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Aims and Scope

FOOD and HEALTH

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

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

The journal's target audience includes specialists and professionals working and interested in all disciplines of food and Nutrition Sciences.

The editorial and publication processes of the journal are shaped in accordance with the guidelines of the International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), and Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), and National Information Standards Organization (NISO). The journal is in conformity with the Principles of Transparency and Best Practice in Scholarly Publishing (doaj.org/bestpractice).

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Research Article

Increased expression of GLUT4, catalase and nitric oxide by a crystalised fraction from ethanolic extracts of oak leaf lettuce and okra seed in C2C12 cell line

Jasadee KAEWSRICHAN[1], Ashara PENGNOO[2]

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ABSTRACT

The fractions obtained from low temperature-induced crystallisation of ethanolic extracts of green- and red-oak leaf and okra seed were accounted for total polyphenol content and the DPPH antioxidant activity, resulting in 5.99, 4.44, and 12.35 mg gallic acid equivalent per g sample, and 0.45, 0.35, and 0.99 mg Trolox equivalent per g sample, respectively. Insulin resistance was the result after incubating C2C12 skeletal muscle cells in high glucose DMEM for 20 h. Oxidative stress and inflammation were triggered by re-incubating these resistant cells with insulin. The expression of p-p38 MAPK and NF-κB proteins and the NF-B p65 transcription factor activity were enhanced. Such inflammatory protein markers were reduced when the crystalised extracts replaced insulin. Increased catalase activity and NO production were also determined for the incubations using the crystalised extracts. It was suitable to include these vegetables in the daily diets of prediabetic individuals for better management of the patient's health status by increasing insulin sensitivity and decreasing inflammation.

Keywords: NF-κB p65 transcription factor, GLUT4, Catalase activity, Nitric oxide, Polyphenols, DPPH antioxidant activity, Oak leaf lettuce, Okra seed, C2C12 cell line

Introduction

The effect on health promotion of vegetables consumed daily has been demonstrated, usually leading to reductions of inflammatory mediators to the levels are not risk for chronic- and degenerative diseases, such as type II diabetes mellitus (T2D), cardiovascular diseases, and neurodegenerative diseases (Stanaway et al., 2022; Godos et al., 2020; Parasoglou et al., 2017). More than 50% of the global deaths have been attributable to these inflammation-related diseases (Furman et al., 2019). Increased consumption of plant-derived flavonoids has improved cognitive function and decreasing risks of age-related neurodegeneration because of intensely suppressing TNF-α and C-reactive protein synthesis (Li et al., 2023). Indeed, there are a variety of secondary metabolites in edible plants that impart health-promoting as well as disease-curing and preventing effects, commonly related to antioxidant, anti-bacterial, anti-cancer, immune-modulating, and/or anti-inflammatory activities (Zheng et al., 2019). However, a plant cultivar has defined sets of secondary metabolites corresponding to plant genetics (Yang et al., 2018). For example, similar phenolic components of green- and red-oak lettuce cultivars have been reported. Apigenin, glucuronide, and dihydroxy-benzoic acid have solely been detected in the green cultivar. In contrast, only luteolin, apigenin conjugates, cyanidin conjugates, and hydroxy-malonyl-hexoside have existed in the red cultivar (Viacava et al., 2017). The pod of *Abelmoschus esculentus* (called okra) is an edible part of the plant, containing ample proteins and fatty acids in the seed (Sami et al., 2013), and mucilage polysaccharides, vitamins, fibres, and minerals in the peel (Zim et al., 2021). In addition, quercetin has been suggested as a major flavonoid in the aqueous extract of okra pod, presenting anti-hyperglycemic activity in the high-fat diet-streptozotocin rat model (Peter et al., 2021). Besides, the mucilage-like substance obtained by boiling okra pods in water has shown anti-diabetic and anti-hyperlipidemic properties in alloxan-induced diabetic mice (Zim et al., 2021). Altogether, the health-beneficial effects of green- and red-oak leaf lettuces and okra seed might be more diverse than expected, leading to an underestimation of their nutritional values. In this research, biological effects concerning anti-oxidation, anti-inflammation, and increased GLUT4 expression of crystallised compounds from ethanolic extracts of green- and red-oak leaf and of okra seed were investigated on C2C12 skeletal muscle cells in normal and high glucose media. The acquired data would be adequately scientific and useful for managing complications related to prediabetes due to increased daily consumption of vegetables (Găman et al., 2020).

Materials and Methods

Preparation of Crystalized Extracts

Okra seeds and green- and red-oak leaves were collected from the Faculty of Natural Resources, Prince of Songkla University, Thailand. The plant materials were dried under the sun to ~95% dryness and ground into fine powder using a blender. Each 50 g was macerated in 150 mL of 95% ethanol (Loba Chemie, India) overnight at room temperature. The supernatant was separated by centrifugation at 6000 x *g* for 15 min. This was 1 cycle, and 3 cycles of the maceration were carried out for a sample. The pooled supernatant of ~400 mL was evaporated using a rotary evaporator at 45°C to obtain viscous extract. This extract was cooled at 6°C overnight for crystallisation induction. The formed crystals were carefully separated, air-dried, and kept in an air-tight container at 6°C until use.

Total Phenolic Content

The folin-Ciocalteu method was applied to determine the total phenolic content of samples (Peter et al., 2021). DMSO (RCI Labscan™) was used as the sample solvent. Solution A was prepared by mixing 2% Na_2CO_3, 2% $CuSO_4$, and 4% sodium potassium tartrate at a 100:1:1 volume ratio. A sample of 100 µl and 100 µl of solution A were mixed and incubated for 5 min. Then, 50 µl of 0.5 M sodium hydroxide was added and incubated for 10 min. Next, 200 µl of 1:1 diluted Folin-Ciocalteu reagent (Loba Chemie) in water was added and incubated for 30 min. The OD_{750} was spectrophotometrically measured using a microplate reader (Varioskan LUX, ThermaFisher, USA). Gallic acid (Sigma-Aldrich, Germany) was used as a positive standard, and the concentration range of 0-2 mg/mL was carried out to draw the standard curve. The total phenolic content of a sample was calculated and reported as mg gallic acid equivalent per gram sample.

DPPH Antioxidant Assay

The antioxidant activity of a sample was accounted for by using a DPPH radical scavenging assay (Zim et al., 2021). Briefly, a 100-µl sample solution was mixed with 0.9 mL of 0.1 mM DPPH reagent in methanol (Sigma-Aldrich) using a vortex. A 100-µl DMSO was of a blank control. After incubating for 30 min at 37°C and protected from light, the OD_{517} was measured against the blank. Trolox (Sigma-Aldrich) was used as a positive standard, and the concentration range of 0.05-0.2 mg/mL in methanol was performed to construct the

standard curve. The DPPH antioxidant activity was calculated and reported as mg Trolox equivalent per gram sample.

C2C12 Cell Culture

C2C12 is a myoblast cell line from ATCC. The cells were routinely grown in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum and 1% antibiotic and antimycotic (called normal medium) in a 5% CO_2 incubator at 37°C. The cells were subcultured when their growth approached 70% confluency. When specified, high-glucose DMEM was used to replace the normal medium. Chemicals and reagents used for the cell culture technique were purchased from Gibco™ (ThermoFisher, USA).

Preparation of Test Solutions for Treating Cells

A stock solution of a test extract of 2 mg/mL in DMSO was prepared. This sample stock solution was diluted to a final concentration of 200 µg/mL in a suitable growth medium to treat cells. Treatments were performed on ~80% confluent cells by incubation with a test solution for 20 h at 37°C in a CO_2 incubator. Insulin at a final concentration of 100 nM was handled as the positive control. These treated cells were next used in experiments including quantitative PCR (qPCR), SDS-PAGE and Western blotting, and determinations of p65 transcription factor activity, catalase activity, and nitric oxide (NO) production, as detailed in the following.

qPCR

The total RNA of cells was extracted using Tri Reagent™ and the RNA concentration was fluorometrically measured using Qubit™ RNA Assay Kit (Invitrogen, USA). Procedures related to the kits were in accord with the manufacturers' recommendations. First-strand cDNA was synthesised in a 25-µl reaction containing 12.5 µl of SensiFAST cDNA Synthesis Master Mix (Meridian Bioscience, USA), 200 pmolar of oligo$(dT)_{20}$ primer, and 500 ng of the RNA template. The qPCR of a sample was done in triplicate on the Magnetic Induction Cycler (Mic qPCR) (Bio Molecular Systems, Australia) by using qRT-PCR Brilliant III SYBR Master Mix (Agilent, CA, USA) and a pair of primers regarding inflammatory genes, such as *TNF-α*, *IL-6*, and *NF-κB*, and of *GAPDH* control gene. The primers were of Origene™ with the codes NM_013693, NM_031168, NM_008689, and NM_008084, respectively. The set-up qPCR cycling was 5 min at 95°C, followed by 40 cycles at 95°C for 30 sec and 60°C for 1 min to acquire Ct data. Primer specificity was confirmed by doing a dissociation curve analysis. For a sample, the Ct value of a test gene was normalised by that of GAPDH, and relative expression in fold change was reported by dividing the normalised Ct of a treated sample by the untreated control.

Protein Electrophoresis and Western Blotting

Cells were lysed on ice using RIPA buffer (Sigma-Aldrich) supplemented with protease inhibitor cocktail (Roche, USA) and centrifuged at 11.000 × g for 15 min at 4°C to remove cell debris. A lysate's protein concentration was quantitated using the Pierce BCA Protein Assay Kit (ThermoFisher). An equal amount of sample proteins was loaded on SDS-polyacrylamide gel for electrophoresis. The separated bands were wet-transferred to the PVDF membrane (Amersham™) at 4°C overnight. The membrane was blocked in Tris-buffered saline containing 0.1% Tween 20 for 2 h at room temperature and probed with 1° antibodies (dilution 1:1000) against NF-κB, p38 MAPK, p-p38 MAPK, GLUT4, or β-Actin protein (Cell Signaling Technology, USA) at 4°C overnight. The binding of the bound 1° antibodies to HRP-conjugated 2° antibody (dilution 1:2000) (Santa Cruz Biotechnology, USA) was carried out at room temperature for 2 h. An ECL reagents kit (ThermoFisher) was used to generate the signal. Band visualisation was accomplished using Luminescent Image Analyzer (GE Healthcare, Sweden), and the bands' density was calculated using ImageQuant™ TL 10.2 analysis software. Variation in the loaded protein concentration was compensated by normalising the band density of a target protein to that of GAPDH.

Preparation of Nuclear Extract

The NE-PER™ Nuclear and Cytoplasmic Extraction Reagents (ThermoFisher) were utilised to separate nuclear extract from cultured cells. In brief, cells in suspension were collected by centrifugation at 500×g for 5 min, washed by resuspending in PBS, transferred of ~5×10^6 cells to a 1.5-mL microcentrifuge tube, and re-centrifuged again at 500×g for 2-3 min. The supernatant was discarded, and the cell pellet was dried as much as possible in the air. Then, the CER I solution was added, mixed vigorously for 15 sec using a vortex and incubated on ice for 10 min. Next, CER II solution was added, mixed for 5 sec, and incubated on ice for 1 min. After centrifugation at 16.000×g for 5 min, the supernatant (the cytoplasmic extract) was separated and stored at -80°C until use. The insoluble fraction in the tube's bottom was resuspended in ice-cold NER solution and mixed for 15 sec for every 10-minute incubation of the total 40 min incubation on ice. The nuclear extract was acquired by centrifugation at 16.000×g for 10 min. The supernatant was immediately aspirated, transferred to a clean pre-chilled tube, and stored at -80°C until use.

Assay of NF-κB p65 Transcription Factor Activity

RayBio® NF-κB p65 Transcription Factor Activity Assay Kit (RayBiotech, USA) was recently used to determine active p65 transcription factors in nuclear extracts. The kit was provided in a 96-well plate format. The wells were pre-coated with double-stranded oligonucleotides containing NF-κB binding sequence (5′-GGGACTTTCC-3′). A sample of nuclear extracts was added to the well and incubated at 4°C overnight before being removed. The primary antibody against p65 was added and incubated for 2 h at room temperature. Unbound antibodies were washed away. Then HRP-conjugated secondary antibody was added, incubated for 1 h at room temperature, and removed. The TMB substrate reagent was added and incubated for colour development at room temperature for 1 h. The stop-solution was added, and the OD_{450} was immediately measured using a microplate reader.

Nitric Oxide (NO) Assay

The principle for assaying NO of Sigma-Aldrich is based on oxidising any present NO to be NO^{2-} (nitrite) and NO^{3-}(nitrate) using a Griess reagent. The reaction products are stable and can be quantitatively measured at OD_{540}. The standard curve can be constructed using accurate concentrations of NO^{2-} for the reaction. NO sample concentration was calculated concerning the standard curve and normalised by the corresponding cells' total protein using Pierce BCA Protein Assay Kit (ThermoFisher). The normalised NO concentration of a test sample was compared with that of the control for reporting the relative NO production.

Catalase Activity Assay

Recently, the determination of catalase activity in samples as cell lysates was carried out by using the Catalase Colorimetric Activity Kit (Invitrogen), according to the manufacturer's protocol. A cell lysate from adhered cells in a well was prepared as follows. The cultured medium was removed, and the cells were gently dislodged with a rubber policeman in 1x assay buffer. After centrifugation of the lysate at 10.000 x g for 15 min at 4°C, the supernatant was collected and used for assaying catalase activity immediately. Catalase standard, as supplied in the kit, was used for constructing the standard curve with a concentration range of 0-5 U/mL, where 1 U of catalase stands for the decomposition of 1.0 µmol H_2O_2 in 1 min at 25°C, pH 7. Samples and catalase standard solutions were separately incubated with mixed hydrogen peroxide reagent at room temperature for 30 min, followed by mixed substrate reagent at room temperature for 15 min before measuring the OD_{560}. Again, the catalase activity determined for a sample was normalised with the corresponding sample protein and compared with the normalised result of the untreated control for reporting the relative catalase activity.

Data Analysis

Each experiment was done in triplicate. Data were reported as Mean ± S.D. Differences between groups were evaluated at $p = 0.05$ using the Student t-test or ANOVA with post hoc test Bonferroni of the IBM SPSS software for multiple comparisons. Results were considered significantly different if p values < 0.05.

Results and Discussion

Extraction Yields, Total Phenolic Content, and DPPH Antioxidant Activity

Amorphous crystals of 3 g, 6 g, and 4.5 g were obtained by extracting 50 g dried powder from okra seed, green-oak leaf, and red-oak leaf with three times repeated 150-mL 95% ethanol. This resulted in a 6%, 12%, and 9% yield. All the extracts were sparingly soluble in ethanol and methanol but freely dissolved in DMSO. Stock solutions of 2 mg/mL in DMSO of the extracts were prepared and diluted in methanol to examine total phenolic content (TPC) and DPPH antioxidant activity using gallic acid and Trolox as the corresponding standards, respectively. The extract from okra seed showed TPC and antioxidant activity of about 2 times and 2.8 times greater than those of green and red leaf, respectively. Moreover, the measured antioxidant activities corresponded with the TPC values (Table 1).

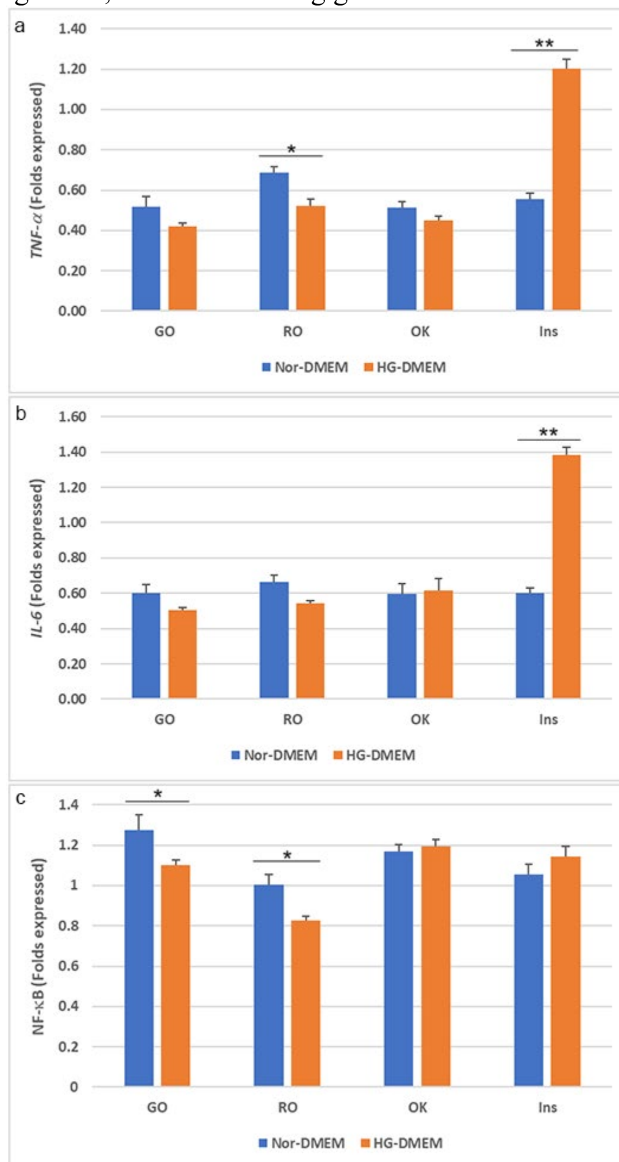
Table 1 The values of TPC and DPPH antioxidant activity for the extracts of okra seed, green-oak leaf and red-oak leaf

The extract origin	TPC (mg gallic acid per g sample)	DPPH antioxidant activity (mg Trolox per g sample)
Green-oak leaf	5.99 ±0.22 ^{a,b}	0.45 ±0.02 ^{c,d}
Red-oak leaf	4.44 ±0.06 ^{a,b}	0.35 ±0.05 ^{c,d}
Okra seed	12.35 ±0.15 ^{b}	0.99 ±0.08 ^{d}

a and c, significant difference with $p < 0.05$; b and d, significant difference with $p < 0.01$

Transcriptions of TNF-α, IL-6, and NF-κB Genes

The transcription of *TNF-α* and *IL-6* genes seemed to be silenced in the normal glucose medium because the folds expressed were less than 1 (Figure 1). Transcriptional silencing of these genes was observed for all incubations whether using insulin or the extracts of green- and red-oak leaf and of okra seed. However, significant elevation of such gene transcriptions was evident after treating the cells with insulin in the high glucose medium (Figure 1a and b). For the *NF-κB* gene transcription in normal glucose, incubations using green-oak leaf and okra seed extracts resulted in transcriptional activation of the gene, leading to the folds increased of > 1 (Figure 1c). This gene transcription was not affected when challenged with red-oak leaf extract and insulin (folds changed ~1). In the high glucose medium, it was noted that the NF-κB transcription was suppressed by green- and red-oak leaf extracts but slightly activated by insulin. Likely, the effect of okra seed extract on the NF-κB transcription in both the normal- and the high-glucose media was equivalent and stimulatory, indicating a 1.2-fold change.

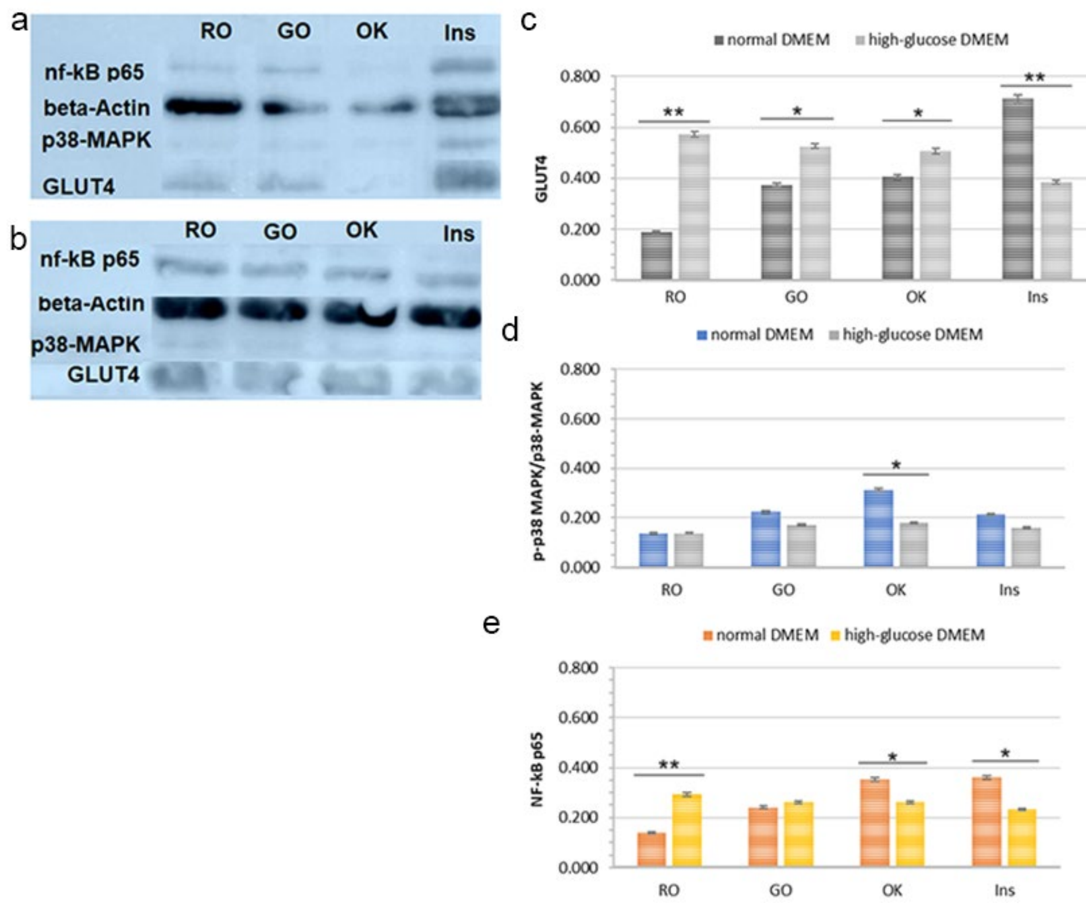


Symbols: $p < 0.05$; **, $p < 0.01$

Figure 1. Transcriptional expression in folds changed of genes *TNF-α*, *IL-6*, and *NF-κB* of C2C12 cells grown in normal- and high-glucose DMEM determined by qPCR method; Insulin of 100 nM (Ins) or 200 µg/mL extracts from green-oak leaf (GO), red-oak leaf (RO), or okra seed (OK) was independently incubated with the cells for 20 h before running the qPCR.

Expression of GLUT4, p38 MAPK, p-p38 MAPK, and NF-κB Proteins

Cells of C2C12 treated with 100 nM insulin or 200 µg/mL extracts of green- and red-oak leaf and of okra seed were subjected to SDS-PAGE and immunoblotting against NF-κB, p38 MAPK, p-p38 MAPK, and GLUT4 proteins. Results are shown in Figure 2. The expressed GLUT4 protein after insulin challenge in the normal medium was higher than that affected by other used extracts. However, such expression was intensely suppressed by insulin in the high-glucose medium. Lower levels of GLUT4 protein in normal glucose were determined for the incubations using green- and red-oak leaf and okra seed extracts compared to that using insulin. Of note, the expression levels were increased while replacing the normal medium with another the containing high glucose (Figure 2c). The expressed ratio of p-p38 MAPK and p-38 MAPK proteins after incubations with green- and red-oak leaf and insulin was not altered whether normal glucose or high glucose medium was supplied (Figure 2d). Instead, such the ratio was decreased after incubated with okra seed extract using the high glucose medium compared to that of the normal one. The expressed levels of NF-κB in the normal medium were arranged according to the treatments as red-oak leaf < green-oak leaf < okra seed = insulin (Figure 2e). When changed to the high glucose medium, decreased expression of NF-κB was examined for treatments using okra seed extract and insulin. However, a similar amount of this expressed protein was indicated after incubation using green oak leaf extract in either the normal or the high glucose medium. Notably, increased expression of NF-κB protein was apparent for red-oak extract treatment in the high glucose culture.



Symbols: *, $p < 0.05$; **, $p < 0.01$

Figure 2. Expressions of GLUT4, p-38 MAPK, p-p38 MAPK, and NF-κB proteins determined by Western blotting method; C2C12 cells grown in normal- or high- glucose DMEM were incubated with 100 nM insulin (Ins) or 200 µg/mL extracts from green-oak leaf (GO), red-oak leaf (RO), or okra seed (OK) for 20 h followed by SDS-PAGE of the cells grown in normal DMEM (a) and those in high glucose DMEM (b); The density of protein bands was calculated by using ImageQuant™ TL 10.2 analysis software and graphed for GLUT4 (c), the p-p38 MAPK/p-38 MAPK ratio (d), and NF-κB (e).

NF-κB p65 Transcription Factor Activity

p65 is one of the five components that form the NF-κB transcription factor family, which is present in most cells and mediates inflammatory responses through the NF-κB signalling pathway (Zhang et al., 2020). In this research, the transcriptional activity of p65 in C2C12 cells was investigated using the kit as specified. Results are shown in Fig. 3. Increased p65 transcriptional activity was found in normal glucose after incubation with green-oak leaf extract compared to that of okra seed and red-oak leaf. Suppression of the activity was suggested for insulin, whether using the normal or the high glucose medium. Instead, reduction of the p65 activity was significant after being treated with the extracts of okra seed and green oak leaf in the high-glucose medium. However, slight decreasing the activity was seen for the incubation using the red oak extract.

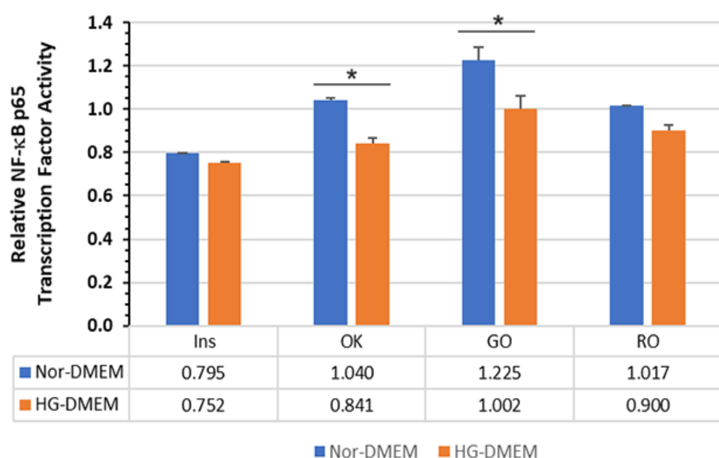


Figure 3. Relative p65 transcription factor activity: C2C12 cells grown in normal- or high- glucose DMEM were treated with 100 nM insulin (Ins) or 200 µg/mL extracts from green-oak leaf (GO), red-oak leaf (RO) or okra seed (OK) for 20 h before the assay using nuclear extracts. The untreated cells were of the control. Symbols: *, $p < 0.05$

NO Production

The production of NO by C2C12 cells grown in the normal or the high glucose medium and treated with insulin or the extract of either green- and red-oak leaf or okra seed was measured using cell supernatants. Results are shown in Fig. 4. NO was actively produced by the cells after incubation with okra seed extract, and the lower producing levels were observed after incubation with green- and red-oak leaf extracts. Meanwhile, the effect of insulin on inducing NO production was insignificant, whether in the normal or the high glucose medium.

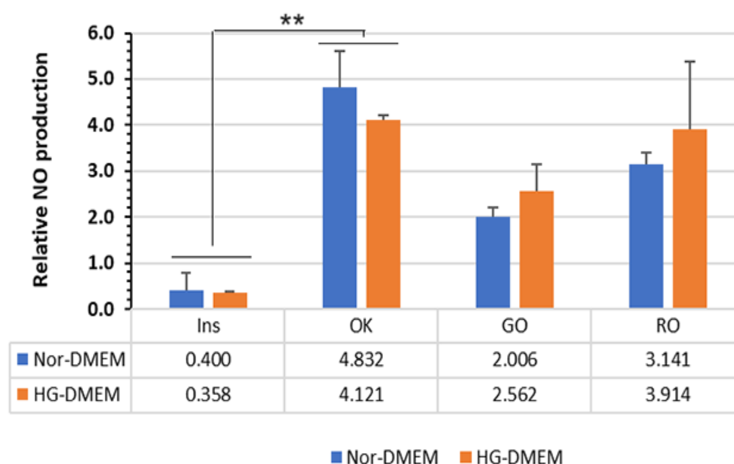


Figure 4. Relative NO production: C2C12 cells grown in normal- or high- glucose DMEM were treated with 100 nM insulin (Ins) or 200 µg/mL extracts from green-oak leaf (GO), red-oak leaf (RO), or okra seed (OK) for 20 h before the assay using cell supernatants. The untreated cells were of the control. Symbols: **, $p < 0.01$

Catalase Activity

Catalase is an enzyme found in nearly all living cells exposed to oxygen and is important by protecting cells from oxidative stress caused by reactive oxygen species (ROS). This enzyme, among others, has the highest turnover rate in converting millions of hydrogen peroxide molecules to water and oxygen in a second. In Fig. 5, increased catalase activity was found for the cells grown in the normal glucose medium and after treatments using okra seed and green- and red-oak leaf extracts. In comparison, less catalase activity was investigated for the cells of insulin challenge. In high glucose cultures, the enzyme activity decreased significantly when incubated with insulin as well as red oak leaf extracts and okra seed extracts. However, the activity disappeared after incubation using green oak leaf extract.

Fasting blood glucose is one of the criteria for diagnosing prediabetes (also called insulin resistance), about which plasma glucose reached in 2 h of an oral 75-g glucose challenge ranges between 101 and 125 mg/dL (5.6–6.9 mM). Importantly, about 50% of the diagnosed prediabetic individuals have been noted to develop T2D within five years and are also at a higher risk of developing other metabolic disorders and cancers (Merz & Thurmond, 2020; Owei et al., 2019). Lifestyle interventions through exercise and diet are the current strategies that are potential to cure prediabetes, including the fact that the co-administration of metformin has improved insulin sensitivity conditions. However, using this drug for prediabetes has not been ratified by the US FDA because it

sounds ineffective compared to lifestyle interventions in addition to the drug's side effects (Centers for Disease Control and Prevention, 2017). The skeletal muscle is the largest organ system of the body, essential for movements in daily life. Over 80% of oral glucose uptake is absorbed and metabolised to ATP by the muscle cells, corresponding to insulin's function. Desensitisation to insulin of these cells impacts health by increasing blood glucose concentrations, the preceding diabetes condition (Khan et al., 2019).

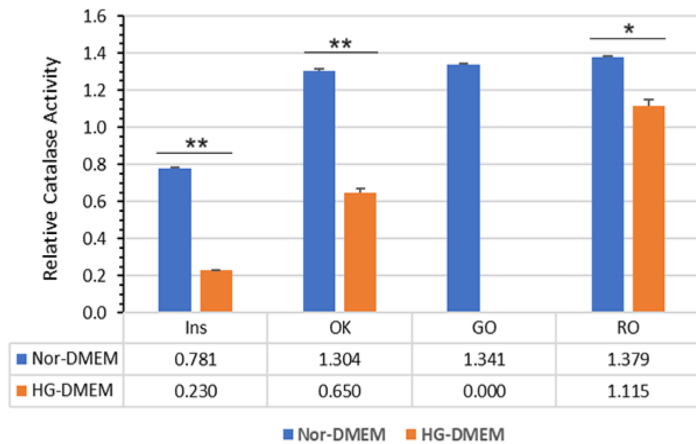


Figure 5. Relative catalase activity: C2C12 cells grown in normal- or high- glucose DMEM were treated with 100 nM insulin (Ins) or 200 µg/mL extracts from green-oak leaf (GO), red-oak leaf (RO) or okra seed (OK) for 20 h before the assay using cell lysates. The untreated cells were of the control. Symbols: *, $p < 0.05$; **, $p < 0.01$

The glucose concentration of the recently used high glucose DMEM has been mimicked to prediabetes's fasting blood glucose level. This was used to desensitise the cultured C2C12 cells to insulin to cause insulin resistance. Accordingly, the responses of these insulin-resistant cells to all test extracts in high glucose mediums would be different from those of the insulin effects. Regarding some inflammatory genes' markers, depletion of *TNF-α*, *IL-6*, and *NF-κB* transcriptions after incubation with green- and red-oak leaf extracts was greater than that of okra seed incubation (Figure 1). However, suppression of p-p38 MAPK, p-38 MAPK, and NF-κB proteins was potential for okra seed extract compared to those of green- and red-oak leaf samples (Figure 2d and e). For insulin effects, the promotion of NF-B transcription was suggested with decreased NF-B translation (Figure 2e). For reliability, the NF-κB signal transduction was validated by measuring NF-κB p65 transcription factor activity using a specific reagent kit. The extracts of okra seed and green-oak leaf, or even of red-oak leaf, were indicated to suppress the p65 transcription factor activity, and these results were different from the impacts of insulin (Figure 3). As such, all of the test extracts would mitigate inflammations as induced by high glucose condition but not by insulin. The promotion of GLUT4 expression was also determined after incubation with the extracts, and the highest protein expression was suggested for the red-oak leaf sample. Again, reduction of the expressed GLUT4 protein was significantly observed for insulin supplement in the high glucose medium (Figure 2c). It was certain that the extracts of green- and red-oak leaf and of okra seed had the potential for improvement of prediabetic conditions due to increasing insulin sensitivity and glucose uptake (Bird and Hawley, 2017).

Hyperosmotic stress of C2C12 cells possibly happened while grown in the high glucose medium, leading to water efflux and cell shrinking. Although oxidative and osmotic stress differ significantly, responses to these stresses by the affected cells have overlapped (Gayathri et al., 2023; Mager et al., 2000). There was a rationale to monitor transcriptional and translational extents associated with inflammations of C2C12 cells after being cultured in iso-osmotic or hyper-osmotic medium recently. In general, cells have defence mechanisms to mitigate stresses and inflammations. NO production and dissipation are parts of biological processes identified to date for stabilising normal cellular oxidative stress. However, depending on its location and concentration, NO has been suggested to elicit pro-inflammatory or anti-inflammatory effects. For instance, localised suppression of NO has been inflammatory by increasing pleural exudate volumes, leukocyte counts, and activities of enzymes related to oxidative stress of rats with carrageenan-induced acute inflammation. However, these signs have been dissipated by supplementing NO locally (Iwata et al., 2020). This project suggested increased NO production by C2C12 cells after incubation with green-oak leaf, red-oak leaf, and okra seed extracts by two, three, and four times, respectively. For insulin-challenged cells, however, very little NO was produced. It was noted that trends of NO production for the cells in the normal- and the high- glucose media were not very varied (Figure 4). Consequently, all test extracts were supposed to be great in resolving oxidative stress and inflammation in the high glucose medium compared to insulin, which might be associated with the existing antioxidant activities (Table 1) and the ability to induce NO production (Figure 4).

Catalase deficiency and malfunctioning have been etiological factors of age-associated degenerative diseases, including T2D (Nandi et al., 2019). A strategy has been proposed to mitigate these diseases using catalase enzymes in food supplement forms (Chandrasekaran et al., 2017). In the present

project, C2C12 cells grown in the normal medium were stimulated to produce catalase after incubations using the extracts from green-oak leaf, red-oak leaf, and okra seed. However, less induction of the enzyme by these extracts was found for the cells in the high glucose medium. The potential of insulin to induce catalase production was much inferior to the extracts (Fig. 5). Although increased catalase expression was liable for C2C12 cells treated with the extracts, mechanisms at molecular levels are further explored.

Conclusion

In summary, the phenolic fractions obtained by cool-induced crystallisation of ethanolic extracts from green- and red-oak leaf and okra seed were determined to present DPPH-radical scavenging activity in a dose-dependent response. Cells of the skeletal muscle cell line, C2C12, were insulin-resistant after being cultured in high glucose DMEM for 20 h. Inflammations were indicated to increase by repeat incubation of these resistant cells with insulin, leading to increased expression of p-p38 MAPK and NF-κB proteins and NF-κB p65 transcription factor activity. Instead, using all of the extracts for the incubation seemed beneficial, as the activations of such inflammatory proteins and the transcription factor were diminished. Besides, increased catalase activity and NO production were apparent by incubations using the extracts. Consequently, these three vegetables might be helpful when included in the daily diets of prediabetic individuals for increasing insulin sensitivity and reducing oxidative stress and inflammation.

Compliance with Ethical Standards

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

Ethics committee approval: The authors declare that this study does not include experiments with human or animal subjects, so ethics committee approval is not required.

Data availability: Data will be made available on request.

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Acknowledgements: -

Disclosure: -

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Effects of cooking techniques on phenolic content and sensory profiles of cauliflower

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ABSTRACT

In this study, the effects of the cooking methods (frying, stir-frying, air-frying, boiling, steaming, baking and sous-vide, and microwaving) on total phenolic content (TPC) and sensory profile of cauliflower have been investigated. Higher cooking temperatures have resulted in higher TPC values, which can be attributed to the generation of new phenolics by the Maillard reaction and cleavage of bound phenolics. The highest TPC and bitterness were obtained when steaming and baking (180°C for 20 min) were applied. While the boiling procedure results in low bitterness and sweetness due to the loss of related compounds in boiling water, the taste of cooked cauliflower was higher when steamed (12 min) and stir-fried (4.5 min). Overall acceptability of the boiled, steamed, and fried samples was higher than in baked and sous-vide treated samples. Steaming was determined as the best technique regarding TPC content and sensory properties.

Keywords: Cauliflower, Total phenolics, Cooking techniques, Sensory analysis, Steaming

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Introduction

Brassicaceae or *Cruciferae* is a family of vegetables in diets and is well known for its health-promoting compounds. Positive health effects of *Cruciferae* are mainly attributed to their high content of phenolic compounds (57.3-230 mg gallic acid equivalence (GAE)/100g) (Ramos dos Reis et al., 2015; Lafarga et al., 2018). Cauliflower (*Brassica oleracea L. var. botrytis*) and broccoli (*Brassica oleracea L. var. italica*) are the most frequently consumed species of Cruciferae, produced over 25 million tons worldwide (FAOSTAT, 2021). While the most predominant phenolic compounds present in cauliflower are protocatechuic acid, quercetin, pyrogallol, vanillic acid, coumaric acid, and kaempferol, other identified phenolic compounds in cauliflower include phenolic acids (gallic acid, 4- amino benzoic, stolleuropein, reversterol, caffein, catechol, alpha-coumaric acid, coumarin, 3-OH-tyrosol, chlorogenic, rosmarinic, caffeic, syringic acid, cinnamic acid, and sinapic acid), flavonoids (naringin, naringenin, 7-hydroxy flavonoid, rutin, quercetrin, apigenin, hespertin, hisperdin, rosmarinic acid, catechin) (Ramos dos Reis et al., 2015; Ahmed & Ali, 2013; Ali, 2015; Gratacós-Cubarsí et al., 2010).

Various kinds of cooking techniques could be evaluated based on their different heat transfer mechanism, such as boiling, frying, stir-frying, steaming, baking, *sous-vide* (SV), grilling, air-frying, and microwaving (MW) to increase the palatability and taste of vegetables and the retention of heat-labile compounds. Heat transfer mechanisms in these techniques depend on the design of the heating cabinet, food surface, contact material type, and fluid medium used (air, oil, water, steam). Cooking may positively or negatively affect total phenolic content (TPC) (Sharma et al., 2015). Some studies support that the TPC of vegetables decreases during boiling, steaming, baking, MW, and SV due to high temperatures (Ramos dos Reis et al., 2015; Lafarga et al., 2018; Sharma et al., 2015; Çubukçu et al., 2019). On the other hand, an increase in the TPC of freeze-dried vegetables has been observed in some studies due to comparing dry basis (d.b.) values (Turkmen et al., 2005; Girgin & El, 2015; Van Boekel, 2001; Cartea et al., 2011). In freeze drying, problems associated with high moisture content during solid mass extraction are reduced. Therefore, the variation in sample weight arising from differences in moisture content is eliminated (Maillard et al., 1996).

This study is a more comprehensive study on cauliflower concerning cooking techniques compared to previous studies mainly focusing on broccoli (Ramos dos Reis et al., 2015; Lafarga et al., 2018; Girgin & El, 2015; Engel et al., 2002; Wieczorek et al., 2018). The primary purpose of this study was to investigate the effects of cooking methods (frying, stir-frying, grilling, air-frying, boiling, steaming, baking and sous-vide, and microwaving) at various time and temperature conditions on TPC and sensory profile (appearance, odour, taste, and texture) of cauliflower, and to find the best cooking method accordingly.

Materials and Methods

Approximately 7 kg of cauliflower (*Brassica oleracea L. var botrytis*) samples were purchased from local markets in Istanbul, Turkey, during September-October 2022. Samples were prepared in the same bite-sized pieces (inflorescences, 5 cm) to eliminate the enzymatic depletion of phenolic compounds due to cutting and slicing (Cartea et al., 2011). The mixed pieces were washed, wiped, and then divided into 200 g portions for processing.

Each of the cooked and raw samples was blended separately (Blendtec Classis 575, USA), then frozen immediately in aluminium bowls at -18°C and freeze-dried at -80°C, below 0.1 mbar for five days (Teknosem TOROS 4/4 DS, Istanbul, Turkey) and ground (IKA A11, China) and stored at -18°C until analysis. Therefore, extraction problems due to high moisture contents were eliminated, as defined by Maillard et al. (1996). Moisture loss was calculated by taking the weight into account.

Cooking Treatments

The cooking techniques and time-temperature parameters, given in Table 1, are determined in preliminary studies that acceptable palatability (bitable firmness and no burnt surface) has been obtained. The same portions (200 g) of cauliflower samples were:

(1) Boiled in 2 L drinking water (B8 and B10).
(2) Steamed 2 L of tap water (S10 and S12). The samples were suspended on a sieve with a lid above the boiling water level.
(3) Stir-fried in Teflon wok panes using 150 mL sunflower oil (F45 and F65).
(4) Baked using two preheated conventional ovens (Unox Cheftop Plus Combi Oven, Italy) (O20-150, O25-150, O20-180, and O25-180).
(5) Sous-vide (SV) cooked in preheated 4 L of tap water by a sous-vide cooker (Proficook pc-sv 1126, Germany) with a vacuum pump (Henkelman Mini Jumbo, Germany) (SV60 and SV70).

(6) Microwave-cooked (MW) using a microwave oven (Arçelik, MD823, Turkey) and sealed microwavable steam cooking bags (Koroplast).
(7) Air-fried (AF) by coating samples with sunflower oil in an air-fryer pan (Mi Smart Air Fryer 3.5 L, MAF02, China). At the 8th minute of each cooking, the air-fryer pan was shaken for 10-20 seconds to turn samples upside down.

Determination of TPC

Six grams of ground sample and 20 mL of 80% aqueous methanol (LC Grade) were added into a 50 mL Falcon centrifuge tube. The mixture was homogenised using an ultrasonic homogeniser (Banderin Sonopuls GM 2200.2) at room temperature and then centrifuged (Cryste Varispin 12R) at 10.000 x g at four °C for 20 minutes. After three extraction steps, supernatants were pooled in 50 mL centrifuge tubes and completed with 80% aqueous methanol to 50 mL in a volumetric flask and stored at -20°C. TPC was determined by the Folin-Ciocalteu method (Ainsworth & Gillespie, 2007) using Folin-Ciocalteu reagent (Sigma-Aldrich), sodium carbonate (ISOLAB Laborgeräte GmbH), gallic acid (Bio Basic) and microplate spectrophotometer (Thermo Scientific Multiskan SkyHigh). The results were reported as mg GAE/100 mg d.b.

Sensory Analysis

The sensory evaluation was performed by 15 semi-trained panellists (20-65 age) using sensorial attributes based on previous studies (Engel et al.,2002; Poelman & Delahunty, 2013) with modifications made according to the sensory panel results. Sensory analysis was conducted on five selected cooking methods, and the time-temperature combination resulted in relatively higher TPC values. MW and AF samples were excluded because they had significantly low TPC values (B8, S12, F45, O20-180, and SV70). About 20 g of floret with a stalk from each sample were placed on polystyrene tables, identified with random 3-digit numbers, and evaluated by a 9-point hedonic scale in an air-conditioned room (21 ±1°C) under white light. The odour of samples was described by odour impact, green odour, cooked cauliflower odour, and sulfur odour; appearance by uneven colour, stalk colour, floret colour, and moistness; taste by taste impact, green taste, cooked cauliflower taste, sweetness, bitterness, and sulfur taste, texture by firmness, moistness, chewing resistance, cohesiveness; finally general acceptability.

Statistical Analysis

The averages and standard deviations were calculated from three measurements of the duplicate samples using Excel software (Microsoft Corporation, Redmond, WA, USA). One-way ANOVA and a post hoc Tukey test with a confidence interval of 95% were used to evaluate the statistical differences (Minitab® 16 Statistical Software, Minitab Inc. State College, Pennsylvania, USA). Correlation analyses were performed using Pearson's correlation coefficient (r) (Excel 2016, Microsoft, Troy, NY).

Results and Discussion

Boiling and steaming processes increased the moisture content, which is expected. On the other hand, other processes decreased when partial steam escaped (Table 1). Stir-fried samples had the lowest moisture content. Moisture affected the appearance of the final cooked cauliflower (Figure 1).

TPC Evaluation

TPC of the processed samples (169.4-637.3 mg GAE/100g d.b.) were significantly higher than those of the raw samples (147.7-224.0 mg GAE/100g d.b., α=0.05) where TPC (mg GAE / 100 g ± SD) and % TPC increase of processed samples were given in Table 1 and Figure S1, which is inconsistent with the previous observations by Turkmen et al. (2005). Higher temperature applications significantly worsen this rise (O20-180, S12, and B8, α=0.05). The effect of higher temperature on TPC content could be explained by the cleavage of bound phenolic compounds: the degradation of the cell wall of the cauliflower and disruption of phenol-protein complexes, which resulted in the release of phenolic compounds, leading to a better extraction (Shahidi & Yeo, 2016). The Maillard reaction at high temperatures may also contribute to the production of some compounds, including phenolic compounds (Sharma et al., 2015; Maillard et al., 1996; Alves et al., 2021). It was declared that above 80°C, isomerisation and degradation reactions of sugar start to become appreciable, which is the initial stage of Maillard reactions (Van Boekel, 2001). Then, the amino group is regenerated, and brown pigments are formed at the intermediate and final stages. In our study, the inner temperature of processed samples was between 65-87ºC, which increased TPC. Application of SV70 cooking showed a significant increase in TPC. On the other hand, no significant change in TPC was observed when SV60 was applied because the sample's inner temperature was probably lower than 60°C, which is not enough for the reactions (Table 1, α=0.05). Because samples were packed during SV cooking for 60 minutes, the inner temperature could not be measured accurately at the end of the process.

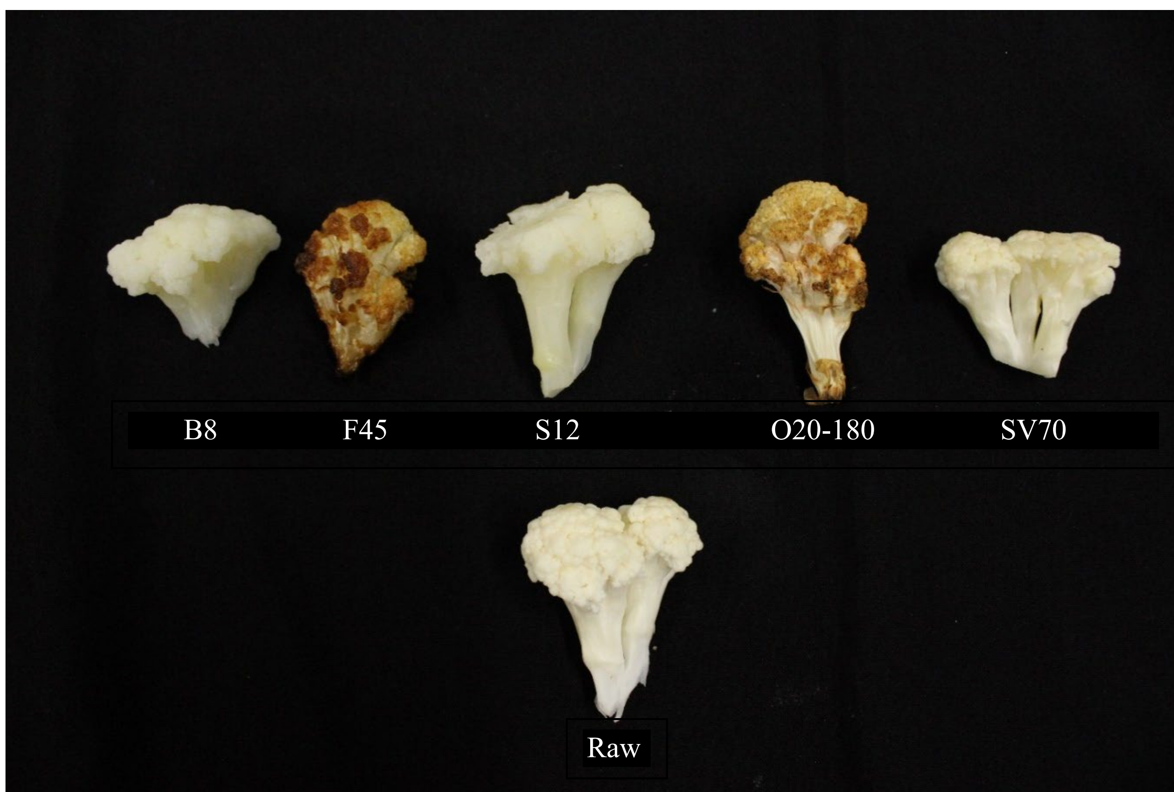

Figure 1. Picture of cooked (top) (B8; F45; S12; O20-180; SV70 from left to right, respectively) and raw (bottom) cauliflowers (B8, boiling for 8 min; F45, stir-frying for 4.5 min, S12, steaming for 12 min, O20-180, Oven cooking at 180°C for 20 min, at 70°C)

Table 1. Moisture content (%), TPC of cauliflower (mg GAE / 100 g ± SD), and % TPC increase of processed samples (% ± SD*)

Conventional Techniques	Sample codes	Moisture Content, %	Final T_{inner} ºC	% TPC increase, db. (%, ±SD)	TPC (mg GAE/100 g ±SD)
Raw sample		93.2	-	-	224.0 ±5.5a
Oven baking					
20 min – 150°C	O20-150	88.3	70	112.2±1.79^{bcd}	475.2 ±4.0^{b}
25 min – 150°C	O25-150	87.1	76	107.0±0.53^{cd}	463.5 ±1.2^{b}
20 min – 180°C	O20-180	88.8	71	**155.0±1.05^{ab}**	571,0 ±2.4^{c}
25 min – 180°C	O25-180	81.1	76	105.4±8.05^{cd}	459.8 ±18.0^{b}
Steaming					
10 min	S10	92.2	86	117.6±1.95^{bcd}	487.3 ±4.4^{b}
12 min	S12	91.4	87	**164.3±28.7^{a}**	591.8 ±64.3^{b}
Boiling**					
8 min	B8	93.4	75	**133.4±13.26^{abc}**	522.7 ±29.7^{b}
10 min	B10	94.1	85	56.8±5.37^{ef}	351.2 ±12,0^{c}
Stir-frying***					
4.5 min	F45	79.3	83	55.2±6.42^{ef}	347.7 ±4,4^{b}
6.5 min	F65	75.3	86	46.5±29.0^{ef}	328.1 ±64,9^{b}
Sous-vide					
60°C - 60 min	SV60	91.6	-	1.4±0.79^{g}	227.0 ±1.8^{a}
70°C - 60 min	SV70	92.5	-	87.9±8.79^{de}	420.8 ± 9.7^{b}

Rapid high-tech Techniques	Sample codes	Moisture Content, %	Final T_{inner} ºC	% TPC increase, db. (%, ±SD)	TPC (mg GAE/100 g ±SD)
Raw sample		91.6		-	147.7 ±2.1^{a}
Microwave					
250 W – 8 min	MW250-8	91.4	85	120.5±8.62^{bcd}	325.7 ±12.7^{b}
250 W –10 min	MW250-10	90.3	81	54.4±3.71^{ef}	228.1 ±5.5^{c}
350 W – 6 min	MW350-6	90.5	80	47.7±1.32^{ef}	218.2 ±1.9^{c}
350 W – 8 min	MW350-8	90.5	75	57.9±13.12^{ef}	233.2 ±19.4^{c}
500 W – 5 min	MW500-5	90.7	85	118.6±4.23^{bcd}	322.9 ±6.2^{b}
500 W – 6 min	MW500-6	90.0	81	105.4±11.57^{cd}	303.4 ±17.1^{b}
700 W – 3 min	MW700-3	90.4	81	59.2±8.18^{ef}	235.1 ±12.1^{c}
700 W – 5 min	MW700-5	89.9	70	56.0±3.99^{ef}	230,4 ±5.9^{c}
Air-frying					
150°C – 10 min	AF150-10	81.8	71	22.9±11.57^{fg}	181.5 ±17.1^{b}
150°C – 15 min	AF150-15	77.1	78	40.1±2.87^{fg}	206.9 ±4.2^{b}
180°C – 10 min	AF180-10	77.5	80	37.1±1.16^{fg}	202.5 ±1.7^{b}
180°C – 15 min	AF180-15	74.2	65	52.5±1.48^{ef}	225.3 ±2.2^{bc}

*SD, standard deviation; $p < 0.05$

** Total phenolic contents (TPC) of boiling water were 0.90 and 0.79 mg Gallic acid equivalents (GAE)/mL dry basis (d.b.) for 8 and 10 min, respectively

*** The temperature of the oil was approximately 180°C

****T_{inner}, cauliflower's centre temperature at the process's end

TPC increase of 10-minute boiled samples was significantly less than steamed ones due to the leaching of phenolics into water (0.90-0.79 mg GAE/mL), which can be explained by osmosis and water solubility of phenolic compounds (Table 1, α=0.05). In addition, the loss of TPC was significantly higher when processing time in boiling was increased (α=0.05). Significant losses of bioactive compounds due to boiling cauliflower were observed in contrast to steaming in literature (Girgin & El, 2015). Despite high-temperature application, a significantly lower increase in TPC was observed in stir-frying and air-frying. This can be explained by higher water loss during cooking and, consequently, heat and mass transfer mechanism due to the impact of water content on the thermophysical properties of foods (density, specific heat, enthalpy, thermal conductivity, and thermal diffusivity). The specific heat of high-moisture foods is primarily dominated by water content (Sahin & Sumnu, 2006a). Air-frying uses a combination of radiation, conduction, and convection heat transfer mechanisms and a narrow cooking chamber, creating a very intense heat transfer medium, which results in more rapid drying than baking. During stir-frying, as soon as the cauliflower is placed in the pan, it encounters both the high heat from the pan through heat conduction and the oil, which is at a much higher temperature than the boiling point of water. Steam, generated into the food pores, moves further and out into the oil. As a result, outward steam pressure prevents oil uptake and increases moisture loss simultaneously. This mechanism explains why the stir-fried samples had the lowest moisture content (Chen et al., 2021).

The TPC of the samples was also increased in MW and AF samples; however, not as much as in baking and steaming (α=0.05). This phenomenon might be due to the rapid drying on the sample's surface during AF, which leads to low thermal diffusivity and lowers the Maillard reaction rate (Van Boekel, 2001).

Heat transfer direction in MW is opposite of that in conventional cooking (Araszkiewicz et al., 2007) (Figure 2). In conventional heating, a hard, heat-resistant surface layer reduces heat transfer and mass transfer of the water in the centre through the surface, requiring longer cooking time. However, in MW, the food material absorbs MW energy. It converts MW energy into the heat generated throughout the product at a rate that depends on the water content of food related to its dielectric properties (ε', ε'') (Sahin & Sumnu, 2006b). As a result, the highest temperature is observed at the centre of the food rather than the surface, and the core and base temperatures drop due to the rapid evaporation of the water (Araszkiewicz et al., 2007). Because the rate of microwave drying is much faster than traditional hot air drying, which is highest at the beginning of the process, rapid drying lowers the rate of Maillard reaction, as described above.

When we examine the effects of MW parameters, lower power and lower process time (250 W—8 min and 500 W—5 min) seem better for microwave-cooking vegetables, resulting in a significantly higher increase of TPC (α=0.05) compared to other techniques.

Finally, O20-180 and S12 were the best cooking techniques for TPC in cauliflower samples.

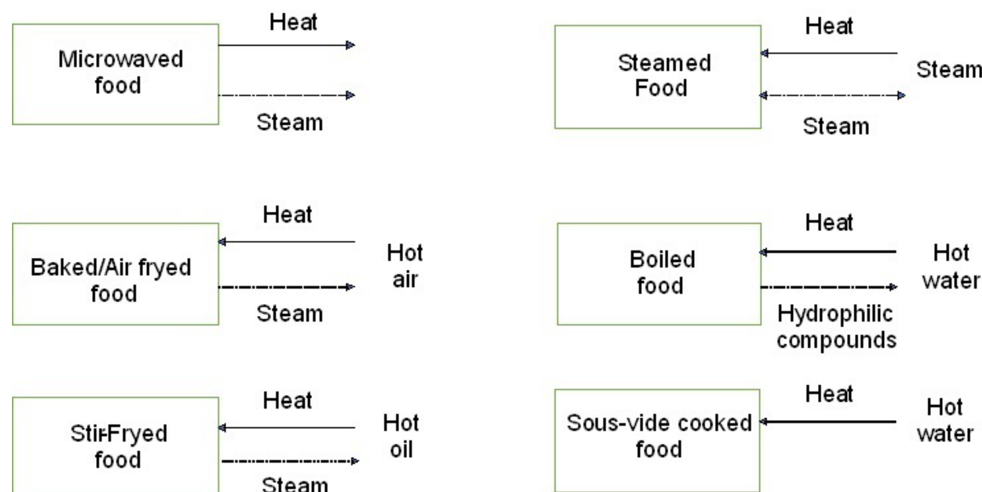

Figure 2 Schematic representation of the heat and mass transfer mechanism of different cooking methods (Araszkiewicz et al., 2007) where the heat transfer direction in microwaved cooking and conventional cooking is opposite

Sensory Analysis

The cooking method affected the samples' appearance, odour, taste, and texture, and these attributes are shown in Figure 3 and Table S1-S5. Both odour impact and cooked cauliflower odour were highest in B8. Similarly, Araya et al. (2009) compared the quality criteria and sensory perception of raw, high-pressure processed, sous-vide cooked, and boiled carrots. They reported that the highest cooked taste and odour impact was obtained in boiled carrots. However, no statistical difference ($p > 0.05$) in odour impact between B8 and F45 existed. Statistical difference in CCO between B8 and S12 was also not observed. There was a high correlation between odour impact and CCO ($r=0.89$), which can be interpreted as the CCO being much more effective than the green odour in the overall odour of the samples. Application of SV resulted in the highest green odour and the lowest CCO. The cellular microstructure of samples obtained by cryo-SEM has shown that SV causes less tissue damage than boiling (Araya et al., 2009). A sulfur odour was found in the O20-180 and F45 samples, with fried samples having the highest score. Compounds such as dimethyl disulfide and trisulfide, minor volatile breakdown products arising from S-methylcysteine sulfoxide, cause sulfur odour at a significant level in processes such as frying and baking, where the water content decreases significantly (Kubec et al., 1998; Marcinkowska & Helen, 2022).

A very high level of uniform colour was observed in B8, S12, and SV70, with B8 being the highest. However, occasional brown spots were observed in O20-180 and F45; thus, the score for uniform colour was lower. Therefore, O20-180 and F45 were also selected as the darkest colour due to Maillard reactions at high temperatures ($p > 0.05$). Navajas-Porras et al. (2022) applied various cooking processes to different foods, including cauliflower, and observed an increase in Maillard reaction products with frying and baking. As expected, according to the water loss, the B8 had the moistest appearance, while the O20-180 had the driest appearance ($p > 0.05$). The lowest moisture content was observed in F45, which O20-180 follows. The discrepancy between moisture values and sensorial perceived moistness may be due to the oiliness of F45, masking the dryness of the sample by coating the mouth and oil droplets on the sample.

The most intense and the highest cooked cauliflower taste was found in F45 and S12 ($p > 0.05$). The lower taste impact of B8, O20-180, and SV70 coincided with less intense cooked vegetable notes. The green taste of O20-180 and SV70 was also intense. Both bitterness and sweetness had the lowest score in B8 due to the transition of soluble sugars and glucosinolates to boiling water. S12 and O20-180 were the most bitter samples ($p > 0.05$). TPC was also highest for S12 and O20-180 and lowest for B8, proving that TPC highly affected the bitterness. Previous studies have also reported that the increase in bitterness is due to the glucosinate level in cauliflowers (Engel et al., 2002; Wieczorek et al., 2022). In a survey of Brussels sprouts, high concentrations of sinigrin and progoitrin were associated with consumer rejection and poor taste (Van Doorn et al., 1998; Wieczorek et al., 2022). In turnip, it was also observed that the higher the glucosinolate level, the higher the bitterness (Nor et al., 2020). There was no difference in sweetness scores of S12, F45, O20-180, and SV70 ($p > 0.05$). Although sulfur taste (2.60 for F45 and 2.00 for O20-180) was rated lower than sulfur odour (4.33 for F45, 3.47 for O20-180), both were higher in samples F45 and O20-180 than in the other samples.

O20-180 and SV70 were very firm, resistant to chewing, and cohesive, meaning that the samples break down into small particles in the mouth that do not combine with saliva and are difficult to swallow. Similar results were observed in B8, S12, and F45 regarding firmness, chewing resistance, and cohesiveness, making them easier to eat.

The general acceptability of the samples prepared was highly affected by cooking methods, as were other sensorial attributes. The highest acceptability value was observed in S12, whereas the lowest was in O20-180 and SV70. Bitterness and sulfur odour/taste did not affect the general acceptance. However, it should be noted that sulfur odour was generally not high in most of the samples. Green taste notes were higher in samples with high firmness and chewing resistance, as well as low cohesiveness and general acceptability. Panellists highly rated samples with high CCO/taste and taste impact with a moist appearance for general acceptability.

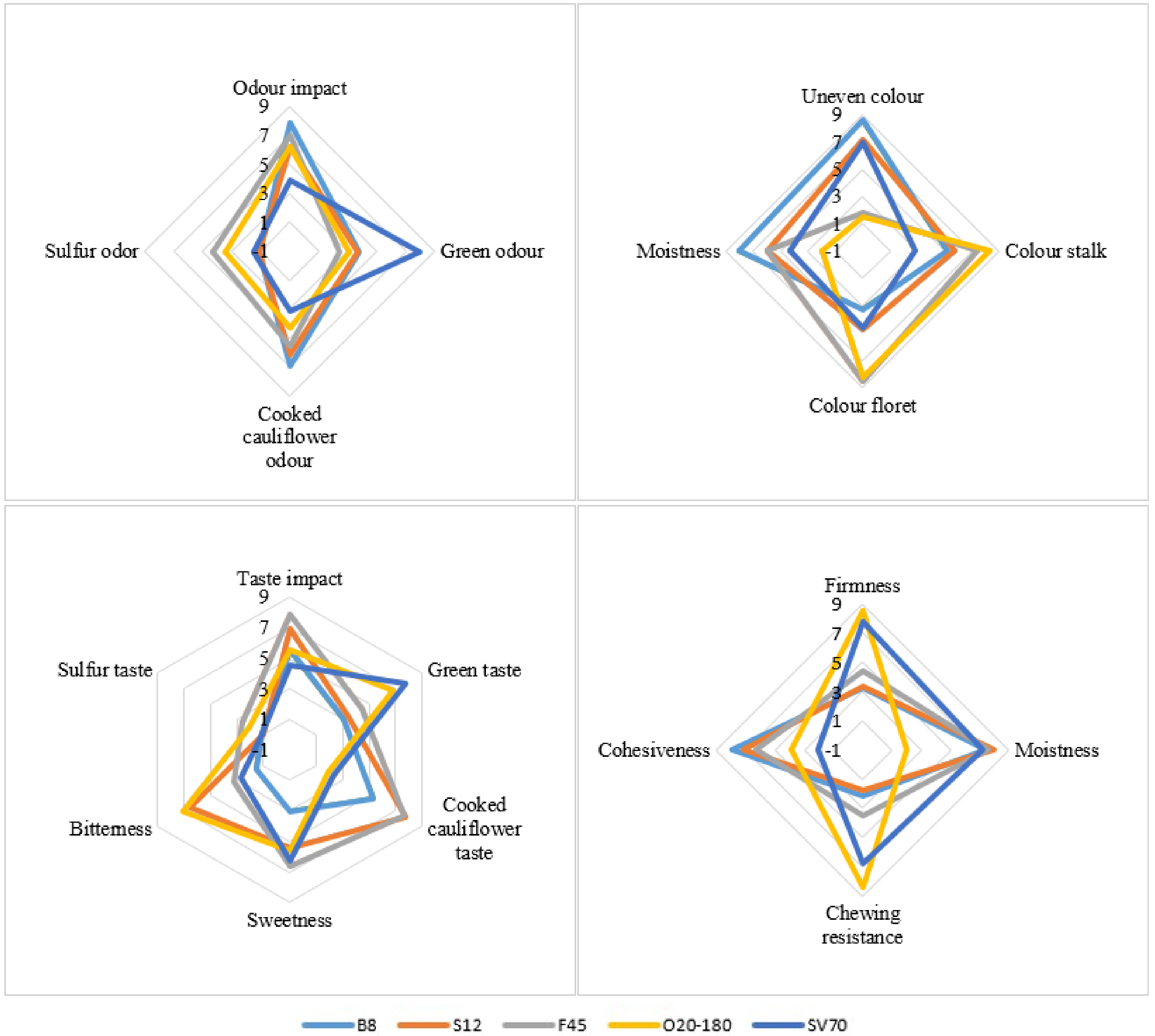

Figure 3. The effect of cooking methods on sensorial properties of cauliflowers (B8, Boiling for 8 min; F45, Stir-frying for 4.5 min, S12, Steaming for 12 min, O20-180, Oven cooking at 180°C for 20 min, SV70, Sous-vide cooking at 70°C)

Conclusions

The highest TPCs were observed in the O20-180 and S12 samples. On the other hand, steamed cauliflower was the most acceptable sample in terms of sensory properties such as higher moistness, chewability, intense and high CCO, and taste. As a heating medium, the change in water amount during cooking treatment was critical for effective heat transfer, sensory properties, and TPC content due to Maillard reactions and the release of bound phenolics by cell wall disruption. In further studies, the changes in certain dominant phenolic compounds and their derivatives of samples could be determined by more sensitive methods. HMF content and colour properties could be measured, and narrower steaming process parameter intervals could be applied to optimise the steaming process. Additionally, thermal probes and flow meters placed in the heating chamber can help track the movement of water, elucidating heat and mass transfer mechanisms. Integrated ovens could also be improved, or different coating formulations could be applied to prevent surface hardening.

Compliance with Ethical Standards

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

Ethics committee approval: The authors declare that this study does not include experiments with human or animal subjects, so ethics committee approval is not required.

Data availability: Data will be made available on request.

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Examination of front-of-packaged (FOP) labelling systems and nutrition-health statements on packaged foods in Türkiye

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ABSTRACT

There is a growing trend worldwide towards greater awareness of food labelling systems in the fight against global challenges such as unhealthy nutrition and obesity. Nearly 30 governments have approved various front-of-package (FOP) labelling systems, and many countries plan to adopt them. However, since there is no legal regulation regarding FOP labelling systems in Türkiye, nutrition and health-related statements appear on many packaged food products. Although these statements enable consumers to make healthy food choices, they also have the potential to mislead consumers by supporting the perception that foods lacking essential nutritional components or containing potentially harmful substances are healthy. This study examined FOP labelling systems used worldwide, and nutrition and health-related statements on the front of 1336 packaged foods in 6 categories in Türkiye were analysed. Although it varies in each category, the most common expressions are generally recommended daily amount (%14.5), fat (%16), sugar (%9.9), vitamin-mineral (%5.1), and protein (%9.4). Additionally, salt (%0,9), vegan (%2), additive (%11.6) and fibre content (%6.8) information is also commonly declared. As a result, the implementation of effective labeling regulations in Turkey can enhance public health and mitigate the adverse effects of unhealthy nutrition on society.

Keywords: Food security, Food labelling, Front of the package (FOP), Nutrition and health

Introduction

Labelling packaged foods is one of the most important tools that enable consumers to make healthier food choices in line with their expectations (Ndanuko et al., 2021). Nowadays, as consumers' concerns about food safety increase, interest in food labelling systems also increases, and the concept of 'nutrition and health-related statements' comes to the fore on labels. "Nutrition and health-related statements" are declarations that food has certain nutritional and/or health-promoting properties, including its energy value, protein, fat, carbohydrate, vitamin, mineral, etc., content. Labelling food products as “high calcium, enriched with vitamins, reduced salt, reduced sugar content, low cholesterol, etc.” is a nutrition statement. In addition, statements such as "it contains calcium, protects bone health or contains low calories, helps lose weight" can be given as examples of statements associated with health (de Boer & Bast, 2015; Singh et al., 2021). Nutrition and health-related statements have the potential to mislead consumers that nutritionally deficient foods are healthy. When it promotes the presence of a beneficial nutrient while not indicating the presence of less beneficial or potentially harmful nutrients in the same product, it has the potential to mislead the consumer. For example, a food with "reduced fat content" statements may contain higher levels of energy, sugar or sodium.

Ingredient information or related statements on packaged foods can be found on the front of the package (FOP) or the back of the package (BOP) (Bryła, 2020). Generally, information such as brand name, product name and design on the outer surface of the packaging is highlighted. In contrast, detailed information such as nutritional values, ingredient list and instructions for use are located on the back surface. BOP is regulated by each country's food labelling regulations and standards. This labelling method allows the front of the product packaging to have a cleaner and more minimalist appearance. On the other hand, FOP summarises the general nutritional profile of the product, which has recently been increasingly seen in packaged foods around the world (Sousa et al., 2023; Temple, 2020). Research shows that FOPs are easier to understand than nutrition fact sheets, provide faster and more accurate information processing, and help consumers better distinguish between healthier and less healthy products (Bayram & Ozturkcan, 2022; Bryła, 2020).

Although there are many types of categorisation of FOP labelling systems, they can generally be divided into "interpretive labels" and "non-interpretive labels". Examples of interpretive labelling systems are the Swedish keyhole, the Finnish Heart Symbol, the Nutri-score system, Warning labels, the Traffic Light Labels, and the Health Star Rating (Batista et al., 2023; Kanter et al., 2018). The Swedish keyhole labelling system has been used in northern European countries since 1989. Foods bearing this symbol have less fat, sugar and salt and more fibre and bran content. The Finnish Heart symbol has been used to indicate products with low fat, salt and sugar content since 2000. The Nutri-Score labelling system has been used in many European countries (France, Belgium, Germany, Spain, Netherlands, Luxemburg, and Switzerland) since 2017. According to literature studies, the Nutri-Score labelling system can be highly distinctive in packaged foods. Foods high in fruits and vegetables are correctly classified into the "healthiest" (A points) categories, while products rich in sugar and animal fats are classified into the "less healthy" (D-E) points" categories. Nutri-Score is a scoring system that takes into account negative qualities (energy, total sugar, saturated fatty acids, sodium content, etc.) as well as positive qualities (fruits, vegetables, nuts, fibre, protein and seed, walnut and olive oil content, etc.) (Muzzioli et al., 2022). The system has been criticised for focusing on the negative effects of nutrients and giving higher negative scores while giving lower scores for foods with positive effects. In this case, the system is thought to focus more on what should not be eaten and leaves what should be eaten as a secondary concern (Carruba et al., 2022). Multiple Traffic Lights have been used in the UK since 2004. In the Traffic Light labelling system, the nutrients in foods are visualised separately as high, medium and low (Bayram & Ozturkcan, 2022). The Warning label system has been used in Chile, Mexico, Israel, Uruguay and Peru since 2016. This system has warning labels on foods such as high calories, high sugar, and high salt (Temple, 2020). The Health Star Rating system it is stated that the summarises the nutritional quality of a product based on its fat, sugar, salt, fibre and energy content, with a rating from 0.5 to 5.0 stars (An et al., 2021). NutrInform Battery labelling system and Guideline Daily Amounts (GDA) can be examples of non-interpretive labelling systems. Each package indicates the energy, fat, saturated fat, sugar and salt content in grams. The "Battery" symbol on the label indicates the percentage of the serving relative to the recommended daily intake. The aim is to "fill up" the battery according to the daily intake recommended in the EU. This system tracks the total amount consumed without exceeding the recommended intake. The GDA system shows the amount of energy, fat, saturated fat, sugar and salt/sodium in a product serving. The reference for these data was to determine the portion size and recommended daily intake values, considering an average

healthy individual (Temple, 2020). Figure 1 shows the front-of-package (FOP) nutrition labelling logos used worldwide.

FOP regulations, in force in nearly 30 countries, play an important role in consumers' healthy food choices. Türkiye has limited regulations regarding certain label statements on some foods. In Türkiye, FOP systems are generally found in international brands' market food products. This study aims to examine the nutritional and health-related statements on the front of packaged foods offered for sale in Türkiye as FOP labelling systems become widespread worldwide.

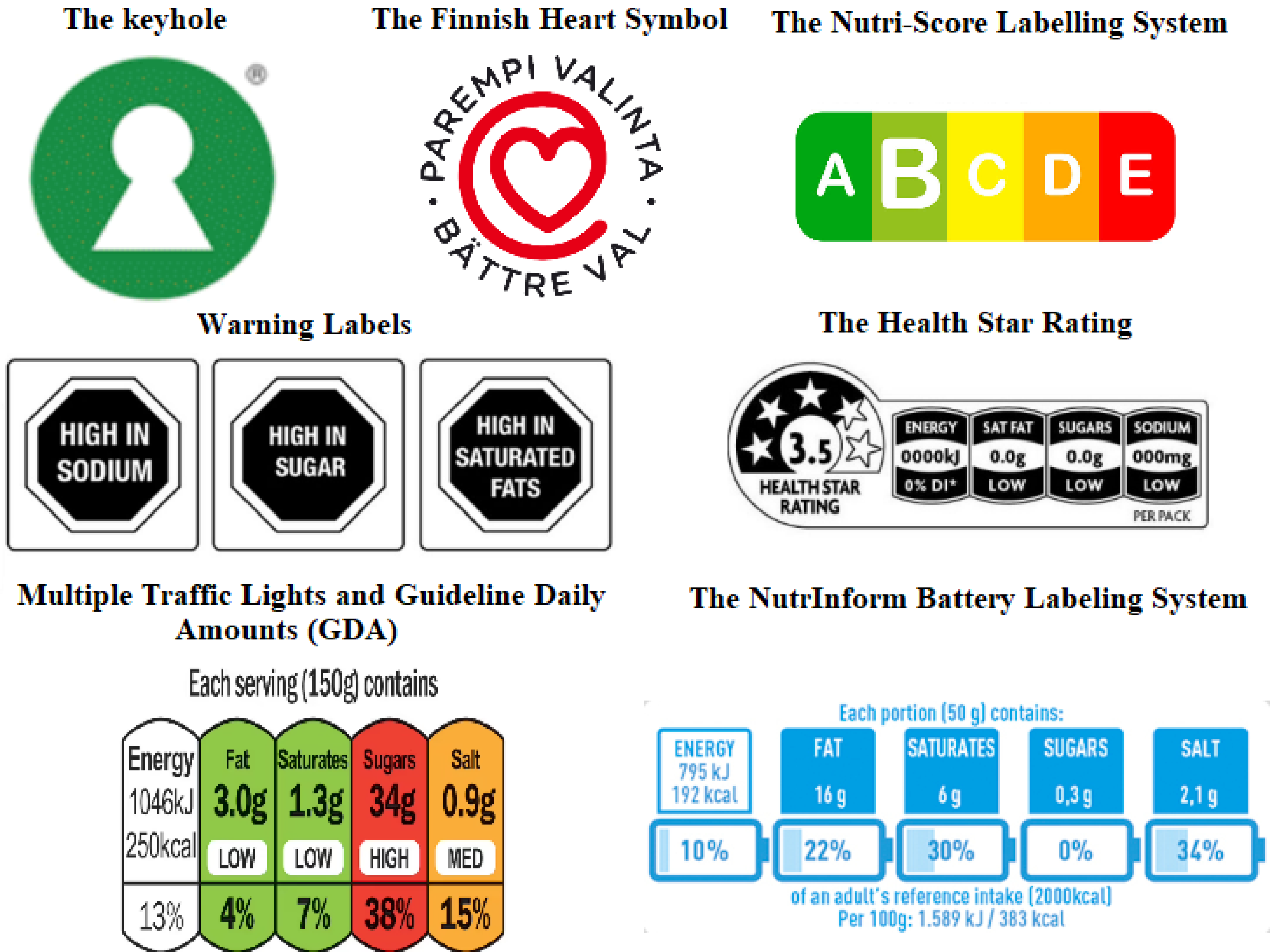

Figure 1. Front-of-package (FOP) nutrition labeling logos used worldwide

Materials and Methods

Data Collection

The research on nutrition and health-related expressions of packaged foods consumed in Türkiye was carried out through ten national supermarket chains determined by the preliminary study. In selecting ten national supermarket chains, the markets with the highest market share, the highest product variety, and the products they sell that reflect consumer preferences across the country were preferred. Official permission and ethics committee approval was obtained from all supermarket chains and administrative authorities in this study.

FOP label notifications of food products in supermarkets were examined under 6 food categories of 27 product groups. These categories include dairy products, beverages, spreadable breakfast products, ready-made foods, snacks, and meat products (Ricardo et al., 2019). Products such as honey, legumes, pasta, etc., without potential FOP label data were not photographed (Table 1).

Table 1. Six packaged food categories reviewed for FOP label notifications

Packaged food categories	Keywords
Dairy products	Milk and milk products (Milks-Flavored-Fruity milk products), yogurt and yogurt products (Yogurt-Buttermilk (Ayran)-Fruity yogurt-Kefir-Fruity kefir- Probiotic yoghurt) and cheeses
Beverages	Fruit juices, Mineral waters, Carbonated drinks-Cold teas, Powdered drinks
Spreadable-Breakfast Products	Hazelnut-Peanut-Pistachio butters, Jams-Marmalades, Halvahs Mulberry-Carob-Grape molasses, Vegetable margarine
Ready-made foods	Canned foods, Pickles Ready foods, Ready soups and bullion, Sauces
Snacks	Chocolates-Wafers, Biscuits, Chips, Ice creams, Breakfast cereals
Processed meat products	Sausages-Pastrami, Salami-Sausage, Döner-Roasting meat

At least 3 photographs were taken of the front of each package and the health statements, if any. In order to ensure the smooth progress of the data collection process, the photographs taken were quickly saved in the data pool. In order to avoid duplication, product information was entered in detail, and codes were entered for each product, taking into account the category to which it belongs. Photographing large-sized products for similar products where the packaging size varies is generally preferred. 2 field workers collected, classified and recorded data for this study. The first of the field workers has expertise in dietetics, and the second has expertise in food engineering. All field employees are trained in food composition and food labelling. The data collection procedure was conducted without disrupting the functioning of the supermarkets, and the photographs were captured during weekdays, specifically on Tuesdays, Wednesdays, and Thursdays, when there was comparatively lower customer traffic. Photographs of the products were taken during daylight hours and 30 minutes so as not to disturb the customers. All photographig operations were completed within 2 months. Field workers kept their identification cards (ID) cards during data collection. Field workers tried not to communicate with customers and emphasised, when necessary, that this was an 'ordinary labelling practice'. As a result of all these studies, 1336 samples were examined individually, and approximately 4551 photographs were taken. All methods used in collecting and classifying labelling data are implemented by adapting the working model developed by Kanter et al. (Kanter et al., 2017).

After the preliminary label information of the 1336 packaged foods examined was recorded in the data pool, the most common nutrition statements were created separately for each category. The most common nutrition statements are fat-free-reduced fat, salt-free-reduced salt, fibre source, protein source, vitamin mineral source, preservative-free, sugar-free-reduced sugar, trans-fat-free, etc. table was determined as common headings. The phrase "traditional" and slogans containing no nutrition statements were not included in the data analysis. The nutrition statements on the front label of each packaged food were examined individually, and the proportions in which they were used were determined according to their categories.

Results and Discussion

In the "Dairy Products" category, where a total of 178 samples were examined, 45% included cheese, and the remaining part included milk (23%) and yoghurt (31.5%) products (Table 2). It was observed that 60.7% of these products had a statement regarding their fat content on the FOP (e.g., light, % fat content, *trans-fat-free*, full fat and low fat). This percentage is 85.4% for milk and milk products, 66.1% for yoghurt and yoghurt products, and 44.4% for cheeses. While fat content statements are present in all milk, they cannot be said to be present in all flavoured and fruity milk products. The

presence of information on *trans* fatty acid content, which occurs naturally in dairy products and is negligible, draws attention as a statement to attract consumer attention. Expressions like full-fat and low-fat are frequently used for milk and yoghurt products. Noteworthy is that approximately one-quarter of dairy product packaging bears the statement 'school food,' which is not used in any other food category. 26.8% of milk and dairy products contain the phrase 'school food. The rate of statements regarding sugar content (e.g., no added sugar, unsweetened, sugar-free, % sugar content, lactose-free), especially seen in flavoured fruit milk and yoghurt products, is 18.5%. While this rate is 53.7% for milk and milk products, it is 19.6% for yogurt and yogurt products. Statements related to vitamin-mineral content (e.g., source of vitamin D, contains Ca, source of Ca, source of Zn, source of vitamin, source of mineral) are present in 14% of all dairy products. These statements are found in 26.8% of milk and milk products and 11.1% of yogurt products. Particularly, 60.7% of yoghurt and yoghurt products contain statements (source of protein, % protein content) regarding protein content. While the rate of statements indicating protein source in cheeses is 3.7%, it is 20.8% for all dairy products. Particularly in kefir and probiotic yoghurts, health statements (e.g., support the immune system, for strong immunity, support the digestive system, contains probiotic, probiotic source) are observed, which are not commonly found in other categories. The proportion of health statements to all dairy products is 9%. The salt content (reduced salt, less salty), which is not declared because it is not found in other dairy products, is declared as 6.2% in cheeses.

In the "Beverages" category, where 192 samples were examined, 38.5% included fruit juices, 12% included mineral waters, 27.1% consisted of carbonated drinks-cold teas, and 22.4% included powdered beverages (Table 3). 25.5% of the products in the beverage category have a %RDA (recommended daily amounts) statement. Approximately half of powdered beverages, 30.8% of carbonated drinks-cold teas, 21.7% of mineral water products and 6.8% of fruit juices have a %RDA statement. In general, these statements on the front label of the packages relate to energy, added sugar, Na, Ca, Zn minerals, C, B3, B6, and B12 vitamins. While powdered drinks generally make statements regarding vitamins and minerals, carbonated drinks-cold tea products make statements regarding their sugar and energy content. Statements regarding the %fruit content of all fruit juices appear in the FOP. In the beverage category, statements regarding vitamin and mineral contents are found in 34.8% of mineral waters, 9.5% of fruit juices and 19.2% of carbonated drinks-cold teas products. Statements such as no added sugar, unsweetened, sugar-free appear on the front of carbonated drinks-cold teas at a rate of 9.6% and on powdered drinks at 23.3%. When the statements regarding sugar beet content are examined, it is found in 60.9% of mineral waters and 17.3% of carbonated drinks-cold tea products.

In the "Spreadable-Breakfast Products" category, where a total of 163 samples were examined, 31.3% were hazelnut-peanut-pistachio butter, 38.7% were jams-marmalades, 9.2% were halvah, 11% were mulberry-carob- grape molasses and 9.8% vegetable margarine (Table 4). 33.1% of the products in this category have content statements such as hazelnut-peanut-pistachio-fruit ratio and fat ratio for margarine. Content information statements are available at different rates for products other than mulberry-carob-grape molasses products. Statements about sugar content on the front label of products in this category are present in 22.7% of all products. Sugar content statements such as No added sugar, Unsweetened, Sugar-free, % sugar content are found at a rate of 27.4% in hazelnut-peanut-pistachio butter products, 26.9% in jams-marmalades products, and 40% in halvahs products. While there are no statements about sugar content in mulberry-carob-grape molasses products, margarines are not expected to contain sugar. When the statements regarding sugar beet content are examined, it is used in 5.5% of all products. Since using glucose and fructose syrup is common in these products, statements such as "produced with sugar beet" are considered a healthier option. These statements are found in 26.6% of halvah products and 6.3% of jams-marmalades products. According to the front-label information in this category, 6.1% of all products have fibre content statements, such as the source of fibre and high fibre content. Halvah products especially have a 40% fibre content statement. When the statements regarding additive inclusion are examined, 15.9% of all products in this category contain statements indicating no preservatives. Mulberry-carob-grape molasses products declare an additive content of 55.5%. All margarines contain a statement regarding their *trans* fat content. The use of these statements in margarine is common because of the health hazards of partially hydrogenated vegetable oils, which are the main source of exposure to *trans* fatty acids. 18.7% of these margarines contain statements such as sources of vitamins and source of minerals.

In the "Ready-Made Foods" category, where a total of 316 samples were examined, 19.3% consisted of canned foods, 13.6% consisted of pickles, 25.3% consisted of ready soups and bullions, and 21.8% consisted of sauces (Table 5). 49.2% of the ready soups and bullion products and 13% of the sauces in this category have the recommended daily amounts statement. This statement usually relates to the energy content of the product. When examining the statements regarding the

protein content in these products, the protein content is found to be 37.7% in canned food products, 30% in ready food products, and 19% in ready soups and bullion products. Overall, 18.6% of all products contain a statement regarding protein content. 37.6% of the products in this category contain statements regarding additive content (no preservative, contains collagen, etc.). There are statements regarding the additive content in 39.3% of canned food products, 37.2% of pickles, 62.5% of ready food products, 26.9% of ready soups and bullion products and 17.3% of sauces. The vegan statement rate for products in this category is 8.5%. Vegan statements are found in 19.6% of canned food products, 16.2% of pickles, and 10% of ready-made products. 10.7% of ready-made foods contain fibre content statements such as the source of fibre and high fibre content. These statements included 21.1% of ready-food products and 19% of soups and bullion products. 2.2% of the products in this category contain statements about salt content, including reduced salt and less salty. Statements about salt content are seen in 6.9% of pickles and 6.3% of ready soups and bullion products. 6.3% of the products in this category contain statements regarding fat content such as light, % fat content, *trans-fat-free*, and high omega-3 sources. In particular, these statements are included in 24.5% of canned food products.

In the "Snacks" category, where 369 samples were examined, 27.6% consisted of chocolates-wafers, 26.5% biscuits, 14% chips, 14.9% ice cream and 16.8% breakfast cereals (Table 6). 28.4% of these products have a recommended daily amounts statement on their front labels. 20.5% of chocolates-wafers, 46.1% of chips, 56.3% of ice creams and 46.7% of breakfast cereals products in this category contain a recommended daily amounts statement. This statement usually relates to the energy content of the product. Among the products in this category, 2% of biscuits and 19.3% of breakfast cereals have statements regarding their protein content. Additionally, 17.7% of breakfast cereal products contain statements about additives such as no preservatives. 4.3% of the products in the snacks category contain statements regarding protein-mineral content. Statements such as the source of vitamins and minerals are found in 1.9% of chocolates-wafer products, 3.6% of ice creams and 19.3% of breakfast cereals products. 4.3% of the products in this category contain statements about sugar content, such as no added sugar or sugar-free. These statements are found mostly in 14.5% of breakfast cereals products. On the front labels of snack products, 13% contain statements about fibre sources and high fibre content. In particular, these statements were used in 51.6% of breakfast cereals products and 16.3% of biscuits. On the front labels of the products in this category, the statements of light, %fat content, and *trans-fat-free* are at 8.9%. However, this rate is found only in 63.4% of chip products.

In the "Meat Products" category, 59 samples were examined; 79.6% consisted of sujuk-pastrami-salami sausage, and 20.3% consisted of doner-roasting meat products (Table 7). The front labels of these products contain 6.7% fat content and 13.5% protein statements (such as source of protein and high protein). Sujuk-pastrami-salami-sausage products feature 8.5% fat content and 17% protein statements. Additionally, these meat products contain an 11.8% gluten statement. These statements make up 14.8% of sujuk-pastrami-salami-sausage products.

When all products in all categories were examined, 1336 products were examined. 14.5% of the products contained a statement of the recommended daily amounts, 16% contained a statement of the fat content, 9.4% contained a statement of the protein content, and 9.9% contained a statement of the sugar content. In addition, statements regarding vitamin and mineral content were found in 5.1% of the products, and statements regarding fibre content were found in 6.8%. At the same time, 11.6% of the products included statements about additives, and 8.9% contained content information. In addition, it was stated that 2% of the products contain vegan content, 0.9% contain salt, and 12.2% do not contain preservatives. Considering all these values, a labelling system such as the Swedish key symbol can be used for products considered to be healthy food. Standard warning statements for foods with relatively unhealthy ingredients or ingredients that may cause problems if consumed in excess are "excessive consumption may cause blood pressure imbalance, cause a rapid increase in blood sugar, etc." statements can be derived. The NutriScore application, which is used in many European countries, can be evaluated in the labelling of packaged foods in Türkiye.

Table 2. Nutrition and health statements for the dairy products category

Dairy Products	Number of Products	Fat Content Statement	School Food Statement	Sugar Statement	Vitamin-Mineral Statement	Protein Statement	Health Related Statements	Salt Statements
Milk and milk products (Milks-Flavored-Fruity milk products)	41 (23%)	35 (85.4%)	11 (26.8%)	22 (53.7%)	11 (26.8%)	0	0	0
Yogurt and yoghurt products (Yogurt-Buttermilk (Ayran)-Fruity yogurt-Kefir-Fruity kefir- Probiotic yoghurt)	56 (31.5%)	37 (66.1%)	2 (3.6%)	11 (19.6%)	5 (8.9%)	34 (60.7%)	16 (28.6%)	0
Cheeses	81 (45.5%)	36 (44.4%)	0	0	9 (11.1%)	3 (3.7%)	0	5 (6.2%)
TOTAL	178	108 (60.7%)	13 (7.3%)	33 (18.5%)	25 (%14)	37 (20.8%)	16 (9%)	5 (2.8%)
		• Light • % fat content • *Trans* fat free • Full-fat, low-fat	• School food	• No added sugar • Unsweetened • Sugar-free • % sugar content • Lactose free	• Source of vitamin D • Contains Ca • Source of Ca • Source of Zn • Source of vitamin • Source of mineral	• Source of protein • % protein content	• Support immune system • For strong immunity • Support digestive system • Contains Probiotic • Probiotic source	• Reduced salt • Less salty

Ca: Calcium, Zn: Zinc

Table 3. Nutrition and health statements for the beverage products category

Beverages	Number of Products	Front Label RDA	Content Statement	Vitamin-Mineral Statement	Sugar Statement	Sugar Beet Statement
Fruit juices	74 (38.5%)	5 (6.8%)	52(70.3%)	7 (9.5%)	0	0
Mineral waters	23 (12%)	5 (21.7%)	0	8 (34.8%)	0	14 (60.9%)
Carbonated drinks-Cold teas	52 (27.1%)	16 (30.8%)	0	10 (19.2%)	5 (9.6%)	9 (17.3%)
Powdered drinks	43 (22.4%)	23 (53.5%)	0	0	10 (23.3%)	0
TOTAL	192	49 (25.5%)	52 (27.1%)	25 (13%)	15 (7.8%)	23 (12%)
		• % RDA per serve energy, added sugar, Na, Ca, Zn minerals, vitamin C, B3, B6, B12	• % fruit content	• Source of vitamin (C, B3, B6, B12) • Source of mineral (Na, Ca, Zn)	• No added sugar • Unsweetened • Sugar-free	• Produced from sugar beet • Sugar beet used

RDA: Recommended daily amounts (based on 2000 kcal diet), Na: Sodium, Ca: Calcium, Zn: Zinc

Table 4. Nutrition and health statements for the spreadable-breakfast products products category

Spreadable-Breakfast Products	Number of Products	Content Statements	Sugar Statement	Sugar Beet Statement	Fiber Content Statement	Additive Statement	*Trans* Fat Statement	Vitamin-Miner: Statement
Hazelnut-Peanut-Pistachio butter	51 (31.3%)	14 (27.4%)	14 (27.4%)	1 (1.9%)	3 (5.8%)	6 (11.7%)	3 (5.8%)	0
Jams-Marmalades	63 (38.7%)	17 (26.9%)	17 (26.9%)	4 (6.3%)	0	10 (15.8%)	0	0
Halvahs	15 (9.2%)	7 (46.6%)	6 (40%)	4 (26.6%)	6 (40%)	0	0	0
Mulberry-Carob-Grape molasses	18 (11%)	0	0	0	1 (5.5%)	10 (55.5%)	0	0
Vegetable margarine	16 (9.8%)	16 (100%)	0	0	0	0	16 (100%)	3 (18.7%)
TOTAL	163	54 (33.1%)	37 (22.7%)	9 (5.5%)	10 (6.1%)	26 (15.9%)	19 (11.6%)	3 (1.8%)
		• % Hazelnut-Peanut-Pistachio-Fruit ratio • % fat ratio (for margarines)	• No added sugar • Unsweetened • Sugar-free • % sugar content	• Produced from sugar beet • Sugar beet used	• Source of fiber • High fiber content	• No preservative	• *Trans* fat free	• Source of vitamin • Source of mineral

Table 5. Nutrition and health statements for the ready-made foods products category

Ready-Made Foods	Number of Products	Front Label RDA	Protein Statement	Additive Statement	Vegan Statement	Fiber Content Statement	Salt Statements	Fat Content Stat
Canned foods	61 (19.3%)	3 (4.9%)	23 (37.7%)	24 (39.3%)	12 (19.6%)	5 (8.1%)	0	15 (24.5%)
Pickles	43 (13.6%)	5 (11.6%)	0	16 (37.2%)	7 (16.2%)	0	3 (6.9%)	1 (2.3%)
Ready foods	80 (25.3%)	0	24 (30%)	50 (62.5%)	8 (10%)	17 (21.2%)	0	3 (3.7%)
Ready soups and bullion	63 (19.9%)	31 (49.2%)	12 (19%)	17 (26.9%)	0	12 (19%)	4 (6.3%)	2 (3.1%)
Sauces	69 (21.8%)	9 (13%)	0	12 (17.3%)	0	0	0	3 (4.3%)
TOTAL	316	40 (12%)	59 (18.6%)	119 (37.6%)	27 (8.5%)	34 (10.7%)	7 (2.2%)	20 (6.3%)
		• % RDA per serve energy	• Source of protein • % protein content	• No preservative • Contains collagen	• Vegan	• Source of fiber • High fiber content	• Reduced salt • Less salty	• Light • % fat content • *Trans* fat free • High omega-3 so

RDA: Recommended daily amounts (based on 2000 kcal diet)

Table 7. Nutrition and health statements for the meat products category

Meat Products	Number of Products	Fat Content Statement	Protein Statement	Content Statement
Sujuk-Pastrami-Salami-Sausage	47 (79.6%)	4 (8.5%)	8 (17%)	7 (14.8%)
Doner-Roasting meat	12 (20.3%)	0	0	0
TOTAL	59	4 (6.7%)	8 (13.5%)	7 (11.8%)
		• % fat content	• Source of protein • High protein	• No gluten • Gluten free

Conclusion

Examining pre-package (FOP) labelling systems for packaged foods in Türkiye and nutrition and health statements for packaged foods sheds light on the broader global trend of increasing transparency and awareness in food labelling. As consumers become increasingly concerned about food safety and nutrition, there is a growing need for clear and informative labelling systems that enable individuals to make informed and healthy food choices. Various FOP labelling systems worldwide have won approval from nearly 30 governments, signalling a concerted effort to address pressing public health problems such as unhealthy diets and obesity. These systems aim to provide consumers with easily understandable information about packaged foods' nutritional content and health effects, ultimately enabling them to make choices that align with their dietary preferences and health goals. However, despite the widespread adoption of FOP labelling systems internationally, there are no specific legal regulations regulating such labelling practices in Türkiye. This regulatory gap highlights the importance of establishing comprehensive guidelines and standards for FOP labelling in Türkiye to ensure consistency, accuracy and consumer confidence in information on packaged foods. Additionally, the presence of nutritional and health-related statements on packaged foods without a standardised FOP labelling system poses the risk of misleading consumers. Without clear guidance, these statements can inadvertently promote the perception that foods that lack essential nutritional components or contain potentially harmful substances are healthy choices. Therefore, Türkiye must enact robust regulations that address FOP labelling and ensure the accuracy and integrity of nutrition and health statements on packaged foods.

Compliance with Ethical Standards

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

Ethics committee approval: The authors declare that this study does not include experiments with human or animal subjects, so ethics committee approval is not required.

Data availability: Data will be made available on request.

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Research Article

Determination of the cyto-genotoxic effects of sodium acetate and sodium sulfite by MTT and comet assays

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ABSTRACT

Sodium acetate (NaA) and sodium sulfite (NaS) are two food additives in the class of preservatives. In this study, 3-(4,5-dimethyl thiazolyl-2)-2,5 diphenyltetrazolium bromide (MTT) assay was established to detect the cytotoxicity, and comet assay was used to determine the genotoxicity of NaA and NaS. For the MTT assay, human hepatocellular carcinoma ($HepG_2$) cells were treated with different concentrations of each preservative (15.63, 31.25, 62.50, 125, 250, 500, 1000 and 2000 µg/mL for NaA; 3.91, 7.81, 15.62, 31.25, 62.50, 125, 250 and 500 µg/mL for NaS, respectively) for 24-h. non-treated wells used as control (only medium) were included. Comet assay was performed on lymphocytes isolated from healthy donors with multiple concentrations of NaA (15.63, 31.25, 62.50, 125, 250 µg/mL) and NaS (3.91, 7.81, 15.62, 31.25, 62.50 µg/mL) for 1 h. A negative (distilled water) and a positive control (100 µM H_2O_2) were also included. Significant cytotoxic activity was detected for NaA and NaS only at the highest concentration. Besides, both substances significantly increased DNA damage compared to the control at almost all concentrations (except at low concentrations). In general, both food preservatives exhibited weak cytotoxic effects in $HepG_2$ cells. These food preservatives showed genotoxic activity, especially at higher concentrations.

Keywords: Comet assay, Food preservative, MTT assay, Sodium acetate, Sodium sulfite

Introduction

Food additives are substances added to foodstuffs to prevent and/or delay product spoilage and to improve or modify its properties, such as flavour and appearance. These substances can be natural or synthetic, ordinarily have no significant nutritional value, and are added to food in specific quantities during production. According to their functions, 25 categories of food additives have been defined. Food preservatives, also called antimicrobial agents, are utilised to prolong the shelf life of food by safeguarding it against spoilage caused by microorganisms, and their use is EU-regulated (Silva & Lidon, 2016; Zang et al.,2023). Microbial spoilage is the gradual decrease in food safety due to the movement of different types of bacteria. Components such as carbohydrates, proteins, vitamins, minerals, and water in foods create a suitable area for bacteria to grow. The oxidation-reduction potential, water activity, and pH value of the food are also significant. The essential foods affected by microbial spoilage are vegetables, fruits, fruit juice, milk, dairy products, cereals and cereal products, and meats (Altuğ, 2009; Yen et al., 2024).

Sodium acetate (NaA, E 262) and sodium sulfite (NaS, E221) are two different food preservatives; NaA is the sodium salt of acetic acid, and NaS is the sodium salt of sulfuric acid (Sallam, 2007). NaA is used effectively in meat, cheese, bakery, snacks, cosmetics, and veterinary chemicals and herbicides. It prevents rotting by reducing the growth of bacteria in products (Mohammadzadeh-Aghdash et al., 2018). NaS preserves food by inhibiting yeast, fungal, and bacterial growth. It is a preservative in products containing egg yolks and foods like beer, salads, caramel, and bread. In addition to its preservative function, it can also be an antioxidant (Silva & Lidon, 2016).

Today, because of the rapid increase in the consumption of chemicals in all fields, it has become essential to determine whether they have a negative effect in terms of toxicology on human health. Cytotoxicity assays are a quick way to assess the impact of a particular chemical compound on a specific human cell line. The most widely used method today is the MTT assay. This method originated as a cell proliferation assay and was used in the same years to investigate the effect of chemotherapeutic drugs on cancer cells (Mamur, 2022a; Ghorbanpour et al., 2024). The MTT assay is based on the conversion of yellow 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide to a water-insoluble purple colour in living cells due to mitochondrial dehydrogenase enzyme. The intensity of the colour is directly related to mitochondrial activity. If there is activity in living cells, formazan crystals are formed. In dead cells, formazan crystals cannot be formed. In summary, the yellow colour of MTT dye turns purple with the addition of solvent due to living activity. This colour change is then read in the microplate reader, and data is obtained using optical density (Mossman,1983). The MTT assay is reliable for measuring cell viability, activation, and growth and is widely used in cytotoxicity studies, which determines the percentage of living cells (Ghasemi et al., 2021).

Genotoxicity tests are performed to determine whether chemical and physical agents cause chromosome abnormalities, mutations, and DNA damage and to understand the mechanisms of action of these agents. Various *in vivo* and *in vitro* test systems are used for this purpose (Unal et al., 2021; Mahoro et al., 2024). The comet assay method is preferred in research studies to determine DNA damage and repair disorders induced by different chemical and physical agents in multiple mammalian cells. It is a preferred biomonitoring method for detecting increased DNA damage in some diseases (such as cancer). On the other hand, it has a widespread framework of use due to its advantages, such as its ability to evaluate genotoxic substances first in their sites of action, its application in almost all eukaryotic cells, its ability to measure low levels of damage, and the fact that it is a fast, practical, simple and convenient method because it requires very few cell samples. This assay quantitatively detects DNA damage (Collins et al., 2014; Costea et al., 2024). In the comet assay, three parameters were used to measure DNA damage levels: tail intensity, length, and tail moment. Enhanced comet tail intensity and tail moment are directly related to increased DNA damage. The most commonly used comet parameters are tail intensity (%DNA in tail) and tail moment, which integrates %DNA in tail and tail length (Azqueta et al., 2019). Comet tail intensity is considered the most helpful comet parameter since it is not affected by experimental conditions and can measure the broadest range of DNA damage (Collins et al., 2014).

Many food additives are categorised as Generally Recognized as Safe (GRAS) in terms of potential risk, including NaS and NaA (EFSA, 2022a, b). However, some studies in the literature showed that these additives may possess a cytogenotoxic risk. For example, Sun et al. (2005) found that NaA at 50-100 mM (24-72 h) caused reduced viability in human gastric adenocarcinoma epithelial cells. In another study, NaA (12.5, 25-, and 50-mM for 24 h) increased the release of LDH, an indicator of cytotoxicity and cell death, in the human gastric adenocarcinoma epithelial cell line (Xia et al., 2016). Similarly, NaA was cytotoxic and genotoxic *in vitro* for lym-

phocytes isolated from adult male Sprague-Dawley; it increased DNA damage percentage, increased LDH release, decreased cell viability and proliferation of lymphocytes at all concentrations (50, 100, and 200 mM/L) (Abd-Elhakim et al., 2018). Mohammadzadeh-Aghdash et al. (2018) indicated that NaA reduced HUVEC cell line growth in a concentration (25, 50, 100, and 200 µM) and time-dependent manner (24 and 48 h). In addition, sulfur dioxide derivatives (125, 250, or 500 mg/kg body wt, intraperitonally for 7 days), including NaS and sodium bisulfite (3:1 M/M), exhibited significant increases in DNA damage in all tested organs (brain, lung, heart, liver, stomach, spleen, thymus, bone marrow and kidney) in male mice (Meng et al., 2004). El-Hefny et al. (2020) demonstrated that NaS (100 mM) has significantly high tumour induction and frequency levels in the SMART assay and a large amount of DNA damage in the comet assay. Finally, in a study conducted in 2023, it was found that NaA (15.63-250µg/mL) and NaS (3.91-62.50 µg/mL) increased the frequency of chromosomal aberrations (CAs) and micronuclei (MN) at higher concentrations (Altunkaynak, 2023).

This study aimed to measure the cytotoxic effects and primary DNA damage capacity of sodium acetate (NaA) and sodium sulfite (NaS). For this purpose, MTT assay in hepatocellular carcinoma ($HepG_2$) cell line, since they are of human origin and retain xenobiotic metabolising enzymes which play a crucial role in the activation/detoxification of genotoxic chemicals (Knasmüller et al., 1998) and comet assay in isolated human lymphocytes, was performed for the first time using various concentrations of NaA and NaS.

Materials and Methods

Concentrations

In this study, MTT assay was used to determine the cytotoxic effect, and comet assay was used to evaluate the genotoxicity of NaA and NaS. The best suitable concentrations were determined using LD_{50} value [NaA: mouse orally 4960 mg/kg (PubChem, 2023) and NaS: rat orally 3560 mg/kg (Sigma-Aldrich, 2023)]. Based on the LD value (mouse orally 4960 mg/kg /4 = 1240 mg/kg ~ 2000 µg/mL) and MTT result for NaA, the highest concentration was chosen as 2000 µg/mL. LD value for NaS (rat orally 3560 mg/kg / 4 = 445 mg/kg ~ 500 µg/mL) was also considered. For the MTT assay, $HepG_2$ cells were treated with eight different concentrations of each preservative (15.63, 31.25, 62.50, 125, 250, 500, 1000 and 2000 µg/mL for NaA; 3.91, 7.81, 15.62, 31.25, 62.50, 125, 250 and 500 µg/mL for NaS) for 24 hours. Additionally, $HepG_2$ cells without any chemical treatment were used as the control group. MTT test results and the previous values (Altunkaynak, 2023) were also used to determine comet assay concentrations. In the comet assay, lymphocytes isolated from blood samples obtained from two healthy donors were treated with NaA concentrations of 15.63, 31.25, 62.50, 125, 250 µg/mL and NaS concentrations of 3.91, 7.81, 15.62, 31.25, 62.50 µg/mL for 1 hour. Distilled water was used as a negative control, and 100 µM H_2O_2 was maintained as a positive control.

$HepG_2$ Cell Line Cultures

The human hepatocellular carcinoma ($HepG_2$) cell line (HB-8065) used in experiments was commercially obtained from the American Type Culture Collection (ATTC, Manassas, USA). Human $HepG_2$ cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum, 1% penicillin/streptomycin, and 2 mM L-glutamine in $75cm_2$ culture flasks under conditions (humidified, 5% CO_2/95% air, 37°C) to obtain the desired count. Cells that reached sufficient numbers two days after being placed in the culture flask were collected by trypsinisation. Subsequently, the cells were transferred to 96 multiwell plates.

MTT Assay

The cytotoxic capability of NaA (CAS. No: 127-09-3, Sigma-Aldrich) and NaS (CAS. No: 7757-83-7, Sigma-Aldrich) were evaluated using MTT assay in $HepG_2$ cells. The MTT assay determining the mitochondrial activity was applied according to Mossman (1983) with some modifications followed by Mamur et al. (2022b) methods. $HepG_2$ cells were grown in 96-well tissue culture plates at a density of 5×10^3 cells per well. Afterwards, the cells were incubated for 24 hours in a CO_2 incubator for the holding wells. Varying concentrations of NaS (3.91-500 µg/mL) and NaA (15.63-2000 µg/mL) were added to each well and incubated for 24 hours. Non-treated cells were used as the negative control (only medium). To complete the incubation, 5 mg/mL MTT solution was added to each well and incubated for 4 hours. A marker of cell viability is the conversion of the tetrazolium salt MTT to a coloured formazan by mitochondrial dehydrogenases. Dimethyl sulfoxide was added to each well to solubilise formazan crystals. Then, the absorbance (Abs) was measured at 570 nm wavelength using a spectrophotometer Molecular Devices M_5 microplate reader. The assay was performed in triplicate. The percentage of cell viability for each concentration and the IC_{50} value were then detected.

Comet Assay

The comet assay applied is based on the technique of Singh et al. (1988) with some amendments. Human peripheral blood samples were taken from two healthy donors, one male and

one female, aged between 22 and 27 years. None of the donors had problems that may have prevented them from being volunteers, such as health, alcohol, cigarettes, and drugs. This study was conducted with permission numbered 2023/121 obtained from the Amasya University Ethics Committee. Lymphocytes (separated from whole blood by biocoll) viability was quantified to be above 96% by the Trypan Blue Exclusion Test. Then lymphocytes were exposed to five concentrations of NaA (15.63, 31.25, 62.50, 125, and 250) and NaS (3.91, 7.81, 15.63, 31.25, and 62.50) and incubated at 37 °C for 1 h. A control (sterile distilled water) and positive control (H_2O_2, 100 µM) were also run. After time off the incubation, the cells were centrifugated at 1348 g, 5 min, resuspended using PBS, and were gently mixed with low melting agarose (0.65%). Next, the suspension was rapidly spread onto slides previously coated with normal melting agarose (0.65%), and coverslips were placed over the slides. The slides were put into a digestion or lysing solution for at least 1 hour at 4°C. Then, the slides were incubated in an electrophoresis solution for 20 min. The electrophoresis was performed at 25 V and 300 mA for 20 minutes. Slides were stained with EtBr (20 µg/mL) after washing in a neutralisation buffer. To determine DNA damage, a total of 200 cells for each concentration (100 cell/donor) were imaged through a dedicated Analysis System called Comet Assay IV (Perceptive Instruments Ltd., UK), with regards to three comet parameters (tail intensity (%), tail length (µm) and tail moment).

Data Analysis

The MTT cell viability assay was analysed using one-way ANOVA followed by Dunnet's multiple comparison tests. Statistical significance was defined as a p-value less than 0.05. To explore statistical significance, a t-test was performed on all comet parameters. A regression analysis was also performed to reveal the dose-response relation. IBM SPSS 22.0 software was used for statistical analysis.

Results and Discussion

Table 1 summarises the results of the MTT assay in $HepG_2$ cells treated with NaA and NaS, respectively. None of the concentrations of either food additive significantly affected the cell viability except the highest concentration. NaA exhibited a significant decrease in cell viability at the highest concentration (2000 µg/mL). Additionally, this concentration was determined as the IC_{50} value (cell viability is 51.57 %) that kills 50% of the population of the cells (Table 1, Figure 1). NaS showed a significant reduction in cell viability only at 500 µg/mL (Figure 2). The cell viability value at 500 µg/mL was 38 %. It was determined that NaA (2000 µg/mL) and NaS (500 µg/mL) showed low mitochondrial activity at the highest concentrations. On the other hand, NaA and NaS did not show cytotoxic effects in $HepG_2$ cells at all concentrations except at the highest concentrations applied.

The effect of NaA and NaS on three comet assay parameters is given in Tables 2 and 3, respectively. At all concentrations except the lowest (15.63 µg/mL), NaA significantly increased the comet parameters compared to the control. These increases were dose-dependent, but the correlation was weak for tail moment (r = 0.48) and tail length (r = 0.44). The correlation was relatively strong for tail intensity (r = 0.75). Similarly, NaS induced significant comet tail intensity at all concentrations except at 3.91 µg/mL (lowest concentration); however, the concentration of 3.91 µg/mL was also statistically significant for the other two comet parameters, tail length and moment, like as being other all concentrations. Also, it was found that a robust dose-dependent correlation between DNA damage induction and concentration for tail intensity (r = 0.76) and tail moment (r = 0.89), while tail length (r = 0.41) showed a weak dose-dependent correlation.

In this study, the possible cytotoxic effect caused by NaA and NaS was evaluated in $HepG_2$ cells by MTT assay, and their potential for damaging DNA was assessed in human lymphocytes using a comet assay. As a result, both food preservatives induced cytotoxic effects at high concentrations. However, at concentrations where DNA damage was observed in human lymphocytes, no cytotoxic effect was determined in $HepG_2$ cells. This result may be due to the difference in the cell group. Notably, a cyto-genotoxic effect with increasing concentration was observed for both test systems.

Table 1. Cytotoxic effect of sodium sulfite and sodium acetate in $HepG_2$ cell line

Test substance	Concentration (μg/mL)	N	Cell viability (24 h) Mean±SD
Control	0.00	3	3.014±0.111
NaA	15.63	3	3.242±0.067
	31.25	3	3.224±0.068
	62.50	3	2.918±0.387
	125	3	3.269±0.265
	250	3	3.195±0.273
	500	3	3.132±0.067
	1000	3	2.973±0.083
	2000	3	1.762±0.566 * #
Control	0.00	3	2.747± 0.163
NaS	3.91	3	2.889±0.084
	7.81	3	2.909±0.230
	15.63	3	3.023±0.247
	31.25	3	3.152±0.036
	62.50	3	2.375±0.090
	125	3	2.909±0.051
	250	3	2.534±0.028
	500	3	1.122±0.540 *

Non-treated cells were used as control. NaA: Sodium acetate; NaS: Sodium sulfite; $HepG_2$: Human Hepatocellular Carcinoma Cell Line; SD: Standard Deviation
* $p<0.05$ statistically significant according to the one-way ANOVA-Dunnet Test)
# IC_{50} value

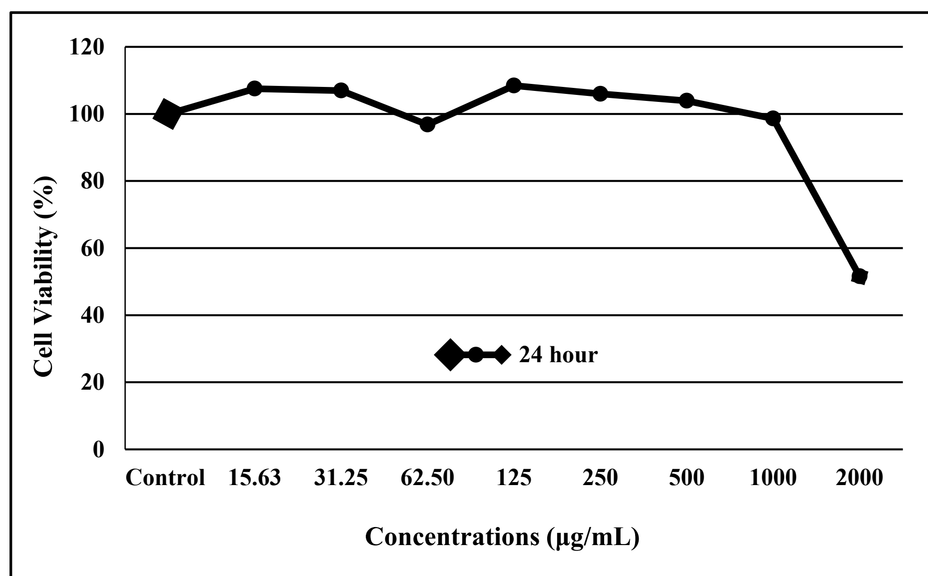

Figure 1. Cell viability result of sodium acetate in $HepG_2$ cell

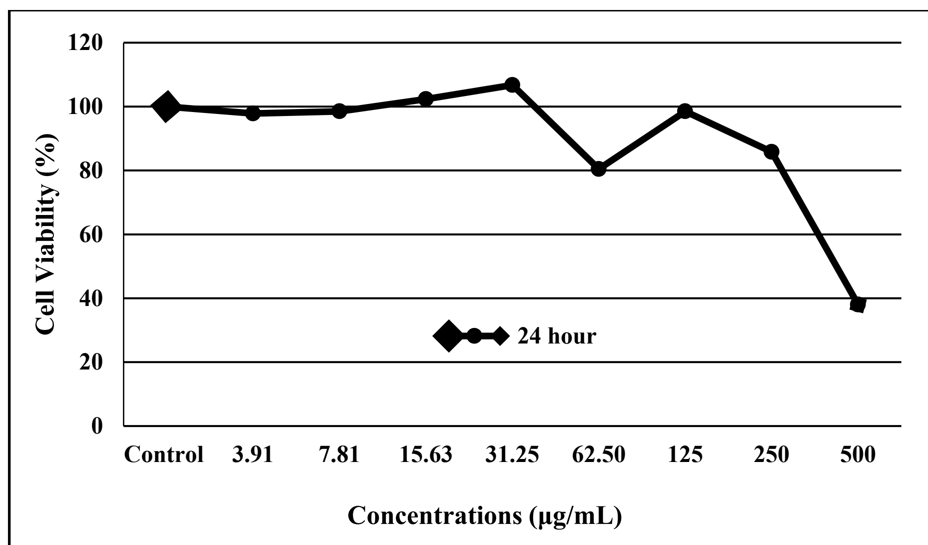

Figure 2. Cell viability result of sodium sulfite in $HepG_2$ cell

Table 2. Levels of DNA damage measured in isolated peripheral lymphocytes that were exposed to different concentrations of sodium acetate (NaA)

Test substance	Exposure time (hour)	Concentration (µg/mL)	Tail intensity (%)	Tail Lenght (µm)	Tail Moment
Control	1	0.00	18.23±1.38	65.91±1.35	4.94±0.40
Positive control (H_2O_2)	1	100 µM	28.82±1.88	198.79±9.20	18.76±2.15
NaA	1	15.63	17.67±1.38	67.96±1.71	5.15±0.59
		31.25	23.21±1.80*	102.08±4.56*	8.65±0.96*
		62.50	22.92±1.57*	87.87±3.20*	7.85±1.03*
		125	22.50±1.27*	79.32±2.65*	6.15±0.45*
		250	22.36±1.44*	83.96±3.00*	7.21±0.81*

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

Table 3. Levels of DNA damage measured in isolated peripheral lymphocytes that were exposed to different concentrations of sodium sulfite (NaS)

Test substance	Exposure time (hour)	Concentration (µg/mL)	Tail intensity (%)	Tail Lenght (µm)	Tail Moment
Control	1	0.00	17.30±1.37	69.76±1.39	4.69±0.43
Positive control (H_2O_2)	1	100 µM	28.57±1.97	156.16±9.56	20.41±2.75
NaS	1	3.91	20.10±1.75	88.64±2.53*	6.50±0.76*
		7.81	22.95±1.73*	94.10±3.45*	8.39±1.05*
		15.63	24.74±1.90*	85.31±2.46*	9.05±1.06*
		31.25	22.03±1.79*	79.43±2.56*	8.15±1.09*
		62.50	23.30±1.99*	90.63±2.85*	9.70±1.28*

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

The potential risks of food additives are regularly reassessed by the European Food Safety Authority (EFSA, 2022a,b). However, many food additives are still categorised as Generally Recognized as Safe (GRAS) and have not been assessed by the regulatory authorities. Much data is based on toxicity tests and assessments commissioned by manufacturers (Maffini et al., 2017; Neltner et al., 2013; Yen et al., 2024). Although both preservatives are Generally Recognized as Safe (GRAS) by EFSA (except for NaS in meats or food recognised as the source of vitamin B1), genotoxic and cytotoxic effects in some cell lines have been reported in the literature. Xia et al. (2016) investigated the NaA on the viability of the human gastric adenocarcinoma epithelial cell line using an LDH release assay. They reported that 6.25 mM NaA for 24 h exhibited a rise in LDH level, but the LDH releasing was fewer than 3%. Moreover, LDH release increased to 2.1, 3.8, and 7.0% after the cells were exposed to 12.5, 25-, and 50-mM NaA for 24 h. The authors concluded that NaA exerted an apoptotic effect in the human gastric adenocarcinoma epithelial cells via a caspase-dependent apoptotic pathway. The results presented in this paper showed that NaA was cytotoxic at higher concentrations, and this effect became more pronounced as the concentration increased. Since the LDH assay is also an indicator of cytotoxicity, the results were similar even though the concentrations used were different. Sun et al. (2005) determined that the lowest concentration of NaA (12.5mM) had increased viability after both treatment periods, 24 and 48 hours. The concentration of 25 mM NaA had almost no effect on the viability of human gastric adenocarcinoma epithelial cells. However, NaA at 50-100 mM caused reduced viability. These results stated that high concentrations of NaA in food have cytotoxic effects (Sun et al., 2005). Similarly, in this study using $HepG_2$ cells, some low NaA concentrations caused an increase in cell viability compared to the control group. Although not significantly reduced, cell viability decreased as the concentrations increased. Indeed, cell viability decreased after 62.50 µg/mL sodium acetate treatment (Figure 1), increased at 125, 250, 500, and 1000 µg/mL concentrations and suddenly decreased at the highest concentration (2000 µg/mL). Similarly, the cell viability, which decreased after 62.50 µg/mL of sodium sulfite treatment (Figure 2), increased with 125 µg/mL treatment and decreased following high concentrations. Therefore, the 62.50 µg/mL concentration may be interpreted as the threshold concentration known as the lowest dose at which the induced effect occurs within the framework of the dose-response relationship. However, these increases and decreases were not statistically significant compared to the control. In addition, as the concentration increases, the observed changes seem small. Meng et al. (2004) evaluated the DNA damaging effects of a mixture containing NaS and sodium bisulfite (3:1 M/M; sulfur dioxide derivatives) on various organs of male mice. Sulfur dioxide (SO_2) derivatives received an intraperitoneal (125, 250, 500 mg/kg body weight) daily for a week. Their results demonstrated that SO_2 and its derivatives significantly increased DNA damage (olive tail moment) in all organs tested from male mice, providing systemic toxicity. Abd-Elhakim et al. (2018) found that NaA increased DNA damage percentage on lymphocytes isolated from adult male Sprague-Dawley rats; tail DNA percentage, length, and moment in a dose-dependent manner at all tested concentrations (50, 100, and 200 mM/L). Additionally, all NaA concentrations decreased cell viability (MTT assay, exposure time: 24 h and 72 h) and proliferation (72 h) of lymphocytes and increased LDH release, concentration-dependently. After all, NaA was suggested to be cytotoxic and genotoxic for lymphocytes isolated *in vitro*. El-Hefny et al. (2020) investigated the DNA-damaging effect of NaS using the SMART and comet assay. In the SMART assay, two different *Drosophila* strains were used, a wild-type strain and a strain carrying *wtsMT*$^{4-1}$ (a lethal wart allele balanced on TM3), and larvae were exposed to NaS (100 mM) for approximately 48 hours. NaS significantly increased the frequency of *warty* tumours in *D. melanogaster* flies. Furthermore, the comet assay evaluated all cell types of the isogenic w^{1118} strain of *D. melanogaster* exposed to NaS (100 mM, 24 h) for DNA breaks. NaS caused significant DNA damage. The researchers commented that NaS has a noticeable genotoxic potential. Remarkably, NaS also appears to cause DNA damage in *in vivo* conditions. In the present study, NaA and NaS increased the comet tail intensity, length, and moment in isolated lymphocytes at almost all concentrations. Both NaA and NaS significantly caused DNA damage. In this respect, the data obtained from the two previous studies are consistent with the present study. Besides, Mohammadzadeh-Aghdash et al. (2018) exhibited that NaA (25, 50, 100, and 200 µM) decreased HUVEC cell line growth in a dose and time-dependent manner (IC_{50} value: 487.71 µM at 24 h and 232.05 µM at 48 h); however, it did not induce a significant effect on DNA fragmentation or smear upon exposure of HUVECs with IC_{50} concentration of NaA using DAPI staining and DNA ladder assays. Moreover, using FITC-labeled Annexin V flow cytometry, IC_{50} concentration of NaA did not lead to considerable apoptosis or necrosis. They concluded that a low concentration of NaA did not have significant cytotoxic and genotoxic effects. On the other hand, the absence of DNA damage and increased cell death in cells exposed to IC_{50} concentration may be due to the difference in the cells used in the experimental sets. Therefore, it is clear that the effects of both NaA and NaS should

be investigated in different cell groups. In a study conducted in 2023, the genotoxic effects of NaA and NaS were examined in human lymphocytes by chromosomal aberration and micronucleus (MN) tests. For this, 15.63-250 µg/mL of NaA and 3.91-62.50 µg/mL of NaS were used. Both food preservatives increased the frequency of CAs and MN at higher concentrations. In the same study, mitotic index values also determined the cytotoxicity of NaA and NaS at the same concentrations in human lymphocytes. The mitotic index, an indicator of cytotoxicity, decreased statistically significantly at the high concentrations of NaA and NaS used in cultured human lymphocytes. In summary, NaA and NaS showed both genotoxic and cytotoxic effects at high concentrations in human lymphocytes (Altunkaynak, 2023). The study's results are consistent with this study regarding both aspects (cytotoxicity and genotoxicity).

According to the recent EFSA report (2022a), sulfites, including NaS, may be unsafe, especially at high concentrations. Ishidate et al., 1984, reported no mutagenicity in the Ames assay and no induction of chromosomal abnormalities in mammalian cells in the *in vitro* chromosomal aberration test. However, in later years, a mutagenic effect was observed in the spore rec test with the *Bacillus subtilis* M45 strain (Ueno et al., 2002). Additionally, it caused gene mutation at the gpt locus of AS52 cells (Meng & Zhang, 1999), which was associated with a cytotoxic effect at the highest concentration used. In this study, the damage at high concentrations was observed more prominently, so the cytotoxic effect observed at high concentrations may be related to DNA damage. Together with the results of the other studies described above, the EFSA report states that there are gaps in the toxicity data of NaS and other sulfites and that the effectivity of this situation on health still needs to be determined. Another report by the same authority announces that acetates (including NaA) used as food additives should be re-evaluated (EFSA, 2022 a, b). Considering the studies conducted, the increase in mutagenicity and genotoxicity, especially at high concentrations, and the finding of different results in different cell groups have caused concern. An example is the finding of completely different mutagenicity results in different prokaryotic organisms in the studies described above. Taken together, as stated in EFSA's recent reports on sulfites and acetates, there is a significant gap in the literature in terms of clarifying their effects and at which concentrations and in which cell groups their effects are more effective. This study will fill the gaps in the literature and contribute to these re-evaluations.

In a study conducted in 2018, the genotoxicity of NaA was investigated under *in vivo* conditions, and a significant genotoxic and cytotoxic effect was detected. It was stated that the release of reactive oxygen species (ROS) by acetate may be a mechanism underlying the cyto-genotoxic effect of NaA (Abd-Elhakim et al., 2018). Therefore, the genotoxic and cytotoxic effects observed in this study may be due to the release of ROS. The resulting ROS and free radicals can react with multiple vital components of the cell, such as proteins, lipids, and DNA, and disrupt the structure and function of these components. Free radicals are unstable, low molecular weight, and very active molecules. Since free radicals are highly reactive compounds, they carry unpaired electrons in their outermost orbitals and quickly react with other organic and inorganic molecules. Free radicals are unstable and react with many organic molecules in the cell, like DNA, to produce various damages that may cause abnormalities (Mercan, 2004; Hou et al., 2024).

Conclusion

Food preservatives are the most commonly used group of food additives. Extending shelf life and reaching the consumer without spoilage are among the most essential concerns in food production. Therefore, food additives have become indispensable in the prepared and packaged food industry. This situation has made exposure to food additives inevitable. However, the potential health risks of these chemicals have always been a matter of debate. The point to be considered is to minimise the hazards as much as possible by ensuring that these substances are used as specified and safely. To protect human health, it is essential to consider the food production chain as a system to continuously control food safety and evaluate scientific evidence and all kinds of risk assessments in all measures taken to protect consumer interests.

Results of this study showed that NaA and NaS exhibited a cyto-genotoxic effect at high concentrations. Different studies indicate that this effect may be due to free radical formation. Free radicals, which are low in most foods, increase depending on the processing technique of foods. In other words, the free radical content of processed food is higher than the content of the raw material. The formed ROS and free radicals can react with multiple vital components of the cell, such as protein, lipid, and DNA, and disrupt the structure and function of these components. Considering other studies with NaA and NaS, caution should be exercised when using NaA and NaS because of the cyto-genotoxic effect in different tissues with different tests supporting each other. However, further studies with various test methods and cell groups are believed to provide direction on the level of hazard associated with using these food additives.

Compliance with Ethical Standards

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

Ethics committee approval: This study was conducted with permission numbered 2023/121 obtained from the Amasya University Ethics Committee.

Data availability: Data will be made available on request.

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Research Article

Bazı popüler diyetlerin besin ögesi içeriklerinin, diyet antioksidan kapasitelerinin, diyet kalitelerinin ve diyet inflamatuar yükünün incelenmesi

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ÖZ

Popüler diyetlerin sağlık üzerine uzun dönemde olası etkilerinin daha iyi anlaşılması için besin ögesi profilinin ayrıntılı olarak değerlendirilmesi gereklidir. Bu çalışmada bazı popüler diyetlerin besin ögesi içeriklerinin, diyet antioksidan kapasitelerinin, diyet kalitelerinin ve diyet inflamatuar yüklerinin incelenmesi amaçlanmaktadır. Popüler diyetleri belirlemek için literatür taranmış ve bu tarama sonucunda Atkins, vegan, Zone, Dukan, Akdeniz, alkali, ketojenik ve Paleo diyet çalışmaya alınmıştır. Popüler diyetlerin Beslenme Bilgi Sistemi (BeBİS) programı kullanılarak besin ögesi içerikleri, antioksidan miktarları ve Oksijen radikali absorbe kapasitesi (ORAC) değerleri belirlenmiştir. Diyet kalitelerini belirlemek için Diyet Kalite İndeksi-I (DKİ-I) ve diyet inflamatuar yükünün belirlenmesi için Diyet İnflamatuvar İndeksi hesaplanmıştır. Tüm diyet modellerinin antioksidan miktarları karşılaştırıl-dığında; antioksidan miktarı en yüksek diyet Paleo diyet (6.0 mmol), en düşük ise klasik ketojenik diyet (1.9 mmol)'tir. Diyet modellerinin ORAC değerleri karşılaştırıldığında, en yüksek ORAC değerine sahip diyet Paleo diyeti (23670.0), en düşük ORAC değerine sahip diyet ise Dukan Seyir diyeti (1828)'tir. En yüksek antiinflamatuar diyet skoruna sahip diyet vegan diyet (-1.5), en yüksek proinflamatuar diyet skoruna sahip diyet ise klasik ketojenik diyet (5.9)'tir. DİI skorlarına göre; vegan, Dukan Seyir ve Akdeniz diyeti antiinflamatuar özelliktedir. En yüksek DKİ-I puanı olan diyet vegan diyeti (74)'dir. Zone diyeti (73) ve Akdeniz diyeti (68) diğer en yüksek puan alan diyetlerdir. En düşük DKİ-I puanına sahip diyetler ise Dukan (atak evresi) (43) ve klasik ketojenik diyet (46)'tir. Bu bulgular, popüler diyetlerin besin yeterliliği hakkında faydalı bilgiler sağlamaktadır. Sonuçlar bitki bazlı ve dengeli diyetlerin düşük karbonhidratlı diyetlere göre daha iyi diyet kalitesine ve daha iyi besin ögesi profiline sahip olduğunu göstermektedir.

Anahtar Kelimeler: Beslenme, Akdeniz diyeti, Ketojenik diyet, Vegan diyet

ABSTRACT

Examination of nutrient content, dietary antioxidant capacity, dietary quality and dietary inflammatory load of some popular diet

This study aimed to examine some popular diets' nutrient content, dietary antioxidant capacity, diet quality, and dietary inflammatory load. **Materials and Method:** Literature was reviewed to identify popular diets, and Atkins, vegan, Zone, Dukan, Mediterranean, alkaline, ketogenic, and Paleo diets were included in the study. Nutrient contents, antioxidant capacity, and oxygen radical absorbance capacity (ORAC) values of popular diets were determined using the Ebispro for Windows (BeBIS) program. Diet Quality Index-I (DQI-I) and Dietary Inflammatory Index were calculated. **Results:** When the antioxidant amounts of all dietary models were compared, the diet with the highest antioxidant content was the Paleo diet (6.0 mmol), and the lowest was the classic ketogenic diet (1.9 mmol). When the ORAC values of the dietary patterns were compared, the diet with the highest antioxidant capacity was the Paleo diet (23670), and the diet with the lowest antioxidant capacity was the Dukan cruise diet (1828). The diet with the lowest DII score was the vegan diet (-1.5), and the diet with the highest DII score was the classic ketogenic diet (5.9). According to the DII scores, vegan, Dukan, and Mediterranean diets have anti-inflammatory properties. Vegan diet had the highest DQI-I score (74). Zone (73) and Mediterranean (68) had the highest scoring diets. The diets with the lowest DQI-I scores were the Dukan (attack phase) (43) and the classic ketogenic diet (46). **Conclusion:** These findings provide valuable information on the nutritional adequacy of popular diets. Plant-based and balanced diets have better quality and nutrient profiles than low-carbohydrate diets.

Keywords: Nutrition, Mediterranean diet, Ketogenic diet, Vegan diet

Giriş

İnsan yaşamında beslenme oldukça önemli bir yere sahiptir. Çağlar boyu insanlar, yaşadıkları dönemin koşullarına uyum sağlamışlar ve bu doğrultuda beslenme tarzlarını değiştirmişlerdir. Göçebelikten yerleşik hayata geçilmesi, ateşin bulunması, farklı araç-gereçlerin icadı ve avcılık, toplayıcılık gibi yaşam tarzı değişiklikleri beslenme alışkanlıklarının şekillenmesine katkı sağlamıştır (Akbulut, 2022). İnsanların benimsedikleri ideolojiler de beslenme tarzlarını etkilemekte ve bu da yeni diyet modellerinin oluşmasına zemin hazırlamaktadır. Örneğin; hayvansal kaynaklı ürünlerinin kullanımına karşı olan veganizm ya da veganlık, vegan diyet modelinin ortaya çıkmasına neden olmuştur (Hassoun ve ark., 2022; Wang ve ark., 2008). İnsanlardaki beden imgesinin ayrı bir boyut kazanması da bireylerin farklı diyet modellerine yönelmesine neden olmaktadır. Beden imgesinin olumlu ya da olumsuz olması bireyin besin seçimini ve beslenme davranışını etkilemektedir (Homan ve ark., 2012). Dünya'da birçok kişi vücut ağırlığını azaltmak veya korumak için hızlı ağırlık kaybı vaat eden, bazı besinleri kısıtlayan çeşitli diyetler uygulamaktadır (Anton ve ark., 2017). İnsanlar tüm bu ve daha başka sebeplerle farklı yaşam biçimlerine uygun diyet modellerini benimsemektedirler. Bu diyet modelleri arasında karbonhidrat alımının azaltılması ilkesine dayananlar oldukça popülerdir. Batılı toplumlarda yüksek yağlı diyetten kaçınan ve yüksek protein alımını teşvik eden uygulamalar da görülmektedir (Navruz ve Acar, 2014). Bu diyet modellerine bakıldığında temelde aynı besin bileşenlerini içerseler de bu bileşenlerin miktarları farklı düzeylerde olabilmektedir. Popüler diyetler, düşük karbonhidratlı/yüksek proteinli, yüksek karbonhidratlı/düşük yağlı veya belirli besinlerin veya besin gruplarının (hayvansal kaynaklı besinler, şeker vs.) kısıtlamasına dayalı diyet modelleri dâhil olmak üzere birkaç ana gruba ayrılabilir (Joshi ve Mohan, 2018). Günümüzde Paleo diyeti (taş devri diyeti), Dukan diyeti, alkali diyet, vegan diyeti, Atkins diyeti, ketojenik diyet, Akdeniz diyeti ve Zone diyeti gibi birçok popüler diyet mevcuttur ve uygulanmaktadır (Anton ve ark., 2017). Popüler diyetlerin içerdikleri besin bileşenlerinin farklılıkları diyet kalitesinin etkilemekte ve bazı diyetler sağlığı iyileştirmeye katkı sağlarken bazıları ise kötü sağlık çıktıları ile ilişkilendirilmektedir (Dinu ve ark., 2020; Huang ve ark., 2020). Daha fazla insanın popüler diyetleri takip etme konusundaki eğilimleri arttıkça, bu diyetlerin diyet kalitesi hakkındaki bilgiler daha da önemli hale gelmektedir (International Food Information, 2023).

Bu çalışmada bireylerin sağlıklı bir yaşam sürdürmek, ağırlık kaybını sağlamak veya çeşitli inançlar sebebiyle uyguladıkları bazı popüler diyetlerin besin ögesi içeriklerinin, diyet antioksidan kapasitesinin, diyet kalitelerinin ve diyet inflamatuar yüklerinin incelenmesi amaçlanmaktadır.

Materyal ve Metot

Bu çalışma günümüzde uygulanan bazı popüler diyetlerin besin öğeleri ve diyet kalitesi açısından kesitsel bir analizidir. Popüler diyetleri belirlemek için literatür taranmış ve bu tarama sonucunda sekiz popüler diyet çalışmaya dahil edilmiştir. Bu diyetler; Atkins diyeti, vegan diyet, Zone diyeti, Dukan diyeti, Akdeniz diyeti, alkali diyet, ketojenik diyet ve Paleo diyeti'dir. Belirlenen diyet modellerinin bazıları aşamalı ya da farklı çeşitleri olan diyetlerdir. Bu nedenle bazı diyetlerden iki farklı diyet modeline çalışmada yer verilmiştir. Atkins diyeti için, başlangıç evresi ve ön koruma evresi; Dukan diyeti için, atak evresi ve seyir evresi şeklinde ikişer farklı diyet modeli çalışmaya alınmıştır. Her bir diyet için bir günlük örnek bir beslenme programı izokalorik olarak tasarlanmıştır. Çalışmaya alınan diyetlerin Beslenme Bilgi Sistemi (BeBİS) programı kullanılarak makro ve mikro besin ögesi içerikleri, antioksidan miktarları ve Oksijen radikali absorbe kapasitesi (ORAC) değerleri belirlenmiş ve diyet kalitelerini belirlemek için ise Diyet kalite İndeksi-I ve Diyet İnflamatuvar İndeksi hesaplanmıştır.

Diyet Modellerinin Tasarlanması

Popüler diyet modellerini her biri için birer günlük bir beslenme programı izokalorik olarak araştırmacı tarafından tasarlanmıştır. Popüler diyetlerin diyet modelleri tasarlanırken örnek menüleri bulunan diyetlerde bu örnek menüler, örnek menüye ulaşılamayan diyetlerde ise diyet ilkeleri göz önünde bulundurularak araştırmacı tarafından hazırlanmış diyet modelleri kullanılmıştır. Popüler diyetlerin örnek menüler incelenmiş bu menüler genellikle 1500±20 kkal içeriğine sahip olduğu için bütün diyet modelleri izokalorik olarak (1500±20 kkal/gün) olarak oluşturulmuştur.

Atkins diyeti, Amerikalı kardiyolog Dr. Robert C. Atkins tarafından geliştirilen, The Atkins Diet Revolution kitabında yayımlanan düşük karbonhidratlı, yüksek proteinli ağırlık kaybını amaçlayan aşamalı bir diyet planıdır. Diyetin başlangıç aşamasında günde 20 gram net karbonhidrat tüketimi ve bunların 12 ila 15 gramının mutlaka temel sebzelerden (salatalık sebzeler ve pişirilerek tüketilen sebzeler) alınması önerilmektedir. Diyette tahıl ve tahıl ürünleri, kurubaklagiller, nişastalı sebzeler gibi yüksek karbonhidrat içeren besinlerin tüketiminden uzak durulmaktadır. Kırmızı et, balık, kümes hayvanlarının ve doğal yağ tüketiminde kısıtlama bulunmamaktadır. Ön koruma aşamasında vücut ağırlık kaybı devam ettiği sürece karbonhidrat alımı haftada 10' ar gram olmak üzere yavaş yavaş arttırılmaktadır. Bu aşamada küçük porsiyonlarda tam yağlı veya kaymağı alınmış süt; orman meyvelerine ilave olarak diğer meyveler de yavaş yavaş eklenebilmektedir. Ayrıca nişastalı sebzeler ve tam tahıllı ürünler çok küçük porsiyonlarda eklenebilmektedir (Westman ve ark., 2014). Vegan diyet hayvansal kaynaklı bütün besinlerin diyetten çıkarıldığı bir beslenme tarzıdır. Diyette hayvansal kaynaklar dışındaki; tüm sebzeler, meyveler, tahıllar ve yağlı tohumlar tüketilmektedir. Karbonhidrat ve protein ihtiyacı sebzeler, tahıllar ve baklagillerden karşılanırken, yağ ihtiyacı bitkisel kaynaklı yağlardan karşılanmaktadırlar (Barnard, 2020). Zone diyeti ağırlık kaybını hedefleyen ve öğün zamanlarının önemini vurgulayan bir diyet planıdır. Günde beş öğün; üç ana öğün ve iki ara öğün şeklinde tüketilmesi önerilmektedir. Her öğünde yeterli miktarda az yağlı protein tüketilmesi önerilmektedir. En iyi protein seçenekleri; derisiz tavuk eti, hindi, balık, yağsız kırmızı et, yumurta akı, az yağlı süt ve süt ürünleri, tofu ve soya ürünleri şeklinde belirtilmektedir. Zone diyetinde karbonhidrat seçimi yaparken; glisemik indeksi yüksek sebzelerin (mısır, havuç vb.) ve meyvelerin (muz, karpuz, kuru meyveler vb.) ve de nişastalı besinlerin tercih edilmemesi önerilmektedir. Yağ grubuna bakıldığında, tekli doymamış yağ asitleri ve uzun zincirli yağ asitleri (omega-3) 'iyi' yağ olarak adlandırılırken; doymuş yağ asitleri, trans yağ asitleri ve araşidik asit 'kötü' yağ olarak adlandırılmaktadır. Diyetlerde kötü olan yağ asitleri yerine iyi olarak adlandırılan yağ asitlerinin tercih edilmesi önerilmektedir (Sears, 2004). Alkali diyetin temeli, vücuttaki asit yükünü azaltmak ve bunu alkali besinler tüketerek sağlamaktır. Alkali diyette sebze, meyve, yağlı tohum ve bakliyat ürünleri arttırılırken, hayvansal kaynaklı; et, süt ve ürünleri, yumurta sarısı, doymuş yağlar, işlenmiş ürünler gibi vücutta asitlenmeyi arttıran besinler azaltılmaktadır (Vasey, 2006). Dukan diyeti, Fransız Dr. Pierre Dukan tarafından geliştirilmiştir. Dukan diyeti, düşük karbonhidrat, düşük yağ ve yüksek protein tüketerek kısa sürede hızlı vücut ağırlığı kaybı sağladığını savunmaktadır. Dukan diyetinin ilk aşaması olan atak evresinde önemli nokta saf protein tüketilmesidir (1–10 gün). Neredeyse diyetin tamamı hayvansal kaynaklı proteinden oluşmaktadır. Bu aşamada yağ içermeyen protein kaynakları tüketilmelidir ve tüketimde sınırlama bulunmamaktadır. Dukan diyetinin seyir evresinde saf proteine ek olarak taze ya da pişmiş sebzeler eklenmektedir. Tüketilen bitkisel besinlerin yüksek nişastalı (patates, enginar, pirinç, mısır, bezelye, nohut, bakla, mercimek ve börülce) olmamasına dikkat edilmelidir. Ayrıca tüketilen çiğ ya da taze sebzeler her öğünde yer almamalıdır. Seyir evresinde hedef vücut ağırlığına ulaşıncaya ve vücut ağırlığı sabitleninceye kadar iki diyet modeli dönüşümlü olarak birbirini takip etmektedir. Bu iki diyet: Protein + sebze diyeti ve saf protein diyeti şeklindedir (Dukan, 2018). Akdeniz diyetinin temel özellkileri; diyette sebze, meyve, kurubaklagiller, yağlı tohumlar, ekmek ve diğer tahıllar gibi bitkisel kaynakların sıklıkla yer alması, temel yağ kaynağı olarak zeytinyağının kullanılması, hayvansal kaynaklı besinlerin bitkisel kaynaklı besinlere oranla daha az tüketilmesidir. Akdeniz diyetinde kırmızı etin ayda 1-2 kez tüketilmesi, haftada 2 defa balık tüketiminin önerilmesi ve de yemeklerin yanında ılımlı kırmızı şarap tüketilmesi tavsiye edilmektedir. Akdeniz diyetinde tam tahıllı ürünlerin 8 porsiyon / gün, kuru baklagil tüketiminin 1-2 porsiyon / hafta, sebze tüketiminin 2-3 porsiyon / gün ve meyve tüketiminin 4-6 porsiyon / gün tüketilmesi önerilmektedir (Özata ve Bektaş, 2021). Ketojenik diyet, ilk kez 1920'lerde Johns Hopkins Tıp Merkezi'nde (Maryland, ABD) çalışan araştırmacılar tarafından epilepsi hastalarının tedavisinde uygulanmıştır. Ancak günümüzde sıklıkla ağırlık kontrolü ve sağlık yararları iddiasıyla kullanılmaktadır. Ketojenik diyet temelde; yeterli protein, düşük karbonhidrat (20-50 gram) ve yüksek yağdan oluşan bir diyet modelidir. Ketojenik diyet, açlık durumunu taklit ederek kanda ketonları arttırmakta ve kan glikoz seviyesini düşürmektedir (Zengin, 2019). Paleo diyeti, asırlar önceki dönemlerdeki beslenme alışkanlıkları baz alınarak şekillenmiş bir diyet modelidir. Paleo diyette, çiğ besinler toplam diyetin en az % 60'ını oluşturmaktadır. Paleo diyetin temeli, yüksek

protein ve yağ, düşük karbonhidrat tüketimine dayanmaktadır. Doğal olarak yetişen sebzeler tercih edilmekte, özellikle yeşil yapraklı sebzelerin çiğ olarak tüketilmesi önerilmektedir. Diyette kimyasal içerikli hiçbir ürün; katkı maddelerin koruyucuların, renklendiricilerin kullanılması önerilmemektedir. Et, tavuk, balık, yumurta, sebze, az şeker içeren meyveler, kabuklu kuruyemişler, zeytinyağı gibi besinlerin tüketiminde sınırlama bulunma-maktadır. Ayrıca şeker, tahıl, un ve bunlardan yapılan besinlerin tüketilmemesi önerilmektedir. Paleo diyette süt ürünlerinin tüketilmesi önerilirken, inek sütünün tüketilmesi önerilmemektedir (Wolf, 2017).

Toplam Antioksidan Kapasite ve Besin Öğelerinin Belirlenmesi

Diyet modellerinin antioksidan miktarlarını, toplam antioksidan kapasitesilerini ve makro – mikro besin öğelerini hesaplamak için Beslenme Bilgi Sistemi (BEBİS) programı kullanılmıştır.

Diyet İnflamatuar İndeksinin (DİI) Hesaplanması

Diyet inflamatuar indeksinin hesaplanmasında çalışmada yer alan her bireyin günlük besin/besin ögesi alım miktarlarından z skor değerleri [(bireyin besin/besin ögesini günlük tüketim miktarı-standart global tüketim miktarı) / besin/besin ögesinin standart sapma değeri] hesaplanmış ve sonrasında persentil skoruna dönüştürülmüştür. Simetrik bir dağılım elde etmek için, her bir persentil skoru iki ile çarpılmış ve daha sonra "1" çıkarılmıştır. Her bir besin/besin ögesi için belirlenen merkezileştirilmiş persentil değerleri, besin/besin ögesi için Shivappa ve arkadaşları tarafından hesaplanmış olan "özelleştirilmiş tam inflamatuar etki skoru" ile çarpılmış, sonuçta elde edilen değerler toplanarak, bireyin günlük diyetinin inflamatuar yükünü temsil eden diyet inflamatuar indeks (DİI) skoru elde edilmiştir.19 Çalışmaya katılan bireylerin besin tüketimine dayalı DİI hesaplaması için olası 45 besin/besin ögesinden, bireylerin besin tüketim kayıtlarından ulaşılabilen 33(Enerji (kkal), Protein (g), Toplam yağ (g), Doymuş yağ (g), Tekli doymamış yağ asitleri (g), Çoklu doymamış yağ asitleri (g), n-3 yağ asidi (g), n-6 yağ asidi (g), Kolesterol (mg), Karbonhidrat (g), Posa (g), Kafein (mg), A vitamini (RE), Beta karoten (µg), D vitamini (µg), E vitamini (mg), Tiamin (mg), Riboflavin (mg), Niasin (mg), B6 vitamini (mg), Folik asit (µg), B12 vitamini (µg), C vitamini (mg), Demir (mg), Magnezyum (mg), Çinko (mg), Selenyum (mg), Yeşil/siyah çay (g), Soğan(g), Sarımsak (g), Biber (g), Kekik (mg), Zencefil (g)) tanesi kullanılmıştır. Pozitif DİI skorları proinflamatuar, negatif DİI skorları antiinflamatuar olarak kabul edilmektedir (Shivappa ve ark., 2014).

Diyet Modellerinin Diyet Kalite İndeksi (DKİ-I)'nin Hesaplanması

Diyet Kalite İndeksi (DKİ-I), Kim ve ark. (2023) tarafından tanımlanan yönteme göre değerlendirilmiştir. DKİ-I; çeşitlilik, yeterlilik, ölçülülük ve genel denge kategorileri olmak üzere diyetin dört temel bileşenine odaklanmaktadır. Bu kategorilerin her biri için değerlendirilecek belirli alt diyet bileşenleri bulunmaktadır. Her bileşenin puanları dört ana kategorinin kendi içerisinde hesaplanmaktadır. Daha sonra dört kategorinin puanları toplanmakta ve sonuçta 0 ile 100 arasında değişen toplam DKİ-I puanı elde edilmektedir. '0' en düşük ve '100' olası en yüksek DKİ-I puanını temsil etmektedir.

Bulgular ve Tartışma

Şekil 1'de diyet modellerinin makro besin ögesi dağılımları verilmiştir. En yüksek karbonhidrat oranı %65 ile vegan diyette iken en düşük karbonhidrat oranı %4 ile klasik ketojenik diyettedir. En yüksek protein oranı ise Dukan diyetlerinde (%40), en düşük protein oranı ise yine klasik ketojenik diyettedir (%10). Klasik ketojenik diyet %86 ile en yüksek yağ oranına sahip iken %19 ile vegan diyet en düşük yağ oranına sahiptir. Akdeniz diyeti (%38-%19-%43) ve alkali diyet (%38-%22-%40) diğer diyet modellerine göre daha dengeli bir makro besin ögesi dağılımları vardır.

Diyet modellerinin bazı makro ve mikro besin ögesi dağılımları Tablo 1'de verilmiştir. Diyet modelleri incelendiğinde, doymuş yağ miktarı en yüksek Atkins Başlangıç diyetinde (39.2 g), en yüksek kolesterol miktarı ise Dukan Seyir diyetindedir (1212.9 mg). Paleo diyeti (6.0 g) en yüksek omega 3 miktarına sahip diyet iken ikinci en yüksek omega 3 miktarı Dukan Seyir diyetidir (5.9 g). En düşük omega 3 içeriği vegan diyetindedir (0.8 g). Diyet posasını en yüksek miktarda içeren diyet Akdeniz diyetidir (49.0 g). Diyet modellerinin vitamin içerikleri incelendiğinde A vitamini en yüksek miktarda Atkins Ön Koruma diyetinde (4086.7 µg) en düşük miktarda Klasik Ketojenik diyettedir (433.4 µg). C vitamini, en yüksek

vegan diyette (339.5 mg); en düşük ise Dukan Atak diyetindedir (16.3 mg). Diyetlerin B12 vitamin içeriğine bakıldığında, en yüksek Dukan Seyir diyetindedir (16.9 µg), vegan diyette ise B12 vitamini bulunmamaktadır. Diyet modellerinin mineral miktarlarına bakıldığında, kalsiyum en yüksek Dukan Atak diyetinde (3154.4 mg), en düşük ise Klasik Ketojenik diyettedir (299.7 mg). Magnezyum en yüksek vegan diyetinde (546.8 mg) ve en düşük miktarda ise Klasik Ketojenik diyettedir (85.4 mg). Diyet modellerinin potasyum miktarları karşılaştırıldığında, en yüksek miktar Dukan Seyir diyetinde (4830.5 mg), en düşük ise Klasik Ketojenik diyettedir (1154.6 mg). Demir miktarı incelendiğinde, en yüksek demir miktarı vegan diyette (19.4 mg), en düşük ise Klasik Ketojenik diyettedir (5.3 mg).

Diyet modellerinin içerdiği antioksidan miktarı Şekil 2'de verilmiştir. Tüm diyet modellerinin antioksidan miktarları karşılaştırıldığında; antioksidan miktarı en yüksek diyet Paleo diyet (6.0 mmol), en düşük ise Klasik Ketojenik diyet (1.9 mmol)'tir.

Diyet modellerinin ORAC (antioksidan kapasite) değerleri Şekil 3'te verilmektedir. Diyet modellerinin ORAC değerleri karşılaştırıldığında, en yüksek antioksidan kapasiteye sahip diyet Paleo diyeti (23670.0), en düşük antioksidan kapasiteye sahip diyet ise Dukan Seyir diyeti (1828)'tir.

Diyet modellerinin Diyet İnflamatuvar İndeks skorları (Dİİ) Şekil 4'te verilmektedir. Popüler diyetlerin Dİİ skorları incelendiğinde; en yüksek antiinflamatuar diyet skoruna sahip diyet vegan diyet (-1.5), en yüksek proinflamatuar diyet skoruna sahip diyetin ise Klasik Ketojenik diyet (5.9)'tir. Diyet İnflamatuvar İndeks skorlarına göre; vegan, Dukan Seyir ve Akdeniz diyeti antiinflamatuar özellikte, Zone, Paleo, klasik ketojenik, Dukan Atak, Atkins Ön Koruma, Atkins Başlangıç ve alkali diyet ise proinflamatuar özelliktedir.

Diyet modellerinin Diyet Kalite İndeksi–I (DKİ-I) skorlarına ait değerlendirme Tablo 2'te verilmiştir. Tüm diyet modelleri değerlendirildiğinde en yüksek DKİ-I puanı olan diyet vegan diyeti (74)'dir. Zone diyeti 73 puan ile en yüksek DKİ-I puanına sahip ikinci diyettir. En düşük DKİ-I puanına sahip diyet ise Dukan (atak evresi) diyeti (43)'dir.

Beden algısı, farklı düşünce yapıları ve sağlıklı beslenme gibi birçok unsur insanları farklı diyet modeli arayışlarına itmektedir. Ancak popüler diyetlerin sağlık üzerine etkileri son derece tartışmalıdır. Bu çalışmada farklı popüler diyet modellerinin besin ögesi içerikleri, diyet antioksidan kapasiteleri, diyet kaliteleri ve diyet inflamatuar yükleri incelenmiştir.

Türkiye Beslenme Rehberi (TÜBER), 2022'de yayınlanan verilerinde, günlük alınan enerjinin %45-60'ının karbonhidratlardan, %10-20'sinin proteinlerden ve %20-35' inin yağlardan karşılanmasını önermektedir (TÜBER, 2022). Bu çalışmada, 11 farklı izokalorik diyet modelinin makro besin öğeleri karşılaştırılmıştır. Akdeniz diyetinin (yağ %38-protein %19-karbonhidrat %43) ve alkali diyetin (yağ %38-protein %22- karbonhidrat %40) TÜBER-2022 (karbonhidrat %45-60, protein %10-20, yağ %20-35) makro besin ögesi dağılımı önerilerine diğer diyet modellerine göre daha yakın olduğu görülmüştür. Klasik Ketojenik diyet (%86) en yüksek olmakla birlikte vegan diyet (% 19) hariç bütün diyet modelleri TÜBER'in önerilerinden daha yüksek yağ oranına sahiptir. Vegan diyet (% 65) ayrıca, diğer diyet modellerine göre en yüksek ve TÜBER'in önerilerinde fazla karbonhidrat içeriğine sahip tek diyet modelidir. Bu durum vegan diyetin tamamen bitkisel kaynaklı besinlere dayanması ve bu besin gruplarının yüksek karbonhidrat ve düşük yağ içeriğine sahip olmasından kaynaklanmaktadır. Vegan diyet hariç tüm diyetler TÜBER'in önerilerinde daha az karbonhidrat içermektedir. En düşük karbonhidrat içeriği ise Klasik Ketojenik (%4) diyettedir. Diyet modellerinin protein içerikleri incelendiğinde Akdeniz (%19), vegan (%16) ve Klasik Ketojenik (%10) diyetin protein içeriğinin TÜBER önerilerine uygun olduğu, diğer tüm diyet modellerinin protein içeriğinin TÜBER önerilerinden yüksek olduğu görülmüştür.

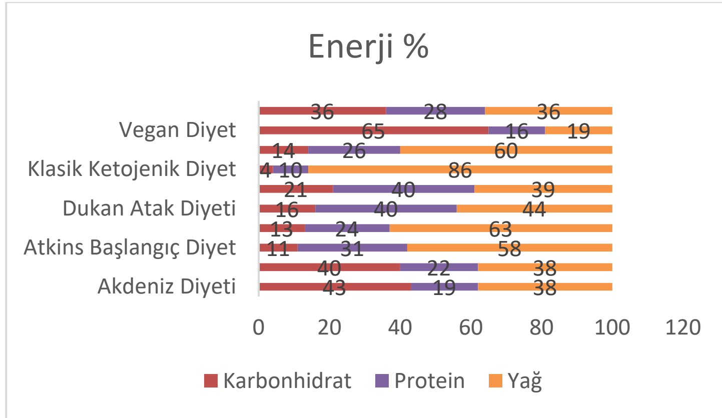
Antioksidanlar serbest radikal oluşumunun azaltılmasıyla da ilişkilidir ve bireylerde antioksidan durumunu iyileştirirler. Dolayısıyla optimum sağlık ve refahın korunmasında ve resbest radikallerin neden olduğu hastalıkların tedavisinde kritik öneme sahiptirler (Sen ve Chakraborty, 2021). Bitkisel kaynaklı besinler bünyesinde bulundurduğu fenolik bileşikler nedeniyle yüksek antioksidan içeriğe sahiptir (Kolaç ve ark., 2017). Bu çalışmada diyet modellerinin antioksidan miktarı değerlendirildiğinde, en yüksek antioksidan miktarı Paleo diyette (6.0 mmol), en düşük antioksidan miktarının ise klasik ketojenik diyette (1.9 mmol) görülmektedir. Klasik ketojenik

diyetin yüksek oranda yağ (%86) içeren bir diyet olması ve diğer diyetlere kıyasla daha düşük miktarda sebze ve meyve içermesi bu durumu desteklemektedir. ORAC, bir besinin serbest radikalleri temizleme kapasitesini ölçme birimidir. Kısacası ORAC, besinin antioksidan kapasitesi anlamına gelmektedir (Vasey, 2006). Diyet modellerinin antioksidan kapasiteleri incelendiğinde, Paleo diyetin en yüksek ORAC değerine sahip olduğu, Dukan diyetin ise en düşük ORAC değerine sahip olduğu görülmektedir. Paleo diyeti esas olarak; doğal otlarla beslenen ve merada yetiştirilen hayvan etleri, çiğ sebzeler, meyveler, mantarlar, kökler ve sert kabuklu yemişlerden oluşur (Cambeses-Franco ve ark, 2021; Frączek ve ark., 2021). Paleo diyetin yüksek oranda içerdiği bu besinler yüksek antioksidan seviyesini açıklamaktadır. Yüksek sebze, meyve, resveratrol ve zeytinyağı tüketimi ile karakterize Akdeniz diyetininin Paleodan sonra en yüksek antioksidan değerine sahip olması ve düşük sebze meyve tüketimiyle karakterize Klasik ketojenik diyetinde en düşük antioksidan değerine sahip olması bu durumu desteklemektedir.

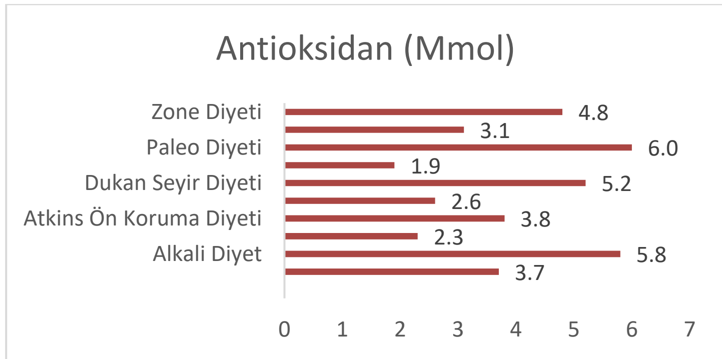
Tablo 1. Diyet modellerinin bazı makro ve mikro besin ögesi dağılımları

Table 1. Some macro and micronutrient distributions of dietary models

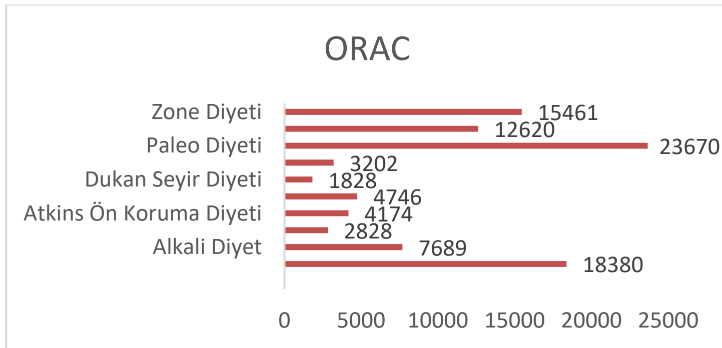
Besin Öğeleri	Akdeniz Diyeti	Alkali Diyet	Atkins Başlangıç Diyeti	Atkins Ön Koruma Diyeti	Dukan Atak Diyeti	Dukan Seyir Diyeti	Klasik Ketojenik Diyet	Paleo Diyeti	Vegan diyeti	Zone Diyeti
Enerji (kkal)	1513.1	1508.2	1519.6	1513.9	1511.9	1511.4	1512.8	1516.9	1513.1	1517.8
Doymuş Yağ (g)	13.5	15.8	39.2	34.1	28.5	21.4	27.8	24.2	4.3	13.5
Tekli Doymamış Yağ (g)	30.8	29.9	44.7	49.4	22.5	24.6	97.0	54.5	17.5	27.7
Çoklu Doymamış Yağ (g)	15.5	14.2	7.8	17.9	17.0	15.1	13.2	19.6	7.3	16.1
Omega-3 (g)	4.2	4.5	1.6	5.1	1.2	5.9	1.5	6.0	0.8	3.9
Omega-6 (g)	11.2	9.6	6.2	12.8	15.7	9	11.6	13.5	6.5	12.2
Kolesterol (mg)	280	371.3	674.1	656.3	675.5	1212.9	286.4	453.7	-	136.2
Diyet Posası (g)	49.0	19.7	21.1	25.2	4.1	11.1	4.3	20.4	35.1	27.5
Betakaroten (mg)	5300.0	2300.0	1200.0	21700.0	300.0	8900.0	800.0	1800.0	9300.0	3500.0
A Vitamini (µg)	1053.5	751.8	831.6	4086.7	657	2227.6	433.4	433.7	1804.7	695.9
D Vitamini (µg)	4.5	6.9	8.7	9.4	2.1	12.1	1.8	14.4	51.4	4.6
E vitamini (mg)	15.9	18.9	10.9	20.6	19.5	13.3	17.4	18.9	26.1	20.2
K Vitamini (µg)	78.9	60.2	125.8	586.6	10.3	833.9	88.3	85.2	643.3	338.9
C Vitamini (mg)	142.0	97.0	122.3	88.1	16.3	213.1	69.3	144.3	339.5	327.2
Folat (µg)	484.0	373.5	286.3	565.8	687.2	798.5	111.7	357.9	675.5	528.5
B12 Vitamini (µg)	4.6	6.5	9.6	9.6	13.1	16.9	3.9	9.0	-	7.3
Tiamin (mg)	1.4	1.1	0.7	1.0	0.9	1.7	0.4	1.1	1.6	1.5
Riboflavin (mg)	1.3	1.4	1.5	1.5	3.8	4.4	1.0	1.6	1.1	2.2
Niasin (mg)	14.6	21.6	26.9	21.7	17.5	33.7	9.6	35.5	13.2	24.6
Kalsiyum (mg)	579.2	316.8	1210.4	931.1	3154.4	2422.8	299.7	379.5	571.5	1062.3
Magnezyum (mg)	361.8	299.2	276.6	320.6	326.3	461.7	85.4	263.2	546.8	326.9
Potasyum (mg)	3665.0	3169.8	2242.7	3437.9	2724.9	4830.5	1154.6	3436.8	4583.6	3634.8
Fosfor (mg)	1446.7	1120.0	1662.2	1526.3	3662.3	3231.3	534.3	1164.8	1134.3	1653.4
Çinko (mg)	7.9	5.7	14.7	9.2	18.7	15.8	5.3	5.5	8.4	8.4
Demir (mg)	13.6	13.7	13.0	12.6	11.1	16.2	4.7	11.1	19.4	11.1



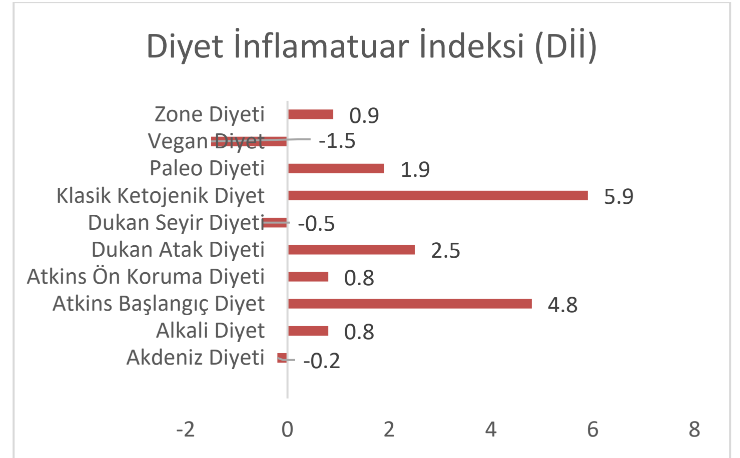
Şekil 1. Diyet modellerinin makro besin ögesi dağılımları
Figure 1. Macronutrient distributions of dietary models



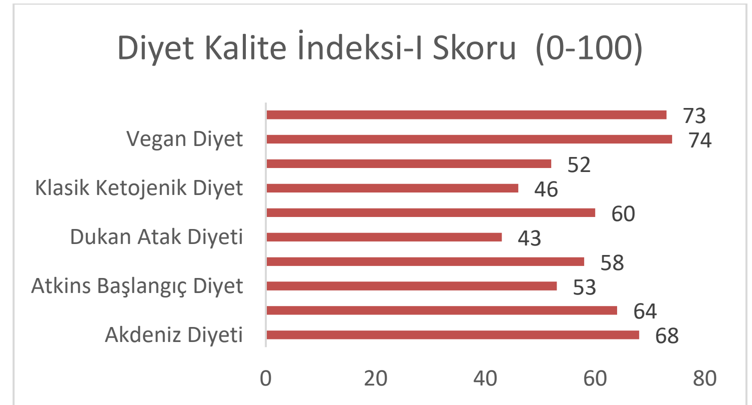
Şekil 2. Diyet modellerinin içerdiği antioksidan miktarları
Figure 2. Antioxidant content of dietary models



Şekil 3. Diyet modellerinin içerdiği ORAC değerleri
Figure 3. ORAC values of dietary models



Şekil 4. Diyet modellerinin Diyet İnflamatuar İndeksi (Dİİ) Skorları
Figure 4. Dietary Inflammatory Index (DII) Scores of dietary models



Şekil 5. Diyet modellerinin Diyet Kalite İndeksi-I Skorları
Figure 5. Diet Quality Index-I Scores of dietary models

Bu çalışmada diyet modelleri Diyet İnflamatuvar İndeksi (Dİİ) skoruna göre incelendiğinde Zone, Paleo, klasik ketojenik, Dukan Atak, Atkins Ön Koruma, Atkins Başlangıç ve alkali diyetin pozitif (+) değer yani proinflamatuar özellik gösterdiği bulunmuştur. Yine bu diyetler arasından klasik ketojenik diyetin en yüksek pozitif (+) skora sahip proinflamatuar diyet olduğu görülmektedir. Diyet İnflamatuvar İndeks (Dİİ) skoruna göre vegan, Dukan Seyir ve Akdeniz diyeti ise negative (-) yani antiinflamatuar bir değer aldığı saptanmıştır. Dİİ skoru en düşük (en antiinfamatuvar) olan diyet modeli ise vegan diyettir (-1.5). Önceki çalışmalarda da bizim çalışmamıza paralel olarak bitki temelli diyet uygulayanların Dİİ skorlarının vejetaryen olmayanlara göre daha düşük olduğu

bulunmuştur (Turner-McGrievy ve ark., 2015; Turner-McGrievy ve ark., 2021). Vegan ve Akdeniz diyeti (meyve ve sebzeler miktarının yüksek, işlenmiş besin tüketiminin az) gibi diyetler daha düşük inflamasyon düzeyleri ile ilişkilendirilirken, batı tarzı diyetler (örneğin, yüksek yağ ve basit karbonhidrat içeren) daha yüksek inflamasyon düzeyleri ile ilişkilendirilmektedir (Whalen ve ark., 2016; Neale ve ark., 2016; Neustadt ve ark., 2006). Ayrıca C, D ve E vitaminleri, betakaroten, omega-3, çoklu doymamış yağ asitleri, flavonoidler ve posa gibi spesifik besinler daha düşük inflamasyon düzeyiyle ilişkilendirilmiştir (Corley ve ark., 2019). Bu çalışmada da en yüksek C, D ve E vitaminine sahip diyet modelinin vegan diyet olduğu görülmektedir (Tablo 1). Bu durum Dİİ skorlarının daha düşük olmasının muhtemel nedenidir. Akdeniz diyetininde, dengeli besin ögesi dağılımına sahip olması, sebze ve meyve tüketiminin yüksek olması ve spesifik (resveratrol, zeytinyağı vs) besinleri içermesi nedeniyle antiinflamatuar diyet olduğunu düşünülmektedir. Bu çalışmada klasik ketojenik diyetin en yüksek Dİİ skoru sahip olmasının nedeni ise düşük meyve ve sebze tüketimini önermesi ve diğer popüler diyetlere göre en düşük A vitamin, D vitamin, folat, B12 vitamini, tiamin, riboflamin, niasin, kalsiyum, magnezyum, potasyum, fosfor, çinko, demir içeriğine sahip olmasından kaynaklanmaktadır (Tablo 1).

Diyet kalitesinin değerlendirilmesi, tek tek besin öğelerinden ziyade bütünsel diyetin kalitesi ve çeşitliliği ile ilgilidir ve diyet örüntülerinin diyet önerileriyle ne kadar uyumlu olduğunun değerlendirilmesine olanak tanır (Marshall ve ark., 2015). Ayrıca diyet kalitesinin ölçülmesi beslenme ile sağlık ve hastalık göstergeleri arasındaki ilişkilerin araştırılmasında önemli yere sahiptir (Román-Viñas. Ve ark., 2009). Bu nedenle diyet modellerinin diyet kalitelerinin belirlenmesi bu diyet modellerinin sağlık üzerine etkilerini anlamamıza yardımcı olacaktır. Diyet kalitesinin Sağlık Yeme İndeksi ile ölçüldüğü bir çalışmada makrobesin öğeleri dengeli dağılmış (86.9 ±7.7) ve bitki bazlı (86.1 ±10.7) diyetlerin Sağlık Yeme İndeksi skorlarının, düşük karbonhidratlı diyetlere (70.6 ±15.0) göre anlamlı derecede daha yüksek olduğu görülmüştür (Turner-McGrievy ve ark., 2021). Popüler diyetlerin diyet kalitesinin incelendiği bir diğer çalışmada ise Zone ve Akdeniz diyeti diyet kalitesi indeksine göre 74 puan ile diyet kalitelisi en yüksek diyetler olurken Atkins diyeti 53 puan ile diyet kalitesi en düşük diyet olarak tespit edilmiştir (Kemaloğlu ve Öner, 2021). Bu çalışmada diyet modelleri değerlendirildiğinde en yüksek DKİ-I puanı olan diyet vegan diyeti (74)'dir. Kemaloğlu ve Öner'in (2021) yaptığı çalışmaya benzer olarak Zone diyeti (73 puan) ve Akdeniz diyeti (68 puan) vegan diyetten sonra en yüksek DKİ-I puanına sahip diyetlerdir. Dukan (atak evresi) diyeti (43 puan) ve Klasik Katojenik diyet (46 puan) ise en düşük DKİ-I puanına sahip diyetlerdir. Ayrıca Kemaloğlu ve Öner'in yaptığı çalışmanın sonuçlarına benzer olarak bu çalışmada Atkins başlangıç diyeti 53 puan, Atkins ön koruma diyeti 58 puan almıştır. Daha az havyansal kaynaklı besinler, daha fazla sebze, meyve, kurubaklagiller, glisemik indeksi düşük karbonhidratlar ve sağlıklı yağlar içeren vegan, Zone ve Akdeniz diyetlerinin diğer diyet modellerine göre daha yüksek diyet kalitesine sahip olduğu görülmektedir. Bununla birlikte bu çalışmada en düşük diyet kalitesine sahip diyet olan Dukan (atak evresi) diyet diğer popüler diyetlere göre daha yüksek protein (%40), omega 6 (15.7 g) ve daha düşük posa (4.1 g) beta karoten (300 mg) ve C vitamini (16.3 mg) içermektedir (Tablo 1). Bu besin öğeleri düşük diyet kalitesinin nedeni olabilir. Klasik Katojenik diyetin ise diğer popüler diyetlere göre bu çalışmada incelenen 17 mikrobesin ögesinden on üçünde (A vitamin, D vitamin, folat, B12 vitamini, tiamin, riboflamin, niasin, kalsiyum, magnezyum, potasyum, fosfor, çinko, demir) en düşük içeriğe, en yüksek yağ (%86) oranına ve TÜBER'e göre günlük 25 g posa tüketimi tavsiyesi edilmesine ragmen 4.3 g posa içeriğine sahip olması düşük diyet kalitesini açıklayabilir (Tablo 1).

Sonuç

Günümüzde popüler diyetlere olan ilgi giderek artmasına rağmen diyetlerin sağlık üzerine etkileri hala tartışmalıdır. Diyet modellerinin makro besin öğelerinin dağılımları, içerdikleri besin ögesi miktarı, antioksidan ve antiinflamatuar içerikleri, diyetlerde oluşturulan besin çeşitliliği gibi unsurlar diyet kalitesini etkilemektedir. Bireylerin diyet modellerini seçerken bu faktörlere dikkat etmesi beslenme ile ilişkili sağlık yararlarını arttıracaktır. On popüler diyet modelini birçok açıdan incelediğimiz bu çalışmanın sonuçlarına göre; farklı kategorilerde farklı diyet modellerinin ön plana çıktığı görülmektedir. Vegan diyet toplam diyet kalitesi ve antiinflamatuar özellikleri açısından diğer diyet modellerine göre daha iyi sonuç-

lar almıştır. Paleo diyet de antioksidan özelliği ile en iyi sonuçları almıştır. Akdeniz diyeti ise tüm kategorilerde (dengeli makro ve mikro besin ögesi dağılımı, yüksek antioksidan içeriği, yüksek antiinflamatuar özellikleri ve yüksek diyet kalitesi) diğer diyet modellerine göre iyi sonuçları ile ön plana çıkmaktadır. Diyet inflamatuar özellikleri, diyet kalitesi, diyet antioksidan ve diğer besin ögesi içerikleri açısından en kötü sonuçları ise ketojenik ve Dukan diyetler almıştır. Bu çalışmanın verileri popüler diyetler arasında Akdeniz diyetinin daha iyi besin öğesi profili ile daha fazla tercih edilmesi gerektiğini, ketojenik ve Dukan diyetlerin ise daha kötü besin öğesi profillerinden dolayı daha az tercih edilmesi gerektiğini vurgulamaktadır. Diyet modellerinin besinsel kalitelerinin belirlenmesi ve toplumsal anlamda kaliteli diyetlere yönelik bilincin oluşturulması sağlık harcamalarının azalmasını ve iş gücüne katılımın artmasını sağlayıp toplumsal refahı arttıracaktır.

Etik Standartlar ile Uyumluluk

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

Etik izin: Araştırma niteliği bakımından etik izne tabii değildir.

Veri erişilebilirliği: Veriler talep üzerine sağlanacaktır.

Finansal destek: Bu çalışma herhangi bir fon tarafından desteklenmemiştir.

Teşekkür: -

Açıklama: -

Ekler

Örnek Diyet Modelleri
Sample Diet Models

1500 KKAL'LİK ATKİNS (BAŞLANGIÇ EVRESİ) DİYETİ	
KAHVALTI	**ARA**
2 adet sahanda yumurta (yağsız) 20g salatalık ½ adet avokado (80g)	35 gram mozarella peyniri 50g kereviz sapı
ÖĞLE	**ARA**
Ton balıklı maş fasulye salatası *- 160g konserve ton balığı (yağ ilavesiz)-* *50g maş fasulyesi* *- 10g yeşil/taze soğan* *- 1 yemek kaşığı limon suyu (10g)-* *1 yemek kaşığı üzüm sirkesi (10g)-* *5g doğranmış dereotu* *- 5 adet siyah zeytin (18g)*	10 adet yeşil zeytin (35g) 60g gouda peyniri
AKŞAM	
85g koyun eti pirzolası (yarım yağlı, ızgara) 30g cheddar peyniri 60g karnabahar 50g yaban turpu 2 yemek kaşığı İtalyan sosu *- limon suyu 3mL* *- sarımsak 1g* *- maydanoz 1g* *- fesleğen 1g* *- pul biber 1g* *- zeytinyağı 20mL* *- üzüm sirkesi 8mL*	

1500 KKAL'LİK ATKİNS (ÖN KORUMA EVRESİ) DİYETİ	
KAHVALTI	**ARA**
2 adet yumurta kızartılmış (yağ ilavesiz) 15 gram taze/krem peynir 37g yaban mersini 5 adet yeşil zeytin 15g tatlı badem (çiğ)	40g İsviçre/emmental peynir 1 orta boy havuç (80g)
ÖĞLE	**ARA**
60g tavuk eti (derisiz) Karışık salata (80g göbekli marul + 100g kereviz kökü + 80g havuç, 2 tatlı kaşığı zeytinyağı ile) Mayonez (25g)	Kaju fıstığı (çiğ) (15g) 1 orta boy nar (170g)
AKŞAM	
Somon balığı (150g) 100g ıspanak 50g nohut 1 tatlı kaşığı zeytinyağı	

1500 KKAL'LİK VEGAN DİYETİ	
KAHVALTI	**ARA**
1 su bardağı portakal suyu (200mL) 20g fındık ezmesi (şeker ilavesi) ¼ adet avokado (40g) Söğüş salata (limonlu) *- Salatalık (120g)* *- Domates (85g)* *- 1 yemek kaşığı limon (10g)* 1 ince dilim tam tahıllı ekmek (30g)	Yeşil Smoothie: *1 su bardağı soya sütü (200mL)* *150g ıspanak* *25 adet üzüm (100g)* *½ adet küçük boy armut (60g)*
ÖĞLE	**ARA**
Fasulye Dürüm: *- lavaş (45g)* *- 4 yemek kaşığı haşlanmış börülce (konserve) (70g)* *- 2 yemek kaşığı pirinç (20g)* *- 2 yemek kaşığı domates sosu (30g)*	1 küçük boy muz (85g) 6 adet badem (6g)
AKŞAM	
1 kâse mısır çorbası (200mL) *- 20g doğranmış kuru soğan* *- 15g doğranmış kırmızı biber* *- 1 gram sarımsak* *- 50g doğranmış patates* *- 5 gram buğday unu* *- 50 mL badem sütü* *- 50g dondurulmuş şeker mısır* *- 1g karabiber* 2 adet yeşil mercimekli biber dolması- *80g kepekli esmer pirinç* *- 20g doğranmış kuru soğan* *- 1g sarımsak* *- 5g kimyon* *- 50g yeşil mercimek konservesi* *- 1g karabiber* *- 20g domates sosu* *- 2 adet (normal/70g) yeşil dolamlık biber*	

1500 KKAL'LİK ZONE DİYETİ	
KAHVALTI (07:00)	**ARA (17:00)**
İspanyol Omleti *- 2 yk ince doğranmış kuru soğan (40g) - İri doğranmış yeşilbiber (20g)* *- 4 yumurta akı (120g)* *- Az yağlı beyaz peynir (30g)* *- ¼ su bardağı konserve börülce (25g) - 1 tatlı kaşığı zeytinyağı* *- 1 yemek kaşığı Ayçiçek yağı (12g)* 60 g yulaf ezmesi 1 orta boy portakal (140g)	50 gram az yağlı beyaz peynir 150g siyah üzüm 3 adet siyah zeytin
ÖĞLE (12:00)	**ARA (23:00)**
Izgara tavuk salatası *-50g göbekli marul* *- 100g brokoli* *- 15g doğranmış yeşilbiber* *- 85g doğranmış domates* *- 1 yk zeytinyağlı sirke sosu (1 çay kaşığı zeytinyağı (5g) + 1 tatlı kaşığı üzüm sirkesi (5g))* *- 1 yk limon suyu (10mL)* *- Tavukgöğsü (80g)* 1 orta boy armut	1 su bardağı yağsız inek sütü (200 mL) 8 adet fındık
AKŞAM (19:00)	
Izgarada somon balığı *- somon balığı (120g)* *- 5mL zeytinyağı* *- ½ çay kaşığı kuru biberiye (2g) - ½ çay kaşığı kuru tarhun otu (1g) - Kuru dereotu (3g)* *- 130g jülyen doğranmış sakız kabağı* 1 orta boy elma	

1500 KKAL'LİK ALKALİ DİYET	
KAHVALTI (09:00)	**ARA (14:00)**
4 yumurta akı ve 1 yumurta sarısı ile omlet (5g tereyağ ile) Lor peynir (yağsız, tuzsuz) (30g) 5 adet tuzsuz siyah zeytin (18g) Tatlı badem (10g) Söğüş salata (limonlu) *- Salatalık (120g)* *- Domates (85g)* *- 1 yemek kaşığı limon (10g)* 3 ince dilim glütensiz ekmek (75g	1 fincan yeşil çay (150mL) 2 parça bitter çikolata (10g) 1 orta boy elma (120g) 1 tatlı kaşığı tarçın katılmış 1 bardak alkali su
ÖĞLE (12:30)	
1 kepçe sebze çorbası (150mL) *- 5g patates* *- 5g havuç* *- 2g kereviz kökü* *- 5g bezelye* *- 7g buğday unu* *- 3g sıvıyağ Ayçiçek* *- 3g taze fasulye* *- 0,1g karabiber* 100-110g fasulye piyazı *- 40g kuru fasulye* *- 0,25g limon* *- 5g elma sirkesi* *- 1g maydanoz* *- 0,2g kırmızı pul biber* *- 10g soğan* *- 7g zeytinyağı* *- 5g çarliston biber* 150g somon balığı ve 60g tatlı patates ile fırınla	
AKŞAM (17:00)	
1 adet zeytinyağlı imambayıldı yemeği *- 150g patlıcan* *- 25g soğan* *- 40g domates* *- 10g çarliston biber* *- 5g domates salçası* *- 1g sarımsak* *- 1g maydanoz* *- 10mL zeytinyağı* *- 0,1g karabiber* 30g tavuk eti (derisiz) 40g esmer pirinç	

1500 KKAL'LİK DUKAN (ATAK EVRESİ) DİYETİ	
KAHVALTI	**ARA (10:00 – 11:00)**
Aspartamlı çay (1g tatlandırıcılı, 120mL) Beyaz peynir (200g) 1 adet haşlanmış yumurta (50g)	150g yağsız yoğurt 1,5 yemek kaşığı yulaf kepeği (22g)
ÖĞLE	**ARA (16:00)**
150g çiğ dil balığı (buharda pişecek) - *10mL limon suyu* *- 0,5g karabiber* *- 1g maydanoz,* piştikten sonra üzerine eklenecek. 175g az yağlı beyaz peynir	150g yağsız yoğurt 30g hindi eti (derisiz)
AKŞAM	
Hardallı tavşan *- 60g tavşan eti (az yağlı, çiğ)* *- 5g toz hardal* *- 2g kuru kekik* *Sosu içim;* *- 24mL Ayçiçek yağı* *- 100g yağsız yoğurt* *- 1g karabiber* Küçük adalar *- 1 adet yumurta (akı ve sarısı ayrı olarak kullanılacak) (50g)* *- 125 mL inek sütü (yağsız)* *- 0,5g tatlandırıcı (aspartam)*	

1500 KKAL'LİK DUKAN (SEYİR EVRESİ) DİYETİ	
KAHVALTI	**ARA (10:00 – 11:00)**
Aspartamlı çay (1g tatlandırıcılı, 120mL) 1 adet haşlanmış yumurta (50g) 250g yoğurt (yağsız) 2 yemek kaşığı yulaf kepeği (30g)	100g az yağlı beyaz peynir
ÖĞLE	**ARA (16:00)**
100g tavuk eti Beyaz soslu ıspanak *- 150g ıspanak* *Beyaz sos için;* *- 2 adet yumurtanın sarısı (40g)* *- 150g yoğurt (yağsız)* *- 1 su bardağı inek sütü (yağsız)(200mL) - 1g karabiber* Küçük adalar *- 1 adet yumurta (akı ve sarısı ayrı olarak kullanılacak)(50g)* *- 125 mL inek sütü (yağsız)* *- 0,5g tatlandırıcı (aspartam)*	60g hindi eti (derisiz) 150g yoğurt (yağsız)
AKŞAM	
200g somon balığı Limonlu brokoli *- 100g brokoli* *- 1 yemek kaşığı limon suyu (10mL)- 15g maydanoz* 150g yoğurt (yağsız)	

1500 KKAL AKDENİZ DİYETİ	
Kahvaltı (07.00 – 08.00)	**Ara (15.00)**
Şekersiz çay 100g %1 yağlı lor peynir 1 adet rafadan yumurta 10 adet çiğ badem 2 dilim tam tahıllı ekmek (60g)	1 orta boy portakal (ort. 160g) 10g ceviz içi 2 tepeleme yemek kaşığı tam yağlı yoğurt (80g) + 25g yulaf ezmesi
Öğle (12.00 – 13.00)	
Zeytinyağlı enginar yemeği *- 150g enginar göbeği* *- 5g soğan* *- 10mL limon suyu* *- 10g patates* *- 10g havuç* *- 10g dondurulmuş bezelye* *- 0,5g buğday unu* *- 1g sarımsak* *- 1g maydanoz* *- 10mL zeytinyağı* *- 1g toz şeker* *- 0,1g karabiber* 80g tam yağlı yoğurt 2 dilim tam buğday ekmeği (60g) 150g üzüm	
Akşam (19:00 – 20:00)	
90g somon balığı Zeytinyağlı barbunya pilaki *- 35g kuru barbunya fasulyesi* *- 8g domates salçası* *- 7g havuç* *- 10g patates* *- 7g soğan* *- 10mL limon suyu* *- 1g maydanoz* *- 10mL zeytinyağı* *- 1g sarımsak* *- 0,2g pul biber* Mevsim salata *- 25g havuç* *- 20g kırmızı kıvırcık marul* *- 10g marul* *- 10mL limon* *- 1g maydanoz* *- 8g turp* *- 5mL zeytinyağı* 1 adet elma (120g)	

1500 KKAL’LİK KETOJENİK (KLASİK KETOJENİK DİYET) DİYET	
KAHVALTI	**ARA**
Kıymalı yumurta *- 50g tavuk yumurtası* *- 24g dana kıyma* *- 40mL zeytinyağı* *- 5 adet siyah zeytin (18g)* *- 20g domates* *- 25g salatalık*	100g tam yağlı yoğurt 25mL zeytinyağı 50g çilek
ÖĞLE	
Kıymalı kabak yemeği *- 70g sakız kabağı* *- 35g dana kıyma* *- 10g kuru soğan* *- 4g domates salçası* *- 15g çarliston biber* *- 40mL zeytinyağı* 60g tam yağlı yoğurt	
AKŞAM	
Kremalı tavuk *- 30g tavukgöğsü* *- 13mL %10 yağlı krema* *- 10g kuru soğan* *- 18mL zeytinyağı* *- 30g kültür mantarı*	

1500 KKAL'LİK PALEO (TAŞ DEVRİ) DİYETİ	
KAHVALTI	**ARA**
1 adet çılbır usulü haşlama yumurta (50g) 10 adet badem 160 gram portakal	30g tavuk eti (derisiz) ½ dilim avokado (80g) 120 g elma 7 adet fındık
ÖĞLE	
Tavuk fajita salatası *- 1 yemek kaşığı zeytinyağı* *- 10 gram doğranmış soğan* *- 150 gram derisiz tavuk* *- ½ çay kaşığı kimyon* *- 1 çay kaşığı kekik (kuru)* *- 10 gram doğranmış kırmızıbiber- 20 g kırmızı marul yaprağı* *- 85g domates* *- ½ adet avokado*	
AKŞAM	
Izgara somon *- 5g hindistancevizi yağı* *- 200 gram somon* *- 10g pikan cevizi* *- 2 çay kaşığı biberiye* Fırında taze fasulye *- 100 gram taze fasulye* *- 1 tatlı kaşığı kekik (2g)* *- 2 tatlı kaşığı zeytinyağı (10mL)*	

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Kış ve ilkbaharda marketlerden toplanan tereyağı örneklerinde Aflatoksin M1 düzeyinin araştırılması

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ÖZ

Hayvan yemlerinde bulunan Aflatoksin B1 (AFB1), hayvanlarda M1'e (AFM1) dönüşerek bu hayvanların sütünden elde edilen tüm süt ürünlerini kontamine eder. Bu çalışmada Türkiye'deki yasal toksin sınırı olan 0.05 µg/kg (50 ng/L) temel alınarak çeşitli marketlerden 42'si Ocak-Şubat ve 42'si Mayıs aylarında toplanan 84 tereyağı örneğinde AFM1 düzeyleri duyarlılığı yüksek olan kompetitif ELISA yöntemiyle araştırılmıştır. İncelenen 84 tereyağı örneğinin 79' unda (%94) farklı düzeylerde AFM1 varlığı saptanırken, bu örneklerinin 67'sinde (%79.7) yasal sınırın aşıldığı belirlenmiştir. Sonuçlar mevsimsel açıdan değerlendirildiğinde, Ocak-Şubat aylarına (kış) ait 42 tereyağı örneğinin 37'sinin (%88.1), Mayıs ayında (ilkbahar) toplanan 42 tereyağı örneğinin tümünün (%100) çeşitli düzeylerde AFM1 ile kontamine olduğu saptanmıştır. Çalışma sonucunda, tereyağlarında belirlenen AFM1 düzeylerinin ciddi halk sağlığı sorunu oluşturabileceği, bu duruma sebep olabilecek hayvan yemlerinin AFB1 düzeylerinin ve bu yemlerin saklandığı depo koşullarının düzenli kontrolünün sağlanması gerektiği düşünülmüştür. İlkbaharda toksin düzeylerinin kış mevsimine göre daha yüksek olması, hava koşulları nedeniyle hayvanların meraya çıkamadığı ve depo yemleri ile beslenmeye devam ettikleri şeklinde yorumlanmıştır.

Anahtar Kelimeler: Aflatoksin, *Aspergillus*, Mikotoksin, AFM1, Tereyağı

ABSTRACT

Investigating the aflatoxin M1 level in butter samples collected from markets in winter and spring

Aflatoxin B1 (AFB1) found in animal feeds is converted to M1 (AFM1) in animals. It contaminates all dairy products made from the milk of these animals. In this study, AFM1 levels were investigated by competitive ELISA, which is a highly sensitive method in 84 butter samples collected from various markets based on the legal toxin limit of 0.05 µg/kg (50 ng/L) in Turkey. While the presence of AFM1 at different levels was detected in 79 (94%) of the 84 butter samples examined, it was determined that the legal limit was exceeded in 67 (79.7%) of these samples. When the results are evaluated from a seasonal perspective, 37 (88.1%) of 42 butter samples collected in January-February and all (100%) in May were contaminated with AFM1 at various levels. As a result of the study, it was thought that the AFM1 levels determined in butter samples may be a severe public health problem and that the AFB1 levels of animal feeds and store conditions should be controlled regularly. The fact that toxin levels were higher in the spring than in the winter was interpreted as the animals being unable to go to the pasture due to weather conditions and continuing to feed on store feed.

Keywords: Aflatoxin, *Aspergillus*, Mycotoxin, AFM1, Butter

Giriş

Mikotoksinler, özellikle tahıllarda filamenöz mantar türleri tarafından oluşturulan düşük molekül ağırlığına sahip ikincil metabolitlerdir. Bu bileşiklerle kontamine olmuş gıdalar ve yemler, insan ve hayvan sağlığına zararlıdır (Cimbalo et al., 2020). Yirminci yüzyılın ortalarına kadar, hayvan ve insanların tükettiği gıdalarda mantarların zararlı rolü üzerine çok fazla çalışma yapılmamıştır. *Aspergillus flavus* tarafından oluşturulan toksinin keşfedilmesine ve aflatoksin olarak isimlendirilmesine yol açan turkey X hastalığı sebebiyle, İngiltere'de binlerce hindinin ölümü sonucunda 1960'lı yıllarda mikotoksin çalışmaları artmıştır. Aflatoksinler (AF), okratoksinler (OTA) ve fumonisinler halk sağlığını tehdit eden başlıca mikotoksinlerdir. En sık karşılaşılan mikotoksinler *Aspergillus* spp. tarafından oluşturulan aflatoksin, okratoksin A ve patulin, *Penicilium* spp. tarafından oluşturulan fumonisin, zearalenon (ZEN), T-2 ve HT-2 toksinleri gibi trikotesenler ve deoksinivalenol (DON) ile *Claviceps purpurea* tarafından oluşturulan ergot alkaloidleri (EA) dir. Mikotoksinler, maruz kalma derecesine ve klinik belirtilere bağlı olarak akut, subakut veya kronik olabilen mikotoksikoz oluştururlar. Aflatoksinlerin vücutta mutajenik, karsinojen, teratojenik ve immünosupresif etkileri vardır. AFB1, aflatoksinler içinde en toksik olandır ve Uluslararası Kanser Araştırmaları Ajansı tarafından yapılan sınıflandırmada Grup 1, AFM1 ise Grup 2 karsinojen olarak bildirilmiştir. Özellikle *A. flavus* ve *A. parasiticus* ve de nadiren *A. nomius*'un oluşturduğu aflatoksinler AFB1, AFB2, AFG1, AFG2, AFM1 ve AFM2 olarak sınıflandırılmıştır (Yang et al., 2020; Waithaka et al., 2022; Monter Arciniega et al., 2022; Ekici et al., 2016; Becker-Algeri et al., 2016; Aranega & Oliveira, 2022).

Aflatoksin M1 (AFM1); aflatoksin B1 (AFB1) ile kontamine olan yemleri tüketmiş olan hayvanların sütlerinde görülen, hepatokarsinojenik etkisi bulunan bir metabolittir. AFM1, AFB1' in hidroksillenmiş ara ürünüdür. AFB1 ile kontamine olan yemlerle beslenen hayvanlarda AFB1 metabolize olarak, AFM1'e dönüşür. Besinle alınan AFB1 yaklaşık % 0.3-6.2 olarak sütlerde AFM1 olarak bulunur. AFM1, sterilizasyona ve pastörizasyona dirençlidir, bundan dolayı AFM1 içeren sütlerden oluşan tüm ürünler bu toksini bulundurur. Bu nedenle özellikle süt ve sütten elde edilen ürünlerin AFM1 analizi ve incelenmesi günümüzde önem kazanmıştır (Monter Arciniega et al., 2022; Ekici et al., 2016; Becker-Algeri et al., 2016).

Birleşmiş Milletler Gıda ve Tarım Örgütü (FAO), dünyadaki mahsullerin takriben %25'inde mikotoksin bulaşının olduğunu belirtmiştir. Bu sebeple, Amerika Birleşik Devletleri (ABD)' nde bulunan Gıda ve İlaç Dairesi (FDA), Avrupa Birliği (AB) ve dünyadaki ülkelerin birçoğu, kontaminasyonu azaltmak ve önüne geçebilmek için gıdalardaki ve yemlerdeki mikotoksinler için bazı limitler belirlemiştir (Monter Arciniega et al., 2022). Türk Gıda Kodeksi Bulaşanlar Yönetmeliği'nde yayımlanan süt ürünlerinde AFM1 limit değeri; çiğ süt, ısıl işlem görmüş süt, süt bazlı ürünlerin üretiminde kullanılan sütte 0,05 µg/kg, bebek formülü ve devam formülünde 0,025 µg/kg ve bebek ve küçük çocuk ek gıdalarında 0 (sıfır) µg/kg' dır (Türk Gıda Kodeksi, 2023). Günümüzde yapılan çalışmalar incelendiğinde, küresel ısınma, daha yüksek sıcaklıklar, daha düşük yağış oranı, su kıtlığı, kuraklık, sel ve atmosferdeki daha yüksek karbondioksit miktarı sonucunda verimin düştüğü, mikotoksin kontaminasyon oranlarının arttığı ve oluşan hastalık sıklığında artış olduğu görülmektedir (Monter Arciniega et al., 2022; Sujayasree et al., 2022) Mevsimsel ve iklimsel değişiklikler, birincil üretimden tüketime kadar gıda zincirinin belirli aşamalarında gıda güvenliğinde tehlike oluşturabilmektedir. Birçok bilim insanı, havanın ve mevsimlerin aflatoksin oluşumu üzerindeki etkisini kabul etmektedir (Ekici et al., 2016; Sujayasree et al., 2022; Ansari et al., 2019). Gıda güvenliğinin sağlanması ve gıda kökenli zehirlenmelerin kontrol altına alınması için mikotoksin teşhisi büyük önem taşımaktadır. Bu nedenle değişik analiz yöntemleri geliştirilmiştir. Mikotoksin düzeylerinin belirlenmesinde kullanılan yöntemlerden bazıları; Enzim Bağlanmış İmmunoabsorbant yöntemi (ELISA–Enzyme Linked Immunosorbent Assay), İnce Tabaka Kromatografisi (İTK), Yüksek Basınçlı Sıvı Kromatografisi (HPLC), Kolon Kromatografisi, Gaz Kromatografisi/Kütle Spektrometresi (GC/MS), Enzim Aktivitesine Bağlı İmmunoteknik (Enzyme Multiplied Immunotechnique/EMIT) dir. Süt ve süt ürünlerinde aflatoksin M1 miktarının belirlenmesi için en fazla tercih edilen yöntem kolay uygulanabilir ve duyarlılığı yüksek olan ELISA'dır. Antikora bağlanmış enzim aktivitesinin araştırılması temeline dayanan kantitatif bir ölçümdür. Kompetitif ELISA yönteminde ise, yüzey yapısı araştırılan toksine özel antikorlarla kaplanmış özel plaklar kullanılır ve yöntemin çalışma metodu serbest antijenlerin ve işaretlenen antijenlerin antikorlara karşı yarışmasına dayanır. Sonuçlar standart gruplar ile karşılaştırılarak değerlendirilir (Aranega & Oliveira, 2022; Açu & Özdestan Ocak, 2019).

Çalışmamızda 42'si ocak-şubat (kış mevsiminde) ve 42'si mayıs (ilkbaharda) aylarında olmak üzere marketlerden farklı lot numaralarına sahip (aynı ürünle tekrar çalışmamak için) 84 tereyağı örneği toplanmış ve örneklerdeki AFM1

düzeylerinin mevsimsel değişiklik gösterip göstermediği kompetitif ELISA yöntemiyle araştırılmıştır. Mevcut yasal toksin düzeyleri göz önünde bulundurularak, beslenmemizde büyük öneme sahip tereyağlarında potansiyel bir risk olup olmadığı ve toksin miktarlarının mevsimlere göre değişkenliği araştırılmış ve epidemiyolojik verilere katkı sağlanması amaçlanmıştır.

Materyal ve Metot

Tereyağı Örneklerinin Toplanması

Yapılan bu çalışmada 42'si ocak-şubat (kış) ve 42'si mayıs aylarında (ilkbahar) olmak üzere marketlerden farklı lot numaralarına sahip 84 tereyağı örneği toplanmış ve örneklerdeki AFM1 düzeylerinin mevsimsel değişiklik gösterip göstermediği kompetitif ELISA (Biopharm Ridascreen Aflatoxin M1, Darmstadt/Germany) (Art No: R1121) yöntemi ile araştırılmıştır. Örnekler soğuk zincir altında laboratuvara getirilerek analizleri gerçekleştirilmiştir.

Kit İçeriği

Analiz amacıyla Biopharm Ridascreen Aflatoxin M1, Darmstadt/Germany test kiti (Art No: R1121) kullanılmış ve ölçümler yapılmıştır (Şekil 1). Kullanılan ELISA kitinin içeriğinde standart solüsyonlar, substrat kromojen, yıkama solüsyonu, konjugat, buffer 1, buffer 2, stop solüsyonu hazır olarak yer almıştır. Bir tane Ridascreen kitiyle standartlar dahil olmak üzere, toplamda 48 tereyağı örneğinde AFM1 düzeyi belirlenmiştir. Sonuçlar değerlendirilirken 0.05 µg/kg (50 ng/L) yasal sınır değer olarak kullanılmıştır.

Şekil 1. Analiz için kullanılan Biopharm Ridascreen Aflatoxin M1 (Darmstadt/Germany) test kiti

Figure 1. Biopharm Ridascreen Aflatoxin M1 (Darmstadt/Germany) test kit used for analysis

Tereyağlarının Analize Hazırlanması

Tereyağı örnekleri hassas terazide 3 gr olacak şekilde tartılmış ve 50 mL'lik falkon tüplere alınarak, 40°C'lik su banyosunda eritilmiştir. Üzerine 3 mL n-hekzan ve 3 mL distile su ile sulandırılmış %70'lik metanol eklenmiştir. Kimyasallar eklendikten sonra örnekler vortekslenmiştir. Karıştırma işlemine tüp tersyüz edilerek 15 dakika daha devamedilmiştir. Yağ tabakalarından ayrılması için örnekler 10°C'de, 4000 rpm'de 10 dakika santrifüj edilmiştir. Santrifüj sonrasında üstte ayrılan süpernatant (krema tabakası) Pastör pipetiyle tereyağı örneğinden ayrılmıştır. Örnekleri seyreltmek için kit içerisinde bulunan buffer solüsyonundan 1.5 mL'lik eppendorf tüplerine 800 µL ve yağı alınan tereyağı örneklerinden 50 µL aktarılmış ve tüp vortekslenmiş, buffer 1 çözeltisi ile 1/17 oranında dilüe edilmiştir.Sonrasında hazırlanan tereyağı örnekleri için dilüsyon faktörü 20 olarak belirlenmiştir. Hazırlanan bu tereyağı örneklerinden mikroplaktaki her kuyucuğa 100 µL ilave edilmiştir.

Yıkama Solüsyonunun Hazırlanması

Kit içerisinden hazır halde bulunan yıkama tozunun üzerine kullanım talimatı doğrultusunda 1 L distile su eklenerek homojen bir karışım elde edilmiştir. Bu karışım mikroplakların yıkanması sırasında kullanılmıştır.

Testin Uygulanması

Çalışılırken kit içerisinde bulunan tüm reaktifler ELISA aşamasından önce oda sıcaklığına getirilmiştir. Test uygulanırken mikro kuyucukların tamamen kurumamasına dikkat edilmiştir.Testin doğru uygulandığı büyük oranda mikro kuyucukların yıkanma aşamasındaki dikkate bağlı olduğu için yıkama işlemlerinde prosedürde belirtilen yıkama aşamalarına dikkat edilmiştir ve plaklara reaktifleri ilave ettikten sonra inkübasyonları esnasında direkt güneş ışığına temasından kaçınılmıştır. Üretici firma yöntemin tespit limitini 5 ng/L, sütte geri kazanım oranını %100 olarak bildirmiştir.

- Her kit için standartlar ve tereyağı örnekleri iki kez çalışılmıştır. Mikroplakların ilk 12 kuyusu altı standart için kullanılıp, diğer kuyular örnekler için kullanılmıştır.

- Her kuyuya 100 µL antikor solüsyonu eklenip sonrasında plaklar yavaşça çalkalanmış ve 15 dakika boyunca oda sıcaklığında (25°C) ışık görmeyecek şekilde inkübe edilmiştir.

- Her bir kuyucuk 250 µL yıkama solüsyonu ile yıkanmış ve bu işlem üç kez tekrar edilmiştir.

- Her standarttan iki kez olacak şekilde, ilk 12 kuyucuğa 100 µL standart solüsyonlarından eklenmiştir.
- Diğer kuyucuklara tereyağı örneklerinden iki defa 100 µL eklenmiştir.
- Plaklar hafif bir şekilde çalkalandıktan sonra oda sıcaklığında (25°C), karanlık ortamda, 30 dakika boyunca inkübe edilmiştir.
- Her kuyucuk 250 µL yıkama solüsyonu ile yıkanmış ve bu işlem üç kez tekrar edilmiştir.
- Her bir kuyucuğa 100 µL konjugat solüsyonu eklenmiş, plaklar yavaşça çalkalanmış ve oda sıcaklığında (25°C), karanlık ortamda, 15 dakika inkübe edilmiştir.
- Her bir kuyucuk 250 µL yıkama solüsyonu ile yıkanmış ve bu işlem üç kez tekrarlanmıştır.
- Her bir kuyucuğa 100 µL kromojen solüsyonu eklenmiş, plaklar yavaşça çalkalanıp oda sıcaklığında (25°C), karanlıkta 15 dakika inkübe edilmiştir.
- Her bir kuyucuğa 100 µL stop solüsyonu eklenmiştir ve plaklar yavaşça çalkalanmış ve örnekler 450 nm dalga boyunda spektrofotometrede (ELx800 BioTek Instruments Inc, USA) analiz edilmiştir.

Bulgular ve Tartışma

Çeşitli marketlerden kış ve ilkbahar mevsimlerinde toplanan farklı lot numaralarına ait 84 tereyağı örneğinin; kış mevsiminde toplanan 42 örneğe ait AFM1 düzeyleri 0.05µg/kg (50 ng/L) yasal sınır değeri temel alınarak Tablo 1'de, ilkbahar mevsiminde toplanan 42 örneğe ait AFM1 düzeyleri Tablo 2'de gösterilmiştir.

Kış mevsiminde (ocak-şubat) toplanan tereyağı örnekleri AFM1 açısından değerlendirildiğinde, 42 tereyağı örneğinin 37'sinin (%88.1) saptanabilir düzeylerde AFM1 ile kontamine olduğu belirlenmiş ve bu örneklerin 32'sinde (%76) sınır değerin aşıldığı belirlenmiştir. Beş örnekte ise sınır değerinin altında AFM1 saptanırken (%12), beş örnekte (%12) toksine rastlanmamıştır (Tablo 1).

İlkbaharda (mayıs) toplanan tereyağı örnekleri AFM1 açısından değerlendirildiğinde, 42 tereyağı örneğinin tümünün (%100) çeşitli düzeylerde AFM1 içerdiği belirlenmiştir. Otuz beş örnekte sınır değerin aşıldığı (%83.3) saptanırken, bu örneklerin 10'unda (%23,8) çok yüksek düzeyde (>100) toksin belirlenmiştir. Yedi örnekte sınır değerin altında AFM1 (%16.6) saptanmıştır (Tablo 2).

Sonuçlar toplu olarak değerlendirildiğinde, 84 tereyağı örneğinin 79' unda (%94) farklı düzeylerde AFM1 miktarı saptanırken, bu tereyağı örneklerinin 67'sinde (%79,7) yasal sınırın aşıldığı belirlenmiş ve beş (%5,9) örnekte toksine rastlanmamıştır.

Kış mevsiminde toplanan tereyağı örneklerinin AFM1 konsantrasyonlarının hesaplanması için kullanılan kalibrasyon grafiği Şekil 2'de, ilkbahar mevsiminde toplanan tereyağı örneklerinin AFM1 konsantrasyonlarının hesaplanmasında kullanılan kalibrasyon grafiği Şekil 3'de gösterilmiştir.

Tablo 1. Ocak-Şubat aylarında toplanan tereyağlarında belirlenen AFM1 düzeylerinin standart aralıklarına göre dağılımı

Table 1. Distribution of AFM1 levels determined in butter samples collected in January-February according to their standard ranges

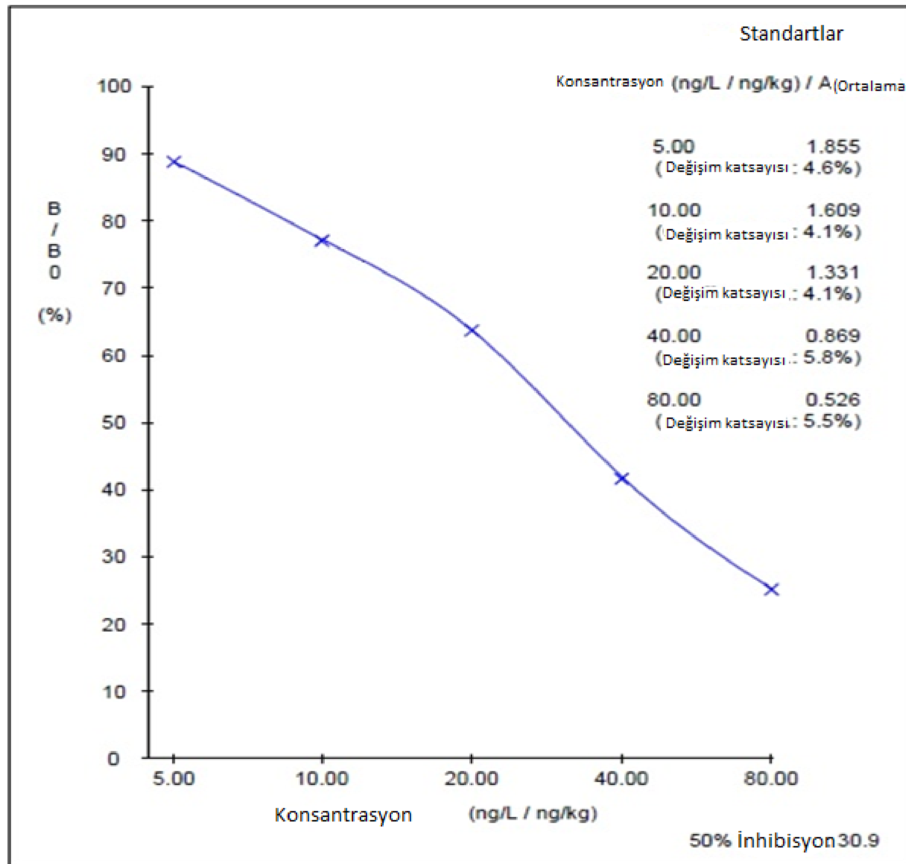
Standartlara göre AFM1 düzeyi (ng/L)	Saptanan AFM1 düzeyi (ng/L)									Toplam örnek sayısı
0	0	0	0	0	0					5
40-80	43.78*	47.34*	47.95*	48.15*	48.97*	50.83	51.67	52.30	52.51	35
	53.37	53.79	55.09	55.31	55.31	57.07	57.07	57.29	58.41	
	58.86	59.76	61.59	63.22	64.40	64.63	65.58	66.06	70.45	
	70.95	72.45	74.48	74.74	76.29	76.81	79.70	61.59		
80-100	80.23	95.39								2

*Sınır değerin altındaki AFM1 düzeyleri (beş örnek)

Tablo 2. Mayıs ayında toplanan tereyağlarında belirlenen AFM1 düzeylerinin standart aralıklarına göre dağılımı.

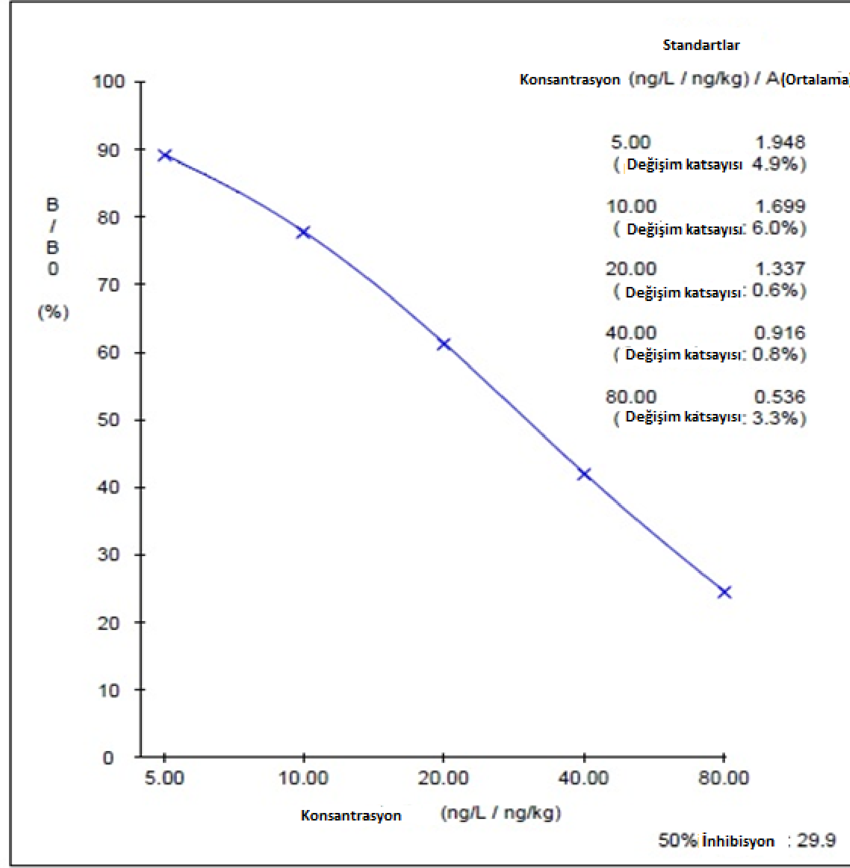
Table 2. Distribution of AFM1 levels determined in butter collected in May according to their standard ranges.
*Sınır değerin altındaki AFM1 düzeyleri (yedi örnek)

Standartlara göre AFM1 düzeyi (ng/L)	Saptanan AFM1 düzeyi (ng/L)									Toplam örnek sayısı
40-80	42.38*	43.22*	45.35*	45.57*	48.65*	49.55*	49.77*	50.68	51.14	26
	52.53	55.83	55.83	57.77	60.25	61.50	62.52	63.55	64.58	
	68.01	69.35	70.98	72.08	72.36	72.36	76.29	78.88		
80-100	82.71	84.51	90.05	91.95	91.95	95.14				6
>100	108.99	111.10	114.67	116.12	117.94	121.99	123.48	123.85	134.54	10
	152.53									



Şekil 2. Ocak-Şubat aylarına ait standart eğri

Figure 2. Standard curve of January-February



Şekil 3. Mayıs ayına ait standart eğri

Figure 3. Standard curve of May

Süt ve süt ürünleri beslenmemizde büyük öneme sahip temel besin kaynaklarındandır. Pastörizasyon ve sterilizasyon gibi yüksek ısıl işlemlere dayanıklı olan AFM1, sütleri ve bu sütlerden üretilen tüm gıdaları kontamine etmektedir. AFM1 insanlarda hepatotoksik, kansirojen, genotoksikdir ve immün sistemi baskılama yeteneğindedir, bu nedenle ülkelerde 0,03 ila 0,250 µg/kg arasında değişen yasal sınırlar içeren uluslararası düzenlemeler yapılmıştır (Monter Arciniega et al., 2022; Aranega & Oliveira, 2022). Türk Gıda Kodeksi'nin Bulaşanlar Tebliği'ne göre süt temelli ürünlerde belirlenen aflatoksin sınırı 0,05 µg/kg (50 ng/L)'dır (Türk Gıda Kodeksi, 2023).

Dünya çapında, her yaşta tüketilen ve beslenmemizde önemli bir yeri olan süt ve bunun ürünü olan tereyağı gibi süt ürünlerinde AFM1 varlığıyla ilgili birçok çalışma yapılmıştır.

Pakistan Lahor'da yapılan bir çalışmada (Gill et al., 2022) süt ve süt ürünlerindeki AFM1 varlığı araştırılmıştır. Toplam 60 süt örneği ve 30 tereyağı, 30 peynir, 30 krema ve 30 yoğurt dahil olmak üzere 120 süt ürünü örneğinin incelendiği çalışmada, tereyağı örneklerinin %16.7'sinde AFM1 düzeyi sınırın üzerinde saptanmıştır.

Hindistan Ludhiana'da yapılan bir çalışmada (Kaur et al., 2021) yedi tesisten 109 süt, 97 tereyağı, 99 peynir ve 103 lor olmak üzere toplam 408 örnek toplanmıştır. Örnekler ELISA yöntemiyle test edilmiştir. Süt ve süt ürünlerinin tümünde (%100) AFM1 saptanmıştır. Çalışmada tüketim miktarları sağlık riski değerlendirmesi yapılmıştır, çalışma alanındaki tüketicilerin, özellikle çocukların, düşük vücut ağırlıkları ve daha yüksek süt alımları nedeniyle AFM1 açısından daha yüksek sağlık riski altında olduğu gösterilmiştir.

Ülkemizde beş şehri içine alarak yapılan bir (İstanbul, Tekirdağ, İzmir, Konya ve Kayseri) çalışmada (Tekinşen& Uçar, 2008), 100 krem peynir ve 92 tereyağı örneğindeki AFM1 miktarları ELISA yöntemiyle belirlenmiştir. Tereyağı örneklerinin %100'ünde 10 ila 7000 ng/kg ve krem peynir örneklerinin %99'unda 0 ila 4100 ng/kg aralığında AFM1 tespit etmişlerdir.

Kayseri'deki bir diğer çalışmada (Özkan & Onmaz, 2019) kaymak ve tereyağı örneklerinde AFM1 varlığı araştırılmıştır. Çalışmada rastgele toplanmış olan 100 örnekte (50 tereyağı ve 50 kaymak) ELISA tekniği kullanılmış, örneklerin tamamında AFM1 saptanmıştır. AFM1 kontaminasyon seviyeleri tereyağı örneklerinde 6.58-128.69 ng/kg arasında tespit edilmiştir. Yalnızca bir (%2) tereyağı örneğinde AFM1 konsantrasyonu Türk Gıda Kodeksi'nin belirlediği yasal sınırın üzerinde çıkmıştır.

Adana'da yapılan bir çalışmada (Var & Kabak, 2009) 20 kaşar peyniri, 20 süt, 10 tereyağı ve 20 beyaz peynir örneğinden oluşan toplam 70 süt ürününde ELISA ile AFM1 analizi yapılmıştır. Yasal limit sütte 50 ng/L olarak ve tereyağı, beyaz peynir ve kaşar peynirinde ise 25 ng/kg olarak kabul edilmiştir. Analiz edilen 70 süt ürününün 49'unda (%70) AFM 1 düzeyinin 10-388 ng/kg arasında olduğu bulunmuştur. On tereyağı örneğinin üçünde AFM 1 kontaminasyon düzeyi 40-70 ng/kg arasında bulunmuş; üç süt, iki tereyağı (% 2.85), bir beyaz peynir ve bir kaşar peyniri örneğinde AFM1 seviyeleri Türk Gıda Kodeksi limitlerinin üzerinde saptanmıştır.

Bu çalışmada İstanbul ilindeki marketlerden kış mevsiminde (ocak ve şubat aylarında) farklı lot numaralarına sahip 42 ve ilkbahar mevsiminde (mayıs ayında) 42 adet tereyağı olmak üzere 84 örnek toplanmış ve ELISA yöntemi kullanılarak analiz edilmiştir. seksendöert tereyağı örneğinin 79' unda (%94) farklı düzeylerde AFM1 varlığı saptanırken, bu tereyağı örneklerinin 67'sinde (%79.7) yasal sınırın aşıldığı belirlenmiş ve beş (%5.9) örnekte toksine rastlanmamıştır. Çalışmamızın sonuçları tereyağı örneklerinin %94'ünün kontamine olduğunu ve %79'unda yasal sınırın aşıldığını göstermektedir. Bu durumda, süt ve süt ürünlerindeki aflatoksin miktarlarının ve en başta yem depolama koşullarının iyileştirilmesi ve daha sıkı kontrol yöntemlerinin uygulanmasının uygun olacağı düşünülmüştür.

Süt ve süt ürünlerindeki AFM1 miktarlarının mevsimsel değişkenlik de gösterdiği birçok çalışmada gösterilmiştir.

Hırvatistan'daki bir çalışmada çiftliklerden ve pazarlardan Şubat-Temmuz 2013 tarihleri arasında toplanan çiğ süt (3716) ve UHT süt (706) örneklerinde AFM1 kontaminasyonu araştırılmıştır. Çiğ sütlerin toplam %27.8' inde ve UHT sütlerin %9.64'ünde yasal sınırı aşan AFM1 seviyeleri belirlenmiştir. Şubat, mart, mayıs ve haziran aylarında toplanan örneklerde, çiğ ve UHT süt örnekleri arasında ortalama AFM1 konsantrasyonlarında istatistiksel farklılıklar gözlenmiştir. Hem çiğ hem de UHT süt örneklerinde AFM1 konsantrasyonları mayıs ayından temmuz ayına kadar olan dönemde kademeli olarak azalmış, yaz döneminde daha düşük AFM1 konsantrasyonları saptanmıştır (Bilandžić et.al., 2022).

Çin'de çiğ sütün AFM1 ile kontamine olma sıklığını belirlemek amacıyla yapılan bir çalışmada (Li et.al., 2018) toplam 5650 çiğ süt örneğinden 267'sinde (%4.7) AFM1 kontaminasyonu tespit edilmiştir. Çiğ süt örneklerinin yalnızca %1.1'i Avrupa Birliği yasal sınırını (50 ng/L) aşmış ve örneklerin hiçbiri Çin ve Amerika Birleşik Devletleri yasal sınırını (500 ng/L) aşmamıştır. Çin'de çiğ süt numunelerinde AFM1 kontaminasyonu kış aylarında %10.2, ilkbahar, yaz ve sonbaharda sırasıyla %3.0, %2.1 ve %4.4 olarak çok daha düşük bulunmuştur.

İran'da yapılan bir çalışmada (Ansari et.al., 2019) ilkbahar, yaz, sonbahar ve kış aylarında geleneksel olarak toplanan 100 pastörize süt örneğinde AFM1 kontaminasyonu değerlendirilmiştir. Örnekler, yüksek performanslı sıvı kromatografisi (HPLC) yöntemi ve florimetrik tespit ile değerlendirilmiştir. Sonuçta, kışın toplanan örneklerin yaklaşık %44'ünün, ilkbaharda toplanan örneklerin %32'sinin, yazın toplanan örneklerin %24'ünün ve sonbaharda toplanan örneklerin yaklaşık %20'sinin İran standartları tarafından belirlenen 50 μg/L olan sınır değeri aştığı gösterilmiştir. İstatistiksel analize göre, farklı mevsimlerde AFM1 düzeyleri arasında anlamlı bir farklılık bulunamamıştır.

Pakistan'da yapılan bir çalışmada (Iqbal et.al., 2013) Kasım 2011- Şubat 2012 (kış) toplam 221 ve Mayıs-Ağustos 2012'de (yaz) 212 süt ve sütten elde edilen ürünler toplanmıştır. Örnekler HPLC yöntemiyle analiz edilmiştir. Sonuçlarda kış mevsiminde süt ve süt ürünleri örneklerinin yaklaşık %45'inin AFM1 ile kontamine olduğu ortaya çıkmış; çiğ sütün %40'ının, UHT sütün %51'inin, yoğurdun %37'sinin, tereyağının %60'ının yasal sınırın üzerinde AFM1 içerdiği belirlenmiştir. Ancak yaz sezonundan itibaren süt ve süt ürünleri örneklerinin %32'sinin kontamine olduğu bulunmuş; yani çiğ sütün %36'sı, UHT sütün %31'i, yoğurdun %29'u, tereyağının %40'ında yasal sınırın üzerinde AFM1 bulunmuştur. Süt ve süt ürünlerindeki kontaminasyon seviyeleri kış mevsiminde yaz mevsimine göre daha yüksek bulunmuştur.

Bu çalışmanın sonuçları mevsimsel açıdan değerlendirildiğinde, kış aylarında toplanan 42 tereyağı örneğinin 37'sinin (%88.1) saptanabilir düzeylerde AFM1 ile kontamine olduğu belirlenmiş ve bu örneklerin 32'sinde (%76) sınır değerin (0,05μg/kg) aşıldığı görülmüştür. Beş örnekte ise sınır değerinin altında AFM1 saptanırken (%12), beş örnekte (%12) toksine rastlanmamıştır. İlkbaharda mayıs ayında toplanan tereyağı örnekleri AFM1 açısından değerlendirildiğinde, 42 tereyağı örneğinin tümünün (%100) çeşitli düzeylerde AFM1 içerdiği belirlenmiştir. Otuz beş örnekte 0,05 μg/kg olan sınır değerin aşıldığı (%83.3) saptanırken, bu örneklerin 10'unda

(%23.8) çok yüksek düzeyde (>100.00) toksin belirlenmiştir. Yedi örnekte sınır değerin altında AFM1 (%16.6) saptanmıştır.

Sonuç

Çalışmamızdaki sonuçlara göre ilkbahar mevsimindeki tereyağı örneklerinde saptanan AFM1 düzeyleri, kış aylarında toplanan örneklerdekinden daha yüksek bulunmuştur. İlkbaharda toksin düzeylerinin kış mevsimine göre daha düşük olması beklenirken yüksek bulunması, hava koşulları nedeniyle hayvanların meraya çıkamadığı ve hala depo yemlerle beslenmiş olabilecekleri şeklinde yorumlanmıştır. Sonuç olarak, tereyağlarında belirlenen AFM1 düzeylerinin ciddi halk sağlığı sorunu oluşturabileceği, bu duruma sebep olabilecek hayvan yemlerinde bulunan AFB1 miktarlarının düzeylerinin ve özellikle bu yemlerin depo koşullarının düzenli olarak kontrol edilmesi/denetlenmesi gerektiği düşünülmüştür.

Etik Standartlar ile Uyumluluk

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

Etik izin: Araştırma niteliği bakımından etik izne tabii değildir.

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Teşekkür: -

Açıklama: -

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A study on the knowledge and attitudes of dietitians and their clients about collagen

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ABSTRACT

This study aimed to assess the opinions of dietitians and their clients regarding their knowledge and use of collagen and to determine their level of knowledge regarding the use of collagen supplements. It was conducted on 30 dietitians and 125 clients in Istanbul, Balıkesir, Antalya and Hatay provinces between March and April 2023. Online data was collected from participants via Google Forms. 56.7% (n=17) of dietitians and 54.4% (n=68) of their clients use collagen (P>0.05). While 36.7% (n=11) of dietitians said, it was beneficial for the skin, 23.3% (n=7) used it for weight control and digestive regulation, 38.4% (n=48) said it was beneficial for the skin and 34.4% (n=43) for joint density and pain relief. There was also no statistically significant difference (P>0.05) between dietitians' and clients' information about collagen use. However, while 17.5% (n=3) of dietitians were concerned about the reliability, certification, and brand of collagen, 22.1% (n=15) of consumers were concerned about its reliability. In addition, the opinions of the dietitians and clients participating in the study regarding the use of collagen were not found to be statistically significant (P>0.05).

Keywords: Collagen, Collagen knowledge level, Collagen use

Introduction

Collagen is the main structural protein in connective tissues such as skin, tendons, cartilage and bone. It accounts for 25-30% of all proteins in the body. Collagen is a component of the extracellular matrix of skin tissue, accounting for 75% of its total weight, and its main function is to provide mechanical support. Together with other extracellular matrix fibres, hyaluronic acid, reticulin and elastin, collagen forms a support network for fibroblasts, keratinocytes, melanocytes and specialised cells of the skin's immune system (de Miranda et al., 2021).

Ageing is a natural process that causes physiological changes in organs, tissues, and cells over time. Ageing leads to a decrease in the synthesis and changes in the arrangement of proteoglycans and collagen in the skin and cartilage, as well as the loss of glycosaminoglycans, which are responsible for the integrity and health of these tissues. Daily oral supplementation with a liquid nutraceutical containing hydrolysed collagen, vitamins, antioxidants and other active ingredients can improve skin texture and elasticity. It may also have a protective effect on joints (Czajka et al., 2018).

Collagen is characterised by a high concentration of three amino acids (glycine, proline and hydroxyproline) that form its characteristic triple helix structure. Therefore, collagen is enzymatically hydrolysed and broken down into smaller bioactive peptides that are easily absorbed in the digestive tract before entering the circulation. Due to hydrolysis, collagen peptides do not have the gelling properties of gelatin and are soluble in cold water (Khatri et al., 2021). Collagen is mostly found in animal tissues, and the amount of collagen naturally present in plant foods is usually limited. Foods rich in collagen are meat, fish, bone broth, seafood, cowhide, milk and dairy products, soy products, egg whites, citrus fruits (orange, tangerine, grapefruit, etc.), red fruits, green leafy vegetables and almonds are foods that support collagen synthesis. As the amount of collagen in food is often insufficient, people prefer to use collagen supplements or cosmetic products containing collagen (Avila Rodríguez et al., 2018). As a source of collagen, bovine collagen has properties such as biocompatibility and low immunogenicity; it is generally well tolerated and does not cause an immune response in most people, except for those with collagen allergy (Davison-Kotler et al., 2019). Porcine collagen is used in industrial applications because of its similarity to human collagen and low antigenicity, but it carries a risk of zoonotic disease. In Middle Eastern countries, the use of porcine collagen is restricted for religious reasons. In East Asian countries such as China and Japan, the use of porcine collagen is an important part of the diet (bone broth, confectionery, etc.) due to the health and beauty benefits of porcine collagen peptides (Bhadra et al., 2021). Marine-derived collagen has attracted attention due to its different chemical, biological and mechanical properties compared to animal-derived collagen (Davison-Kotler et al., 2019). Marine-derived collagen has attracted attention due to its different chemical, biological, and mechanical properties compared to animal-derived collagen (Davison-Kotler et al., 2019). Marine collagen is reported to have no risk of infectious disease, is generally recognised as safe by the US Food and Drug Administration (FDA), and is inexpensive to produce (Avila Rodríguez et al., 2018). Collagen has been produced from fish since 1957 (Eastoe, 1957). To date, collagen has been produced from carp, catfish, sardines, perch and sargo scum, snails, perch skin and bones. It has been reported that puffer fish and fish offal have a good potential as a source of collagen and can be used in functional foods. In cosmetics and pharmacology, obtaining collagen from fish offal has important advantages in terms of economic contribution and prevention of environmental pollution (Nagai et al., 2004; Liu et al., 2007; Huang et al., 2011; Duan et al., 2018).

Collagen hydrolysate is a water-soluble protein hydrolysate produced by enzymatic or acid hydrolysis of collagen protein from animal skin and bone. Collagen hydrolysate, or collagen peptides, contains high levels of type 1 and 2 collagen from bovine, porcine, and fish hides. The average molecular weight of collagen is between 2000 and 5000 Daltons. As a result, its digestibility and bioavailability are very high. Collagen hydrolysate, or collagen peptides, is made from type 1 collagen from bovine, porcine, fish bones, tendons, skin, and ligaments. The molecular weight of collagen hydrolysate is broken down by acid and enzymatic hydrolysis and is commonly used as a protein. They are additives for food supplements and cosmetic applications (Edward, 1987).

The aim of this study was to assess and compare the opinions of dietitians and their clients about their knowledge and frequency of collagen use and to determine the level of knowledge about the use of collagen supplements.

Materials and Methods

Type of Research

This descriptive study was conducted between March and April 2023 with 30 dietitians and 125 clients in private clinics in Istanbul, Balıkesir, Antalya and Hatay provinces using Google Form. Based on the literature, it has been reported

that individuals are familiar with the term 'dietary supplements', but they have difficulty classifying these products into appropriate categories. More than 55% consider dietary supplements as food (Yetim, 2011). Considering the perception of collagen consumption, known as dietary supplements, at 70%, it was calculated that a total of 155 dietitians and clients should be included in the R (software/programming - version 3.6.2 - CRAN) programme according to 90% power (Wierzejska et al., 2014). This investigation was conducted in the private clinics that agreed to participate in the current study in the Istanbul, Balıkesir, Antalya and Hatay provinces.

Study population, exclusion criteria

Data were collected from the following clinics using Google Forms for the study. The forms, which were prepared for purposes such as data collection, recording, grouping, and transfer, were preferred because the clinics are located in different cities, some clients are served online, and the participants can be reached in a short time and with less cost. Istanbul, Balıkesir, Antalya, and Hatay were identified as the clinics that could reach the sample size for the study.

Children and adolescents under the age of 18, people with chronic kidney or liver failure, pregnant or breastfeeding women, vegans or vegetarians, as collagen supplements, are usually derived from animal sources, and people with allergies, as they may contain allergens such as fish, were not included in the study.

Data Collection Method

This descriptive study collected online data from dietitians and their clients. The data collection form administered to the participants asked questions to determine demographic characteristics, knowledge and usage status of collagen, purpose of use, purchase preferences and feelings of trust in these products.

Data Collection Form

The demographic characteristics of the participants were collected using a 6-item 'socio-demographic data form'. Based on the literature, the researchers developed a 13-item 'Collagen Use Information Data Form' and a 27-item 'Collagen Information Form'.

In the 'Collagen Use Information Form', participants were asked if they had used collagen before, in what form, what they looked for when obtaining it, and if they experienced any side effects after using it. The researchers developed these questions to collect data. In the 'Collagen Information Data Form', the participants were asked questions in the form of a 5-point Likert scale about the structure of collagen, its form, and who is recommended to use it and when. A previous study conducted at Necmettin Erbakan University Institute of Science and Technology was used as an example in the design of this form (Avcı C, 2022). These forms were administered to the participants via Google Forms.

Statistical Analysis

After all the data were collected, SPSS (Statistical Package for Social Science) Statistics 15.0 was used in a Windows environment for statistical evaluation. In the statistical data analysis, percentage ratio was used for qualitative variables; mean, standard deviation and minimum-maximum parameters were used for quantitative parametric variables; median, lower and upper-value parameters were used for quantitative non-parametric variables. Chi-square analyses were used to examine relationships between categorical variables using cross-tabulations. This study was approved by the Biruni University Non-Interventional Studies Ethics Committee (decision no: 2023/76-01).

Results and Discussion

The study was conducted with 155 dietitians and their clients, 104 women and 51 men. When the educational status of the individuals was examined, it was found that 11% (n=17) were primary school graduates, 17.4% (n=27) were high school graduates, 45.8% (n=71) were undergraduate graduates, and 25.8% (n=40) were postgraduate graduates. The occupations of the individuals were 38.1% (n=59) workers, 18.7% (n=29) students, 6.5% (n=10) academics, 17.4% (n=27) health professionals and 19.4% (n=30) dietitians. Individuals spent most on food (34.8% (n=54) and clothing (22.6% (n=35)), education (7.1% (n=11), health (9% (n=14), care (9% (n=14), entertainment (8.4% (n=13) and other (9% (n=14)). When the difference between the collagen knowledge of all participants in the study was evaluated, it was found that while there was a statistically significant difference according to gender and educational status ($P<0.05$), there was no significant difference according to occupation ($P>0.05$). The study found that while there was a statistically significant difference ($P<0.05$) between the knowledge level of individuals using collagen according to gender, educational status and collagen use status, there was no significant difference ($P>0.05$) according to the occupations of the individuals. It was found that there was a statistically significant difference ($P<0.05$) in the opinions of individuals about collagen use according to gender, educational status, occupation and collagen use status. As a result of comparing the descriptive characteristics of individuals according to their collagen use status, it was found that there was no statistically significant difference in any parameter

(P>0.05) (Table 1). It was found that there was no statistically significant difference between the collagen knowledge levels of the dietitians and their clients included in the study (P>0.05) (Table 2).

When comparing the descriptive characteristics of the dietitians and their clients (Table 1), a statistically significant difference in educational status was found (P<0.05). While 40.8% (n=51) of the clients were university graduates, 13.6% (n=17) were primary school graduates. While 66.7% (n=20) of the dietitians were undergraduate graduates, 33.3% (n=10) were postgraduate graduates.

When comparing the collagen knowledge of dietitians and their clients (Table 2), there was no statistically significant difference (P>0.05). 56.7% (n=17) of the dietitians and 54.4% (n=68) of their clients used collagen. While 36.7% (n=11) of the dietitians used collagen for skin benefits and 23.3% (n=7) for weight control and digestive system regulation, 38.4% (n=48) of the clients used collagen for skin benefits and 34.4% (n=43) for joint density and pain relief.

In the present study, 38.1% of participants reported using collagen for its effects on the skin (Table 2). A systematic review of the dermatologic effects of oral collagen use evaluated 11 studies and 805 patients. According to two studies, 3 g/day of collagen tripeptide for 4-12 weeks improved skin elasticity and hydration. One study suggested that the anti-ageing effect of collagen is proportional to the collagen dipeptide content (Choi et al., 2019). Additionally, 31.6% of participants reported using collagen for its effects on joint health (Table 2). In a randomised controlled trial of the efficacy and tolerability of undenatured type II collagen supplementation in knee osteoarthritis symptoms, 191 subjects were divided into three groups. One of the three groups received 40 mg/day of undenatured collagen for 180 days, one received glucosamine hydroxide plus chondroitin sulfate, and the other received placebo. The results showed that participants using undenatured type II collagen were effective and well-tolerated for knee joint symptoms (Lugo et al., 2016).

Table 1: Comparison of descriptive characteristics of dietitians and their clients

		Dietitian (n=30)		Client (n=125)		Total (n=155)		χ^2	*P*
		n	%	n	%	n	%		
Gender	**Female**	22	73.3	82	65.6	104	67.1	0.655	0.418
	Male	8	26.7	43	34.4	51	32.9		
Education Status	**Primary School**	-	-	17	13.6	17	11.0	14.911	**0.002**
	Highschool	-	-	27	21.6	27	17.4		
	Bachelor	20	66.7	51	40.8	71	45.8		
	Postgraduate	10	33.3	30	24.0	40	25.8		
Area with the highest monthly expenditure	**Food**	11	36.7	43	34.4	54	34.8	13.128	0.057
	Clothing	7	23.3	28	22.4	35	22.6		
	Education	6	20.0	5	4.0	11	7.1		
	Health	1	3.3	13	10.4	14	9.0		
	Care	3	10.0	11	8.8	14	9.0		
	Entertainment	1	3.3	12	9.6	13	8.4		
	Other	1	3.3	13	10.4	14	9.0		

χ^2 : Chi-Square Analysis, P level of significance <0.05

According to the analysis, no statistically significant difference (P>0.05) was found when comparing the knowledge of collagen users between dietitians and clients (Table 3). While 23.5% (n=4) of the dietitians use collagen in liquid or tablet form, 35.3% (n=24) of their clients use collagen in powder form. While 17.5% (n=3) of dietitians are concerned about the reliability, certification and brand of collagen, 22.1% (n=15) of clients are concerned about its reliability.

It was found that there was no statistically significant difference in the opinions of dietitians and their clients regarding the use of collagen (P>0.05). As a result of this research, 33.3% (n=10) of the dietitians agreed with using collagen in ageing, while 23.2% (n=29) of their clients disagreed. A Japanese study investigated the effects of daily oral supplementation with collagen hydrolysate on UV-B-damaged skin. Using in vivo methods to measure water-holding capacity, collagen hydrolysate was shown to be nutritionally beneficial, improve skin defects caused by UV-B damage and photoaging, and increase skin water-holding capacity (Tanaka et al., 2009). Another study conducted in Japan found that women who consumed collagen hydrolysate in their diet increased collagen water retention compared to women who did not (Huang et al., 2011). In a study of rats, rats were given three different daily doses of collagen: 0.166 g/kg, 1.66 g/kg and 16.6 g/kg for 4 weeks. The effect of collagen on body weight and bone density was assessed. It was observed that collagen increased total body weight and bone density more than other ratios in the 16.6 g/kg daily collagen group (Eastoe, 1957).

Table 2: Comparison of Collagen Knowledge of Dietitians and Their Clients

		Dietitian (n=30)		Client (n=125)		Total (n=155)		χ^2	P
		n	%	n	%	n	%		
Collagen knowledge	**Yes**	22	73.3	81	64.8	103	66.5	0.791	0.374
	No	8	26.7	44	35.2	52	33.5		
Collagen use	**Yes**	17	56.7	68	54.4	85	54.8	0.051	0.823
	No	13	43.3	57	45.6	70	45.2		
Collagen prices	**Cheap**	6	20.0	25	20.0	31	20.0	0.071	0.966
	Normal	12	40.0	47	37.6	59	38.1		
	Expensive	12	40.0	53	42.4	65	41.9		
Reasons to use collagen	**Beneficial for skin health**	11	36.7	48	38.4	59	38.1	4.141	0.247
	Beneficial for hair care	6	20.0	19	15.2	25	16.1		
	Joint density and pain relief	6	20.0	43	34.4	49	31.6		
	Weight control and digestive system regulator	7	23.3	15	12.0	22	14.2		
Side effects of collagen	**Yes**	10	33.3	51	40.8	61	39.4	0.565	0.452
	No	20	66.7	74	59.2	94	60.6		
Benefits of collagen	**Yes**	23	76.7	89	71.2	112	72.3	0.361	0.548
	No	7	23.3	36	28.8	43	27.7		
How to obtain collagen	**Herbalist**	5	16.7	15	12.0	20	12.9	1.661	0.798
	Television	1	3.3	7	5.6	8	5.2		
	Internet	5	16.7	21	16.8	26	16.8		
	Apotheke	15	50.0	72	57.6	87	56.1		
	Market	4	13.3	10	8.0	14	9.0		

χ^2 : Chi-Square Analysis, P level of significance <0.05

Table 3: Comparison of Dietitians' and Clients' Knowledge of Collagen Users

		Dietitian (n=30)		Client (n=125)		Total (n=155)		χ^2	*P*
		n	%	n	%	n	%		
Preferred form of collagen use	Liquid	4	23.5	13	19.1	17	20.0	0.724	0.868
	Dust	7	41.2	24	35.3	31	36.5		
	Sachet	2	11.8	13	19.1	15	17.6		
	Tablet	4	23.5	18	26.5	22	25.9		
Attention to the source of collagen	Yes	14	82.4	52	76.5	66	77.6	0.271	0.603
	No	3	17.6	16	23.5	19	22.4		
Frequency of collagen use	Once a day	5	29.4	28	41.2	33	38.8	2.655	0.618
	2 times a day	2	11.8	10	14.7	12	14.1		
	3 times a day	3	17.6	7	10.3	10	11.8		
	1 times a week	4	23.5	8	11.8	12	14.1		
	Other	3	17.6	15	22.1	18	21.2		
Factors considered in the procurement of collagen	Being local	2	11.8	2	2.9	4	4.7	7.333	0.501
	Halal	-	-	6	8.8	6	7.1		
	Reliable	3	17.6	15	22.1	18	21.2		
	Being cheap	-	-	4	5.9	4	4.7		
	Certified	3	17.6	14	20.6	17	20.0		
	Brand	3	17.6	5	7.4	8	9.4		
	Source from which it was obtained	2	11.8	11	16.2	13	15.3		
	Easy accessibility	2	11.8	4	5.9	6	7.1		
	Other	2	11.8	7	10.3	9	10.6		
Problems after collagen use	Yes	5	29.4	21	30.9	26	30.6	0.014	0.906
	No	12	70.6	47	69.1	59	69.4		
Paying attention to the expiry date of collagen	Yes	13	76.5	56	82.4	69	81.2	0.308	0.579
	No	4	23.5	12	17.6	16	18.8		

χ^2 : Chi-Square Analysis, *P* level of significance <0.05

According to the study's results, 36.7% (n=11) of the dietitians who agreed that 'the collagen content of the supplement should be 10 g or at least 5 g' in the collagen knowledge survey were undecided. In comparison, 41.6% (n=52) of their clients were undecided. In a study investigating the effects of collagen hydrolysate on osteoarthritis and osteoporosis, 10 g of collagen hydrolysate per day for sixty days was effective in treating osteoarthritis and osteoporosis. Collagen hydrolysate increases protein synthesis in cartilage tissue. It has an anabolic effect that stimulates tissue growth, so it can improve joint structure and integrity in athletes to reduce joint pain (Moskowitz, 2000). In a study on adult athletes at the German Olympic Centre, 79% showed improved joint mobility and flexibility after taking 10g of collagen hydrolysate daily for 12 weeks of training (Clark et al., 2008).

In a post-study article about the benefits of collagen, 40% (n=12) of the dietitians who agreed that 'collagen is only good for the skin' disagreed, while 20% (n=25) of their clients were undecided. A study conducted in Thailand on 36 postmenopausal women aged 50-60 years evaluated the effect of oral intake of 5g of collagen hydrolysate for 4 weeks on skin elasticity. In addition, the effects of collagen hydrolysate supplementation on skin elasticity were compared between sun-exposed and sun-protected areas. Participants were assessed for skin elasticity using a cutometer at baseline, after 2 and 4 weeks of collagen hydrolysate supplementation, and 4 weeks after collagen hydrolysate supplementation was discontinued. An increase in skin elasticity measured on the left and right cheeks was observed in participants using collagen hydrolysate compared to placebo. In addition, skin elasticity

measured on the left and right cheeks was found to be different between the collagen hydrolysate and placebo groups at week 4 after stopping collagen hydrolysate. In conclusion, a significant improvement in skin elasticity in sun-exposed areas was demonstrated after 4 weeks of use of marine-derived collagen hydrolysate. The improvement in elasticity was observed to continue for 4 weeks after cessation of collagen hydrolysate use. No serious adverse effects were observed during the study (Sangsuwan & Asawanonda, 2021). randomised controlled trial involved 60 healthy female participants. Participants were divided into groups of 20, including topical collagen users, oral collagen supplement users and oral placebo users. Stratum corneum water content, skin viscoelasticity, dermal echogenicity and skin pore parameters were assessed. The topical collagen group showed a significant increase in stratum corneum water content and skin elasticity at 28 days, and the peptide formulation was also effective on dermis echogenicity at 90 days. Oral collagen supplements effectively improved skin elasticity and showed a more pronounced effect on dermal echogenicity, reducing skin pores after 90 days (Maia Campos et al., 2019).

When asked about the ingredients in collagen supplements, 23.3% (n=7) of dietitians disagreed, were undecided or strongly agreed that the supplement should contain copper, zinc and vitamin C to support collagen synthesis. In comparison, 36.8% (n=46) of their clients agreed. In the study, 72 healthy female participants aged 35 and over living in Germany were given the test product for twelve weeks. The test product was a specially developed blend of 2.5 g collagen peptides, 666 mg acerola fruit extract, 80 mg vitamin C, 3 mg Zinc, 2.3 mg vitamin E and 50 µg biotin. Participants were dermatologically assessed at baseline, after 12 weeks of supplementation, and after a 4-week follow-up period without supplementation. In addition, tolerability and efficacy data were collected. The study's results showed that using the test product improved skin hydration, elasticity and density and reduced skin roughness. All test parameters differed between the intervention and placebo groups, which was also observed during follow-up (Bolke et al., 2019).

On a question related to the weight of collagen in collagen supplements, 46.7% (n=14) of the dietitians were undecided about the correct choice of low molecular weight collagens (around 3000 Daltons). In comparison, 53.6% (n=67) of their clients were undecided. A randomised controlled trial was conducted in Korea in 64 healthy women aged 40-60. Subjects received a placebo or 1000 mg of low molecular weight collagen peptide once daily for 12 weeks. Skin hydration, wrinkle and elasticity parameters were assessed at baseline and after 6 and 12 weeks. Compared to the placebo group, skin hydration scores were significantly higher in the collagen group at 6 and 12 weeks. After 12 weeks, the visual assessment score and three parameters of skin wrinkles were significantly improved in the collagen group compared to the placebo group. In the intervention group, skin hydration scores improved at weeks 6 and 12. Significant improvements were seen in the intervention group's three parameters of skin wrinkles compared to placebo. These results suggest that collagen can be a healthy functional ingredient that provides hydration and elasticity and prevents wrinkles in human skin (Do-Un Kim, 2018).

Conclusion

Collagen is an essential protein for body structure and is very important for the health of bones, joints, skin and tendons. Individuals must know their collagen intake and where they may lack knowledge about supplements. As a result of the research, it is clear that people's opinions on the use of supplemental collagen vary, and there is no clear consensus. While male participants were generally undecided, female participants expressed an opinion. The study also found no significant difference in the knowledge of collagen users according to their occupation. It was observed that the use and knowledge of dietitians about supplemental collagen is insufficient and that dietitians should have more information about collagen through more studies. As the research was conducted on dietitians in private hospitals and clinics, the use of collagen was generally recommended, and most clients were observed to be using collagen supplements. These results are based on private hospitals and clinics partnering with food supplement companies. In contrast, a similar study should be conducted on dietitians and their clients working in public institutions to address the deficiencies. Different opinions and experiences were observed between dietitians and clients. The results of this study highlight the complexity of knowledge and individual differences in the use of collagen. Lack of scientific evidence and conflicting information make reaching a consistent consensus between dietitians and clients difficult. This situation highlights the importance of scientific research and reliable sources. It shows that dietitians and clients need more information and reliable research to make informed decisions about collagen consumption. At this stage, the scientific accuracy of health industry claims needs to be investigated and communicated. It is also important to assess the appropriateness of using collagen supplements, considering customers' needs and their body composition.

Compliance with Ethical Standards

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

Ethics committee approval: The Biruni University Non-Interventional Studies Ethics Committee approved the study (decision no: 2023/76-01, date: 29.03.2023).

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Instructions to Reviewers and Authors

The journal "**FOOD and HEALTH**" establishes the highest standards of publishing ethics and benefits from the contents of the **International Committee of Medical Journal Editors (ICMJE), World Association of Medical Editors (WAME), Council of Science Editors (CSE), Committee on Publication Ethics (COPE), European Association of Science Editors (EASE), Open Access Scholarly and Publishers Association (OASPA),** and **Directory of Open Access Journals (DOAJ).**

The "**FOOD and HEALTH**" journal provides a platform for the open public discussion of the journal contents. To secure accountability and to encourage sincere professional inputs without incivilities, the system is set up to require registration and logging for the recording of inputs. Some website contents will be available without logging, but no peer review comments can be posted without disclosing the reviewer's identity to the journal editors.

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Journal Publisher Policy

1. Aims and Scope

Our journal started its publication life as "Journal of Food and Health Sciences" between 2015-2017. In 2018, its name was changed to "Food and Health". The journal "**FOOD AND HEALTH**" publishes peer-reviewed (double-blind) original research, communication and review articles covering all aspects of food science and its effects on health. Our journal will be published quarterly in English or Turkish language**.**

2. Scientific Quality and Objectivity

The journal evaluates and publishes research articles and reviews, adhering to high scientific standards. Adhering to the principle of impartiality, it strictly complies with ethical rules to prevent conflicts of interest among editors, referees, and authors.

3. Open Access

The journal adopts an open-access policy that supports open and free access to information. This aims to increase access to scientific knowledge in society at large by making science available to a wider audience.

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5. **Ethical Standards**

   The journal maintains a rigorous attitude towards upholding ethical standards among authors and reviewers. The processes of evaluating the effects of research on humans, animals and the environment are carried out in full compliance with national and international ethical rules.

6. **Peer Review**

   The journal employs a double-blind referee system. Referees are selected among experts and experienced people in their fields. The peer review process involves subjecting articles to rigorous review in terms of scientific content, methodology and ethics.

7. **Author Rights and Licensing**

   The journal respects the property rights of authors and grants appropriate licenses to articles. It allows articles to be freely shared and used by others using appropriate licensing models, such as Creative Commons licenses.

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   The journal encourages diversity among authors, editors, and reviewers. It fights against inequalities in the scientific world, considering gender, geographical origin, discipline, and other elements of diversity.

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   The journal promotes open communication between authors, reviewers, and readers. Publisher policies, article evaluation processes and other important information are transparently published on the journal's website.

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1. **Scientific Neutrality and Objectivity:**

   All publications must reflect an impartial and objective perspective. If there are any conflicts of interest, authors must clearly state these conflicts of interest.

2. **Scientific Soundness:**

   Articles should be based on a solid methodology and reliable results. The accuracy of statistical analyses should be at the forefront.

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   The journal supports the principles of the Basel Declaration (**https://animalresearchtomorrow.org/en**) and the guidelines published by the International Council for Laboratory Animal Science (**https://iclas.org/**). In this regard, the research must fully comply with the relevant ethical rules and standards. International ethics committees must conduct studies on humans, animals, or the environment and must be confirmed by the authors of the journal.

   For research submitted to this journal, authors are advised to comply with the **IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on Trade in Endangered Species of Wild Fauna and Flora for research involving plants.**

4. **Originality and Plagiarism:**

   Publications must be original, and appropriate attribution must be made when quoting other sources. In our journal, plagiarism is considered a serious crime. For this reason, all articles submitted to the "Aquatic Research" journal must undergo a preliminary evaluation. Advanced Plagiarism Detection Software (iThenticate, etc.) tools will be used.

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   The journal adopts open access principles to promote open and free access to information and complies with the **Budapest Open Access Initiative** (BOAI) definition of open access. Open-access articles in the journal are licensed under the terms of the Creative Commons Attribution 4.0 International (CC BY 4.0) license.

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1. **Confidentiality:**

   The peer review process should be carried out per the principles of double-blind refereeing. Reviewers and authors should not know each other's identities.

2. **Expertise:**

   Referees should be selected among experts and experienced people in relevant fields. Referees must be trusted to make an impartial and ethical assessment.

3. **Timely Evaluation:**

   The peer-review process must be completed on time to publish the articles quickly. Time limits should be set for referees to evaluate within a certain period.

4. **Open Communication:**

   Reviewers should be encouraged to provide open and constructive feedback to authors and editors.

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1. **Article Format:**

   Authors must write in the article format determined by the journal. Sections such as title, abstract, keywords, introduction, method, findings, discussion, and references should be included. All submissions are screened by similarity detection software. The similarity rate in the articles sent to the journal should be below 20%.

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   Authors must appropriately cite the sources used by scientific standards.

3. **Submission Process:**

   Authors must comply with the specified submission process when submitting their articles to the journal. This process should include evaluating, editing and publishing the article.

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   "**FOOD and HEALTH**" journal requires corresponding authors to submit a signed and scanned version of the copyright transfer, ethics, and authorship contribution form (available for download at **https://dergipark.org.tr/tr/download/journal-file/19582**)

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1. **Maintaining High Scientific Standards:**

   To ensure that the articles published in the journal comply with high scientific standards.

   To ensure full compliance with ethical rules and journal policies.

2. **Managing the Article Evaluation Process:**

   To effectively manage the article evaluation process and support a rapid publication process.

   To adopt the principles of double-blind arbitration and maintain the principles of expertise and impartiality in selecting arbitrators.

3. **Making Editorial Decisions:**

   Consider referee evaluations to make decisions about accepting or rejecting articles for publication.

   Maintaining transparency and openness in the editorial process.

4. **Contact with Authors:**

   Maintaining effective and constructive communication with authors.

   They provide authors with regular updates on the status of their articles, correction requests, and publication dates.

5. **Managing Journal Policies:**

   Keep the journal's policies and guidelines updated and revise them as needed.

   To provide a reliable platform between readers and writers.

Responsibilities of Referees

1. **Objectivity and Expertise:**

   To comply with the principles of double-blind refereeing and to evaluate articles impartially.

   Evaluating articles by focusing on areas of expertise on the subject.

2. **Privacy and Reliability:**

   To protect the confidentiality of the article evaluation process.

   Provide reliable and constructive feedback to authors, journal editors, and other reviewers.

3. **Timely Evaluation:**

   Evaluating articles by the timelines determined by the journal.

   Informing editors promptly in case of delays.

4. **Compliance with Ethical Rules:**

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   Clearly express conflicts of interest and withdraw from the evaluation process when necessary.

5. **Constructive Feedback to Writers:**

   Provide clear and constructive feedback to authors and suggest improving the article when necessary.

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Preparation of the Manuscript

Manuscripts prepared in Microsoft Word must be converted into a single file before submission. Please start with the title page and insert your graphics (schemes, figures, *etc.*) and tables in the one main text (Word Office file).

Title (should be clear, descriptive, and not too long)

Full Name(s) and Surname (s) of author(s)

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

Authors complete correspondence Address (es) of affiliations and e-mail (s)

Abstract

Keywords (indexing terms), usually 3-6 items

Introduction

Material and Methods

Results and Discussion

Conclusion

Compliance with Ethical Standards

- **Conflict of Interest:** When you (or your employer or sponsor) have a financial, commercial, legal, or professional relationship with other organisations or people working with them, a conflict of interest may arise that may affect your research. A full description is required when you submit your article to a journal.
- **Ethics committee approval:** Ethical committee approval is routinely requested from every research article based on experiments on living organisms and humans. Sometimes, studies from different countries may not have the ethics committee's approval, and the authors may argue that they do not need support for their work. In such situations, we consult COPE's "Guidance for Editors: Research, Audit, and Service Evaluations" document, evaluate the study with the editorial board, and decide whether or not it needs approval.
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One of the following should be selected and stated in the submitted article;

1. No data was used for the research described in the article.
2. The data that has been used is confidential.
3. The authors do not have permission to share the data.
4. Data will be made available on request.
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6. Other (please explain; for example, I have shared the link to my data in the attached file step).

- **Funding:** If there is any, the institutions that support the research and the agreements with them should be given here.
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- **Disclosure:** Explanations about your scientific / article work that you consider ethically important.

References

Tables (all tables given in the main text)

Figures (all figures/photos shown in the main text)

Manuscript Types

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

Statistical analysis to support conclusions is usually necessary. International statistical reporting standards must conduct statistical analyses. Information on statistical analyses should be provided with a separate subheading under the Materials and Methods section, and the statistical software used during the process must be specified.

Units should be prepared by the International System of Units (SI).

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Review Articles: Reviews prepared by authors with extensive knowledge of a particular field and whose scientific background has been translated into a high volume of publications with a high citation potential are welcomed. The journal may even invite these authors. Reviews should describe, discuss, and evaluate the current knowledge level of a research topic and should guide future studies. The main text should start with the Introduction and end with the Conclusion sections. Authors may choose to use any subheadings in between those sections.

Short Communication: This type of manuscript discusses important parts, overlooked aspects, or lacking features of a previously published article. Articles on subjects within the journal's scope that might attract the readers' attention, particularly educative cases, may also be submitted as a "Short Communication". Readers can also comment on the published manuscripts as a "Short Communication". The main text should contain **"Title", "Abstract", "Introduction", "Materials and Methods", "Results and Discussion", "Conclusion", "Compliance with Ethical Standards**", and **"References"** sections.

Table 1. Limitations for each manuscript type

Type of manuscript	Page	Abstract word limit	Reference limit
Original Article	≤30	200	40
Review Article	no limits	200	60
Short Communication	≤5	200	20

Tables

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

Figures and Figure Legends

Figures, graphics, and photographs should be submitted through the submission system in the main document's Word files (in JPEG or PNG format). Any information within the images that may indicate an individual or institution should be blinded. The minimum resolution of each submitted figure should be 300 DPI. To prevent delays in the evaluation process, all submitted figures should be clear in resolution and large (minimum dimensions: 100 × 100 mm). Figure legends should be listed at the end of the primary document.

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

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

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

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

References

The citation style and methods that comply with the scientific standards that should be used in the "FOOD and HEALTH" journal for the sources used by the authors in their works are given below.

Reference System is APA 6th Edition (with minor changes)

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

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number, appears only in a citation to a direct quotation.

....(Erkan, 2011).

....(Mol and Erkan, 2009).

....(Özden et al., 2021).

....(Mol and Erkan, 2009; Erkan, 2011; Özden et al., 2021).

Citations for a Reference Section:

An article

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https://doi.org/10.3153/FH21025
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Harrigan, W.F. (1998). Laboratory Methods in Food Microbiology. Academic Press, pp. 308. ISBN: 9780123260437

A book chapter

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

A webpages

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

Revisions

When submitting a revised version of a paper, the author must submit a detailed "Response to the reviewers" that states point by point how each issue raised by the reviewers has been covered and where it can be found (each reviewer's comment, followed by the author's reply and line numbers where the changes have been made) as well as an annotated copy of the primary document. Revised manuscripts must be submitted within 15 days from the date of the decision letter. If the revised version of the manuscript is not submitted within the allocated time, the revision option may be cancelled. If the submitting author(s) believe that additional time is required, they should request this extension before the initial 15-day period is over.

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