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Antimicrobial Activity of Edible Surface-active Xanthan Gum Coating Enriched with Different Extracts and Effect on Quality of Rainbow Trout (*Onchorhyncus mykiss*)

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Abstract

The aim of this study was to determine the antibacterial effects of daphne (*Laurus nobilis* L.) and basil (*Ocimum basilicum* L.) in coating formulation edible film-based xanthan gum on the shelf-life of rainbow trout (Oncorhynchus mykiss) for 10 days storage at 4 ± 1°C. The most effective concentration of the extracts used in the edible coating against seven fish pathogenic bacteria was determined as 1 mg ml^{-1}. While the use of antimicrobial edible film in trout significantly inhibited bacterial growth; the lactic acid bacteria increased during storage. It was clear that the best fish and plant odor were detected in the coated groups, coated with daphne extract groups were also judged as stronger than basil extract groups. Although the control groups deteriorated on the 7th day, the edible film-coating groups were finished sensory and microbiological on the 10th day. Throughout the storage period in this study, 14 different bacteria and 3 different yeasts were identified via Bacterial Identification API Kits.

Keywords: Edible surface-active coating, xanthan gum, plant extract, antimicrobial coating, *Oncorhynchus mykiss*

Research article
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INTRODUCTION

The rainbow trout (*Oncorhynchus mykiss*) contains a high percentage of polyunsaturated fatty acids (PUFAs), resulting in rapid microbial spoilage and chemical oxidation (Baron et al., 2007). Considering that rainbow trout fillets have a short shelf-life, but there is a growing market for fresh fish, new preservation methods need to be developed to extend the shelf-life of fresh fish (Aspevik et al., 2021; Hosseini et al., 2016). In order to meet consumers demands for more disposable, biodegradable, natural and recyclable food package material, nowadays, research have focused on the incorporation of natural antimicrobial compounds such as bacteriocins and plant extracts in the bio-based sustainable package materials in place of petroleum based plastic films (Díaz-Montes et al., 2021; Irkin and Esmer, 2015; Weber et al., 2002).

Therefore, it has recently become important to edible films/coatings with essential oils or plant extracts to extend the shelf life of fish and fishery products (Bensid et al., 2014; Gao et al. 2014; Gomez-Estaca et al., 2007; Mei et al., 2019; Ong et al., 2021).

Antimicrobial edible films made from biopolymers can improve food quality and increase shelf life by providing a slower respiration rate and controlled microbial growth (Garcia et al., 2010). Xanthan gum (XG), one of the most important commercial microbial hydrocolloids used in the food industry, is a high molecular weight extracellular polysaccharide produced by the bacterium *Xanthomonas campestris* (Zheng et al., 2019). Bio-based food packaging with antimicrobial properties derived from xanthan gum is a promising material for food preservation application (Sapper et al., 2019). Although there are many studies on the antibacterial activities of plant essential oils and extracts, the use of xanthan in edible film coatings in fish has not been seen. This is the main constituents of medicinal plants vary greatly depending on nutritional status, geographical origins, extracted plants (stem, leaf and flower), genetic factors and/or extraction methods (Burt, 2004; Costa et al., 2015; Lang and Buchbauer, 2012). Basil species contain 0.2 - 1% extract, the main ingredients are linalool and estragole (methyl chavicol), and camphene, o-cymene, beta-pinene, citral, alpha-pinene, geraniol (Sakkas and Papadopoulou, 2017). Furthermore, daphne species have been reported in previous studies to contain various secondary metabolites such as flavonoids, coumarins, diterpenes lignans and triterpenoids. It has been determined that these plant species have many biological activities including antimicrobial, antioxidant, cytotoxic, antitumor and anti-inflammatory activities (Asong et al., 2019; Balkan et al., 2017; Biswas et al., 2014; Hashemi and Khaneghah, 2017).

The aim of this study was (1) to evaluate the antibacterial potential of xanthan gum based edible coating containing extracts from daphne (*Laurus nobilis* L.) and basil (*Ocimum basilicum* L.) against fish pathogens and (2) to determine effectiveness of these extract containing surface-active coating on microbiological characteristics of rainbow trout (*Oncorhynchus mykiss*) fillets during refrigerated storage (4 ± 1°C).

MATERIAL and METHOD

Materials

Rainbow trout fillets were transfered to Ege University Laboratory of Fisheries Processing Technology via frigorific transport by Bağcı Alabalık A.Ş. (Izmir). Food grade biopolymers: Xanthan gum, glycerol and ethanol and other reagents were purchased from Merck (Germany). Extracts were prepared from two natural plants as daphne (*L. nobilis*) and basil (*O. basilicum*).

Preparation of plant extracts

A total 50,0 ± 0,1 g of dried/granulated daphne or basil was extracted with 300 mL of organic ethanol by shaking (Dragon Lab SK-330 model, Beijing, China) for 24 h at room temperature (Baytop, 1999). Afterwards the obtained solution was extracted using rotary evaporator (Stuart, RE300DB) at 45 °C.

Preparation of xanthan coating solution

Edible coating solution was prepared according to Sothornvit (2011) with slight modifications. Xanthan gum powder samples (1 g 100 ml^{-1} distilled water) were hydrated at 25 °C for 1 hour. The edible coating solutions were homogenized by stirring for 30 min at 90 °C.

Then glycerol (30 % w w^{-1} of the total hydrocolloid content) was added as a plasticiser. Plant extracts (0,5 % w v^{-1}), dispersed in an aqueous solution, were stirred for 15 min (Hotplate stirrer, Wissestir MSH-20A, Kore) at 25 °C. The most appropriate formulation for coating fish by the tested xanthan gum hydrocolloid(s) was detected when the latter's concentration was equivalent to 0.5 % (w w^{-1}).

Antibacterial activity of extracts and edible coating

Microbial strains

Gram-positive bacteria (*Bacillus cereus* ATCC 7064, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 43300, *Staphylococcus epidermidis* ATCC 12228, Gram-negative (*Escherichia coli* ATCC 29998, *Escherichia coli* 0157:H7 RSKK 234 and *Salmonella typhimurium* CCM 5445) as well as *Candida albicans* ATCC 10231 were used for the antimicrobial activity studies. Yeast and the lyophilized bacteria were obtained from Ege University, Faculty of Science, Department of Basic and Industrial Microbiology.

Minimum inhibitory concentration (MIC) by micro-dilution susceptibility test

MICs were determined according to the National Committee for Clinical Laboratory Standards (CLSI, 2011). Test microorganisms had grown in Mueller Hinton Broth (MHB) for 5 h (exponential phase) and adjusted to 0.5 McFarland. These test organisms and MHB were transferred to each well of a standard 96-well microplate. MIC values were determined after 20-24 hours of incubation at 37ºC. Positive control consisted of different Ampicillin (AMP) (Sigma) and flucytosine (FC) (Sigma), which are standard antibacterial and antifungal agents respectively, at concentrations 0.5 – 256 μg ml^{-1}. The same procedure was repeated three times.

Agar well diffusion test

The antimicrobial activity of xanthan coating solutions containing daphne and basil extracts were tested against eight food pathogen microorganisms using agar well diffusion assay. Test organisms were activated in Mueller-Hinton Agar (MHA) (Oxoid) for 18-24 hours. Test organisms established according to the 0.5 McFarland standard were inoculated into the media. 30 μl of extracts and control antibiotics were absorbed into discs and placed in petri dishes. The petri dishes were kept at 4 ^{o}C for 2 hours before incubation, and then after 24 hours of incubation at 37 ^{o}C. The effect of the extract rich xanthan surface-active coating was compared to that without extract. Antimicrobial activity was assessed by measuring the inhibition zone (mm). Ceftazidime (Oxoid, 30μg) and Nystatin (Oxoid, 30μg) were used as positive controls. The inhibition zone result represents the average of 3 trials (CLSI, 2011).

Edible coating application and fish fillets quality analysis

Treatment of fish fillets

Samples were coated with daphne (DE) and basil (BE) extracts. Xanthan gum coated sample without extract was used as control (C). The trout fillets were coated by dipping xanthan gum solution for 2 min at room temperature (25 °C), and then dried for 2 min. A total 50 ± 5 g of fish fillets were aseptically placed into trays and covered with stretch film. The coated and uncoated trout fillets were stored at 4 ± 1 °C for 10 days. Analysis was carried out on the 1st, 3rd, 5th, 7th and 10th day of storage.

Microbiological analysis and bacteria identifications

Three fish were evaluated from each designated sampling point and 10 g samples were taken from each group. Samples (10 g) were added aseptically to 90 mL of sterile Buffered Peptone Water solution (1 g mL^{-1} bacteriological peptone, Merck, Germany) and mixed in a Stomacher (IUL, Barcelona, Spain) at high speed for 1 min. Plate Count Agar (PCA, Merck, Germany) was used to evaluate the total viable count (TVC) and and total aerobic psychrophilic bacteria (TAP). Violet Red Bile Dextrose Agar (VRBD-A, Merck, Germany), Baird Parker Agar (BPA, Merck, Germany) and De Man Rogosa Sharpe Agar (MRS, Merk, Germany) were used to assess *Enterobacteriaceae* (ENT), *Staphylococcus* (SPH), and lactic acid bacteria (LAB) respectively. On the other hand, Yeast Extract Glucose Chloramphenicol Agar (YGC, Merck, Germany) were used to grow Molds and yeasts (MY). Plates were incubated at 30 °C for 24 h; 37 °C for 24 h; 30 °C for 72 h respectively for TVC and ENT, SPH, LAB. TAP were proceeded after incubation at 7 °C for 10 d. Yeasts and fungi were incubated at 25 °C for 72 h (Harrigan and Mc Cance, 1976). After incubation, the means of counts with standard deviations, were reported as logarithms of the number of colonies forming units (log CFU g^{-1}). Furthermore, the isolated microorganisms were identified with API test kits with 20 NE (30 °C for 24 h), 20 E (36 °C for 24 h), 29 C AUX (30 °C for 48 h), 50 CH and 50 CHL (30 °C for 24 h) (Biomérieux, France) from bacterial flora. These kits were used according to the instructions of the manufacturer and the database provided by BioMerieux.

Sensory evaluation of raw and cooked fish fillets

Sensory evaluation was performed by seven trained panellists. Two different sensory analyses were carried out as raw and cooked trout. The fillets samples were cooked at 170 °C for 15 min in a preheated conventional electric oven. A scale ranging from 1 (bad) to 9 (perfect) was used by the panellists to evaluate the three groups according to hedonic scale parameters which include colour, odour, texture and general acceptability. However, taste was not evaluated in raw products. Sensory analyses were also performed on the 1st, 3rd, 5th, 7th and 10th days of storage. The panellists were also asked on daily basis to evaluate the fish whether they were still fit for consumption or not (Bonilla et al., 2007).

pH analysis

The pH value was measured directly from the homogenate (5 g fish 5 ml^{-1} distilled water) using a Hanna 211 model pH meter (Cluj-Napoca, Romania). The experiment was proceeded in triplicate (Lima Dos Santos et al., 1981).

Statistical analysis

The data were analysed by one-way ANOVA. Tukey's multiple range test was applied for determining group differences at 95% significance level. Analysis was performed using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS and DISCUSSION

Antimicrobial assays

Antimicrobial activity by MIC

The production yield of daphne and basil extracts was 5.8 % and 7.8 % respectively. The effect of different extraction methods solvent systems on plant extracts yields and their antimicrobial activity have been reported in many studies (Dirar et al., 2019; Do et al., 2014; Hayouni et al., 2007). Moreover, the antimicrobial activity can be exploited in edible coating to protect seafood. The antimicrobial activity result of the extracts against some target microorganisms were given by using MIC and disk diffusion method (Table 1 and 2).

Table 1. The minimum inhibitory concentration (MIC) of daphne and basil extracts against pathogen bacteria. Data shown are the means ± standard deviation.

Test bacteria	MIC (mg ml^{-1})			
	Daphne	**Basil**	**AMP**	**FC**
***E. coli* ATCC 29998**	0.90 ± 0.8^{aA}	0.73 ± 0.5^{aB}	0.78 ± 0.2^{aB}	R
***E. coli* O157:H7 RSKK 234**	0.45 ± 0.25^{bB}	0.73 ± 0.2^{aA}	R	R
***S. aureus* ATCC 43300**	0.23 ± 0.3^{cC}	0.73 ± 0.1^{aA}	0.39 ± 0.3^{bB}	R
***S. typhimurium* CCM 5445**	0.23 ± 0.12^{cB}	0.73 ± 0.5^{aA}	0.78 ± 0.4^{aA}	R
***E. faecalis* ATCC 29212**	0.11 ± 0.55^{dC}	0.73 ± 0.35^{aA}	0.39 ± 0.15^{bB}	R
***B.cereus* ATCC 7064**	0.11 ± 0.4^{dC}	0.73 ± 0.25^{aA}	0.39 ± 0.12^{bB}	R
***S. epidermidis* ATCC 12228**	0.23 ± 0.33^{cB}	0.73 ± 0.1^{aA}	0.19 ± 0.5^{cB}	R
***C. albicans* ATCC 10231**	0.11 ± 0.3^{dC}	0.73 ± 0.1^{aB}	R	0.78 ± 0.8^{A}

AMP: Ampicillin, FC: Flucytosine, R: No inhibition zone.
*Lowercase letters indicate significant differences in a column ($P < 0.05$). Uppercase letters indicate significant differences in a row ($P < 0.05$).

In our study, the MIC values of daphne and basil extracts against test microorganisms ranged between 0.90 mg ml^{-1} and 0.11 mg ml^{-1}. 0.90 mg ml^{-1} demonstrated the maximum antimicrobial activity, therefore, a value of 1 % (mg 100 ml^{-1}) was added to the edible coating solution. The highest antibacterial activity exhibited by daphne was recorded against *E. faecalis, B.cereus* and *C. albicans* with MIC value 0.11 µg ml^{-1} ($P < 0.05$). Basil extract exhibited the same antimicrobial activity (MIC = 0,73 µg ml^{-1}) against all the tested strains ($P > 0.05$). The antibiotics and extracts had higher microbial activity on Gram-positive bacteria when compared to that on Gram-negative (*E. coli* ATCC 29998, *E. coli* 0157:H7 RSKK 234 and *S. thyphimurium* CCM 5445). Tayoub et al,. (2012) were determined the leaves ethanolic extract had the highest effect on pathogenic bacteria, compared with other plant parts extracts. Other studies on the genus Daphne and basil showed that the ethanol extract contains compounds with antibacterial properties (Adamczak et al., 2020; Manojlovic et al., 2012).

Agar well diffusion test

The xanthan-daphne coating displayed significant antimicrobial activity with inhibition zone ≥ 15 mm when a concentration was used 500 µl ml^{-1}. Inhibition zones measured against the tested strains were as follows: *S. epidermidis* (21.6 mm), *S. aureus* (16.2 mm), *E. coli* O157:H7 (16.1 mm), *B.cereus* (15.2 mm) and *E.faecalis* (15.0 mm).

At the same time, the xanthan-basil coating displayed significant antimicrobial activity against *S. typhimurium* (17.5 mm) and *E. coli* O157:H7 (15.7 mm).

However, DE and BE did not show any activity against *S. typhimurium* at any of the employed concentrations expect to Ceftazidime chemical agent (12.2 mm). All the extracts obtain edible coating displayed significant zone of inhibition against *S. aureus* and *S. epidermidis* at all concentrations (Table 2).

Table 2. The inhibition zone diameter of xanthan surface-active coating with daphne and basil extract.

Test bacteria	Agar well diffusion (mm)							
	Daphne			Basil			Standard antibiotics	
	0.50 mg ml^{-1}	0.25 mg ml^{-1}	0.10 mg ml^{-1}	0.50 mg ml^{-1}	0.25 mg ml^{-1}	0.10 mg ml^{-1}	CF	NYS
***E. coli* ATCC 29998**	12.9 ± 0.3^{cC}	R	R	15.7 ± 0.1^{bA}	14.0 ± 0.2^{aB}	R	14.2 ± 0.2^{dB}	R
***E. coli* O157:H7 RSKK 234**	16.1 ± 0.1^{bA}	14.3 ± 0.1^{aB}	10.1 ± 0.3^{cC}	R	R	R	R	R
***S. aureus* ATCC 43300**	16.2 ± 0.2^{bB}	15.6 ± 0.4^{aC}	13.2 ± 0.2^{aD}	14.0 ± 0.4^{cD}	13.1 ± 0.4^{bD}	12.7 ± 0.1^{aDE}	20.5 ± 0.3^{aA}	R
***S. typhimurium* CCM 5445**	R	R	R	R	R	R	12.2 ± 0.1^{eA}	R
***E. faecalis* ATCC 29212**	15.0 ± 0.0^{bB}	12.8 ± 0.2^{bC}	R	12.2 ± 0.4^{cdC}	R	R	17.0 ± 0.2^{bA}	R
***B.cereus* ATCC 7064**	15.2 ± 0.2^{bA}	14.4 ± 0.3^{aAB}	11.4 ± 0.2^{bCD}	13.1 ± 0.1^{cB}	12.1 ± 0.2^{bC}	10.4 ± 0.2^{bD}	15.4 ± 0.2^{cA}	R
***S. epidermidis* ATCC 12228**	21.6 ± 0.2^{aA}	14.2 ± 0.0^{aC}	11.6 ± 0.2^{bD}	17.5 ± 0.1^{aB}	14.5 ± 0.2^{aC}	R	14.5 ± 0.5^{cdC}	R
***C. albicans* ATCC 10231**	13.0 ± 0.2^{cB}	11.9 ± 0.1^{cC}	R	11.2 ± 0.3^{dC}	R	R	R	18.5 ± 0.1^{A}

CF:Ceftazidime, NYS: Nystatine, R: No inhibition zone.

* Lowercase letters indicate significant differences in a column ($P < 0.05$). Uppercase letters indicate significant differences in a row ($P < 0.05$).

Bodini et al., (2013) had studied gelatine based edible films with ethanol propolis extract at different (0, 5, 40 and 200 g of extract 100 g^{-1} of gelatine) concentrations. The study showed only the films with propolis extracts, whose concentrations ranged between to 40 and 200 g, inhibited the growth of *S. aureus*.

Such results can be possibly attributed to the increase in polyphenol concentrations. Fish covered with edible film caused the inhibition of the microbial growth because of being created a good barrier of oxygen transfer to the fish (Song et al., 2011). However, Flores et al., (2010) reported that xanthan gum surface-active coating material had no positive effect on the inhibition of the microbial activity.

Microbiological quality

On first day was detected 3.0 (log CFU g^{-1}) and 3.5 (log CFU g^{-1}) from control group in total viable count (TVC) and and total aerobic psychrophilic bacteria (TAP). On the other hand, 3.05 (log CFU g^{-1}), 2.01 (log CFU g^{-1}) 2.5 (log CFU g^{-1}), 2.5 (log CFU g^{-1}) were detected for ENT, SPH, MY and LAB respectively. There was not statistically significant (*P>0.05*) difference between the groups on the first day of storage. During storage time, TVC and TAP of basil and daphne xanthan coated samples demonstrated an increase and reached to the maximal levels at day 10 of chilled storage. The uncoated fish samples from experiment group were completely spoiled at day 7. The microbial growth in trout fillets coated with extracts was lower than uncoated samples (*P > 0.05*). Extract coatings were effective in slowing down the bacterial growth, but the two extracts (daphne and basil) were not differences (*P > 0.05*). On the other hand, DE and BE resulted in good preservation in coated samples when compared with uncoated samples after 7 days of storage. Therefore, the odour and appearance of xanthan coated samples were not only better than the uncoated samples but also the inhibition of the microbial growth was in xanthan coated samples.

According to International Commission for Microbiological Specifications for Foods (ICMSF, 1986) the limits for mesophilic bacteria counts should not exceed equivalent to 10^7 CFU g^{-1} in fresh fish for human consumption. According to Figure 1A uncoated samples attained 7 log CFU g^{-1} on day 7, thus this time was taken as the maximum shelf-life of C group. However, coating with xanthan delayed and restricted the growth of microorganisms. The antibacterial extracts on fish samples can function as a barrier against oxygen diffusion and bacterial proliferation. The lowest microbial count, after 7 days of storage was recorded with DE coated fish. In fact, the TVC of fish coated with DE was equivalent to 5.3 log CFU g^{-1}, which is significantly lower (*P < 0.05*) than that recorded for both the control group (7.95 log CFU g^{-1}) and fish coated with BE group (5.51 log CFU g^{-1}). Andevari and Rezaei (2011) indicated that a cinnamon oil coated gelatine films extended the shelf-life of fresh rainbow trout fillets in cold storage. Gelatine coatings with cinnamon oil reduced the total bacteria growth for 15 days.

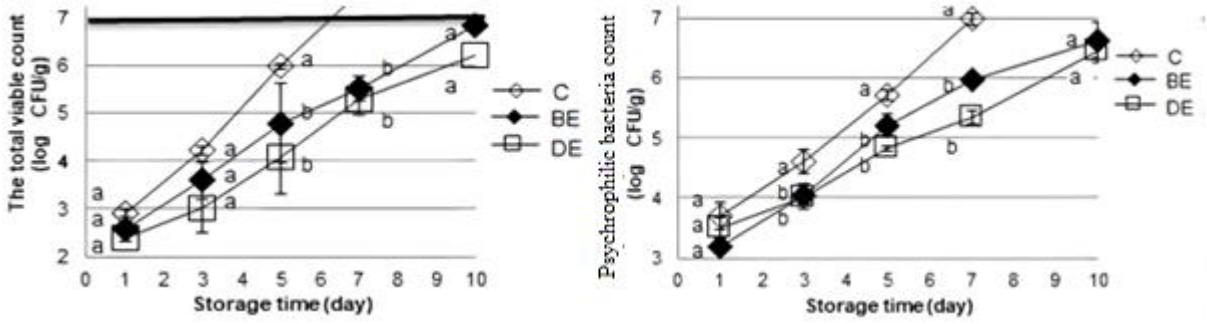

Figure 1. Changes in (A) total viable counts (log CFU g^{-1}) and (B) psychrophilic microorganisms (log CFU g^{-1}) during cold storage (4 °C) of uncoated and coated rainbow trout fillets. Lowercase letters difference between groups in the same day.

The changes in TAP of the uncoated and coated fish fillets are presented in (Figure 1B). Especially in the control group, the number of psychrophilic bacteria increased in the storage time and had the fastest bacterial growth rate ($P < 0.05$). Although extract coatings were effective in slowing down the bacterial growth on fillets, but no significant difference was recorded between the two extracts (DE and BE) ($P > 0.05$). In general, the most effective inhibition of psychrophilic bacteria was obtained when coated fillets by DE.

Ojagh et al. (2010) reported that the counts of psychrophilic bacteria obtained from coated trout fillets with chitosan enriched with cinnamon (2.88 log CFU g^{-1}) and chitosan (3.85 log CFU g^{-1}) were very close to each other at the beginning of storage. The study also mentioned the growth and cell counts in PVC and TVC were similar.

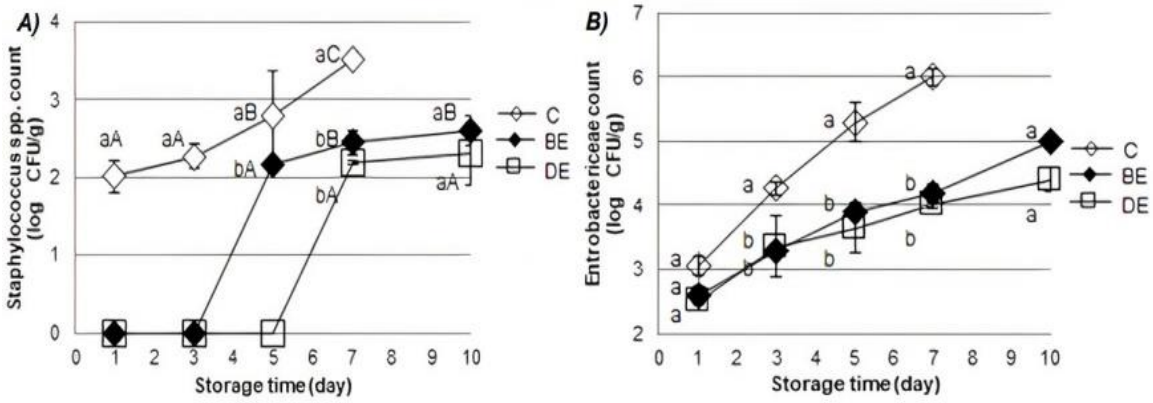

Figure 2. Changes in (A) *Staphylococcus* spp. (SPH) (log CFU g^{-1}); (B) *Enterobacteriaceae* (ENT) microorganisms (log CFU g^{-1}) during cold storage (4 °C) of uncoated and coated rainbow trout fillets. Lowercase letters difference between groups in the same day. Uppercase letters difference in the same group during storage.

At the beginning of storage period, SPH were not detected in DE and BE (<10 CFU g^{-1}), yet an increase was recorded during the last days of storage (Figure 2A). The growth of *Staphylococcus* bacteria started after the 5th (2.16 log CFU g^{-1}) and 7th (2.8 log CFU g^{-1}) days in the samples coated with BE and DE. The highest slope of increment in population of *Staphylococcus* bacteria observed was uncoated samples ($P > 0.05$). The lowest growth of *Staphylococcus* bacteria was that of the sample DE ($P > 0.05$). Stojanovic-Radic et al., (2018) reported that treatment of meat pieces with basil essential oils (BEO) demonstrated bactericidal effect against the entire bacteria population and resulted in reduced the growth of *S. aureus* by 2.74 log CFU g^{-1}.

Significant reduction (more than 2 log CFU g^{-1}) in the *Enterobacteriaceae* count (ENT) was observed in treatments with extracts ($P < 0.05$) (Figure 2B). Both DE and BE samples resulted in a significant reduction in bacterial counts equivalent to (4.00 log CFU g^{-1}) and (4.20 log CFU g^{-1}) respectively when compared to the control samples (6.00 log CFU g^{-1}) at the 7th day ($P < 0.05$). In daphne treatment also demonstrated bactericidal effect (almost 1 log) which was higher than that recorded by basil coated samples at the end of storage ($P > 0.05$).

This result is agreement with that of Volpe et al., (2015) regarding the *Enterobacteriaceae* counts. The *Enterobacteriaceae* counts at the end of the experiment were equivalent to 2 log CFU g^{-1}, 3.5 log CFU g^{-1} and 5 log CFU g^{-1} when the trout fillets were coated with carrageenan film containing lemon essential oil (1 % g ml^{-1}), carrageenan film and uncoated samples respectively. Frangos et al. (2010) reported that addition of 0.4 % (v w^{-1}) oregano essential oil in vacuum-packaged rainbow trout fillets significantly decreased the lactic acid bacteria, *Enterobacteriaceae*, *Pseudomonas* spp. and H_2S producing bacteria during storage at 4°C.

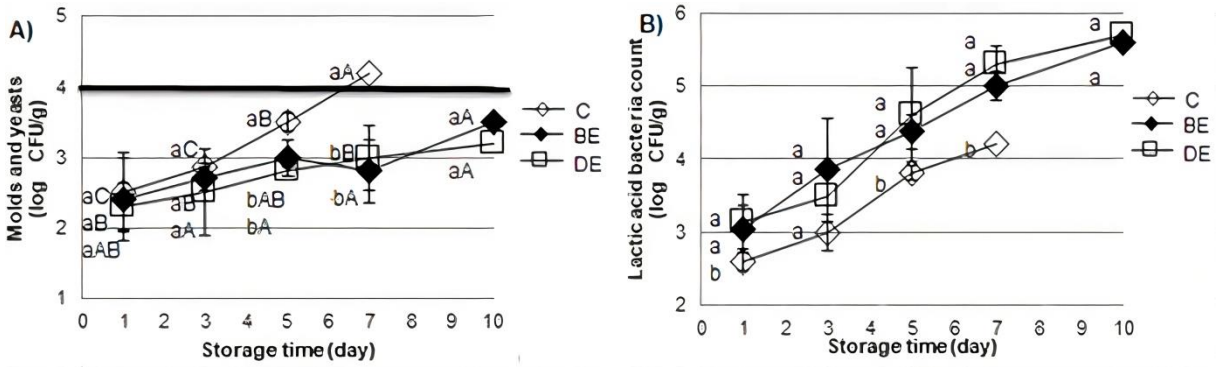

Figure 3. Changes in (A) Molds and yeasts (MY); (B) Lactic acid bacteria (LAB) (log CFU g^{-1}) during cold storage (4 °C) of uncoated and coated rainbow trout fillets. Lowercase letters difference between groups in the same day. Uppercase letters difference in the same group during storage.

Molds and yeast(s) exhibited similar growth trends during storage (Figure 3A). The increment in the Molds and yeast populations of uncoated sample was equivalent to 2.50 (log CFU g^{-1}), 2.85 (log CFU g^{-1}), 3.50 (log CFU g^{-1}), 4.20 (log CFU g^{-1}) during the storage period ($P > 0.05$). Molds and yeast count of daphne and basil extract coated fillets recorded 1.5 log lower than control samples in the last day of storage ($P < 0.05$). Xanthan film coating reduces contact with oxygen of microorganisms. The microbial population were observed reduction in the trout presented in this study agrees with other studies that used plant essential oils and/or extracts (Ojagh et al., 2010, Pyrgotou et al., 2010).

The LAB count was equivalent to 2.60 log CFU g^{-1} (on day 1) which increased to 4.20 log CFU g^{-1} on day 7 of storage for the uncoated samples (Figure 3B). On the same day, the use of daphne and basil extracts resulted in a rise of LAB by 0.55 and 0.44 log CFU g^{-1}, respectively ($P > 0.05$). The increment of LAB was also observed (López de Lacey et al., 2014) in hake fillet coated agar green tea film and agar probiotic green tea film during storage. The study was reported that green tea and probiotic edible agar films not only increased the shelf-life of hake fish for 1 week but also increased the number of beneficial lactic acid bacteria in fish. Similar results were reported by Raeisi et al., (2015) when rainbow trout treated with carboxymethyl cellulose-based coatings incorporated with *Zataria multiflora Boiss.* essential oil and grape seed extract. Previous studies also reported the enzyme produced by lactic acid bacteria can reduce the number of food spoilage microorganisms and inhibit the growth of pathogenic microorganisms (Adams et al., 1987; Gómez-Estaca et al., 2010; Ostergaard et al., 1998).

Bacterial strain identification

Dominant bacteria definitions in fish consumed as human food determine the bacteriological quality. Throughout the storage period in this study, 14 different bacteria and 3 different yeasts were identified via Bacterial Identification API Kits. The identified yeast isolates were *Candida calliculosa* (84.5 %), *Candida zeylanoides* (98.3 %), *Trichospora inkin* (92.7 %). These microorganisms are generally of soil origin. It is stated that it can be transmitted from plant extracts used in fish or from tools and materials used after catching. The lactic acid bacterial isolates were *Lactobacillus salivarius* (99.9 %), *Lactobacillus brevis* (99.6 %), *Lactobacillus acidophilus* (89.1 %), *Lactococcus lactis* ssp. (81.8 %). *Serratia liquefaciens* (98 %), *Serratia marcescens* (98 %), *Erwinia* spp. (85 %) which belong to *Enterobacteriaceae*, weren't detected only in xanthan-coated groups.

Serratia and *Erwinia* species are opportunistic pathogens and can be isolated from water, soil, plants, and air (Mossad, 2000). In our study, the identification of these species in coated trout samples with daphne and basil extract was thought that these species were come from the plants. On the other hand, *Moraxella* (76 %) was detected all groups. Mesophilic bacterial species were also identified those includes *Pseudomonas fluorescens* (99.9 %), *Pseudomonas luteola* (99.7 %), *Vibrio metschnikovii* (99.4 %), *Pasteurella multocida* (98%), *Pseudomonas putida* 96.8% and *Ralstonia picketti* (91.1%).

Sensory evaluation

Figure 4 shows the scores of the treated fish fillets in the sensory evaluation. Appearance and colour, odour, texture and in general acceptability were considered to evaluate the total acceptance (Figure 4).

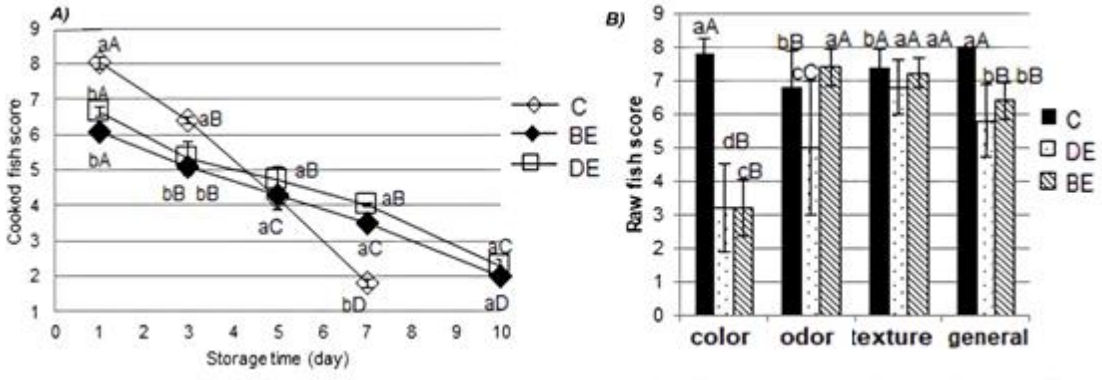

Figure 4. The sensory scores of cooked (A) and raw (B) fish fillets uncoated and coated with xanthan surface-active coating daphne and basil extract. Lowercase letters difference between groups in the same day. Uppercase letters difference in the same group during storage.

This study selected natural extracts such as daphne and basil due to their theoretically compatible organoleptic properties (odour, taste) with fish products. Even though the counts of treated samples were different from those of the control ($P < 0.05$), the sensory scores, which were analysed daily, demonstrated almost no significant differences between the treated groups ($P > 0.05$). In general, initial sensorial quality was good for all fish samples. Aroma and flavour due to treatment with plant extracts was evaluated by panellists. Superficial moisture was given negative evaluation when the control fish fillets were assessed at the 5th day. Yet severe liquid loss wasn't detected xanthan surface-active coating samples during the same storage period.

The colour scores, of the control groups at the last day of storage, were significantly lower when compared to those coated with DE and BE ($P < 0.05$) (Figure 4A). Samples of all groups tended to slowly deteriorate in their sensory scores up to day 5 ($P > 0.05$), after which a sudden and significant drop in sensory scores was recorded on the day 7 ($P < 0.05$). The texture parameters were stable over the entire storage period, with no significant difference between all groups during the first 7 days of storage. The C groups were evaluated as unsuitable for consumption on the 7th day, while the treated groups got discarded on the 10th day of storage (Figure 4B). Significant differences between the plant extracts became more evident in the end of week. It was clear that the best fish and plant odour were detected in the coated groups, DE were also judged as stronger than BE. Higher deterioration of sensory characteristics was detected by the panellists in control groups when compared to the treated groups on the 7th day. Subsequently, lower score in “cooked fish flavour” was recorded for the xanthan coated samples.

In a study performed by Mexis et al. (2009) the shelf-life of trout fillets was determined as 4 days for the control samples when stored at 4 ºC, 7–8 days for samples containing oregano essential oil, 13–14 days for samples containing O_2 absorber and 17 days for samples containing O_2 absorber and oregano oil. They reported that both odour and taste are determinant factors effecting the quality of rainbow trout fillet ($P < 0.05$).

pH

Figure 5 presents the pH values of the xanthan coated (DE and BE) and uncoated control samples (C) for 10 days storage at 4 °C. The initial pH of all samples was founded 6.2. Coating the surface of fish samples by xanthan gum was resulted in decrease of pH values ($P > 0.05$), it's, as low of pH in xanthan coating and plant extracts. The values of pH in coated samples were no significant difference ($P > 0.05$) between the groups. Similarly, change in pH values were also reported in other previous studies (Arashisar et al., 2004; Chytiri et al., 2004; Kakaei and Shahbazi, 2016).

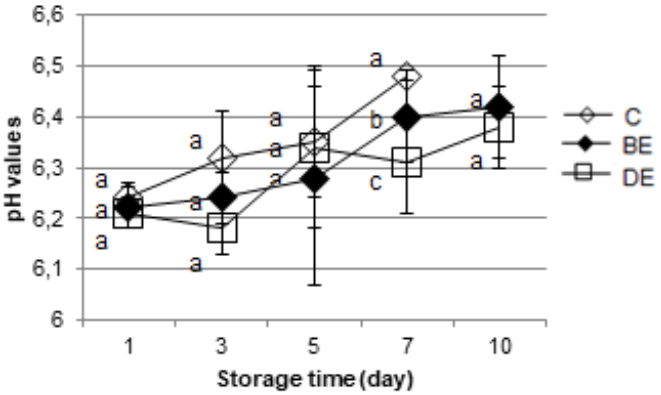

Figure 5. Changes in pH values of uncoated and coated rainbow trout fillets. Lowercase letters difference between groups in the same day.

CONCLUSION

The results of the study proved that xanthan gum-based edible film coatings with antimicrobial extracts are a successful and effective method to extend the shelf life of fish fillets for 10 days storage at 4 °C. All the coated with basil and daphne extract samples showed lower degradation in total viable counts, total aerobic psychrophilic bacteria, Molds – yeasts and pH as opposed to coated without extract samples. Significant delays were also found in weight loss, *Staphylococcus* spp. and *Enterobacteriaceae* microorganisms. The use of 1% daphne and basil extracts in the edible coating combination shows that it is the best coating combination as it preserves the microbial quality in the fish. The lactic acid bacteria were more increased in all coated with extract samples due to anaerobic condition and plant extracts. While the samples coated with the extract were less preferred due to the different colour formation in the fish, especially the products with daphne extract were appreciated in terms of flavour and texture properties. In general, all coated with extract samples were acceptable at the end of the 10-day storage run, while only xanthan gum coated samples were acceptable up to day 7. In future studies, it is necessary to be improved the colours of the extracts used in fish edible surface coatings, containing natural antimicrobials. Thus, BE and DE could be used as an active coating to maintain the quality and extend the shelf-life of the rainbow trout fillets under refrigerated storage.

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Serotonin Values According to Nutritional Habits of University Students and Its Effect on Academic Achievement, Quality of Life

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Abstract

Objective: The study was planned to determine the effect of nutrition on serotonin and, consequently, the effect of different eating styles on students' academic achievement and quality of life. Method;The study was conducted with 155 students studying at Afyon Health Sciences University Faculty of Health Sciences, Nutrition and Dietetics, Physical Therapy and Rehabilitation, Nursing and Health Management departments. Blood samples and questionnaires taken from students were used in the study. Personal and nutritional status information form and World Health Organization Quality of Life Scale-Short Form (WHOQOL-BREF) were used in the questionnaire. Results; (31.6%) of the students participating in the research are students of the Department of Nutrition and Dietetics. BMI ($\bar{X}$ = 22.52**)** of the students is between normal values. The grade point average of the female students participating in our study is 2.83, and the average of the male students is 2.62. The students' serotonin levels ($\bar{X}$=37.12 ng/dl) were generally lower than the reference values. It was found that general stress level moderately affected academic achievement and general quality of life, and personality type moderately affected quality of life and general stress level ($p<0.05$). Accordingly, as the stress level of the students and consumption of fruits, vegetables and legumes increase, their academic success increases, but their quality of life decreases ($p<0.05$). In addition, it was determined that the quality of life of the students whose personality type was open to life, extrovert, and agreeable was stronger than those who were emotional ($p<0.01$). Conclusion; It was determined that students' diets affect serotonin levels and academic success. A continuous "Proper Nutrition Program" should be carried out in universities on the nutrition patterns and effects of university students, and thus both the educational success of the students should be supported, and their quality of life should be improved by improving their well-being.

Keywords: Nutrition, Serotonin, academic success, quality of life

Research article
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INTRODUCTION

It is accepted that inadequate and unbalanced nutrition affects public health negatively and slows down social and economic development (Özmert, 2005; Odhiambo et. al., 2015; Türkmen ve Karaca Sivrikaya, 2020). With the beginning of the university period, the lifestyles of the students have changed and this has led to a change in the nutritional habits of the students. Changing nutritional behaviors can affect the mental and physical state of university students, as well as indirectly affect their academic success (Erten, 2006; Muslu, 2023).

Serotonin is mainly synthesized in the enterochromaffin cells in the intestinal mucosa, serotonergic nerve endings and pineal gland (Kuhn ve Lovenberg, 1983; Vanhoutte et.al., 1988). Although only 1% of the total serotonin in the body is found in the central nervous system, it plays a role in many important events such as blood pressure regulation, sleep, thermo-regulation, behavior, memory, eating and drinking. Some diseases are associated with pathological changes in serotonin metabolism. For example, measurement of serotonin is valuable in diagnosing carcinoid syndrome (Keller et. al., 1987).

Academic success has a significant impact on university students' future plans. University education plays a major role in guiding the future lives of individuals. For this reason, it is of great importance to know the factors affecting the success of students. Many factors affect the success of students. In order for students to be successful, they should not be adversely affected by these factors. In recent studies, different variables such as non-cognitive factors affecting students' academic success, the relationship between students' cognitive consistency and academic success, the effect of special talent exams on academic success, the relationship of nutrition, physical activity and sports with academic success have begun to be investigated. In this context, our study was planned to determine the effects of diet and serotonin levels on students' academic achievement and quality of life.

MATERIAL and METHOD

The universe of the study consists of all 3rd year students (206 students) in a state university, Faculty of Health Sciences, Nutrition and Dietetics, Physical Therapy and Rehabilitation, Nursing and Health Management departments. The study was completed with 155 students by excluding students who have any chronic disease, regularly use drugs, vitamins or minerals and do not want to participate in the study. In the study “G. Power-3.1.9.2” using the program, the sample size was calculated using the sample calculation method with a known universe at 95% confidence level. As a result of the analysis, the sample size was calculated as 155 with a theoretical power of 0.80 (α= 0.05, 1-β= 0.80).

The study is a cross-sectional study. Ethics committee approval was obtained for the study from Afyon Kocatepe University Faculty of Medicine Clinical Research Ethics Committee with the decision dated 04.08.2017 and numbered 2017/8-219. In the study, the principles of informed consent, autonomy, confidentiality and protection of confidentiality, fairness, non-harming/beneficialness were taken into consideration. Supported by Afyon Kocatepe University Scientific Research Projects Unit as Project No. 18.Kariyer.08.

Students who were eligible and volunteered for the study were informed about the study and their voluntary consent was obtained. Afterwards, the questionnaire forms were filled in and blood samples were taken in the morning while fasting. Blood samples were taken by a nurse in the nursing practice laboratory, in accordance with aseptic techniques.

Questions about nutritional status of students, socio-demographic, socio-economic, socio-cultural status (11 questions), questioning information about nutritional status (16 questions), general stress levels (1 question), questions about personality type (1 question) were reviewed by the researcher. (Erten, 2006; Dağ, 2013).

The questions of the World Health Organization Quality of Life Short Form (Whoqol Bref) (27 questions) scale (Fidaner ve ark. 1999; Aydemir ve Köroğlu, 2006; Eser ve ark., 1999) were applied. The academic success of the students was determined regarding their weighted Grade Point Average (GPA) scores obtained from Student Affairs Office. The data were analyzed using the SPSS statistics program. The significance level was taken as $p< 0.05$ for all statistical analyzes. Frequency and percentage calculations, one way analysis of variance (ANOVA) and Pearson correlation analysis were used to analyze the data.

World Health Organization Quality of Life Scale, short form (WHOQOL-BREF):

The validity and reliability of the Turkish version was shown by Eser et al. It contains 26 items, measuring general health (0-15 points), physiological health (9-35 points), psychological health (6-30 points), social relationships (3-15 points), and environmental health (16-40 points). Each sub-domain provides an independent measure of the quality of life, with a total score range of 49 to 120. Higher scores indicate a better quality of life. The reported alpha-coefficients for reliability for the above-listed subdomains in the Turkish version are 0.83, 0.66, 0.53, 0.73, and 0.73, respectively (Fidaner ve ark. 1999; Eser ve ark., 1999; Eser ve ark. 2010; Avcı ve Pala, 2014). In our study, alpha values were determined as 0.81, 0.67, 0.53, 0.75, respectively.

RESULTS and DISCUSSION

The students, 20.6% were Nursing, 17.4% Health Management formal education (FE), 14.2% Health Management evening education (EE), 16.1% Physical Therapy and Rehabilitation and 31,6% Nutrition and Dietetics, 73.5% were girls and 26.5% were boys.

The students, 59.4% stayed in the dormitory, 56.1% had a mother's education level of primary school or below, 40.0% had a father's education level at Secondary education and 44.5% of the students spent most of their lives in provincial centers determined. It is seen that 81.3% of the students do not sports regularly, 54.8% sleep 7-8 hours at night, 80% do not smoke and 84.5% do not drink alcohol, 85.2% did not follow a diet and 48.3% of them consumed a snack once a day (Table 1).

Table 1. Findings regarding the socio-demographic and nutritional characteristics of the students

Variables	**Groups**	**f**	**%**
Department	Nursing	32	20,6
	Formal Health Management (FE)	27	17,4
	Evening Health Management (EE)	22	14,2
	Physical Therapy Rehabilitation	25	16,1
	Nutrition and Dietetics	49	31,6
Gender	Female	114	73,5
	Male	41	26,5
Shelter	Family	15	9,7
	Country	92	59,4
	home with friends	48	31,0
Mother education	Elementary school or below	87	56,1
	secondary education	52	33,5
	High school education	16	10,3
Father education	Elementary school or below	51	32,9
	secondary education	62	40,0
	High school education	42	27,1
Where life takes place	city	69	44,5
	District	66	42,6
	Town- Village	20	12,9
Regular sport	No	126	81,3
	Yes	29	18,7
Night sleep	4 hours or less	14	9
	5-6 hours	53	34,2
	7-8 hours	85	54,8
	9 hours or more	3	1,9
Smoking	No	124	80
	Yes	31	20
Alcohol Use	No	131	84,5
	Yes	24	15,5
Diet in the past year	No	132	85,2
	Yes	23	14,8
Snack	None	29	18,7
	Once a day	75	48,3
	Twice a day	41	26,4
	Three times a day or more	10	6,4
Total		155	100

While the average GPA of the female students participating in our study is 2.83, the average age is 20.84, and the Body Mass Index (BMI) is 21.62, the average GPA of the male students is 2.62, the average age is 21.45, and the BMI is 23.43 (Table 2).

Table 2. Descriptive statistics on quantitative variables

Variables	FEMALE				MALE			
	Min.	**Max.**	**Ort.**	**SS**	**Min.**	**Max.**	**Ort.**	**SS**
GPA	2,12	3,65	2,83	0,35	1,89	3,40	2,62	0,31
Age	18,00	27,00	20,84	1,43	19,00	26,00	21,45	1,46
Size	152,00	177,00	163,30	5,12	164,00	190,00	176,95	5,46
Weight	42,00	95,00	57,68	9,71	58,00	110,00	73,35	10,77
BKI	15,79	35,32	21,62	3,47	18,79	33,95	23,43	3,25

It was determined that there was a statistically significant ($p<0.01$) difference between the students quality of life sub-dimensions. Accordingly, while the physical health dimension ($\overline{X}=3.59$) of the students is the highest, the mental health dimension ($\overline{X}=3.41$) is high, the social relations dimension ($\overline{X}=3.37$) is high, and the environmental health dimension ($\overline{X}=3.35$) is high found (Table 3).

Table 3. Findings Regarding the Sub-Dimensions of Students Quality of Life

WHO-SF Sub-Dimensions	$\overline{X}$	**SS**	**F**	**P**
Physical Health Dimension	3,59 a	0,56	9,608	0,000*
Mental Health Dimension	3,41 b	0,65		
Social Relations Dimension	3,37 b	0,76		
Environmental Health Dimension	3,35 b	0,43		
General	**3,43**	**0,48**		

*p<0,01

The students serotonin levels ($\overline{X}=37.12$ ng/dl) were determined to be low, and there was no statistically significant difference between serotonin levels and gender ($p>0.05$) (Table 4).

Table 4. Findings Regarding the Seratonin Values of the Students

Serotonin value	$\overline{X}$	**SS**	**p**	**The reference range**
Female	35,19	22,62	0,562	50-250 ng/dl
Male	42,23	75,01		
General	37,12	43,52		

When the correlation coefficients were examined, it was found that there was a moderately strong positive relationship between the general stress level and academic achievement. In other words, as the general stress level increases, academic achievement increases. A negative and moderate correlation was found between general quality of life and its sub-dimensions and general stress level.

This means that as the general quality of life and sub-dimensions scores increase, the general stress level will decrease moderately. Accordingly, those with good mental health have the least stress, followed by social relations, physical health and environmental health at least. As a result, it is revealed that as the general quality of life increases, the general stress level will decrease moderately. Apart from this, although there was a weak negative relationship between serotonin hormone, general stress level and academic achievement, no statistical significance was found (p>0.05) (Table 5).

Table 5. Correlation coefficients for the relationships between General Stress Level, WHO-SF and Serotonin hormone

Variables	**General Stress Level**	**GPA**	**Serotonin**
General Stress Level	-	0,229*	-0,060
Physical Health Dimension	-0,326*	-0,017	0,045
Mental Health Dimension	-0,420*	-0,050	0,052
Social Relations Dimension	-0,350*	-0,151	0,020
Environmental Health Dimension	-0,296*	0,018	0,032
General WHO-SF	-0,445*	-0,078	0,045
GPA	0,229*	-	-0,012

**p<0,01*

A statistically significant relationship was found between personality type, quality of life and general stress level (p<0.01). It was revealed that the students who stated that their personality type was open to life ($\bar{X}$ =3.58), extrovert ($\bar{X}$=3.52) and agreeable ($\bar{X}$ =3.50) had a stronger positive quality of life than those who were emotional ($\bar{X}$ =3.26). In addition, it was found that the general stress levels of those with emotional ($\bar{X}$=6.80) and extrovert ($\bar{X}$ =6.43) personality types were higher than those whose personality type was agreeable ($\bar{X}$ =5.58) and open to life ($\bar{X}$=4.71) reached (Table 6).

Table 6. Correlation between General WHO-SF and General Stress Levels by Personality Types

Personality Types		**General WHO-SF**		**General Stress Level**	
	n	$\bar{X}$	**SS**	$\bar{X}$	**SS**
Extrovert	23	3,52 a	0,56	6,43 a	2,46
Agreeable	46	3,50 a	0,43	5,58 b	1,85
Emotional	57	3,26 b	0,40	6,80 a	1,94
Open to life	29	3,58 a	0,54	4,71 c	2,51
p		**0,009***		**0,000***	

**p<0,01*

It was found that there was a moderately strong positive relationship between the weekly consumption of fruits, vegetables and legumes and their academic achievement ($p<0.05$). In other words, as the consumption of fruits, vegetables and legumes increases, academic success increases. In addition, it is seen that there is a moderately strong positive relationship between yogurt consumption and general quality of life ($p<0.05$). Accordingly, as yogurt consumption increases, the overall quality of life also increases (Table 7).

Table 7. Serotonin values according to nutritional status and related factors correlation coefficients

Variables	**GPA**	**General WHO-SF**	**General Stress Level**	**Serotonin**
Daily water consumption	0,059	-0,78	0,229	-0,081
Other fluid consumption per day	-0,123	-0,101	0152	0,013
Weekly milk consumption (lt)	0,024	0,046	-0,052	-0,055
Weekly cheese consumption (slice)	0,080	0,059	0,006	-0,44
Weekly yogurt consumption (bowl)	0,077	**0,175***	0,014	-0,017
Weekly red meat consumption (portion)	0,149	0,020	-0,004	0,002
Weekly chicken consumption (portion)	-0,003	0,039	-0,001	-0,029
Weekly fish consumption (piece)	-0,005	0,144	0,043	-0,050
Weekly bread consumption (piece)	-0,073	0,075	-0,009	-0,006
Weekly cake consumption (portion)	-0,022	0,047	0,075	0,066
Weekly fruit consumption (portion)	**0,167***	0,133	-0,061	-0,006
Weekly vegetable consumption (portion)	**0,203***	0,037	-0,036	-0,005
Weekly legume consumption (portion)	**0,185***	0,000	-0,034	0,003
Weekly grain consumption (portion)	0,143	0,080	-0,034	-0,035

**p<0,05*

According to the validity and reliability analysis results of the World Health Organization Quality of Life Scale-Short Form (WHO-SF), “Physical Health” (ability to carry out daily activities, dependence on drugs and treatment, vitality and fatigue, mobility, pain and discomfort, sleep) and rest, ability to work) sub-dimension of "Mental Health" (body image and appearance, negative emotions, self-esteem, positive emotions, spirituality/religion/personal beliefs and thinking/learning/memory/attention) sub-dimension, "Social Relationships" (relationships with other people, social support and sexual life) and "Environmental Health" (financial resources, physical safety and security, health services and social assistance, accessibility and quality, home environment, acquiring new knowledge and skills) opportunities, listening and leisure time opportunities and being able to participate in them were determined to be higher than the physical environment (pollution/noise/traffic/climate) and transportation sub-dimension.

When the correlation coefficients between the students general stress level, general quality of life and sub-dimensions and academic achievement were examined, it was found that there was a moderately strong positive relationship between the general stress level and academic achievement. In other words, as the general stress level increases, academic achievement increases. The results of Özgan ve ark. (2008) research analysis results of the study titled "Causes of stress perceived by education faculty students in the classroom and the effect of personal variables on stress show that there is a significant difference between the stress and achievement status of Education Faculty students [F(2-107)=3,80, p0.05]. According to the results of the test conducted to determine between which groups the differences among students are, students with a grade point average between 3.00 and 4.00 (X = 63.91) have a grade point average between 2.00 and 2.99 (X = 55,94) were found to be more stressed than students. Contrary to this finding of the study, in the study conducted by Çakmak and Hevedanlı (2005), no statistically significant difference was found between the anxiety levels of students according to their perception of their success. Students with a high grade point average tend to work harder and show a greater desire to be successful. These students may have a more stressful structure than other students because they want to be more successful in their lessons and therefore work harder. At the same time, this situation can make them more stressed, as they are in the desire and effort to maintain the state of success they have. When various definitions of stress are examined, it is seen that stress is mostly handled in a negative and harmful way. However, even if stress puts the individual in difficulties, jeopardizes his harmony, causes pain and anxiety, it also has a feature that takes the person forward, happiness and success when stress is coped with. Stress and possible difficulties in daily life can increase the level of burnout of the individual; high burnout can negatively affect the academic adjustment of the individual. In other words, it can be expected that higher burnout level is associated with lower academic achievement level (Belkıs ve ark. 2011). This finding supports Yang (2004)'s view that it is necessary to take measures to reduce burnout levels and stress at the same time in order to increase students' academic success and learning motivation. A negative and moderate correlation was found between general quality of life and its sub-dimensions and general stress level. This means that as the general quality of life and sub-dimensions scores increase, the general stress level will decrease moderately. Accordingly, those with good mental health have the least stress, followed by social relations, physical health and environmental health at least. As a result, it is revealed that as the general quality of life increases, the general stress level will decrease moderately. The quality of life of people can be affected by the stress they are exposed to in the work environment as well as their individual characteristics (Hoffman ve Scott, 2003; Bjørk et. al., 2007).

According to the correlation coefficients between the students quality of life and general stress levels according to their personality types, it was found that the students who stated that their personality type was open to life, extrovert, and agreeable had a stronger positive quality of life than those who were emotional. In addition, it was found that the general stress levels of those whose personality type is emotional and extroverted are higher than those whose personality type is agreeable and open to life. Literature data showing that there is a positive correlation between personality type and quality of life (Demirci ve ark., 2016; Bal ve Şahin, 2011) is consistent with the study result. When the correlation coefficients of the Serotonin values and the factors affecting the students according to their nutritional status are examined, the academic success of the students increases as the consumption of fruits, vegetables and legumes increases. The health and school success of students who are malnourished and unbalanced are negatively affected (Uçar ve Hasipek, 2008).

Similar to the results of the study, it was determined that as the risk related to eating habits of university students increased, their BMI increased and their academic achievement decreased (Aktaş, 2019; Carrillo-López, 2023). In addition, as yoghurt consumption increases, the overall quality of life also increases. There is no overlapping study on this subject. However, it is thought that the obtained findings can be a source in terms of literature knowledge.

Although studies on the positive effects of adequate, balanced and varied nutrition on health (Karadeniz ve ark. 2008; Hawkins ve Stewart, 2012; Mikolajczyk et. al. 2010; Türkmen ve Karaca Sivrikaya, 2020) support our findings, during the period when blood samples were collected, students were exposed to sunlight, season, fruits and vegetables included in the diet. It is thought that it may vary due to variables such as the type of vegetables.

CONCLUSION

As a result, students

- Serotonin levels are low and this negatively affects academic success,

- As the general stress level increases, academic success increases,

- As the general quality of life increases, the general stress level decreases

- People whose personality type is emotional and extrovert have higher general stress levels than those whose personality type is agreeable and open to experience,

- As the consumption of fruits, vegetables and legumes increases, academic success increases.

- Quality of life scores are high and as yoghurt consumption increases, the overall quality of life score increases.

has been seen.

In addition, a continuous "Proper Nutrition Program" should be carried out in universities on the nutrition patterns and effects of university students, thus supporting the educational success of students and improving their well-being and quality of life.

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The Effect of New Concepts Used for Meat on Neophobia

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Abstract

The objective of this research is to examine the responses of students enrolled in the Faculty of Health Sciences at Afyonkarahisar University of Health Sciences when asked with meat substitutes which suggested as an alternative to traditional meat products. The study involved 294 participants who were randomly selected from the student population. These participants have an average age of 21.3 ± 7.66 years. The majority of the participants are women, accounting for 86.1% of the sample. Among the students, the Nutrition and Dietetics Department is the most heavily represented, making up 61.6% of the participants. The research explores participants' perceptions of novel meat substitutes and their reactions to these alternatives. A significant portion of the participants expressed the belief that these new meat concepts are unnatural. Interestingly, when comparing the responses across different academic departments, there were no noticeable differences in attitudes toward these novel meat concepts, and this finding was statistically significant ($p < 0.05$). The study also delves into the participants' level of food neophobia, which refers to the fear or reluctance to try new foods. The average score on the food neophobia scale was found to be 35.46±5.646. This score serves as an indicator of the participants' overall attitude towards trying unfamiliar foods. Notably, there was no significant difference in the degree of food neophobia when considering the participants' academic fields.

Keywords: Artificial Meat, Meat, New Food Phobia, Purchasing.

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INTRODUCTION

Protein is a component that is the basic building block of cells and is very necessary for life, it is one of the most important building blocks that make up the human body. Proteins, which have a high number of important activities in living metabolism, are found in foods of plant and animal origin.

Proteins are important macronutrients for life. The protein rate in the body of an adult is around 16% and the amount of storage is very small. It is recommended that 10-20% of the energy used daily should come from protein (TÜBER, 2019).

In recent years, with the increasing human population and differentiating consumer orientations, there has been an increase in different research and studies on alternative protein sources. The world population is expected to be around 10 billion people by 2050. It is foreseen that with the increase in population, the need for meat consumption will increase and meat production will be at the highest level. Among different foodstuffs, some products of animal origin, such as meat and dairy products, consume more natural resources than foods of plant origin (Molfetta et al., 2022). For this reason, it was foreseen that the classical method would not be able to respond to the increasing meat consumption need and it was thought that the production cost would increase even more. Therefore, it is seen that meat consumption is a luxury need (Pandurangan and Kim, 2015). In addition to having a large ecological footprint, meat production, and consumption, another important reason for reducing meat consumption, especially red meat consumption, is directly related to the potential negative effects of this consumption on human health. For example, associations have been reported between red and processed meat consumption and an increased risk of developing colon, breast, pancreatic, and prostate cancers, and a high prevalence of chronic diseases. Recently, epidemiological evidence has concluded that it is strong enough to confirm that red meat or processed meat intake increases cancer risks, and more specifically, colorectal cancer risk. Although the exact mechanisms underlying the relationship between meat consumption and the risk of developing cancer are still not clarified, red meat consumption, in general, should be reduced to less than 50 g/day to avoid an increased risk of prostate cancer, breast cancer, or colorectal cancer (González et al., 2020). In 2019, a consortium of researchers from 42 universities or research centers in 8 European countries launched the SYSTEMIC project (an integrated approach to the challenge of sustainable food systems: adaptive and mitigating strategies to address climate change and malnutrition). The project aims to develop ways to transform a food system that is climate-resilient and capable of meeting societal challenges. Provides information on proven options that provide sustainable and nutritious food, such as alternative protein-rich foods (e.g., vegetables, fibrous fungi, algae, microalgae, and other microorganisms, insects, and "cultured" meat) that could possibly replace meat (SYSTEMIC, 2023).

Meat substitution is still rather murky as a concept that may relate to the historical development of the need to provide protein and later meat substitution. Terms used for meat substitutes should be outlined. "Meat alternative" is a general term denoting any source of protein (plant, animal, fungal, or microalgae) that can be used as a meat substitute in a meal. The protein sources used as the first choice instead of meat are plants and mushrooms. Insects are also one of the important protein sources with a very high protein content. In addition, it forms the basis of protein sources in laboratory-produced meat or artificial meat, as well as algae. However, various meat analogs on the market do not include insects, microalgae, and other meat bases (Smetana et al., 2023). One proposed solution to reduce our consumption of animal meat is the development and use of cultured meat that can be grown from animal cells without the need for slaughter. In addition to eliminating the need for slaughter, cultured meat is much less harmful to the environment in terms of greenhouse gas emissions and land and water use. Cultured meat may become commercially available within a few years. In recent years, research on consumer acceptance of cultured meat has increased, but there is no technique yet to achieve this (Bryant, 2020). The term “cultured meat” will be used here as it seems to be the most widely used and accepted term, but alternative terms include “synthetic meat”, “in vitro meat”, “lab-grown meat”, “cured meat” and sometimes “artificial meat”.

The two main stem cells considered most suitable for meat culture are embryonic stem cells and satellite cells (Reddy et al., 2022). Artificial meat made from stem cell cultures has a different production process among meat options. Artificial meat is like normal meat not only in appearance and form but also in content. For lab-grown meat, stem cells from a live cow are harvested and fed in the lab to build muscle tissue. Lab-grown meat is not yet available to consumers as the technology remains cost-prohibitive but is expected to be available in the coming years. Besides the technical feasibility challenge of successfully producing large quantities of affordable lab-grown meat, another major challenge is consumer acceptance of new products. For these reasons, more development and promotion work will be needed for artificial meat (Van Loo et al., 2020).

Food choice is based on social, cultural, and individual factors of which to eat or avoid. Today's consumers are increasingly acquainted with a variety of food options as a result of advances in food production, marketing, and transportation (Okumus et al., 2021). While new and different foods are mostly attracted by individuals, some individuals may approach these new and different foods cautiously for different reasons. Firstly, it is the sensory attitudes (disliking the features such as the appearance and smell of the food), second, the expectations about whether the consumption of the food will be beneficial or harmful in the long run, and third, the information that may cause the food to be evaluated positively or to be called disgusting (Kol and Akçil Ok, 2020). This situation is explained by the term “Neophobia”. neophobia; It is used to characterize fear and similar reactions to the novelty of something. This fear may arise against new objects, places, sounds, and other types of impulses. If this new resource is food, it is described as “food neophobia”, that is, “Fear of Innovation in Food” (Dağ and Tabak, 2021). In the study of Pliner and Hobden (1992), the concept of food neophobia is defined as "avoidance of new food or foods" (Pliner and Hobden, 1992). Food neophobia has been defined as a personal reluctance to accept and/or enjoy new or unfamiliar foods (Rabadán and Bernabéu, 2021). Factors affecting food choices can be both innate and learned. That is, although neophobia is largely hereditary, it can also be a result of the environment in which individuals grow up. Imitation of parents or peers and parent's educational practices can be given as examples of environmental factors (Soylu et al., 2021). Food neophobia causes a decrease in the variety of food consumed and the inability to provide people with as much nutrition as they need. The excess of unconsumed foods or the long duration of this process can negatively affect the health of people (de Oliveira Torres et al., 2020). In the study, it was aimed to evaluate university students' knowledge and preference of the concept of artificial meat and food neophobia against this concept.

MATERIAL and METHOD

Participants

The research is a descriptive type of observational study. The population of the research consists of 2137 students studying at Afyonkarahisar Health Sciences University Faculty of Health Sciences (AFSU, 2023). The data of the study were obtained from the online questionnaire form applied to 294 students who attended school between January and May of the 2022-2023 academic year and agreed to participate in the study. Written informed consent was obtained from the participants before starting the study. Clinical Research Ethics Committee of Afyonkarahisar Health Sciences University granted approval for the study with the code 2023/1 and the date 06.01.2023. Since it was aimed to reach high participation in the study, the sample was not selected, and all students studying at the faculty were tried to be reached. In the research, 13.8% of the population was reached.

Measure

As a data collection tool, a questionnaire developed as a result of the researchers' literature review was used (Duman et al., 2020; Gençel, 2021). The developed questionnaire consists of three main parts. In the first part, the socio-demographic and economic characteristics of the students were examined, then the meat consumption habits and purchasing characteristics and the factors affecting them were analyzed. In the second part, the "Food Neophobia Scale" was used to describe the avoidance, fear, or avoidance of the students towards food items that they have not encountered before or are unfamiliar with. The Food Neophobia Scale (FNS) questionnaire was developed by Pliner and Hobden in 1992 (Pliner and Hobden, 1992), and its Turkish adaptation was done by Duman et al. in 2020 (Duman et al., 2020). The Food Neophobia Scale was evaluated with a single factor and 10-item 5-point Likert scale. The options are as follows: "Totally Agree" is 5 points, "I agree" is 4 points, "Neither agree nor disagree" is 3 points, "Disagree" is 2 points, and "Totally Disagree" is 1 point (Gençel, 2021). Items 2, 3, 5, 7, 8 and 9 of the scale are evaluated as "trust in new foods", and items 1, 4, 6 and 10 are reverse scored and evaluated as "willingness to try new foods" (Uçar, 2018). Total scores can vary between 10 and 50 due to the use of a 5-point Likert scale for scoring. Participants were divided into two separate groups neophiliac and neophobic. High scores between 33 and 50 obtained from the Food Neophobia Scale indicate food neophobia (fear of food), and low scores between 10 and 25 indicate food neophilia (enjoyment of foods) (Gençel, 2021). In the third part, a 7-question survey prepared by the researchers was used to measure the attitudes of the participants towards different concepts used for artificial meat (Ede and Yalçın, 2023).

Statistical analysis

In the evaluation of the data, standard deviation (X±SD), frequency, and percentage values were used as descriptive statistical methods. Whether the relationship between categorical variables was significant was evaluated with the Chi-square test. For non-normally distributed variables, the Kruskal-Wallis test was used to examine differences between groups. To test the likely correlation between independent variables, the bivariant Pearson correlation was used ($p < 0.05$). While examining the hypothesis tests, $\alpha=0.05$, and accordingly the confidence interval was determined as 95%, and the significance was evaluated at the $p<0.05$ level. Statistical analysis of the data was performed in the SPSS v26 (IBM Inc., Chicago, IL, USA) package program.

RESULTS and DISCUSSION

The study involved a total of 294 participants, comprising 86.1% females (n=253) and 13.9% males (n=41). The participants' average age was 21.3 ± 7.66 years. Among the participants, a significant portion, 61.6% (n=181), were enrolled in the nutrition and dietetics department. Regarding their living arrangements, 41.5% (n=122) of the respondents reported residing in dormitories, 35.0% (n=103) lived in homes with their families, 13.9% (n=41) in student housing, and 5.4% (n=16) stated that they lived alone. When considering monthly income levels, 42.5% (n=125) of the participants reported having incomes between 0-1500 TL. Among those with these monthly incomes, 48.6% (n=143) allocated 25-50% of their income towards food expenditures, while 33.3% (n=98) allocated 50-75%, as shown in Table 1.

Table 1. Distribution of their descriptive characteristics of the participants

Variable	**Categories**	**n (294)**	**% (100)**
Age	18-21	141	48.0
	21-26	134	45.6
	26+	19	6.5
Mean ($\bar{X}$± SD)		21.3 ± 7.66	
Sex	Male	41	13.9
	Female	253	86.1
Education Department	Nutrition and Dietetics	181	61.6
	Physical Therapy and Rehabilitation	32	10.9
	Nursing	51	17.3
	Healthcare Management	30	10.2
Household	At home with family	103	35.0
	At dorm with friends	122	41.5
	Apartment	16	5.4
	At home with friends	41	13.9
	Alone	12	4.1
Monthly income	0-1500	125	42.5
	1501-3000	78	26.5
	3000 and above	91	31.0
What percent of your monthly food expenses income	%0-%25	38	12.9
	%25-%50	143	48.6
	%50-%75	98	33.3
	%75-%100	15	5.1

n: Frequency, %: percent, $\bar{X}$: Mean, SD: Standard deviation.

Table 2. Distribution table of participants' thoughts on new meat concepts

	Artificial Meat		**In-vitro Meat**		**Cultured Meat**		**Non-animal meat**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Tasty	12	4.1	6	2.0	55	18.7	6	2.0
Disgust	24	8.2	14	4.8	12	4.1	42	14.3
Not tasty	19	6.5	27	9.2	33	11.2	53	18.0
Unnatural	151	51.4	93	31.6	73	24.8	77	26.2
Anxious	19	6.5	48	16.3	46	15.6	42	14.3
Pose a threat to health	66	22.4	61	20.7	42	14.3	53	18.0
Other	3	1.0	45	15.3	33	11.2	21	7.1
Total	294	100	294	100	294	100	294	100

n: Frequency, %: percent

As presented in Table 2, participants were queried about their opinions on various meat-related concepts, including artificial meat, in-vitro meat, cultured meat, and meat produced without animals. The findings indicate that the participants largely perceive these products as unnatural. Specifically, 51.4% (n=151) find artificial meat to be unnatural, while 31.6% (n=93) feel the same way about in-vitro meat. For cultured meat, 15.6% (n=73) consider it unnatural, and for meat produced without animals, 26.2% (n=77) share this perspective.

As depicted in Table 3, participants were surveyed about their viewpoints on different meat concepts—artificial meat, in-vitro meat, non-animal meat, and cultured meat—based on their academic departments.

However, the analysis indicates that the participants' opinions regarding these novel meat concepts did not exhibit any statistically significant differences across the various departments they were enrolled in ($p > 0.05$). The findings demonstrate that, regardless of the academic department, all participants share the perception that the concepts of artificial meat, in-vitro meat, cultured meat, and non-animal meat are characterized as unnatural forms of meat.

Table 3. Distribution of thoughts on new meat concepts according to the section read by the participants.

Education Department		Nutrition and Dietetics		Physical Therapy and Reh.		Nursing		Healthcare Man.		Total		p
		n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	
Artificial Meat	Tasty	7	3.9	1	3.1	3	5.9	1	3.3	12	4.1	0.883 χ^2=11.253
	Disgust	14	7.7	2	6.3	3	5.9	5	16.7	24	8.2	
	Not tasty	13	7.2	2	6.3	3	5.9	1	3.3	19	6.5	
	Unnatural	95	52.5	19	59.4	23	45.1	14	46.7	151	51.3	
	Anxious	13	7.2	2	6.3	2	3.9	2	6.7	19	6.5	
	Pose a threat to health	36	19.9	6	18.8	17	33.3	7	23.3	66	22.4	
	Other	3	1.6	0	0	0	0	0	0	3	1.0	
In-vitro meat	Tasty	3	1.7	0	0	3	5.9	0	0	6	2.0	0.436 χ^2=18.306
	Disgust	9	5.0	1	3.1	2	3.9	2	6.7	14	4.8	
	Not tasty	20	11.0	1	3.1	3	5.9	3	10.0	27	9.2	
	Unnatural	55	30.4	10	31.2	17	33.3	11	36.6	93	31.6	
	Anxious	34	18.8	8	25.0	4	7.9	2	6.7	48	16.3	
	Pose a threat to health	32	17.7	6	18.8	15	29.4	8	26.7	61	20.8	
	Other	28	15.4	6	18.8	7	13.7	4	13.3	45	15.3	
Cultured meat	Tasty	35	19.3	6	18.8	9	17.7	5	16.6	55	18.7	0.906 χ^2=10.723
	Disgust	6	3.3	1	3.1	3	5.9	2	6.7	12	4.1	
	Not tasty	17	9.4	6	18.8	8	15.7	2	6.7	33	11.2	
	Unnatural	47	26.0	8	25.0	12	23.5	6	20.0	73	24.8	
	Anxious	28	15.5	5	15.6	5	9.8	8	26.7	46	15.7	
	Pose a threat to health	26	14.4	4	12.5	7	13.7	5	16.6	42	14.3	
	Other	22	12.1	2	6.2	7	13.7	2	6.7	33	11.2	
Non-animal meat	Tasty	4	2.2	1	3.1	1	2.0	0	0.0	6	2.0	0.281 χ^2=20.971
	Disgust	24	13.2	6	18.8	7	13.7	5	16.7	42	14.3	
	Not tasty	35	19.3	8	25.0	7	13.7	3	10.0	53	18.0	
	Unnatural	41	22.7	5	15.6	22	43.1	9	30.0	77	26.2	
	Anxious	30	16.6	3	9.4	4	7.9	5	16.7	42	14.3	
	Pose a threat to health	32	17.7	5	15.6	8	15.7	8	26.6	53	18.0	
	Other	15	8.3	4	12.5	2	3.9	0	0.0	21	7.2	

n: Frequency, %: percent, *p<0.05

When the new food fear level of the participants was evaluated, a minimum of 18 and a maximum of 48 points were determined, and the average was determined as neophobic with 35.46 ± 5.646. New food fear levels according to the departments studied by the participants; The states of being neophiliac, neutral, or neophobic are shown in Table 4. When the levels of neophobia (fear of new foods) were investigated in relation to the academic department the participants were enrolled in; it was determined that neophobic, that is, afraid to try new food, with the highest rate in all departments. The relationship between the department they read, and their new food fear levels was not found statistically significant ($p > 0.05$).

Table 4. Distribution of Participants' New Food Fear Levels

Education Department	Neophiliac (10-25)		Neutral (26-32)		Neophobic (33-50)	
	n	%	n	%	n	%
Nutrition and Dietetics	5	50.0	45	62.5	131	61.8
Physical Therapy and Rehabilitation	3	30.0	8	11.1	21	9.9
Nursing	1	10.0	12	16.7	38	17.9
Healthcare Management	1	10.0	7	9.7	22	10.4
Total	10	3.4	72	24.5	212	72.1

p=0.649 $\chi 2$=4.205

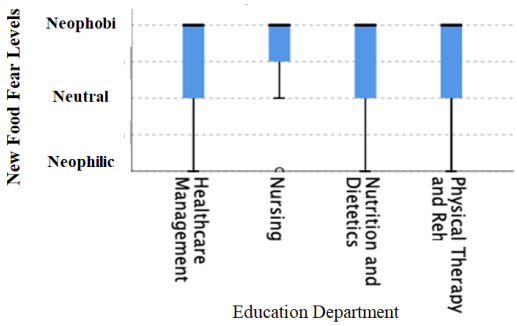

Figure 1. New Food Fear Level Distribution by Education Department

Bryant and Barnett (2019) examined familiarity and attitudes towards terms such as 'cultured meat', Neophilic ıt', 'lab-raised meat' and 'animal free meat'. They found that the term 'clean meat' is less weıı known than other terms. They concluded that 'non-animal meat' led to more positive attitudes towards meat in-vitro. The term 'lab grown meat' has had negative connotations (Bryant and Barnett, 2019). Hocquette eı ıl. (2015) found in their study that more than 50% of the participants believed that "artif ial meat" technology was realistic. Younger women and older men have been found to be more positive towards this technology than they are. Many of the respondents argued that there are environmental, animal welfare and productivity issues in the meat industry.

Gómez-Luciano et al. (2019) conducted research focusing on consumer perceptions and preferences for a variety of alternative protein sources, including plant-based proteins, cultured meat, and insect-based proteins, covering various countries. It revealed that participants' beliefs about the nutritional benefits of alternative protein sources differed between countries. The belief that alternative proteins can match the benefits of meat has been found to range from low percentages in some countries to higher percentages in others. British respondents felt that meat was less important for a balanced diet compared to respondents from other countries. A negative correlation was found between the belief that meat provides essential nutrients and the belief that alternative proteins can match the benefits of meat. It has been observed that participants from different countries perceive various features of protein sources differently. The UK, Spain and Brazil have found insect-based proteins to be healthier, safer, and more sustainable than cultured meat. However, the Dominican Republic has been found to prefer plant-based proteins. The research highlights the complex interplay between cultural, perceptual, and practical factors when it comes to consumer preferences for alternative protein sources.

De Oliveira Padilha et al. (2022), in an Australian study, found that plant-based meat alternatives were consumed less than once a month. While 31% of the participants stated that they had never heard of the term meat produced in the laboratory, 5% of them heard the term but knew little or nothing about it.

After the concept of lab-grown meat was explained, about a quarter of consumers indicated that they were willing to eat lab-raised chicken or lab-raised beef. When comparing conventionally raised chicken to Lab-raised chicken and plant-based meat alternatives, consumers rated conventionally raised chicken as the healthiest, most affordable, and enjoyable to eat, but least animal-friendly. Plant-based products scored the highest among protein alternatives as animal-friendly and environmentally friendly. In the study of Slade (2018), consumers were given the option to buy burgers made from beef, plant-based protein, or cultured meat, and found that consumers prefer beef burgers even if they all taste the same. Preferences are linked to age, gender, views on food technologies, and environmental attitudes. He stated that if the prices were equal, 65% of the consumers would buy the meat burger, 21% would buy the veggie burger, 11% would buy the cultured meat burger, and 4% would not buy it at all (Slade, 2018).

Bryant and Sanctorum (2021) conducted a study to identify Belgian consumer attitudes in 2019 and 2020, and respondents were asked online questions about their diet, their attitudes towards available plant-based meat alternatives, and their attitudes towards cultured meat. They found increased satisfaction with plant-based alternatives on a yearly basis. There was no significant change in attitudes towards cultured meat. While plant-based products are more attractive to women and vegetarians; it has been determined that cultured meat is more interesting to men.

Hartmann et al. (2015) reported that developing familiarity with Western cultures is crucial for overcoming behavioral barriers such as negative taste expectations and neophobic responses through taste training and placing insects as a food source. The major challenge to insect consumption has been noted to be the public's strong, socio-culturally defined prejudice against insects. Insect-based foods and dishes that are Westernized and adapted to European taste profiles, textural preferences, and food aesthetics are more likely to enter consumers' diets, as evidenced by the widespread adoption of other ethnic foods.

Dupont and Fiebelkorn (2020) investigated the acceptance of insect-based foods and cultured meat in German children and adolescents. It has been determined that German children are more willing to consume cultured beef instead of insect burgers. Similar rated for insect-based food and cultured meat. Attitude toward insect burgers and food neophobia were the two most important determinants of the desire to consume insect burgers, while age was also identified as an influencing factor. In addition, it was observed that the attitude towards meat type and food neophobia affect preferences. Research by Gravel and Doyen (2020) concluded that edible insects can be a source of protein for the global population. Insect protein can be concentrated as an alternative protein in food formulation. Comparative studies on insect protein functionality are needed and it is thought that it can replace expensive, environmentally harmful conventional proteins in the future. In summary, studies investigate consumers' perceptions of alternative meat sources; familiarity, attitudes, and factors affecting preferences are emphasized.

CONCLUSION

The current study, students' perceptions of alternative meat concepts and food neophobia levels were examined. It was found that the participants largely thought these concepts were unnatural and exhibited varying degrees of food neophobia.

The research contributes to understanding consumer acceptance of alternative foods by shedding light on students' attitudes toward new protein sources. Traditional livestock farming requires significant amounts of land, water, and feed. Artificial meat production requires significantly fewer resources, potentially helping to reduce pressure on land and aquatic ecosystems. Traditional livestock farming causes greenhouse gas emissions and water pollution.

In addition, reduced reliance on traditional livestock can put less pressure on natural ecosystems, helping to conserve biodiversity. So artificial meat can significantly reduce these negative environmental impacts. As the world population continues to increase, there are concerns about meeting the increasing demand for meat. Artificial meat production could offer a way to produce more food with fewer resources. Alternative meat sources will minimize dependence on land and water resources and make it a sustainable food source. However, the technology you mentioned is still in its infancy. There is a need for study in terms of production, technology, and preferability.

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Reduction of Arsenic Level in Rice by Different Preparation and Cooking Methods

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Abstract

Rice, a crop that grows underwater, can absorb more arsenic and cause dietary exposure to arsenic. Arsenic levels can be reduced by using different methods. This study aimed to determine the arsenic level in rice by different preparation and cooking methods. Rice was prepared by 4 different preparations and 7 different cooking methods, and arsenic levels were analysed by ICP-MS method. Arsenic level was 0.05 ± 0.009 mg/kg in raw rice, while arsenic levels varied between 0.03 - 0.04 mg/kg in different preparation methods and 0.01 - 0.02 mg/kg in different cooking methods. Arsenic levels decreased with cooking, and the highest arsenic removal percentage was achieved in cooking by filtration and steaming methods (for both 80%). In conclusion, the arsenic level in raw rice was below the reference limits, with the appropriate preparation and cooking methods this amount can be reduced.

Keywords: Arsenic, cooking methods, preparation methods, rice.

Research article
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INTRODUCTION

Agricultural practices such as the widespread use of fertilizers and pesticides, the increase in industrialization, and industrial urban wastes accelerate the heavy metal pollution in the soil and the environment (Sönmez and Kılıç, 2021). With the use of arsenic-contaminated groundwater in agricultural practices, plants become contaminated with arsenic and arsenic reaches the table through the food chain. One of the foods affected by this condition is rice (Öztürk et al., 2017).

Rice, which has an important place in human nutrition, also has a very important place in world grain production. It is the main food source for more than half of the world's population. Per capita consumption in the Far East and South Asian countries is up to 200 kilograms (Semerci and Everest, 2021). The Republic of China ranks first in world rice production. This country is followed by India, Indonesia and Bangladesh. These are the Asian countries with the largest paddy cultivation area in the world and meet about 68% of the world's rice production. According to the 2021 report of the Ministry of Agriculture and Forestry, it was reported that 600 thousand tons of rice were obtained from 126 thousand hectares of land in Turkey (TEGPE, 2021). Although paddy is produced in 31 provinces in our country, Edirne ranks first in paddy production (Hayati et al., 2015).

It is stated that the arsenic concentration is high in rice grown in wetlands (Öztürk et al., 2017). Rice has a relatively higher tendency to absorb inorganic arsenic because it is grown in submerged soil conditions. Among populations not exposed to inorganic arsenic in drinking water, rice contributes significantly to inorganic arsenic uptake (Davis et al., 2017). People who are exposed to high amounts of arsenic experience disorders such as blood circulation disorders, dermatological problems, nervous system disorders, gastrointestinal system problems, weakness, loss of appetite, fatigue, and loss of consciousness. As a result of long-term exposure, serious health problems such as lung, skin, kidney, and liver cancer are observed (Akbal et al., 2013; Sinczuk et al., 2010).

Due to the effects of arsenic on human health, research has shown that preparation and cooking methods are also effective in reducing arsenic level in rice (Rahman et al., 2006; Sengupta et al., 2006; Raab et al., 2008; Mihucz et al., 2007). In these studies, arsenic levels in rice were examined after soaking in cold and hot water Mihucz et al., 2007), washed or unwashed cooking (Sengupta et al., 2006), low and high-volume cooking (Rahman et al. 2006; Sengupta et al., 2006; Raab et al., 2008), and boiling was used as the cooking method.

. A comprehensive study investigating the arsenic levels in rice that was soaked in water at different pHs during the preparation stage and cooked by different methods such as steaming, boiling, and roasting could not be found. Therefore, preparation in different kinds of water and cooking with different methods should also be evaluated. In this study, it was aimed to analyse and compare arsenic levels in rice made with different preparation and cooking methods.

MATERIAL and METHOD

Sample Selection and Collection

In January 2023, a total of 3 kg of rice (3 packets of 1 kg with the same serial number) were purchased from a local market in the Maltepe district of Istanbul. The production place of those rice was Edirne – Turkey. It has been reported that Edirne alone has more than 40% of the paddy agricultural areas of Turkey (TEPGE, 2021). The type of rice was “Osmancık-97” which has long, wide and glassy grains with matte appearance. It is an ideal type of rice for cooking pilaf due to its easy consistency (TFC, 2010).

Sample Preparation

Four different preparation methods and seven different cooking methods were used (Figure 1).

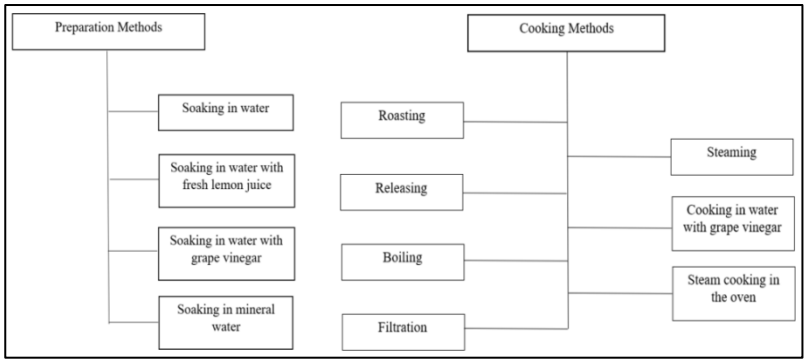

Figure 1. Preparation and cooking methods

Preparation Methods

To investigate the effect of changing the pH value of the water, the rice was kept in waters of different pH values as follows:

1. Soaking in water: 200 g of rice was soaked in 400 ml of water for 45 minutes at room temperature (25 to 32 °C) and the pH was measured as 7.8.

2. Soaking in water with fresh lemon juice: 200 g of rice was soaked in 400 ml of water containing fresh lemon juice (5%) for 45 minutes at room temperature (25 to 32 °C) and the pH of the water was measured as 1.95.

3. Soaking in water with grape vinegar: 200 g of rice was soaked in 400 ml of water containing vinegar (5%) for 45 minutes at room temperature (25 to 32 °C) and the pH of the water was measured as 3.90.

4. Soaking in mineral water: 200 g of rice was soaked in 400 ml of mineral water for 45 minutes at room temperature (25 to 32 °C). The pH value of the mineral water was measured as 5.55.

Cooking Methods

1. Roasting: 200 g of rice was added to 20 g of butter melted in the pan and roasted at 180 °C. When the rice grains started to become transparent, 400 ml of water and 2 g of salt were added. It was cooked on low heat with the lid of the pot closed. When the rice dried up, the stove was turned off and the rice was left to infuse for 30 minutes.

2. Releasing: 400 ml of water, 30 g of sunflower oil and 2 g of salt were boiled in the pot, then 200 g of rice was added to the pot and cooked at 100 °C until the water evaporated. It was infused for about 30 minutes after the stove was turned off.

3. Boiling: 200 g of rice was added to 400 ml of boiling water (with 2 g of salt) and cooked at 100 °C until the water evaporated. 20 g of butter were added, and the stove was turned off. Then it was infused for about 30 minutes.

4. Filtration: After washing 200 g of rice, the water was filtered. Two of salt, 30 g of sunflower oil and rice were added to 1000 ml of boiling water. After boiling for 5 minutes at 100 °C, the water was filtered in a strainer. 20 g of butter was added to drained rice and the lid of the pot was closed and cooked on low heat for 10 minutes. It was then left to infuse for 30 minutes.

5. Steaming: Two gram of salt and 200 g of rice were added to 1000 ml of boiling water and boiled at 96 °C for 10 minutes and then the water was filtered. Some boiling water was placed in the pot and a strainer with rice was placed on it. The lid was closed and steamed for 10 minutes.

6. Cooking in water with grape vinegar: Four hundred ml of water and 20 g of grape vinegar were boiled in a pan at 100 °C. Then 200 g of rice was added and boiled for 10 minutes. Before the rice was fully softened, the bottom of the stove was turned off and the rice was filtered. The rice was roasted in a pan with 1 g of salt and 30 g of sunflower oil for 5 minutes and rested for 30 minutes.

7. Steam cooking in the oven: Four-hundred ml of water, 1 g of salt, 30 g of sunflower oil and 200 g of rice were added to a Pyrex and the lid was closed. It was steam-cooked in the oven at 200 °C for 35 minutes. It was taken out of the oven when the water was completely absorbed.

Arsenic Analysis

Samples of 200 g were taken from raw rice, rice prepared with different preparation methods, and rice cooked with different cooking methods, and placed in zip-lock storage bags. Analysis of the samples was carried out in A&T Food Control Laboratory which has been accredited by TÜRKAK according to AB-0509-T and TS EN ISO/IEC 17025 standards. 0.5 g were taken from the samples and dissolved in the microwave with 6 mL of nitric acid (HNO3, 65%) and 2 mL of hydrogen peroxide (H2O2, 30%). The samples were homogenized using shredder at 10.000 rpm for 2 minutes. Then, water was added and the solution was completed to 50 ml and the inorganic arsenic level was measured in the ICP-MS device.

Samples were first analyzed using ICP-MS (Thermo-Fisher Scientific iCAP-Q and iCAP-TQ; Thermo Fischer Scientific). For this analysis, a certain amount of rice flour from each sample was digested in HNO3 in perfluoroalkoxy (PFA) containers. Milli-Q was diluted with water before elemental analysis by inductively coupled plasma mass spectrometry (ICP-MS). The device was operated using a collision cell (Q cell) using He (He-cell) with kinetic energy discrimination to eliminate polyatomic interferences. Samples were included from an autosampler (Cetac ASX-520) incorporating an ASXpress quick retrieval module. Internal standards are introduced to the sample stream on a separate line via the ASXpress unit. Sample processing was performed using Qtegra software (Thermo-Fisher Scientific) using external cross-calibration between pulse counting and analogue detector modes as needed. The expanded measurement uncertainty, using a coverage factor k=2 which gives a level of confidence of approximately 95%. All the analyzes were carried out in triplicate.

Calculation and Comparison

The inorganic arsenic values found were compared with the reference limits of Codex Alimentarius, Joint FAO/WHO Expert Committee on Food Additives (JECFA) and EU Commission Regulation (Wu, 2014; JECFA, 2010; EU, 2015).

The percentage of inorganic arsenic removal for each different preparation and cooking technique was calculated by using the formulation below (Mihucz et al., 2007):

As Removal % = [(As value before processing – As value after processing) / As value before processing]

RESULTS and DISCUSSION

Inorganic arsenic levels in rice samples were given in Table 1, and inorganic arsenic removal percentages were shown in Figure 2. None of the rice samples exceeded the reference limits.

The inorganic arsenic level in raw rice was 0.05 ± 0.009 mg/kg. It was determined that inorganic arsenic levels in raw rice soaked in water with different pH values decreased slightly.While the inorganic arsenic level was the same (0.03 ± 0.005 mg/kg) both in water with grape vinegar, mineral water and water solely, it was higher in the water with fresh lemon juice (0.04 ± 0.007 mg/kg).

Inorganic arsenic levels decreased in rice samples with cooking. Among the cooking methods, the highest level of inorganic arsenic removal was achieved in cooking by filtration (80%) and steam cooking (80%).

Inorganic arsenic levels were found to be the same (0.02 ± 0.004 mg/kg) in the releasing, roasting, boiling, steam cooking in the oven and cooking in water with grape vinegar and 60% of the inorganic arsenic level was removed by these methods.

Table 1. Inorganic arsenic levels in rice samples

	Analysis result (mg/kg) Mean ± SD
Raw rice	0.05 ± 0.009
Preparation methods	
Soaking in water	0.03 ± 0.005
Soaking in water with grape vinegar	0.03 ± 0.005
Soaking in mineral water	0.03 ± 0.005
Soaking in water with fresh lemon juice	0.04 ± 0.007
Cooking methods	
Releasing	0.02 ± 0.004
Roasting	0.02 ± 0.004
Boiling	0.02 ± 0.004
Steam cooking in the oven	0.02 ± 0.004
Cooking in water with grape vinegar	0.02 ± 0.004
Filtration	0.01 ± 0.002
Steaming	0.01 ± 0.002

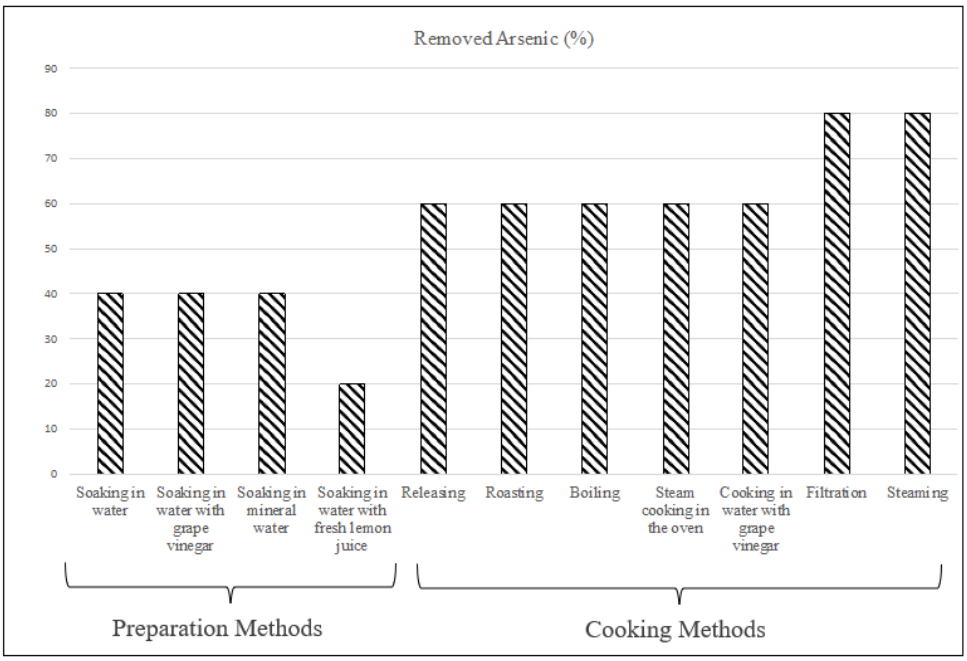

Figure 2. Inorganic arsenic removal by different methods

Rice is one of the most consumed foods in our country and in the world and is prepared and cooked in different methods. Studies in the literature show that different preparation and cooking methods can be effective in removing arsenic from rice (Diaz et al., 2004; Cubadda et al., 2003; Misbahuddin, 2003; Rahman et al., 2006; Sengupta et al., 2006; Signes-Pastor et al., 2008; Raab et al., 2008; Mihucz et al., 2007).

In this study, the effects of different preparation and cooking methods on inorganic arsenic levels in rice were investigated.

Comparing with the reference limits

In the Turkish Food Codex, there is no upper limit for arsenic in terms of heavy metal contamination in rice in the "Regulation on Maximum Limits of Contaminants in Foodstuffs" dated May 17, 2008 and numbered 26879 (TFC 2008). When the international legislation on heavy metal pollution in rice is examined; in the Codex Alimentarius, only the upper limit value was given for inorganic arsenic among the arsenic species found in rice. The maximum inorganic arsenic level for husked rice was 0.35 mg/kg, and the maximum inorganic arsenic level for polished rice was 0.2 mg/kg (Wu, 2014). The arsenic limit set by the European Union for rice in 2015 is 0.2 mg/kg (EU, 2015). It was determined that all the rice samples used in the study met the Codex Alimentarius and European Union standards. The type of rice used in the study was Osmancık-97, and the arsenic level of raw rice was found to be 0.05 ± 0.009 mg/kg.

Effect of pH

A study on arsenic removal in drinking water at different pH values was investigated by using Fe and Al electrodes. Depending on the pH value of the aqueous medium, arsenic levels were found to change through coprecipitation and adsorption reactions. It was determined that arsenic removal increased when the pH value of the water was changed from 4.5 to 8.5. It was stated that the optimum pH value for arsenic removal was between 6.5 and 7.0 (Kobya et al., 2011). In this study, rice was soaked in water with different pH values (mineral water: 5.55, water with grape vinegar: 3.90, water with fresh lemon juice: 1.95, water = 7.8) and it was found that the inorganic arsenic level in rice decreased in all methods. Moreover, inorganic arsenic removal was found to be the lowest in the water with fresh lemon juice.

Effect of preparation and cooking

Cooking affects arsenic absorption in rice (Sengupta et al., 2006; Signes-Pastor et al., 2008). Raab et al. (2008) were investigated the effects of rinse washing on the arsenic level of rice. While approximately 10% of total and inorganic arsenic was removed from basmati rice by rinse washing, this process was less effective in other types of rice. In another study, three samples of West Bengal rice were rinse-washed 5-6 times until the water was clear and then cooked in a high volume of water. Rinse washing removed 28% of the arsenic in raw rice (Sengupta et al., 2006).

Furthermore, in Mihucz et al.'s (2007), most of the arsenic was removed by cooking in water (26-49%), while the amount of removed arsenic was found to be less by washing (8-17%). In the present study, rinse washing was done with safe drinking water in boiling, roasting and filtration methods. While 60% of the inorganic arsenic was removed by boiling and roasting methods, 80% of arsenic was removed in the filtration method in which rice is cooked with a higher water volume. Rinse washing was not done in low-volume water-cooking methods such as releasing, steam cooking in the oven, and cooking in water with grape vinegar; rinse washing was done in boiling and roasting methods. All these methods removed 60% of the inorganic arsenic in rice. No effect of rinse washing was found on the inorganic arsenic level of rice cooked in low-volume water.

The water used in the cooking process is an important factor in the arsenic level in rice. Rice cooked with uncontaminated water has a lower arsenic level (Diaz et al., 2004; Cubadda et al., 2003). In areas with high arsenic pollution in Bangladesh, the amount of arsenic in cooked rice was approximately 10-35% higher than in raw rice. It has been stated that the reason for this is cooking water with arsenic contamination (Misbahuddin, 2003).

Since the arsenic concentration of groundwater used as cooking water in South Asian countries is well above the maximum allowable limit (10 μg/L) set by the World Health Organization (WHO). The effect of arsenic concentration in cooking water on arsenic retention in cooked rice is significant in these countries (Sengupta et al., 2006; Signes-Pastor et al., 2008). Rahman et al. (2006) observed that when there is arsenic contamination in cooking water, the arsenic concentration in cooked rice increases as the rice absorbs the arsenic in the water. In this study, inorganic arsenic levels decreased as a result of cooking. This is thought to be due to the use of safe drinking water as cooking water.

Effect of water volume

The effects of rinse cooking in low volume (2.5:1 water: rice) and high volume (6:1 water: rice) water on different rice varieties were investigated and it was found that cooking with low volume water did not remove arsenic. In the method of cooking in high-volume water, the inorganic arsenic level of long grain and basmati rice decreased by 45% compared to raw rice (Raab et al., 2008). In another study, three different types of rice were used. When cooking with high-volume water (6:1, water:rice), a 42-63% decrease was found in the total amount of arsenic in cooked rice (Mihucz et al., 2007).

Sengupta et al. (2006) investigated the arsenic load in rice cooked with traditional and modern methods (2006), using low As-containing water (As <3 mcg/L). By using the traditional method of the Indian subcontinent (wash until clear; cook with rice: water 1:6; discard excess water) the arsenic was removed by 57% from the rice. Using low-As water in the traditional preparation of arsenic-contaminated rice reduced the arsenic load. Cooking unwashed rice until there is no water left, with a rice:water ratio of 1:1.5-2.0, which is the frequently used method nowadays, did not change the arsenic content despite using low-As water (Signes-Pastor et al., 2008).

Similar to the previous studies, high-volume water was used in filtration and steaming methods in this study. In addition, rinse washing was done with safe drinking water in the filtration method. In the current study, among the cooking methods, filtering and steaming methods were minimized the inorganic arsenic level and 80% of arsenic was removed from rice cooked by these methods. The limitation of this study is that the arsenic level of the water used in the preparation and cooking of rice was not measured. Another limitation is the use of only one type of rice. In addition, the use of many preparation and cooking methods is the strength of the study.

CONCLUSION

The most effective methods of removing inorganic arsenic are filtration and steaming methods. Unlike other methods, the use of high volumes of safe drinking water in filtration and steaming has a significant effect on reducing the inorganic arsenic level.

In addition, rinse washing with drinking water in the filtration method was effective in reducing the inorganic arsenic level. Although the inorganic arsenic level in raw rice is below the reference limits in the study, this amount can be further reduced with the right preparation and cooking methods. With the use of appropriate preparation and cooking methods, caution can be taken to protect food safety and public health.

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An Investigation of Consumers' Consciousness Level About Food Safety in Milk and Dairy Consumption in Urban Area of Niğde Province

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Abstract

This study examined consumers' food safety consciousness and purchasing behavior for milk and dairy products. The data was gathered through the application of a questionnaire developed for food safety in milk and dairy products and purchasing preferences of consumers via face-to-face questionnaires with 272 consumers residing in the urban area of Nigde province. As a result of this study, the milk and dairy products purchased by the majority of consumers were reported, such as cow milk, cow milk products, and butter. It was determined that consumers buy an average of 13.5 liters of milk, 3 kg of cheese, about 10.5 kg of yogurt, and 1.5 kg of butter per month. It was found that 82% of consumers know the concept of food safety, and they stated that they were familiar with the Ministry of Agriculture and Forestry and the Ministry of Health as the food safety supervisory organizations. The almost half of consumers purchased sheep and goat milk and dairy products directly from farmers or at open-air markets. This means that the consumers are more vulnerable in consuming for sheep and goat unsafe dairy products. In the study, it was concluded that consumers consider the good for the product's health to be purchased as the most important criterion when purchasing milk and dairy products.

Keywords: Food safety, milk and dairy products, food safety in dairy products, consumer

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INTRODUCTION

Today, society has become more conscious about healthy lifestyles and safe diets. Consumers demand healthy and safe foods because they will be more effective in achieving a good quality of life than measuring the calories and basic needs of the body. Therefore, food safety, which has become one of the determining factors in the demand for all food products due to increasing the sensitivity of consumers to food production and consumption, is also constantly on the agenda. Generally, food safety includes particular assays to ensure the foods that reach consumers are healthy and safe, preserving their physical, biological, chemical, and sensory qualities for consumption (farm to fork) after production. Hence, food safety requires proper control from production to consumption.

Some factors negatively affect food safety in Turkey. These factors are the high population, the high number of small-scale agricultural farms, risky food consumption habits and socio-economic structure of consumers (Anonymous, 2017). Consumers constitute the latest link in the food safety chain. Consumer purchasing power and awareness level are important factors in ensuring food safety. Low purchasing power and low education level of most of the consumer potential, the lack of consumer awareness, and insufficient knowledge of food safety are also seen as important social weaknesses in ensuring food safety. This adversely affects public health and its industry's quality and safe food production (Alpoğuz et al., 2009).

Besides, food safety issues are determined by the behavior and actions of various stakeholders, such as farmers, food handlers and distributors, food manufacturers, and food service operators. Moreover, they are also affected by consumer awareness and practices. In return, consumers' demand for food safety depends on their food safety consciousness (Thapa et al., 2020). Comprehension of consumers' knowledge regarding food safety and their food preparation practices has become an important subject for food producers, retailers, politicians, and health promoters (Wilcock et al., 2004).

Milk is one of the nutrients which is necessary for the growth and development of the human body, which has an important place in the entire life period of a person from infancy to old age, contains protein, fat, lactose, vitamins, and minerals of animal origin, and it is necessary for adequate and balanced nutrition. While many foodstuffs can meet only a part of the nutritional elements of human being, milk, with its unique composition, is the only nutrient that contains all the factors of nutrition, namely protein, fat, carbohydrate and mineral substances, enzymes, antibodies, vitamins in a balanced and sufficient amount (Pereira, 2014). Nevertheless, food safety of milk and dairy products has been an increasing concern in Türkiye. There are food safety challenges in milk production because milk has a very favourable nature for growth of microorganisms in it. Therefore, because of reaching the safe dairy products from the production process to consumers, dairy industries began to implement global food safety management systems and standards such as HACCP (Hazard Analysis and Critical Control Points), G.A.P (Global Good Agriculture Practices), G.M.P (Good Manufacturing Practice), ISO 22000 (International Organization for Standardization).

Although Turkey has made significant strides in food safety in recent years and progress has been made in the awareness level of the society, unfortunately not all segments of the society have food safety awareness. Accordingly, many research and studies have been carried out on the purchasing behavior and knowledge of consumers on food safety in the world (Bruhn and Schutz, 1999; Mutlu, 2007; Gündüz and Aydoğan, 2015; Sopi et al., 2015; Eryılmaz et al., 2018; Öztürk et al., 2019; Yüksel, 2019; Kırmacı and Özçelik, 2021). Studies have become widespread with the increasing importance of the subject. Especially, there are some studies about consumers' knowledge of food safety and purchasing attitude toward milk and dairy products in Turkey according to different consumers' segment and region (Mutlu and Berk,2004; Bozoğlu et al., 2014; Can, 2020; Başer et al., 2022). However, no study has been found to determine consumers' consciousness regarding food safety in milk and dairy products in Niğde province.

The main objective of this study is to determine the effective factors of consumers' purchasing milk and dairy products and focus on food safety knowledge of consumers in the case of Niğde, Turkey. Besides, the specific objectives of this study include to determine the milk and dairy products consumption level of consumers in the urban area of Niğde province and to determine what is the level of consumers' food safety consciousness and purchasing preferences in terms of dairy products.

MATERIAL and METHOD

The study's primary data were obtained from surveys using a structured questionnaire. The research was conducted among the urban residents of Center and Bor districts of Niğde province of Türkiye, which constitute 91,7% (199.545 persons) of total urban population (217.640 persons) in Niğde (TURKSTAT, 2020). The survey was conducted in July 2021.

The number of consumers to be surveyed was determined with the "Proportional Sample Size" formulated by Newbold et al (2013), and as a result of the calculation a 90% confidence level, and 5% margin of error, it was found that 272 surveys were required. The unit of analysis for the study was the household, with the assumption that the household is where one can get most of information with regard to the study objectives. The sample size was calculated using the following formula:

$$n = \frac{\mathrm{Np(1-p)}}{(\mathrm{N}-1)\sigma_{px}^2 + p(1-p)} \quad (1)$$

Where n = sample size, N= population (consumer numbers), p= proportion of sampled consumer, σ^2px = variance attribute of interest). Because the general prevalence rate of the key variable was not known, the value of p was set at 50% (0.5) to maximize the impact of this variable on the sample size.

Within the scope of the research, characteristics of consumers and consumption were measured with basic statistical methods (frequency distribution and average). Besides, the opinions of the consumers within the scope of the research on the factors affecting their purchases of milk and dairy products were determined using a 5-point Likert Scale. In this study, taking into account income groups, whether there was a difference between the data series showing the scaling of a certain expression depending on the subcategories/groups was determined with the Kruskall-Wallis test (Laerd Statistics, 2014), which is the non-parametric equivalent of one-way ANOVA.

RESULTS and DISCUSSION

Socio-demographic and economic characteristics of households

Socio-demographic and economic characteristics of the consumers were evaluated for comprehension relationship between consumers' purchasing behavior and food safety awareness. Statistics describing the socio-demographic characteristics of the individuals interviewed were given in Table 4.1. According to the table, of the 272 individuals, 148 (54.41%) were males, and 124 individuals (45.59%) were females. In addition, the average age of individuals was 31 years, and the majority (62.5%) were between 18 ages and 34 ages. In this study, it was found that 52.2% of interviewed individuals were single and 36.8% of individuals had bachelor's education level. In the households interviewed, 24.3% of the individuals were students, 17.6% were freelancers, and 16.3% were employed. This was followed by unemployed individuals with 12.5%. Proximately 5% of individuals in the position were retired, and approximately 10% were civil workers. 27% of males were student, while 29.8% (37 Persons) of females were housewife. The average number of individuals living in a household was about 4.46 persons, and the average number of children was 1.62. In a similar study which conducted in Tekirdağ province, 11.8% of consumers had bachelor's degree, the average number of people living in a household was 3, and 2.9% of individuals were unemployed, and 28% of them were housewife (Can, 2020).

Table 1. Socio-demographic characteristics of the interviewed households

Socio-demographic factors	Categories for variables	Male		Female		Overall	
		n	%	n	%	n	%
Age	18-24	53	36	31	25	84	30.9
	25-34	46	31	40	32	86	31.6
	35-44	24	16	26	21	50	18.4
	45-54	16	11	17	14	33	12.1
	55-64	4	3	8	6	12	4.4
	65 and older	5	3	2	2	7	2.6
	Total	148	100	124	100	272	100.0
Marital status	Married	64	43	66	53	130	47.8
	Single	84	57	58	47	142	52.2
	Total	148	100.0	124	100.0	272	100.0
Education level	Literate	6	4	7	6	13	4. 8
	Primary education	7	5	12	10	19	7.0
	Secondary education	17	11	11	9	28	10.3
	High School	43	29	34	27	77	28.3
	Bachelors	62	42	38	31	100	36.8
	Postgraduate	13	9	22	18	35	12.9
	Total	148	100.0	124	100.0	272	100.0
Occupation	Freelancer	39	26	9	7	48	17.6
	Civil Worker	15	10	13	10	28	10.3
	Employee	26	18	19	15	45	16.5
	Student	40	27	26	21	66	24.3
	Housewife	0	0	37	30	37	13.6
	Retired	11	7	3	2	14	5.1
	Unemployed	17	11	17	14	34	12.5
	Total	148	100.0	124	100.0	272	100.0
Number of household members (average)							4.46
Number of children in the household (average)							1.62

In this study, household income levels may differ. For this purpose, consumer income groups were created, and monthly total household incomes were calculated from the smallest to the largest. According to the income levels of the households they ranked into 3 groups: high-income, middle-income, and low-income groups. Accordingly, households with a monthly income of up to 3000 TL were low-income households. Those with an income between 3000 TL- 4999 TL were called the middle-income group and those with more than 5000 TL income were called high income households. Monthly household income varied between 900 TL and 20000 TL, and the average monthly income of households was determined as 4221.5 TL in the study. Households with low income constituted 30.1% of the total sample, middle income households constituted 39.0% of the total, and 30.9% were high income households.

Besides, the average monthly food expenditure for the total sample was calculated as 1591.07 TL, and the share of monthly food expenditures in monthly income was 37.69%. The share of food expenditure in total income was the highest with 41.23% in the middle-income group, while it was the lowest with 34.55% in the high-income group. Furthermore, the share of total dairy products expenditures in total food expenditures was 23.68% (28.57% in the low-income group, 25.18% in the middle-income group, and 20.84% in the high-income group) (Table 2).

Relationship of average monthly food expenditure of households and average monthly dairy products expenditure along with income groups were investigated using Kruskall Wallis Test. There were statistically significant differences between income groups on the average monthly food expenditure and average monthly dairy expenditure (p-value = 0.000).

Table 2. Economic status of households by income groups (monthly average)

Income Groups	Average of Monthly Income (TL)	Average of Monthly Food Expenditure (TL) *	% of Monthly Income	Average of Monthly Dairy Expenditure (TL) *	% of Monthly Food Expenditure
Low Income	2073.47	838.41	40.44	239.51	28.57
Middle Income	3680.37	1517.36	41.23	382.12	25.18
High Income	7001.19	2418.81	34.55	504.05	20.84
Total	4221.48	1591.07	37.69	376.78	23.68

*According to the Kruskall Wallis Test, the difference between the means was statistically significant ($p<0.05$)

Food safety knowledge and consciousness of consumers

Among surveyed consumers, approximately 82% admitted that they are aware of food safety concept, while about 18% of respondents did not know about food safety. In accordance with a study investigated in Samsun province of Turkey, it has been reported that 84% of the consumers who participated in the survey stated that they had heard of the concept of food safety; however, 16% stated that they had not heard of the concept of food safety (Yalçın and Kızılaslan, 2013).

The definition of food safety was asked to the consumers in the study. Accordingly, while 82% of the respondents answered the question about the definition of food safety, 18% of them did not answer the question. It has been demonstrated that 16.3% of consumers defined the concept of food safety as safe food in terms of health with nutritionally value, 12.8% as food produced with hygienic and safety quality standards, 8.7% as food that compliance with essential food safety regulations, and 40.4% of consumers responded as all the definitions given. At this point, it appears that the concept of food safety has been widely considered.

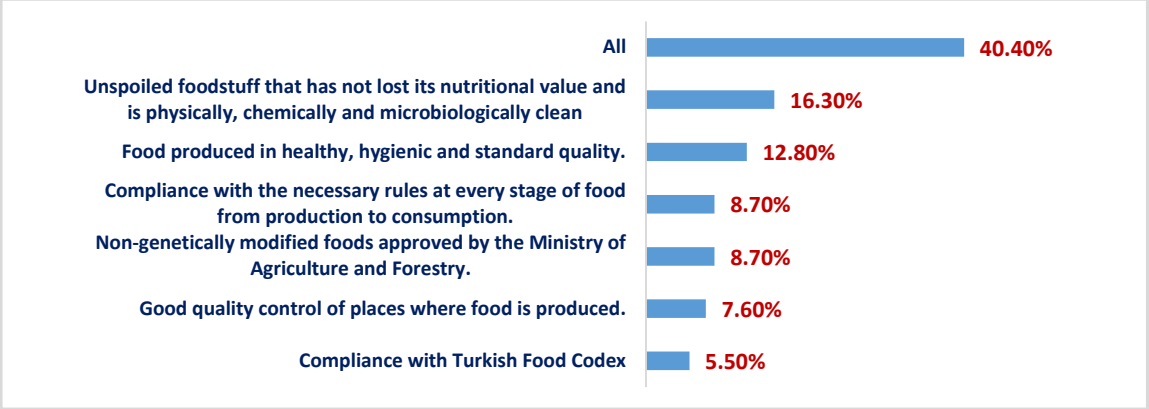

Figure 1. Definition of food safety according to the consumers

Consumers were asked which food safety assurance systems they were familiar. As a result, 88% of surveyed individuals responded to this question, and 12% did not answer, since they did not have knowledge of food safety systems. Though, the food safety system most recognized by consumers was TSE (Turkish Standards Institution) with a rate of 24.8%. Organic or Ecological Products Certificate has the second rank with an 18.8% rate, which was followed by HALAL Islamic standards with 18.1% and by Standards of the International Standards Organization (ISO) with 17.1%. HACCP (Hazard Analysis at Critical Control Points for Processed Agricultural Products) ranked 5th with at 11.1% and European Good Agricultural Practices Document (EUROGAP) ranked the last one with at 10%.

The most well-known food safety supervisory organization by consumers was stated as the Ministry of Agriculture and Forestry with 23.70%, which was followed by the Ministry of Health with 22.70%. 15.50% of individuals claimed that they were aware of the Turkish Standards Institute (TSE) as safety supervision competent of foodstuff. Moreover, municipalities (15.4%) and Alo 174 Food Line (15.4%) were equally rated as the responsible institutions for food safety by consumers. Provincial Hygiene Centers were found to be the least known institution responsible for food safety with 7.40%.

Food safety perceptions of consumers on milk and dairy products

The consumers' purchasing behaviors for milk and dairy products were investigated in terms of average consumption quantity according to income groups, and the places they prefer to buy, and the factors affecting the choice of the consumers' purchasing place. Besides, consumers' preferences in choosing drinking milk and packaging types for milk and dairy products were examined.

As a result of the consuming amounts of dairy products, cow milk products were found higher than other milking animals' products, this proves that cow milk and cow milk products are quite accessible and commonly used in individuals' daily life. The average monthly cow-milk, cow-cheese and cow-yogurt households interviewed within the scope of the study consumption was found as 11.23 kg, 2.31 kg, and 8.31 kg, respectively (Table 3). The consumption quantity of milk and dairy products differs in accordance with individuals' diet preferences, availability of milk products in their neighborhood, and lifestyle. In line with the study of Can (2020), the average for monthly milk consumption was found as 9.5 liters in Tekirdağ province.

Table 3 indicates that the consumption amount of milk and dairy products of the households increase as the income levels increases. It has been determined that there is a considerable difference in the amount of cow milk and cow milk products in the surveyed households; those had consumed more than other milk products. Pursuant to the Kruskall Wallis Test statistic between consumption and income groups in milk and dairy products, a significant difference was found between the average consumption amounts of cow milk (p-value= 0.013) and goat milk (p-value= 0.009), and households income groups.

Table 3. Average amounts of milk and dairy product consumption from different source of households considering the income groups (kg/month)

Products	Low Income	Middle Income	High Income	Overall
Cow Milk*	9.56	11.06	13.07	11.23
Sheep Milk	1.00	1.49	1.15	1.23
Goat Milk*	0.35	1.59	1.12	1.07
Cow Cheese	1.98	2.38	2.53	2.31
Sheep Cheese	0.35	0.40	0.73	0.49
Goat Cheese	0.16	0.31	0.42	0.30
Cow Yogurt	7.60	8.10	9.25	8.31
Sheep Yogurt	0.71	0.73	1.56	1.00
Goat Yogurt	0.52	1.37	1.74	1.23
Butter	1.25	1.65	1.58	1.51
Cream	0.72	0.73	0.79	0.74
Kefir	0.13	0.32	0.22	0.23

*According to the Kruskall Wallis Test, the difference between the means was statistically significant ($p<0.05$)

Consumers preferences of drinking milk preferences according to processed types were given in Table 4. It has shown that households mostly consider sterilized by boiling type in total which has the highest rank 41.5%. This determines that consumers are willing to sterilize milk they bought at home. Approximately 30.5% of consumers prefer UHT (Ultra-high temperature/ ultra-pasteurized) milk and followed by 27.9% pasteurized milk. It was interested in whether the drinking milk processed type was related to the income groups of respondents. This relationship was verified by a Chi-square test. Although there was an insignificant association at 5% significance level according to test results ($\chi 2 = 6.013$; p-value = 0.198), it was found that sterilization of milk by boiling at home was less preferred in high-income households (34.5%) compared to low-income (50.0%) and middle-income (40.6%) households.

Table 4. Drinking milk preferences of consumers according to income status (%)

Income Groups	Pasteurized	UHT	Sterilized By Boiling
Low Income	28.0	22.0	50.0
Middle Income	28.3	31.1	40.6
High Income	27.4	38.1	34.5
% of Total	27.9	30.5	41.5

As shown in Figure 2, consumers regularly preferred cardboard boxes in milk packaging types which have 32.4%, while glass bottles are almost 30% and plastic bottles with 11.4%. However, 21.7% of individuals evident that they do not consider specific milk packaging during purchasing milk, and the lowest preferred milk package types were the plastic bags with lids (2.6%) and cut open plastic bags (2.2%).

The reason of less preferences of those were being not secure and safe as much as other packages are and challenging to carry and keep it through consumption time.

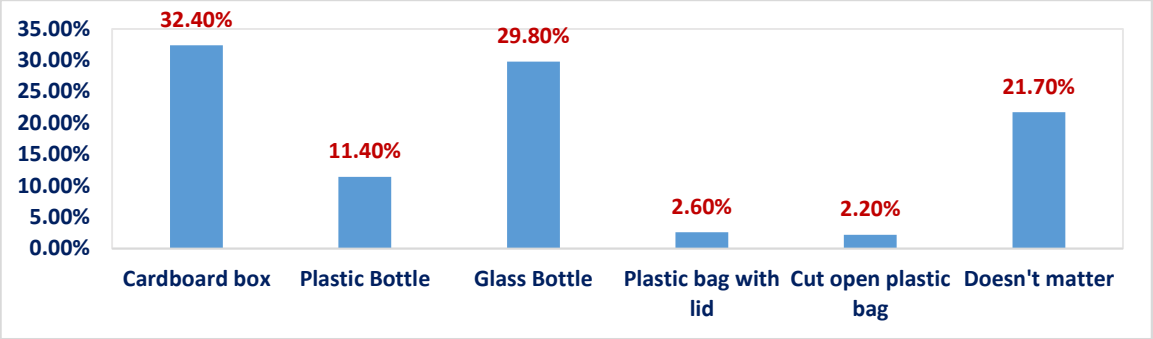

Figure 2. Preferences of milk package types by consumers

Within the scope of consumer behavior, it is also considerable to elaborate the consumers purchasing place preferences. The places they use to buy dairy products regularly are examined in Table 5. Supermarkets and hypermarkets got first rank (cow milk, cow cheese, goat cheese, cow yogurt, butter, and cream) almost in all dairy products, while for purchasing fresh milk they still prefer directly from producers and traditional retailers such as delicatessens and street milkmen. However, purchasing sheep milk directly from producers rated first, and street milkman for goat milk, due to the rarity of these products in supermarkets and hypermarkets. This shows that in the urban population, consumers mostly prefer to purchase milk and dairy products from supermarkets, the following directly from the producers, and delicatessens, street milkmen are preferred by consumers. As determination in similar investigation, it has also indicated that consumer preferred the supermarket for purchasing all the dairy products, while none of consumers prefer purchasing milk and dairy products from internet (Can, 2020).

Table 4.5. Consumers purchasing place preferences in milk and dairy products

Products	Hyper/Super Market	Grocery Shop	Delicatessen Dairy	Street Milkman	Directly from Producer	Total
Cow Milk	112 (41.95%)	47 (17.60%)	31 (11.61%)	28 (10.49%)	49 (18.35%)	267 (100%)
Sheep Milk	16 (13.68%)	18 (15.38%)	25 (21.37%)	25 (21.37%)	33 (28.21%)	117 (100%)
Goat Milk	17 (17.53%)	13 (13.40%)	20 (20.62%)	28 (28.87%)	19 (19.59%)	97 (100%)
Cow Cheese	125 (52.30%)	28 (11.72%)	41 (17.15%)	13 (5.44%)	32 (13.39%)	239 (100%)
Sheep Cheese	30 (27.52%)	11 (10.09%)	31 (28.44%)	12 (11.01%)	25 (22.94%)	109 (100%)
Goat Cheese	22 (26.19%)	9 (10.71%)	19 (22.62%)	16 (19.05%)	18 (21.43%)	84 (100%)
Cow Yogurt	108 (49.77%)	32 (14.75%)	28 (12.90%)	17 (7.83%)	32 (14.75%)	217 (100%)
Sheep Yogurt	21 (26.25%)	12 (15.00%)	15 (18.75%)	11 (13.75%)	21 (26.25%)	80 (100%)
Goat Yogurt	14 (17.50%)	11 (13.75%)	20 (25.00%)	13 (16.25%)	22 (27.50%)	80 (100%)
Butter	105 (44.49%)	29 (12.29%)	37 (15.68%)	19 (8.05%)	46 (19.49%)	236 (100%)
Cream	62 (38.04%)	26 (15.95%)	32 (19.63%)	15 (9.20%)	28 (17.18%)	163 (100%)

The factors affecting the choice of the consumers' purchasing place of milk and dairy products were evaluated in Figure 3. Accordingly, the sale places hygiene conditions, having a good relationship between quality and price, and having a quality warranty were the main criterions. Furtherly, in other side being close to the consumers' home and workplace, as well as the advertising and promotion have not much effect on consumers' choices. In many studies, in general, for food purchasing, choosing the place to buy the products; proximity, advertisement, price and shopping hours were important in milk and dairy products were found as factors (Okumuş and Bulduk, 2003). In dairy products, health and quality are the prioritized criteria.

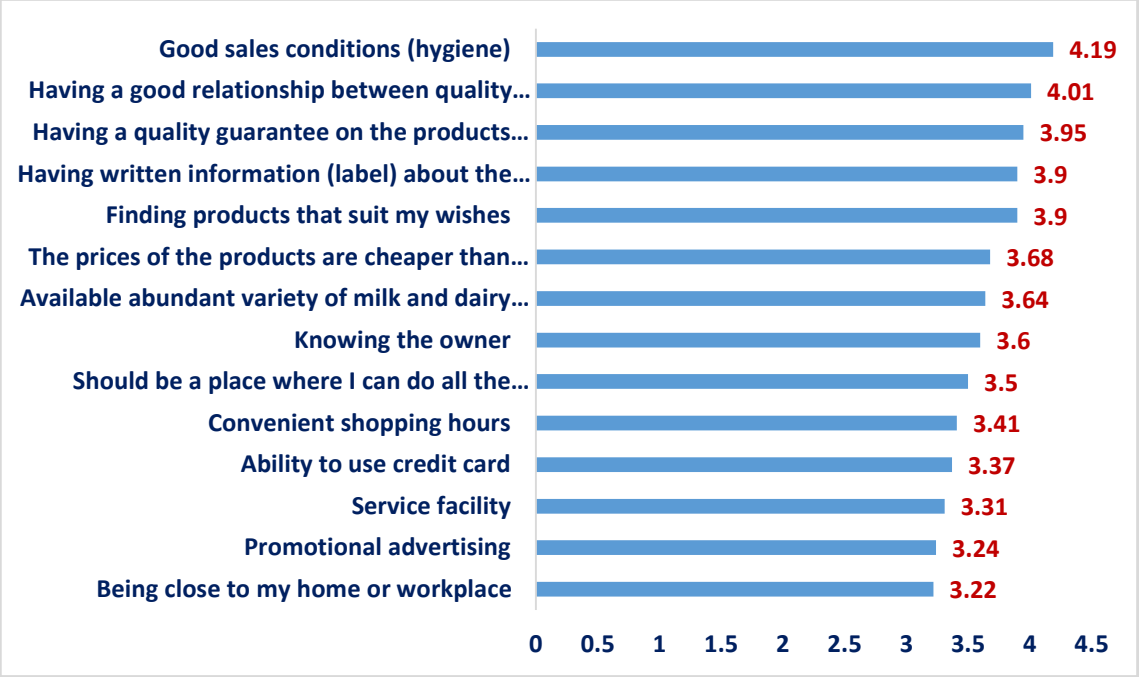

Figure 3. Factors affecting the choice of the consumers' purchasing place of milk and dairy products (1= not important, 5= very important)

An investigation of consumers' attitudes about milk and dairy products' reliability and safety compared to the previous years, it has been observed that almost 40% of answers given by individuals were less reliable than in previous years, regarding increasing the number of additives in dairy products and medicines taken by animals. At the same time, some of them complained about the water addition to the unpacked milk. On the other hand, around 31% of consumers believed that the reliability of dairy products had not differed from past years, followed by near to 28% responses over more reliable and safer than in previous years, owing to the yearly up-to-dates in food safety regulations and expansion of food safety importance. In this study, consumers' awareness of food safety news and the consequences of food safety news about dairy products on the consumption habits of consumers were investigated. Approximately 62% of surveyed citizens declared that food safety news affected their dairy products consumption, whereas about 38% did not notice any changes recently caused by food safety news. 62.5% of surveyed individuals answered the consequences of food safety news regarding milk and dairy products which were heard. Accordingly, 32% of consumers claimed that food safety news had a positive effect, and their dairy consumption increased; however, almost 16.2% of consumers reported that no variation occurred in their dairy consumption habit, and particularly 14.4% of those stated a decrease as a result the convenient news influence their dairy consumption.

It was determined from which sources consumers obtained food safety information about milk and dairy products in the last six months. Accordingly, it was determined that 23.3% of consumers asked for information from where they purchased, followed by 20.1% from media, magazines, internet and television. While 16.8% of those got information from their close circle and friends, 15.4% followed on social media and 14.7% of reading product labels. Following expert' recommendations by consumers was observed as only 9.7%.

78.3% of consumers stated that they made a complaint to the competent authorities when they encountered a spoiled dairy product, while 21.7% stated that they did not make a complaint. Among 78.3% of consumers who had made a complaint, 73% obtained result, and 27% did not. Here, it has emerged that there is a need to create a public opinion to report a defective product intended for consumers to the competent authorities.

Approximately 21.7% of consumers did not make a complaint to the competent authorities when they encounter spoiled dairy products, as a reason for not applying the competent authorities, 31% of those stated that they did not want to deal with, 25% stated that they would not get any results, and 19% did not make a complaint instead they preferred to change the place of purchase, while 17% of consumers believed that there were no sufficient controls, 8% of consumers claimed, they did not know the relevant authorities to the registration of complaints. In a study investigated in Tokat Province, it was determined that 49% of consumers had thought of not obtaining the result, 46% stated that they did not want to deal with it, while 4% did not make a complaint about the concept of giving harm to producers (Can, 2020).

The food chain consists of production and its components up to consumption, such as production, processing, distribution, packaging, storage, and preparation of successive steps and processes. Food can have contaminated at every stage of production till consumption. While the primary food safety responsibility belongs to food producers, many food-borne diseases are caused by improperly prepared or mishandled food at home, in food service establishments, markets or even on farms (WHO, 2002). In the study, it was determined that consumers generally found all processes applied as moderately reliable from milk and dairy products were milked to processed. All the processes of packaging, storage, selling places, and keeping the milk at home were generally considered as quite reliable by consumers.

Table 4.6. Food safety at production chain of dairy products

Criteria	Percentage (%)						Mean
	1	2	3	4	5	6	
Production of milk	10.3	21.7	**29.4**	12.9	15.8	9.9	3.32
Milking (by hand, with machine)	4	21.3	**26.8**	21	16.9	9.9	3.55
Transportation of milk	4.8	14.7	**27.6**	19.99	21.3	11.8	3.74
Processing of milk	9.6	13.6	**24.3**	17.6	21	14	3.69
Packaging	6.3	11	22.4	20.6	**24.6**	15.1	3.92
Storage	6.3	11.8	23.5	16.9	**25.4**	16.2	3.92
Sale places	3.7	11.4	22.8	19.9	**32.4**	9.9	3.96
Keeping inside house	3.7	7	17.3	21.7	41.2	9.2	4.17

1: Not Safe, 2: Less Safe, 3: Moderately Reliable, 4: Quite Reliable, 5: Very Reliable, 6: Do not have any idea

Consumers' attitudes toward hygiene and safety of milk and dairy products sale places were examined in Figure 4. As a result of this examination, consumers believed that sale places were moderately sufficient with 27.8%, followed by 26.8% fairly sufficient and 20.6% very sufficient rates, even though on the other hand about 14.0% were not sure about sufficiency and 10.7% of interviewers pointed out that sale places were not sufficient at all.

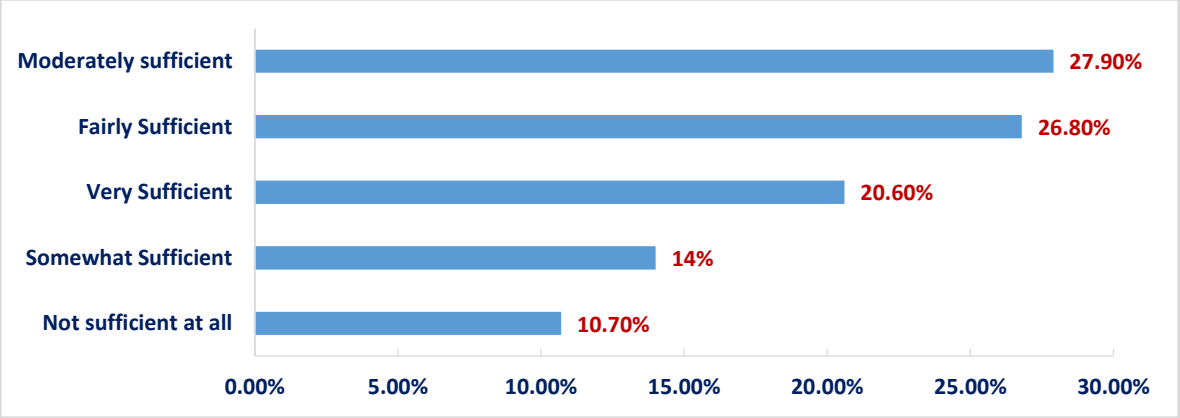

Figure 4. Consumers' attitudes toward hygiene and safety of milk and dairy products sale places

Consumers who had admitted that milk and dairy product production processes are not safe enough due to food safety standards were asked what the reason for this opinion was. In the Figure 5, consumers' answers are demonstrated. Approximately 38.6% of consumers stated that dairy products contain additives, followed by about 25.7% lack enough hygiene in the place of production, and 21.6% claimed the chemical includes either chemical contamination and lastly microbiological development, rated 14.1%. In some studies, up to a third of all raw milk samples contained pathogens, even when they were sourced from clinically healthy animals or from milk that appeared to be of good quality.

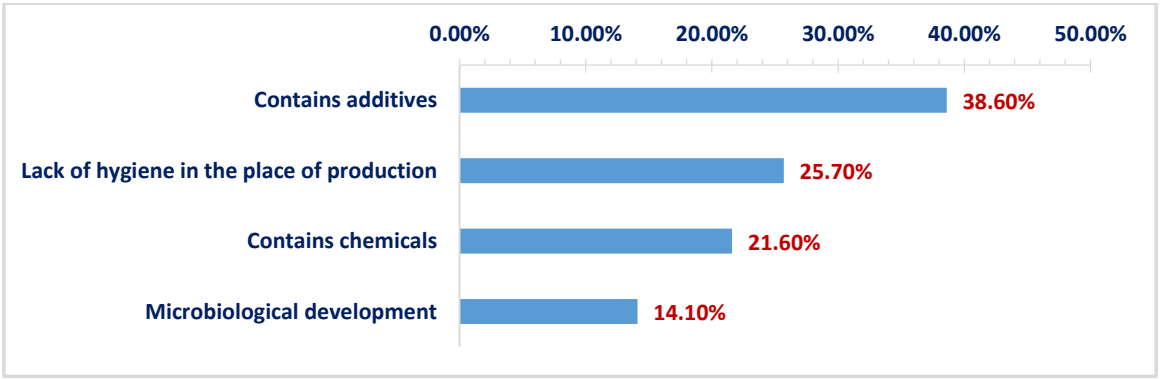

Figure 5. Reasons why the dairy production chain is not safe

Consumers were asked about their considerations regarding milk and dairy products producing manufacturers, which sold in markets sufficiently controlled by supervisory institutes. 49.6% of consumers stated that enough controls and supervision are applied in producing milk and dairy products, and on the other side, 50.4% of individuals admitted there is no appropriate supervision from dairy production companies. Furtherly, consumers were asked about the reason for this opinion in terms of dairy production companies; 27.4% of consumers thought that the penalties were not a deterrent, while 22.6% of consumers thought there were deficiencies in the legislation for supervision. Besides, 19.1% of consumers thought that those working in supervisory institutions abused this issue, 16.6% thought that the authorities of those working in supervisory institutions were limited. 14.3% of consumers thought the laboratory facilities were limited for controls.

Food safety is everyone's business and responsibility. Food safety is not the only duty of government or food producers (WHO, 2019). Food businesses are responsible for complying with food safety legislation, regulations, and standards. Who is the most responsible one in the matter of food safety, as a result of consumer rates, among the first three, the business owners or production employees have a 31%, the Ministry of Agriculture and Forestry has almost 28%, and municipalities with approximately 19% come (Figure 6).

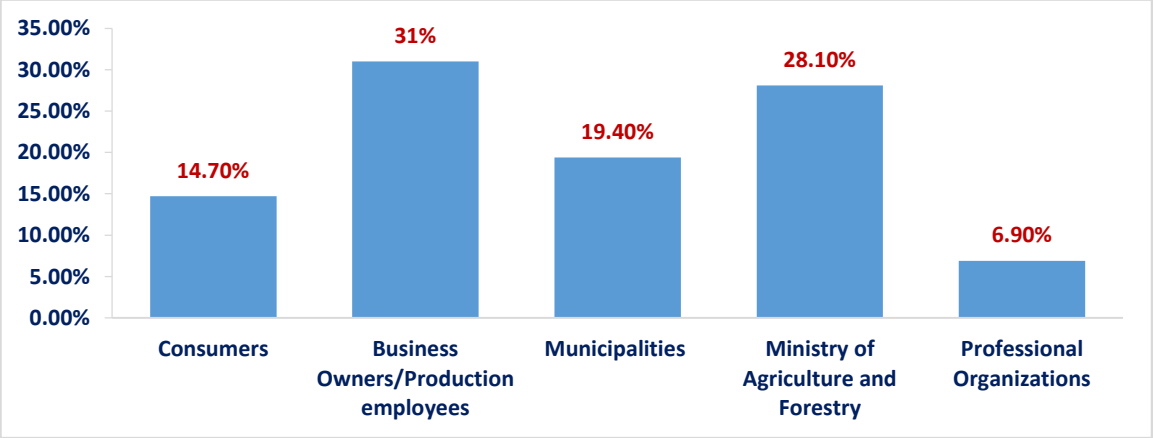

Figure 6. Responsible for food safety of foodstuffs

When shopping for food, consumers were asked which products were important in terms of food safety. According to the data given in Table 7, milk and dairy products took first place with a rate of 32.9%, meat and meat products ranked second with a rate of 22.6%, and bakery products were in third grade with 12.5%. In a similar study regarding the collected data, meat and meat products rated 92.1%, while milk and dairy products ranked 79.1% (Can, 2020).

Table 4.7. Importance of food safety while purchasing foodstuffs

In which products is food safety important to you when shopping for food?	**N**	**%**
Meat and meat products	125	22.6
Milk and dairy products	182	32.9
Dried Legumes	53	9.6
Bakery products	69	12.5
Dried Fruits and Vegetables	45	8.1
Spices	33	6.0
Hot and cold Drinks	47	8.5

CONCLUSION

Within the scope of this study, the consumers in Niğde province's purchasing tendencies of milk and dairy products and their perceptions of food safety of these products were examined. As a result, it was determined that cow's milk and products ranked first in the preference of consumers for milk and dairy products in the province of Niğde, while the healthiness of the product was the first reason for the consumers' purchasing preference. It was concluded that consumers consider the healthiness of the product as main factor affecting the choice of purchasing place for milk and dairy products, which were the sale places hygiene conditions, having a good relationship between quality and price, and having a quality warranty as main criterion.

An important finding of this study is that the almost half of consumers purchased sheep and goat milk and dairy products directly from farmers or at open-air markets. This means that the consumers are vulnerable in consuming of unsafe dairy products. Therefore, it is important to educate and convince people who are used to open-air markets or purchasing dairy products directly from farmers to switch to shopping in markets or supermarkets, where they can get dairy products that are safer.

The vast majority of consumers stated that they know the concept of food safety with a rate of 82%, and that they know the Ministry of Agriculture and Forestry and the Ministry of Health the most among the food safety supervisory organizations. Consumers stated that they know the TSE (Turkish Standards Institution) standards and Organic or Ecological Product Certificate the most among the food safety assurance systems. It was determined that consumers consider food safety the most when purchasing milk and dairy products and meat and meat products.

The rate of consumers in Niğde province looking for the HACCP certification when purchasing milk and dairy products was found to be 11%. This situation shows that consumers in the province do not pay much attention to the HACCP certification criteria for purchasing dairy products. The government is in a regulatory and supervisory position with the power of law for people's access to safe food within the scope of food security. In this respect, the most important duty of the state is to implement the legal regulations that will ensure food safety and to supervise the applicability of these activities through supervisory institutions.

The government is also responsible for raising public awareness about food safety. It should also ensure that people prioritize food safety in food shopping. In this regard, activities such as organizing seminars, preparing brochures, providing food safety training in schools, as well as preparing advertisements such as public service ads can be implemented.

However, as food safety is the responsibility of the government and food producers, it is also the responsibility of consumers and households. Therefore, everyone is responsible in terms of food safety for reducing food borne diseases and food poisoning. However, it has been observed that consumers' priorities regarding food safety are not sufficient. This situation shows that consumers should be more informed about food safety through activities such as seminars, congresses, and workshops.

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