotiğin canlılık çalışması. *Food and Health,* 6(2), 98-109.
https://doi.org/10.3153/FH20011

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Submitted: 04.08.2019
Revision requested: 05.11.2019
Last revision received: 15.11.2019
Accepted: 06.12.2019
Published online: 14.02.2020

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ÖZ

Son yıllarda tüm dünyada sağlık problemlerinin ve hastalıkların artışıyla beraber tüketicinin fonksiyonel gıdalara olan talebi de artış göstermiştir. Probiyotikli ürünlerin üretiminde hammadde olarak sütün kullanımıyla probiyotikli yoğurt, probiyotikli kefir, probiyotikli peynir, probiyotikli dondurma, probiyotikli ayran, hatta probiyotikli dondurulmuş sütlü tatlılar ve probiyotikli peynir altı suyu içeren içecekler üretilmiştir. Bu şekilde bu ürünlerden vücudun temel besin öğeleri gereksinimini karşılamanın dışında insan fizyolojisi ve metabolik fonksiyonları üzerinde faydalar sağlaması ve hastalık riskinin azaltması gibi faydalı etkiler beklenmektedir. Yukarıda belirtilen faydalı etkilerinden dolayı bu çalışmada, probiyotik olarak liyofilize formdaki *Bifidobacterium animalis* subsp. *lactis* (BB-12) ve prebiyotik olarak fruktooligosakkarit keçi sütü bazlı devam formülüne eklenmiş ve devam formülünün nem oranları, pH değeri ve *B. animalis* subsp. *lactis'in* canlılığı 3, 15 ve 30 gün oda sıcaklığında depolama sırasında araştırılmıştır. 30 günlük çalışmada, nem ilk gün %2.79 ve son gün %2.78 bulunmuştur. Ürünün pH değeri ilk gün 6.97; son gün 6,86'dır ve anlamlı farklılık göstermemiştir. Canlılık çalışmasında *B. animalis* subp. *lactis* (BB-12)'in ilk sayımları 7.53 log kob/g'dır, son gün ise 7.42 log kob/g'dır ve anlamlı farklılık göstermemiştir. Sayımların sağlık yararlarını sağlamak için önerilen ekleme seviyesinin (6 log kob/g) üzerinde kaldığı saptanmıştır. Ayrıca, nem oranı ve pH değerleri anlamlı farklılık göstermemiştir

Anahtar Kelimeler: Devam Sütü, Keçi Sütü, Fruktooligosakkarit, *Bifidobacterium animalis* subsp. *lactis* (BB-12)

ABSTRACT

Vitability study of probiotic in probiotic follow-up formula

In recent years, with increasing health problems and diseases all over the world, consumer demand for functional foods has increased. By using milk as a raw material in the production of probiotic products, probiotic yoghurt, probiotic kefir, probiotic cheese, probiotic ice cream, probiotic buttermilk, probiotic frozen milk desserts and drinks that contain probiotic whey have been produced. In this way, these products are expected not only to meet the body's essential nutrient requirements but also provide beneficial effects on human physiology and metabolic functions, and as well as reducing the risk of disease.Due to the above mentioned beneficial effects in this study, lyophilized *Bifidobacterium animalis* subsp. *lactis* (BB-12) as probiotic and fructooligosaccharide as prebiotic were added to a goat milk based follow-up formula and the moisture content, pH value and the viability of the *B. animalis* subsp. *lactis* during storage of 3, 15 and 30 days at room temperature were determined. In the 30-day study, moisture was 2.79% on the first day and 2.78% on the last day. The pH value of the product was 6.97 on the first day; 6.86 on the last day and showed no significant difference. In the viability study, *B. animalis* subsp. *lactis* was 7.53 log cfu/g on the first day and 7.42 log cfu/g on the last day and showed no significant difference. The counts were found to be above the recommended addition level (6 log cfu/g) to provide health benefits. In addition, the moisture contents and pH values did not show a significant difference.

Keywords: Follow-Up Formula, Goat Milk, Fructooligosaccharide, *Bifidobacterium animalis* subsp. *lactis* (BB-12)

Giriş

Bebekler için anne sütü ideal besin kaynağıdır ve doğumdan sonraki ilk 6 ay boyunca tek besin kaynağı olarak kullanılmaktadır. Fakat bazen annenin bebeği emziremediği ya da emzirmenin yetersiz olduğu durumlarla karşılaşılabilir. Böyle durumlarda bebek formülleri ve devam formüllerinden yararlanılmaktadır.

Devam formülleri, 6 ay ve üzeri bebeklerin ve küçük çocukların dengeli beslenmesi ve diyetin bir parçası olarak kullanılmaktadır. Altıncı aydan sonra tamamlayıcı beslenmeye başlama kararı bebeğin büyüme ve gelişim ihtiyaçları doğrultusunda verilmelidir (TGK, 2014).

Probiyotikler; bağırsak patojenlerinin inhibisyonu, bağışıklık sisteminin aktivasyonu ve bağırsaklarda mikrobiyal dengenin düzenlenmesi gibi sağlığı teşvik edici etkileri nedeniyle son yıllarda önem kazanan bakterilerden birisidir (Liong, 2007).

Oligosakkaritler, prebiyotik olarak bilinir ve kalın bağırsakta patojen bakterilerin sayısını sınırlayan, probiyotik bakterilerin gelişimini teşvik eden ve sindirilemeyen karbonhidratlar olarak tanımlanırlar (Chandan, 1997; Roberfroid, 2000; Shah, 2001; Holzapfel ve Schillinger, 2002).

Keçi sütünün ve probiyotiklerin beslenmedeki önemi bilinmektedir ve bu ürünlere ilgi günden güne artış göstermektedir. Probiyotiklerin iyi bir gelişme ve asitlendirme performansı gösterebilmesi için ona uygun bir prebiyotik seçilmesi de önemli bir noktadır. Ancak piyasada bulunan devam formülleri incelendiğinde çoğunun ham maddesinin inek sütü olduğu ve keçi sütüne dayalı formülasyona sahip sınırlı sayıda ürün bulunduğu saptanmıştır. Bu ürünlerin yine büyük bir çoğunluğu probiyotik ilavesiz olduğu bilinmektedir.

Laktik asit bakterileri; laktik asit, asetik asit üretimlerinin yanısıra bakteriyosin de üretmelerinden dolayı antibakteriyel etkiye sahip bakterilerdir. Oluşturdukları asitten dolayı bağırsak ortamının pH'sını düşürerek patojen mikroorganizmaların çoğalmasını engellerler. *Bifidobacterium* ve *Lactobacillus* özellikle anne sütü ile beslenen bebek bağırsak mikroflorasında yaygın olarak bulunmakta ve bu bölgede toplam bakteri popülasyonunun büyük bölümünü oluşturmaktadır. Ayrıca kompleks oligosakkaritleri karbon ve enerji kaynağı olarak kullanmaktadırlar (Garrido vd., 2013).

Araştırma bulguları; oligosakkaritler, prebiyotikler ve probiyotikler gibi çeşitli fonksiyonel gıda bileşenlerinin, bebeklerin bağırsak mikroflorasının bileşimi ve aktivitesinde yararlı etkilerinin bulunduğunu göstermiştir (Mountzouris vd., 2002). En çok kullanılan fonksiyonel bileşenlerden birisi de probiyotiklerdir. Probiyotikler, yeterli miktarda alındığında konakta bir sağlık yararı sağlayan canlı mikroorganizmalar olarak tanımlanırlar (Guarner vd., 2005). Probiyotiklerin yaygın kullanım alanlarından birisi gastrointestinal sistemdir.

Bebeklerde akut ishal sık görülen enfeksiyonlar arasındadır. Yapılan birçok kontrollü çalışma, probiyotiklerin çocukluk çağı akut ishallerinin süresini kısalttığını göstermiştir (Vanderhoof ve Young, 2004; O'Sullivan vd., 2005). Nekrotizan enterokolit (NEK) kanlı dışkılama ile karakterize edilen, özellikle 1500 gramın altındaki prematüreler olmak üzere yeni doğan döneminde görülen ve hayatı tehdit eden bir enfeksiyondur (Martin ve Walker, 2008). Düşük doğum ağırlıklı prematüre bebeklerin bağırsak florasında *Clostridium perfringens ve Escherichia coli* baskın florayı oluşturmaktadır. Nekrotizan enterokolit (NEK) teşhisi konan bebeklerin %40'nın bağırsaklarında *Lactobacillus* cinsine ait türlerin oranının azalmış olduğu saptanmıştır. Bu bulgu bizlere *Lactobacillus* oranının azalması ile nekrotizan enterokolit arasında bir bağlantı olabileceğini göstermektedir (Bin-Nun vd., 2005). Probiyotiklerin antibiyotik kullanımı sonucu oluşan diyare, *Helicobacter pylori* enfeksiyonları, alerji, yüksek kolesterol üzerine de faydalarının olduğu da çeşitli çalışmalarda rapor edilmiştir (Macfarlane ve Cummings, 2002).

Ayrıca gıdalardaki canlı probiyotik organizmaların, farklı kökenli diyarelerin azaltılması ve önlenmesi, bağırsak mikrobiyal dengesinin iyileştirilmesi, laktoz intoleransının hafifletilmesi, bağışıklık sisteminin güçlendirilmesi, antitümör ve antihipertansif özellikleri gibi bilimsel olarak belirlenmiş ve/veya klinik olarak kanıtlanmış sağlık etkilerinin olduğu da tespit edilmiştir (Liong, 2007). Probiyotikler potansiyel sağlık yararları nedeniyle, genellikle süt preparatlarına sağlıklı fonksiyonel ürünler elde etmek için ilave edilmektedir (Pinto vd., 2006).

Probiyotiklerin yararlı etkiler sergilemeleri için, probiyotik organizmaların sayısının en az 10^6 kob/g olması gerekir (Yeo ve Liong, 2010). Minimum terapötik doz ise günlük olarak 1 gramda 10^8-10^9 canlı hücredir ve bu da 10^6–10^7 kob/g canlı hücre içeren 100 g ürün tüketilerek karşılanabilmektedir (Yeo ve Liong, 2010). Tüketicinin sağlık konusundaki farkındalığının artması, probiyotik içeren fonksiyonel gıdalara olan talebi artırmıştır (Liong, 2007).

Prebiyotikler, konakçı sağlığını artırabilen, kolonda bir veya sınırlı sayıda bakterinin büyümesini ve/veya aktivitesini seçici olarak uyararak konakçıya fayda sağlayan sindirilemeyen gıda bileşenleridir (Gibson ve Roberfroid, 1995). Prebiyotikler, mide ve ince bağırsakta sindirilmeden kalın bağırsağa geçer ve burada bulunan *Lactobacillus* ve *Bifidobacterium* gibi probiyotiklerin gelişimlerini ve aktivitelerini desteklerler (Yıldırım vd., 2003). Prebiyotik özelliğe sahip en önemli gıda bileşenleri fruktooligosakkarit, inülin, galaktooligosakkarit,

glukooligosakkarit, ksilooligosakkarit, izomaltooligosakkarit, gentiooligosakkarit, laktuloz, laktosukroz, polidekstroz, pirodekstrin ve rafinozdur (Holzapfel ve Schillinger, 2002; Gibson ve Roberfroid, 1995; Ziemer ve Gibson, 1998).

Oligosakkaritler prebiyotik özelliğe sahiptir ve kalın bağırsakta patojen bakterilerin sayısını sınırlamaktadırlar. Fruktooligosakkaritler, galaktooligosakkaritler, ksilooligosakkaritler, laktuloz gibi pek çok ürün prebiyotik özelliğe sahiptir. Bu ürünler arasında gıda endüstrisinde özellikle bebek formüllerinde kullanılanlar çoğunlukla galaktooligosakkaritler ve fruktooligosakkaritlerdir (Garrido vd., 2013). Türk Gıda Kodeksi Devam Formülleri Tebliği'nde de (TGK, 2014) devam formüllerine fruktooligosakkaritler ve galaktooligosakkaritlerin ilave edilmesine izin verilmiştir. İlave edilmesi durumunda, bunların miktarı, kullanıma hazır üründe 0,8 g/100 ml'nin üzerinde olmaması gerektiği belirtilmiştir.

Galaktooligosakkarit, substrat olarak laktoz kullanılarak, maya veya bakterilerden elde edilen β-galaktosidazlar ile enzimatik transgalaktosilasyon yoluyla sentezlenen bileşiklerdir (Bode, 2012).

Fruktooligosakkarit (FOS) ve inülin son zamanlarda prebiyotik olarak dikkat çekmektedir. FOS, glukozidik bağlarla bağlanmış 2–10 fruktoz ünitesini içerirken, inülin, 3-60 birim arası zincirlere sahip çeşitli fruktanlar içermektedir (Rossi vd., 2005). FOS, çoğunlukla hindiba gibi *Compositae* ailesine ait bitkilerden enzimatik sentez yoluyla elde edilir ve çoğunlukla β-2,1 bağlı inülin tipli fruktoz oligomerleridir (Bode, 2012). Fruktooligosakkarit, kalsiyum ve minerallerin emilimi, şeker ve yağın gıda ürünlerinde yer değiştirmesi, kolestrolün azaltılması, çeşitli hastalıkların kontrol altına alınması gibi bilimsel olarak kanıtlanmış sağlık yararları olan, karsinojenik olmayan, düşük kalorili bir prebiyotiktir (Bali vd., 2015).

Memeli sütleri karşılaştırıldığında oligosakkarit içeriği en yüksek olan insan sütüdür (700-1200 mg/100 mL). Keçi sütü (25-30 mg/100 mL), inek sütüyle (2-3 mg/100 mL) karşılaştırıldığında daha yüksek oranda oligosakkarit içeriğine sahip olup, bu özelliği bakımından anne sütüne daha yakındır. Keçi sütünde bulunan oligosakkaritler miktar olarak fazla olmalarının yanında, çeşitlilik açısından da inek ve koyun sütüne göre daha zengindir (Martinez, 2006).

Son yıllardaki araştırmalar, insan sütü oligosakkaritlerinin bioaktivitesinin anahtar yapısal unsurlarının keçi sütü içerisinde de bulunduğunu göstermektedir. Bu nedenle keçi sütünün fonksiyonel bir gıda olduğu düşünülmektedir (Bode, 2012).

Dünya piyasasını araştırdığımızda keçi sütü bazlı birçok ürün bulunmaktadır. Örneğin tam yağlı mineral ve vitamin katkılı keçi sütü tozları, koyulaştırılmış keçi sütü, keçi sütü yoğurdu tozu, çikolata ve meyve aromalı keçi yoğurdu, anne sütünü artırıcı takviye keçi sütü ve çocuklar için keçi sütü bazlı çikolatalar gibi ürünler teknolojik olarak üretilmektedir.

Ancak piyasadaki mevcut bebek formülü ve devam formüllerine bakıldığında üretimlerinde genellikle inek sütü kullanılmaktadır (Gürsel, 2007).

İnek sütüne kıyasla keçi sütünün αs_1-kazein seviyesi daha düşük olduğu için daha yumuşak kazein pıhtısı oluşturur. Bu sebeple keçi sütü proteinleri, inek sütü proteinlerinden daha kolay sindirilir (Raynal vd., 2008; Ribeiro, 2010). Ayrıca keçi sütü, inek sütüne göre daha küçük çaplı yağ globülleri içerir. Bu küçük çaplı yağ globülleri de sütte daha homojen bir yağ dağılımı sağlar. Keçi sütü kısa ve orta zincirli tekli ve çoklu doymamış yağ asitleri bakımından zengin olup, 6-10 karbonlu olanların oranı inek sütündekinin iki katı kadardır (Alichanidis ve Polychroniadou, 1996; Alonso vd., 1999). Orta zincirli yağ asitlerinden, özellikle kaprilik (C8:0) ve kaprik (C10:0) asitler, emilim ve metabolizma bozuklukları, kolesterol problemleri ve yetersiz beslenme bulguları sergileyen hastalarda tedavi edici nitelikler taşıdığı rapor edilmiştir.

Ayrıca keçi sütü inek sütünden daha etkili olarak bağışıklık sistemini güçlendirir ve antibakteriyel etkilere de sahiptir (Oliveira vd., 2015). Keçi sütü özellikle laktozdan türetilmiş kompleks oligosakkaritlerce zengindir. Keçi sütünde işlevsel oligosakkaritlerin keşfedilmesi, insan sağlığını iyileştirmede olanak sağlamaktadır. Özellikle bazı oligosakkaritlerin varlığı özel önem taşımaktadır çünkü bu maddeler prebiyotik özellikleri ve patojen bağlama aktiviteleriyle sağlık avantajlarına sahiptir. Bazı çalışmalarda keçi sütü oligosakkaritlerinin *in vivo* ortamda faydalı etkilerinin olduğu gösterilmiştir. Keçi sütü insan sütüne oranla daha az miktarda laktoz içeriğine sahip olmasına rağmen, keçi sütünün insan sütü oligosakkarit profiline inek veya koyun sütlerinden daha yakın olduğu bildirilmiştir. Bu bulgular, keçi sütü oligosakkaritlerinin, özellikle bebek formüllerinin takviyesi için çok umut verici bir fonksiyonel gıda özelliği taşıdığını göstermektedir.

Probiyotik gıdalar, genel olarak içerisinde raf ömrü sonuna kadar yeterli miktarda canlı probiyotik mikroorganizma (10^6 kob/g) içeren ve bu canlılığı muhafaza eden ürünler olarak ifade edilir (TGK, 2006). FAO ve WHO tarafından hazırlanan rapora göre de gıdalarda kullanılan probiyotikler sindirim sistemi boyunca canlı kalabilmeli, gastrik sıvılara ve safra tuzlarına karşı dayanıklı olmalı, hızlı gelişebilmeli ve bağırsak sisteminde kolonize olabilmelidir. Ayrıca, probiyotikler etkili ve güvenli olmasının yanı sıra bu etkinliğini ürünün raf ömrü boyunca da sürdürebilmelidir (Tsuda ve Miyamoto, 2010).

Süt ürünlerinde bulunan probiyotiklerin canlılık kaybına neden olan başlıca etkenler, bakterilerin çoğalmasına bağlı olarak ortamda organik asitlerin birikmesi ile ürün pH'sının düşmesidir (Shah ve Jelen, 1990). Ağız yolu ile alınan probiyotiklerin intestinal bölgelere ulaşmadan önce midenin gastrik asit ortamından (pH 1,5-3,0) canlı olarak geçmesi gerekmektedir. Probiyotik bakterilerin düşük pH da özellikle de meyve sularında gelişimi oldukça güçtür. Bifidobakteriler laktobasillere göre asidik ortamda gelişmeye karşı daha hassastırlar ve asidik ortamda canlılıklarını kolayca kaybedebilmektedirler. Büyüme faktörlerinin mevcudiyeti, süt ürünlerinde probiyotik bakterilerin canlılığını da etkilemektedir (Pérez-Conesa vd., 2005).

Bifidobakteriler anaerobik koşullarda gelişen mikroorganizmalardır ve gıdalardaki sayılarında hızlı düşüşün, çözünmüş oksijen, hidrojen peroksit, süperoksit ve hidroksil radikalleri (Carlsson vd., 1978) gibi oksijen türevlerine veya ambalajın oksijen geçirgenliğine bağlı olabileceği saptanmıştır.

Probiyotik gıdaların canlılıklarını etkileyen en önemli faktörlerden biri ürünün nem almasıdır. Probiyotik bakteri içeren gıdalarda nem oranının artması sonucu suyun doku içinde hareketliliği artarak probiyotiklerin canlılıklarında kayıplar meydana gelmektedir (Ying vd., 2010)

Weinbreck vd., (2010) tarafından yapılmış bir çalışmada süt ve süt ürünlerinde sık kullanılan bir probiyotik bakteri olan *Lactobacillus rhamnosus* GG (LGG) suşunun enapsüle edilmiş ve edilmemiş formda üretildikten sonra yüksek oranda su aktivitesine maruz bırakılmış ve bunların depolama süresince canlılıkları tespit edilmiştir. Sonuçlar yüksek su aktivitesinin (0.7) enkapsüle edilmemiş LGG'nin canlılık oranını azalttığını ve 2 hafta içinde hücre canlılığında 10 logdan fazla bir kayba yol açtığı gösterilmiştir.

Probiyotik bakterilerin depolama koşullarına bağlı olarak canlılıklarını olumsuz yönde etkileyen bir diğer koşul depolama ortamındaki oksijen varlığı ve redoks potansiyelidir. Bu çalışmada bu faktör üzerine odaklanılmamış olmakla beraber probiyotiklerin ambalaj açıldıktan sonra oksijene maruz kalmaları durumunda canlılıklarının azalacağı da göz ardı edilmemelidir.

Bu çalışmanın amacı, keçi sütünün ve probiyotiklerin kullanım olanaklarının artırılmasına yönelik olarak probiyotik ve oligosakkarit ilaveli keçi sütü bazlı devam formülünde depolama süresince probiyotiğin canlılık, nem, pH değerlerini saptamaktır.

Materyal ve Metot

Materyal

Prebiyotik olarak fruktooligosakkarit (Sigma-Aldrich) kullanılmıştır.

Test bakterisi olarak *Bifidobacterium animalis* subsp. *lactis* (BB-12) kullanılmıştır.

Besiyeri olarak De Man, Rogosa and Sharpe (MRS) Agar (Merck Ürün Kodu: 1.10660.0500) kullanılmıştır. Ayrıca bacteriological peptone (Oxoid Ürün Kodu: LP0037), Phosphate buffered saline tablet (Sigma-Aldrich Ürün Kodu: P4417), L-sistein HCl anhydrous (Sigma-Aldrich Ürün Kodu: C1276), anaerobic jar (Merck Ürün Kodu: 1.16387.0001), Microbiologia Anaerocult A (Merck Ürün Kodu: 1.13829.0001), Microbiologie Anaerotest (Merck Ürün Kodu: 1.15112.0001) kullanılmıştır.

Ambalaj materyali olarak hermetik olarak kapatılmış teneke kutu kullanılmıştır. Ambalajlar, Silgan Öntaş Amb. San. ve Tic. A.Ş. (İzmir)'den temin edilmiştir.

Devam formülü üretimi, Keçicik Süt Endüstrisi ve Gıda Sanayi Ticaret A.Ş. (Aydın) firmasının bebek formülü ve devam formülü fabrikasında gerçekleştirilmiştir. Devam formülü üretiminde kullanılan keçi sütü ve diğer ham maddeler yine Keçicik firması tarafından sağlanmıştır.

Yöntem

Keçicik Süt Endüstrisi ve Gıda Sanayi Ticaret A.Ş. tarafından üretilen devam formülüne *Bifidobacterium animalis* subsp. *lactis* (BB-12) ve fruktooligosakkarit ilave edilerek probiyotikli devam formülü üretimi gerçekleştirilmiş ve hermetik olarak kapatılmış 400 gramlık teneke kutular halinde ambalajlanmıştır. Probiyotikli devam formülünün 3 gün, 15 gün ve 30 gün süresince nem ve pH analizleri gerçekleştirilmiş ve devam formülünde probiyotiğin canlılığı test edilmiştir.

Üretilen toz devam formülünün kullanımı için hazırlanan örnek beslenme tablosu Tablo 1'de görüldüğü gibidir. 1 ölçek toz devam formülü yaklaşık 4.3 gramdır.

Tablo 1'deki örnek beslenme tablosuna göre günlük devam formülü tüketim miktarı; 4.3 gram x 8 doz x 4 günlük öğün sayısı = 137.6 gram / gün'dür. Ambalajlı 400 gramlık probiyotikli devam formülünün bitme süresi yaklaşık 3 gündür.

Tablo 1. Örnek beslenme tablosu

Table 1. Table of example nutrition

Yaş (Ay)	Su (mL)	Ölçek sayısı	Günlük Öğün Sayısı
6-12 ay	240	8	4

Üç günlük çalışmada probiyotikli devam formülü tüketimi, evde annelerin devam formülünü kullanımı taklit edilerek tasarlanmıştır. Birinci gün, kutu açılmıştır ve takip eden her gün, bebek tarafından günlük olarak tüketilmesi gereken miktarda probiyotikli devam formülü kutudan alınmış ve tüm ambalaj (400 g) bitinceye kadar yani 3 gün boyunca devam formülünün nem analizleri, pH analizleri ve devam formülünde probiyotiğin canlılık analizleri gerçekleştirilmiştir.

İkinci bir çalışmada kutu açıldıktan sonra 15 gün süresince devam formülünün nem analizleri, pH analizleri ve devam formülünde probiyotiğin canlılık analizleri gerçekleştirilmiştir. Analiz edilecek bir numune alındıktan sonra kalan probiyotikli devam formülü oda sıcaklığında muhafaza edilmiştir.

Üretilen probiyotikli devam formülünün 400 gramlık kapalı teneke kutuda 1 ay boyunca depolanması süresince de devam formülünün nem analizleri, pH analizleri ve devam formülünde probiyotiğin canlılık analizleri gerçekleştirilmiştir. Analiz edilecek bir numune alındıktan sonra kalan probiyotikli devam formülü oda sıcaklığında muhafaza edilmiştir.

Canlılık analizleri için probiyotikli devam formülünden 10 g alınıp 90 mL PBS içerisinde (Macfarlane ve Englyst 1986; Ingham 1999) süspanse edilmiş (ilk dilüsyon) ve 30 dakika oda sıcaklığında numunenin dağılımına kadar tutulmuştur. Daha sonra bu ilk dilüsyondan başlayarak % 0.1 lik peptonlu su içerisinde seri dilüsyonlar hazırlanmış ve numunenin seri dilüsyonlarından 3'er paralel olarak çalışılmıştır.

Bifidobacterium animalis subsp. *lactis* (BB-12) sayımı L-sistein HCI ile desteklenmiş MRS agar ile 2 katlı dökme plak yöntemi kullanılarak gerçekleştirilmiştir. Petriler, 37°C sıcaklıkta 48 saat anaerobik kavanoz ve anaerocult A yardımıyla sağlanan anaerobik ortamda inkübe edilmiştir. Böylece test bakterisi sayımları gerçekleştirilmiştir. Sonuçlar log kob/g olarak belirlenmiştir.

200 mL saf suya 1 adet Phosphate buffered saline (PBS) tablet eklenmiş, magnetik karıştırıcıda homojen olarak karıştırma sonucu PBS sıvı ortamı elde edilmiştir.

0.1 g bakteriyolojik pepton tartılıp 100 mL balonjojede saf su ile tamamlanmıştır. Magnetik karıştırıcıda homojen dağılımı sağlanıp %0.1 (w/v)'lik bakteriyolojik peptonlu su elde edilmiştir. Daha sonra 9 mL'lik tüplere dağıtılıp otoklavlanmıştır.

Infrared nem tayin cihazı (Sartorius MA35) kullanılarak belirlenmiştir. Cihazın alüminyum ölçüm kabının darası alınarak 2.5 g numune tartılıp 105°C'de cihaz çalıştırılmıştır. Numunenin nemin uçurularak sabit ağırlığa ulaşması ilkesine dayanarak termogravimetrik yöntem ile % nem olarak üründeki nem miktarı belirlenmiştir.

Ürünün pH ölçümleri, pH metre (Mettler Toledo seven2go, Almanya) ile yapılmıştır.

Sonuçlar ANOVA analizi gerçekleştirilerek SPSS paket programı (IBM SPSS Statistics V21 x 86, Chicago, IL) kullanılarak incelenmiştir. Elde edilen verilere Duncan Çoklu Karşılaştırma testi uygulanmıştır.

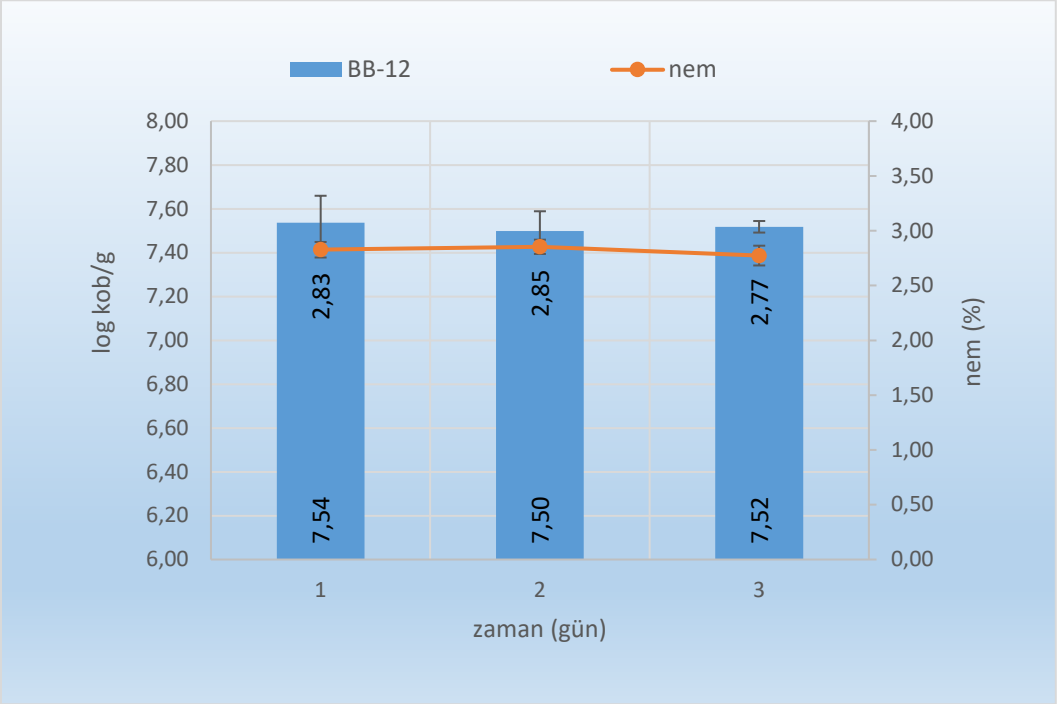
Bulgular ve Tartışma

Üç günlük çalışmada birinci gün devam formülü kutusu açılmıştır ve takip eden her gün, bebek tarafından günlük olarak tüketilmesi gereken miktarda (137,6 g) probiyotikli devam formülü kutudan alınmış ve tüm ambalaj (400 g) bitinceye kadar yani 3 gün boyunca devam formülünün nem analizleri, pH analizleri ve devam formülünde probiyotiğin canlılık analizleri gerçekleştirilmiştir. Probiyotikli devam formülünde *Bifidobacterium animalis* subp. *lactis* (BB-12)'in 3 gün boyunca canlılığı ve probiyotikli devam formülünün nem değerleri Şekil 1'de gösterilmiştir. Üç günlük çalışmada probiyotikli devam formülünün pH değerleri Şekil 2'de gösterilmiştir.

Üç gün boyunca yapılan çalışmada, probiyotikli devam formülünden örnekler alınmıştır. Devam formülünün nem içeriğinde bir artış olup olmadığını belirlemek için numunelerin nemi ölçülmüştür. Nem % 2.77 ile % 2.83 arasında değişmiş, istatistiksel açıdan anlamlı fark göstermemiştir. Bifidobakterinin canlılığını etkileyen diğer bir faktör, probiyotik bakterileri içeren ürünün pH'sıdır. Ürünün pH değeri ilk gün 6.92 ve son iki gün 6,89 bulunmuştur ve anlamlı farklılık göstermemiştir. Canlılık çalışmasında *Bifidobacterium animalis* subp. *lactis* (BB-12)'in ilk sayımları 7.54 log kob/g iken 3. gün bu değer 7.52 log kob/ml olarak saptanmış olup istatistiki açıdan anlamlı farklılık göstermemiştir. Sayımların sağlık yararlarını sağlamak için önerilen ekleme seviyesinin (6 kob/g) üzerinde kaldığı saptanmıştır.

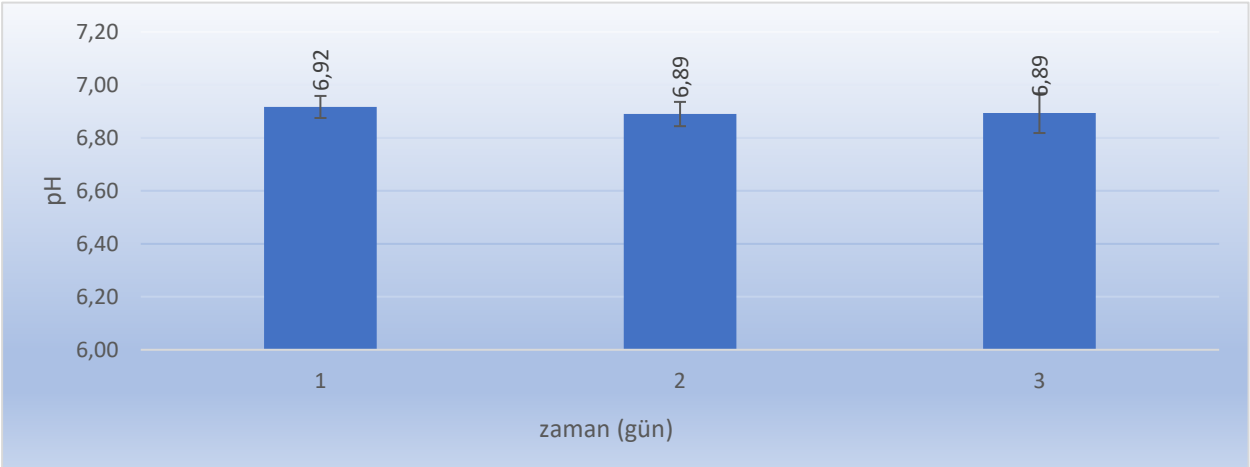
Bir başka deneme olarak da kutu açıldıktan sonra 15 gün süresince devam formülünün nem analizleri, pH analizleri ve

devam formülünde probiyotiğin canlılık analizleri gerçekleştirilmiştir. Analiz edilecek bir numune alındıktan sonra kalan probiyotikli devam formülü oda sıcaklığında muhafaza edilmiştir. Probiyotikli devam formülünde *Bifidobacterium animalis* subp. *lactis* (B- 12)'in 15 gün boyunca canlılığı ve devam formülünün nem değerleri Şekil 3'te görüldüğü gibidir. 15 günlük çalışmada probiyotikli devam formülünün pH değerleri de Şekil 4'te gösterilmiştir.



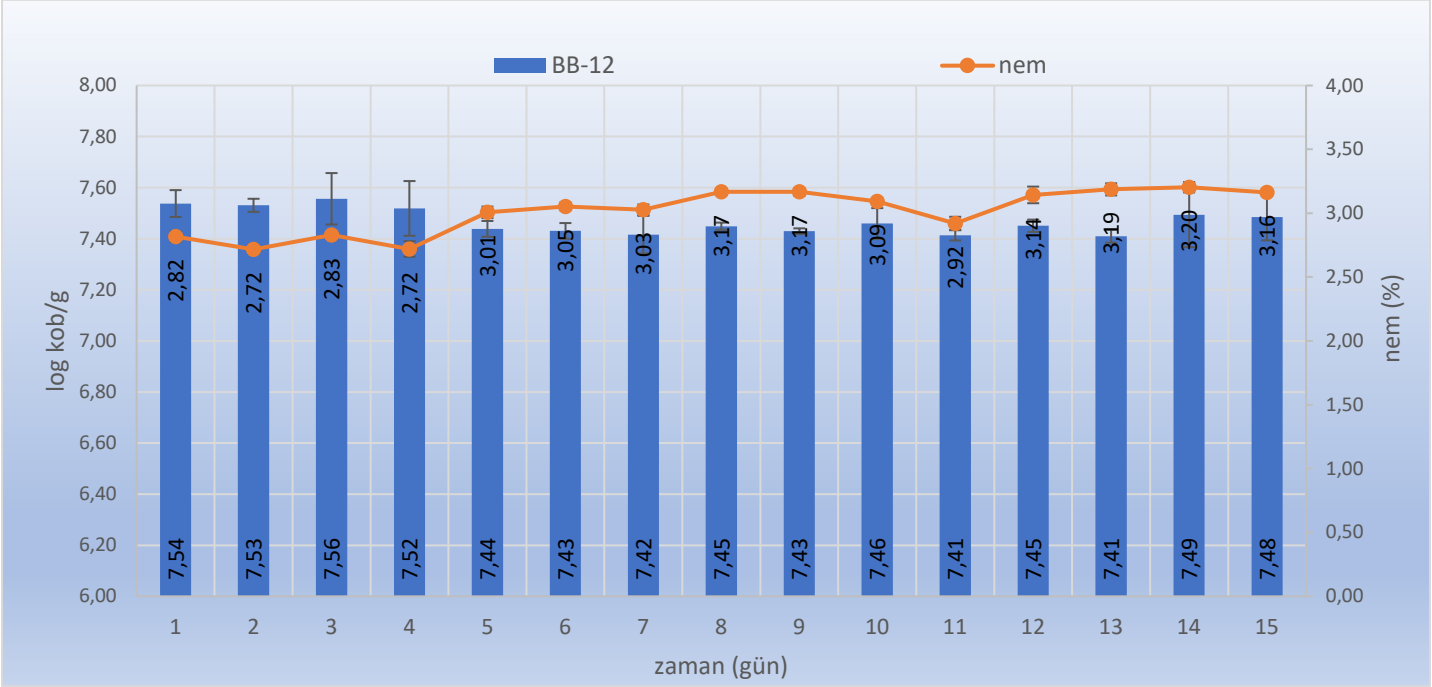
Şekil 1. Fruktooligisakkarit ve *Bifidobacterium animalis* subs. *lactis* (BB-12) ilaveli devam formülünün kutu açıldıktan sonra 3 günlük depolama sürecinde canlılık ve nem değerleri

Figure 1. The moisture and the viability values of the follow-up formula supplemented with *Bifidobacterium animalis* subs. *lactis* (BB-12) and fructooligosaccharide after opening the box during 3 days of storage



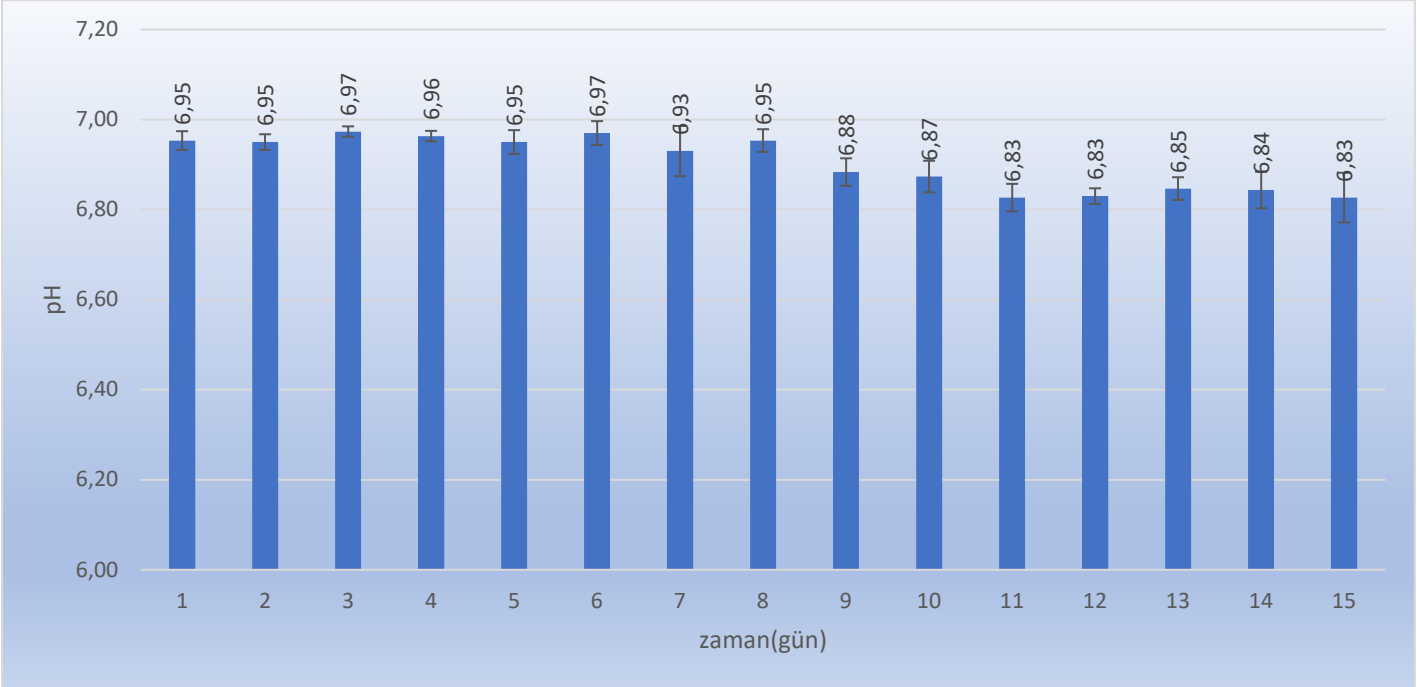
Şekil 2. Fruktooligisakkarit ve *Bifidobacterium animalis* subs. *lactis* (BB-12) ilaveli devam formülünün kutu açıldıktan sonraki 3 günlük depolama sürecinde pH değerleri

Figure 2. pH values of the follow-up formula supplemented with *Bifidobacterium animalis* subs. *lactis* (BB-12) and fructooligosaccharide after opening the box during 3 days of storage



Şekil 3. Fruktooligisakkarit ve *Bifidobacterium animalis* subs. *lactis* (BB-12) ilaveli devam formülünün 15 günlük depolama sürecinde canlılık ve nem değerleri

Figure 3. The moisture and the viability values of the follow-up formula supplemented with *Bifidobacterium animalis* subs. *lactis* (BB-12) and fructooligosaccharide during 15 days of storage



Şekil 4. Fruktooligisakkarit ve *Bifidobacterium animalis* subs. *lactis* (BB-12) ilaveli devam formülünün 15 günlük depolama sürecinde pH değerleri

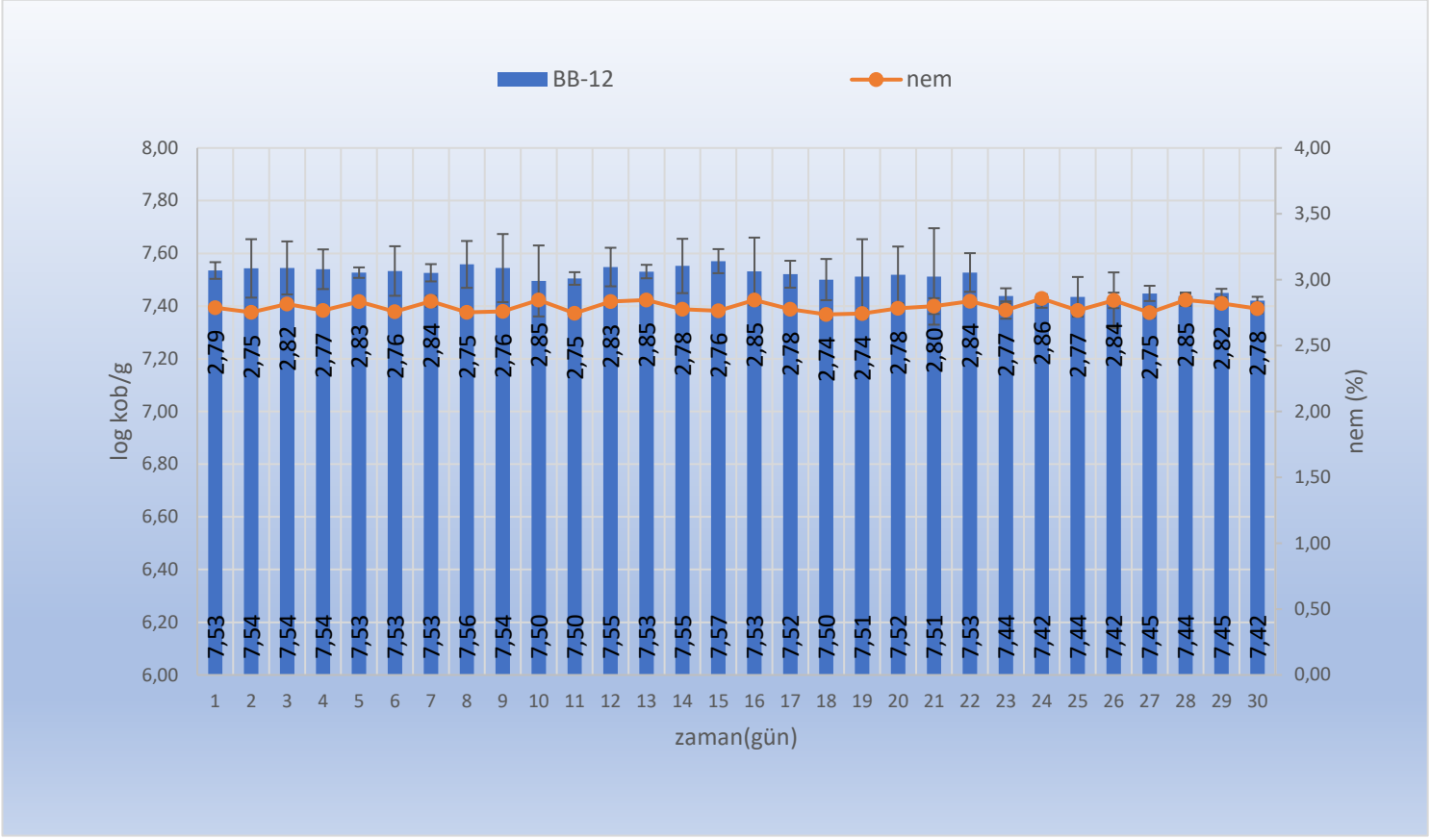
Figure 4. pH values of the follow-up formula supplemented with *Bifidobacterium animalis* subs. *lactis* (BB-12) and fructooligosaccharide during 15 days of storage

3 ve 15 günlük depolama analizleri için birer adet paket açılmış ve kapakları açıldıktan sonra 3 ve 15 günlük periyotlar halinde ürün oda sıcaklığında depolanmış ve canlılık, pH ve nem değerleri tespit edilmiştir.

Şekil 3'te görüldüğü gibi nem oranlarının 15 günlük çalışmada % 2.72 ile % 3.2 arasında değiştiği saptanmıştır. Ürünün pH değeri de 6.83 ile 6.97 arasında değişmiştir. On beş gün boyunca yapılan çalışmada (Şekil 3), beşinci günde probiyotik sayımları azalmış, 7.44 log kob/g'a kadar düşmüş, ancak sağlık yararları sağlamak için önerilen ekleme seviyesinin (10^6 kob/g) üzerinde kalmıştır. Takip eden günlerde, sayımlar, 7.41 log kob/g'a kadar gerilemiştir. Ancak 3 günlük çalışmada da olduğu gibi, sayım son günde de önerilen ekleme seviyesinin üzerinde kalmıştır ve anlamlı farklılık göstermemiştir.

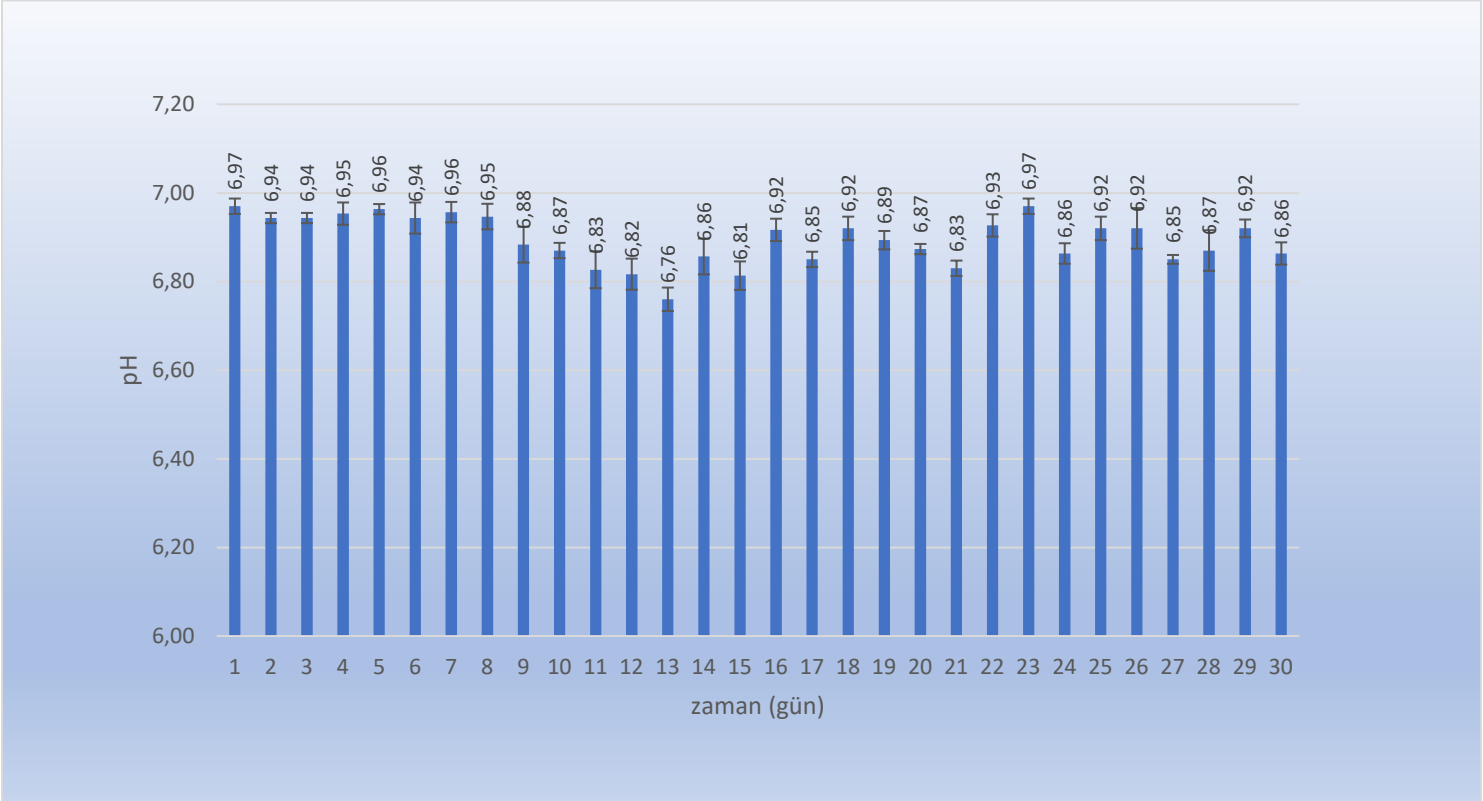
Üretilen probiyotikli devam formülünden 400 gramlık 30 adet ürün kapalı teneke kutuda ambalajlanmış ve her gün yeni bir kutu açıldıktan sonra günlük devam formülünün nem analizleri, pH analizleri ve devam formülünde probiyotiğin canlılık analizleri gerçekleştirilmiştir. Probiyotikli devam formülünde *Bifidobacterium animalis* subp. *lactis* (BB-12)'in 30 gün boyunca canlılığı ve devam formülünün nem değerleri Şekil 5'te görüldüğü gibidir. 30 günlük çalışmada probiyotikli devam formülünün pH değerleri Şekil 6'da gösterilmiştir.

Nem 30 günlük çalışmada ise % 2.75 ile % 2.86 arasında değişmiştir. Ürünün pH değeri 6.76 ile 6.97 arasında saptanmıştır. 30 gün boyunca yapılan çalışmada, sayımlar 7.42 log kob/g'a kadar düşmüştür. Ancak *Bifidobacterium animalis* subp. *lactis* (BB- 12) sayısı son günde de önerilen ekleme seviyesinin üzerinde kalmıştır ve anlamlı farklılık göstermemiştir.



Şekil 5. Fruktooligisakkarit ve *Bifidobacterium animalis* subs. *lactis* (BB-12) ilaveli devam formülünün 30 günlük depolama sürecinde canlılık ve nem değerleri

Figure 5. The moisture and the viability values of the follow-up formula supplemented with *Bifidobacterium animalis* subs. *lactis* (BB-12) and fructooligosaccharide during 30 days of storage



Şekil 6. Fruktooligisakkarit ve *Bifidobacterium animalis* subs. *lactis* (BB-12) ilaveli devam formülünün 30 günlük depolama sürecinde pH değerleri

Figure 6. pH values of the follow-up formula supplemented with *Bifidobacterium animalis* subs. *lactis* (BB-12) and fructooligosaccharide during 30 days of storage

Süt ürünlerinde bifidobakterilerin yaşayabilirliği hakkında bilimsel birçok çalışma, çeşitli sürelerde buzdolabında saklanan yoğurtlar veya fermente süt ürünlerinde araştırılmıştır. Akalın vd. (2004)'ün çalışmasında yoğurt FOS ile takviye edilmiştir. FOS içeren yoğurtta *Bifidobacterium longum*'un canlılığı, 21 güne kadar 10^6 kob/g'ın üzerinde kalırken, herhangi bir prebiyotik içermeyen yoğurtta sadece 7 gün boyunca muhafaza edilmiştir. Bizim çalışmamızda, ürün pH değeri hiçbir zaman 6.81'in altına düşmemiş ve *Bifidobacterium animalis* subp. *lactis* (BB-12) sayısı önerilen ekleme seviyesi olan 6 log kob/ml'nin üzerinde kalmıştır.

Jayamanne ve Adams (2004)'ün çalışmasına göre plastik kaplarda fermente edilmiş manda sütlerinde, cam şişelerde fermente edilenlere kıyasla *Bifidobacterium longum* NCTC11818'in canlılığı önemli ölçüde azalmıştır. Bizim çalışmamızda ise hermetik olarak kapatılmış teneke kutu kullanılmış, *Bifidobacterium animalis* subp. *lactis* (BB-12) sayısı önerilen seviyenin üzerinde kalmıştır.

Tedarik zincirinin (üretim, depolama, dağıtım araçları, ticari depolama yerleri ve ev koşulları) *Bifidobacterium animalis* subsp. *lactis* BB-12'nin canlılığını etkileyebileceği hesaba katılmıştır. Bu çalışmada elde edilen sonuçlar, probiyotikli devam formülünde *Bifidobacterium animalis* subsp. *lactis* (BB-12) sayısının, probiyotikler için önerilen ekleme seviyesinin 10^6 kob/g üzerinde kaldığını göstermiştir. Bu duruma, muhtemelen toz formdaki devam formülünün oda koşullarında muhafaza edilmesi, pH stabilitesi, koruyucu atmosferde paketlenmesi, düşük nem seviyesi ve oksijen geçirimsiz ambalaj materyali kullanılması katkıda bulunmuştur.

Sonuç

Anne sütünün kullanılmasının mümkün olmadığı ya da yetersiz olduğu durumlarda buna alternatif gıdalar da bebek ve devam formülleri gibi özel ürünlerin kullanımı gündeme gelmektedir. Piyasada bulunan formüller incelendiğinde marketlerde ve eczanelerde bulunan bebek ve devam formüllerinin

çoğunun hammaddesinin inek sütüne dayalı olduğu görülmektedir. Sınırlı sayıda keçi sütüne dayalı formülasyona sahip ürün bulunmaktadır. Bu ürünlerin de yine büyük bir çoğunluğu probiyotik ilavesizdir. Son yıllarda keçi sütünün insanların beslenmesinde düşük alerjik etkileri nedeniyle daha yoğun kullanılması ve probiyotiklerin bilinen yararlı özelliklerinin bebek formülasyonlarında kullanılması bu çalışmanın ortaya çıkmasını sağlamıştır. Bundan dolayı son yıllarda probiyotik bakteri içeren gıdaların sağlık üzerine olan olumlu etkilerinin tespit edilmesi bu ürünlere olan talebin artış göstermesi bu bakterilerin bebek mamasına ilave edilebileceğini de gündeme getirmiştir. Bu çalışmada fruktooligosakkarit ve *Bifidobacterium animalis* subsp. *lactis* (BB-12) keçi sütü bazlı devam formülüne ilave edilmiş ve tüketileceği süre üzerinden canlılık çalışması yapılmıştır. Fermente süt ürünlerinde bifidobakterinin canlılığı için en önemli dezavantajlardan biri ürünün pH değerinin düşmesidir. Bizim çalışmamızda toz haldeki devam formülü, fermente edilmiş ürünlerden farklı olarak rekonstitüe edilip beklemeden hemen tüketildiği için pH değişimi çok olmamıştır.

Fruktooligosakkaritin daha karmaşık bir bakteriyel ortam üzerindeki etkisini değerlendirmek için daha fazla canlı modeli çalışması gerekmektedir. İleriki çalışmalarda canlı hücre hatları kullanılarak, hücre kültür analizleri ile *in vivo* çalışmaların yapılması oligosakkaritlerin test bakterileri üzerindeki etkisini değerlendirmek için daha uygun bir ortam sağlanmış olacaktır.

Etik Standart ile Uyumluluk

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

Etik izin: Araştırma niteliği bakımından etik izin gerektirmemektedir.

Finansal destek: Bu çalışma Aydın Adnan Menderes Üniversitesi Bilimsel Araştırma Projeleri Koordinatörlüğü tarafından desteklenmiştir (Proje No: MF 18003)

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 6(2), 110-116 (2020) • https://doi.org/10.3153/FH20012 Research Article

Antioxidant, total phenolic, ascorbic acid and color changes of *Ocimum bacilicum* L. by sun and microwave drying

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Cite this article as:
Turgay, Ö., Esen, Y. (2020). Antioxidant, total phenolic, ascorbic and changes of *Ocimum basilicum* L. by sun and microwave drying. *Food and Health,* 6(2), 110-116. https://doi.org/10.3153/FH20012

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Submitted: 02.07.2019
Revision requested: 10.11.2019
Last revision received: 02.12.2019
Accepted: 15.12.2019
Published online: 24.02.2020

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ABSTRACT

Basil is a plant consumed fresh and dried in Mediterranean cuisine. The effect of drying on antioxidant activity, total phenolic content, ascorbic acid content and color of *Ocimum bacilicum* L. from Turkey was investigated. The samples were dried by two methods: sun drying (RH 43-55% for 2 days) and microwave drying (400 watts for 8 min). Total phenolics contents were ranged from 34.1 to 62.2 mg GAE/g DM). The antioxidant activity based on the DPPH IC_{50} assay of the basil extracts varied from 104 to 149.7. The initial content of ascorbic acid in basil was 134.3 mg/ 100g. The ascorbic acid content of sun and microwave dried samples were 32.5, 25.5 mg/ 100g, respectively. The values for the L^*, a^* and b^* coordinates of the fresh basil were 55.14, -17.13 and 27.76, sun and microwave dried basil samples were 45.63, -1.73, 16.79, and 39.17, -12.19, 21.62, respectively. As a result of this study, it was determined that dried basil samples contain more total phenolic component and have more antioxidant activity than fresh basil samples. It is also possible to deduce from the results that heat-drying causes high levels of ascorbic acid degradation and causes significant changes in color materials.

Keywords: *Ocimum bacilicum* L., Antioxidant activity, Total phenolic content, Ascorbic acid content

Introduction

Polyphenols are responsible for various health benefits and suggested to be a major bioactive compound of plants. Antioxidants play a major role to avoid food degradation, prevent many diseases and aging. Recent researches have shown that the consumption of plant polyphenols may protect against the cardiovascular disease and certain forms of cancer (Gross, 2004; Neuhouser, 2004). Intake of natural antioxidants could be increased body defense mechanism as anticarcinogen (Ames, 1983). Lots of plants, spices and herbs contain antioxidative, antimicrobial, antimutagen and other nutritional constituents in their tissues (Ateş and Erdoğrul, 2003; Erdoğrul, 2002; Simon et al., 1999).

Basil is known for its substantial genetic heterogeneity with between 65 and 150 species (Makri and Kintzios, 2007). *Ocimum bacilicum* L. or basil is a worldwide cultivated plant under a variety of ecological conditions but originating in warm tropical climates of India, Africa and southern Asia (Putievsky and Galambosi, 1999). Basil can used traditionally as culinary in the Mediterranean and Southeast Asian foods. It is using in the treatment of a headache, cough, diarrhea and kidney malfunctions, against insect bites, acne and it has long been used to flavor foods, as well as dental and oral products (Simon et al., 1984). Local sweet basil, lemon basil, purple ruffle and mintier Egyptian basil are most commonly used in European and American cuisine.

One of the oldest methods of preserving foods is drying or desiccation. The preservation of foods by drying is a direct removal or binding of moisture, which stops the growth of microorganisms. The content of the moisture of the vegetables should be reduced below 4% to have satisfactory storage life and quality (Jay, 2000). Fruits, vegetables and herbs are often dried by sunlight. Studies about the different drying methods on different matrixes have been attracting the attention of scientists.

Main objectives of this work were to study the antioxidant activity, total phenolic content, ascorbic acid content and color of *Ocimum bacilicum* L. from Turkey and effect of drying to these parameters were also determined.

Materials and Methods

All parts of cultivated *Ocimum bacilicum* L. were purchased from Kahramanmaraş local markets during summer (June-July 2018). Fresh, sun and microwave dried leaves of basil samples were analyzed according to their antioxidant activity, ascorbic acid content, total phenolic content and color changes in three replicates. Taxonomic identification of the samples was conducted by the KSU Department of Biology.

Extraction

For both fresh and dried samples, 1 g of basil samples extracted into 20 mL of acidified (with 1% hydrochloric acid, v/v) methanol (80 %) on a shaker (170 rpm) for 2 h and filtered by Whatman paper (No:4). The same extract was used for both total phenolic content and antioxidant activity analysis.

Total Phenolic Content

The concentration of total phenols in extracts was measured by UV spectrophotometer (PG Instruments 25 UV/VIS), based on a colorimetric oxidation/reduction reaction. The oxidizing agent was Folin-Ciocalteu reagent (Merck) (Ranilla et al., 2010). For this purpose, 0.1 mL of diluted methanol extract (1%) and 2 mL of Na_2CO_3 (2%) was added and incubated for 5 minutes, 0.1 mL of Folin-Ciocalteu reagent was added and incubated for 60 min at room temperature in the dark. Distilled water was used for a control sample. The absorbance was measured at 760 nm. The results are expressed as mg gallic acid equivalents/1 g in dry matter (mg GAE/g DM).

DPPH Scavenging Activity

The capacity to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma) free radical was monitored according to a method reported before (Hatano et al., 1988). Various concentrations of sample extracts (0.1, 0.2, 0.3 mL) were mixed with (2.9, 2.8, 2.7 mL) methanol and 1 mL of methanolic solution containing DPPH radicals (0.1mM) added to the mixture. The mixture was shaken hard and left in the dark until stable absorption values were obtained. The reduction of the DPPH radical was measured by monitoring continuously the decrease in absorption at 517 nm. DPPH scavenging effect was calculated as a percentage of DPPH discoloration using the equation: % scavenging effect = $[(A_{DPPH} - A_S)/A_{DPPH}]$ x100, where A_S is the absorbance of the solution when the sample extract has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution. Scavenging activity in this assay was expressed as IC50, which represents the concentration of the extract (mg/mL) required to inhibit 50% of the free radical-scavenging activity. Butylated hydroxytoluene (BHT) was used as a positive control.

Ascorbic Acid Content

A 10 mL aliquot of the sample was placed into a 100 mL volumetric flask and brought to volume with 0.4% oxalic acid solution. The solution was filtered by Whatman No. 4 filter paper. A 10 mL aliquot of the filtered solution was pipetted into a conical flask along with 15 mL of 0.4% oxalic acid solution. The solution was titrated, using a microburette, with

0.04% aqueous sodium dichlorophenolindophenol solution to the first pink shade. The sodium dichlorophenolindophenol solution was standardized with sodium thiosulfate 0.01 N, in a matrix of potassium iodide (50%) and HCl 1 N using starch as an indicator. The absorbance was measured at 518 nm. The results were expressed in mg/ 100g for both fresh and dried samples (Hışıl, 1993).

A 10 mL portion of the sample was put in a 100 mL volumetric flask and completed to the target volume with an oxalic acid solution that is 0.4%. The solution was filtered through Whatman No 4 filter paper. A 10 mL aliquot of the filtered solution was pipetted into the erlenmeyer with 15 mL of 0.4% oxalic acid solution. The solution was titrated by a microburette with 0.04% aqueous sodium dichlorophenolindophenol solution until the appearance of the first pink tone.

Drying of the Basil Samples

Samples were dried by using two methods, sun drying (RH 43-55% for 2 days) and microwave drying (400 watts for 8 min).

Color Measurement

Color measurement was made before drying and after other drying procedures a Konica Minolta CR-400 model colorimeter. The instrument was standardized each time with a white and a black ceramic plate. The color values were expressed as *L* (whiteness or brightness/darkness), *a* (redness/greenness) and *b* (yellowness/blueness) at any time, respectively (*L*= 97.45, a*=0.00, b*=1.77*). The total color change ($\Delta E = \sqrt{(L_0 - L_1)^2 + (a_0 - a_1)^2 + (b_0 - b_1)^2}$), was the parameter considered for the overall color difference evaluation (Demirhan and Özbek, 2009).

Statistical Analysis

The results of the analysis were subjected to one-way analysis of variance (ANOVA) using a general linear model (GLM) procedure in the SPSS software (SPSS Inc., Chicago, IL). The means were compared for significance at the 5% level using Duncan's multiple range tests.

Results and Discussion

The average concentration of total phenolic content, DPPH scavenging activity and ascorbic acid content of the basil samples were presented in Table 1. Color measurement of the fresh, sun and microwave dried samples were presented in Table 2.

The amount of total phenolics ranged from 34.1 to 62.2 mg GAE/g DM. Fresh basil' s total phenolic content was determined as 34.1 mg GAE/g DM and consistent with the results of Gajula et al. (2009), Javanmardi et al. (2003) and Hossain et al. (2010) studies on different types of fresh basil samples. However, it was seen that it is higher than the results of Śledź et al. (2013), Bušić et al. (2014), Siti Mahirah et al. (2018) studies. The highest total phenolic content was detected in microwave dried samples as 62.2 mg GAE/g DM. It was determined that this result was higher than the results of Bušić et al. (2014).

The antioxidant activity based on the DPPH IC_{50} assay of the basil extracts varied from 104 to 149.7. The antioxidant activity of fresh basil was determined as 149.7. This result was consistent with the results of Gajula et al. (2009) and Bayala et al. (2014) studies. But it was found that higher than the results of Bušić et al. (2014). The highest antioxidant activity was detected in microwave dried samples as 104 (Table1). All of the total phenolic contents and antioxidant activity results were in the range of the previous similar studies.

Table1. The Average Concentration of Total Phenolic Content (TPC), DPPH Scavenging Activity (DSA), Ascorbic Acid Content (AAC) of Basil Samples

Sample	TPC (mg GAE/g DM)	DSA (IC_{50})	AAC (mg/100g)
Fresh basil	34.1[a]	149.7[b]	134.3[a]
Sun-dried	48.2[c]	129.5[b]	32.5[b]
Microwave-dried	62.2[b]	104[a]	25.5[c]

Table 2. Color measurement of the fresh, sun and microwave dried basil samples

Sample	L*	a*	b*	Chroma	Hue	Δ*E*
Fresh basil	55.14	-17.13	27.76	32.62	-35.46	
Sun-dried	45.63	-1.73	16.79	16.88	-5.85	21.16
Microwave-dried	39.17	-12.19	21.62	24.82	-32.29	17.81

L (whiteness or brightness/darkness), *a* (redness/greenness), *b* (yellowness/blueness)

Average antioxidant capacities for the fresh, sun and microwave dried basil samples in this study were determined using DPPH free-radical scavenging assay. It is one of the most commonly used methods to evaluate antioxidant capacity. The 2,2-diphenyl-2-picrylhydrazyl radical has been used to evaluate the free radical scavenging capacity of antioxidants (Yu, 2001). It is possible to determine the antiradical power of an antioxidant activity by measurement of the decrease in the absorbance of DPPH- at 517 nm. The color change from purple to yellow, the absorbance decreased when the DPPH- was scavenged by an antioxidant, through the donation of hydrogen to form a stable DPPH- molecule. This molecule had an absorbance at 517 nm in the radical form which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule (Matthäus, 2002)

The Folin-Ciocalteu assay to determine total phenolic concentrations is based on an electron transfer mechanism, and typically has a high degree of linear correlation with DPPH antioxidant capacity. This result also seemed in this study. Furthermore, it has been found that the total amount of phenolic content is better preserved in microwave type drying than in natural drying methods (sun) (Açıkgöz et al., 2015). As reported in a study about the effects of drying on total phenolics of grape skins (Chism et al.,1996), phenolic compounds are frequently found in the outer areas of vacuoles. Thus, phenolic compounds stored outside the organelles are also more susceptible to degradation due to degradation of the cell structure during the drying process (de Torres et al., 2010).

Medicinal plants are used in different types of products as fresh, dried and stored forms (Lin et al., 2011). Methods of using different drying can be performed. Dehydration is one of important preservation method, because it inhibits enzymatic degradation and limits microbial growth (Harbourne et al., 2009; Muller and Heinds, 2006). Normally, the antioxidant (phenolic compounds, vitamins, etc.) content of fresh plant materials is higher than that of dried plant materials, these are degraded during drying. Some recent studies have shown that dried plant materials contain higher antioxidants, such as polyphenols, and antioxidant activity as compared to fresh plant materials (Chang et al., 2006; Choi et al., 2006). Drying also affected the antioxidant activity of fruits and vegetables differently (Choi et al., 2006; Kuljarachanan et al., 2009). The initial content of ascorbic acid in fresh basil was 134.3 mg/ 100g. This result was consistent with the result of Bušić et al. (2014) study. The ascorbic acid content of sun and microwave dried samples were 32.5, 25.5 mg/ 100g, respectively and the differences were statistically important. The degradation of ascorbic acid is considerably affected by the drying conditions, causing losses in almost every case. In addition, Bušić et al. (2014) also reported a decrease in ascorbic acid content of about 22% with drying. Generally, every kind of processing procedure has been decreased nutritional value of fruits and vegetables compared to the fresh samples (Barbosa-Canovas et al., 2008). The decrease of ascorbic acid occurs as the oxidation of ascorbic acid to dehydroascorbic acid (DHAA) (Singh and Rajini, 2004)

Despite a decrease in the ascorbic acid, the increase in the total phenolic content and DPPH scavenging activity has been reported in the present study. A decrease of ascorbic acid, an increase of antioxidant activity was also reported in tomato products processed at high temperatures (Nicoli et al., 1997; Dewanto et al., 2002) and ascorbic acid content was negatively correlated with FRAP values in berries (Pantelidis et al., 2007). Decreased ascorbic acid content accompanied with high antioxidant activity in thermal dried sweet potatoes could be joined with ascorbic acid oxidation and phenol regeneration (Pantelidis et al., 2007; Yang et al., 2010).

Drying of basil samples resulted change in color. The average values of the color parameters for basil in fresh, after sun and microwave-drying are presented in Table 2 for L^* (brightness), a^* (redness), b^* (yellowness), chroma, hue angle and color change. The L^* value represents the change in the lightness level of a sample and is useful to judge the brownness and darkness of leaves after drying. The values for the L^*, a^*, and b^* coordinates of the fresh basil were 55.14, −17.13 and 27.76, respectively. The values for the L^*, a^* and b^* coordinates of the sun-dried and microwave dried basil samples were 45.63, -1.73, 16.79, and 39.17, -12.19, 21.62, respectively. Microwave drying produced no remarkable changes in the color parameters of basil samples as compared with the fresh basil. However, sun drying allowed both coordinates L^* and a^* to rise and b^* coordinate to decrease. The total color difference ΔE, which is a combination of the L^*, a^* and b^* values is a colorimetric parameter extensively used to characterize the variation of color in foods during processing.

The fresh basils results were consistent with the results of Śledź et al. (2013), but the ΔE result of the microwave dried sample which in the present study was higher than it. However, similarly, a decrease was observed when the results of microwave dried samples were compared. L^*, a^* and b^* values found in another study on Basil were lower than the results obtained in the present study. But the Chroma value was approximately the same. In the same study, the ΔE result for the sun dried sample coincided with the result of the present study (Bušić et al., 2014). The decrease of a^* and b^* values may be due to decomposition of chlorophyll and other pigments and non-enzymatic reactions (Maskan, 2001). The

browning reactions occurring during drying can have a significant impact on the final color of the product. The enzymatic reaction due to PPO and Maillard reaction are the contributing factors to this change in color. The total color difference ΔE, which is a combination of the L^*, a^* and b^* values is a colorimetric parameter extensively used to characterize the variation of color in foods during processing. The color difference parameter had a value of 21.16 to the basil sun-dried and decreased to 17.81 the microwave dried samples.

Conclusion

Studies about health promotion by plant phytochemicals have been increasingly attracting the attention of scientists. The results of the present work showed that dried plant materials contain higher total phenolics and antioxidants, drying enhanced antioxidant activity by the increasing of phenolic compounds rate. However, from the results of the present study, it was possible to conclude that thermal drying caused a serious degradation of ascorbic acid and made color changes in basil samples.

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: No animals are used in this study

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 6(2), 117-127 (2020) • https://doi.org/10.3153/FH20013 Research Article

Extracellular phytase activites of lactic acid bacteria in sourdough mix prepared from traditionally produced boza as starter culture

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Cite this article as:
Doğan, M., Tekiner İ.H. (2020). Extracellular phytase activites of lactic acid bakteria in sourdough mix prepared from traditionally produced Boza as starter culture. *Food and Health,* 6(2), 117-127. https://doi.org/10.3153/FH20013

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Submitted: 01.11.2019
Revision requested:
no revision requested
Last revision received: -
Accepted: 23.01.2020
Published online: 27.02.2020

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ABSTRACT

Fermentation using Lactic Acid Bacteria (LAB) and LAB species can exhibit extracellular activities such as decreasing of antinutritional factors, in particular phytic acid (PA) or phytate. The objective of this study was to assess extracellular phytase activities of LAB in sourdough mix prepared from traditionally produced boza as starter culture. To do this, thirthy-five boza samples were collected from Central Anatolia, Marmara and Eastern Anatolia regions in Turkey to be used as starter culture for preparing sourdough mix. In each mixture, LAB strains and phytase (+) ones were screened by culture-based examination, characterized by VITEK® MS, and extracellular phytase activity of each LAB strain was determined by spectrophotometry. Overall, 29 presumptive strains of LAB were isolated. Of them, 21 were found to be phytase (+). The average extracellular phytase activity was 656.8±188.1 U//mL, and a *Pediococcus pentosaceus* EK1 isolate showed the highest activity as 1285.5 U/mL. In conclusion, the traditionally produced bozas have been found as potential starter culture reservoirs for sourdough fermentation with significantly higher extracellular phytase activities, thus challenging opportunitites to lower antinutritional factors, in particular phytic acid (PA) or phytate in the foods for the consumers.

Keywords: Boza, Fermentation, Health, Lactic Acid Bacteria, Phytic acid, Phytase, Sourdough

Introduction

Cereals and cereal-based products are a good source of phenolic compounds, lignans, phytosterols, phytic acid, fiber, vitamins, minerals and other biologically active compounds. However, they are rich in phytic acid (myo-Inositol (1, 2, 3, 4, 5, 6)-hexakisphosphate, InsP6) or salts, also known as phytates. Phytic acid (PA) is a naturally occurring compound found in all seeds and cells of plants. It accumulates up to seed ripening during development, and phosphorus is its main form of storage accounting for 60% of total phosphorus content in cereals, legumes, nuts and oil seeds (Lott et al., 2000; Grases et al., 2017).

Many studies show that a diet based on foods with high phytate content may cause anemia and deficiencies in mineral absorption. Phytate levels can be reduced by phytases, which are the valuable enzymes by phtate hydrolysis. Phytate hydrolysis produces low myo-inositol phosphates by enzymatic degradation. This enzymatic degredation can be achieved by increasing activity of phytase, or adding phytase active microorganisms (Hurrell et al., 2003; Shi et al., 2004; Nuobariene et al., 2015; Moll & Davis, 2017).

Traditional cereal-fermented products are widely consumed all over the world, in particular in Asia and Africa. For instance, boza is one of the well-known fermented cereal-based beverages. To make boza, a ground amount of different cereals such as millet, corn, rice, rye, oats, and wheat is cooked with water, and the mixture is allowed for fermentation by adding sugar. There exist diverse microorganisms in the boza occurring from raw materials, production process and storage conditions. On the other hand, the dominant microflora mainly include LAB (Osimani et al., 2015; Petrova & Petrov, 2017).

The food industries and scientific related areas are emphasizing the capacity of fermentation using LAB species to improve the nutritive quality of cereals and cereal-based foods by decreasing of some antinutritional factors such as PA or phytate, tannins and enzyme inhibitors. The activities of LAB sepcies during cereal fermentation produce a broad range of metabolites and compounds, including organic acids, exopolysaccharides, antimicrobial compounds, and useful enzymes LAB species encoding phytases may be utilized as starter culture suitable for legume and cereal fermentations (Sumengen et al., 2013; Rollán, Gerez, & LeBlanc, 2019).

Only a few strains of LAB have been reported to show intracellular phytase activity (Lopez et al., 2000; De angelis et al., 2003; Reale et al., 2004), whereas there have been other studies reporting that LAB involved in sourdough fermentation exhibits extracellular phytase activities (Cizeikiene et al., 2015; Karaman et al., 2018; Yildirim and Arici, 2019).

In this study, we aimed to assess extracellular phytase activities of LAB in sourdough mix prepared from traditionally produced boza as starter culture.

Materials and Methods

Collection of Boza Samples

During the year 2019, thirty-five traditionally produced boza samples were collected from the boza producers located in the Regions of Marmara (n=15), Central Anatolia (n=10) and Eastern Anatolia (n=10) in Turkey. All the collected samples were taken to the laboratory under sterile conditions at 4°C until further analysis.

Chemicals and Reagents

The chemicals and reagents used in this study were DeMan, Rogosa and Sharpe (MRS) agar (Merck 1.10660, Germany), MRS Broth (Merck 1.10661), M17 agar (1.15108 Merck) and M17 broth (Merck 1.15029) for cultural examination, preidentification and storage of LAB strains from sourdoughs; crystal violet, safranin and lugol dyes for biochemical and morphological tests; physiological saline solution (PSS) (8.5 g NaCl dissolved in water, autoclaved 15 minutes at 121°C, and cooled to room temperature) for dilution, and 20% glycerol (Merck 10494) for store of culture, respectively. 0.1% sodium phytate (Sigma Aldrich 68388, Germany) and 0.2% glucose to MRS/M17 Broth medium (52.2 g/L) was used for phytase (+) LAB strains (Media with a pH of 6.2 sterilized at 121°C for 15 minutes at 1.2 atm. In the identification of phytase (+) LAB strains, *Escherichia* (*E.*) *coli* ATCC® 25922™ for positive testing control, and 1 µL alpha-cyano-4-hydroxycinnamic acid (CHCA) matrix solution for crystallization of the strain to be tested according to the instructions by VITEK® MS (bioMerieux, Marcy I'Etoile, France). Finally, 100 mM sodium acetate (Sigma Aldrich W302406) - acetic acid (Sigma Aldrich W200603) buffer, and 500 µl of 10% (w/v) trichloroacetic acid solution (TCA) (Sigma Aldrich T3399) for determination of extracellular phytase activities of the phytase (+) LAB. All the chemicals and reagents were prepared according to the Instructions by ISO 11133 (2014), Songré-Ouattara et al. (2008), Raghavendra & Halami (2009), and Dubois et al. (2012).

Preparation of Sourdough

Ten grams of boza sample were initially mixed with 150 g of whole-wheat flour, 2 g of table salt and 350 mL of drinking water in a mixer for 5 minutes. Subsequently, the blend was allowed for fermentation at 35°C for 24 hours. At the end of the duration, 50 g of whole-wheat flour and 25 mL of drinking water more were added to the dough, the dough was kneaded for 1 minute, refreshed, and left to ferment again at

35°C during 10 days. At every 24 hours, 50 g whole-wheat flour and 25 mL of drinking water were added to the dough, and the dough was kneaded for 1 minute as previously suggested by Menteş al. (2007).

Culture-Based Analysis and Isolation of LAB Strains

The cultural examination of the suspected LAB strains were made according to the Instructions by ISO 11133 (2014) and ISO 6887-6 (2013). Ninety mL of PSS was added to 10 grams of the homogenized and fermented sample to prepare serial dilutions of 10^{-2} and 10^{-3}, respectively. After that, 1 mL of the diluted suspension was transferred to MRS agar or M17 agar, allowed for incubation at 37°C for 24-48 hours (NÜVE EN-500, Ankara, Turkey). At the end of the incubation, suspected LAB colonies were examined morphologically under microscope. To ensure the purity of the suspected colonies, MRS and/orM17 were inoculated into broth tubes, and activated at 37°C for 24 hours under aerobic/anaerobic conditions. Then, the matte-cream colored colonies were evaluated as LAB strains. Dyeing was performed for pure cultures; Gram (+), cocci and rods were determined under the light microscope, and followed by the catalase test. Those negative for catalase test were selected.

Detection and Enumeration of Phytase (+) LAB Strains

To detect phytase (+) LAB strains, sodium phytate MRS/M17 broths were prepared to inoculate the suspected LAB strains with 200 µl active cultures. Then, the suspensions were allowed for incubation at 37°C for 24 hours (NÜVE EN-500, Ankara, Turkey). After incubation, 100 µl of the incubated culture were inoculated into MRS/M17 agar containing sodium phytate, and left for incubation at 37°C for 24 hours, 48 hours, and 96 hours. Phytase production of the strain was determined by production of clear zones (in millimeters) around the colonies on the sodium phytate containing medium as previously described by Bae et al. (1999) and Songré-Ouattara et al. (2008). For enumeration of phytase (+) LAB strains, 100 µl of the MRS/M17 broth suspension containing sodium phytate were pipetted, and transferred to an Eppendorf tube containing 900 µl of PSS to obtain a diluted culture of 10^{-1}. Following that, 100 µl of the homogenized sample were taken, and serial dilutions from 10^{-1} to 10^{-7} were prepared. Among these dilutions, 100 µl of each dilution from 10^{-4} to 10^{-7} were spreaded on MRS/M17 agar. After incubation at 37°C for 24 hours, the viable bacterial were counted in 30-300 colony-containing petri dishes (Songré-Ouattara et al., 2008; Tharmaraj and Shah, 2003).

Characterization of Phytase (+) LAB Strains Using MS

The phytase (+) LAB strains were characterized using VITEK® MS according to the manufacturer's instructions. A reference strain of *E. coli* ATCC® 25922™ was used for the positive test control (Dubois et al., 2012).

Determination of Extracellular Phytase Activity

One unit of phytase activity (U) is defined as the amount of enzyme producing one nmol of inorganic phosphorus per minute at 50°C. Phytase enzyme activity was calculated by incubating the sourdough mix prepared with 250 µl cell suspensions and 250 µl of 2 mM substrate in 100 mM sodium acetate-acetic acid buffer for 15 minutes at 50°C (NÜVE EN-500, Ankara, Turkey). A blind tube was prepared by adding 10% TCA solution before adding the substrate. Then, reaction was stopped by adding 500 µl of 10% (w/v) TCA. Finally, inorganic phosphate was calculated at 700 nm using iron sulfate-ammonium molybdate method by a UV-VIS spectrophotometer (Shimadzu UV-1280, Kyoto, Japan) (Raghavendra & Halami, 2009).

Results and Discussion

In this study, the extracellular phytase activity of the LAB strains isolated from the sourdough mix prepared from the traditionally produced boza samples as starter culture were assessed. Our study showed that 29 presumptive strains of LAB were isolated. Of them, 21 (1 *Enterococcus faecium,* 5 *Lactobacillus casei*, 1 *Lactobacillus fermentum*, 4 *Lactobacillus pentosus*, 3 *Leuconostoc lactis*, and 7 *Pediococcus pentosaceus*) were found to be phytase (+). The average extracellular phytase activity was 468.2 U/mL and 1285.5 U/mL, and a *Pediococcus* (*P.*) *pentosaceus* EK1 strain showed the highest activity as 1285.5 U/mL.

Sourdough has been produced since 3.000 BC by fermentation method. Since the 19th century, its use has decreased due to faster production and faster consumption habits, and replaced with commercial baker's yeasts, i.e., *Saccharomyces* (*S.*) *cerevisiae*. However, the use of sourdough has started increasing in the recent years due to public interest in healthy eating and artisanal products. Sourdough is a specific ecosystem inhabited by mainly heterofermentative LAB species such as *L. fermentum, L. paralimentarius, L. plantarum*, and *L. sanfranciscensis* and yeasts. The diverse compositions of sourdough microbiota is affected by the diversity of fermentation processes. Sourdough has diverse contributions to the foods, such as improvement of nutritional properties, extension of shelf life, and enhancement of sensory characteristics (De Vuyst et al., 2014; Gänzle & Ripari, 2016; De Vuyst et al., 2017; Kourkouta et al., 2017; Papadimitriou et al., 2019; Catzeddu, 2019). In this study, we prepared the sourdough

mix using the traditionally produced boza as starter culture, instead of utilizing a starter culture such as *S. cerevisiae*, or other sourdough food. This way of fermentation is one of the most widely preferred approaches to making fermented food. The distiributions of the collected boza samples based on the geographical region in Turkey were 28.6% Central Anatolia, 42.9% Marmara, and 28.6% Eastern Anatolia.

Boza is one of the most well known cereal-based fermented drinks. Its pleasant taste, flavor, and nutritional value have made it a very popular beverage among the people of all ages. It is normally produced by fermentation involving mixed cultures of LAB and yeasts. However, LAB is always the basic microflora in the boza with an average LAB/yeasts ratio of 2.4 (Erkmen & Bozoğlu, 2016). Differences between the microflora of boza are related to production processesse, storage temperature and period, and raw materials. The lactic acid fermentation is one of the two different simultanesously occurring types of fermentation in the boza production, which produces lactic acid, and determines the acidic character of this traditional beverage. Vast majority (96.3%) of the strains common in boza were the multiple LAB species (25.6% *Leuconostoc* (*L.*) *paramesenteroides*, 21.9% *L. sanfrancisco* and 18.6% *L. mesenteroides*) (Hancioglu & Karapinar, 1997; Petrova & Petrov, 2017; Irkin, 2019). On the other hand, *L. plant*arum (24%), *L. acidophilus* (23) and *L. fermentum* (19%) were dominant in the Bulgarian boza, whereas *L. plantarum* was the major species isolated from Turkish boza samples (Gotcheva et al., 2001; Kivanc et al., 2011; Lokumcu Altay et al., 2013). A recent study in Turkey by Borcaklı et al. (2018) showed that various LAB involving *Lactococcus lactis*, *leuconostocs* (*L. pseudomesenteroides*, *Lc. lactis*, *Lc. citreum*), and *Lactobacillus* spp. (*L. plantarum*, *L. paracasei*, *L. brevis*, *L. delbrueckii* subsp. *delbrueckii*) were identified as the common members of the microbial community in the boza samples (Borcaklı, Öztürk, & Yeşilada, 2018). In our study, initial cultural examination revealed 29 presumptive LAB strains (1 *E. faecium*, 11 *L. casei*, 1 *L. fermentum*, 6 *L. pentosus*, 3 *L. lactis* and 7 *P. pentosaceus*) from the fermented sourdoughs. Of them, 21 (1 *E. faecium*, 5 *L. casei*, 1 *L. fermentum*, 4 *L. pentosus*, 3 *L. lactis* and 7 *P. pentosaceus*) were found to be phytase (+), whereas 8 (6 *L. casei* and 2 *L. pentosus*) were phytase (-). Our results showed that multiple LAB strains were common in the sourdoughs, and similar to those previously conducted nationall and international works (Table 1 & Figure 1).

Table 1. Results of phytase screening in culturally isolated presumptive LAB strains

No	Name of strain	Result of phtase screening (n)		Origin of boza *
		Phytase (+)	Phytase (–)	
1	*E. faecium*	1	0	CA
2	*L. lactis*	3	0	CA
3	*P. pentosaceus*	7	0	CA, M, EA
4	*L. casei*	5	6	M, EA
5	*L. fermentum*	1	0	M
6	*L. pentosus*	4	2	M, EA
	Total	**21**	**8**	

**CA: Central Anatolia, M: Marmara, EA: Eastern Anatolia*

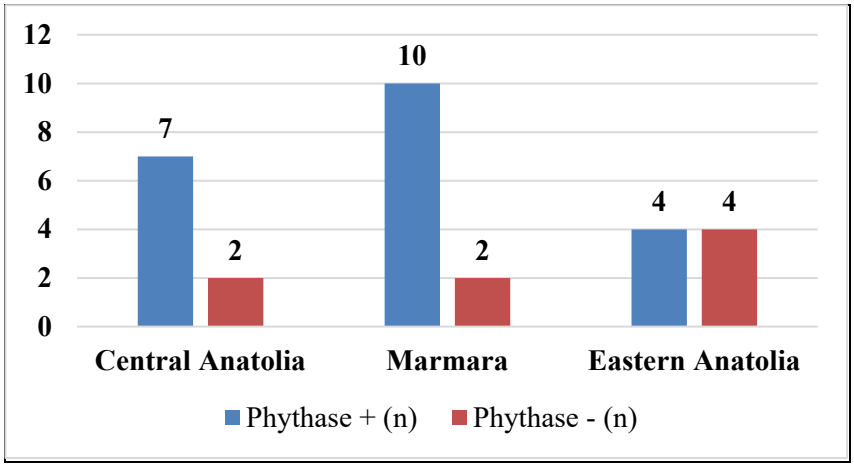

Figure 1. Distribution of phythase (+) and phythase (-) LAB strains based on the origins of the collected boza samples

Phytic acid is an antinutrient because of its ability to bind nutrients such as minerals and proteins, either directly or indirectly, and thus adversely affect their solubility, functionality, absorption, and digestibility (Damayanti et al., 2017). The organisms, including plants, microorganisms, and animal cells have the ability to synthesize phytases. Generally, fungi produce extracellular phytases, whereas bacteria produce the cell-associated enzymes mostly. In the literature, only bacteria exhibiting extracellular phytase activity are those of the genera *Bacillus* and *Enterobacter.* LAB were within the first bacteria to be evaluated because of their involvement in food fermentations and in the human health. However, not all LAB are linked to food fermentations (Papadimitriou et al., 2016). Phytases have gained great interest for biotechnological applications, in particular for the reduction of phytate content in feed and food (Konietzny & Greiner, 2004). Sumengen et al. (2013) studied phytase produced from *L. plantarum* isolated from a fermented food (Shalgam), and determined extracellular and intracellular enzyme activities of *L. plantarum* to be 984.50 U/mL and 494 U/g, respectively (Sumengen et al., 2013). Metabolism of sourdough microbiota and the activity of cereal enzymes are interdependent (Gänzle, 2014). According to Reale et al. (2007), the extent of phytate degradation is mostly independent from LAB strain used for fermentation, and phytate degradation during cereal dough fermentation is positively correlated with endogenous plant phytase activity. Lactic acid fermentation significantly decreases phytate content in plant-based foods. It is widely believed that this reduction is because of the activity of the intrinsic plant phytases, and LAB strains provide suitable conditions for the endogenus cereal phytases by lowering pH value in the medium. So far, only *L. amylovorus* and *L. plantarum* were reported to produce significant extracellular phytase activities. On the other hand, Reale et al. (2007) claims that if a wild-type LAB strain produces extracellular phytase activity, its production can be sufficient for the phytate dephosphorylation during fermentation (Reale et al., 2007). Similarly, Leenhardt et al. (2005) reported that a moderate drop of the dough pH (around 5.5) was sufficient to lower significantly the phytate content of a wholemeal flour (Leenhardt et al., 2005). However, a few strains of LAB have shown consistent phytase activity to degrade phytate by producing extracellular phytases (Anastasio et al., 2010). Therefore, there has been a growing interest in deriving alternate strategies of phytate utilization by probiotics in the human, as they are capable of producing phytase to combat mineral deficiency of zinc and iron (Priyodip, Prakash, & Balaji, 2017). During boza fermentation, phytic acid is catalyzed by the activation of phytase enzyme in LAB, resulting in cause and upsurge of mineral absorption (Borcaklı, Öztürk, & Yeşilada, 2018). Zamudio et al. (2001) investigated the intracellular and extracellular phytase activities of six LAB (*Ped. pentosaceus*, *Leuc. mesenteroides*, *Lact. casei*, *Lact. fermentum*, *Lact. delbrueckii* and *Lact. plantarum*). There was no intracellular phytase activity, whereas *L. plantarum* showed the highest extracellular phytase activity (6.3 mU/mL) (Zamudio et al., 2001). Khodaii et al (2013) reported that *L. casei* from dairy products exhibited higher phytase activity (> 0.004 U) than those isolates from pharmaceutical products (40% versus 27%) (Khodaii et al., 2013). Cizeikiene et al. (2015) showed that the highest extracellular phytase activity produces *Pediococcus pentosaceus* strains from rye sourdough with 32 to 54 U/mL, respectively, under conditions similar to leavening of bread dough (Cizeikiene et al., 2015). On the other hand, a study by Goswami et al. (2017) did not show phytases activity of the LAB strains in the extracellular medium. The specific activities of the studied lactobacilli against phytate varied from 0.03 U/mg to 0.43 U/mg proteins, being the lowest in *L. fermentum* and the highest in *L. Plantarum* (Goswami et al., 2017). In this study, we detected 21 phytase isolates out of 29 presumptive LAB strains in the prepared sourdoughs. At the end of 24 hours, the vitabilities of the phytase (+) isolates varied between 8.52 log cfu/g (*P. pentosaceus* EK1 from Marmara Region) and 3.60 log cfu/g (*P. pentosaceus* NB32 from Central Anatolia region). Phytase production of each strain was mainly determined by production of clear zones around the colonies on the sodium phytate containing medium (Sümengen, Dinçer, & Kaya, 2012). Phytase activity of each strain at the end of 24 hours were changed from 6 mm (*P. pentosaceus* EK1 from Marmara) down to 3 mm (*L. casei* strains from Marmara and Eastern Anatolia, and *P. pentosaceus* from Central Anatolia), respectively. Accordingly, the average extracellular phytase activity was found to be 656.8±188.1 U//mL, and a *P. pentosaceus* EK1 isolated from the sourdough prepared using the boza from Marmara region as starter culture showed the highest activity as 1285.5 U/mL among them, as similar to that reported by Cizeikiene et al. (2015) (Table 2 & Figure 2). Our results showed that the a LAB strain, *P. pentosaceus* EK1, isolated from sorudough mix prepared using traditionally produced boza from Marmara Region as starter culture yieleded a performance of extracellular phytase activity better than the previously identical strains isolated from different sources of foods.

Table 2. Viability and phytase activities of LAB strains

	Isolate type / code	Origin*	V(log cfu/g)**		PA (mm)***			EPA (U/mL)****
			0 h	24 h	24 h	48 h	96 h	24 h
1	*E. faecium* NB32A	CA	4.30	5.93	3.5	4	4	548.2
2	*L. casei* B21	M	3.54	5.69	3	4	4	594.6
3	*L. casei* B31A	M	3.99	5.99	4	5	5	682.7
4	*L. casei* K11	EA	4.41	5.51	3	3	4	487.3
5	*L. casei K22*	EA	3.92	5.11	3	4	4	506.4
6	*L. casei* K32	EA	3.72	5.97	3	4	4	635.3
7	*L. fermentum* B1A	M	3.57	6.93	5	5.5	6	743.7
8	*L. pentosus* B1	M	4.68	5.54	4.5	5	5	678.5
9	*L. pentosus* B31	M	4.30	7.69	4	5	5	763.0
10	*L. pentosus* B33	M	3.96	6.62	5	5.5	7	634.4
11	*L. pentosus* B33A	M	4.53	6.86	5	7	9	714.7
12	*L. lactis* B11	CA	4.96	5.46	4	5	5	463.6
13	*L. lactis* B12	CA	4.93	5.48	5	6	6	943.1
14	*L. lactis* B32	CA	4.46	6.92	5.5	6	8	810.5
15	*P. pentosaceus* EK1	M	3.80	8.52	6	7.5	11	1285.5
16	*P. pentosaceus* EK2	M	3.94	5.40	3	3.5	4	559.4
17	*P. pentosaceus* EK3	M	4.36	5.71	3	4	4	576.3
18	*P. pentosaceus* K33	EA	3.89	4.90	4	4	5	603.6
19	*P. pentosaceus* NB1	CA	4.46	5.98	3.5	4	4	497.6
20	*P. pentosaceus* NB32	CA	3.56	3.60	3	3	4	521.7
21	*P. pentosaceus* NB34	CA	4.81	6.00	4	4	4	532.8

**CA:Central Anatolia, M: Marmara, EA: Eastern Anatolia, **V: Viability, ***PA: Phytase Activity, ****EPA: Extracellular Phytase Activity*

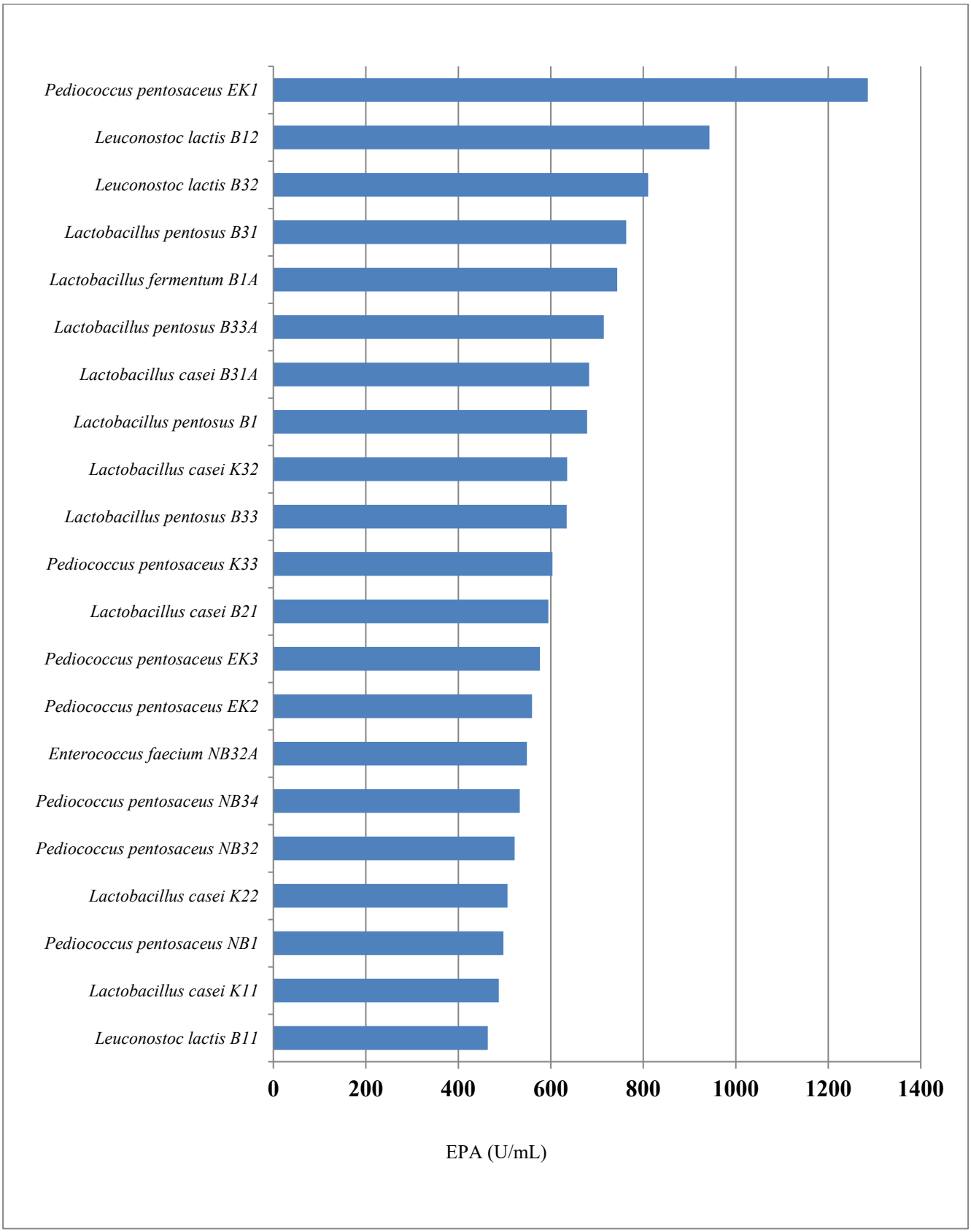

Figure 2. Extracellular phytase activity (EPA) of phytase (+) LAB isolates

Conclusion

In conclusion, the traditionally produced bozas have been found as potential starter culture reservoirs for sourdough fermentation with significantly higher extracellular phytase activities, thus challenging opportunitites to lower antinutritional factors, in particular phytic acid (PA) or phytate in the foods for the consumers.

Compliance with Ethical Standard

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

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FOOD and HEALTH

FOOD and HEALTH
E-ISSN 2602-2834

Food Health 6(2), 128-139 (2020) • https://doi.org/10.3153/FH20014 *Review Article*

Sokak gıdalarının güvenliği için risk faktörlerinin değerlendirilmesi

Öznur Cumhur

Cite this article as:
Cumhur, Ö. (2020). Sokak gıdalarının güvenliği için risk faktörlerinin değerlendirilmesi. *Food and Health,* 6(2), 128-139.
https://doi.org/10.3153/FH20014

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Submitted: 20.10.2019
Revision requested: 16.12.2019
Last revision received: 25.12.2019
Accepted: 27.12.2019
Published online: 27.02.2020

ÖZ

Sokak gıdaları ucuz, besleyici, lezzetli, her zevke uygun, otantik ve kültürel olduğu kadar erişimi kolay olduğu için toplumlar tarafından kabul görerek tüketilmektedir. Çok fazla çeşitlilikte olan sokak gıdalarında uygulanan çeşitli işlemlerin hepsi veya bir bölümü sabit, yarı sabit veya gezici olarak servis alanlarında yapılmaktadır. Sokaklarda satılan ve izlenebilirliği sağlanamayan gıdaların mikrobiyolojik ve kimyasal kontaminasyonu tüketiciler için risk oluşturmaktadır. Bu çalışmada, sahip olduğu koşullar çerçevesinde sokak gıdalarının güvenlik ve hijyen durumları ele alınmıştır. Satış yerlerindeki eksiklikler, kullanılan eşya ve ekipmanlar, sokak satıcılarının hijyen problemleri, kalitesiz hammaddeler, gıdaların taşıma, depolama, hazırlama, işleme ve servisindeki aksaklıklar sokak gıdalarının kalitesi ve güvenliğinden sorumlu ana risk etmenleri olarak belirlenmiştir. Sokak gıdalarına yönelik yapılacak değerlendirmeler ve düzenlemelerle iyileştirici eylemlerin geliştirilmesi, hijyen esaslı ve Tehlike Analizi ve Kritik Kontrol Noktası ilkelerine dayalı bir gıda güvenlik sisteminin kurulması için gerekli ortamın oluşturulması gıda kaynaklı hastalık riskini önemli ölçüde azaltacaktır. Bu noktada, ilgili düzenlemelerin gerçekleştirilerek tabana yayılması için tüm paydaşların aktif işbirliği önerilmektedir. Böylece sokaklarda güvenli gıdaların üretilmesi ve satılmasıyla halk sağlığının korunmasında önemli bir adım atılmış olacaktır.

Anahtar Kelimeler: Sokak gıdaları, Güvenli gıda, Gıda güvenliği riskleri

ABSTRACT

Evaluation of risk factors for the safety of street foods

Street foods are accepted and consumed by societies as they are cheap, nutritious, delicious, suitable for all tastes, authentic, cultural and as well as easy to access. All or part of the various processes applied in a wide variety of street foods is carried out in fixed, semi-fixed or mobile service areas. Microbiological and chemical contamination of foods that are sold on the streets and whose traceability cannot be established poses a risk to consumers. This study explores the safety and hygiene status of street foods within the scope of their conditions. Deficiencies in sales areas, goods and equipment used, hygiene problems of street vendors, poor quality raw materials, and troubles in transportation, storage, preparation, processing, and service of food were determined as the main risk factors for the quality and safety of street foods. Developing remedial actions through assessments and arrangements to be made for street foods and setting up an environment for establishing a food safety system based on hygiene and Hazard Analysis and Critical Control Point will significantly reduce the risk of foodborne disease. At this point, active cooperation of all stakeholders is proposed to ensure that related regulations are made and spread to the floor. Thus, the production and sale of safe foods in the streets will be an important step in the protection of public health.

Keywords: Street foods, Safe food, Food safety risks

Giriş

"Sokak gıdaları" veya "sokakta satılan gıdalar", daha fazla işlenmeden veya hazırlanmadan hemen veya daha sonraki bir zamanda tüketim için sokaklarda ve benzeri kamusal alanlarda satıcılar tarafından hazırlanan ve/veya satılan yiyecek ve içecekler olarak tanımlanır (WHO, 1996). Sokak gıdaları içerik, işleme, pazarlama, tüketim ve temsil ettikleri kültür bakımından geniş bir çeşitlilik gösterirler. Tüm sosyal sınıflardan insanların tükettiği sokak gıdalarının renkli ve uzun bir geçmişi vardır. Ülkemizde sokakta satılan gıdaların büyük bir bölümü geleneksel bilgiye göre hazırlanıp tüketilir ve genellikle bölgesel ürün çeşitliliğinden etkilenir.

Sokak gıdaları satıcılar ya da aileleri ya da başka bir küçük ölçekli girişimci tarafından sokak gıda ticareti içinde işlenir veya ticari üreticiler tarafından belirli üretim yapılan merkezlerde işlenerek satış alanlarına getirilir (Al Mamun ve Turin, 2016). Çok fazla çeşitlilikte olan sokak gıdaları pişirmenin gerekli olmadığı yemeye hazır gıdalar (meyveler, salatalar, taze sıkılmış meyve ve sebze suları vb.), önceden hazırlanarak satış alanına getirilen ve servis edilen gıdalar (simit, börek, tavuklu/nohutlu pilav, midye dolma, kağıt helva, elma şekeri, tulumba/lokma tatlısı, boza, salep, dondurma vb.), satış alanında hazırlanan ve servis edilen gıdalar (köfte ekmek, balık ekmek, döner, ciğer, ıslak hamburger, kumru, kokoreç, kestane, mısır, kumpir, kahve, pamuk şekeri vb.) şeklinde sınıflandırılabilir. Sokak gıdaları ucuz, besleyici, lezzetli, her zevke uygun, otantik ve kültürel olduğu kadar erişimi de kolay olduğu için toplum tarafından kabul görmektedir (Malhotra, 2016). Özellikle ülkemizdeki sokak lezzetleri şehirlerin temel dayanağı haline gelmiş olup, hem büyük şehirlerde hem de küçük kasabalarda gıda endüstrisinin bir parçası olmuştur.

Sokak gıdaları genellikle insanların alışveriş ya da rekreasyon amaçlı toplandığı bir yerde satıldığı için, bu yiyeceklerin sunulduğu yerler sadece sokaklar değil, aynı zamanda farklı malların satıldığı açık ve kapalı pazarlar ile fuar ve festival alanları da olabilir. Sokak gıdaları satış biçimi açısından genel olarak sabit, yarı sabit veya gezici olarak motorlu araçlarda, bisikletlerde, seyyar arabalarda, sepetlerde, stantlarda, çadırlarda, büfelerde veya etrafı kalıcı olarak kapatılmamış tezgâhlarda satılmaktadır (Kraig ve Sen, 2013). Etrafında kalıcı bir korunağı olmayan açık alanlarda satış yapılması; sokak gıda satıcılarını kafe, bar, restoran gibi diğer yiyecek ve içecek hizmeti veren işletmelerden ayırmaktadır.

Dünya Sağlık Örgütü ile Gıda ve Tarım Örgütü, sokak gıdalarının güvenliğini sağlamak ve arttırmak için çok sayıda program geliştirmiştir. Sokak gıdalarına yönelik rehber niteliğindeki bu programlarda; gıda güvenliği gereksinimleri ve uygulamaları, tehlike analizi tabanlı gıda güvenliği stratejileri ve sokak satıcılarına yönelik gıda güvenliği önlemleri konusunda yönlendirmeler genel ve bölgesel olarak yer almaktadır (WHO, 1996; FAO/WHO, 2001; FAO, 2009; FAO/WHO, 2010; FAO/WHO, 2013; FAO/WHO, 2017).

Sokak gıdalarının güvenliği sadece yerel nüfusu değil, aynı zamanda seyahat eden insanları ve turistleri de etkilemektedir. Özellikle gezginler ve turistler için sokak lezzetleri yerel mutfağı yansıtan çekici ve farklı lezzetler olarak dikkat çekerken, yeni bir deneyim sunmakta ve yerel alanla ilişkilerde bir anı temsil etmektedir (Privitera ve Nesci, 2015). Şehirlerdeki sokak lezzetlerinin genel olarak bahsedildiği Ağustos 2018 tarihli CNN raporunda, sokak gıdaları için dünyanın en iyi yirmi üç şehri arasında İstanbul yer almaktadır (Shea, 2018).

Sokak gıdaları konusunda oldukça fazla çeşitliliğe sahip olan ülkemizde, gıdalardan izole edilen patojen mikroorganizma ve toksinlerden bağımsız olarak gıda kaynaklı hastalıklar özellikle sokak gıdalarıyla ilişkilendirilmemiştir. Bunun başlıca nedenleri, gıda kaynaklı hastalıkların vaka temeline göre tespit edilmesi ve sebepleriyle somut bağlantılarının kurulmasına yönelik etkin bir programın olmayışı ve sokak gıdaları özelinde izlenebilirliğin sağlanamamasıdır. Ancak gıda kaynaklı hastalıklar, hem gelişmekte olan hem de gelişmiş ülkelerde yaygın olarak görülen ve büyüyen bir halk sağlığı sorunudur (Carbas vd., 2013). Bu bağlamda sokaklarda satılan, kaynağı ve üretimi belli olmayan gıdaların mikrobiyolojik ve kimyasal kontaminasyonu tüketiciler için büyük risk oluşturmaktadır. Ülkemizde sokaklarda ve pazarlarda açıkta satılan yiyeceklerden alınan örneklerin laboratuvar analizi patojenlerin varlığını ve toksinlerin oluştuğunu göstermiştir (Tablo 1).

Kavramsal bir çalışma olan bu araştırmada, sokak gıdalarıyla ilgili hammadde temininden servis edilmesine kadar potansiyel tehlike kaynakları çeşitli yönleriyle ele alınmaya çalışılmıştır. Çok fazla çeşitlilikte olan sokak gıdalarında üretim zincirinin her aşamasında gıda güvenliğini tehlikeye sokabilecek risk etkenlerinin belirlenmesi ve bu noktalarda tehlikelerin oluşması ve nihai ürüne geçmesini önlemeye yönelik yapılacak uygulamaların ortaya konularak sokak gıdalarına yönelik gıda güvenliğinin sağlanması ve sistemin inşa edilmesine katkıda bulunulması amaçlanmıştır.

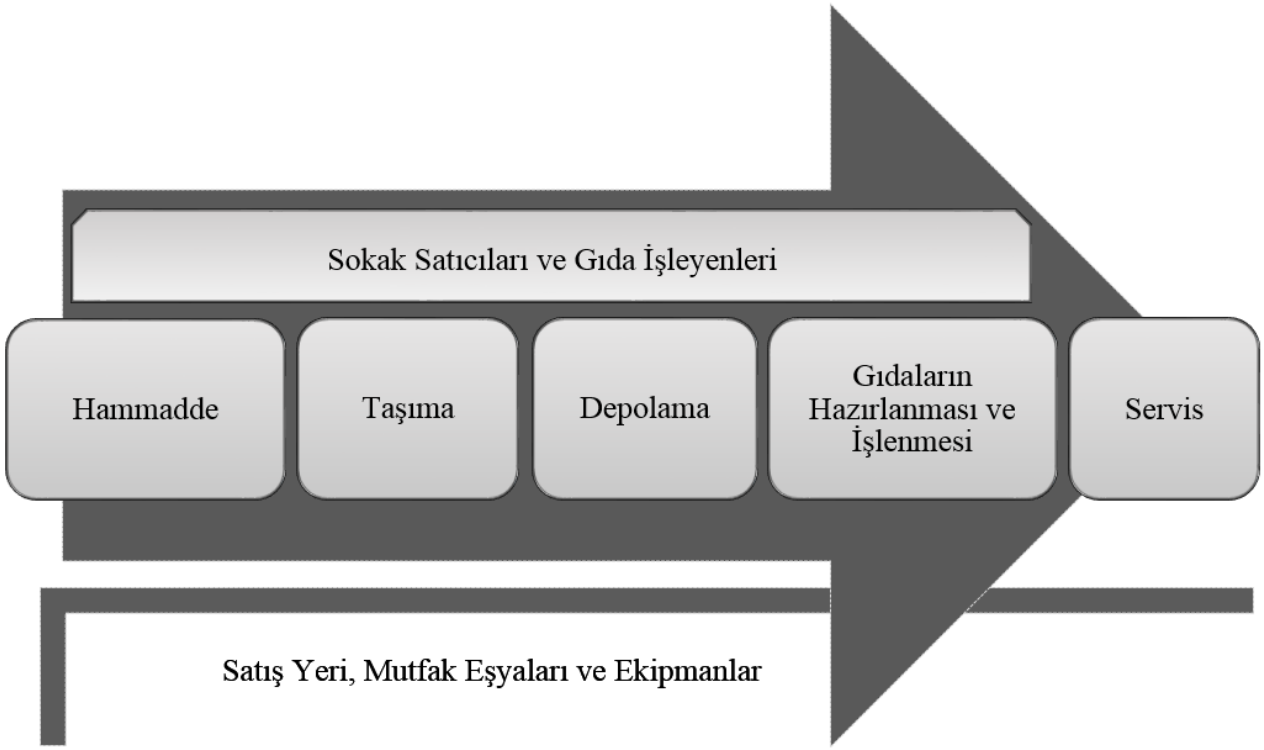
Tablo 1. Bazı sokak gıdalarında tespit edilen mikroorganizmalar ve toksinler.

Table 1. Microorganisms and toxins detected in some street foods.

Gıda Ürünü		Mikroorganizmalar veya Toksinler	Kaynaklar
Izgara Et ve Et Karışımları	Izgara Et	Koliform bakteri	Hampikyan vd. (2008)
	Döner	*Escherichia coli,* Enterobakteri	Bostan vd. (2011)
		Escherichia coli, Koliform bakteri	Hampikyan vd. (2008)
		Bacillus cereus, Salmonella spp., Stafilokokal enterotoksin	Tutuş vd. (2016)
	Kebap	*Escherichia coli, Staphylococcus aureus,* Koliform bakteri	Hampikyan vd. (2008)
	Kokoreç	*Escherichia coli, Staphylococcus aureus,* Koliform bakteri	Hampikyan vd. (2008)
	Köfte	*Escherichia coli, Staphylococcus aureus,* Koliform bakteri	Hampikyan vd. (2008)
	Çiğ Köfte	Koliform bakteri, *Stafilokok*	Delikanlı vd. (2014)
		Escherichia coli, Staphylococcus aureus, Salmonella, Koliform bakteri	Hampikyan vd. (2008)
	Midye Dolma / Midye Tava	*Bacillus cereus, Clostridium perfringens, Escherichia coli, Staphylococcus aureus*	Ates vd. (2011)
		Escherichia coli, *Vibrio* spp., Koliform bakteri	Kocatepe vd. (2016)
		Bacillus cereus, Staphylococcus aureus, Vibrio spp., Koliform bakteri	Kök vd. (2015)
		Staphylococcus aureus, Koliform bakteri	Öztürk ve Gündüz (2018)
		Escherichia coli, Staphylococcus aureus, Koliform bakteri	Hampikyan vd. (2008)
Süt ve Süt Ürünleri	Sokak Sütü	Koliform bakteri	Göncü vd. (2017)
	Kaymak	*Escherichia coli,* Enterobakteri, Koagülaz pozitif stafilokok, Koliform bakteri	Pamuk ve Gürler (2009)
	Dondurma	*Staphylococcus aureus*	Ede (2016)
		Staphylococcus aureus	İşleyici vd. (2016)
	Peynir Türleri	*Listeria monocytogenes*	Elmas (2014)
		Escherichia coli, Koagülaz pozitif stafilokok	Kirdar (2012)
	Yoğurt	*Escherichia coli, Staphylococcus aureus,* Koliform bakteri	Ertaş vd. (2014)
Çoban Salata /Yeşil Salata / Rus Salatası		*Escherichia coli, Staphylococcus aureus,* Koliform bakteri	Hampikyan vd. (2008)
Sandviç		*Bacillus cereus, Escherichia coli, Staphylococcus aureus, Listeria* spp.	Büyükyörük vd. (2014)
Boza		Koliform bakteri	Meriç (2010)
		Koliform bakteri, Okratoksin A	Uysal vd. (2009)
Şalgam Suyu		*Escherichia coli*	Gök (2017)
Kurutulmuş Meyveler		Aflatoksin	Hal (2014)
Baharatlar		*Salmonella* spp.	Kızıl vd. (2015)
		Aflatoksin	Ekici (2018)

Sokakta Satılan Gıdalarda Temel Risk Faktörleri

Sokakta satılan gıdalar görüntüsü, kokusu, lezzeti, kolaylığı, ekonomik oluşu ve toplumların kültürel mirasında oynadıkları rol ile sunduğu tüm avantajlara rağmen birçok tehlikeyi bünyesinde barındırmaktadır. Sokak gıdalarında hastalığa, yaralanmaya, yaşam kalitesini düşürmeye ve hatta ölüme neden olabilecek fiziksel, kimyasal ve biyolojik tehlikeler olabilir. Sokak gıdalarında üretim zincirinin herhangi bir aşamasında uygun olmayan bir işlem yapılması veya ihmal olması gıda tehlikelerini ortaya çıkarmaktadır. Bu bağlamda sokak gıdalarından kaynaklanan tehlikelerin analiz edilmesinde göz önünde bulundurulması gereken temel risk faktörleri Şekil 1'de yer almaktadır.



Şekil 1. Sokak gıda üretim zincirinde risk faktörleri

Figure 1. Risk factors in the street food production chain

Satış Yeri, Mutfak Eşyaları ve Ekipmanlar

Gıda güvenliğinin sağlanması, gıdaların temas ettiği ortamdan ayrı değerlendirilemez. Sokak gıdalarının hazırlandığı, üretildiği ve tüketildiği yerler her zaman temiz ve kirlilik kaynağından uzak olmadıkları için dikkat edilmesi gereken risk noktalarından bir tanesi olmaktadır. Sokak gıdalarının satış alanlarıyla ilgili olarak satıcılarının çalıştığı fiziksel ortamlarda tezgahların çevresel kirliliğe açık olduğu, yiyeceklerin ve ekipmanların yerleştirildiği ve saklandığı yerlerin zeminden yeterince yüksek olmadığı, tezgahların kolayca temizlenip dezenfekte edilebilecek malzemeden yapılmadığı, tezgahların ihtiyaçlar doğrultusunda çapraz bulaşmayı önleyecek şekilde dizayn edilmediği, kirli mutfak ekipmanlarının uygun olmayan koşullarda bekletildiği, tuvalet, el yıkama lavaboları ve temiz suyun sağlanması gibi bir alt yapının olmadığı, katı ve sıvı atık bertarafı için bir tesis ve drenaj sisteminin olmadığı, tesislerin olduğu yerlerde ise uygunsuz atık yönetiminin olduğu, kirli atıkların tezgahlara yakın yerlerde olduğu, sağlıksız çevre nedeniyle haşere ve kemirgenlerden korunmak için önlemlerin alınmasında zorlanıldığı ve genel çevresel kirliliklerden korunmak için korunaklı bir alana sahip olmadığı tespit edilmiştir (Mensah vd., 2002; Muyanja vd., 2011). Bu alanlardaki genel düzen ve hijyen eksikliği sokak gıdalarının çeşitli kirliliklerle karşı karşıya kalmasına neden olmaktadır. Sokak gıdalarını güvenli bir şekilde sunmak için korunaklı bir alan ve altyapı oluşturulması ve bu alanda gıda türüne göre gerekliliklerin yerine getirilmesi esastır. Bu

noktada sokak satıcıların satış yaptığı konumlar göz önünde bulundurularak altyapının ve ortak kullanım alanlarını sağlandığı tesisler kentsel gelişim programlarına dahil edilmelidir.

Satış alanlarının bir parçası olan mutfak eşyaları ve ekipmanları ile yiyecek hazırlama yüzeyleri, sokak gıdalarının güvenliği için kritik öneme sahip olan bir diğer noktadır. Mutfak ve servis ekipmanlarının gayri resmi imalatçılardan gelmesi, uygun olmayan koşullarda kullanılması veya zarar görmüş olması neticesinde bu ekipmanlardan gıdalara bir dizi toksik bileşik geçebilmektedir (Proietti vd., 2014). Ayrıca kullanılan eşya ve ekipmanlar ile yiyecek hazırlama yüzeyleri, yüzey malzemesinden veya yetersiz temizlikten dolayı bakteri yoğunluğu artışına sebebiyet verdiği için çapraz bulaşma kaynağı olarak gösterilmektedir (Kotzekidou, 2016). Mutfakta gıdayla temas eden malzemeler toksik bileşen içermeyen, koku veya tat vermeyen, emici özelliği olmayan, fiziksel bir parça veya kimyasal maddeyle bulaşmaya neden olmayan, korozyona karşı dayanıklı, temizlik ve dezenfeksiyonu kolay, erişilmeyen ölü noktaları olmayan ve gıdalarla kullanılabilecek özellikte olmalıdır (WHO, 1996; Rane, 2009).

Mutfak ekipmanı ve eşyalarıyla ilgili olarak bir diğer önemli husus çapraz bulaşmayı önlemek için yeterli sayıda olmaları ve uygun kullanımlarının sağlanmasıdır. Asogwa ve diğerleri (2005) sokak satıcılarına yönelik hijyen değerlendirilmesi yaptıkları çalışmalarında, kullanılan kapların yüzeylerinin ve bıçakların mikrobiyal bulaşma kaynağı olduğunu tespit etmişlerdir. Yapılan başka bir çalışmada ise; mutfakta veya serviste temizlik için kullanılan bez ve süngerlerin gıda kalıntılarını barındırması, nemli olması ve oda sıcaklığında saklanması sebebiyle yüksek oranda mikroorganizma içerdiği ve çapraz bulaşma kaynağı olduğu gösterilmiştir (Chavatte vd., 2014).

Su sokak gıdalarında el, ekipman, mutfak eşyası ve gıda bileşenlerinin temizlik ve dezenfeksiyonunda kullanılırken; buhar ve buz fazındaki su gıdanın ısıtılması veya soğutulmasında, bir bileşen olarak gıdanın üretiminde veya içme amaçlı kullanılmaktadır. Sular ciddi sağlık sorunlarına neden olan mikroorganizmalarla, toksik kimyasallarla, organik, inorganik ve radyoaktif bulaşanlarla kirlenebilir. 2015 yılında Uganda'nın başkenti olan Kampala'da bir kişinin ölümü ve onlarca kişinin hastanelik olmasına neden olan tifo salgının sebebi olarak korunmamış kaynaklardan gelen kirlenmiş su ve bu suyla yapılıp sokaklarda satılan içecekler gösterilmiştir (Kabwama vd., 2017). 2018 yılı Ağustos ayında Sakarya'nın Karasu ilçesinde çeşitli şikâyetlerle sağlık kuruluşlarına başvuran 7154 kişide sebep olan etkenin norovirüs olduğu tespit edilmiş ve resmi kanallardan yapılan açıklamalarda alt yapı sorunu ve kuyu suyunun salgına neden olduğu belirtilmiştir. (CNN Türk Haber, 2018). Gıda güvenliği için gıda üretim ve satış alanlarında kullanılmak üzere fiziksel, kimyasal ve mikrobiyolojik açıdan içme suyu kalitesinde, yeterli miktarda sıcak ve soğuk su temini sağlanmalıdır (Mahmutoğlu, 2010). Sokak gıdaları satış alanında kullanılan suyun kaynağına, taşınma ve depolama koşullarına bakılmalı ve kalitesi periyodik olarak test edilmelidir.

Gıda Üretim Basamakları

Sokak gıdalarının sahip olduğu koşullar çerçevesinde güvenli bir şekilde hazırlanarak tüketiciye sunulması için üretim akışının değerlendirilmesi gerekir. Çok fazla çeşitlilikte olan sokak gıdalarının üretimini değerlendirdiğimizde hammaddelerin satın alınması, taşıma, depolama, gıdaların işlenmesi ve hazırlanması, bekletme ve servis olarak üretim basamaklarını sıralayabiliriz.

Hammaddeler: Gıda güvenliği açısından sokak gıdalarının hazırlanmasında kullanılan tüm hammaddelerin ve gıda bileşenlerinin geçerli standartları karşılaması beklenmektedir. Ancak sokak gıdalarında kullanılan bileşenlerin kontaminasyonunun türü ve kaynağı, diğer gıda sektörü içerisindeki işletmelerde olabilecek kontaminasyondan biraz farklı olabilir. Çünkü bazı sokak satıcıları kâr maksimizasyonu ve gıda güvenliğine dair yeterli bilgiye sahip olmamaları sebebiyle yetkisiz tedarikçilerden riski daha da artıracak olan hammaddeler ve gıda bileşenleri ile izinsiz katkı maddeleri satın alabilmektedir (Alimi, 2016).

Sokak gıdaları, hammaddelerin ve gıda bileşenlerinin üretiminde yer alan uygulamalardaki sorunlar nedeniyle mikrobiyal patojenler, bunların toksinleri veya toksik kimyasallar içerebilir. Sebzeler patojenler, gübre veya tarım ilaçlarıyla kontamine olabilirken; etler standart olmayan kesim tesislerinden dolayı patojen mikroorganizmalarla; baharat, kuru meyveler, kahve ve çerezler sporlu bakteriler ve mikotoksinlerle, yağlı et ve süt ürünleri kalıcı organik kirleticilerle kontamine olabilir. Örneğin, 2005 yılında İzmit'te Atık ve Arıtma Yakma Tesisi çevresindeki bölgeden toplanan yumurta örneklerinde Avrupa Birliği sınırlarını aşan dioksin seviyeleri tespit edilmiştir (Yarman ve Bumerang, 2006). Kırıkkale'de iki yıl boyunca yapılan başka bir çalışmada sığır karkasının %4.6'sında ve kesimhanedeki atık su örneğinin %20.8'inde *Escherichia coli* O157:H7 patojeni izole edilmiş olup, karkastaki patojen bakterinin çapraz bulaşma kaynağı olarak kesim tesisleri gösterilmiştir (Ayaz vd., 2014). 2004 yılında ise İzmir'de enfekte domuz etinin kullanılması neticesinde sokakta satılan çiğ köfte tüketimi trichinellosis salgınına neden olmuştur (Turk vd., 2006; Akkoc vd., 2009). Bu bağlamda, çiğ ve az pişmiş gıdaların popülerlik kazandığı günümüzde gıda kaynaklarının güvenliği giderek önem kazanmaktadır. Gıdalarda kullanılan bileşenlerde dikkat edilmesi

gereken bir başka nokta ise; sokak gıdalarında ilgili yönetmeliklere göre izin verilmeyen gıda katkı maddelerinin, özellikle yetkisiz renklendiricilerin kullanılması veya izin verilen katkı maddelerinin de belirtilen seviyeleri aşan miktarlarda kullanılması söz konusu olabilmektedir (Malhotra, 2016). Yiğit ve İnanç (2017) tarafından yapılan çalışmada, baharatlarda kullanılmasına izin verilmeyen renklendirici katkı maddelerinin açıkta satılan baharatlık kırmızıbiberlerde ambalajlı ürünlere göre daha yüksek olduğu tespit edilmiştir.

Taşıma ve Depolama: Birçok sokak satıcısı hammaddelerini saklayabilecekleri uygun depolama alanlarına ve gıdaları çalışma alanlarına taşımak için uygun ekipman ve koşullara sahip olmayabilir. Hammaddelerin veya işlenmiş gıdaların taşıma ve depolama koşulları sokak gıdalarının güvenliğinde önemli rol oynamaktadır. Gıdaların taşınması ve muhafaza edilmesi esnasında kullanılan alanların ve kapların bu iş için uygunluğuna, hijyen ve sanitasyon standartlarının sağlanmasına, periyodik bakımlarının yapılmasına, gıdaya herhangi bir madde geçişi veya bulaşmasına izin verilmemesine, stok rotasyonunun sistematik olmasına, ayrı tutulması gereken gıda ve malzemelerin aynı yerde depolanmamasına, gıdaların özelliklerine göre taşıma ve depolama gerekliliklerinin sağlanmasına, gıdaların raf ömrü içerisinde tüketilmesine dikkat edilmelidir (Alimi, 2016; Proietti vd., 2014). Mol ve Tosun (2011)'un İstanbul'da sabit pazarlarda satılan balıkların kalite düzeylerini belirlenmek amacıyla yaptıkları çalışmalarında, taşıma ve depolama aşamalarında uygun olmayan koşullar tespit edilmiş ve sonuçları ortaya konulmuş; taşımada kullanılan araçların soğutma sistemi ile donatılmasına ve tüketiciye ulaşıncaya kadar soğutma koşullarının oluşturulmasının gerekliliğine vurgu yapılmıştır.

Gıdaların Hazırlanması ve İşlenmesi: Hazırlık aşaması ve işleme, gıdaların satış ve tüketiminden önce tabi tutulduğu ve gıda güvenliğinin sağlanmasında önemli olan yıkama, kesme, dilimleme, doğrama, karıştırma, marine etme, dinlendirme, dondurma, çözündürme, pişirme, süsleme, porsiyonlama gibi bir dizi aşamadan oluşan kritik bir alandır. Bu aşamada yapılan işlemler yiyecek ve içeceklerin türüne göre gerçekleşmekte ve çeşitlenmektedir. Bazı sokak gıdaları için satıcılar kızartma, ızgara, fırınlama, haşlama, kavurma gibi pişirmeyle ilgili veya harmanlama, soslama gibi diğer son işlemleri müşterilerin önünde açık tezgâhlarda gerçekleştirirler. Bu son işlemler tüketiciler açısından güven duyma eğilimini ve yiyeceklerin lezzetini geliştirmektedir (WHO, 1996).

Genel olarak gıda kaynaklı hastalıkların ortaya çıkmasında gıdaların hazırlık ve işleme aşamasında yapılan hatalar başlıca rol oynamaktadır. Çok fazla çeşitlilikte işleme sahip olan sokak gıdalarının hazırlanması ve işlenmesinde gıdaların uygun olmayan koşullarda uzun süre bekletilmesi, dondurulmuş gıdaların bir defadan fazla çözündürülmesi, pişirilecek gıdaların yetersiz ısıl işleme tabi tutulması, daha önce pişirilmiş ve soğutulmuş gıdaların yetersiz şekilde yeniden ısıtılması, gıdaların açıkta bekletilmesi, çapraz bulaşmanın önlenememesi, kızartma yağlarının tekrar tekrar kullanılması, gıdalarla birlikte sürekli servise sunulan sos ve baharatların uzun süre uygunsuz koşullarda bekletilmesi belirlenen belli başlı risk faktörleri arasındadır (Alimi, 2016; Callano, 2012; FAO/WHO, 2017; Kotzekidou, 2016). Amerika Birleşik Devletleri (ABD) Hastalık Kontrol ve Korunma Merkezleri'nin 2016 yılı verilerine göre ABD'nde 14259 gıda kaynaklı hastalık olduğu ve yaklaşık 17 ölüm olduğu bildirilmiştir. Vakaların büyük çoğunluğu yetersiz pişmiş veya yanlış işlenmiş gıda ürünlerinin tüketilmesiyle ilişkilendirilmiştir (Centers for Disease Control and Prevention, 2018). Simit ve benzeri unlu mamullerin mikrobiyolojik büyüme için uygun olmadığı düşünüldüğü halde; Var ve diğerleri (2015)'nin yaptığı çalışmada *Staphylococcus aureus*'un gıda güvenliği için bir tehdit olabileceği gösterilmiş ve fermantasyon sırasında simit hamurunda çoğalabileceği bildirilmiştir. Midye, döner gibi popüler sokak lezzetleriyle ilgili yapılan çalışmalarda ise ısıl işlem aşaması dikkat edilmesi gereken kontrol noktalarından bir tanesi olarak gösterilmiş ve gıda kaynaklı hastalığa neden olmaması için yeterli ısıl işlem uygulanmasının gerekliliği belirtilmiştir (Ates vd., 2011; Haskaraca vd., 2015; Hewitt ve Greening, 2006).

Pişirilen sokak yemeklerinde gıda güvenliğini sağlamak için pişirme sıcaklıkları ve sürelerine dikkat edilmeli, sıcak yemekler pişirildikten hemen sonra tüketilmeli, hemen tüketilmeyecekse mikroorganizmaların hızlı çoğalabildikleri tehlikeli sıcaklık bölgesinde (5-65 °C) uzun süre bekletilmemeli ve hızla soğutulmalı, servis yapmadan önce yemekler sadece bir kez ısıtılmalı ve yeterli ısıtma sağlanmalı, yeni hazırlanmış yemeğin içerisine artan yemek karıştırılmamalıdır (Artık vd., 2017). Sokak gıdalarında ortaya çıkan bir diğer tehlike ise, kontrolsüz ısıl işlem veya uygun olmayan sıcaklık ve sürede gerçekleştirilen pişirme işlemleri neticesinde gıdalarda doğal olarak bulunan belirli bileşiklerden kanserojen olarak kabul edilen bir takım ısıl işlem bulaşanlarının oluşumudur. Örneğin; gıdaların ızgarada ve yüksek sıcaklıklarda işlenmesiyle polisiklik aromatik hidrokarbonlar (Lee vd., 2016), et ve balık gibi protein bakımından zengin gıdaların pişirilmesi sebebiyle heterosiklik aromatik aminler (Gibis, 2016; Özsaraç vd., 2019) ve kızartılmış patates, unlu mamüller, kavrulmuş kahve gibi gıdaların ısıl işlemi sonucunda akrilamid oluşumu (Gökmen, 2016) görülmektedir.

Servis: Sokak gıdalarında satış miktarları tam olarak tahmin edilemediği için büyük miktarlarda önceden hazırlanan birçok gıda uygun olmayan koşullarda ve çevresel bulaşmaya

karşı etkin koruma sağlanamadan uzun süre bekletilebilmektedir (Fellows ve Hilmi, 2011). Japonya'daki bir üniversite festivalinde, önceden hazırlanarak pişirilen kreplerin yetersiz soğutulması ve uzun süre bekletilmesi gıda zehirlenmesinin ana nedeni olarak belirtilmiştir (Kitamoto vd., 2009). Izgara et ürünleriyle ilgili yapılan çalışmalarda, ürünlerin önceden pişirilerek ızgara kenarlarında tutulduğu tespit edilirken, bu bekleme sıcaklıklarının patojenlerin çoğalması için uygun ortam oluşturduğu belirtilmiştir (Hampikyan vd., 2008; Tidjani vd., 2013). Uygun olmayan koşullarda satış alanına sahip, soğutma ve ısıtma sistemi olmayan, çevresel bulaşmaya karşı korunmayan, hazırlık veya bekleme süreleri düzenlenmeyen sokak gıdalarında mikroorganizmaların bulaşması ve çoğalması yoluyla gıda kaynaklı hastalığa katkıda bulunulmaktadır. Bu aşamada servis edilene kadar sıcak yiyecekler 65°C ve üzeri sıcaklıklarda, soğuk yiyecek ve içecekler ise 10°C ve altı sıcaklık derecelerinde muhafaza edilmeli ve servis edilmelidir. Pişmiş sokak yemeklerinin servis tezgahlarında kalma süresi 2 saati geçmemeli, ancak daha sonra tüketilecekse kısa sürede soğutulmalı ve uygun koşullarda muhafaza edilmesi sağlanmalıdır (Mahmutoğlu, 2010).

Sokak gıdaları ve satıcılarına yönelik yapılan çalışmalarda, gıdaların servis aşamasında personel veya bilgi eksikliği kaynaklı kötü hijyen koşulları söz konusu olabilmektedir (Cortese vd., 2016; Liu vd., 2014). Sokak gıda satıcılarının yiyecek hazırlama ve servis hizmetlerinde gıda güvenlik uygulamalarını izlemeleri tavsiye edilirken; ambalajlama ve servis amacıyla gazete, uygun olmayan kâğıt veya servis malzemesinin kullanılmaması gerekmektedir (FAO, 2009).

Sokak Satıcıları ve Gıda İşleyenleri

Sokak gıdalarında satış alanları, ekipmanları ve malzemeler ne kadar gelişmiş, modern ve iyi organize edilmiş olsa da gıda güvenliği için buradaki en önemli unsur insan, yani gıda satıcıları ve işleyenleridir. Sokak gıdalarını işleyen ve satanların sağlıklı olması, kişisel hijyen gerekliliklerini yerine getirmesi, temizlik ve hijyen esaslarını sağlaması gerekmektedir. Sokak gıda satıcılarının ve işleyenlerin eğitim seviyeleri genellikle düşük olup, hijyen ve gıda güvenliğiyle ile ilgili yeterli ve etkin bilgiye sahip olmadıkları yapılan çalışmalarda görülmektedir (Ercan vd., 2012; Basch vd., 2015; Samapundo vd., 2016; Trafialek vd., 2018). Gıda kaynaklı hastalıkların neden hala devam ettiğine ilişkin olarak gıda güvenliğiyle ilgili bilgi ve uygulamaların eksikliği ile geçici gıda tedarik ortamlarında etkin gıda güvenliği eğitiminin verilmemesi gösterilmektedir (Cortese vd., 2016). Bu noktada verilen eğitimlerin içeriği, çıktısı ve bilginin uygulamaya dönüştürülememesi tartışılmaktadır. Yapılan çalışmalarda gıda güvenliği ve hijyenik uygulamalar için gıda çalışanlarına verilen eğitimin bilgi, beceri ve tutum düzeylerinde olumlu değişikliklere neden olduğu vurgulanırken; eğitimin tüm davranışlara yansımadığı belirtilmektedir (Acikel vd., 2008; Roberts vd., 2008). Eğitimin etkinliğinin zayıf kalmasındaki temel sebeplerden bir tanesi satıcıların riskten haberdar olup, yine de onlar için hiçbir şeyin olmayacağını varsayarak yani iyimser önyargıya dayalı davranmasıdır (Lange, 2017). Ayrıca yapılan araştırmalarda, etkili gıda güvenliği eğitimi için bilgiyi geliştirmeye yönelik sadece teorik öğelere odaklanılmaması gerektiği belirtilmektedir. Gıda güvenliği eğitiminin faaliyetin türüne ve koşullarına uygun olması, içeriğinde gıda güvenliği sorunlarına vurgular yapılması gerekmektedir (Howells vd., 2008). Örneğin, sokak gıdaları yetersiz alan ve altyapıya sahip ve çevresel kirliliklerle direkt karşı karşıyadır. Dolayısıyla sokak gıdalarını işleyenler ve satanlara yönelik sahip olunan koşullardaki engeller ve sokak gıdalarındaki riskler göz önünde bulundurularak eğitim programları geliştirilmelidir. Satıcıların gıda güvenliğinin sağlanması için gerekli temel ilkeler ve önlemler konusunda daha fazla bilinçlendirilmesi, sokak gıdalarının oluşturduğu sağlık risklerini azaltmak için en uygun maliyete sahip seçeneklerden biridir (FAO/WHO, 2010). Genel olarak değerlendirildiğinde, gıda kaynaklı tehlikelerin çoğu bahsi geçen kontrol noktalarında tedbirlerin alınması ve bu tedbirlerin sokak satıcıları tarafından uygulanmasıyla önlenebilir.

Sonuç

Sokak gıdaları sosyoekonomik olarak, gıda sektörünün kayıt altına alınamayan önemli bir bileşenini oluşturmaktadır. Bu bağlamda sokak gıdalarının üretim zincirinde bir takım eksikliklerin olması ve yanlış uygulamaların yapılması halk sağlığını tehdit etmektedir. Gıda güvenliğinin yeni teknolojilerle dijitalleşmesine yönelik çalışmaların gelişerek ilerlediği günümüzde, sokak gıdalarının üretim ve satışında eksikliklerin olması ve bir takım sorunların yaşanması oldukça manidardır. Bu noktada sokak gıdaları ve satıcıları için temel gereksinimlerin geliştirilmesi gerekmektedir. Yapılacaklar çok karmaşık veya masraflı olmamalı, ancak sokak satıcılarının gıda güvenliği sorunlarını tanımasında ve bunlara pratik çözümler bulmasında yardımcı olacak bir yapıda olmalıdır.

Sokak gıdalarıyla ilgili minimum alt yapıyı sağlayarak, çevre koşullarını ve tesisleri iyileştirerek, güvenli gıda üretmek için ihtiyaç duyulan kaynakları elde ederek, gıda üretim aşamalarında önerilen risk azaltma işlemlerini yerine getirerek ve beklenen faaliyetler doğrultusunda gıda işlemesinde ve satışında çalışanların eğitim ihtiyaçlarını karşılayarak güvenli gıdalar sokaklarda hazırlanabilir. Tüm bu gerekliliklerin yerine getirilmesi için; sokak gıdalarının güvenliğiyle ilgili veri toplamak, risk analizi yapmak, yaygın uygunsuzluk durumlarını

belirlemek, gıda güvenliği sistemini sokak gıdalarının işleyişine uyarlamak, gıda mevzuatındaki eksiklikleri gidermek, izlenebilirliği sağlamak ve denetlemek gerekmektedir. Bu bağlamda ilgili kurum ve kuruluşların, sokak gıda satıcılarının hijyen esaslı ve Tehlike Analizi ve Kritik Kontrol Noktası ilkelerine dayalı bir gıda güvenlik sistemini uyarlaması için gerekli ortamın oluşturulmasında yardımcı olması ve gıda güvenliğine yönelik pratik uygulama çalışmaların yapılmasında öncü olması önerilmektedir. Tüm paydaşların katılımıyla, sokak gıdalarına yönelik yapılacak değerlendirmeler ve düzenlemelerle iyileştirici eylemler geliştirmek, ülkemizde gıda güvenliği standartlarının tabana yayılmasına katkıda bulunacak ve ticaretin doğru bir şekilde yönetilmesini sağlayacaktır. Böylece sokaklarda güvenli gıdaların üretilmesi ve satılmasıyla halk sağlığının korunmasında önemli bir adım atılmış olacaktır.

Etik Standart ile Uyumluluk

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

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Tables

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An eBook	**Millbower, L. (2003).** *Show biz training: Fun and effective business training techniques from the worlds of stage, screen, and song*. p. 92-90. Retrieved from http://www.amacombooks.org/ (accessed 10.10.2015).
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Websites - professional or personal sites	***The World Famous Hot Dog Site.* (1999, July 7).** Retrieved January 5, 2008, from http://www.xroads.com/~tcs/hotdog/hotdog.html (accessed 10.10.2015).
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