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1. Abstracts on Hygiene and Communicable Diseases
2. Academic Index
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4. Academic Search Ultimate
5. Academindex
6. Advanced Science Index (ASI)
7. AgBiotech News and Information
8. AgBiotechNet
9. Agricultural Economics Database
10. Agricultural Engineering Abstracts
11. Agroforestry Abstracts
12. Animal Breeding Abstracts
13. Animal Production Database
14. Animal Science Database
15. Asian Science Citation Index (ASCI)
16. Asos İndeks
17. Biocontrol News and Information
18. Biofuels Abstracts
19. Botanical Pesticides
20. CAB Abstracts
21. CAB Direct
22. Cite Factor
23. Crop Science Database
24. CrossRef
25. Dairy Science Abstracts
26. Directory of Research Journals Indexing (DRJI)
27. EBSCO - Academic Search Ultimate Database
28. Environmental Impact
29. Environmental Science Database
30. Eurasian Scientific Journal Index
31. EuroPub
32. Field Crop Abstracts
33. Food Science and Technology Abstracts (FSTA)
34. Forest Science Database
35. Global Health
36. Google Scholar
37. Horticultural Science Abstracts
38. Horticultural Science Database
39. Impact Factor Services for International Journals (IFSIJ)
40. International Innovative Journal Impact Factor (IIJIF)
41. International Institute of Organized Research (I2OR)
42. İdeal Online
43. Maize Abstracts
44. MIAR (Information Matrix for the Analysis of Journals)
45. Nutrition Abstracts and Reviews Series A:Human and Experimental
46. Nutrition Abstracts and Reviews Series B: Livestock Feeds and Feeding
47. Nutrition and Food Sciences Database
48. Ornamental Horticulture
49. Parasitology Database
50. Plant Breeding Abstracts
51. Plant Genetic Resources Abstracts
52. Plant Genetics and Breeding Database
53. Plant Protection Database
54. Postharvest Abstracts
55. Potato Abstracts
56. Poultry Abstracts
57. Protozoological Abstracts
58. Review of Agricultural Entomolog
59. Review of Aromatic and Medicinal Plants (RAMP)
60. Review of Medical and Veterinary Entomology
61. Review of Medical and Veterinary Mycology
62. Review of Plant Pathology
63. Rice Abstracts
64. Rural Development Abstracts
65. Science Library Index
66. Scientific Indexing Services (SIS)
67. SCOPUS (Elsevier)
68. Seed Abstracts
69. Scilit
70. Soil Science Database
71. Soils and Fertilizers Abstracts
72. Soybean Abstracts
73. Sugar Industry Abstracts
74. Systematic Impact Factor (SIF)
75. The Belt and Road Initiative Reference Source
76. The Turkish Academic Network and Information Centre Life Sciences Database (TÜBİTAK-ULAKBİM Yaşam Bilimleri Veritabanı, TR-DİZİN)
77. Tropical Diseases Bulletin
78. Veterinary Science Database
79. VetMed Resource
80. Weed Abstracts
81. Wheat, Barley and Triticale Abstracts
82. World Agricultural Economics and Rural Sociology Abstracts (WAERSA)

Akademik Gıda 22 (3) (2024)

İÇİNDEKİLER / CONTENTS

■ **Editörden** / *Editorial* — VII-VIII

■ MAKALELER / PAPERS

■ **Araştırma Makaleleri / Research Papers**

Optimization of Spray Drying Parameters and Wall Material Composition of Juniper (Juniperus drupacea L.) Extract Using Response Surface Methodology / *Ardıç (Juniperus drupacea L.) Ekstrakt Tozu Üretiminde Taşıyıcı Bileşimi ve Püskürtmeli Kurutma Parametrelerinin Yanıt Yüzey Yöntemiyle Optimizasyonu* / *Yusuf Yılmaz, Ecem Vural, Ayhan Topuz* — 167-178

Bioactive Content and in vitro Antioxidant and Enzyme-Inhibitory Potential of Leaf and Fruits of Parsnip (Pastinaca sativa L. subsp. Urens) / *Şeker Havucu (Pastinaca sativa L. subsp. urens) Yaprak ve Meyvelerinin Biyoaktif İçeriği ile in vitro Antioksidan ve Enzim-İnhibitör Potansiyeli* / *Nuraniye Eruygur, Yavuz Bağcı, Süleyman Doğu, Nevin Tuzcu* — 179-185

Effect of Different Drying Methods and Distillation Times on Essential Oil Composition and Antioxidant Content of Rosemary / *Farklı Kurutma Yöntemi ve Damıtma Süresinin Biberiyenin Uçucu Yağ Bileşimi ve Antioksidan İçeriği Üzerine Etkisi* / *Muharrem Gölükcü, Orçun Çınar, Haluk Tokgöz, Fatma Uysal Bayar, Temel Özek* — 186-194

Effect of Cold PlasmaTreatment on Physicochemical and Microbiological Properties of Clotted Cream / *Soğuk Plazma Uygulamasının İnek Kaymağının Fizikokimyasal ve Mikrobiyolojik Özellikleri Üzerine Etkisi* / *Gökhan Akarca, Betül Özkan, Azize Atik, İlker Atik* — 195-204

Comparison of Chemical Properties and Fatty Acid Composition of Artisanal and Commercial Butters / *Ticari ve Ev Yapımı Tereyağlarının Kimyasal Özellikleri ve Yağ Asidi Kompozisyonlarının Karşılaştırılması* / *Oğuz Gürsoy, Kübra Kocatürk, Ayşe Deniz, Hande Özge Güler Dal, Yusuf Yilmaz* — 205-214

Quality Assessment and Bioactive Component Analysis of Honey from Different Geographical Regions in Erzurum, Türkiye / *Erzurum'un Farklı Coğrafi Bölgelerinden Elde Edilen Balların Kalite Değerlendirmesi ve Biyoaktif Bileşen Analizi* / *Adem Savaş, Halil İbrahim Binici, İhsan Güngör Şat, Mustafa Kılıç* — 215-223

Regression Tool in MS Excel® Spreadsheets for Biological Data: R-BioXL / *Biyolojik Veriler İçin MS Excel® Hesap Çizelgesi Aracı: R-BioXL* / *Hasan Basri Öksüz, Sencer Buzrul* — 224-235

Alabaş (Brassica oleracea var. gongylodes) Sebzesi ile Zenginleştirilmiş Erişte Üretimi / *Production of Turkish Noodles Enriched with Kohlrabi (Brassica oleracea var. gongylodes) Vegetable* / *Songül Çoban, Ayşe Nur Tonay* — 236-243

■ **Akademik Gıda Dergisi Yazım Kuralları** / *Guidelines to Authors* — IX-XII

■ **Etik Beyanı** / *Ethics and Publication Malpractice Statement* — XIII-XVIII

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Saygılarımla.

Prof. Dr. Oğuz Gürsoy
Baş Editör

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3. Uluslararası Geleneksel Gıdalar ve Sürdürülebilir Beslenme Sempozyumu

Toros Üniversitesi tarafından düzenlenen 3. Uluslararası Geleneksel Gıdalar ve Sürdürülebilir Beslenme Sempozyumu 3-4 Ekim 2024 tarihlerinde online olarak araştırmacı ve akademisyenlerin katılımıyla gerçekleştirilecektir. Sürdürülebilirliğe yönelik küresel zorluklarla mücadele etme yöntemlerinin ana konu olarak belirlendiği sempozyum ile ilgili bilgilere **https://food24.toros.edu.tr/** adresinden ulaşılabilir.

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7. Uluslararası Malzeme ve Polimer Bilimi Kongresi

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Research Paper / Araştırma Makalesi

Optimization of Spray Drying Parameters and Wall Material Composition of Juniper (*Juniperus drupacea* L.) Extract Using Response Surface Methodology

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ABSTRACT

The aim of this study was to produce a juniper extract powder rich in some bioactive and volatile components such as phenolics, α-pinene and d-limonene with a high yield. For this purpose, the juniper extract, which can be used in various food formulations, was spray-dried under optimized conditions. In this optimization, inlet air temperature (120°C - 180°C) in the spray drying process and the carrier composition were selected as independent variables, while dependent variables included drying efficiency and the total phenolic (TPC), and α-pinene contents of the extract powder. Response surface methodology was used to maximize product yield, TPC and volatile levels, especially α-pinene. The optimum inlet air temperature and carrier ratio were 180ºC and 15 g gum Arabic (GA) per 100 mL extract, respectively. The highest powder yield (37.92%), TPC (9.91 mg GAE/g dm powder) and α-pinene content (peak area 1.3×10^7) were obtained under the optimum conditions while the bulk and compressed bulk densities, TPC and antioxidant activity of the extract powder were 0.39±0.01 g/cm^3 and 0.51±0.02 g/cm^3, 9.89±0.27 g gallic acid equivalent (GAE)/100 g (dm) and 4.12±0.14 g Trolox® equivalent antioxidant activity (TEAC)/100 g dm, respectively. The particle size of the powder produced under optimum conditions ranged from 1.09 to 22.39 µm. Fifteen volatiles in both juniper extract and the reconstituted form of the extract powder were identified, and the major components of juniper extract were d-limonene, α-pinene and γ-muurolene.

Keywords: Juniper, Spray drying, Optimization, TPC and volatile components

Ardıç (*Juniperus drupacea* L.) Ekstrakt Tozu Üretiminde Taşıyıcı Bileşimi ve Püskürtmeli Kurutma Parametrelerinin Yanıt Yüzey Yöntemiyle Optimizasyonu

ÖZ

Bu çalışmada, fenolikler, α-pinen, d-limonen gibi bazı biyoaktif ve uçucu bileşenler bakımından zengin ve yüksek toz verime sahip ardıç (andız) ekstrakt tozu üretimi amaçlanmıştır. Bu amaçla farklı gıda formülasyonlarında kullanılabilir nitelikte toz ürün optimize edilmiş bir koşulda püskürtmeli kurutucuda kurutulmuştur. Bu optimizasyonda taşıyıcı bileşimi ve püskürtmeli kurutucuya giriş havası sıcaklığı bağımsız değişken; kurutma verimi, toplam fenolik madde ve α-pinen içerikleri bağımlı değişkenler olarak seçilmiştir. Ürün verimini, toplam fenolik madde içeriği ve uçucu bileşen seviyeleri, özellikle de α-pinen içeriğini maksimize etmek için yanıt yüzeyi yöntemi kullanılmıştır. Optimum kurutucu giriş havası sıcaklığı ve taşıyıcı oranı sırasıyla 180ºC ve 15 g Arap zamkı/100 mL ekstrakt olarak belirlenmiştir. Optimum koşullarda gerçekleştirilen kurutma işleminde en yüksek toz verimi (%37.92), toplam fenolik madde içeriği

(9.91 mg GAE/g toz) ile α-pinen içeriği (pik alanı 1.3×10^7) elde edilirken tozun yığın ve sıkıştırılmış yığın yoğunlukları ile toplam fenolik madde içeriği ve antioksidan aktivitesi sırasıyla 0.39±0.01 ve 0.51±0.02 g/cm^3, 9.89±0.27 g gallik asit eşdeğeri (GAE) /100 g (km) ve 4.12±0.14 g Trolox® eşdeğeri antioksidan aktivite (TEAC) /100 g km olarak belirlenmiştir. Optimum koşullarda üretilen tozun partikül boyutu dağılımı 1.09 ile 22.39 µm arasında değişmiştir. Hem andız ekstraktında hem de sulandırılmış tozda on beş uçucu bileşen tanımlanırken andız ekstraktının ana bileşenleri d-limonene, α-pinene ve γ-muurolene olmuştur.

Anahtar Kelimeler: Andız, Püskürterek kurutma, Optimizasyon, TPC ve uçucu bileşenler

INTRODUCTION

Juniper (*Juniperus drupacea* (Labill) Antoine and Kotschy) is a perennial tree that is distributed in the Mediterranean countries at altitudes of 600 – 1750 m and constitutes 4% of Türkiye's forests [1-3]. The berries of junipers contain a high amount of reducing sugar (22%), sucrose (11%), oil (4%), protein (2.5%), minerals (2.5%), and essential oils (0.38%) [4-6]. In addition, the fruit extracts of junipers which is mostly used in the production of pekmez [7-9], contain many phenolics such as protocatechuic acid, gallic acid, *p*-hydroxybenzoic acid, catechin, tyrosol, rutin and *m*-coumaric acid, [1, 10] and terpenoids such as α-pinene, camphor and thymol [11].

Juniper berries are commonly used in the form of molasses after extraction, however, there are limited uses in the form of tar and, essential oils for various medical and cosmetic purposes [10-15]. The extract of Juniper berries shows significant antimicrobial effects against some pathogens and molds, especially due to its volatile components. [11, 16, 17]. This extract is widely used in the treatment of helminth infections, stomach pain, hemorrhoids, and diarrhea [7, 18-21]. It has also antioxidant, anticarcinogenic, anti-inflammatory [22], antiarthritic [23], antiviral [24], antitumor [25] and antihyperglycemic [26] effects.

Ripe fruits are unsuitable for direct consumption or addition in food formulations due to their hard texture and extremely astringent taste. For this reason, it can be considered as an alternative that juniper fruit extract can be turned into powder with innovative food processing methods and can be used as a natural food additive to add functionality and enrichment to different products. Powdering of juniper extract, either directly or with carriers, provides various advantages such as increased chemical and microbiological stability due to the decrease in moisture content and water activity (a_w) and decreasing transportation costs by reducing weight and volume [27-29]. The addition of a carrier is a common practice in spray drying of extracts or juices. In this application, both the product yield is increased, and the active ingredients are better preserved.

Interest in the use of fruit and fruit extracts in powder form is increasing. There are various on the use of extract powder in a number of foodstuffs about this subject [30-34]. However, to the best of our knowledge, no study has been found on the optimization of the spray drying conditions of juniper berry extract.

Various processes are applied to obtain the product in powder form; however, spray drying, one of these processes, is frequently used in the food and pharmaceutical industries to convert the liquid product into powders. Spray-dried powders have lower water activity, are easier to process, and are stable against environmental factors such as heat, oxygen, light, etc. [35]. The quality of the powders depends on the feeding solution properties, the contact patterns of the hot air with the droplets in the drying chamber, the type of atomizer, also the temperature, pressure, and flow rate of air in the dryer [36]. Solutions such as extracts and fruit juices with high sugar content can be transformed to powders with lower volume and longer shelf life using this method, but problems with sticking to the spray cylinder occur when drying such solutions. Feed solutions containing low molecular weight sugars and high concentrations of organic acids are the solutions where this problem is most commonly encountered. Sugars and organic acids in such feeding solutions reduce the temperature of glass transition and cause it to stick to inner surface of the drying chamber, resulting in reduced efficiency [37]. By adding carriers such as maltodextrin (MD), β-cyclodextrin (BCD), modified starches and gum arabic (GA) to the solution, this problem can be avoided, and it may be possible to protect sensitive components in foods [38].

The main goal of this study was to produce TPC and α-pinene rich juniper extract powders with high production yield for use in the food industry, by optimizing the inlet air temperature of the spray drying process and the carrier composition. For this purpose, 15 different powders containing MD, GA and BCD at different ratios and inlet temperatures of 100-180°C were prepared according to an optimal (combined) experimental design obtained from the Design Expert 13.0 statistical program. Juniper extract powder produced under optimum conditions was evaluated for quality characteristics.

MATERIALS AND METHODS

Materials

The ripe fruits of juniper trees were obtained from around the same local plantation (920 m altitude) around Gümüşdamla Village, Akseki (Antalya, Türkiye) in November 2020. Akseki is the most intensive juniper plantation in Türkiye. The GA (Alfasol, Istanbul, Türkiye) MD (9 DE) and BCD (Smart Kimya, Izmir, Türkiye), were procured from a local company in Türkiye. The other chemicals (Sigma-Aldrich, Darmstadt, Germany) used in the analyses were purchased from a local distributor.

Experimental Design

Optimization was carried out using response surface methodology with the software of Design Expert 13.0 (Stat-Ease Inc., Minneapolis, MN, USA). An optimal (Combined) experimental design was used to plan the experiments and identify optimum variables, with fifteen treatments carried out for optimization. The independent variables selected were wall material composition and inlet air temperature (120-180°C). The carrier composition is composed of a mixture of MD and GA with a fixed proportion of BCD (1 g/100 mL) in extracts. MD:GA ratio, as determined from preliminary experiments, was adjusted so that its total in the extract was 15 g/100 mL extract. The dependent variables as response selected for optimization were product yield and α-pinene peak area.

Preparation of Juniper Extract

The ripe, brownish fruits were harvested from trees. They were then crushed with a hammer and placed in deionized water (1:4, w/w) for about 3 days until the soluble solids of extract reached 15 °Bx. The extraction process was performed in a jacketed vessel (İldam, Kahramankazan, Ankara, Türkiye) at room temperature with continuous stirring using an overhead propeller type stirrer (Jeio Tech Lab companion MSD-0420, Yuseong-gu, Daejeon, South Korea). The final mixture was filtered through cheesecloth to obtain a particle-free extract [4].

Spray Drying

The mixture of MD/GA (total of 15 g) and 1 g BCD were added to 100 mL of extracts as indicated in the experimental design to give a total weight of 16 g. The obtained solution was then homogenized using an Ultra-Turrax (IKA T25 Digital, Staufen, Germany) at rotation speed of 15,000 rpm for 5 min to ensure uniformity and then spray dried.

In this study, a mini spray dryer (Buchi, B-290, Flawil, Switzerland) equipped with a dual fluid pneumatic nozzle with a diameter of 0.5 mm was co-currently used with compressed air at a flow rate of 600 L/h. The heated air flow rate was maintained at 30 m^3/h and the drying air inlet temperatures of the drying processes were set to the experimental temperature. The feed solution was pumped at an ambient temperature by a peristaltic pump after conditioning. The flow rate was regulated to provide an exact outlet temperature of 80±5°C, corresponding to 240 - 640 mL/h, depending on the soluble solids of the feed solution. During the drying process, the solutions were continuously stirred with a magnetic stirrer at 25°C. At the end of the drying experiments, the powders collected in the collection vessel were transferred to sealed bottles and stored at 4°C for subsequent analyses.

Product Yield

The product yield (PY), calculated using the weight of the solid in the feed solution and the weight of the powder obtained from the collection vessel, was determined gravimetrically as described in Equation (1).

$$PY = \frac{\text{weight of collected powder (g)}}{\text{weight of solid in feed liquid (g)}} \times 100 \quad (1)$$

Moisture Content and Water Activity

The moisture content of samples was determined gravimetrically using a moisture analyzer (Kern DBS, Balingen, Germany) by running samples at 105 °C until constant weight was reached, as described in the instrument manual. Meanwhile, the water activity (a_w) of samples was determined at 25 °C using a a_w analyzer (Aqualab 4TE: Decagon Devices, Pullman, WA, USA).

Color Values

The L* (lightness - darkness), a* (redness - greenness) and b* (yellowness - blueness) color parameters of the samples were determined with a tristimulus colorimeter (CR 400, Konica Minolta Corp., Tokyo, Japan) calibrated with a standard plate (L = 97.02, a* = 0.08, b* = 1.75). The color parameters of the samples (with a minimum sample height of 1 cm) were measured individually in the instrument's special holder for powder samples by placing them on the head of the light source [30,53]. Hue angle, chroma and browning index (BI) values were determined using Equations (2-5).

$$Hue\ angle = \frac{180}{\pi}\ x\ arctan\frac{b^*}{a^*} \quad (2)$$

$$Chroma = \sqrt{b^{*2}+a^{*2}} \quad (3)$$

$$BI = \frac{100(x-0.31)}{0.17} \quad (4)$$

$$x = \frac{a^*+1.75L^*}{5.645L^*+(a^*-3.012b^*)} \quad (5)$$

Solubility

The solubility values were determined according to a previous study [39]. Exactly 0.50 g of the samples were dissolved in 50 mL of distilled water and stirred at 600 rpm for 5 minutes using a magnetic stirrer (Jeio Tech Lab companion, MS-32M, Yuseong-gu, Daejeon, South Korea). Then, the slurry was centrifuged (Sigma 3 K-18, Osterode am Harz, Germany) at 3000g for 5 min. The supernatant (25 mL) was transferred to a Petri dish and kept at 70°C until a constant weight was reached. The percentage solubility (%) was determined from the measurements of water-soluble samples to total samples.

Bulk and Tapped Density

The bulk density (ρ_b) and tapped density (ρ_t) of the samples were determined according to the methods used [40]. The bulk density was determined by measuring the weight and the volume of the powder and then proportioning them to each other. Similarly, the tapped density was determined by measuring the volume of 2 g of powder after tapping on a hard surface until the volume remained constant. The results are expressed in units of g/cm^3. Carr index and Hausner ratio values were calculated by using Equations (6) and

(7) with the values of bulk density and tapped bulk density.

$$CI = \frac{\rho_t - \rho_b}{\rho_t} \quad (6)$$

$$HR = \frac{\rho_t}{\rho_b} \quad (7)$$

Total Phenolic Content

Phenolic substances were extracted from samples according to a method used in a previous study [41] with slight modifications. Specifically, 0.5 grams of the samples were dissolved in 9.5 mL of 80% aqueous methanol solution and homogenized with a homogenizer (Ultraturrax, T 25 IKA Labortechnic, Staufen, Germany) for 10 minutes. The extraction process was performed for 2 hours on an orbital shaking water bath (GFL 1092, Burgwedel, Germany) operating at 150 rpm, set at 50°C. The extracts cooled to room temperature were filtered through filter paper (Whatman No. 42) to obtain clear extracts.

The TPC of clear extracts was determined using the Folin–Ciocalteu method reported in a previous work [42]. The volume of 0.5 mL extract was added into the solutions of 2.5 mL of 0.2 N Folin–Ciocalteu reagent and 2 mL of a 75 g/L Na_2CO_3. The mixture was incubated at 50°C for 5 minutes, then allowed to cool to room temperature. The absorbance of the final mixture was recorded with a spectrophotometer (Shimadzu UV Vis 160 A, Kyoto, Japan) at a wavelength of 760 nm. The aqueous methanol solution (80%) was used as the blank. The results were expressed as milligrams of gallic acid per gram of the powders.

DPPH Radical Scavenging Activity

The radical scavenging activity (%) of the powders were assessed using the DPPH radical inhibition method (1,1-diphenyl-2-picrylhydrazyl, Sigma-Aldrich Chemie, Steinheim, Germany) [43]. In this procedure, 950 µL of a 60 µM DPPH solution was mixed with 50 µL of the diluted extract and allowed to incubate in a dark place at ambient temperature for 30 minutes. The initial absorbance of the DPPH solution was measured against methanol at 516 nm. After incubation for 30 minutes, absorbance of the samples was recorded and the differences in absorbance were calculated relative to the DPPH solution. The antioxidant activity of the samples was then determined based on these absorbance differences. The results were calculated as grams of Trolox equivalent per 100 grams of dry matter (g TEAC/100 g dm).

The percent inhibition of DPPH radicals by each extract was calculated using the following Equation (8).

$$\textit{Radical Scavenging Activity}\ (\%) = [\ Abs_{blank} - (Abs_{sample} / Abs_{blank})\ ] * 100 \quad (8)$$

Volatile Composition

Volatile component analyses of *Juniper extract* powder, feeding solution and rehydrate powder were conducted using the GC/MS (QP 2000, Shimadzu, Kyoto, Japan) with the solid phase microextraction (SPME) method by using an autosampler (Combi PAL, CTC Analytics, Zwingen, Switzerland).

Volatile compounds were extracted from the dissolved powders. The powders were rehydrated to a final concentration of 20°Bx. Exact amount of 2 g rehydrated powders were transferred into 20 mL screw-cap vials and they were sealed with PTFE septum. Then the reconstituted samples were incubated in the vials at 40°C for 15 minutes while being agitated at 250 rpm. During the incubation, a fiber coated with 50/30 µm DVB/CAR/PDMS (Supelco, Pennsylvania, USA) was used to absorb the volatile compounds. After incubation, the fiber was desorbed in the injection port of the GC for 10 minutes at 200°C, with a split ratio of 1:5. Separation of volatile compounds was carried out using a fused silica column (TRB5-MS, 30 m x 0.25 mm i.d. x 0.25 µm film thickness). Helium (99.99% purity) was used as the carrier gas at a constant flow rate of 1.78 mL/min. Temperature of the column was programmed to hold at 40°C for 3 min, then increase to 100°C at 4°C/min (held for 3 min), and then to 200°C at 10°C/min (held for 3 min). The ion source, injection port, and detector temperatures were maintained at 200°C, 200°C, and 275°C, respectively. The MS conditions were set to electron impact (EI) at 70 eV, with scanning range of 30–500 m/z and scanning rate of 769 scans/s. Volatile compounds were identified by comparing mass spectral data with the Wiley 10 and NIST libraries, as well as retention indices [11, 44].

Particle Size

The particle size of the powder was assessed using the light scattering technique with a particle size analyzer (Mastersizer 2000 instrument, Malvern, Worcestershire, UK). This instrument was equipped with a liquid dispersion unit called Hydro 2000S. 2-Propanol was used as a dispersant to disperse the spray-dried powder. The particle size measurements were conducted with this dispersion at a temperature of 25°C. The refractive index of the particles was 1.52, while the dispersant refractive index was 1.39. The particle size analysis yielded two parameters: the surface mean diameter (D_{32}) and the volume mean diameter (D_{43}). Additionally, the particle size distribution of the powder was assessed using the span, calculated according to Equations (9-11) [45, 52]

$$D_{32} = \frac{\Sigma n_i d_i^3}{\Sigma n_i d_i^2} \quad (9)$$

$$D_{43} = \frac{\Sigma n_i d_i^4}{\Sigma n_i d_i^3} \quad (10)$$

$$Span = \frac{d_{90} - d_{10}}{d_{50}} \quad (11)$$

Where n_i is the number of particles of diameter d_i and d_{90}, d_{50}, and d_{10} are the equivalent volume diameters at 90%, 50%, and 10% cumulative volumes, respectively.

Statistical Analyses

The experiments were carried out in duplicates. All analyses were performed in triplicates. The results were evaluated in statistical analyses in an optimal (Combined) experimental design, while the response variables were estimated by a polynomial Equation (11) given below:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{123} x_1 x_2 x_3 + \beta_{133} x_1 x_3 x_3 + \beta_{233} x_2 x_3 x_3 \quad (12)$$

where x_1, x_2, and x_3 are independent variables corresponding to MD, GA and the inlet air temperature. Y and β are the optimized dependent variables and constant of coefficient, respectively. The values of probability (*p*) were used to determine the significance and effective levels of the model. Regression analyses were performed using Design Expert 13.0 (Stat-Ease Inc., Minneapolis, MN, USA) software to determine the coefficient of determination (R^2), analysis of variance (ANOVA), F-tests (lack of fit). The response surface methodology (RSM) was carried out according to the desirability function as given in following Equation (13):

$$D = (d_1 \times d_2 \times \ldots \times d_m)^{1/m} \quad (13)$$

where, m is the number of responses and d is the desirability of individuals. Individual desirability (d) evaluates how the settings optimize a single response while composite desirability (D) evaluates how the settings optimize a set of responses overall. The desirability functions were estimated as the product yield and α -pinene were maximum. The optimum points were determined by considering the highest values among the calculated desirability functions.

RESULTS and DISCUSSION

Optimum Drying Conditions for Juniper Extract

The juniper extract was dried with the mixtures of carrier materials to produce juniper extract powder with high phenolic content, α-pinene content and product yield. The ratio of the MD, GA, and optimum inlet air temperature were selected as independent variables. BCD proportion was fixed at 1 g/100 mL for each extract due to solubility concern. In the optimization experiments, it was decided to use a total of 15 g/100 mL of carrier materials, as the combinations of MD and GA in the proportions determined by the program (Table 1). The limit values of the independent variables were determined as regard to literature review and some preliminary tests. Three-dimensional surface graphs illustrating binary interactions of the variables were generated using Design Expert 13.0 software.

Table 1. The levels of factors and their effect on response variables (product yield, α–pinene area and total phenolic contents) used in the optimization of spray drying conditions

Run*	MD* (g/100 mL extract)	GA (g/100 mL extract)	Inlet Temperature (°C)	PY (%)	α-pinene (Peak area × 10^6)	TPC (mg GAE /g dm)
1	11.25	3.75	165	35.44	6.67	9.09
2	7.50	7.50	180	39.39	5.30	9.89
3	7.50	7.50	150	30.67	6.69	9.14
4	0.00	15.00	120	31.22	12.00	9.95
5	15.00	0.00	150	27.12	4.06	8.81
6	0.00	15.00	180	35.82	14.14	10.39
7	0.00	15.00	120	28.90	9.94	9.87
8	3.75	11.25	135	34.09	6.11	9.42
9	15.00	0.00	180	33.48	1.25	8.20
10	15.00	0.00	180	34.24	2.83	8.46
11	11.25	3.75	135	34.86	3.03	8.95
12	7.50	7.50	120	33.96	7.41	8.93
13	15.00	0.00	120	33.95	2.18	8.52
14	0.00	15.00	150	26.31	5.43	9.32
15	0.00	15.00	180	38.81	1.33	10.00

* For all runs, the amount of β-cyclodextrin was 1 g per 100 mL extract. **MD: Maltodextrin, GA: Gum Arabic, PY: Product Yield, TPC: Total Phenolic Content

The impact of the three variables on the response is depicted in Figures 1-3. According to the statistical analysis presented in Table 2; MD x AG, AG x T_{inlet} and T_{inlet} x T_{inlet} interactions were identified as the significant factors ($P < 0.05$) affecting the product yield. As seen in Figure 2, a saddle formation is present in the graph. The saddle peak is observed in the region where the ratios of the carriers in the mixture are close to each other, but the gum Arabic is relatively higher at the drying temperature of 180°C. This may be related to the shorter drying time of the particles in the drying chamber and the fact that mixtures containing GA adhered less to the glass drying chamber of the unit.

Table 2. The result of response surface methodology analysis for spray drying optimization

Legends		Product Yield (%) KCV model × KCV model		α - Pinene (area) Linear × Quadratic		TPC (mg/g) Linear × Quadratic	
		Coefficients	*P*	Coefficients	*P*	Coefficients	*P*
B_0	Constant	0	0.0045*	0	0.0001*	0	0.0002*
B_1	MD	28.42		4.68×10^6		8.89	
B_2	AG	27.98		6.01×10^6		9.41	
B_{12}	MD × AG	18.21	0.0064*				
B_{13}	MD × T_{inlet}	0.11	0.9225	-8.51×10^4	0.9149	0.0231	0.8628
B_{23}	AG × T_{inlet}	3.59	0.0048*	1.11×10^6	0.1345	0.2231	0.0794
B_{33}	T_{inlet} × T_{inlet}	5.41	0.0023*				
B_{133}	MD × T_{inlet} × T_{inlet}			2.79×10^6	0.0780	-0.4445	0.0914
B_{233}	AG × T_{inlet} × T_{inlet}			6.15×10^6	0.0016*	0.6852	0.0163*
	Lack of Fit		0.2973		0.3002		0.3264
R^2	Adjusted R^2	0.8106	0.7053	0.9187	0.8735	0.9112	0.8619
A.P.	Predicted R^2	9.2523	0.5427	12.6573	0.8173	12.5024	0.7652

*Values with *P*<0.05 are statistically significant. (A.P.: Adequate precision)

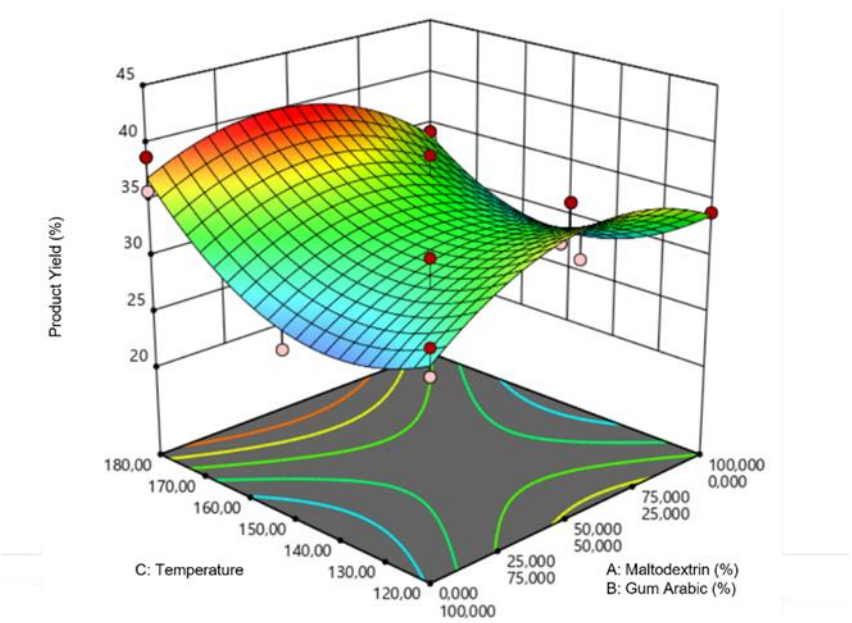

Figure 1. The effect of temperature, maltodextrin and gum *Arabic* amounts on product yield

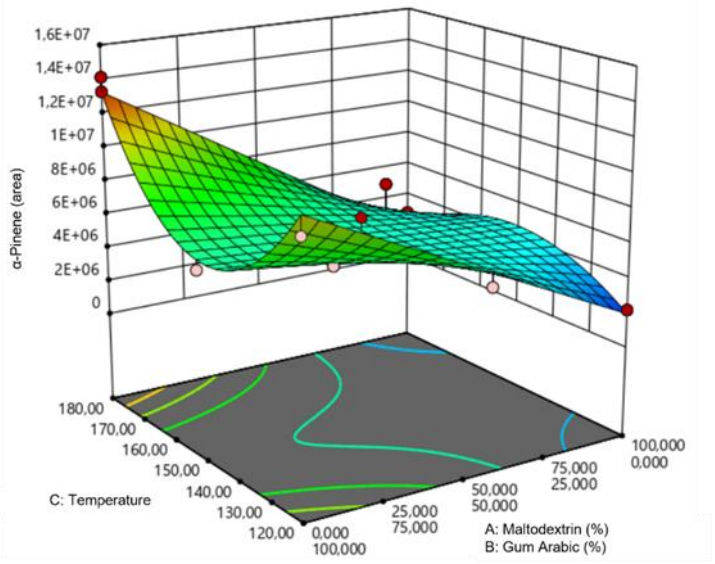

Figure 2. The effect of temperature, maltodextrin and gum Arabic amounts on α-pinene area

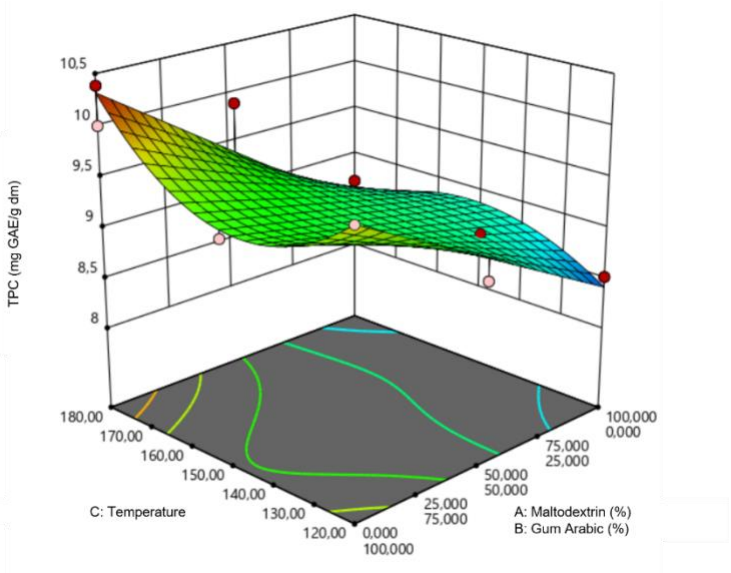

Figure 3. The effect of temperature, *maltodextrin* and *gum Arabic* amounts on total phenolic contents

According to the statistical analysis presented in Table 2, the interaction of MD × AG × T_{Inlet} was determined as the significant factors (P<0.05) affecting the α-pinene area. As seen in three-dimensional surface graphics illustrated in Figure 2, the α-pinene area increased as the amount of gum Arabic increased. In terms of temperature changes, an inverted hump formed in the graph; α-pinene area, which was at a minimum at the midpoint, increased with both an increase and a decrease in temperature. The increase in temperature boosted the α-pinene area more significantly. It is evaluated that this is due to the reduction in the loss of volatile components caused by the shortened drying time in the drying chamber with the increase in temperature.

The effects of independent variables on the TPC of the powder can be clearly seen in three-dimensional surface graph (Figure 3). The TPCs of the powder increased with increasing GA in the carrier mixture, but decreased with increasing MD. However, the effect of the carrier composition on the TPC was also associated with the drying temperature. Indeed, at drying temperatures around midpoint, the TPC of the powder decreased with higher AG content in the carriers, but it increased with the lower AG content. This interaction of AG × T_{Inlet} × T_{Inlet} was determined to be significant (p<0.05) on the TPC content (Table 2).

Optimized Spray Drying Conditions

Spray drying temperature and carrier composition were optimized to achieve maximum yield, α-pinene area and TPC. The optimum inlet air temperature, and the ratios of MD and GA were estimated as 180°C, 0 and 15 g/100 mL extract, respectively with a maximum soluble amount of BCD (1 g/100 mL extract). Using this condition, PY, α-pinene area and TPC were theoretically estimated as 36.89%, 13267352 and 10,32 mg/g dm, respectively. The theoretical results were experimentally verified and the difference between the results of the optimized dependent variables was below 6% (Table 3).

Table 3. Theoretical optimal spray drying conditions and experimental validation

	MD* (g/100 mL extract)	GA (g/100 mL extract)	T_{Inlet} (°C)	PY (%)	α – Pinene (peak area)	TPC (mg/g dm)
Theoretical	0	15	180	36.892	13.27×10^6	10.317
Experimental	0	15	180	35.284	12.49×10^6	9.886
Error (%)				4.557	5.890	4.178

*MD: Maltodextrin, GA: Gum Arabic, PY: Product Yield, TPC: Total Phenolic Content

Powder Analysis

Moisture Content and Water Activity

The moisture content and a_w of the juniper extract powder produced through the spray drying process were determined as 0.24±0.01 and 4.44±0.33 g/100 g, respectively (Table 4). Similar moisture content and a_w results of spray-dried fruit powders were reported in range of 2–5% and 0.2–0.6, respectively [46]. Based on the classification reviewed by [34], food powders are considered hygienic if they have a moisture content of less than 10% and a a_w of less than 0.6. Therefore, the current moisture content and a_w results of the juniper extract powder correspond to a stable and hygienic product.

Color Values

The color of many products mostly changes by thermal food processes, and they are simply characterized by L*, a*, b*, Hue Angle, and Chroma values. In this respect, the color properties of the juniper extract powder were measured as 87.15±0.30 for L*, 1.25±0.19

for a*, 16.32±0.85 for b*, 85.65±0.43° for hue angle, and 16.37±0.86 for chroma (Table 4). These color parameters indicate a light, slightly reddish yellow with moderate saturation. It is close to a pure and pale yellow but has a hint of red which makes it warm. The high lightness and moderate chroma of the color give it a vivid but not overwhelming appearance.

Table 4. Quality properties of juniper extract powder

Quality properties (unit)		Values
Product yield (%)		35.28±3.85
Moisture content (%)		4.44±0.33
Water activity (a_w)		0.24±0.01
Color values	L*	87.15±0.30
	a*	1.25±0.19
	b*	16.32±0.85
	h°	85.65±0.43
	C*	16.37±0.86
Browning index		21.34±1.42
Solubility (%)		78.30±0.54
Bulk density (g/cm^3)		0.39±0.01
Taped density (g/cm^3)		0.51±0.02
Carr index		23.81±0.81
Hausner ratio		1.31±0.01
Particle distribution	Span	2.15±0.05
	D_{43}	11.30±0.30
	D_{32}	3.49±0.15
HMF (mg/kg)		21.37±0.61
TPC (g GAE/100 g dm)		9.89±0.27
Antioxidant activity		4.12±0.35

Solubility

The water solubility value of juniper extract powder was determined to be 78.3% which indicates moderate solubility characteristic in water (Table 4). This result is in agreement with a number of previous work which indicated that the solubility is related to the use of 100% GA [33, 47].

Solubility is a critical quality parameter of powdered products that can significantly affect the reconstitution behavior of food powders. High solubility is particularly desired in food powders, especially for instant products. This property is primarily influenced by the main constituents, moisture content and particle size of the powders [48]. Previous studies reported that fruit powders produced by spray drying have high or moderate solubilities with the solubility values ranging from 70% to 99% [49, 50].

Bulk and Tapped Density

The bulk and tapped densities of the obtained juniper extract powders were determined as 0.39±0.01 g/cm^3 and 0.51±0.02 g/m^3, respectively (Table 4). In previous studies on spray drying of different raw materials, bulk densities were calculated between 0.20 - 0.60 g/cm^3 [49, 51-53]. The results of the present study are consistent with the findings of several previous studies. As these parameters affect the storage, stability and flow behavior of powdered products, they are important for food powders.

TPC

The TPC of the juniper extract powder was determined as 9.89±0.27 g GAE/100 g dm (Table 4). This result is relatively higher than that of a previous study (4.81 g GAE/100 g extract) performed on extract of juniper by [10], [54]. The higher value of the TPC result is most likely attributed to the calculation in dry matter. It may also be related to the origin of the fruits, harvesting time and the processing conditions of the powder.

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity method relies on the reduction of the DPPH radical by antioxidants that donate hydrogen and electrons. The antioxidant activity of juniper extract powder was determined to be 4.12 g of TEAC per 100 grams of dry matter. These antioxidant compounds determining the DPPH radical scavenging activity can mitigate the detrimental effects of free radicals in cellular systems [55].

Volatile Composition

The juniper extract powder produced in optimized condition were tested in characteristic volatile compounds to compare feeding solution, reconstituted powder and powder. Fifteen volatile compounds were identified for both extract and reconstituted powder. d-limonene, α-pinene and γ–muurolene were determined to as the main components of the extract. These main components were also determined as the main volatile components of the reconstitute powder, but their peak areas percentage were determined to be lower in comparison to the feeding extract. The optimized encapsulation process performed by the spray drying process enabled to keep 80.98% of the volatile component. The results obtained from the reconstituted samples showed that about 20% of the total volatiles were lost during the drying process. It is noteworthy that the highest encapsulation among the identified volatile

components could be provided in the β-pinene component, followed by α-selinene, d-limonene, β-myrcene and α-pinene. On the contrary, the highest losses among the volatile components were determined in α-muurolene, α-copaene and δ-cadinene. The volatile release from powder form of samples was 0.53%, indicating that an effective encapsulation was achieved. In terms of volatile release from the powders, the other components were not detected in the headspace of the samples.

Particle Size Index

The particle sizes of the juniper extract powder were measured and calculated as both volume-weighted mean representing the average diameter of a sphere with equivalent volume, and surface-weighted mean representing the average diameter of a sphere with equivalent surface area. The particle sizes were determined in range between 1.091±0.21 and 22.390±0.40 µm (Figure 4). The surface mean diameter (D_{32}), volume mean diameter (D_{43}), and span values of the powder were calculated as 3.495±0.15 µm, 11.298±0.30 µm, and 2.150±0.05, respectively (Table 4).

The particle size values were mostly influenced by wall and core materials composition, type of atomizer and spray drying conditions. The particle size of the juniper extract powder obtained in this study were significantly smaller than those reported in a previous study [56] performed on spray dried berry.

Table 5. Volatile composition of feeding solution, powder (aroma gain) and powder (aroma release)

RI TRB5-MS	Compounds	Feeding solution		Powder (Volatile Gain)		Powder (Volatile Release)	
		Area	%	Area	%	Area	%
910-925	α-pinene	3.22×10^7	24.30	2.56×10^7	19.30	1.01×10^5	0.08
952	β- pinene	1.86×10^6	1.41	1.74×10^6	1.32	-	
968	β- myrcene	8.15×10^6	6.15	6.54×10^6	4.94	-	
1007	D- limonene	5.31×10^7	40.08	4.72×10^7	35.62	-	
1321-1349	α- cubebene	4.55×10^5	0.34	7.06×10^5	0.53	-	
1343-1391	ylangene	1.65×10^6	1.24	8.86×10^5	0.67	-	
1349-1401	α-copaene	7.03×10^6	5.30	3.74×10^6	2.82	5.52×10^4	0.04
1391-1445	β- ylangene	4.65×10^5	0.35	7.46×10^5	0.56	-	
1401-1448	β- cubebene	6.32×10^5	0.48	8.83×10^5	0.67	-	
1427-1452	α- humulene	2.12×10^5	0.16	3.90×10^5	0.29	-	
1468-1491	α - selinene	3.57×10^5	0.27	3.30×10^5	0.25	-	
1452-1468	α- amorphene	3.57×10^5	0.27	2.74×10^5	0.21	-	
1448-1485	γ - muurolene	1.49×10^7	11.27	1.15×10^7	8.68	3.59×10^5	0.27
1471-1493	α- muurolene	2.78×10^6	2.10	1.45×10^6	1.09	-	
1485-1508	δ- cadinene	8.34×10^6	6.19	5.35×10^6	4.04	1.92×10^5	0.15
	Total	1.33×10^8	100.00	1.07×10^8	80.98	7.07×10^5	0.53

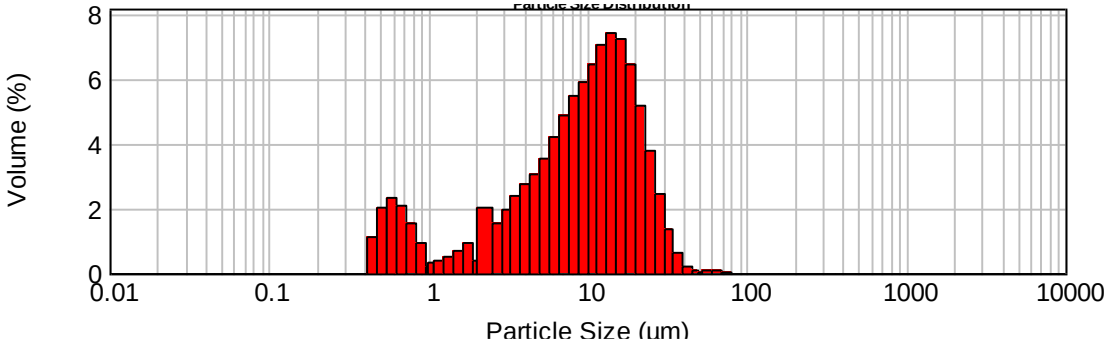

Figure 4. Particle distribution of juniper extract powder

CONCLUSION

In this study, for the first time, the production conditions of high-yield juniper extract powders rich in some bioactive and volatile components such as phenolics and α-pinene were optimized using spray drying. The sample using only GA as a wall material and powdered at an inlet temperature of 180°C maximized the responses obtained. The optimized spray drying encapsulation process enabled 80.98% of the volatile component to be retained. The volatile release in the final powder was 0.53%, indicating that an effective encapsulation was achieved. Fifteen volatile compounds were identified for both extract and reconstituted powder. d-limonene, α-pinene and γ–muurolene were determined to as the main components of the extract. Considering the physicochemical properties, juniper extract powder produced under optimized spray-drying conditions can enrich many food formulations in terms of flavor and bioactive components. Nevertheless, further studies should be carried out to improve particle distribution and increase product yield.

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Research Paper / Araştırma Makalesi

Bioactive Content and *in vitro* Antioxidant and Enzyme-Inhibitory Potential of Leaf and Fruits of Parsnip (*Pastinaca sativa* L. subsp. *Urens*)

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ABSTRACT

Parsnip *(Pastinaca sativa* L. subsp. *urens)* is one of the species of *Pastinaca* genus. This plant has been traditionally used worldwide for the treatment of various diseases and cultivated for its nutritional values. The aim of this study was to determine the antimicrobial and enzyme-inhibitory potentials of the fruits and leaves of parsnips as well as their bioactive properties such as their antioxidant activities, total phenolic and flavonoid contents in their hexane and ethanol extracts. The total phenolic and flavonoid contents were determined using Folin-Ciocalteu and aluminum chloride colorimetric methods, respectively. The antioxidant activities of extracts were determined by three methods of DPPH and ABTS radical scavenging activities and iron-chelating ability. In the leaves, the highest total phenolic content (60.94 mg GAE/g extract) was found in ethanol extracts while the highest total flavonoid content (21.47 mg RuE/g extract) was determined in hexane extracts. Ethanol extracts of leaves showed the highest radical scavenging activities in both assays of DPPH and ABTS with the IC_{50} values of 1039±1.35 and 150.7±0.81 µg/mL, respectively. Growth inhibition zone diameters (mm) of PSFE, PSLE, PSFH, PSLH (2 mg/mL) against reference microorganisms were -/-/15/15, -/-/15/14, 10/10/22/15, -/-/15/14 and MIC values -20>/20>/20>/20>, 20>/20>/20>/2.5, 0.625/10/-/-, 20>/20>/20>/20> mg/mL for *Staphylococcus aureus ATCC6538, Micrococcus luteus ATCC9341, Bacillus subtilis ATCC6633, Candida albicans ATCC14053,* respectively. Antimicrobial activity was not determined against other reference microorganisms.

Keywords: Antioxidant, Enzyme inhibition, *Pastinaca sativa,* DPPH•, Antimicrobial

Şeker Havucu (*Pastinaca sativa* L. subsp. *urens*) Yaprak ve Meyvelerinin Biyoaktif İçeriği ile *in vitro* Antioksidan ve Enzim-İnhibitör Potansiyeli

ÖZ

Şeker havucu (*Pastinaca sativa* L. subsp. *urens*), *Pastinaca* cinsinin türlerinden biridir. Bu bitki, dünya çapında geleneksel olarak çeşitli hastalıkların tedavisinde kullanılmakta ve besin değerleri nedeniyle yetiştirilmektedir. Bu çalışmanın amacı, şeker havucu meyve ve yapraklarının antimikrobiyal ve enzim inhibe edici potansiyelleri ile bunların antioksidan aktivitesi, toplam fenolik madde ve flavonoid içerikleri gibi biyoaktif özelliklerini belirlemektir. Toplam fenolik ve flavonoid içerikleri sırasıyla Folin-Ciocalteu ve alüminyum klorür kolorimetrik yöntemleri kullanılarak belirlenmiştir. Yaprak örnekleri arasında en yüksek toplam fenolik madde içeriği (60.94 mg GAE/g özüt) etanol özütünde bulunurken, en yüksek toplam flavonoid içeriği (21.47 mg RuE/g özüt) hekzan özütünde belirlenmiştir. Yaprak etanol özütü sırasıyla 1039±1.35 ve 150.7±0.81 µg/mL IC_{50} değeriyle en yüksek DPPH ve ABTS radikal

süpürücü aktivite göstermiştir. Meyve ve yaprakların etanol ve hekzan özütlerinin *Staphylococcus aureus ATCC6538, Micrococcus luteus ATCC9341, Bacillus subtilis ATCC6633, Candida albicans ATCC14053* mikroorganizmalara karşı büyüme inhibisyon zon çapı (mm) 2 mg/mL konsantrasyonda -/-/15/15, -/-/15/14, 10/10/22/15, -/-/15/14 ve MIC değerleri ise sırasıyla -20>/20>/20>/20>, 20>/20>/20>/2.5, 0.625/10/-/-, 20>/20>/20>/20> mg/mL olarak tespit edilmiştir. Diğer mikroorganizmalara karşı herhangi bir antimikrobiyal aktivite tespit edilmemiştir.

Anahtar Kelimeler: Antioksidan, Enzim inhibisyon, *Pastinaca sativa,* DPPH•, Antimikrobiyal

INTRODUCTION

Despite the great successes of herbal active substances (alkaloids, glycosides and others) and synthetic chemical compounds in the field of medicine, the medicinal plants still have an important place in the treatment of some diseases. According to the report of the World Health Organization, 80% of the world's population uses herbal treatment as a primary treatment method. Compared to before the 90s, it is seen that new herbal medicines have entered the treatment rapidly in the last 20-25 years and their use has become very common all over the world and in Türkiye. Developments in the pharmaceutical industry accelerated the production of synthetic drugs and the information about herbal medicines started to decrease. However, in the following years, because of the side effects in the use of synthetic preparations, the interest in the use of herbal medicines has gained momentum with the adoption of the philosophy of returning to nature. Parallel to this, pharmacological and clinical studies on herbal medicines have increased rapidly [1].

Vegetables and herbs used as spices are important in terms of protecting our health due to the secondary metabolites they contain. Especially vegetables and spices in the Apiaceae family have various health benefits due to the rich essential oil and coumarin compounds they contain [2]. *Pastinaca* is a genus of 15 species endemic to Eurasia that belongs to the Apiaceae family and the Tordylieae tribe. 'Pastus' (Latin) denotes food, nourishment, or something grown for eating [3]. Parsnip (*Pastinaca sativa*) is an edible root that has long been used in the cooking and preparation of infant food and livestock feed [4]. Parsnip root is a popular vegetable that may be eaten fresh, boiled, baked, fried, or roasted. According to previous studies, the important phytochemical components detected in parsnips are: coumarins, furanocoumarins, polyacetylenes, essential oils, terpenes and flavonoids [5-7]. Topical and oral use of parsnip is recommended for treatment of headaches, stomatitis, ophthalmitis, dermatitis and fever in traditional medicine [8]. Different parts of parsnip were used in folk medicine, for example, the root and leaf infusions were used for improve appetite, milk production as well as digestive and diuretic properties in Serbia [9], while in Italy the infusion of root and leaves was used for dietetic, cholagogue and diuretic purposes [10]. In the previous pharmacological activity studies on *P. sativa* species, there are studies such as antimicrobial and cytotoxicity [11]. Certain skin illnesses, such as vitiligo, mycosis fungoides and psoriasis are treated with a combination of psoralen or xanthotoxin with UV radiation (PUVA). Furanocoumarins are effective in the treatment of diseases such as psoriasis and mycosis fungoides due to their anti-proliferative effect, which causes selective photo-induced lesions to DNA [12]. In this context, *P. sativa* plant gains importance because it is rich in furanocoumarins. However, the studies on biological activities of different parsnip species were very limited.

The aim of this study was to analyze and statistically compare the antioxidant, antimicrobial and enzyme inhibitory activity of ethanol and hexane extracts prepared from leaves and fruits of *P. sativa* subsp. *urens*, which is a wild- growing relative of *P. sativa.*

MATERIALS and METHODS

Plant Materials

Pastinaca sativa L. subsp. *urens* (Req. Ex Gren. & Godr.) Čelak*,* as a whole plant was harvested from Beyşehir, located in the Konya province of Turkey (C4: Konya, Beyşehir, Beyşehir-Konya road's 1st km. stream side, 1200 m, 17.07.2018). The plant material was determined by a botanist specialist (Süleyman Dogu, Assoc. Professor at Department of Biology), works in Necmettin Erbakan University. Voucher specimen was kept in Herbarium of Necmettin Erbakan University (herbarium code: S DOĞU 3087). After the plant material was dried in the shade, it was pulverized into fine powder by laboratory type miller and sieved through a mesh size 80 to obtain a fine powder of uniform size.

Preparation of Plant Extracts

Twenty grams of each plant powder (fruits and leaves) was extracted with 300 mL of hexane and ethanol for 3 h in Soxhlet apparatus separately. The extracts were concentrated under vacuo using a rotary evaporator (Buchi, Switzerland) at 40°C to yield hexane and ethanol extract (Table 1). The extracts were kept in the refrigerator until utilization for *in vitro* assays.

Bioactive Contents of Plant Extracts

The total phenolic (TPC) and flavonoid contents (TFC) of the ethanol and n-hexane extracts of parsnips were determined using spectrophotometric methods, such as Folin-Ciocalteu [13] and aluminum chloride [14] methods, respectively. The total phenolic content was expressed as milligram of gallic acid equivalents per gram of extract (mg GAE/g extract). The quantities of total flavonoids were expressed as mg equivalence of rutin over gram of extract (mg RuE/g extract).

The antioxidant capacity of plant-derived compounds or extracts must be assessed using techniques that

consider the mechanism of antioxidant action. Therefore, in this study, the iron chelating activity (ICA), 2,2-diphenyl-1-picrylhydrazyl (DPPH•) and 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonate) radical ($ABTS^{+}$•) free radical scavenging assays were used to assess the antioxidant capacity of the extracts. The DPPH radical scavenging activity was measured according to the method of Clarke [13]. The ABTS model was used as an alternative radical scavenging capacity test and was estimated using method of Re [15] with some modifications. The iron chelating activities of the extracts were determined by their interaction with the formation of the ferrozine-Fe^{2+} complex [16].

Enzyme Inhibition Potentials

Anticholinesterase effects were assessed by slight modifying the Ellman's method in advance [17]. The tyrosinase inhibition effect was also carried out using the reported earlier technique, with L-dopa serving as the substrate, and kojic acid serving as a standard agent [18]. Moreover, as initially disclosed, α-glucosidase inhibition effect was measured by microplate assay [19]. Additionally, the α-amylase inhibition experiment was subjected by adapting the procedure used by Caraway-Somogi iodine/potassium iodide method [20].

Antimicrobial Activity

The antimicrobial activity of the ethanol and n-hexane extracts of parsnips at different concentrations was determined by disc diffusion and broth microdilution method. *Escherichia coli ATCC8739, Salmonella typhimurium ATCC14028, Pseudomonas aeruginosa ATCC9027, Staphylococcus aureus ATCC6538, Staphylococcus epidermidis ATCC12228, Micrococcus luteus ATCC9341, Bacillus subtilis ATCC6633, Candida albicans ATCC14053* were used as the reference microorganisms in these tests.

In the disk diffusion assay, reference microorganisms at a density of 0.5 MacFarland were spread on Mueller Hinton Agar (Neogen, Lot No:UK305171/109) and Sabouraud Dextrose Agar (Neogen, Lot No:209791/190) surface respectively bacterial and yeast strain. 10 µL different concentrations of the ethanol and n-hexane extracts of parsnips (2-1-0.5 mg/mL) were dropped onto the surface of medium and incubated for at 37°C 18-20 hours for bacteria and 22°C 48 hours for yeast. Gentamicin (10 µg) and Fluconazole (25 µg) were used as positive controls for bacterial and yeast strains, respectively. Sterilizing distilled water was used as a negative control. Formed that growth inhibition zone diameters were measured after incubation [21]. This test was repeated three times.

Broth microdilution method was used to determine the minimal inhibitory concentrations (MICs), and different concentrations of the ethanol and n-hexane extracts of *P. sativa L.* subsp. *urens* (50-0.05 mg/mL), Mueller Hinton Broth (MHB) (Liofilchem, Lot No: 080718502) for bacteria and Sabouraud Dextrose Broth (SDB) (Neogen, Lot No: UK318137/333) for yeast were used. First wells and other wells of microplate were filled 50 µL of MHB or SDB. The ethanol and n-hexane extracts of *P. sativa L.* subsp. *urens* were dissolved in DMSO and added to the first well (50 µL). Then serial two-fold dilutions were made. Microorganism suspensions were prepared at a density of 0.5 MacFarland ($1.5x10^{8}$ cfu/mL). These were subsequently diluted to 10^{5} cfu/mL- 10^{3} cfu/mL respectively bacteria and yeast strains. Then microorganism suspensions were added to all wells (50 µL). Microplates were incubated at 37°C, 18-20 hours for bacteria and 22°C 48 hours for yeast and read at a OD_{620nm}. Each test included growth and sterility control. The lowest concentration without growth was determined as MIC [22]. This test was repeated 3 times. Vancomycin and Fluconazole were used as positive control bacteria and yeast strain, respectively. Those with only medium in the wells were considered as sterility control, and those with medium and bacteria/yeast strains were considered as growth control.

To determine minimal bactericidal (MBC) and fungicidal concentrations (MFC), microplate wells in which no growth was observed were inoculated on 100 µL Nutrient Broth (NB) and SDB for bacteria and yeast strains, respectively. Liquid cultures were incubated 18-24 hours for bacteria and 48 hours for yeast strain. The lowest concentration with no visible growth was defined as MBC/MFC [23].

Statistical Analysis

Each bioactivity test was conducted in triplicate during the experimental procedures. Three parallel assessments' mean were utilized to summarize the results. The significance was analyzed using one-way ANOVA followed by Tukey's test. If the p value was less than 0.05, the difference was statistically viewed as significant.

RESULTS and DISCUSSION

In this study, the bioactive compounds of the hexane and ethanol extract prepared from the fruit and leaves of parsnips were determined in terms of TPC and TFC, the results are shown in Table 1. Among the extracts, the highest TPC was observed in the PSLE extract (60.94 mg GAE/g extract) followed by the PSFE extract (41.75 mg GAE/g extract). However, the TFC was highest in PSLH extract (21.47 mg RuE/g extract) followed by the PSFH extract (12.34 mg RuE/g extract). According to the results, while ethanol solvent is the best solvent for obtaining phenolic compounds from this plant, hexane seems to be a better solvent for obtaining flavonoids. The Folin-Ciocalteu method, that used for the determination of total phenol content, may cause the results obtained to be higher than the actual value due to the reaction of reducing agents as well as phenolic compounds. Furthermore, the fact that the method is carried out in aqueous media limits the measurement of lipophilic phenolic compounds. To overcome these limitations, the use of chromatographic assays is recommended for future studies.

Many disorders, including autoimmune diseases, inflammation, Parkinson's and neurodegenerative

diseases, aging, cataracts, arteriosclerosis, and cancer, are influenced by oxidative stress [24]. There are reports about the high correlation of phenolic compounds with their antioxidant potential [25]. Therefore, plant phenolics have a great interest in developing natural antioxidants. In a study, the total phenolic content of root and fruit extracts of *Pastinaca sativa* L. subsp *urens* was found as (67.86±1.02 mg GAE/g extract) and (50.40±1.40 mg GAE/g extract), respectively [26]. In another study, the TPC was reported for methanol extract of *Pastinaca ferulacea* as 65.1 mg/g gallic acid [27].

Antioxidants prevent cell damage by reducing oxidative stress caused by free radicals. Therefore, determining the antioxidant capacity of herbal extracts may provide important health benefits. The high phenolic content of parsnips reveals the potential for potent antioxidant activity.

Table 1. Extract yield, total phenolic and flavonoid contents, and antioxidant activities of *Pastinaca sativa L.* subsp. *urens* hexane and ethanol extracts[a]

Extract/ Reference*	Extract yield (%, g/g)	Total phenolic (*m*g GAEs/g)[b]	Total flavonoids (*m*g RuEs/g)[c]	Antioxidant activity (IC_{50} µg/mL)		
				DPPH	ABTS	Iron chelating
PSFH	5.50	33.30±0.44	12.34±1.98	45303±0.61	1142±0.79	-
PSFE	10.73	41.75±4.07	3.98±0.49	2365±0.92	280.4±0.79**	8130±0.60
PSLH	1.82	32.32±1.77	21.47±8.37	-	585.5±0.84*	1090±0.77
PSLE	13.48	60.94±3.44	0.07±3.09	1039±1.35*	150.7±0.81**	4816±1.35
Quercetin		-	-	168.5±1.99*	-	
BHT		-	-	-	163.4±1.24**	
EDTA		-	-	-	-	822.6±2.46

a: The data was presented as the averages ± standard deviations of three parallel calculations. b: GAEs. Gallic acid equivalents (y = 0.0027x + 0.0084 gallic acid (mg) (r^2 = 0.997)). c: REs. Rutin equivalents (y = 0.0056x + 0.1313 rutin (mg) (r^2 = 0.993)). *PSFH: Hexane extract *of P. sativa* fruit; PSFE: Ethanol extract *of P. sativa* fruit; PSLH: Hexane extract *of P. sativa* leaves; PSLE: Ethanol extract *of P. sativa* leaves

Enzyme Inhibitory Activity

Enzyme inhibitors play an important role in the treatment of various diseases. In particular, α-glucosidase inhibitors are used in the treatment of diabetes. Therefore, the study of enzyme inhibitory activities of parsnip extracts is critical for the discovery of potential therapeutic agents. Acetylcholine is a neurotransmitter found at the intersections of nerves and muscles, lymph nodes of the motor systems of internal organs, and various parts of the central nervous system. The reduction of acetylcholine in the brain causes Alzheimer's disease. Therefore, it is an important agent for this disease. Studies have reported that increases in acetylcholine levels due to cholinesterase inhibition is a target therapeutical strategy for Alzheimer's disease [28]. Cholinesterase inhibitory activities of *P. sativa* L. subsp. *urens* ethanol and hexane extracts were screened according to the Ellman method and the results are given in Table 2. PSLE displayed the best inhibitory activity against AChE (IC_{50}: 798.4±1.15 µg/mL), while the PSFH extract showed the best inhibitory activity on the BChE (IC_{50}: 242.9±2.25 µg/mL).

The dopachrome method was used to test tyrosinase inhibitory activities of *P. sativa* L. subsp. *urens* ethanol and hexane extracts. As it is given in Table 2, PSFE extract showed inhibitory activity against tyrosinase with the IC_{50} value of 999.2±0.98 µg/mL while the reference compound kojic acid was exhibited tyrosinase inhibitory activity with the IC_{50} value of 190.7±0.79 µg/mL. The other extract exhibited lower tyrosinase inhibitory activity in the following order: PSLE>PSLH>PSFH.

Table 2. Enzyme inhibition effects of *Pastinaca sativa* L. subsp. *urens* extracts[a] (IC_{50} µg/mL)

Extract/ Reference[b]	AChE	BChE	Tyrosinase	α-glucosidase	α-amylase
PSFH	2115±2.58	242.9±2.25**	3781±0.47	2644±0.92	22338±0.42
PSFE	23702±0.62	660.6±1.44*	999.2±0.98	233.0±3.12*	13775±0.41
PSLH	8515±0.12	22556±0.32	1800±0.66	2184±0.73	845.9±1.44**
PSLE	798.4±1.15*	545.6±2.28*	1557±0.55	5.38±0.29***	1585±4.40
Galanthamine	132.4±2.24**	54.00±0.26**	-	-	-
Kojic acid	-	-	190.7±0.79*	-	-
Acarbose	-	-	-	1794±0.88	5705±0.27

[a]:IC_{50} values were presented as the averages plus standard deviations of three parallel calculations. [b]:PSFH: Hexane extract *of P. sativa* fruit; PSFE: Ethanol extract *of P. sativa* fruit; PSLH: Hexane extract *of P. sativa* leaves; PSLE: Ethanol extract *of P. sativa* leaves. *, **, *** means for marking significance levels of P<.05, P<.01, P<.001.

The α-glucosidase enzyme is one of the important target enzymes that is used in antidiabetic therapeutic. The enzyme converts the polysaccharide into monosaccharide in the intestine, which is the main reason for raising postprandial glucose level. Antidiabetic activities of *Pastinaca sativa* L. subsp.

urens extracts on a-amylase and a-glucosidase were determined by spectrophotometric method. As it presented in Table 2, the highest α-amylase inhibitory activity was found in PSLH extract (IC_{50}: 845.9±1.44 µg/mL) while the best α-glucosidase inhibitory activity was observed in PSLE extract (IC_{50}: 5.38±0.29 µg/mL). In a former study, the crude extract of *P. sativa* showed α-glucosidase and α-amylase inhibitory activity with the IC_{50} value of 88.05±1.25 µg/mL and 91.69±1.5 µg/mL, respectively [29].

Antimicrobial Activity

Antimicrobial agents help to control infections by inhibiting the growth of pathogenic microorganisms. In this study, determination of the antimicrobial activity of Parsnip is important for the discovery and development of natural antimicrobial agents (Figure 1). Growth inhibition zone diameters (mm) of PSFE, PSLE, PSFH, PSLH against reference microorganisms and MIC values were shown in Table 3. As can be seen from the results, growth inhibition zone diameters (mm) of PSFE, PSLE, PSFH, PSLH (2 mg/mL) against reference microorganisms were -/-/15/15, -/-/15/14, 10/10/22/15, -/-/15/14 and MIC values were found as 20>/20>/20>/20>, 20>/20>/20>/2.5, 0.625/5/20>/20>, 20>/20>/20>/20> mg/mL and MBC/MFC values were found as 20>/20>/20>/20>, 20>/20>/20>/2.5, 0.625/10/-/-, 20>/20>/20>/20> mg/mL for *Staphylococcus aureus ATCC6538, Micrococcus luteus ATCC9341, Bacillus subtilis ATCC6633, Candida albicans ATCC14053,* respectively.

Table 3. The growth inhibition zone diameter of PSFE, PSLE, PSFH and PSLH* determined by disc diffusion method

Reference Microorganisms	Growth Inhibition Zone (mm) 1-2-3-4 (PSFE, PSLE, PSFH, PSLH)				MIC (mg/mL) 1-2-3-4	MBC/MFC (mg/mL) 1-2-3-4
	2 mg/mL	1 mg/mL	0.5 mg/mL	Positive Control		
Escherichia coli ATCC8739	-/-/-/-	-/-/-/-/	-/-/-/-	7	-	-
Salmonella typhimurium ATCC14028	-/-/-/-	-/-/-/-	-/-/-/-	7	-	-
Pseudomonas aeruginosa ATCC9027	-/-/-/-	-/-/-/-	-/-/-/-	16	-	-
Staphylococcus aureus ATCC6538	-/-/-/-	-/-/-/-	-/-/-/-	22	-	-
Staphylococcus epidermidis ATCC12228	-/-/15/15	-/-/12/14	-/-/-/-	32	20>	20>
Micrococcus luteus ATCC9341	-/-/15/14	-/-/14/12	-/-/13/10	32	20>/20>/20>/2.5	20>/20>/20>/2.5
Bacillus subtilis ATCC6633	10/10/22/15	-/6/15/13	-/-/-/11	30	0.625/5/20>/20>	0.625/10/-/-
Candida albicans ATCC14053	-/-/15/14	-/-/12/12	-/-/-/-	15	20>	20>

*PSFH: Hexane extract *of P. sativa* fruit; PSFE: Ethanol extract *of P. sativa* fruit; PSLH: Hexane extract *of P. sativa* leaves; PSLE: Ethanol extract *of P. sativa* leaves

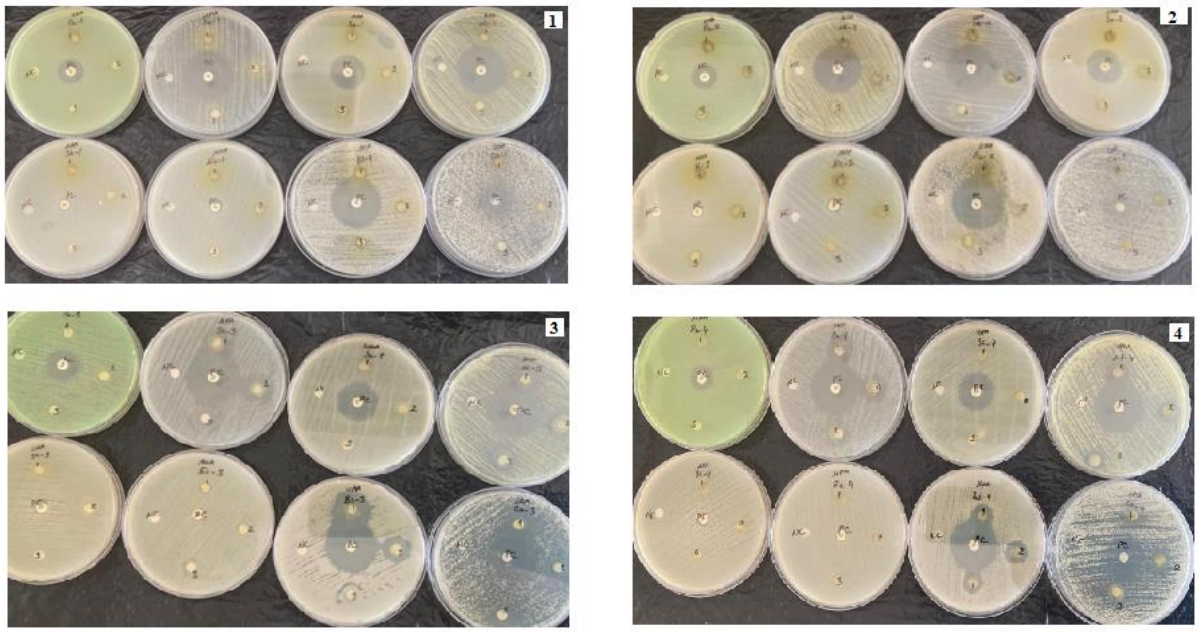

Figure 1. Antimicrobial activity of PSFE (1), PSLE (2), PSFH (3), PSLH (4) (2-1-0.5 mg/mL) by disc diffusion method (PSFH: Hexane extract *of P. sativa* fruit; PSFE: Ethanol extract *of P. sativa* fruit; PSLH: Hexane extract *of P. sativa* leaves; PSLE: Ethanol extract *of P. sativa* leaves)

Determination of the MIC is important for diagnostic laboratories due to it helps in confirming resistance of microorganism to an antimicrobial agent. It is a highest dilution or least concentration of the extract that inhibit

growth of microorganism. Therefore, the lower the MIC value of the plant extract, the higher its antimicrobial activity. According to some authors, plant extracts with MICs <100 µg/mL were considered highly active antimicrobial agents; MICs ranging from 100 to 500 µg/mL were classified as active; MICs ranging between 500 and 1000 µg/mL were considered moderately active; MICs ranging from 1000 to 2000 µg/mL were considered to have low activity; and MICs >2000 µg/mL were classified as inactive [30]. When evaluated accordingly, since the MIC value of the PSFE extract was 0.625 mg/mL, it showed moderate antimicrobial activity against *Bacillus subtilis*, while other extract inactive against the tested microorganisms.

In a previous study, the essential oil of three parsnip species were investigated for antimicrobial activity using microdilution method against *Candida tropicalis, C. parapsilosis, C. krusei, C. glabrata, C. albicans, Staphylococcus aureus, Bacillus cereus, Listeria monocytogenes, Escherichia coli, Salmonella typhimurium* and *Enterobacter cloacae;* with the minimum inhibition concentration (MIC) between 0.25–8 mg/mL and minimum bactericidal concentration (MBC) between 0.5–16 mg/mL [31].

CONCLUSION

Antioxidant, antimicrobial, and enzyme inhibitory activities of hexane and ethanol extracts of parsnip fruits and leaves were investigated with total phenolic and flavonoid contents in this current study. This study is the first investigation of the antimicrobial and enzyme inhibitory activities of parsnip. This shows that the study makes an important contribution to the literature. Moreover, In the study, the activities of compounds with different polarities were compared using both hexane and ethanol extracts. This allows the biological activities of the parsnip to be evaluated from a broader perspective. It was determined that PSLE extract with the highest total phenolic contents had the best antioxidant activity in all studied assays except iron chelating assay. When the extracts showed moderate enzyme inhibitory activities, PSLE extract showed superior inhibitory activity against α-glucosidase. Also, PSFE extract showed highest antimicrobial activity on *Bacillus subtilis* with the MIC value of 0.625 mg/ml, while the PSLH extract showed highest antimicrobial activity on *Micrococcus luteus* with the MIC value of 2.5 mg/ml. This study can be considered as the first investigation on antimicrobial and enzyme inhibitory activities of *P. sativa*. In the continuation of this study, it is thought that these plants, which are used for different purposes in folk medicine, will contribute more to the research of biological activity and the production of products that can be used as food support.

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Research Paper / Araştırma Makalesi

Effect of Different Drying Methods and Distillation Times on Essential Oil Composition and Antioxidant Content of Rosemary

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ABSTRACT

The aim of this study is to determine the effect of different drying methods and distillation times on the antioxidant and essential oil contents of rosemary. The essential oil content of rosemary extracts was determined using hydrodistillation and its composition by GC-FID/MS. The antioxidant content of deoiled materials was quantified on the LC-MS/MS. Carnosol and carnosic acid contents (LC/MS-MS) and essential oil compositions (GC-MS) were determined after each treatment. The highest essential oil (2.34%), carnosol (0.62%), and carnosic acid (0.85%) contents of dried samples were determined at 45°C. Some variations were also observed in the essential oil composition of dried samples. These quality parameters showed significant variations over different distillation times. And, the distillation time of 120 min was determined more appropriate to obtain essential oils and antioxidant compounds at a high yield. To produce rosemary extracts with a high content of essential oils and antioxidant compounds, it is highly recommended that the fresh plants should be first dried at 45°C, and then dried samples should be processed into essential oils by hydrodistillation for 120 min. The remnants of plant materials from the production of rosemary essential oil could be used to produce carnosol and carnosic acid. Eventually, essential oils and antioxidant compounds should be extracted from rosemary in an integrated manner.

Keywords: *Rosmarinus officinalis*, Drying, Distillation time, Essential oil, Antioxidant

Farklı Kurutma Yöntemi ve Damıtma Süresinin Biberiyenin Uçucu Yağ Bileşimi ve Antioksidan İçeriği Üzerine Etkisi

ÖZ

Bu çalışmanın amacı farklı kurutma yöntemleri ve distilasyon sürelerinin biberiyenin antioksidan ve uçucu yağ içeriği üzerine etkilerini belirlemektir. Biberiye özütünün uçucu yağ içerikleri hidrodistilasyon yöntemi, uçucu yağların bileşimi de GC-FID/MS cihaz ile belirlenmiştir. Uçucu yağı alınanan örneklerin antioksidan bileşen analizleri LC-MS/MS kullanılarak tespit edilmiştir. Her uygulamadan sonra karnosol ve karnosik asit içerikleri (LC/MS-MS) ve uçucu yağ bileşen (GC-MS) analizleri gerçekleştirilmiştir. Kurutulan örneklerde en yüksek uçucu yağ (%2.34), karnosol (%0.62) ve karnosik asit (%0.85) içerikleri 45°C'de kurutlmuş örneklerde belirlenmiştir. Kurutma sıcaklığına göre örneklerin uçucu yağ bileşiminde bazı farklılıklar tespit edilmiştir. Örneklerin analizi yapılan kalite parametreleri distilasyon sürelerine göre de önemli farklılıklar göstermiştir. Distilasyon sürelerine göre en yüksek uçucu yağ oranı ve antioksidan içeriği 120 dakikalık süresinde tespit edilmiştir. Sonuç olarak biberiyeden hem uçucu yağ hem de antioksidan (karnasol ve karnosik asit) üretmek için 45°C'de kurutmanın uygun olduğu, kurutulan örneklerden uçucu

yağ elde etmek için de 120 dakikalık hidrodistilasyon uygulamasının yeterli olduğu ortaya konulmuştur. Biberiye uçucu yağı üretiminden arta kalan bitkisel materyalin daha sonra karnosol ve karnosik asit üretiminde kullanılabileceği tespit edilmiştir. Sonuçlar biberiyeden uçucu yağ ve antioksidan üretiminin entegre bir şekilde yapılabileceğini ortaya koymuştur.

Anahtar Kelimeler: *Rosmarinus officinalis*, Kurutma, Distilasyon süresi, Uçucu yağ, Antioksidan

INTRODUCTION

Rosemary (*Rosmarinus officinalis* L.) is a valuable essential oil and spice plant of the Lamiaceae family. It grows in mainly Mediterranean countries and its dried or fresh form is used as a spice in culinary. Rosemary is also processed for essential oil and antioxidant production [1].

Chemical composition is the main characteristics of rosemary essential oil [2]. As it is well known, the essential oil composition of MAPs (medicinal and aromatic plants) depends on physiological variations, genetic factors, environmental conditions, post-harvest processing and storage conditions [3]. Drying and distillation among post-harvest processes have generally significant effects on essential oil yield and composition of MAPs [4]. Fresh MAPs commonly contain 75-80% moisture, and if they are not processed fresh, moisture levels must be lower then 15% for protection from biochemical alteration andmicrobial spoilage of MAPs. Drying is a considerably used treatment for this purpose. On the other hand, essential oil yield and composition of any MAPs could be varied depending on drying methods and process parameters [5]. Significant variations were examined in the essential oil content and composition of rosemary according to applied drying methods [6]. The drying process has also important effects on the concentration of compounds with the antioxidant effects like rosmarinic acid, carnosol and carnosic acid in rosemary depending on the applied method parameters [7]. Commonly, low drying temperatures (30-50°C) are advised to preserve susceptible active constituents of MAPs [8]. Then, dried plant materials could be processed for essential oil or phenolic compounds. Rosemary essential oil is commonly obtained by steam or hydro-distillation methods [9]. Essential oil yield and composition of any MAPs could also be varied significantly according to distillation time [10]. Essential oil composition (active constituents' concentration) is an important factor to determine the commercial economic value for any essential oil. Furthermore, the usage area and activity performance of any essential oil could be varied in relation to active components concentration [11]. The plant residue after essential oil production is not generally evaluated effectively. It is thought that the marc of rosemary leaves, after essential oil production, can be a rich source of some antioxidants, especially water-insoluble ones. Rosemary is also used in rosmarinic acid (water soluble), carnosol and carnosic acid (water insoluble) production. These natural antioxidants contents in rosemary could be varied according to the growing region, harvesting time, plant parts [12, 13]. Moreover, essential oil distillation parameters could also influence their concentration. Together with these compounds, there has been increasing interest in natural biologically active compounds. Rosmarinic acid which is naturally occurring in several plants including rosemary, displays several biological activities together with anti-oxidant. There are many studies, including anti-oxidant properties of rosmarinic acid. However, the changes in the tricyclic carnosol and carnosic acid in rosemary due to different process parameters and the situation in the activities are not considered in detail. Additionally, there is no comprehensive study on both essential oil and diterpenoids (carnosol and carnosic acid) contents of rosemary concerning drying processes and distillation time. Hence, the objective of this paper is to assess the impact of drying temperatures and distillation time intervals on essential oil profile and carnosol and carnosic acid contents for rosemary leaves. The goal of this study is to present essential oil and antioxidants (carnosol and carnosic acid) which could be produced from the same raw material.

MATERIALS and METHODS

Materials

The rosemary (*Rosmarinus officinalis*) leaves were obtained from Tarsus-Mersin (coordinates: 36°58'05″ N; 34°48'30″ E, altitude: 254 m), located in the Mediterranean region of Turkey, in September 2017. The samples were brought to the Medicinal and Aromatic Plants Laboratory of Western Mediterranean Research Institute as soon as possible after harvesting and then they were allowed for drying processes afterward separating leaves from stems. In order to avoid raw material differences, all leaves were mixed and then randomly divided into three batches for each replication.

Methods

The drying process was carried out in the shade and tray dryer at three different temperatures (35, 45 and 55°C) using the convective hot-air oven up to the remaining moisture content of about 10%.

Essential Oil Content and Composition

The essential oil content of the samples was determined with hydrodistillation by using the Clevenger apparatus (Isotex, 98-IV-B, China). Deionized water (300 mL) was added to the Clevenger apparatus which contained a 20 g sample and extraction was carried out for 3 hours. The results were expressed as mL volatile oil content of 100 g sample (%, v/w) [14].

The essential oil composition of samples was analyzed by gas chromatography (Agilent 7890A, USA) coupled to a flame ionization detector and mass spectrometry (Agilent 5975C, USA) using capillary column (HP Innowax Capillary; 60.0 m × 0.25 mm × 0.25 µm) [15]. Essential oils were diluted at a 1:100 ratio with hexane. GC-FID/MS analysis was carried out at a split mode of 50:1. Injection volume and temperature were adjusted to 1 µL and 250°C, respectively. Helium (99.9%) was the carrier gas at a constant flow rate of 0.8 mL/min. The oven temperature was programmed as follows; 60°C for 10 min, increased at 4°C min^{-1} to 220°C and held for 8 min at 220°C. MS-spectra were monitored between 35-450 amu and EI ionization mode was used at 70 eV. The relative percentage of the components was calculated from GC-FID peak areas. The components were identified by using Wiley7n, NIST05 and Adams mass spectrum libraries. Some pure standards (*α*-pinene, 1,8-cineole, borneol, etc.) were also injected at the same procedure to compare and check the results for correct identification of the compounds. Moreover, the identification of each component was evaluated both by matching their mass spectra in the libraries and retention index values were calculated against to C_8-C_{24} alkane series.

Carnosol and Carnosic Acid Contents

Before the extraction, dried materials were ground by a grinder (Retsch GM200, Germany) at 10000 rpm for 20 s. Thereafter, the sample was extracted by ethyl alcohol in an ultrasonic water bath (Bandelin Sonorex, Germany) for 15 min. Then, centrifuged by ultracentrifuge (Sigma, 2-16KL,) at 5000 rpm at 4°C for 5 min. Finally, the supernatant was filtered from a 0.45 µm membrane filter and transferred to a liquid chromatography (Agilent, 1290 Infinity, USA)-mass spectrometer (Agilent 6430 Triple Quad, USA) (LC-MS/MS) for analysis [16].

Antioxidant compounds were quantified according to the method by Fischer *et al*. [17] by using the Zorbax RRHD Eclipse Plus C18 column (3 µm, 100 × 2.1 mm) on the LC-MS/MS (Agilent. First of all, MS optimization parameters of carnosol and carnosic acid were determined (Table 1). Then, calibration solutions for each component (1, 2, 5, 10 and 20 ppm) were prepared and the calibration curve was drawn using these determined parameters.

Table 1. MS optimization parameters for the antioxidant compounds.

Parameter	Compound	
	Carnosol	Carnosic acid
Polarity	+	-
Precursor ion	330.8	330.9
Fragmentor voltage	110	90
Product ions	266.8, 284.9	286.9, 243.9
Collision energy	18, 10	18, 20

LC-MS/MS Analysis Parameters

The HPLC elution was carried out at 35 °C with an gradient flow of mobil phases (Table 2) at a flow rate of 0.3mL $minute^{-1}$ and injection volume of 3 µL. Mobil phase: A; methanol:water (5:95(v/v), 0.01% formic acid, 5 µM ammonium formate), B; methanol (0.01% formic acid, 5 µM ammonium formate). Total analysis duration was 15 minute and, ionization was done by ESI source at 70 eV.

Table 2. Gradient elution program used in chromatographic analyses

Time (minute)	A (%)	B (%)
0.00	95	5
3.00	95	5
8.00	20	30
12.00	10	95
15.00	5	5

Statistical Analyses

The experiment was conducted in a randomized design with three replications and the results of the analyses were reported as mean ± standard error (SE). Significant differences were calculated by analysis of variance using SAS software (SAS Institute Inc., Cary, NC). Furthermore, Duncan's multiple range test was performed to determine the significance of differences between variances ($P<0.05$).

RESULTS and DISCUSSION

Rosemary leaves are dried conventionally in shade at atmospheric conditions. Otherwise, they are dried by different drying methods especially air-circulated oven dryers at different temperatures concerning raw material status. Figure 1 shows the variation of essential oil and antioxidant component contents of rosemary leaves depending on the drying processes.

The essential oil yields of the samples showed statistically significant differences ($P<0.05$) compared to the drying methods. The essential oil content of the samples dried in the shade had the lowest value at 1.84%. This process duration was one week to obtain about 10% moisture level. The second lowest value was determined as 2.03% at 55°C. The former has a long drying time and this may lead to essential oil loss. The latter has a high drying temperature compared to other applications. Thus, this may lead to similar results because of evaporation. Previous studies stated that drying at high temperatures or long time may cause a decrease in essential oil content [18, 19]. In order to avoid this result, drying is generally carried out at 30-50°C and as much as possible a short time for MAPs. Our findings showed that the highest essential oil content was determined at 35°C and 45°C. MAP's drying is very important in terms of preserving the quality and aromatic properties. As the drying temperature is increased, the amount of essential oil decreases [20]. Piga *et al*. [21] examined the effect of different drying temperatures (30°C, 38°C and 45°C) and air flow rates (300, 1250 m^3 h^{-1}) on the amount of essential oil for rosemary grown in Italy. Essential oil yields were determined as 4.05%, 3.69% and 2.98% for each temperature, respectively. Thus, the best drying temperature for the rosemary plant was mentioned as

45°C for fast hot-air flow and at 38°C for low-speed hot-air flow. Jalal *et al*. [22] examined the effect of different drying practices on the essential oil content of rosemary. The highest rate of essential oil was determined as 0.9% in shade dried sample. In another study the essential oil ratios were 1.00%, 0.14% and 0.12% for mint (*Mentha piperita* L.), and 2.13% 1.62% and 1.09% for rosemary at 40, 60 and 80 °C, respectively [6]. Our results are in line with the literature concerning temperature effects. While Piga *et al*.'s [20] findings are higher than our results, Jalal *et al.* [22] report's value is lower than the present study. These differences could result from raw material (genetic factors, harvesting time, etc.), applied temperature, instrument properties (air flow, temperature increment etc.) differences as mentioned by Hernandez *et al.* [2].

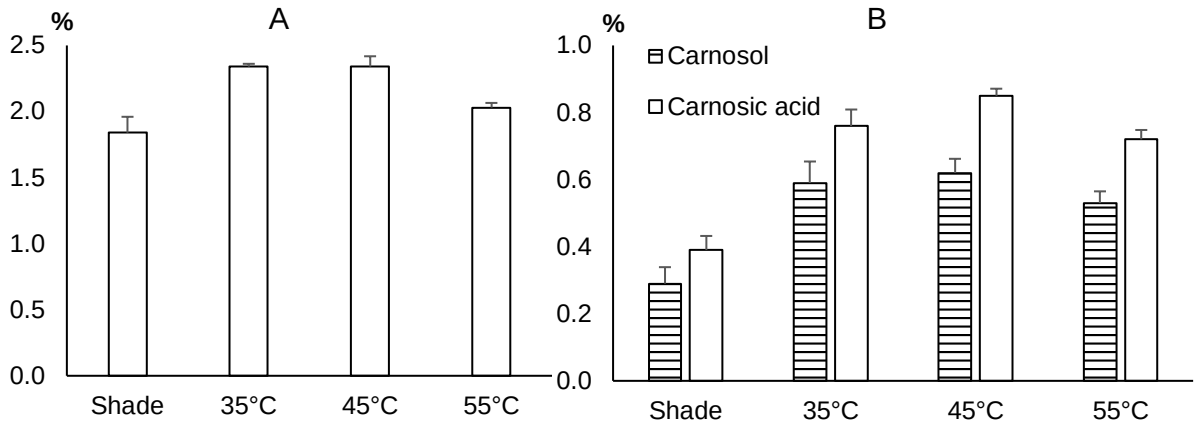

Figure 1. Essential oil (A), carnosol and carnosic acid (B) contents of the samples dried with different drying methods (means±standard deviation)

The drying process may also have an effect on some other phytochemicals like antioxidants. The main antioxidant compounds are carnosol and carnosic acid for rosemary [23]. It was observed that there were statistically significant differences in the amounts of carnosol and carnosic acid in the samples depending on the drying conditions. The lowest carnosol and carnosic acid were determined at 55°C followed by room temperature dried (in shade) sample. And, the highest values for these components were determined at 45°C. Similar results were observed in essential oil yield as aforementioned. Research showed that drying conditions may affect the chemical composition of MAPs. It has been reported that the antioxidant properties of MAPs and their extracts could be changed concerningdrying methods. Hence, it is important to choose appropriate drying methods for obtaining targeted phytochemicals with high yields [24]. Such as Orphanides *et al.* [25] found that there was a decrease in phenolic amounts when high temperatures were applied to mentha. However, the drying effects on the antioxidant compounds of rosemary are very limited. Hussain *et al*. [26] determined the total phenolic matter, rosmarinic acid content and antioxidant activity (ORAC) of six Lamiaceae herbs, including rosemary, dried by air, freeze and vacuum oven. Air-dried one had the highest values for these parameters. Mulinacci *et al*. [7] compared the antioxidant compounds content of the freeze-dried and air-dried (shade) rosemary. Freeze-dried samples had higher carnosol, carnosic acid and rosmarinic acid than shade-dried ones. Present findings showed significant differences from the literature because of process parameters and raw material differences. It is concluded that the most suitable drying process among the applied processes is 45°C oven drying in terms of carnosol, carnosic acid and essential oil yields. Within the scope of the research, the effects of drying on the essential oil composition were also examined. The essential oil composition changes of the samples are depicted in Table 3.

Results showed that the essential oil composition of rosemary was significantly affected ($P<0.05$) by the drying applications. The main component of rosemary essential oil is 1,8-cineole. There are two types of rosemary in the European pharmacopoeia, and their chemical composition differed from each other with respect to the ratio of main components. The main components of rosemary essential oil were 1,8-cineole and camphor. There are two types of rosemary essential oil. One of them called Spanish and the other one is Moroccan and Tunisian in European Pharmacopoeia. There are major differences in essential oil composition and also some other characteristics of them [28]. Napoli *et al.* [29] classified rosemary in three chemotypes as cineoliferum (1,8-cineole rich), camphoriferum (camphor > 20%), verbenoniferum (verbenone > 15%), considering essential oil composition. The rosemary used in the present study is close to Moroccan and Tunisian types with respect to the European Pharmacopoeia and cineoliferum according to essential oil composition. The highest 1,8-cineole content was found in shade-dried sample as 58.91% followed by 55°C. The sample dried at 45°C had the lowest 1,8-cineole content among the oven-drying applications. Additionally, the highest *α*-terpineol and *δ*–terpineol contents were found in shade-dried samples, and the lowest value was obtained at 45°C. On the other hand, *α*-pinene, camphene and borneol were found as the highest at 45°C, and the lowest values were found in shade-dried samples. The data obtained showed that there were partial changes in the other essential oil

components depending on the drying process. The change in the ratio of the essential oil component during drying depends on the type of compounds, the drying time and temperature [19]. Szumny *et al*. [30] reported drying methods have significant effects on the essential oil composition of rosemary. Convective and vacuum-microwave drying combination was found more appropriate because of the short time process to decrease the adverse impact of the drying on the product. Verma and Chauhan [31] compared shade and oven drying (50°C) effects on rosemary essential oil composition. 1,8-cineole concentration was found to be 31.8% in shade drying and 32.9% in oven drying. Other major constituents, camphor and *α*-pinene yields were found to be 26.6%, 13.4% in shade drying and 31.7% and 9.2% in oven drying, respectively. The difference between our research findings and literature values is thought to be due to the difference in herbal material. However, as stated by Verma and Chauhan [31], the similarity between 50°C applications in the shade and in the oven was also seen in our findings.

Table 3. Essential oil composition of the samples dried with different drying methods (%, mean±SE)

Compounds	RI†	RI‡	Drying Conditions			
			Shade	35°C	45°C	55°C
α-pinene	1024	1025	8.20^{b}±0.268	8.67^{ab}±0.778	9.53^{a}±0.297	9.48^{ab}±0.255
Camphene	1068	1069	2.49^{b}±0.085	2.88^{a}±0.099	3.15^{a}±0.057	3.02^{a}±0.156
β-pinene	1111	1110	6.39^{b}±0.085	7.42^{b}±0.057	6.17^{b}±0.099	5.80^{c}±0.099
β-myrcene	1165	1161	1.80^{a}±0.085	1.74^{a}±0.071	1.76^{a}±0.042	1.51^{b}±0.028
α-terpinene	1182	1178	0.74^{a}±0.014	0.76^{a}±0.042	0.73^{a}±0.014	0.71^{a}±0.007
Limonene	1202	1198	1.99^{ab}±0.057	1.94^{b}±0.014	2.08^{a}±0.042	1.74^{c}±0.042
1,8-cineole	1214	1211	58.91^{a}±0.750	54.20^{b}±1.287	52.63^{b}±0.962	58.23^{a}±1.153
γ-terpinene	1248	1245	1.26^{c}±0.085	1.72^{a}±0.099	1.55^{ab}±0.085	1.41^{bc}±0.071
p-cymene	1274	1270	1.03^{a}±0.014	0.87^{b}±0.071	0.98^{ab}±0.042	0.96^{ab}±0.057
Camphor	1533	1515	2.03^{b}±0.113	2.36^{a}±0.042	1.95^{b}±0.085	1.07^{c}±0.057
Bornyl acetate	1591	1579	2.20^{c}±0.085	3.03^{a}±0.085	2.66^{b}±0.127	2.16^{c}±0.156
Terpinen-4-ol	1613	1601	3.15^{b}±0.141	4.10^{a}±0.113	3.81^{a}±0.226	2.64^{c}±0.170
δ-terpineol	1679	1679	0.91^{a}±0.042	0.81^{ab}±0.057	0.71^{b}±0.028	0.75^{b}±0.014
α-terpineol	1704	1694	4.71^{a}±0.113	4.20^{b}±0.127	3.94^{b}±0.255	4.09^{b}±0.085
Borneol	1709	1700	4.21^{d}±0.127	5.30^{c}±0.057	8.34^{a}±0.099	6.41^{b}±0.339

In each row different letters mean significant differences between applications (P<0.05).
† Calculated from retention times of each compound compared by alkane series' retention time.
‡ Retention indices mentioned in Babushok *et al*. [27].

Distillation time is another factor that has an effect on essential oil, carnosol and carnosic acid amounts. For this purpose, samples were taken and analyzed at 10, 20, 30, 60, 120 and 180 min intervals from the beginning of boiling in the hydrodistillation process to compare the findings. Carnosol and carnosic acid were determined in the plant marc (waste for essential oil process) during this process. Figure 2 shows the essential oil, carnosol and carnosic acid contents of each sample with respect to distillation time.

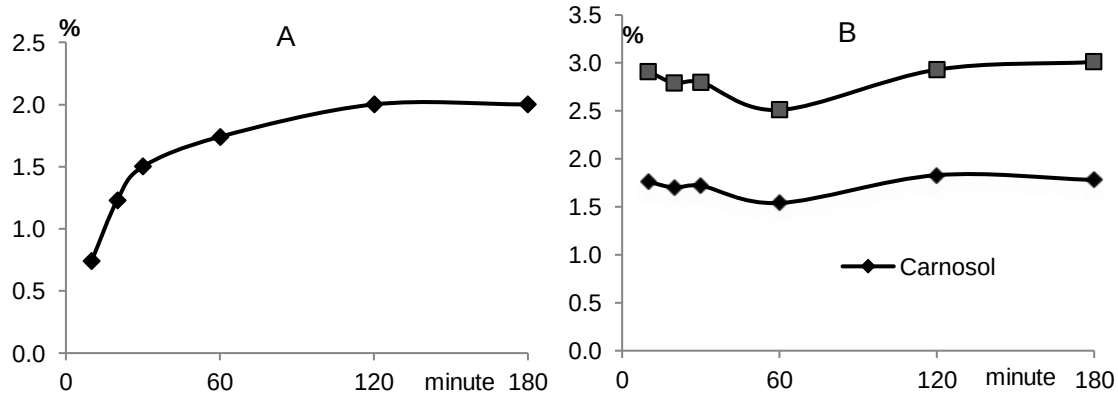

Figure 2. Essential oil (A), carnosol and carnosic acid contents (B) of the samples with respect to distillation time

Some differences were observed in each antioxidant component depending on the distillation times. While these differences were statistically insignificant for carnosol (P > 0.05), 60 min of distillation for carnosic acid differed statistically from other applications. The highest carnosol content was determined in 120 min of distillation, followed by 180 min. Along with this, the highest carnosic acid was found in 180 and 120 min applications. On the other hand, statistically significant differences were found in essential oil content according to the distillation times. When the essential oil amounts are compared, 37% of the total essential oil is in the first 10 min, 61.5% in 20 min, 75% in 30 min, 87% in 60 min, and the whole in 120 min. As a result, it was determined that the 120 min distillation time is sufficient for the production of essential oil from rosemary during this

process. Essential oil content could be decreased with increasing distillation times because of the essential oil volatile characteristics. According to the encountered literature, there are no available records on together with antioxdant eficiency and essential oil contents of rosemary leaves with respect to distillation times. However, detailed research has been carried out on the essential oil and antioxidant content of rosemary individually. Zhelijazkov *et al*. [10] studied the effect of steam distillation time on essential oil yield and composition of rosemary. They reported that there were no increments in essential oil yield after 40 min distillation. Essential oil yield was found as 0.51% at this time and then started to decrease to 0.38% at 160 min. Jalal *et al.* [22] determined the essential oil yield of rosemary with respect to distillation time. They found the total essential oil yield as 1.8% at 3 hours hydro-distillation. And, 75% of the oil was obtained in 20 minutes. The essential oil content was somewhat higher than the reported by Zhelijazkov *et al.* [10] and Jalal *et al*. [22] This could be sourced from rosemary genetic differences and also distillation procedure differences (boiling rate, size reduction, plant/water ratio etc). Hence, important differences were seen in essential oil composition for each material and also in distillation methods. According to Wollinger *et al*. [32] distillation time also affects antioxidant compounds concentration in both water and leaves residue. They found that the highest rosmarinic acid in the water residue for 150 minutes of distillation due to its water solubility. On the other hand, carnosic acid content of the water residue was low because of the low solubility of this compound in water. These findings show that it would be appropriate to produce essential oils and antioxidants from rosemary in an integrated manner.

Essential oil compositions were determined to ascertain the effect of distillation times on the quality of the oil. Duncan's results of essential oil components are given in Table 4.

Table 4. Essential oil composition of the samples with respect to distillation time (%, mean±SE)

Compounds	Distillation time (min)					
	10	20	30	60	120	180
α-pinene	12.70^{a}±1.259	10.44^{bc}±1.032	9.21^{c}±0.339	10.41^{bc}±0.608	12.13^{ab}±0.354	13.33^{a}±0.099
Camphene	4.55^{a}±0.453	3.35^{b}±0.325	3.21^{b}±0.311	3.31^{b}±0.368	3.69^{ab}±0.368	3.87^{ab}±0.339
β-pinene	4.21^{a}±0.410	3.25^{b}±0.325	3.15^{b}±0.311	3.44^{ab}±0.339	3.91^{ab}±0.382	3.97^{ab}±0.269
β-myrcene	1.26^{b}±0.127	1.26^{b}±0.127	1.27^{b}±0.127	1.35^{b}±0.127	1.57^{ab}±0.156	1.67^{a}±0.057
α-terpinene	0.54^{ab}±0.057	0.50^{b}±0.049	0.51^{b}±0.057	0.53^{ab}±0.057	0.66^{ab}±0.071	0.67^{a}±0.071
Limonene	1.84^{bc}±0.184	1.75^{c}±0.170	1.92^{bc}±0.184	1.94^{bc}±0.198	2.31^{ab}±0.226	2.47^{a}±0.184
1,8-cineole	57.79^{abc}±1.344	60.96^{a}±1.018	57.62^{abc}±1.895	60.05^{ab}±1.711	56.39^{bc}±1.442	54.72^{c}±1.739
γ-terpinene	1.04^{a}±0.141	0.86^{a}±0.127	0.90^{a}±0.099	0.83^{a}±0.113	1.06^{a}±0.156	1.03^{a}±0.141
p-cymene	1.81^{a}±0.170	1.80^{a}±0.255	1.95^{a}±0.276	1.94^{a}±0.269	2.11^{a}±0.184	2.26^{a}±0.325
Camphor	1.73^{a}±0.240	1.66^{a}±0.240	1.92^{a}±0.269	1.53^{a}±0.212	1.52^{a}±0.212	1.36^{a}±0.198
Bornyl acetate	2.15^{a}±0.141	1.92^{a}±0.269	2.26^{a}±0.325	1.75^{a}±0.247	1.95^{a}±0.276	1.65^{a}±0.141
Terpinen-4-ol	3.95^{a}±0.141	3.12^{ab}±0.438	4.13^{a}±0.580	2.55^{b}±0.354	3.31^{ab}±0.283	3.74^{a}±0.523
δ-terpineol	0.44^{b}±0.057	0.67^{ab}±0.099	0.83^{a}±0.113	0.72^{a}±0.099	0.65^{ab}±0.092	0.63^{ab}±0.085
α-terpineol	1.97^{b}±0.283	3.57^{a}±0.509	4.61^{a}±0.651	4.04^{a}±0.566	3.62^{a}±0.481	3.60^{a}±0.297
Borneol	4.02^{b}±0.566	4.88^{ab}±0.905	6.49^{a}±0.919	5.60^{ab}±0.792	5.13^{ab}±0.240	5.03^{ab}±0.849

In each row different letters mean significant differences between applications (P<0.05).

The essential oil compositions are significantly affected by the distillation times. 1,8-Cineole, having a monoterpene ether structure and also named eucalyptol, is the main component of rosemary essential oil. The biological activities of the essential oil are attributed to mainly monoterpenes such as 1,8-cineole, borneol, pinene [33]. In particular, 1,8-cineole is widely used for cough treatment, muscular pain, neurosis, rheumatism, asthma, and urinary stones and also in the cosmetic industry [34]. It ranged from 54.72% (180 min) to 60.96% (20 min) depending on distillation time. There is a decrease in the amount of this main component after 20 min of distillation time. The second highest component was found as *α*-pinene for this oil. Contrary to 1,8-cineole, the highest value for *α*-pinene was determined at 180 minutes' distillation time. The average concentration of *α*-pinene decreased to a minimum degree of 9.21% at the 30th min and then started to increase, reaching 13.33% at the 180th min. The other major components were camphene, *β*-pinene, terpinen-4-ol, *α*-terpineol and borneol. There was a change in camphene and *β*-pinene similar to the *α*-pinene concentration with regard to distillation time. On the other hand, terpinen-4-ol, *α*-terpineol and borneol amounts varied irregularly considering distillation times. The concentrations of *β*-myrcene, *α*-terpinene, limonene, *p*-cymene components in the essential oil varied between 1.26-1.67%, 0.50-0.67%, 1.75-2.47%, 1.80-2.26%, respectively. These component concentrations increased by 33%, 24% 34%, and 25% from the beginning to the end of the distillation period, in the same order. This is related to the boiling point and solubility degree in water of each component of essential oil [35]. Sadeh et al. [36] reported that essential oil components were separated with respect to water solubility degree than boiling points.

Zheljazkov *et al.* [10] applied eight distillation times from 1.25 min to 160 min with a steam distillation method to compare the yield and composition of rosemary essential oil. Major components were determined as *α*-pinene, 1,8-cineole and camphor. They reported that, while *α*-pinene, 1,8-cineole yields decreased with increasing in distillation time, camphor increased during distillation. Likewise, 1,8-cineole content ranged between 18.9-23.3%, and the highest ratio was determined at 2.5 min distillation time. Jalal *et al.* [22] reported that 75% of the total essential oil was obtained

at 20 minutes' distillation time and highest 1,8-cineole with 60,13% at this time for rosemary. There is no more available record about essential oil composition and antioxidant content for rosemary applied at different distillation time according to encountered literature. However, some research has been conducted on this topic for different MAPs. One of them is on bay laurel and its main component is 1,8-cineole. For this purpose, the effect of five different hydro-distillation times (10-120 min) on the essential oil composition of bay laurel was investigated. It was found that the distillation time was quite effective in the laurel essential oil composition. It was determined that 1,8-cineole, which is one of the important components of laurel essential oil, varies between 57.7-79.4% depending on the application time, the highest 1,8-cineole ratio was determined with 79.4% in 10 min of distillation time application [37]. Toker *et al*. [38] determined differences in essential oil composition for oregano as to distillation times. The main component is carvacrol for this plant, ranging between 62.92-84.35%, and the highest value was determined for 30 minutes' distillation. Some changes were also observed in studies on the effect of distillation time on plants such as fennel [39]. There are some differences between the present study and the literature. This could be the result of raw materials, processing techniques and process parameters. To compare the yield of antioxidant components, there are no available results on these compounds regarding distillation times.

CONCLUSION

Drying is the most common and fundamental method for post-harvest preservation and processing of any MAP's. It was determined that the most suitable drying temperature for rosemary was 45°C in an air circulation oven of essential oil and antioxidant content. Rosemary can be evaluated in different ways following the drying process, and one of the products that can be produced in this sense is essential oil. Within the scope of the study, depending on the duration of the distillation process used in the production of essential oil, the essential oil yield and composition, as well as carnosol and carnosic acid contents, were analyzed and the optimum distillation time was tried to be revealed. In this sense, it was observed that a two-hour hydrodistillation application under laboratory conditions gave the most successful results in terms of essential oil. Besides, the biomass residue after hydro-distillation (essential oil production) could be evaluated to obtain antioxidant substances called carnosol and carnosic acid. Moreover, results revealed that compositional differences for the essential oil could be obtained by accounting for distillation process times. As a conclusion, essential oil and antioxidant production from rosemary should be done in an integrated manner.

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Research Paper / Araştırma Makalesi

Effect of Cold PlasmaTreatment on Physicochemical and Microbiological Properties of Clotted Cream

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ABSTRACT

The aim of this study was to determine the effect of cold plasma treatment, which was applied by using two different gases (O_2 and Ar) and their mixtures onto sample surfaces for different intervals, on the physicochemical and microbial parameters of clotted cream during storage for up to 10 days. Cold plasma treatment caused a decrease in pH, aw, L*, and b* values of clotted cream samples and an increase in their a* and TBA values. The pH values of samples ranged from 4.68 to 6.67, and the range for a_w values was between 0.903 and 0.803. For TBA, the highest change was observed in the K2 sample with 0.479 mg malondialdehyde/kg. The color L* values of all samples were between 99.79 and 93.82, a* values between 1.82 and 1.22, and b* values between 6.22 and 4.74. Among the treatments, the treatment of O_2-Ar gas mixture (50-50%) resulted in the highest decrease in the total counts of aerobic mesophilic bacteria (TMAB), total aerobic psychrophilic bacteria, proteolytic bacteria, yeast-molds, coliform group bacteria, and *Staphylococcus aureus*. Compared to the control sample, at the end of 10 days of storage, a decrease of 2.41 for TMAB, 3.64 for total yeast-mold, 4.23 for coliform group bacteria, and 4.72 log cfu/g for *S. aureus* was achieved. Results indicated that the cold plasma treatment did not cause significant changes in the physicochemical values of clotted cream samples but reduced their microbial load.

Keywords: Cold plasma, Clotted cream, Argon, Pathogen, Quality

Soğuk Plazma Uygulamasının İnek Kaymağının Fizikokimyasal ve Mikrobiyolojik Özellikleri Üzerine Etkisi

ÖZ

Bu araştırmanın amacı, iki farklı gaz (O_2 ve Ar) ve bunların karışımının farklı sürelerde örnek yüzeyine uygulanmasıyla gerçekleştirilen soğuk plazma işleminin, kaymağın fizikokimyasal ve mikrobiyal parametrelerine etkilerinin 10 günlük depolama süresince değişiminin incelenmedir. Soğuk plazma uygulaması, kaymak örneklerinin pH, a_w, L*, b* değerlerini azaltırken, a* ve TBA değerlerini arttırmıştır. Örneklerin pH değerleri 6.67-4.68 arasında, a_w değerleri ise 0.903-0.803 arasında değişmiştir. TBA için en fazla değişim 0,479 mg malonaldehit/kg ile K2 örneğinde olmuştur. Tüm örneklerin L* değerleri 99.79-93.82, a* değerleri 1.82-1.22 ve b* değerleri 6.22-4.74 aralığında belirlenmiştir. Toplam aerobik mezofil bakteri sayısı (TMAB), toplam aerobik psikrofil bakteri sayısı, proteolitik bakteri sayısı, toplam maya-küf sayısı, koliform grubu bakteri sayısı, *Staphylococcus aureus* sayısı ve üzerinde uygulamalar arasında en fazla azalmaya gaz karışımı uygulaması neden olmuştur. Kontrol örneğine göre 10 günlük depolama sonunda, TMAB 2.41, toplam maya-küf 3.64, koliform grubu bakteri 4.23 ve *S.aureus* 4.72 log kob/g azalmıştır. Sonuçlar, soğuk

plazma uygulamasının, kaymağın fizikokimyasal özelliklerinde önemli bir değişime neden olmadığı ancak mikrobiyal yükü azalttığını göstermiştir.

Anahtar Kelimeler: Soğuk plazma, Kaymak, Argon, Patojen, Kalite

INTRODUCTION

Milk, a necessary food at all stages of human life, is an essential food product, especially for children, pregnant women, and older people, especially for balanced nutrition and improving and protecting bone health. Dairy products, as well as milk, play important roles in ensuring that people have a balanced diet and live a healthy life. Dairy products with high nutritional value contain primarily protein, fat, calcium, phosphorus, vitamin A, vitamin B12, and riboflavin [1]. Among all these components, milk fat is ranked after milk protein in importance. Milk fat, one of the leading quality criteria in the production of various dairy products, is used as a raw material in some products. The most important of these products are cream and butter [2].

As is known, due to the density difference between the phases of milk fat (0.93 g/cm^3) and serum (~ 1.036 g/cm^3), when kept for a while, the fat particles will accumulate on the surface and move upwards [3]. Over time, the layer accumulating on the surface becomes rich in fat and forms the "cream layer" structure, mainly consisting of milk fat. The cream is produced by passing this layer through different stages of the process. According to the Turkish Food Codex Cream and Clotted Cream Communiqué (2009/5), creams containing 60% milk fat are called clotted cream [4].

Clotted cream is a product with a slightly acidic taste and creamy consistency [5]. Today, clotted cream can be produced industrially or using traditional methods. The rich nutritional value and high moisture content of clotted cream are the most important factors limiting its shelf life [6]. In addition, the absence of fermentation in the process of clotted cream and its higher pH value compared to other fermented dairy products cause its shelf life to be shorter [7]. In addition, the microbiological quality of clotted cream is another factor that affects its shelf life [2].

The microbiological and sensory properties of clotted cream may vary depending on the quality of the milk used and the conditions at the production stage [2]. Most of the enterprises producing clotted cream in our country are local small family enterprises, and they become vulnerable to microbial contamination due to non-compliance with hygiene and sanitation rules, lack of standard production, and failure to create hygienic conditions during packaging and storage [8]. One of the main concerns in cream production is the presence of pathogenic bacteria, particularly *Staphylococcus aureus* and *Enterococcus* species. Studies [9] have shown that methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium* [10] were isolated from clotted cream samples. The presence of these bacteria requires implementing effective microbial control measures during production. Processing techniques such as pasteurization to reduce the microbial load in the cream [11], the use of natural preservatives such as nisin, a bacteriocin produced by Lactococcus lactis [12], as well as the integration of good hygienic practices and good manufacturing practices during production are recommended to minimize contamination risks [13]. Practices such as biological preservation and advanced sanitation programmes are used to prevent microbial spoilage in dairy products [14]. In addition, non-thermal minimal processes such as ionizing radiation, cold plasma, and high hydrostatic have begun to be used as an alternative to traditional methods [15]. Non-thermal techniques have emerged as promising alternatives to traditional thermal processing methods to reduce microbial loads while preserving the sensory and nutritional qualities of food products. Cold plasma technology is a promising, innovative, non-thermal method for microbial inactivation. Cold plasma produces reactive species that can damage microbial cell membranes and inactivate pathogens [16,17]. This technique has been investigated for application in various food products, including fruits and vegetables, and its potential use in dairy products is being investigated. The ability of cold plasma to decontaminate surfaces without the need for heat makes it an attractive option for preserving clotted cream quality [16].

The cold plasma has become a useful and technological method that is important for the surface sterilization of food products [18]. Plasma is defined as ionized gas or the fourth state of matter [19], which consists of reagents that contains photons, electrons, positive-negative ions, free radicals, and neutral atoms, including sufficient amounts of electrical energy to multiply chemical reactions that break covalent bonds of different types [20, 21]. Cold plasma is produced between electrodes using radio frequency (RF), dielectric barrier discharge (DBD), and microwaves (MW) using various gases such as oxygen, argon, and helium [22]. Inactivation of microorganisms on the food surface can be achieved with cold plasma [23].

Previous studies have primarily focused on the application of non-thermal processes to milk. Notably, there is a lack of research on the use of cold plasma technology in clotted cream. This study investigates the microbiological and physicochemical quality changes in clotted cream by applying cold plasma with different gases to its surface during various storage periods.

MATERIALS and METHODS

Materials

Clotted cream samples used in the research were obtained from a producer in Afyonkarahisar (Türkiye)

province. Clotted cream samples were brought to the Afyon Kocatepe University, Faculty of Engineering, Department of Food Engineering microbiology laboratory under a cold chain and kept in the refrigerator at 4°C until the analysis was completed. The gases used in the research were purchased from a company operating in Afyonkarahisar province.

Methods

Cold Plasma Treatment

The cold plasma system used in the study was generated by modifying the method used by Aktop [24]. (Figure 1). To produce the required plasma, a power source with a power of 25 kV and a frequency of 42 kHz was used. The system used two different gases (argon and oxygen) and their mixtures. The gas flow was determined as 1 L/min, and the application was made for two different periods (20 and 40 min). In the study, 7 pieces of 1 mm tungsten steel electrodes were used, and one of the electrodes was placed horizontally across the other 6 electrodes to produce plasma between the anode and cathode ends. The distance between the ends was set to 13 mm. Before the treatment, the samples obtained from the market were mixed homogeneously for 5 min with the help of a sterile mixer (Arçelik K 9250) at the lowest speed to prevent the creams from undergoing any physical changes. In this way, a homogeneous distribution of the microbial load was ensured. Then, 100 g of the samples were weighed separately into sterile Petri dishes using a sterile spatula and a Bunsen burner flame on a precision scale. The control sample was not subjected to any cold plasma treatment and was prepared by taking it from the mixture with a sterile spatula, like the other samples. All samples were adjusted to 100 g. Samples were placed in sterile Petri dishes and subjected to cold plasma treatment. The samples were prepared with a diameter of 90 mm and a height of 5 mm. The distance between the point where the plasma was formed and the sample surface was adjusted to 60 mm. Although this distance could be adjusted, it was set as standard in all samples

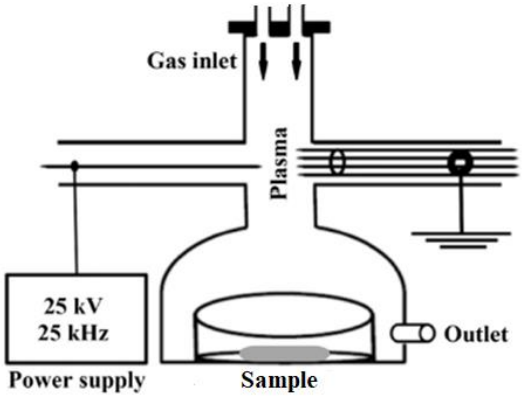

Figure 1. The cold plasma system [23]

Physicochemical Analyses

The pH values of the clotted cream samples were determined with the Cyberscan 300 model pH according to AOAC 981.12, and the a_w values were determined with Novasina LabTouch-a_w (Novasina AG, Lachen, Switzerland) according to AOAC 978.18 [25,26]. The color of the samples was measured using a colorimeter (Minolta Co., Osaka, Japan). CIE L*a*b* color space gives the lightness (L*, from 100 for white to 0 for black), redness (a*, from +120 for red to −120 for green) and yellowness (b *, from +120 for yellow to −120 for blue) values of the color [27].

2-Thiobarbituric acid (TBA) values of clotted cream samples were determined by spectrophotometric method [28]. For this purpose, absorbance values were determined with a standard spectrophotometer (Shimadzu UV-1800 Spectrophotometer, Kyoto, Japan) against the blank sample at a wavelength of 538 nm, and TBA values were calculated by multiplying the absorbance values by 7.8.

Microbiological Analyses

In clotted cream samples, total aerobic mesophilic bacteria (TAMB), total aerobic psychrophilic bacteria (TAPB), lipolytic bacteria, proteolytic bacteria, yeast/mold, total coliform group bacteria (TCGB), lactic acid bacteria (LAB), *Staphylococcus aureus* counts were determined using the spread plate technique [29].

10 g of the samples were taken under sterile conditions and transferred to sterile stomacher bags (Spa-174538, Lp Italiana, Milan, Italy), and serial dilutions up to 10^{-4} were prepared [30]. TAMB count analysis was performed using Plate Count Agar (PCA) (Merck, 1.05463, Germany). The cultivated petri dishes were incubated in an incubator (MM Incucell 55, Germany) for 48-72 hours at 30°C under aerobic conditions for TAMB counting [31, 32]. For TAPB analysis, Plate Count Agar (PCA Merck, 1.05463, Germany) medium was used, and the cultivated petri dishes were incubated at 4°C for 5-7 days. Colonies larger than 0.5 mm that grew on the medium were counted and recorded [33]. Tributyrin Agar (TBA, Merck 1.01957, Germany) medium was used to analyze lipolytic bacteria. Counts were taken after 2-3 days of incubation at 35-37°C [33]. Proteolytic bacteria analysis was performed using Plate Count Agar (PCA, Merck 1.05463, Germany) medium. After inoculation, the petri dishes were inverted and incubated at 35-37°C for 24-72 hours. After incubation, 1% HCl acid was poured into the petri dishes, waited for 1 minute, and after removing the excess acid from the petri dishes, colonies with light-colored zones around them were counted [34]. Potato Dextrose Agar (PDA Merck 1.10130, Germany) medium was used for yeast/mold count analysis, and the cultivated petri dishes were incubated in an incubator (MMM Incucell 55, Germany) for 5-7 days at 22°C under aerobic conditions [35]. Violet Red Bile Agar (VRB, Merck 1.01406, Germany) medium was used for total coliform group bacteria count, and the petri dishes were incubated in an incubator (MMM Incucell 55, Germany) for 24-48 hours at 30°C under aerobic conditions [36]. For the count of lactic acid bacteria, Man Rogasa and Sharpe Agar (MRS, Merck 1.10660, Germany) medium was used, petri dishes were placed in jars (Merck

1.16387, Germany) and incubated in an incubator (Daihan, IG50, Malaysia) under anaerobic conditions at 30°C for 24-48 hours [37]. The medium prepared using Baird-Parker Agar (BPA, Merck, 1.05406, USA) and Egg Yolk Tellurite Emulsion (Merck 1.03785, USA) was used to determine the count of *S. aureus*. The cultivated petri dishes were left to incubate for 24-48 hours in an incubator (Nüve, FN 500, Turkey) at 37°C. At the end of the period, bright black colonies with clean zones that developed on the media and had a thin white precipitation ring around the edges were marked and counted [38]. After marking the colonies, the petri dishes were subjected to a second incubation for 18 hours. At the end of the incubation, typical *Staphylococcus* colonies with white precipitation rings and bright black colonies that do not form zones were counted separately, a coagulase test was applied to 5 of each type of colony, and the count of *S. aureus* was determined by counting the colonies with positive results [39].

Statistical Analyses

A total of seven samples were studied, including six different cold plasma treatments and a control sample. Additionally, there were four different storage times within the scope of the study, and each sample was studied in two parallels. Accordingly, there were a total of fifty-six samples in the study. The analysis results obtained were statistically evaluated using the Duncan multiple comparison test using the IBM SPSS V. The 23.0 package program.

RESULTS and DISCUSSION

The changes in pH, a_w and TBA values of clotted cream samples after cold plasma treatment during storage and the variation and correlation analysis of the effects of sample type and storage time are shown in Table 1. According to the results of the variation analysis, it was revealed that storage time and sample type and storage time x sample type interactions were very highly significant on pH and TBA values ($p<0.0001$). According to the results of the correlation analysis, storage time and sample type interactions showed negative, very highly correlational effects on the pH value. It was determined that all electrical field applications used in the study reduced the pH value ($p<0.05$). Among the samples, the lowest pH value was 6.11 in the application of using the mixture of 50% oxygen and argon gases for 40 minutes (K6), followed by values of 6.23 in the application of using 100% argon gas for 40 minutes (K4) and 6.30 in the application of using 100% oxygen gas for 40 minutes (K2) at the beginning of the storage period.

Additionally, the pH values of all samples decreased in parallel with the cold plasma application during storage ($p<0.05$). The samples whose pH values decreased the most during storage were K6, K4, and K2, respectively. Compared to the control sample, the pH value of the K6 sample decreased by 0.93 units more during storage.

Table 1. Changes in pH, a_w and TBA values of clotted cream samples during storage

Sample	pH				a_w			TBA (mg MDA/kg)			
	Storage Time (Day)										
	0.	4.	7.	10.	0.	4.	7.	10.	0.	4.	7.	10.
K0	6.67±0.056^{Aa}	6.57±0.028^{Aa}	6.35±0.070^{Ba}	6.17±0.098^{Ba}	0.903±0.005^{Aa}	0.887±0.002^{Ba}	0.871±0.001^{Ca}	0.854±0.005^{Da}	0.303±0.005^{Da}	0.401±0.004^{Cc}	0.588±0.004^{Ba}	0.630±0.001^{Ac}
K1	6.56±0.042^{Aa}	6.37±0.070^{Bb}	6.12±0.028^{Cb}	6.04±0.028^{Ca}	0.875±0.007^{Aa}	0.866±0.005^{ABb}	0.853±0.002^{BCb}	0.841±0.001^{Cab}	0.252±0.002^{Dd}	0.367±0.004^{Cd}	0.508±0.004^{Bb}	0.682±0.001^{Ab}
K2	6.30±0.070^{Aa}	5.90±0.113^{Bc}	5.37±0.028^{Ce}	5.11±0.014^{Dd}	0.841±0.002^{Aa}	0.834±0.005^{Acd}	0.831±0.005^{Ade}	0.827±0.007^{Ab}	0.264±0.002^{Dc}	0.476±0.001^{Ca}	0.592±0.004^{Ba}	0.743±0.002^{Aa}
K3	6.47±0.028^{Aa}	6.25±0.070^{Bb}	5.92±0.028^{Cc}	5.80±0.056^{Cb}	0.869±0.008^{Aa}	0.859±0.004^{ABb}	0.845±0.005^{Bbc}	0.839±0.009^{Bb}	0.251±0.002^{Dd}	0.299±0.004^{Cf}	0.378±0.004^{Be}	0.456±0.007^{Af}
K4	6.23±0.042^{Aa}	5.64±0.056^{Bd}	4.99±0.056^{Cf}	4.85±0.070^{Ce}	0.832±0.002^{Aa}	0.825±0.007^{Ade}	0.819±0.002^{ABef}	0.811±0.004^{Bc}	0.289±0.002^{Dc}	0.342±0.002^{Ce}	0.388±0.004^{Bd}	0.431±0.001^{Ag}
K5	6.42±0.028^{Aa}	6.05±0.056^{Bc}	5.72±0.014^{Cd}	5.29±0.098^{Dc}	0.852±0.002^{Aa}	0.842±0.002^{Bc}	0.837±0.002^{BCcd}	0.832±0.004^{Cb}	0.296±0.005^{Dab}	0.399±0.004^{Cc}	0.477±0.005^{Bc}	0.620±0.002^{Ad}
K6	6.11±0.056^{Aa}	5.58±0.042^{Bd}	4.81±0.014^{Cg}	4.68±0.042^{Df}	0.826±0.004^{Aa}	0.819±0.002^{ABe}	0.809±0.011^{ABf}	0.803±0.005^{Bc}	0.301±0.004^{Da}	0.455±0.004^{Cb}	0.597±0.001^{Ba}	0.608±0.004^{Ae}
Interactions			P value	r	Interactions		P value	r	Interactions		P value	r
Sample Type (S)			<0.0001	-0.534**	Sample Type (S)		0.024	-0.403**	Sample Type (S)		<0.0001	-0.069
Storage Time (T)			<0.0001	-0.622**	Storage Time (T)		0.400	-0.185	Storage Time (T)		<0.0001	0.845**
S X T			<0.0001	--	S X T		0.804	--	S X T		<0.0001	--

MDA: malondialdehyde, K0: Control, K1: %100 O_2 gas 20 minutes, K2: %100 O_2 gas 40 minutes, K3: %100 Ar gas 20 minutes, K4: %100 Ar gas 40 minutes, K5: %50 O_2 - %50 Ar gases 20 minutes, K6: %50 O_2 - %50 Ar gases 40 minutes. A – D (→):Values with different capital letters in the same row differ significantly ($p<0.05$) among storage days.a - g (↓):Values with different lowercase letters in the same column differ significantly ($p<0.05$) among the samples. Statistical significance: $p<0.0001$: very highly significant,, $p<0.01$: highly significant, $p<0.05$: significant, $p>0.05$: not significant. Correlation significance is indicated by asterisks: **, significant at the 0.01 level (2-tailed).

In the correlation analysis, the sample type had a negative and very highly correlational effect on the a_w value (r: -0.403) (Table 1).

The increase in the concentration of H^+ ions during cold plasma application was effective in reducing this decrease in pH values. Reactive species with mainly acidic properties, such as nitric acid (HNO_3) and nitrous acid (HNO_2) produced by the plasma, are responsible for the pH decrease [40].

It was determined that all electrical field applications used in the study had a reducing effect on the a_w value of the samples ($p<0.05$). The highest reducing effect on the a_w value was detected in the K6 (50% Ar – 50% O_2 / 40 min) sample, whereas the highest a_w value was detected in the K0 (control) sample.

The a_w values of all samples decreased during the subsequent ten-day storage ($p<0.05$). During 10 days of storage, the highest change was observed in the values of the K0 sample, in the range of 0.903-0.854, and the least change was observed in the values of the K2 sample, in the range of 0.841-0.827 (Table 1). The decrease in a_w decreased with increasing plasma exposure time ($p<0.05$). The decrease in a_w values can be attributed to the ability of O_2 and Ar gases used in the plasma process to retain free water molecules on the clotted cream surface [41].

According to the results of the correlation analysis, the storage time showed a positive and very highly correlational effect on the TBA value (Table 1). Cold plasma treatment had a decreasing effect on the TBA values of the samples ($p<0.05$). The greatest effect was revealed in the K3 sample (100% Ar / 20 min) with a value of 0.251 mg malondialdehyde/kg, and the least effect was in the K6 sample with a value of 0.301 mg malondialdehyde/kg. TBA values of all samples increased during storage ($p<0.05$). After ten days of storage, the highest increase rate was in the K2 sample with a difference of 479 mg malondialdehyde/kg, and the least increase rate was in the K4 sample with a difference of 142 mg malondialdehyde/kg. Of the two different gases used and the mixture of these gases, the most effective application was O_2 gas application. The resulting effect increased depending on the application time. A similar study [42] reported that in the cold plasma treatment applied to the surface of cheddar cheese, the TBA value, which was initially 0.132 mg malondialdehyde/kg, decreased to 0.141, 0.161 and 0.183 mg malondialdehyde/kg, respectively, after 2.5, 5 and 10 minutes of plasma application. In addition [43], it was observed that the TBA value of the 10-minute plasma application on sirloin increased with the duration of cold plasma.

The change of color values, one of the qualities that affects the admiration of foods during storage after cold plasma treatment, is shown in Figure 2. Storage time and sample type were very highly significant ($p<0.0001$) on L*, a*, and b* values. While the sample type had a negative effect on the L*, a*, and b* values, storage time had a negative, very highly correlational effect on the L* and b* values and a positive, very highly correlational effect on the a* value.

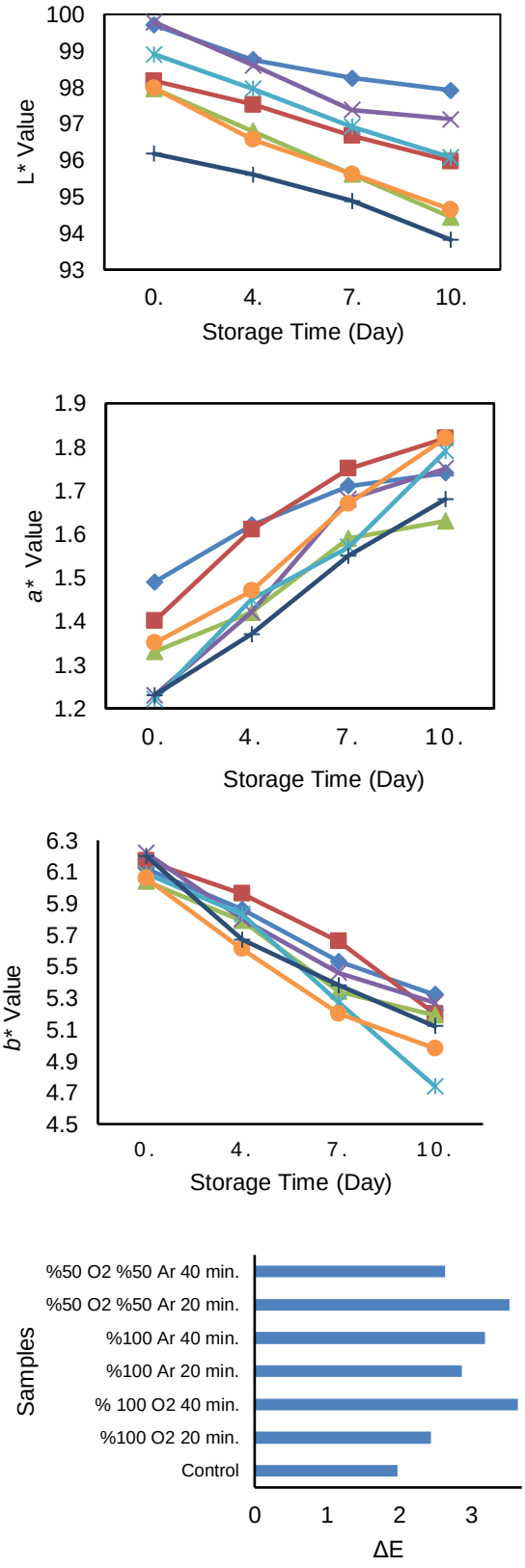

Figure 2. Changes in color (*L**, *a**, *b** and ΔE) values of samples during storage (K0: Control (◆), K1: 100% O_2 20 min (■), K2:100% O_2 40 min (×), K3: 100% Ar 20 min (▲) K4:100% Ar 40 min (✱), K5:50% O_2 and 50% Ar 20 min (●) and K6: 50% O_2 and 50% Ar 410 min (+))

L* value, an indicator of brightness in foods, decreased with cold plasma application ($p<0.05$). The highest L* value was measured as 99.71 in the control sample, and the lowest L* value was 96.18 in the K6 sample. Among the two different gases used during the application, the L* value decreased the most in the K6 sample. During 10 days of storage, the maximum change was observed between the range of 97.97-94.44 in the K2 sample, and the least change was observed between the range of 99.71-97.92 in the K0 sample. It is thought that drying on the surface is effective in decreasing L* values. Additionally, lipid oxidation can cause browning in foods, and brown oxypolymers obtained from milk proteins are responsible for the decrease in the L* value [42].

When the change in a* values was examined, it was seen that there was a decrease in a* value due to cold plasma application ($p<0.05$). During 10 days of storage, there was an increase in the a* value. Of the two different gases used and the mixture of these gases, the most effective one was determined to be Ar gas, and the resulting effect increased depending on the application time. Among the two different gases used during the application, the a* value increased the most in the K4 sample. During 10 days of storage, the highest change was observed in the K4 sample values in the range of 1.79-1.22, and the least change was observed in the K0 sample values in the range of 1.74-1.49.

While cold plasma application did not cause a significant change in the b* value ($p>0.05$), a decrease in the b* value was detected during storage ($p<0.05$). Among the two different gases used during the application, the highest effect on the b* value was detected on the K4 sample. After storage, the maximum change in b* values was determined in the K4 sample with a change of 1.35 units, and the minimum change was determined in the K0 sample with a change of 0.80 units.

When the total color change ΔE values of the samples (Figure 2) were examined, it was determined that cold plasma application was effective on the color of the samples. The highest color changes were observed in the samples K2 (100% O_2 40 min) and K5 (50% O_2 and 50% Ar 20 min). The color change was minimal in the control sample (K0).

The change in TAMB and TAPB counts of clotted cream samples after cold plasma application during storage and the variation and correlation analysis regarding the effects of sample type and storage time are shown in Table 2. It was determined that the storage time and sample type, and storage time x sample type interactions were very highly significant on TAMB and TAPB counts ($p<0.0001$). It was determined that storage time had a positive, very highly correlational effect on the counts of TAMB and TAPB, and the sample type interaction had a negative, highly correlation for TAMB and a positive, very highly correlation for TAPB (Table 2). Cold plasma treatment had a reducing effect on the initial TAMB counts of the samples ($p<0.05$). It was determined that the application using 100% Ar gas for 40 minutes (K4) was the one that reduced the TAMB count the most. In addition, although the TAMB counts of all samples increased during storage, electrical field applications had a slowing effect on this increase ($p<0.05$). The K6 sample was determined to have the lowest TAMB count among the samples on the last day of storage (5.14 log cfu/g) (Table 2). Ulbin-Figlewicz et al. [44] reported that the total count of microorganisms, which was initially 5.45 log cfu/cm^2, decreased to 3.13 log cfu/cm^2 by using argon gas in a 10-minute plasma application.

Table 2. Change in TAMB and TAPB counts of clotted cream samples during storage (log cfu/g)

Sample	TAMB				TAPB			
	Storage Time(Day)							
	0.	4.	7.	10.	0.	4.	7.	10.
K0	3.27 ± 0.098^{Dab}	5.01 ± 0.212^{Ca}	6.97 ± 0.084^{Ba}	7.55 ± 0.353^{Aa}	3.10 ± 0.056^{Dab}	4.89 ± 0.014^{Ca}	6.11 ± 0.042^{Ba}	7.35 ± 0.056^{Aa}
K1	3.34 ± 0.028^{Da}	4.88 ± 0.056^{Cab}	5.32 ± 0.028^{Bc}	6.11 ± 0.014^{Ac}	3.20 ± 0.084^{Da}	4.67 ± 0.028^{Cb}	5.92 ± 0.042^{Bb}	6.45 ± 0.042^{Ab}
K2	3.29 ± 0.063^{Da}	4.81 ± 0.028^{Cabc}	5.27 ± 0.07^{Bcd}	6.02 ± 0.282^{Ac}	3.01 ± 0.028^{Dbc}	4.44 ± 0.056^{Cc}	5.79 ± 0.014^{Bc}	6.09 ± 0.084^{Ac}
K3	3.25 ± 0.042^{Dab}	4.67 ± 0.028^{Cabc}	6.01 ± 0.028^{Bb}	6.82 ± 0.014^{Ab}	2.80 ± 0.042^{Dd}	3.96 ± 0.028^{Cd}	5.38 ± 0.042^{Bd}	5.75 ± 0.028^{Ad}
K4	3.14 ± 0.042^{Cb}	4.40 ± 0.565^{Bbcd}	4.83 ± 0.042^{Be}	5.67 ± 0.028^{Ad}	2.97 ± 0.014^{Dc}	3.81 ± 0.049^{Ce}	5.24 ± 0.028^{Be}	5.57 ± 0.014^{Ae}
K5	3.20 ± 0.056^{Dab}	4.29 ± 0.042^{Ccd}	5.19 ± 0.028^{Bd}	5.97 ± 0.07^{Acd}	2.81 ± 0.014^{Dd}	4.11 ± 0.028^{Cd}	4.67 ± 0.042^{Bf}	5.04 ± 0.028^{Af}
K6	3.27 ± 0.028^{Dab}	4.11 ± 0.014^{Cd}	4.84 ± 0.028^{Be}	5.14 ± 0.042^{Ae}	2.79 ± 0.028^{Cd}	4.10 ± 0.141^{Bd}	4.29 ± 0.084^{Bg}	4.73 ± 0.028^{Ag}

Interactions	P value	r	Interactions	P value	r
Sample Type (S)	<0.0001	-0.299*	Sample Type (S)	<0.0001	0.368**
Storage Time (T)	<0.0001	0.883**	Storage Time (T)	<0.0001	0.881**
S X T	<0.0001	--	S X T	<0.0001	--

K0: Control, K1: %100 O_2 gas 20 minutes, K2: %100 O_2 gas 40 minutes, K3: %100 Ar gas 20 minutes, K4: %100 Ar gas 40 minutes, K5: %50 O_2 - %50 Ar gases 20 minutes, K6: %50 O_2 - %50 Ar gases 40 minutes A – D (→):Values with different capital letters in the same row differ significantly ($p<0.05$) among storage days. a - g (↓):Values with different lowercase letters in the same column differ significantly ($p<0.05$) among samples. Statistical significance: $p<0.0001$: very highly significant;, $p<0.01$: highly significant, $p<0.05$: significant, $p>0.05$: not significant. Correlation significance is indicated by asterisks: **, significant at the 0.01 level (2-tailed); *, significant at the 0.05 level (2-tailed).

Cold plasma also had a reducing effect on the TAPB count. The highest effect was detected in the K6 sample. After 10 days of storage, the highest TAPB count was determined as 7.35 log cfu/g in the K0 sample, and the lowest TAPB count was 4.73 log cfu/g in the K6 sample.

The changes in the lipolytic and proteolytic bacterial counts of the clotted cream samples after cold plasma treatment during storage and the variation and correlation analysis regarding the effects of sample type and storage time are shown in Table 3. According to variation analysis, sample type, storage time, and sample type x storage time were very highly significant on the counts of both lipolytic bacteria and proteolytic bacteria ($p<0.0001$). Although the type of sample showed a negative correlational effect, storage time showed a positive correlation effect (Table 3).

Table 3. Change in lipolytic and proteolytic bacteria counts of clotted cream samples during storage (log cfu/g)

Sample	Lipolytic Bacteria				Proteolytic Bacteria			
	Storage Time (Day)							
	0.	4.	7.	10.	0.	4.	7.	10.
K0	3.07±0.098^{Da}	4.78±0.056^{Ca}	5.52±0.014^{Ba}	6.25±0.042^{Aa}	2.28±0.056^{Db}	3.80±0.028^{Ca}	5.22±0.028^{Ba}	6.52±0.028^{Aa}
K1	3.05±0.056^{Da}	4.21±0.042^{Cc}	4.77±0.028^{Bc}	5.33±0.028^{Ab}	2.45±0.042^{Da}	3.62±0.056^{Cb}	4.97±0.028^{Bb}	5.23±0.042^{Ab}
K2	3.02±0.028^{Da}	4.42±0.014^{Cb}	4.86±0.056^{Bb}	5.42±0.028^{Ab}	2.38±0.056^{Dab}	3.56±0.014^{Cb}	4.86±0.014^{Bc}	5.11±0.028^{Ab}
K3	3.11±0.014^{Da}	4.41±0.028^{Cb}	4.75±0.042^{Bc}	5.15±0.056^{Ac}	2.11±0.042^{Dc}	3.31±0.042^{Cc}	3.72±0.014^{Bd}	4.68±0.084^{Ac}
K4	3.15±0.042^{Da}	4.36±0.07^{Cb}	4.69±0.028^{Bc}	5.11±0.042^{Ac}	2.14±0.042^{Dc}	3.22±0.028^{Cc}	3.67±0.028^{Bd}	4.51±0.014^{Ad}
K5	3.14±0.042^{Da}	4.29±0.028^{Cc}	4.47±0.028^{Bd}	4.86±0.042^{Ad}	2.47±0.014^{Da}	3.12±0.056^{Cd}	3.48±0.084^{Be}	4.21±0.014^{Ae}
K6	3.09±0.042^{Da}	4.12±0.028^{Cc}	4.39±0.042^{Bd}	4.62±0.056^{Ae}	2.29±0.028^{Db}	3.08±0.028^{Cd}	3.28±0.056^{Bf}	3.96±0.014^{Af}
Interactions		P value	r	Interactions		P value	r	
Sample Type (S)		<0.0001	-0.252	Sample Type (S)		<0.0001	-0.382**	
Storage Time (T)		<0.0001	0.896*	Storage Time (T)		<0.0001	0.860**	
S X T		<0.0001	--	S X T		<0.0001	--	

K0: Control, K1: %100 O_2 gas 20 minutes, K2: %100 O_2 gas 40 minutes, K3: %100 Ar gas 20 minutes, K4: %100 Ar gas 40 minutes, K5: %50 O_2 - %50 Ar gases 20 minutes, K6: %50 O_2 - %50 Ar gases 40 minutes A – D (→):Values with different capital letters in the same row differ significantly (p<0.05) among storage days. a - f (↓):Values with different lowercase letters in the same column differ significantly (p<0.05) among samples. Statistical significance: p<0.0001: very highly significant;, p<0.01: highly significant, p<0.05: significant, p>0.05: not significant. Correlation significance is indicated by asterisks: **, significant at the 0.01 level (2-tailed); *, significant at the 0.05 level (2-tailed).

The application of cold plasma did not cause a significant change in the count of lipolytic bacteria in the clotted cream samples (p>0.05) at the first day of storage. However, it had a decreasing effect on the rate of increase in the count of lipolytic bacteria during storage. In particular, the highest effect was determined in the K6 sample. At the end of the 10-day storage period, the highest count of lipolytic bacteria was detected in control sample (6.25 log cfu/g) and the lowest in K6 sample (4.62 log cfu/g).

Cold plasma treatment on proteolytic bacteria had a reducing effect on the count of bacteria. It had a slowing effect on the increase in the count of proteolytic bacteria during storage. At the end of storage, the highest change in the count of proteolytic bacteria was detected in the K0 sample with 4.24 log cfu/g, and the least change was detected in the K6 sample with 1.67 log cfu/g.

The changes in the TYM (Total Yeast/Mold) and LAB (Lactic Acid Bacteria) counts of the clotted cream samples after cold plasma treatment during storage and the variation and correlation analysis regarding the effects of sample type and storage time are shown in Table 4.

Table 4. Change in yeast-mold and lactic acid bacteria counts of clotted cream samples during storage (log cfu/g)

Sample	Yeast-Mold				Lactic Acid Bacteria			
	Storage Time (Day)							
	0.	4.	7.	10.	0.	4.	7.	10.
K0	2.93±0.042^{Da}	4.23±0.014^{Ca}	5.67±0.042^{Ba}	7.78±0.113^{Aa}	2.13±0.042^{Dcd}	4.60±0.056^{Ca}	5.90±0.070^{Ba}	6.57±0.042^{Aa}
K1	2.42±0.028^{Dd}	3.55±0.07^{Cd}	4.23±0.042^{Bcd}	5.05±0.07^{Ac}	2.21±0.028^{Dbc}	4.22±0.028^{Cb}	5.22±0.028^{Bb}	5.80±0.042^{Ab}
K2	2.64±0.056^{Dc}	3.97±0.028^{Cb}	4.66±0.056^{Bb}	5.41±0.014^{Ab}	2.06±0.028^{Dd}	4.11±0.028^{Cc}	5.01±0.028^{Bc}	5.25±0.070^{Ad}
K3	2.86±0.042^{Dab}	3.65±0.056^{Ccd}	4.11±0.028^{Bd}	4.75±0.028^{Ad}	2.54±0.028^{Da}	3.41±0.028^{Ce}	4.97±0.028^{Bc}	5.11±0.042^{Ae}
K4	2.83±0.042^{Dab}	3.72±0.028^{Cc}	4.29±0.127^{Bc}	5.07±0.028^{Ac}	2.46±0.070^{Da}	3.32±0.014^{Cf}	4.72±0.042^{Bd}	5.17±0.028^{Ade}
K5	2.75±0.07^{Db}	3.40±0.056^{Ce}	3.77±0.028^{Be}	4.61±0.014^{Ae}	2.27±0.070^{Db}	4.01±0.028^{Cd}	4.22±0.028^{Be}	4.51±0.028^{Af}
K6	2.61±0.021^{Dc}	3.29±0.056^{Ce}	3.53±0.042^{Bf}	4.14±0.028^{Af}	2.18±0.028^{Dbc}	3.96±0.014^{Cd}	4.09±0.014^{Bf}	5.43±0.028^{Ac}
Interactions		P value	r	Interactions		P value	r	
Sample Type (S)		<0.0001	-0.367**	Sample Type (S)		<0.0001	-0.226	
Storage Time (T)		<0.0001	0.812**	Storage Time (T)		<0.0001	0.903**	
S X T		<0.0001	--	S X T		<0.0001	--	

K0: Control, K1: %100 O_2 gas 20 minutes, K2: %100 O_2 gas 40 minutes, K3: %100 Ar gas 20 minutes, K4: %100 Ar gas 40 minutes, K5: %50 O_2 - %50 Ar gases 20 minutes, K6: %50 O_2 - %50 Ar gases 40 minutes A – D (→):Values with different capital letters in the same row differ significantly (p<0.05) among sample days. a - f (↓):Values with different lowercase letters in the same column differ significantly (p<0.05) among samples. Statistical significance: p<0.0001: very highly significant;, p<0.01: highly significant, p<0.05: significant, p>0.05: not significant. Correlation significance is indicated by asterisks: **, significant at the 0.01 level (2-tailed); *, significant at the 0.05 level (2-tailed).

It was revealed that sample type, storage time, and storage time x sample type interactions were very highly significant on both TYM and LAB counts (p<0.0001). In addition, while the sample type showed a negative, correlational effect on the counts of TYM and LAB, storage time showed a positive, very highly correlational effect (Table 4). Cold plasma treatment reduced the count of TYM. After 10 days of storage, lower TYM counts were detected in the cold plasma applied samples compared to the control group. The highest TYM was detected in the K0 sample with 7.78 log cfu/g, and the lowest TYM was detected in the K6 sample with 4.14 log cfu/g. Ulbin-Figlewicz et al. [44] first reported that the total count of yeast and mold on the meat surface, which was initially 4.43 log cfu/cm^2, decreased to 3.28, 2.31 and 3.37 log cfu/cm2, respectively, depending on the type of argon, helium and nitrogen gases in a 10-minute plasma application. In their study [45], on cold plasma application in mold species, inoculated on kashar cheese, found that there was a 3-4 log reduction in all mold species. In the present study, cold plasma treatment had an increasing effect on the LAB count, except for the K2 sample (p<0.05) at the first day of storage. The count of LAB increased in all samples during storage, but the increase rate was lower in the samples treated with cold plasma. The lowest effect on the count of LAB was detected on the K5 sample. During 10 days of storage, the most change was observed in the range of 6.57-2.13 log cfu/g in the

K0 sample, while the least change was observed in the range of 4.51-2.27 log cfu/g in K5 sample.

The changes in the counts of coliform group bacteria and *S. aureus* during storage and the variation and correlation analysis regarding the effects of sample type and storage time are shown in Table 5. According to the variation analysis, after the cold plasma treatment applied to the surface of the clotted cream, sample type, storage time and storage time x sample type interactions were very highly significant on both the counts of coliform group bacteria and *S. aureus* ($p<0.0001$). Sample type had a negative, very highly correlational effect ($p<0.01$) and storage time had a positive, very highly correlational effect on both the coliform group bacteria and *S. aureus* counts (Tablo 5). Cold plasma treatment caused a decrease in the counts of coliform group bacteria ($p<0.05$). At the end of storage, the highest count of coliform group bacteria was detected in the K0 (5.45 log cfu/g) sample and the lowest count of coliform group bacteria was detected in the K6 (1.22 log cfu/g) sample. Cold plasma treatment applied to clotted cream samples was effective on *S. aureus* ($p<0.05$). The most effective was the 40-minute application (K6) in which 50% O_2 and 50% Ar gas mixtures were used. At the end of storage, the highest count of *S. aureus* was detected in sample K0 as 5.54 log cfu/g, and the lowest count of *S. aureus* was detected in sample K6 as 0.82 log cfu/g. In a similar study [46], reported that in a 10-minute plasma treatment using helium and argon gases, the counts of *S. aureus* inoculated into the agar medium decreased to a highest of 2.02 and 0.96 log cfu/g, respectively.

Table 5. Change in total coliform group bacteria and *S.aureus* counts of clotted cream during storage (log cfu/g)

Sample	Total Coliform Group Bacteria				*S. aureus*			
	Storage Time (Day)							
	0.	4.	7.	10.	0.	4.	7.	10.
K0	1.79 ± 0.028^{Da}	3.59 ± 0.028^{Ca}	4.18 ± 0.028^{Ba}	5.45 ± 0.028^{Aa}	1.54 ± 0.014^{Da}	2.81 ± 0.056^{Ca}	4.11 ± 0.028^{Ba}	5.54 ± 0.063^{Aa}
K1	1.53 ± 0.056^{Cb}	1.88 ± 0.07^{Bb}	2.02 ± 0.014^{Bb}	2.24 ± 0.084^{Ad}	0.46 ± 0.028^{Dd}	0.64 ± 0.042^{Cc}	0.98 ± 0.014^{Bd}	1.12 ± 0.028^{Ac}
K2	1.49 ± 0.042^{Bb}	1.28 ± 0.042^{Cc}	1.57 ± 0.07^{Bc}	1.82 ± 0.014^{Ae}	0.27 ± 0.014^{De}	0.58 ± 0.056^{Cc}	0.76 ± 0.014^{Be}	0.92 ± 0.070^{Ad}
K3	1.57 ± 0.084^{Bb}	1.75 ± 0.028^{ABb}	2.30 ± 0.42^{Ab}	2.36 ± 0.042^{Ac}	0.68 ± 0.028^{Db}	1.02 ± 0.042^{Cb}	1.54 ± 0.028^{Bc}	1.97 ± 0.028^{Ab}
K4	1.50 ± 0.141^{Db}	2.00 ± 0.07^{Cb}	2.42 ± 0.07^{Bb}	2.72 ± 0.014^{Ab}	0.55 ± 0.07^{Dc}	1.10 ± 0.028^{Cb}	1.77 ± 0.028^{Bb}	2.04 ± 0.042^{Ab}
K5	0.84 ± 0.056^{Bc}	1.2 ± 0.282^{ABc}	1.37 ± 0.098^{Acd}	1.62 ± 0.042^{Af}	0.31 ± 0.028^{Da}	0.47 ± 0.028^{Cd}	0.77 ± 0.028^{Be}	0.91 ± 0.014^{Ad}
K6	0.44 ± 0.028^{Dd}	0.67 ± 0.042^{Cd}	1.09 ± 0.014^{Bd}	1.22 ± 0.056^{Ag}	0.23 ± 0.042^{Da}	0.39 ± 0.028^{Cd}	0.69 ± 0.028^{Bf}	0.82 ± 0.028^{Ad}

Interactions	P value	r	Interactions	P value	r
Sample Type (S)	<0.0001	-0.647**	Sample Type (S)	<0.0001	-0.520**
Storage Time (T)	<0.0001	0.422**	Storage Time (T)	<0.0001	0.429**
S X T	<0.0001	--	S X T	<0.0001	--

K0: Control, K1: %100 O_2 gas 20 minutes, K2: %100 O_2 gas 40 minutes, K3: %100 Ar gas 20 minutes, K4: %100 Ar gas 40 minutes, K5: %50 O_2 - %50 Ar gases 20 minutes, K6: %50 O_2 - %50 Ar gases 40 minutes A – D (→):Values with different capital letters in the same row differ significantly ($p<0.05$) among storage days. a - g (↓):Values with different lowercase letters in the same column differ significantly ($p<0.05$) among samples. Statistical significance: $p<0.0001$: very highly significant;, $p<0.01$: highly significant, $p<0.05$: significant, $p>0.05$: not significant. Correlation significance is indicated by asterisks: **, significant at the 0.01 level (2-tailed); *, significant at the 0.05 level (2-tailed).

In cold plasma treatment, the type of gas used and the exposure time are effective in microbial inactivation [47]. Reactive oxygen (ROS) and reactive nitrogen (RNS) species formed during treatment play roles in inactivation [48]. ROS produced during plasma production causes strong oxidative stress. Cells are damaged by enzyme inactivation, lipid peroxidation, and DNA fragmentation. RNS is toxic and can cause cell death by damaging DNA. In general, when the results of microbiological analysis are evaluated, the cold plasma technique, in which a 50% O_2 + 50% Ar gas mixture is applied for 40 min, is sufficient for microbial inactivation.

CONCLUSION

In this research, the effects of the cold plasma technique on the clotted cream surface by applying two different gases (O_2 and Ar) and mixtures of these gases for different periods and their effects on the physicochemical and microbial parameters of the clotted cream were examined during the storage period (10 days).

In order to increase the shelf life of the clotted cream and improve its quality properties, the cold plasma technique was used with different gas and time applications. It has been observed that cold plasma application significantly reduces the microbial activity of clotted cream. In particular, cold plasma treatment using a 50% O_2 – 50% Ar gas mixture for 40 minutes (K6) was the most effective application. In line with this result, it can be recommended to use the cold plasma technique in the food industry for foods with high microbial activity. When applying the cold plasma technique to foods with high amounts of fat, preliminary studies should be conducted to determine the gas and time that will not accelerate lipid oxidation. It can be used by choosing the appropriate gases and times determined as a result of these preliminary studies. For this reason, studies can be conducted with different gas compositions and durations to determine the process parameters that will cause a minimum change in physicochemical properties. Furthermore, it has been observed as a very suitable technique for foods with high microbial activity or foods whose microbial activity increases rapidly.

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Research Paper / Araştırma Makalesi

Comparison of Chemical Properties and Fatty Acid Composition of Artisanal and Commercial Butters

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ABSTRACT

This study compares the chemical properties, fatty acid composition, and conjugated linoleic acid (CLA) levels of artisanal (homemade) (n=10) and commercial butter samples (n=10) produced from cow's milk. Artisanal butters were collected from local producers who maintain their own livestock across various villages within the Burdur province in Türkiye, while commercial samples were acquired from various national and local markets. On average, the dry matter content was significantly higher in commercial butters (84.15%) than artisanal samples (82.60%) ($p<0.05$). However, the difference in the mean fat contents of commercial (84.08%) and artisanal (82.98%) butter samples was insignificant ($p>0.05$). Furthermore, the mean titratable acidity values of commercial and artisanal butters were found as 0.30% and 0.51% (percent lactic acid), respectively ($p<0.05$). Interestingly, artisanal butters exhibited a significantly higher mean CLA content (6.89 mg/g fat) compared to their commercial counterparts (4.11 mg/g fat) ($p<0.05$). However, there were no statistically significant differences in the relative ratios of polyunsaturated fatty acids between commercial and artisanal butters, with respective values of 3.32% and 4.01% ($p>0.05$). In conclusion, this study showed significant differences in the dry matter content, titratable acidity, and CLA content between artisanal and commercial butter samples, indicating potential nutritional and quality variations between the two types of butter, particularly emphasizing the higher CLA content observed in artisanal butter despite comparable fatty acid composition.

Keywords: Butter, Artisanal, Commercial, Fatty acid, Conjugated linoleic acid

Ticari ve Ev Yapımı Tereyağlarının Kimyasal Özellikleri ve Yağ Asidi Kompozisyonlarının Karşılaştırılması

ÖZ

Bu çalışmada, inek sütünden elde edilen ev yapımı (n=10) ve ticari tereyağı örneklerinin (n=10) kimyasal özellikleri, yağ asidi kompozisyonu ve konjuge linoleik asit (KLA) seviyeleri karşılaştırılmıştır. Ev yapımı tereyağı örnekleri, Burdur ilindeki çeşitli köylerde kendi hayvanlarını besleyen yerel üreticilerden, ticari tereyağı örnekleri ise çeşitli ulusal ve yerel marketlerden temin edilmiştir. Ortalama kuru madde içeriğinin ticari tereyağlarında (%84.15) ev yapımı örneklerden (%82.60) önemli ölçüde daha yüksek ($p<0.05$) olduğu, ticari (%84.08) ve ev yapımı (%82.98) tereyağı örneklerinin ortalama yağ içerikleri arasındaki farkın ise önemsiz olduğu bulunmuştur ($p>0.05$). Bu çalışma, kuru madde içeriği, titre edilebilir asitlik ve KLA içeriği bakımından ev yapımı ve ticari tereyağı örnekleri arasında önemli farklılıklar olduğunu

ortaya koymuştur. Özellikle benzer yağ asidi bileşimlerine rağmen, ev yapımı tereyağının daha yüksek KLA içeriğine sahip olması, bu tereyağı türleri arasındaki potansiyel beslenme ve kalite farklılıklarını öne çıkarmaktadır.

Anahtar Kelimeler: Tereyağı, Ev yapımı, Ticari, Yağ asidi, Konjuge linoleik asit

INTRODUCTION

Milk and dairy products play an essential role in human nutrition and well-being. Butter, a widely traded dairy product, exhibits varying consumption patterns across countries, with a global per capita consumption on the rise. In Asia, butter holds the distinction of being the most consumed processed dairy product, constituting nearly half of all processed dairy consumption in terms of milk solids. While Europe and North America currently dominate butter consumption, consumption rates in Asia are witnessing the strongest growth. Statistical data from 2023 indicates that approximately 2.1 million metric tons of butter were produced within the European Union alone [1]. Butter, the most widely consumed animal fat in Türkiye, is rich in short-chain fatty acids and omega-9, making it a significant component of human nutrition due to its nutritional value and sensory attributes. In addition to commercial production, butter is also produced domestically and sold in public markets across Türkiye [2-4]. According to the Turkish Food Codex [5], butter is defined as a product containing a minimum of 80% and a maximum of 90% milk fat, with a maximum of 2% non-fat milk solids and 16% water by weight. Butter production typically involves two methods: churning and emulsification. Traditionally, butter is produced by separating milk fat into cream, followed by churning, crystallization, and finally, kneading of pasteurized cream with added cultures [6].

The fat content and fatty acid composition of dairy products are major quality indicators that significantly impact human health, food quality, and product pricing. Fatty acid profiles in milk are influenced by various factors such as genetics, diet, lactation stage, and seasonal variations, and processing parameters for milk, including heat treatments, addition of starter cultures, maturation conditions (temperature and duration), and storage temperatures, also influence the fatty acid composition of dairy products [3, 7-9]. Monounsaturated oleic acid is recognized for its health benefits as it helps in reducing plasma cholesterol, LDL cholesterol, and triacylglycerols [10]. Studies suggest that the texture and spreadability of butter are positively associated with the proportion of unsaturated fatty acids in its composition [11].

Long-chain polyunsaturated fatty acids (PUFAs) offer numerous potential health benefits. Despite milk fat containing 5% saturated fat, it contributes positively to health due to its constituents such as conjugated linoleic acid (CLA), sphingomyelin, butyric acid, and myristic acid, which have been linked to mitigating chronic diseases [12]. CLA, a mixture of conjugated, positional, and geometric isomers of linoleic acid with 18 carbon atoms and two double bonds (C18:2, cis-9, trans-12), is particularly noteworthy for its unique array of positive effects [15]. Research studies have increasingly explored the beneficial impact of this biologically active compound on human health. CLA is known to significantly reduce the risk of cardiovascular diseases by lowering total plasma cholesterol, triglycerides, and low-density lipoproteins (LDL) [13]. Additionally, it exhibits antioxidant properties and contributes to combating obesity by reducing fat tissue while increasing protein, mineral, and water accumulation in the body [14-18]. The beneficial effects of CLA vary depending on the isomer type, dosage, and metabolic context in which it is administered [19]. Generally, CLA in milk originates from rumen bacteria, and its presence in fermented milk products depends on the activities of the lactic starters employed. The ripening conditions of the products and the starters used can be pivotal in CLA formation [17]. Milk and dairy products represent one of the richest dietary sources of CLA, accounting for approximately 70% of total CLA intake [19]. The average CLA content in milk ranges widely from 2 to 30 mg/g fat [20]. To harness the health benefits associated with CLA, it is recommended that a healthy individual weighing 70 kg consume 1.3-3.0 g of CLA per day [21].

To the best of our knowledge, there is currently no study comparing the CLA contents of artisanal and commercial butters. This study aimed to compare several chemical properties (including CLA content, fat content, dry matter content, fat in dry matter content, titratable acidity, acid degree values, and fatty acid profiles) of commercial butter samples with those of artisanal ones obtained from the city of Burdur, Türkiye. Additionally, the results of the present study were compared with the regulatory standards.

MATERIALS and METHODS

Materials

Various brands of commercial butter (designated as C1-C10) were purchased from national or local markets, while artisanal butter samples (designated as A1-A10) were obtained from local producers in different villages within the Burdur province of Türkiye. All butter samples, whether commercial or artisanal, were derived from cow's milk. The commercial butter brands selected for this study were representative of the prominent brands available in the Turkish market. Subsequently, the butter samples were stored under refrigerated conditions at 4±1°C until they were analyzed.

Methods

Chemical Analyses

The dry matter contents of butter samples were determined by using a rapid moisture analyzer (Kern DBS 60-3, Kern & Sohn GmbH, Balingen, Germany). The fat contents of butter samples were determined by the

Gerber method, which is a widely used reference method for the determination of fat content in milk and milk products, and the fat content was expressed as g/100 g butter [22]. The titratable acidity of butter samples was determined according to Metin and Öztürk [23] by using Eq. 1.

$$\text{Percent acidity (\% lactic acid)} = \frac{\text{V} \times \text{F} \times 0.009}{\text{m}} \times 100 \qquad (1)$$

where V is the amount of NaOH solution (mL) consumed in titration, m is the weight of the sample used in titration (g), F is factor of NaOH solution and 0.009 is the milliequivalent grams of lactic acid.

Lipid Extraction and Acid Degree Values

To extract lipids and determine the total free fatty acid values (acid degree value, ADV), the methods outlined by Renner [24] were used. Initially, butter samples were thoroughly crushed in a beaker along with an ample amount of kieselguhr (Fluka Chemie GmbH, Buchs, Switzerland). Diethyl ether (Fluka Chemie GmbH, Buchs, Switzerland) was subsequently added to the mixtures and thoroughly mixed. The mixture underwent filtration through coarse filter paper to separate butter particles and kieselgur from the solvent. This process was repeated multiple times to ensure complete extraction of lipids into the solvent, and the solvent-lipid mixture was collected in a volumetric flask. The diethyl ether was then removed from the solvent-lipid mixture at 45°C using a rotary evaporator (Heidolph, Schwabach, Germany). The lipid extract was then dried completely under nitrogen flushing and stored at -20°C until further analysis.

To determine the ADV, a procedure involving the addition of 40 mL of an ether-alcohol mixture (1:1) to the weighed lipid extract in an Erlenmeyer flask was employed. The mixture was then titrated with potassium hydroxide (KOH) solution (0.1 N) using phenolphthalein (1%) as an indicator. The total free fatty acid values were calculated using Eq. 2, and the results were expressed as grams of oleic acid per 100 grams of milk fat.

$$\text{Percent oleic acid}\left(\frac{\text{g}}{100\ \text{g}}\right) = \frac{282 \times \text{n} \times \text{F}}{\text{E} \times 100} \qquad (2)$$

where n is the volume of KOH solution consumed (mL), 282 is the molecular weight of oleic acid (g/moL), F and E are the factors of 0.1 N KOH solution and the weight of butter samples (g), respectively.

Preparation of Fatty Acid Methyl Esters and their Chromatographic Analyses

To prepare fatty acid methyl esters (FAMEs) from the lipid extracts prior to gas chromatographic analyses, the method proposed by Yılmazer and Seçilmiş [24] was followed. Initially, 1 mL of 1.5 M methanolic HCl was added to a lipid extract (200 µL) and maintained at 80°C for two hours. Subsequently, the mixture was cooled to room temperature, and 0.5 mL of water was added. The FAMEs were then extracted using 1 mL of hexane.

The fatty acid and CLA compositions of the butter samples were analyzed using an Agilent 7890A gas chromatography (GC) unit, coupled with a Agilent 5975C quadrupole mass spectrometer detector (MS). Electron ionization at 70 eV energy was used in the GC-MS analyses, with fragment ions analyzed in scanning mode within the mass range of 30-500 m/z. FAMEs were separated using a fused silica capillary column (DB WAX, 50 m × 0.20 mm, 0.20 µm film thickness; Chrompack, Midelburg, Netherlands). Injector and detector temperatures were set to 240°C, with an injection volume of 1 µL. Helium served as the carrier gas at a flow rate of 1 mL/min, with a split ratio of 1/20 in the analyses. Fatty acids and CLAs were identified using a standard mixture of FAMEs (Supelco® 37 Component FAME Mix, Catalog No: 47885 U, Sigma-Aldrich, St. Louis, MO, USA) and a CLA standard (Sigma Chemical Company, P Code: 1002398739, Sigma-Aldrich St. Louis, MO, USA).

Statistical Analysis

The experimental data were analyzed using the SAS package program (The SAS System for Windows 9.0, Chicago, USA) employing the analysis of variance (ANOVA) and Duncan's multiple comparison test as a post-ANOVA analysis. The relative fatty acid ratios (%) in the artisanal and commercial butter samples in Table 4 were compared using a t-test. Results were presented as mean ± standard deviation, with a significance level of α=0.05 considered.

RESULTS and DISCUSSION

Chemical Properties of Butter Samples

The fat and water contents, acidity and ADV values of butter samples are presented in Table 1. The moisture content of commercial and artisanal butter samples ranged from 13.25 to 25.00%. The mean dry matter content of commercial butters (84.15%) was found significantly higher than that of the artisanal ones (82.60%) ($p<0.05$). Three commercial butters (C1, C9 and C10) and five artisanal butters (A3, A7, A8, A9 and A10) exceeded the maximum water limit (16%) regulated by the Turkish Food Codex. Fat content of butter samples ranged from 75.00 to 86.75%, with one artisanal butter (A8) falling below the specified codex limit (>80% fat). However, the difference in the fat contents of commercial (84.08%) and artisanal (82.98%) butters was found insignificant ($p>0.05$). Additionally, Tahmas Kahyaoğlu and Çakmakçı [26] determined the dry matter and fat contents ranging from 82.77 to 83.00% and 81.50 to 81.90%, respectively, in butters produced from different animal milks during a 90-day storage period. Tavella et al. [27] reported a fat content of 85% in butter samples sold in Argentina.

Acidity value serves as a crucial parameter reflecting the oxidative stability of butter, with higher values indicating a faster oxidation process [28]. Titratable acidity values of the butter samples in this study ranged from 0.21% to 1.35% lactic acid. Furthermore, the mean titratable acidity values were found significantly higher in artisanal butters (0.51% lactic acid) compared to commercial butters (0.30% lactic acid) ($p<0.05$). This suggests that artisanal

butters are more susceptible to oxidation reactions than their commercial counterparts. Similarly, Tahmas Kahyaoğlu and Çakmakçı [26] reported titratable acidity values ranging from 0.13% to 0.51% lactic acid in butters produced from different animal milks. Akgül et al. [29] found that the titratable acid values of butter samples produced in Trabzon, Türkiye, ranged between 0.32-3.37%.

The ADV of lipids serves as a measure of their free fatty acid content, reflecting the degree of rancidity and lipolysis. ADVs of the butter samples ranged from 0.57% to 5.75% oleic acid. Commercial butters exhibited significantly lower ADV values (0.50%) compared to artisanal butters (0.94%), indicating that commercial butter is less susceptible to rancidity. Berhe et al. [30] determined the acid degree value of butter made from camel milk to be 6.7 mg KOH g^{-1}. Similar to the results of our study, Demirkol [31] found that the acidity values of butters sold in Çanakkale, Türkiye, varied between 0.55-1.22 mg KOH g^{-1}.

Ozkan et al. [31] reported fat, water, and titratable acidity values of butters containing *Satureja cilicica* essential oil as 84.16%, 14.30%, and 0.03%, respectively. In a study on commercially available butter samples, Keskin Çavdar [33] found that moisture and fat contents were 20.67% and 74.53%, respectively. Okur and Seydim [34] assessed the quality characteristics of commercial milk and dairy products sold in Isparta (Türkiye), noting that dry matter and fat contents of butters ranged from 82.09% to 86.86%, and from 83.50% to 86.75%, respectively. Seçkin et al. [33] determined the fat content of commercial butter samples (n=8) between 82.00% and 83.00%. The reults of the present study were in a good agreement with the literature data. Similar total solid contents (84.2-95.7%) and fat contents (81.4–92 g/100 g of total solids) were also reported in butters by Méndez-Cid et al. [34].

Table 1. Results of chemical analyses in commercial and artisanal butter samples

Sample	Fat (%)	Water (%)	Fat (%, dry matter basis)	Titratable Acidity (lactic acid%)	Acid Degree Value (% oleic acid)
C1*	83.00±1.41^{EDF**}	17.00±1.41^{CDE}	490.280±49.10^{FED}	0.33±0.01^{EDF}	0.57±0.04^{I}
C2	84.25±0.35^{EBDFC}	15.75±0.35^{GCFDE}	535.08±14.26^{FBEDC}	0.25±0.01^{IH}	0.62±0.03^{HI}
C3	85.00±0.71^{BDAC}	15.00±0.71^{GHFE}	567.41±31.47^{BDC}	0.28±0.03^{GHF}	0.82±0.10^{HIGF}
C4	84.75±0.35^{EBDAC}	15.25±0.35^{GHFDE}	555.92±15.21^{BEDC}	0.27±0.01^{GH}	0.89±0.11^{HIGEF}
C5	84.00±1.41^{EBDFC}	16.00±1.41^{GCFDE}	527.46±55.46^{FBEDC}	0.28±0.00^{GHF}	0.90±0.05^{HIGEF}
C6	86.75±1.77^{A}	13.25±1.77^{H}	661.50±101.59^{A}	0.33±0.01^{ED}	1.34±0.11^{DE}
C7	84.50±0.71^{EBDC}	15.50±0.71^{GFDE}	545.84±29.47^{FBEDC}	0.34±0.01^{D}	1.27±0.04^{DEF}
C8	86.00±0.00^{BA}	14.00±0.00^{GH}	614.29±0.00^{BA}	0.21±0.01^{I}	0.79±0.08^{HIGF}
C9	80.00±1.41^{G}	20.00±1.41^{B}	401.26±35.45^{G}	0.27±0.01^{GH}	0.89±0.02^{HIGEF}
C10	82.50±2.12^{EF}	17.50±2.12^{CD}	475.66±69.78^{FEG}	0.42±0.00^{C}	1.83±0.08^{C}
A1	84.00±0.00^{EBDFC}	16.00±0.00^{GCFDE}	525.00±0.00^{FBEDC}	0.43±0.03^{C}	1.13±0.01^{HDGEF}
A2	84.25±0.35^{EBDFC}	15.75±0.35^{GCFDE}	535.08±14.26^{FBEDC}	0.22±0.01^{I}	1.16±0.10^{DGEF}
A3	83.25±0.35^{EDFC}	16.75±0.35^{CFDE}	497.15±12.60^{FED}	0.30±0.02^{EGF}	1.41±0.05^{DC}
A4	85.50±0.71^{BAC}	14.50±0.71^{GHF}	590.48±33.67^{BAC}	0.29±0.00^{EGF}	0.89±0.49^{HIGEF}
A5	85.75±0.35^{BA}	14.25±0.35^{GH}	601.98±17.42^{BAC}	0.44±0.01^{C}	0.61±0.01^{I}
A6	84.25±1.06^{EBDFC}	15.75±1.06^{GCFDE}	536.37±42.86^{FBEDC}	0.45±0.03^{C}	1.30±0.07^{DEF}
A7	82.00±0.71^{GF}	18.00±0.71^{CB}	455.99±21.84^{FG}	0.30±0.01^{EGDF}	0.75±0.02^{HIG}
A8	75.00±0.00^{H}	25.00±0.00^{A}	300.00±0.00^{H}	1.08±0.04^{B}	4.47±0.74^{B}
A9	83.75±0.35^{EBDFC}	16.25±0.35^{GCFDE}	515.53±13.39^{FEDC}	1.35±0.01^{A}	5.75±0.20^{A}
A10	82.00±0.00^{GF}	18.00±0.00^{CB}	455.56±0.00^{FG}	0.30±0.04^{EGF}	1.17±0.06^{DGEF}
Mean Values					
Commercial	84.08±2.05^{A}	15.85±1.15^{A}	537.47±79.08^{A}	0.30±0.06^{A}	0.50±0.19^{A}
Artisanal	82.98±3.01^{A}	17.40±3.23^{B}	501.31±84.64^{A}	0.51±0.37^{B}	0.94±0.86^{B}

*C and A letters in the column represent commercial and artisanal butter samples, respectively. **A-J: Different letters in the same column represent significant differences between the means ($p<0.05$).

Fatty Acid Composition of Butter Samples

The fatty acid compositions of both commercial and artisanal butter samples are detailed in Tables 2 and 3, respectively, with an overview of the average fatty acid composition presented in Table 4. Across all butter samples, palmitic, oleic, and myristic acids emerged as the dominant fatty acids, collectively constituting over 58% of the average total fatty acids in both types of butter. These results confirm prior studies, which highlighted that artisanal butter contains approximately 40% short-chain fatty acids (C4–C14), predominantly palmitic and oleic acids [4]. In a study on the effect of cream cooling temperature and acidification methods on some technological properties of butters, Ceylan and Ozcan [37] identified palmitic, stearic, and myristic acids as the primary saturated fatty acids, with oleic acid being the dominant unsaturated fatty acid. Similarly, Okur and Seydim [33] studied the fatty acid compositions of various butter samples and reported the concentrations of myristic, palmitic, stearic, and oleic acids as 11.47, 32.06, 15.07, and 26.89 mg/g fat, respectively. In a study on the effect of salt addition, storage temperature, and duration on fresh butter, palmitic acid was identified as the predominant fatty acid (33.0%), followed by oleic acid (24.8%), myristic acid (11.5%), and stearic acid (9.8%) [37].

In a study by Tavella et al. [27], palmitic acid (16:0) was found to be the predominant fatty acid in butter, comprising 30.88% of the total fatty acid content, followed by oleic acid at 29.51%, stearic acid at 14.59%, and myristic acid at 11.12%. Another study on butter samples from Pakistan [38] reported saturated fatty acid contents ranging from 63.7% to 68.5% and *cis* polyunsaturated fatty acids ranging from 1.20% to 2.94%. Additionally, Serim [39] determined that butter contains 31% oleic acid, 23% palmitoleic acid, 3% linoleic acid, and 2% linolenic acid, with the highest saturated fatty acid contents attributed to palmitic (28%), myristic (11%), and stearic acids (10%).

Table 2. Changes in the fatty acid compositions of commercial butter samples (relative ratio, %)

Compound	Butter Sample									
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10
C4:0	4.00±0.03	3.46±0.05	4.23±0.06	4.86±0.04	3.61±0.03	4.18±0.03	4.41±0.06	2.98±0.02	2.98±0.02	4.80±0.03
C6:0	5.08±0.04	4.16±0.06	6.14±0.09	5.46±0.04	5.46±0.04	4.48±0.03	5.13±0.07	5.00±0.04	3.91±0.03	5.74±0.04
C8:0	3.06±0.02	2.40±0.03	3.87±0.06	3.53±0.03	3.25±0.02	2.64±0.02	3.66±0.05	3.79±0.03	2.94±0.02	4.29±0.03
C10:0	4.49±0.03	5.13±0.07	6.85±0.10	4.65±0.03	6.65±0.05	6.10±0.04	4.68±0.07	6.06±0.04	4.82±0.04	6.50±0.05
C11:0	0.89±0.01	0.65±0.01	0.55±0.01	0.59±0.00	0.52±0.00	1.03±0.01	0.49±0.01	1.73±0.01	0.83±0.01	0.79±0.01
C12:0	5.04±0.04	4.79±0.07	5.96±0.09	4.77±0.03	5.41±0.04	5.04±0.04	4.92±0.07	5.53±0.04	5.47±0.04	5.54±0.04
C14:0	14.99±0.11	13.91±0.20	15.01±0.61	15.33±0.11	13.52±0.10	13.17±0.10	13.58±0.20	13.41±0.10	14.53±0.11	14.86±0.11
C14:1n-5	4.22±0.03	3.20±0.05	4.17±0.06	3.22±0.02	4.13±0.03	3.88±0.03	3.29±0.05	3.10±0.02	3.04±0.02	2.59±0.02
C15:0	1.06±0.01	0.86±0.01	0.85±0.01	0.88±0.01	0.59±0.00	1.03±0.01	0.59±0.01	0.44±0.00	0.29±0.00	0.19±0.00
C16:0	28.04±0.20	30.53±0.44	22.83±0.37	26.88±0.20	27.58±0.20	24.88±0.18	25.21±0.37	24.93±0.18	26.16±0.19	24.11±0.09
C16:1n-7	0.41±0.00	0.42±0.01	0.49±0.01	1.03±0.01	1.17±0.01	0.72±0.00	0.82±0.01	1.97±0.01	1.40±0.01	0.56±0.00
C17:0	0.24±0.00	0.46±0.01	0.33±0.00	0.41±0.00	0.46±0.00	3.48±0.03	1.17±0.02	0.70±0.00	0.28±0.00	0.38±0.00
C18:0	5.45±0.04	7.47±0.11	5.31±0.08	4.66±0.03	4.32±0.03	4.00±0.03	5.04±0.07	5.45±0.04	5.11±0.04	4.09±0.03
C18:1	17.11±0.12	15.12±0.22	15.96±0.23	17.42±0.13	15.98±0.12	17.52±0.13	20.36±0.30	16.76±0.12	18.81±0.14	17.96±0.57
C18:2n-6	1.11±0.01	1.97±0.03	1.86±0.03	1.18±0.01	1.63±0.01	2.89±0.02	1.51±0.02	1.41±0.01	2.02±0.01	2.14±0.02
C18:3n-6	0.99±0.01	1.01±0.01	1.01±0.01	0.61±0.00	0.65±0.00	0.77±0.01	0.80±0.01	0.67±0.00	1.23±0.01	0.62±0.00
C18:3n-3	0.41±0.00	0.65±0.01	0.79±0.01	1.03±0.01	0.72±0.01	0.57±0.00	0.46±0.01	0.67±0.00	1.38±0.01	0.49±0.00
C20:1	0.02±0.00	0.02±0.00	0.05±0.00	0.05±0.00	0.10±0.00	0.06±0.00	0.09±0.00	0.05±0.00	0.05±0.00	0.09±0.00
Others	3.39±0.69	3.81±1.39	3.74±0.12	3.44±0.71	4.23±0.71	3.57±0.70	3.80±1.40	5.36±0.68	4.75±0.70	4.28±0.27
SFA*	72.34±0.52	73.81±1.07	71.92±1.47	72.03±0.53	71.38±0.53	70.03±0.51	68.87±1.00	70.02±0.50	67.32±0.49	71.29±0.43
MUFA	21.75±0.16	18.76±0.27	20.67±0.30	21.71±0.16	21.39±0.16	22.18±0.16	24.55±0.36	21.88±0.16	23.30±0.17	21.19±0.60
PUFA	2.51±0.02	3.62±0.05	3.67±0.05	2.81±0.02	3.00±0.02	4.22±0.03	2.78±0.04	2.74±0.02	4.62±0.03	3.24±0.02

*SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Poly-unsaturated fatty acids. Values represent means ± SD.

Table 3. Changes in the fatty acid compositions of artisanal butter samples (relative ratio, %)

Compound	Butter Sample									
	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10
C4:0	3.94±0.06	3.45±0.13	4.33±0.06	1.68±0.01	5.96±0.04	4.27±0.03	3.19±0.05	3.77±0.03	4.72±0.03	1.81±0.01
C6:0	5.00±0.07	4.13±0.15	5.39±0.08	2.80±0.02	4.83±0.04	5.27±0.04	4.20±0.06	3.07±0.02	3.89±0.03	2.41±0.02
C8:0	3.01±0.04	2.39±0.09	3.17±0.05	1.19±0.01	2.67±0.02	3.52±0.03	3.89±0.06	3.07±0.02	2.46±0.02	2.37±0.02
C10:0	4.42±0.07	5.11±0.19	5.05±0.07	2.27±0.02	3.95±0.03	5.20±0.04	6.60±0.10	5.85±0.04	4.48±0.03	3.60±0.03
C11:0	0.87±0.01	0.65±0.02	0.63±0.01	0.23±0.00	0.60±0.00	0.54±0.00	0.77±0.01	0.73±0.00	0.59±0.00	0.20±0.00
C12:0	4.97±0.07	4.76±0.17	5.67±0.08	3.89±0.03	4.16±0.03	4.94±0.04	2.89±0.04	6.05±0.04	4.53±0.03	7.08±0.05
C14:0	14.76±0.22	13.84±0.50	15.48±0.23	8.78±0.06	8.29±0.06	14.34±0.10	16.55±0.24	12.42±0.09	15.34±0.11	10.91±0.08
C14:1n-5	4.15±0.06	3.18±0.12	3.02±0.04	1.95±0.01	1.88±0.01	1.57±0.01	1.95±0.03	1.12±0.01	0.98±0.01	0.96±0.01
C15:0	1.05±0.02	0.85±0.03	1.10±0.02	0.34±0.00	0.66±0.00	0.42±0.00	0.99±0.01	0.73±0.00	0.46±0.00	0.81±0.01
C16:0	27.61±0.40	29.07±0.73	28.37±0.51	39.66±0.29	22.39±0.17	23.62±0.17	27.20±0.40	23.40±0.17	23.77±0.17	34.65±0.45
C16:1n-7	0.41±0.01	0.42±0.01	1.44±0.02	1.28±0.01	1.58±0.01	2.57±0.02	1.08±0.02	1.03±0.01	0.68±0.00	0.40±0.00
C17:0	0.24±0.00	0.46±0.02	0.59±0.01	0.28±0.00	0.20±0.00	0.45±0.00	0.33±0.00	0.14±0.00	0.63±0.00	0.20±0.00
C18:0	5.37±0.08	7.44±0.27	3.63±0.05	3.20±0.02	6.87±0.05	4.10±0.03	3.52±0.05	3.47±0.02	3.34±0.02	3.80±0.03
C18:1	16.85±0.25	15.04±0.55	15.85±0.42	23.21±0.17	25.71±0.19	21.32±0.15	17.54±0.26	26.51±0.19	26.16±0.19	24.04±0.17
C18:2n-6	1.09±0.02	1.96±0.07	1.56±0.02	3.24±0.02	2.41±0.02	1.98±0.01	1.92±0.03	2.29±0.02	2.36±0.02	2.31±0.02
C18:3n-6	0.98±0.01	1.01±0.04	0.46±0.01	1.25±0.01	1.18±0.01	0.89±0.01	1.04±0.02	1.52±0.01	1.18±0.01	0.73±0.00
C18:3n-3	0.41±0.01	0.65±0.02	0.62±0.01	0.98±0.01	1.14±0.01	1.05±0.01	0.97±0.01	1.05±0.01	1.11±0.01	0.76±0.01
C20:1	0.02±.00	0.02±0.00	0.02±0.00	0.13±0.00	0.09±0.00	0.16±0.00	0.06±0.00	0.18±0.00	0.09±0.00	0.02±0.00
Others	4.88±1.39	5.59±1.65	3.61±0.17	3.66±0.71	5.45±0.70	3.81±0.70	5.30±1.38	3.60±0.69	3.24±0.71	2.96±0.00
SFA*	71.22±1.04	72.14±2.30	73.42±1.16	64.31±0.47	60.57±0.45	66.67±0.48	70.13±1.02	62.70±0.45	64.19±0.47	67.83±0.69
MUFA	21.42±0.31	18.67±0.68	20.33±0.49	26.56±0.19	29.26±0.22	25.61±0.19	20.63±0.30	28.84±0.21	27.91±0.20	25.42±0.18
PUFA	2.47±0.04	3.61±0.13	2.63±0.04	5.47±0.04	4.73±0.03	3.91±0.03	3.94±0.06	4.86±0.03	4.66±0.03	3.79±0.03

*SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Poly-unsaturated fatty acids. Values represent means ± SD.

Table 4. Comparison of the fatty acid relative ratios (%) in artisanal and commercial butter samples

Fatty Acid	Commercial Butter	Artisanal Butter
C4:0	3.95±0.68^{A*}	3.71±1.29^{A}
C6:0	5.06±0.70^{A}	4.10±1.06^{B}
C8:0	3.34±0.59^{A}	2.77±0.75^{A}
C10:0	5.59±0.93^{A}	4.65±1.21^{A}
C11:0	0.81±0.37^{A}	0.58±0.22^{A}
C12:0	5.25±0.39^{A}	4.89±1.18^{A}
C14:0	14.23±0.80^{A}	13.07±2.88^{A}
C14:1n-5	3.48±0.57^{A}	2.08±1.06^{B}
C15:0	0.68±0.30^{A}	0.74±0.27^{A}
C16:0	26.12±2.22^{A}	27.97±5.50^{A}
C16:1n-7	0.90±0.50^{A}	1.09±0.68^{A}
C17:0	0.79±0.98^{A}	0.35±0.17^{A}
C18:0	5.09±1.00^{A}	4.47±1.54^{A}
C18:1	17.30±1.52^{A}	21.22±4.52^{B}
C18:2n-6	1.77±0.53^{A}	2.11±0.57^{A}
C18:3n-6	0.84±0.21^{A}	1.02±0.29^{A}
C18:3n-3	0.72±0.29^{A}	0.87±0.25^{A}
C20:1	0.06±0.03^{A}	0.08±0.06^{A}
Others	4.04±0.63^{A}	4.21±0.99^{A}
SFA	70.90±1.88^{A}	67.32±4.35^{B}
MUFA	21.74±1.53^{A}	24.47±3.88^{B}
PUFA	3.32±0.70^{A}	4.01±0.96^{A}

*$^{A-B}$: Different letters given for C (commercial) and A (artisanal) in each fatty acid represent the significant differences of the mean values ($p<0.05$).

According to Table 4, minor differences were observed in the fatty acid compositions between commercial and artisanal butters. The total SFA content was 70.90% for commercial butters and 67.32% for artisanal butters. However, the percentage of oleic acid (C18:1) was significantly higher in artisanal butters compared to commercial butters ($p<0.01$). Conversely, caproic acid and myristoleic acid contents were significantly higher in commercial butters than in artisanal butters. The difference in mean SFA values between commercial (70.90%) and artisanal (67.32%) butters was statistically significant ($p<0.05$). There was a significant difference in the monounsaturated fatty acid (MUFA) contents between commercial and artisanal butter samples, with artisanal butters being more advantageous from a nutritional standpoint. However, no statistically significant difference was found in the PUFA contents between commercial (3.32%) and artisanal (4.01%) butter samples ($p>0.05$). Similarly, Keskin Çavdar [33] reported average SFA, MUFA, and PUFA contents of commercial butters as 68.30%, 27.90%, and 2.98%, respectively. In a study by Draman [38], MUFA contents of butter samples (n=5) ranged from 58.22% to 66.76%, while MUFA and PUFA contents varied between 28.48%-37.12% and 4.25%-4.57%, respectively. Anwar et al. [38] found SFA contents of commercial butter samples from ten different brands between 63.7% and 68.8%. The PUFA C18:2 and C18:3 contents were reported to be between 0.50 and 2.00%, and 0.20 and 1.40%, respectively, which aligns with our results. Seçkin et al. [35] determined the fatty acid composition of various Turkish dairy products, including butter (n=10), where the most abundant saturated fatty acids were palmitic, stearic, and myristic acids. The average SFA, MUFA, and PUFA contents of butter were reported as 71.25, 27.70, and 0.38% of fatty acids, respectively. In the present study, variations found in fatty acid composition of the butter samples, could be attributed to a variety of factors. The absence of a standardized production method for artisanal butters, variations in production process conditions, diverse feeding methods of dairy animals, and discrepancies in storage and packaging conditions of the final products might be potential reasons for these differences [41]. In summary, while minor differences were noted in the fatty acid compositions of commercial and artisanal butters, significant distinctions emerged in their saturated and monounsaturated fatty acid profiles. Artisanal butters exhibited higher proportions of oleic acid and lower levels of caproic and myristoleic acids compared to their commercial counterparts. These findings emphasize the potential nutritional advantages of artisanal butter, particularly in terms of monounsaturated fatty acid content. However, no significant variance was observed in polyunsaturated fatty acid levels between the two types of butter. These results corroborate previous studies and provide valuable insights into the compositional variations of butter samples in the market.

CLA Contents of Butter Samples

Figure 1 illustrates the CLA contents of both commercial and artisanal butter samples. The average total CLA content was notably higher in artisanal samples (6.89 mg/g fat) compared to commercial ones (4.11 mg/g fat), with a significant difference observed ($p<0.05$).

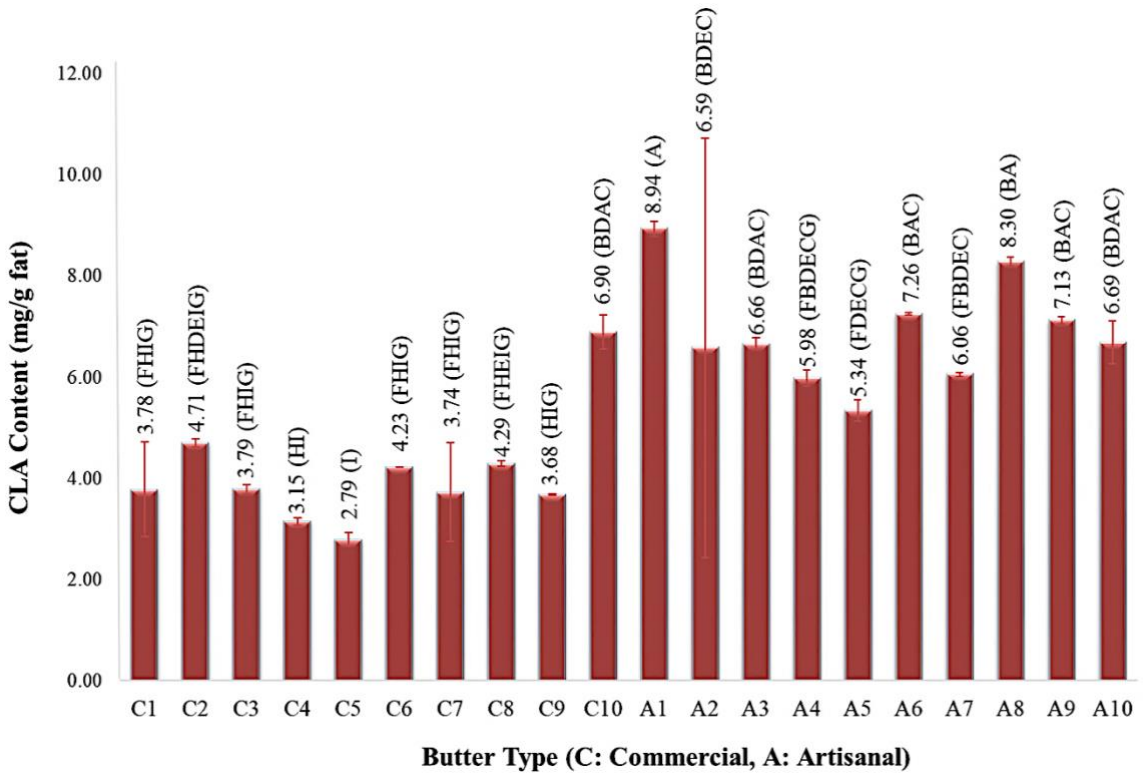

Figure 1. Conjugated linoleic acid (CLA) content (mg/g fat) contents of commercial and artisanal butter samples (**$^{A-I}$: Different letters in the same column represent the significant differences of the mean values ($p<0.05$)).

In line with our results, Shantha et al. [42] studied the effect of storage and processing on CLA content in salted and unsalted butters, revealing CLA contents ranging from 6.39 to 8.11 mg/g fat. Similarly, Draman [40] reported CLA levels in butter samples averaging between 0.72% and 0.86% of fatty acids. In a study by Okur and Seydim [34], the total CLA content of butter was reported as 0.94 mg/g fat. Seçkin et al. [35] observed CLA contents ranging from 2.85 to 4.67 mg/g fat in butters. Méndez-Cid et al. [36] found CLA content in butters to be between 0.65% and 0.83% of total methyl esters, noting that increasing storage temperature generally led to higher CLA content and that elevated temperatures along with salt addition increased oxidative and lipolytic changes in butters. Furthermore, Ledoux et al. [3] analyzed the fatty acid composition, particularly CLA isomers, of butters collected from France throughout different seasons. They reported an average CLA level in butters ranging from 0.45 to 0.80 g CLA/100 g butter, emphasizing regional variations in CLA content. Collectively, these studies underscore the variability in CLA content across different butter samples and highlight the multifaceted influences of storage conditions, processing methods, and regional factors on CLA levels.

In milk and milk products, a myriad of factors may contribute to variations in CLA content, including the inherent properties of raw materials influenced by animal feed composition and seasonal fluctuations, as well as process-related variables such as oxidative reactions, processing methods, and storage conditions [17]. Oxidative reactions play a significant role in CLA concentration, as they accelerate the formation of free radicals of linoleic acid, subsequently facilitating the transformation of double bonds into conjugated structures, thereby increasing CLA levels. However, oxidative reactions can also lead to structural deteriorations of conjugated double bonds, thereby altering CLA content [42]. The discrepancies observed between commercial and artisanal butters in CLA content could be attributed to these various factors, primarily influenced by differences in the diets of the cows producing the milk.

CONCLUSION

The study aimed to compare artisanal and commercial butters and assess their compliance with regulatory standards. Results indicated that some butter samples from various artisanal sources and industrial markets did not meet the criteria outlined in the Turkish Food Codex, primarily due to their high moisture or low fat contents. Moreover, titratable acidity and ADVs were notably higher in artisanal butters compared to commercial ones, suggesting increased susceptibility to oxidative processes. Analysis of fatty acid compositions revealed variations among the butter samples, with artisanal butter demonstrating a CLA content higher than commercial counterparts. Additionally, moisture content in artisanal butters was significantly elevated compared to commercial varieties. These differences may also stem from non-standardized processing techniques, variable salting practices, disparate animal diets, regional climatic influences, and other pertinent factors.

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CONFLICT of INTEREST

The authors declare no conflict of interest.

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Research Paper / Araştırma Makalesi

Quality Assessment and Bioactive Component Analysis of Honey from Different Geographical Regions in Erzurum, Türkiye

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ABSTRACT

In recent years, there has been an increasing interest on foods that are perceived to be healthy and functional among societies. The composition of honey, which plays an important role in human nutrition, is influenced by a number of factors. The aim of this study was to analyze fifteen different honey samples according to various quality criteria and to determine their 5-hydroxymethyl furaldehyde (HMF) and phenolic contents. In this context, honey samples were obtained from five distinct geographical regions within the Erzurum city area in Türkiye. There were statistically significant ($P<0.05$) differences in the pH, moisture, total sugar, reducing sugar, sucrose, proline, 5 (HF) and phenolic content values among the honey samples. HMF contents of samples varied between 5.20 and 108.12 mg/kg, and their phenolic contents ranged from non-detected to 202.95 mg/kg. While the HMF contents of honey samples were in accordance with the Turkish Food Codex Honey Communiqué (Communiqué No: 2020/7),with an exception of only one sample in terms of its proline and HMF contents.

Keywords: Honey, 5-Hydroxymethylfurfural, Proline, Phenolic components, Principal component analysis

Erzurum'un Farklı Coğrafi Bölgelerinden Elde Edilen Balların Kalite Değerlendirmesi ve Biyoaktif Bileşen Analizi

ÖZ

Son yıllarda, toplumlar arasında sağlıklı ve işlevsel olarak algılanan gıdalara olan ilgide kayda değer bir artış olmuştur. İnsan beslenmesinde önemli bir rol oynayan balın bileşimi çeşitli faktörlerden etkilenmektedir. Bu çalışmanın amacı, on beş farklı bal örneğini çeşitli kalite kriterlerine göre analiz etmek ve 5-hidroksimetilfurfural (HMF) ve fenolik bileşen içeriklerini belirlemektir. Bu kapsamda Erzurum ili sınırları içerisinde yer alan beş farklı coğrafi bölgeden bal örnekleri temin edilmiştir. pH, nem, toplam şeker, indirgen şeker,sükroz, prolin, 5-hidroksimetilfurfural (HMF) ve fenolik bileşen değerleri arasında istatistiksel olarak önemli ($P<0.05$) bir fark olduğu tespit edilmiştir. Bal örneklerinin HMF içeriklerinin 5.20-108.12 mg/kg arasında, fenolik bileşen içeriklerinin ise nd-202.95 mg/kg arasında değiştiği belirlenmiştir. Bal örneklerinin HMF içeriklerinin Türk Gıda Kodeksi Bal Tebliği'ne (Tebliğ No: 2020/7) uygun olduğu belirlenirken, pirolin ve HMF içerikleri bakımından sadece bir örneğin kodeksle uyumlu olmadığı tespit edilmiştir.

Anahtar Kelimeler: Bal, 5-hidroksimetilfurfural, Pirolin, Fenolik bileşen, Temel bileşen analizi

INTRODUCTION

The global population is expanding at an accelerated rate, with projections indicating that it will reach 10 billion by 2050. In light of these demographic projections, it is reasonable to anticipate an expansion in food demand in conjunction with population growth. In the era, there is a discernible inclination towards the consumption of safe food, particularly in light of the growing awareness among consumers. In order to safeguard consumer interests and facilitate the sound advancement of the food industry, the battle against food fraud represents a pivotal element of food quality control. One of the most significant challenges facing the food industry is the issue of food fraud. Fraudulent practices have the potential to disrupt the chemical composition and bioactive components of food products, thereby reducing their structural integrity and overall quality. One of the most susceptible products to counterfeiting is honey [1-7].

Honey is a sweet, natural product derived from animal sources that offers a high level of nutritional value [8, 9]. The majority of honey is composed of carbohydrates, which are further subdivided into macromolecules and micromolecules. The composition of honey, which has a viscous structure, is subject to variation depending on a number of factors. It is evident that the geographical location of the bee and the floral composition of the region are among the most significant factors [5, 7]. Honey and honey products, which have a complex chemical composition, are employed in the treatment of a range of diseases. Additionally, honey and its derivatives have been demonstrated to possess a range of beneficial properties, including antibacterial, antiviral, and antioxidant effects [8, 10-12].

One of the key elements in the evaluation of honey quality is the measurement of HMF. HMF can be formed as a result of the Maillard reaction or the dehydration of hexoses. The formation of HMF in honey products is dependent on a number of factors. The aforementioned factors include the composition of the honey, the temperature at which it is stored, the processing techniques employed, and numerous other variables [13, 14].

Honey is a natural food product obtained by bees from the nectar of flowers or honeydew. The composition and content of honey is directly related to environmental conditions under which it is produced. It is stated that honey has been used as a source of healing since ancient times. Especially honey has many positive effects on health. In this context, antioxidants assume particular significance in honey products. One of the important antioxidant compounds in honey products is phenolic components [15, 16].

Honey and its derivatives have been employed for a multitude of purposes throughout history. As a natural food product, honey is of significant nutritional value. However, the quality of honey is susceptible to a number of factors, which can impact its nutrition.The quality of honey and its products can vary considerably. In particular, the geographical location and floral flora directly affect the quality of honey. In the evaluation of the quality parameters of honey, a range of analytical techniques are employed, including pH measurement, electrical conductivity, colour assessment, sugar analysis, diastase activity determination, water content estimation, ash analysis, proline quantification and 5-hydroxymethylfurfural detection [17]. The present study analysed fifteen samples of flower honey obtained from different regions of Erzurum in terms of pH, moisture, total sugar, reducing sugar, sucrose, HMF, proline and phenolic components contents. The objective was to determine the quality criteria and qualities of honey obtained from various regions of Erzurum.

MATERIALS and METHODS

Fifteen flower honey samples from the 2022 flower season were obtained from beekeepers in Erzurum, Türkiye. The honeys used in the analysis were obtained from five different locations in Erzurum. Honeys obtained from different regions of Erzurum were stored at 24±1°C and dark environment until analysis.

Some Physicochemical Parameters

pH and moisture analysis were performed according to Cemeroğlu [18]. While Ohaus-Starter 3100 (Switzerland) pH meter device was used for pH analysis, Binder BD53 (Tuttlingen, Germany) oven device was used for moisture analysis.

Determination of Proline Contents

Proline analysis of honey samples was determined per International Honey Commission IHC [19]. Five g of honey sample was made homogeneous by dissolving in distilled water. Then, it was taken into 3 test tubes and sample, water and proline (0.5 mL of each) were added to each tube, respectively. And 1 mL each of formic and ninhydrin was added to each tube, and shaken in a water bath for 15 minutes. Then, it is kept in a 70°C water bath for another 10 minutes. At the end of the period, 5 mL of 2-propanol (50%) was added to each tube and subjected to a water bath at 70°C for 45 minutes. Then absornance reading was obtanied at 510 nm. Using Equation 1, proline contents were calculated:

$$\text{Proline (mg/kg)}=((E_s/E_a)\times(E_1/E_2))\times 80 \quad (1)$$

where E_s: sample absorbance, E_a: Absorbance value of proline standard solution, E_1: mg proline standard solution, E_2: value of honey in g, and 80: dilution factor

Determination of Total Sugar, Reducing Sugar and Sucrose

The total, reducing and sucrose sugars were determined in accordance with the methodology set forth by Cemeroğlu [18], which employs the Lane-Eynon method. The determination of sugar by this method is based on the reduction of $CuSO_4$ in Fehling's solution of invert sugar to $Cu(OH)_2$ (insoluble in water), in an

alkaline medium and at boiling temperature. Five gram of honey samples were collected and transferred to a 250-milliliter measuring balloon. Two mL of a saturated neutral lead acetate solution were added, and the solution was completed with distilled water up to the balloon line. Subsequently, sodium oxalate was added and filtered once more, following the filtration of the sample through ordinary filter paper. A volume of 50 mL of the filtrate was then transferred to two separate 250 mL flasks. The initial balloon was filled to the 250 mL line with distilled water, while the second was combined with 10 mL of 1/1 HCl and inverted for five minutes in a water bath maintained at a temperature between 67 and 70 °C. Following inversion, neutralisation was achieved through the addition of a few drops of phenol-phthalene and 4N sodium hydroxide, with the solution completed to the line with distilled water. The prepared sample solutions were subjected to titration by boiling with Fehling-I copper sulfate pentahydrate ($Cu_2SO_4 \bullet 5H_2O$) and Fehling-II sodium potassium tartrate tetrahydrate ($KNaC_4H_4O_6 \bullet 4H_2O$) solutions. The results were calculated in accordance with the following formula:

$$\text{Total sugar (g/100g)} = (f/M_1) \times 100 \quad (2)$$

$$\text{Reducing sugar (g/100g)} = (f/M_2) \times 100 \quad (3)$$

$$\text{Sucrose (\%)} = (\text{Total sugar} - \text{reducing sugar}) \times 100 \quad (4)$$

where f: The amount of invert sugar determined in the adjustment, equivalent to 10 mL of Fehling's solution mixture, g, M_1: The actual sample amount contained in the spent sample solution in the titration before inversion, g, and M_2: The actual amount of sample contained in the amount of sample solution spent in the titration after sample inversion, g.

Extractions and HPLC Conditions

The phenolic component and HMF contents of the samples were determined in accordance with the methodology outlined by Alwazeer et al. [20]. The honey samples were subjected to analysis for a range of phenolic components, including epicatechin, caffeic, rutin, *p*-coumaric, *e*-ferrulic, syringic, gallic, chlorogenic acid, and catechin.

The process of HMF extraction was initiated by weighing 2 g of honey and subsequently adding 20 mL of pure water. Subsequently, the solution was mixed in a shaker until dissolution was complete, after which it was filtered through a 0.45 µm filter. In order to ascertain the phenolic components, 15 g of honey was shaken with methanol at room temperature and filtered using filter paper. Once the filtrate had been completed with methanol, it was filtered through a 0.45 µm filter and placed in vials. The Dioder Array Detector (DAD) was employed for HPLC analysis (Agilent 1260 Infinity series, USA). The flow rate employed for the analysis of HMF was 0.6 mL/min, with a total analysis time of 25 minutes. For the phenolic components, a flow rate of 0.8 mL/min was used, with a total analysis time of 40 minutes.

Statistical Analysis

One-way analysis of variance (ANOVA) to determine the significance difference ($P<0.05$) of honey samples. It was also subjected to PCA and Pearsen Correlation analyzes to evaluate differences between honey samples.

RESULTS and DISCUSSION

The pH values and moisture contents of the honey samples are presented in Table 1. Their pH values exhibited considerable variation, with a range of 3.55 to 4.19. These findings were statistically significant ($P<0.05$). The highest pH value was observed in the "g" sample, while the lowest was observed in the "c" sample. Furthermore, it was established that all the honeys subjected to analysis exhibited an acidic structure. It is stated that the acidic structure observed in these honeys is a consequence of the fermentation of sugars and organic acids [21]. Conversely, while there is no restriction on pH value in the legal legislation, it is stated that the total amount of acidity can be 50 meq/kg at most [22]. These findings are corroborated by the literature [21, 23-25, 27].

Table 1. pH and moisture content (%) results of different flower honey samples

Code of Honey Samples	pH±SD	Moisture Content ±SD (%)
a	$3.95\pm0.01^{d*}$	15.77 ± 0.01^{b}
b	3.75 ± 0.04^{j}	19.50 ± 0.00^{gh}
c	3.55 ± 0.01^{k}	16.65 ± 0.21^{c}
d	3.87 ± 0.01^{e}	19.04 ± 0.05^{fg}
e	3.76 ± 0.01^{j}	20.55 ± 0.07^{i}
f	3.86 ± 0.02^{ef}	17.60 ± 0.14^{e}
g	4.19 ± 0.01^{a}	17.35 ± 0.21^{de}
h	3.78 ± 0.01^{ij}	22.25 ± 0.07^{j}
i	3.95 ± 0.01^{d}	17.00 ± 0.71^{cd}
j	3.83 ± 0.01^{gh}	19.75 ± 0.35^{h}
k	4.05 ± 0.01^{c}	18.75 ± 0.07^{f}
l	4.14 ± 0.02^{b}	14.75 ± 0.04^{a}
m	3.81 ± 0.01^{hi}	23.20 ± 0.14^{k}
n	3.84 ± 0.02^{fgh}	19.00 ± 0.28^{fg}
o	3.86 ± 0.01^{efg}	18.57 ± 0.04^{f}

*Different letters (a-l) in the same column are significantly different ($P<0.05$)
Abbreviations: SD: standard deviation.

The moisture content of the honeys varied ranged between 15.77 and 23.20% (Table 1) and were statistically very significant (P<0.05). In addition, the highest moisture content was determined in the “m” sample, while the lowest was determined in the “a” sample. It was detected that only three of the analyzed honey samples did not comply with the Turkish Food Codex (20%). Kayacıer and Karaman [21] reported that the moisture values of different honeys varied ranged beetwen 16.30 and 17.90. Nouri [28] determined that the moisture values of different honeys varied among 16.16-19.08%.

The total sugar content of the honeys varied between 70.57-80.24 g/100g (P<0.05) (Table 2). In honey samples, the highest total sugar content was determined in the “l” sample, while the lowest was determined in the “o” sample. In another study on total sugar, they reported that the total sugar ratio varied between 68.1 and 86 g/100g [24]. Khalil et al. [25] found the total sugar values in Algerian honeys between 62.80 and 70 g/mL. Nouri [28], in his study on different honey, determined that the total sugar content of the samples varied between 72.74 and 73.63%.

The reducing sugar contents of honeys were determined to vary between 56.30 and 78.00 g/100g (P<0.05) (Table 2). In honeys, the highest reducing sugar ratio was determined in the “l” sample, while the lowest was determined in the “o” sample. According to the Turkish Food Codex, it is indicated that the reducing sugar ratio for flower honey should be at least 60 g in 100 g. Ajlouni and Sujirapinyokul [24] determined that the reducing sugar level in honey samples varied between 57.3 and 73.6 g/100g. Oroian et al. [29] reported that the reducing sugar level in different honey samples varied between 65.00 and 70.52 g/100g. Again, Gültekin-Özgüven et al. [30] reported that the honey obtained from various regions of Turkey varies between 56.31 and 81.61%. Ucar and others detected that the reducing sugar ratios in honey samples varied between 63.72 and 71.94%.

Table 2. Total sugar, reducing sugar, sucrose, proline and 5-HMF contents of different flower honey samples

Code of Honey Samples	Total sugar (g/100g)	Reducing sugar (g/100g)	Sucrose (%)	Proline (mg/kg)	5-HMF (mg/kg)
a	73.03 ± 0.37^{f}*	70.370 ± 0.00^{fg}	2.52 ± 0.35^{fgh}	459.54 ± 8.94^{g}	6.25 ± 1.76^{f}
b	73.67 ± 0.20^{ef}	69.76 ± 0.18^{g}	3.72 ± 0.01^{d}	390.15 ± 6.33^{ij}	11.70 ± 1.55^{e}
c	74.10 ± 0.30^{ef}	71.83 ± 0.28^{ef}	2.16 ± 0.01^{ghi}	505.80 ± 4.10^{d}	7.50 ± 1.41^{f}
d	74.15 ± 0.23^{ef}	69.78 ± 0.13^{g}	4.15 ± 0.08^{cd}	412.26 ± 2.69^{h}	18.60 ± 2.54^{c}
e	70.59 ± 0.21^{g}	67.36 ± 0.19^{h}	3.07 ± 0.01^{e}	467.91 ± 2.89^{fg}	13.55 ± 1.55^{de}
f	77.61 ± 0.21^{cd}	72.36 ± 0.16^{de}	4.98 ±0.04^{b}	379.57 ± 9.73^{j}	23.5 ± 0.70^{b}
g	76.52 ± 0.40^{d}	74.70 ± 0.03^{c}	1.72 ± 0.35^{i}	479.25 ± 4.86^{ef}	15.75 ± 1.06^{d}
h	76.68 ± 0.76^{cd}	72.03 ± 0.54^{de}	4.42 ± 0.21^{c}	403.66 ± 6.63^{hi}	5.70 ± 0.14^{f}
i	79.37 ± 0.55^{ab}	76.32 ± 0.59^{b}	2.90 ± 0.04^{ef}	576.02 ± 6.99^{c}	6.45 ± 0.70^{f}
j	76.84 ±1.39^{cd}	73.58 ± 1.27^{cd}	3.09 ± 0.11^{e}	486.93 ± 1.38^{e}	11.50 ±0.71^{e}
k	74.72 ± 0.79^{e}	72.51 ± 1.00^{de}	2.11 ± 0.19^{hi}	636.85 ± 2.62^{b}	6.30 ± 0.71^{f}
l	80.24 ± 0.58^{a}	78.00 ± 0.91^{a}	2.13 ± 0.30^{hi}	363.62 ± 3.06^{k}	19.15 ± 0.49^{c}
m	78.22 ± 0.84^{bc}	75.13 ± 0.61^{bc}	2.94 ± 0.22^{ef}	396.91 ± 2.18^{i}	6.40 ± 0.14^{f}
n	73.03 ± 0.37^{f}	70.28 ± 0.82^{fg}	2.62 ± 0.43^{efg}	726.00 ± 5.23^{a}	5.20 ± 0.56^{f}
o	70.57 ± 1.53^{g}	56.30 ± 1.44^{i}	13.56 ± 0.08^{a}	244.79 ± 13.61^{l}	108.12 ±1.48^{a}
Significance	**	**	**	**	**

*Different letters (a-l) in the same column are significantly different (P<0.05) Abbreviations: SD: standard deviation.

It was detected that the sucrose contents of honey samples varied between 1.72-13.56% (P<0.05) (Table 2). As per Turkish Food Codex, it is stated that 5g/100g should be used for flower honey. According to Khalil and others the sucrose content of Algerian honey samples was 1.80-2.54%, Oroian et al. [29] determined the sucrose content of different honey samples as 1.76 g/100g, while Gültekin-Özgüven et al. [30] determined the sucrose content of the samples between non-detected and 3.43% in honey obtained from various regions of Turkey, and Nouri [28] determined that the sucrose content of the samples in his study on different honeys varied between 0.5 and 3.48%.

Proline, one of the important amino acids, is mostly derived from the salivary secretions of *Apis mellifera* during the transformation of nectar into honey [23]. The main amino acid in honey products is proline [15]. Therefore, the amount of proline in honey samples has an important role in determining sugar adulteration. Proline constitutes the largest part of the amino acid composition of honey (~ 85%). It is stated that the proline value of adulterated honey is low [15, 29]. As per the TFC, the proline content of honey varies depending on the honey types. It is stated that the proline content of flower honey should be at least 300 mg/kg [30]. In the study, it was determined that the proline value of honeys varied between 244.79 and 726 mg/kg (Table 2). It is also observed that the “n” group with the highest proline value has the lowest 5-HMF level. Similarly, Gültekin-Özgüven et al. [30] determined the proline value of 271-928.2 mg/kg in honey obtained from various regions of Turkey. Ucar et al. [14] detected that the values of proline in honeys varied between 657.39 and 1974.23 mg/ kg. Machado and others determined that the proline values of honey samples varied ranged between 0.2 and 2.2 mg/g. Ecem Bayram et al. [31], in his study, determined that the proline content of forty different honeys varied between 384.41 and 1271.56 mg/kg.

HMF is one of the important parameters in point of purity and quality of honey. HMF, which can be found at very

low levels even in fresh honey, is known to increase with storage and heat treatment of honey [23]. Due to high exposure to HMF, it can cause various health problems [33]. It is stated that the recommended level of HMF in all fresh honey samples is 40 mg/kg [24]. As per the results of the research, it was determined that the HMF values of honey samples varied between 5.20 and 108.12 mg/kg and were within the limits determined by the Turkish Food Codex except for only one sample (P<0.05) (Table 2). As a matter of fact, there are studies in the literature with similar and different results. Silva et al. [23] found HMF values between 17 and 51.5 mg/kg in honey samples. In their study, Ajlouni and Sujirapinyokul [24] determined that the HMF values of commercial and fresh honeys in which they applied different temperatures (65.75 and 85 °C) varied between 0.36 and 74.9 mg/kg. Khalil et al. [25] found HMF values between 15.23 and 24.21 mg/kg in Algerian honey samples. In another study, they determined that HMF values varied between 8.8 and 400 mg/kg [8]. Tomczyk et al. [34] determined the HMF levels of honey samples as 5.03-22.98 mg/kg in his study.

One of the important bioactive compounds in honey products is their phenolic components. In honey products, phenolic compounds has an affect sensory properties as well as antioxdidant activity. Phenolic components commonly found in honey products are gallic acid, *p*-coumaric acid, caffeic acid, chlorogenic acid, vanillic acid and syringic acid [35]. Phenolic substance contents vary in honey products. It is stated that these differences are especially due to climatic conditions, geography and honey types [6, 36]. Phenolic contents of the analyzed honey samples are given in Table 3. Epicatechin, caffeic, routine, *p*-coumaric, *e*-ferrulic and syringic acid were not detected in the study. However, gallic acid was determined as 194.72 µg/g in all samples, chlorogenic acid as 21.47 µg/g in only one sample, and catechin as 202.96 µg/g in three samples.

Table 3. Phenolic components of different flower honey samples (µg/g)

Code of Honey Samples	Gallic acid	Chlorogenic	Catechin
a	58.42±1.16^{g*}	21.47±0.5	nd
b	53.66±1.38^{i}	nd	nd
c	88.73±0.73^{d}	nd	160.86±0.01^{c}
d	53.65±2.85^{i}	nd	nd
e	58.15±0.65^{gh}	nd	nd
f	49.33±1.94^{jk}	nd	nd
g	41.00±0.50^{l}	nd	nd
h	45.44±2.01^{k}	nd	nd
i	103.59±2.27^{c}	nd	190.23±9.76^{b}
j	85.82±0.46^{e}	nd	nd
k	150.85±3.47^{b}	nd	202.95±7.49^{a}
l	54.03±5.55^{h}	nd	nd
m	49.79±5.21^{jk}	nd	nd
n	194.72±0.48^{a}	nd	nd
o	84.92±1.37^{f}	nd	nd

*Different letters (a-l) in the same column are significantly different (P<0.05), Abbreviations: SD: standard deviation.nd: not determined.

Gallic acid was the most abundant phenolic component in the analyzed honey samples. The gallic acid contents of the samples were determined between 41.00 and 194.72 µg/g. Different levels of gallic acid contents have been determined in the studies conducted in the literature. Alshammari et al. [37] determined the gallic acid contents of the samples between non-detected and 1.14 mg/100g, Andrade et al. [38] determined 237.20 mg phenolic acid /100g in different honey samples. In another study, gallic acid contents in different Turkish honey samples were reported to be between non-detected and 82.49 µg/g [39]. Pham et al. [40] determined gallic acid between 0.28 and 12.50 mg/100g in honey samples.

Chlorogenic acid contents of honeys were determined between non-detected and 21.47 µg/g (Table 3). Pham et al. [40] determined chlorogenic acid between 0.28-12.50 mg/100g in honey samples. On the other hand, Can et al. [39] reported that they could not detect chlorogenic acid in any sample in different Turkish honeys. Catechin contents of the samples were determined between non-detected and 202.95 µg/g. Pham et al. [40] determined catechin contents between 9.51 and 104.40 mg/100g in honey samples. In another study, catechin contents of different honey samples were found to vary between non-detected and 23.07 µg/g [39].

Correlation Among Quality Characteristics

When the results are analyzed, there are negative and positive correlations between pH, total sugar, reducing sugar and sucrose. On the other hand, 5-HMF, which is very important for honey samples, was negatively correlated with total sugar (P<0.05, r=-0,38*), reducing sugar (P<0.01, r=-0.80**) and pyroline (P<0.01, r=-0.60**), while it was positively correlated with sucrose (P<0.01, r= 0,95**). In addition, it was determined that there was a positive correlation between proline and reducing sugar (P<0.05, r=0.365*), while there was a negative correlation with sucrose (P<0.01, r=-0.60**) and HMF (P<0.01, r=-0.60**). Among the phenolic components, gallic acid (P<0.01, r=0.78**) and catechin (P<0.01, r=0.84**) showed a positive correlation with proline (Figure 1).

	Moisture (%)	pH	Total Sugar (g/100g)	Reducing sugar (g/100g)	Sucrose (%)	Pyroline (mg/kg)	HMF (mg/kg)	Gallic acid	Catechin	Chlorogenic
Moisture (%)	1,00	0,41*	0,13	0,15	-0,12	0,07	0,08	0,07	0,18	0,35
pH	0,41*	1,00	0,39*	0,32	-0,15	0,06	0,03	-0,02	-0,07	0,11
Total sugar (g/100g)	0,13	0,39*	1,00	0,84**	-0,41*	0,00	-0,38*	-0,22	-0,03	-0,21
Reducing sugar (g/100g)	0,15	0,32	0,84**	1,00	-0,84**	0,37*	-0,80**	-0,09	0,15	-0,05
Sucrose (%)	-0,12	-0,15	-0,41*	-0,84**	1,00	-0,61**	0,95**	-0,07	-0,27	-0,12
Pyroline (mg/kg)	0,07	0,06	0,00	0,37*	-0,61**	1,00	-0,60**	0,78**	0,84**	-0,01
HMF (mg/kg)	0,08	0,03	-0,38*	-0,80**	0,95**	-0,60**	1,00	-0,07	-0,27	-0,12
Gallic acid	0,07	-0,02	-0,22	-0,09	-0,07	0,78**	-0,07	1,00	0,89**	-0,13
Catechin	0,18	-0,07	-0,03	0,15	-0,27	0,84**	-0,27	0,89**	1,00	-0,16
Chlorogenic acid	0,35	0,11	-0,21	-0,05	-0,12	-0,01	-0,12	-0,13	-0,16	1,00

+1

-1

Figure 1. Correlation among quality parameters of honey samples (significant at *P<0.05 and **P<0.01)

Discrimination of Honey Samples by Principal Component Analysis

PCA has recently been widely used in the literature for the discrimination of different food samples [4, 41, 42, 43]. PCA analysis was used to determine the differences between honey samples. In addition, the differences between the applied analyzes were determined. The score scatterplot, loading scatter plot, biplot and hierarchical clustering are shown in Figure 2 A–D, it can be seen that two principal components accounted for 69.3 % of the variance. Honey samples were divided into 5 different groups as group1 (o), group 2 (n), group 3 (c, k, i), group 4 (l, g, m) and group 5 (a, e, j, d, b, h, f). The analyses are clustered in two regions, right and left. Moisture, 5-hydroxymethylfurfural and sucrose values clustered on the right side, while catechin, gallic acid, cholorocenic acid, proline, reducing sugar, total sugar and pH clustered on the left side.

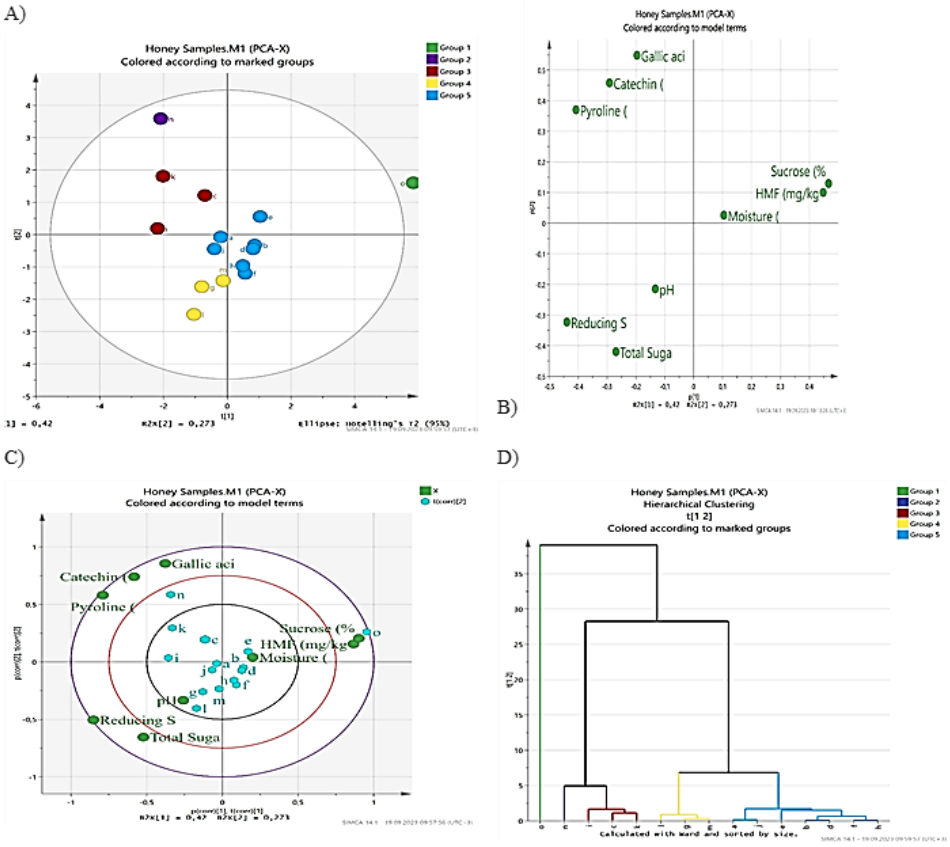

Figure 2. S-S-P (A), L-S-P (B), B (C) and H-C (D) of PCA analysis (PC1 versus PC2) for the components in the honey samples

CONCLUSIONS

Honey and its derivatives represent a significant component of the human nutrition. The present study investigated the quality parameters and the contents of HMF, proline and phenolic compounds of honey samples collected from various regions of the Erzurum province of Turkey. The results demonstrated that three of the fifteen honey samples exhibited moisture values that did not meet the national standards, while one sample displayed reducing sugar and proline values that did not align with the national standards. In general, the honey samples collected from Erzurum province were found to comply with the national standards. The adulteration of honey gives rise to unfair competition and has the potential to have adverse effects on consumer health.

Consequently, food adulteration represents not only an economic loss but also a significant threat to public health. In order to protect themselves from such adulteration, consumers should endeavour to purchase products from reliable brands, to examine product labels carefully and to avoid cheap products that are below market value. In this context, it is recommended that honey producers should be continuously inspected to maintain quality.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

A. Savaş conceived and designed the evaluation and collected the data. H.İ. Binici participated in designing the evaluation, re-evaluating the data, and revising the manuscript. İ.G. Şat helped drafted the manuscript and performed parts of the statistical analysis, and revised the manuscript.M. Kılıç conceived and designed the evaluation, re-evaluated the data, and revised the manuscript.

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Research Paper / Araştırma Makalesi

Regression Tool in MS Excel® Spreadsheets for Biological Data: R-BioXL

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ABSTRACT

A user-friendly MS Excel® spreadsheet as a freeware (R-BioXL) was developed to fit mathematical models to experimental data. (R-BioXL is available to everyone at https://drive.google.com/drive/folders/1GyjT3Z_CJQZu6ASb4LQBlS-ajLa_nF6X?usp=sharing) Initially, users are expected to enter their *X-Y* data and define their parameters of the model. Then, a model equation should also be entered again by users. Users can visualize data (scatter plot) and model fit (line plot) with the defined initial estimates of parameters on the same graph by default. Squared differences between experimental data and model estimates are calculated automatically. Users can change the initial estimates of the parameters to make the model closer to the data instantly, and Solver Add-In of Excel® should be used to minimize the sum of squared error by changing the parameter values. After the parameters are obtained, standard errors (by using "SolverAid" macro), 95 and 99% confidence intervals of the parameters, p values to determine the statistical significance of the parameters, and goodness-of-fit indices are calculated as the last step. All results can be saved on a different Excel® working page. Whole procedure takes a couple of minutes (~3 to 10 min) depending on the Excel® experience of the user. The utility, accuracy and reliability of the spreadsheet was shown by applying two-parameter (non-linear) Michealis-Menten equation for enzyme kinetics, three-parameter (linear) van Deemter equation for chromatography, and four-parameter (non-linear) modified Gompertz equation for microbial growth. In conclusion, R-BioXL can be safely and freely used to describe the experimental data with Excel® knowledge, without any skills in programming and without additional cost for other software package.

Keywords: Curve fitting, Freeware, Mathematical modeling, Solver, SolverAid

Biyolojik Veriler İçin MS Excel® Hesap Çizelgesi Aracı: R-BioXL

ÖZ

Deneysel verilere matematik modelleri uydurmak için ücretsiz yazılım olarak kullanıcı dostu bir MS Excel® hesap çizelgesi aracı (R-BioXL) geliştirilmiştir. (R-BioXL https://drive.google.com/drive/folders/1GyjT3Z_CJQZu6ASb4LQBlS-ajLa_nF6X?usp=sharing bağlantısından herkese açıktır) Başlangıçta, kullanıcıların *X-Y* verilerini bu araca girmeleri ve model parametrelerini tanımlamaları beklenmektedir. Daha sonra model denkleminin de yine kullanıcılar tarafından girilmesi gerekmektedir. Kullanıcılar verileri (dağılım grafiği) ve model uyumunu (çizgi grafiği) girilen ilk parametre değerleri ile aynı grafik üzerinde varsayılan olarak gözlemleyebilmektedirler. Deneysel veriler ve model tahminleri arasındaki farkların karesi otomatik olarak hesaplanmaktadır. Kullanıcılar modeli verilere anında daha yakın hale getirmek için parametrelerin ilk değerlerini değiştirebilir. Excel®'in Çözücü eklentisi parametre değerlerini değiştirerek hataların karesinin toplamını en aza indirmek için kullanılmalıdır. Parametreler elde edildikten sonra son adım olarak standart hatalar ("SolverAid"

makrosu kullanılarak), parametrelerin %95 ve %99 güven aralıkları, parametrelerin istatistiksel anlamlılığını belirlemek için p değerleri ve uyum iyiliği indeksleri hesaplanır. Tüm sonuçlar farklı bir Excel® çalışma sayfasına kaydedilebilir. Tüm bu prosedür, kullanıcının Excel® deneyimine bağlı olarak birkaç dakika (~3 ila 10 dakika) sürebilir. Aracın kullanımı, doğruluğu ve güvenilirliği enzim kinetiği için iki parametreli (doğrusal olmayan) Michealis-Menten denklemi, kromatografi için üç parametreli (doğrusal) van Deemter denklemi ve mikrobiyal büyüme için dört parametreli (doğrusal olmayan) modifiye Gompertz denklemi uygulanarak gösterilmiştir. Sonuç olarak, R-BioXL, Excel® bilgisi ile deneysel verileri tanımlamak için, herhangi bir programlama becerisi gerektirmeden ve diğer yazılım paketleri için ek maliyet olmadan güvenle ve serbestçe kullanılabilir.

Anahtar Kelimeler: Eğri uydurma, Ücretsiz yazılım, Matematik modelleme, Çözücü, SolverAid

INTRODUCTION

Mathematical models are used to describe the experimental data in many fields and biological sciences (agriculture, biology, biochemistry, biotechnology, environment, food science etc.) are no exception. To estimate the parameters of a model, one should fit linear or non-linear functions to data and many software packages are available for this purpose. However, spreadsheet techniques are less effortless because in general, no programming skills are required [1]. That is why, not only undergraduate and graduate students but also scientists and researchers from various disciplines prefer highly available and user-friendly Microsoft Excel® for such data analysis [2].

If a model is linear in its parameters that is, if the derivative of the model equation with respect to a parameter does not contain that parameter, then linear regression is applied and it is possible to obtain the parameters together with the uncertainties (standard errors or confidence intervals) of the parameters by using Excel®. This can be done by using "Data Analysis" tool (under the "Data" menu of Excel®) and "Regression" application [3]. Excel® gives a detailed "Summary Output" where parameters, standard error of the parameters, 95% confidence intervals (by default) and/or any confidence intervals such as 99% (User should define it.) are listed. Moreover, coefficient of determination (R^2), adjusted coefficient of determination (R^2_{adj}) and standard error of the estimate, which is also known as root mean square error (RMSE) are calculated to judge the goodness-of-fit of the linear model. However, most of the models used in biological sciences are non-linear in parameters [1] and hence, non-linear regression is required. Excel® "Solver" routine can be used for this purpose [4–6] but, only parameters (not the uncertainties in those parameters) would be obtained which is unacceptable [7]. Parameters are uninterpretable without their uncertainties and uncertainties are as important as the parameters themselves [8] and therefore, they should be obtained as well in case of a non-linear regression.

It should be noted that depending on the non-linearity of the equation, uncertainties are not symmetric [8]. On the other hand, many software packages calculate the asymptotic standard errors or confidence intervals. Asymptotic standard errors can also be calculated by using Excel®: first, a matrix so-called "Jacobian matrix" (***J***) should be constructed and this can be done by taking the partial derivative of the model equation with respect to each parameter. Then, by using the parameter values obtained from the non-linear regression, each value of the partial derivatives for every *X* (independent variable) are calculated. Taking the transpose of the Jacobian matrix ($\boldsymbol{J}^T$) and taking the inverse of the multiplication of transpose of Jacobian matrix by Jacobian matrix itself $[(\boldsymbol{J}^T \cdot \boldsymbol{J})^{-1}]$ will give $p \times p$ variance-covariance matrix, where p is the number of parameters in the model. By using the diagonal element of this matrix, asymptotic standard errors of the parameters and confidence intervals could be calculated. This calculation may take several minutes even for the experienced Excel® users. Moreover, if the number of parameters is high in a model (≥ 4), calculations become cumbersome. Alternatively, standard errors of the parameters could also be calculated in Excel® by using macros such as the "SolverAid" provided by De Levie [9], but unfortunately it seems that it is not widely be used [7].

The objective of this study is to present a user-friendly Excel spreadsheet where the user can enter his/her model by himself/herself and minimize the sum of squared error (SSE) by changing the parameter values of the non-linear model in Excel® with the Solver function. Then, the asymptotic standard errors, 95% and 99% confidence intervals as well as the goodness-of-fit indices (R^2, R^2_{adj} and RMSE) can be calculated automatically (with a little effort by the user). Usefulness of the Excel® spreadsheet is described by using three different models and the results are compared with other statistical software packages such as SigmaPlot 12.0, MATLAB R2017b and SPSS 22.0.

METHODOLOGY

R-BioXL

R-BioXL (Regression tool for biological data in Excel®) is a user-friendly spreadsheet application to describe the biological data in various disciplines (biology, biotechnology, biomedicine, environmental sciences, food sciences etc.). R-BioXL is a freeware and can be found at https://drive.google.com/drive/folders/1GyjT3Z_CJQZu6ASb4LQBlS-ajLa_nF6X?usp=sharing. It contains 5 working pages where users can enter 5 different models to each. Alternatively, users can use the same page by deleting the previous models or the tool can be downloaded again to use it for the next 5 models. Furthermore, users need not know any programming

skills, being familiar to some basic functions of Excel® is enough to use the tool – see below.

Figure 1 shows the blank page of R-BioXL. Buttons to be used to calculate the squared differences between the experimental data and model fit, sum of squared differences (error), and regression statistics, and to save the results are all inactive. User should enter the *X*-*Y* data to Columns A and B (starting from A2-B2), respectively where *X* is the independent variable (generally time) and *Y* is the dependent variable. Definitions of all necessary cells are also given for the users. It is possible to see the data on the graph as a scatter plot as soon as the data are entered. Then, user should enter the name(s) of the parameter(s) to Column G (starting from cell G2) and in the next cell (Column H – starting from cell H2) initial value(s) of the parameter estimate(s). Initial values for all parameters can be entered as "1" as the starting point of the iteration, but these are subjected to change before using the Solver function – see below. Before entering the model to Column C, users have two options: (i) defining the name of the parameters i.e., naming the cells, (ii) using "$" sign to fix the cells of the parameters so that using the same values of the parameters even after dragging or copying. Naming the cells are very easy and explained in different studies [4–6]. Moreover, it allows users to see the parameters on the model equation, not the name of the cells, which is easy to interpret. Nevertheless, second option is still valid: in the model equation, users should select the initial estimate of the parameters and enter "$" before and after the letter of the cell as such "$H$2". Both cases are demonstrated in the next section.

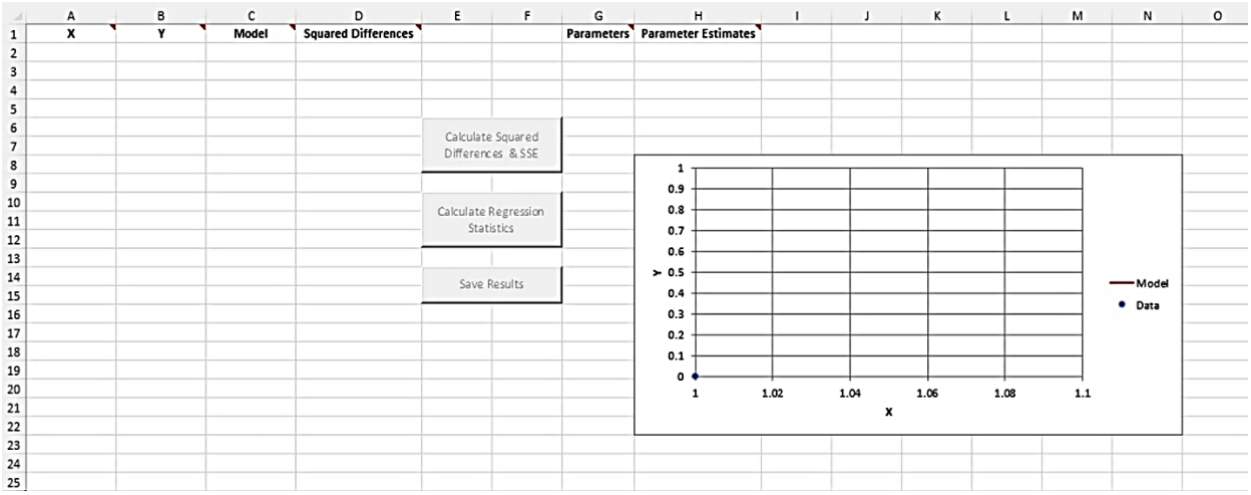


Figure 1. Blank page of R-BioXL. Buttons will be all inactive unless the necessary parts are filled in

RESULTS

Case Study I: Enzyme Kinetics

Data of initial rates of sucrose hydrolysis by the enzyme (yeast) invertase as a function substrate concentration are given in Table 1. Data were originally published by Chase et al. [10] and were also used by van Boekel [11]. Henri-Michealis-Menten equation or more widely known as Michealis-Menten equation [Equation (1)] was used to describe the data:

$$V = \frac{V_{max} \cdot S}{K_M + S} \qquad (1)$$

where *V* is the (initial) rate (dependent variable) and *S* is the sucrose concentration (independent variable). The model has two parameters: V_{max} is the maximum rate, and K_M is the substrate concentration where rate is equal to $0.5 \cdot V_{max}$.

Table 1. Initial rates of hydrolysis of sucrose by the Invertase enzyme. Original data are from Chase et al. [10]

Sucrose (M)	Initial rate (min^{-1})
0.0292	0.182
0.0580	0.258
0.0584	0.265
0.0876	0.311
0.1170	0.330
0.1170	0.342
0.1460	0.349
0.1750	0.372
0.2050	0.347
0.2340	0.371

Figure 2 shows entering the data into columns A and B. As the data are entered, graph is updated simultaneously, and data are seen as blue circles (Figure 2a). At this stage, buttons are still inactive. Then, the parameters (column G) and their corresponding (initial) values (column H) are entered. Model equation should be written to column C. When these steps are completed, model fit appears as the red line on the graph and the first button becomes active (Figure 2b). The equation can be written in Excel® as such: "=V_{max}*A2/(K_m+A2)" where V_{max} and K_m are the defined parameters and A2 is the first cell where S (substrate

concentration) was inserted. Note that, parameters were defined i.e., the cells were named (cells H2 and H3 not G2 and G3!): Formulas > Define name, so that the names of the parameters appear on the model equation (column C) [4–6].

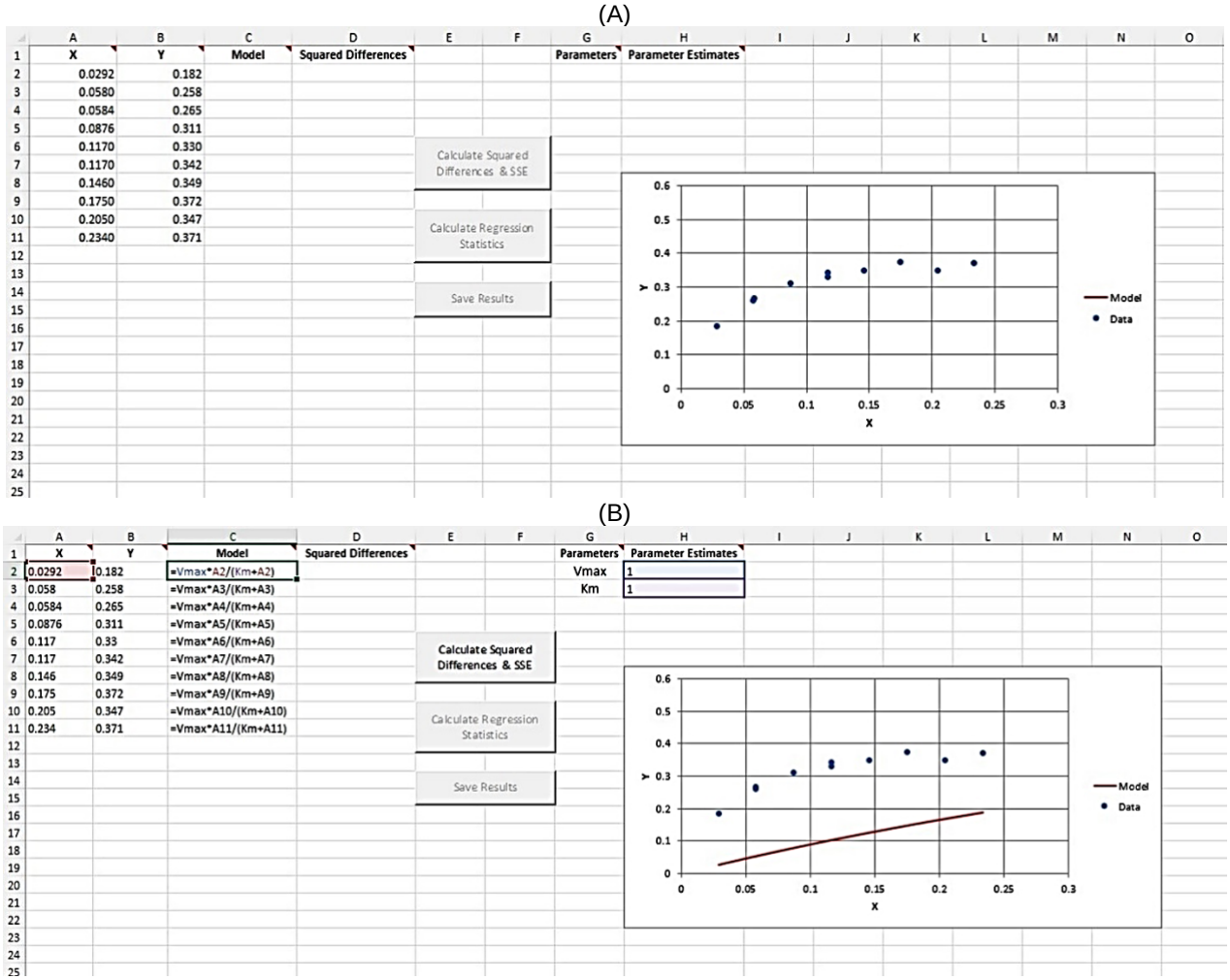


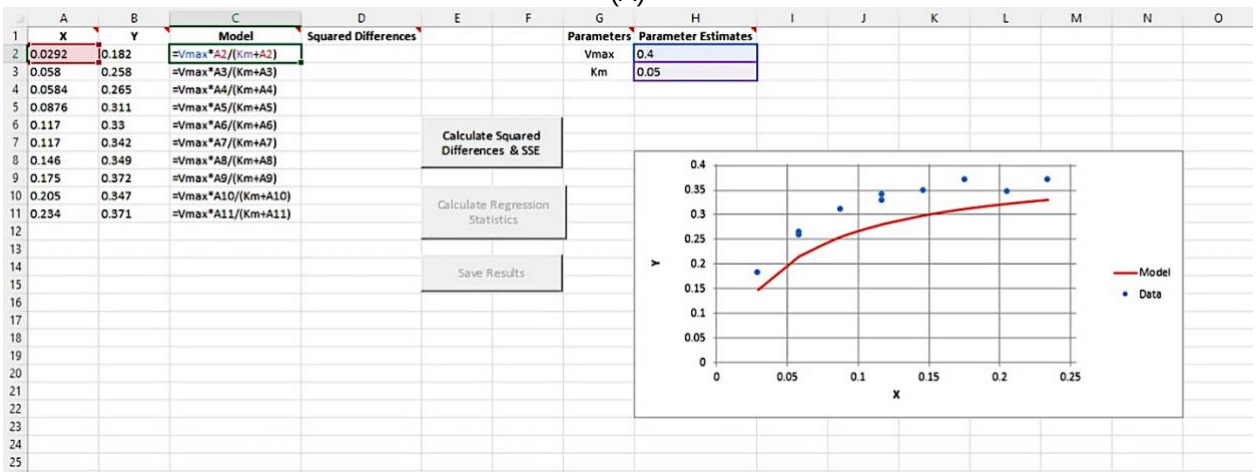
Figure 2. Entering the data given in Table 1 to R-BioXL. Data are observed as blue circles on the graph (A). Entering the model parameters (cells G2 and G3), initial estimates of the model parameters (cells H2 and H3) and the model equation [Equation (1)] (column C, starting from cell C2). Model fit is observed as the red line on the graph (B). Note that cells H2 and H3 are named.

Initial values which were entered as 1, can be changed and the updated model fit according to the new initial values is also observed instantly (Figure 3a). User can select better values and make the model fit closer to the data before using the Solver function. Then, by clicking the "Calculate Squared Differences & SSE" button, all calculations are done automatically and instantly (Figure 3b). At this stage, second button is active; however, using this button before using the Solver function for obtaining the best parameter estimates ends up with the wrong results. Therefore, a warning will appear if the user clicks this button.

Figure 4a shows the best parameter estimates by using Solver function. As soon as the "Calculate Regression Statistics" button is hit, a window asking the number of parameters in the model equation appears (Figure 4b). Since there are two parameters (V_{max} and K_M) in the equation, "2" was entered. Then, standard errors and confidence intervals of the parameters, p values, and goodness-of-fit indices were calculated and tabulated automatically (Figure 4c).

Note that the standard errors and the confidence intervals are asymptotic meaning that V_{max} = 0.4367 ± 0.0122 min^{-1} (standard error), V_{max} = 0.4367 ± 0.0282 min^{-1} (95% confidence interval) or V_{max} = 0.4367 ± 0.0411 min^{-1} (99% confidence interval). Both parameters were statistically significant since p values were ≤ 0.05 or ≤ 0.01. Moreover, high R^2 and R^2_{adj}, and low RMSE values revealed a good fit. These were compared with some other software packages such as SigmaPlot, MATLAB and SPSS and identical results were obtained (results not shown).

(A)



(B)

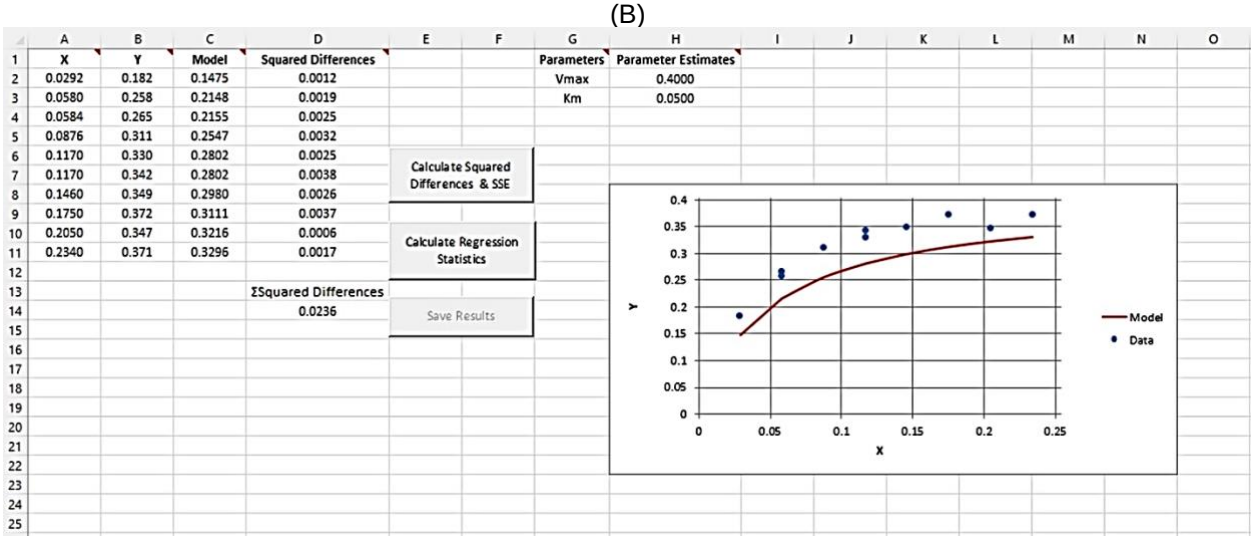


Figure 3. Changing the initial estimates of the parameters and making model fit closer to the experimental data given in Table 1 (A). Calculating the squared differences and sum of squared error (SSE) by clicking the "Calculate Squared Differences & SSE" button (B).

"Save Results" button can be used after all calculations to save and extract the output to another Excel® page. User can now arrange the graph (scaling and naming the axes, changing the color etc.).

Case Study II: Gas Chromatography

Experimental gas chromatography data published by Moody [12] are given in Table 2. Data were described by van Deemter equation [Equation (2)]:

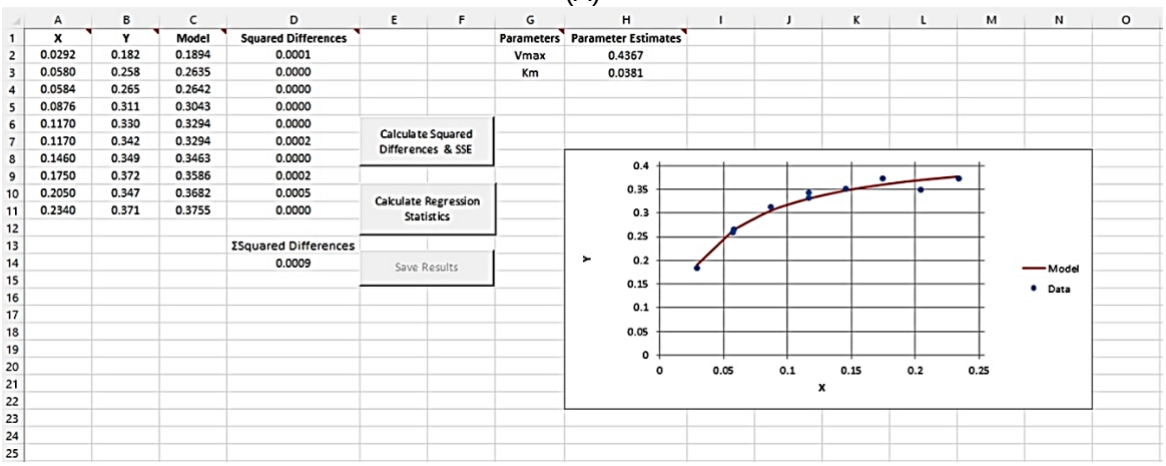
$$H = A \cdot \dot{F} + B/\dot{F} + C \qquad (2)$$

where *H* is the plate height (dependent variable) and $\dot{F}$ is the volumetric flow rate (independent variable). Note that the model has three parameters (*A*, *B* and *C*) and the parameters are linear. Therefore, linear regression can be used to obtain the parameter values; however, this example was used to show that R-BioXL could also be used linear models as well as non-linear models.

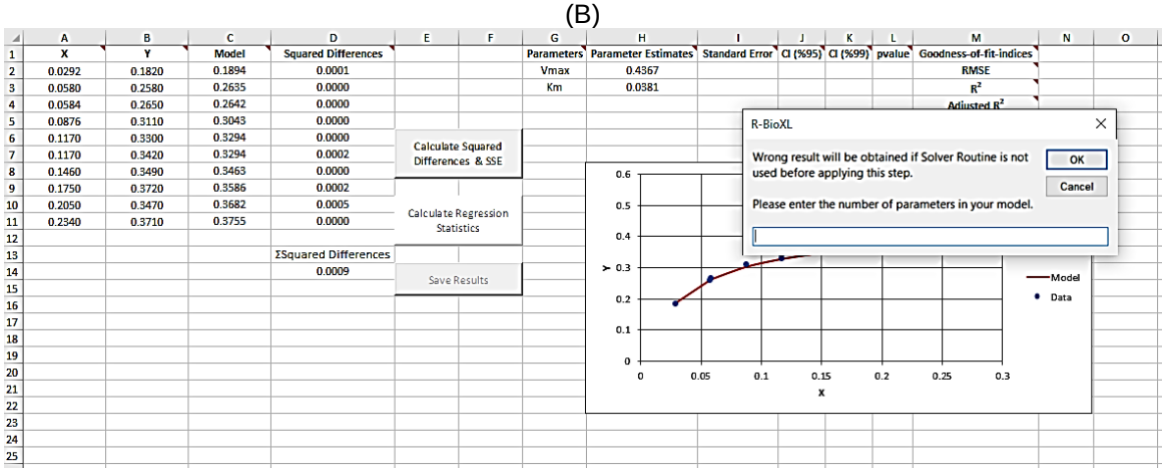
Table 2. Gas chromatography data from Moody [12]

Plate height (mm)	Flow rate (mL/min)
3.4	9.59
7.1	5.29
16.1	3.63
20.0	3.42
23.1	3.46
34.4	3.06
40.0	3.25
44.7	3.31
65.9	3.50
78.9	3.86
96.8	4.24
115.4	4.62
120.0	4.67

(A)



(B)



(C)

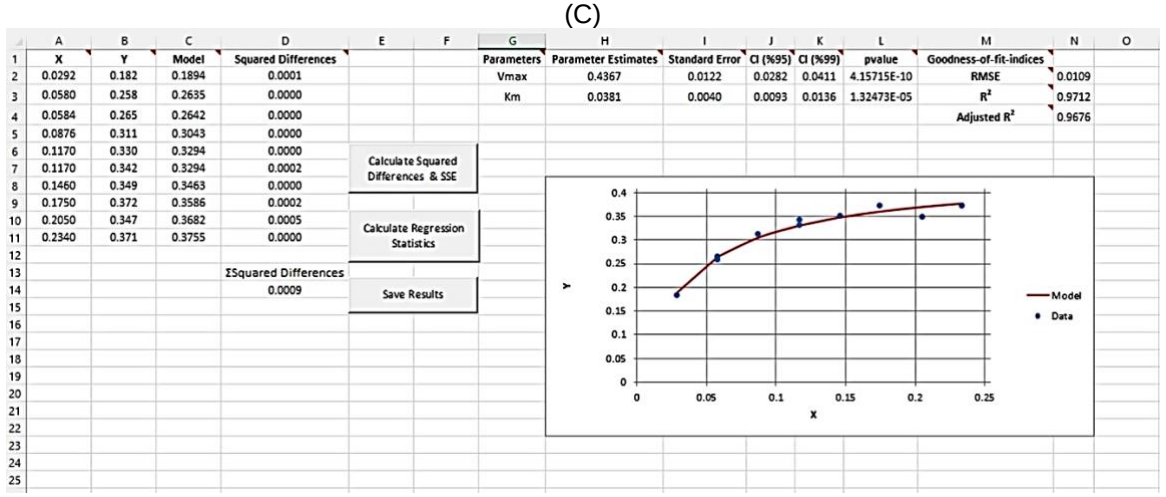


Figure 4. Obtaining the best-fitted parameter values by using Solver (A). Entering the number of parameters in the model after clicking the "Calculate Regression Statistics" button (B). Results of the model fit [Equation (1)] which can be saved and extracted to another Excel® working page by clicking the "Save Results" button (C).

Figure 5 shows entering the parameters, the initial estimates of the parameters as "1" and the model equation. The equation was written in Excel® as such: "=$H$2*A2+$H$3/A2+$H$4" where $H$2, $H$3 and $H$4 are the values of the parameters *A*, *B* and *C*, respectively in Equation (2), and again A2 is the is the first cell where $\dot{F}$ (volumetric flow rate) was inserted (Figure 5a). Note that cells were not defined but "$" ($H$2, $H$3 and $H$4) was used to fix the values of the parameters in the model equation (Figure 5a). If the Solver function is used with these initial estimates, no results will be obtained (convergence failure). Therefore, initial estimates of the parameters should be selected wisely (Figure 5b) and after obtaining the best estimates of the parameters, the necessary steps could be followed (calculating SSE, entering the number of parameters as "3") to conclude the process (Figure 5c). Another important remark, since the van Deemter

equation [Equation (2)] is linear in parameters there is no need for initial estimates of the parameters. Data analysis > Regression tool of Excel® can be used [3]. The results of the linear regression are presented in Figure 6. The same results were also obtained for applying linear regression by using Data Analysis > Regression application of Excel®. Output gives the confidence intervals as upper and lower limit; however, in R-BioXL they are given as plus/minus the best fit value and therefore, they can be calculated by subtracting the best-fitted parameter value from the upper limit or subtracting lower limit value from the best-fitted parameter value. For example, for the parameter *C* in van Deemter equation [Equation (1)] 95% confidence interval is whether 1.7365 – 1.5681 = 0.1684 or 1.5681 – 1.3997 = 0.1684 (Figure 6) and this was the same result with the R-BioXL (Figure 5c).

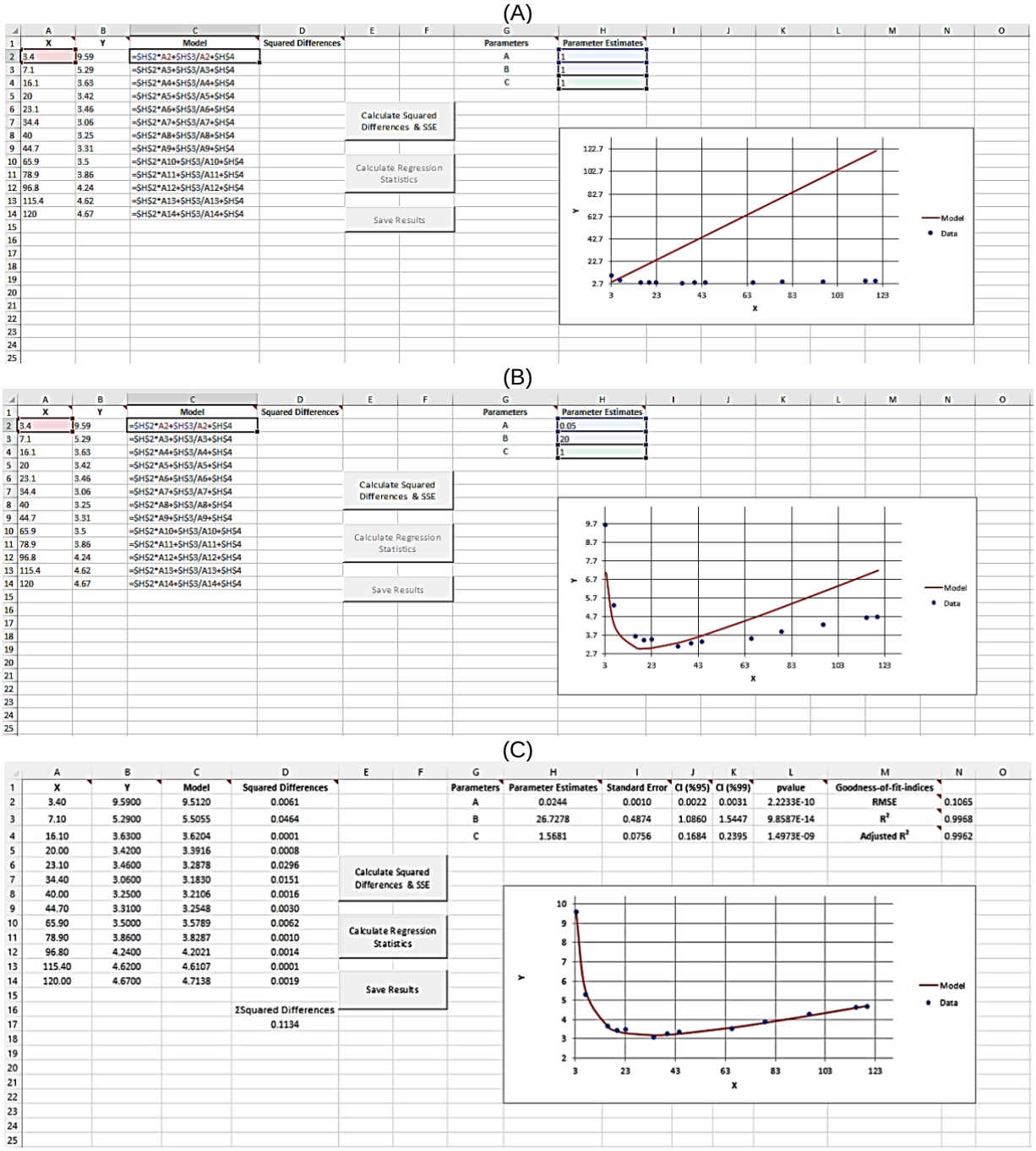


Figure 5. Entering the data given in Table 2, the model parameters (cells G2 to G4), initial estimates of the model parameters (cells H2 to H4) and the model equation [Equation (2)] (column C, starting from cell C2). (A). Changing the initial estimates of the parameters and making model fit closer to the experimental data given in Table 2 (B). Results of the model fit [Equation (2)] which can be saved and extracted to another Excel® working page by clicking the “Save Results” button (C).

	A	B	C	D	E	F	G	H	I	J
1	SUMMARY OUTPUT									
2										
3	*Regression Statistics*									
4	Multiple R	0.9984								
5	R Square	0.9968								
6	Adjusted R Square	0.9962								
7	Standard Error	0.1065								
8	Observations	13								
9										
10	ANOVA									
11		*df*	*SS*	*MS*	*F*	*Significance F*				
12	Regression	2	35.4764	17.7382	1563.7258	3.2894E-13				
13	Residual	10	0.1134	0.0113						
14	Total	12	35.5898							
15										
16		*Coefficients*	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 99.0%*	*Upper 99.0%*	
17	Intercept (C)	1.5681	0.0756	20.7494	1.4974E-09	1.3997	1.7365	1.3286	1.8076	
18	X Variable 1 (A)	0.0244	0.0010	25.1942	2.2232E-10	0.0222	0.0265	0.0213	0.0274	
19	X Variable 2 (B)	26.7279	0.4874	54.8363	9.8585E-14	25.6418	27.8139	25.1831	28.2726	
20										
21										

Figure 6. Summary output of the fit of van Deemter equation [Equation (2)] to the data given in Table 2 by linear regression tool (Data > Data Analysis > Regression) of Excel®.

Case Study III: Microbial Growth

Growth of *Listeria monocytogenes* at 30°C in high salt media was published by Lambert et al. [13] are given in Table 3. Data were described by modified Gompertz equation [Equation (3)] which was proposed by Zwietering et al. [14]:

$$\log_{10}N(t) = \log_{10}N_0 + (\log_{10}N_{max} - \log_{10}N_0) \cdot \exp\left\{-\exp\left[\frac{\mu_{max} \cdot e/2.303}{(\log_{10}N_{max} - \log_{10}N_0)} \cdot (\lambda - t) + 1\right]\right\} \quad (3)$$

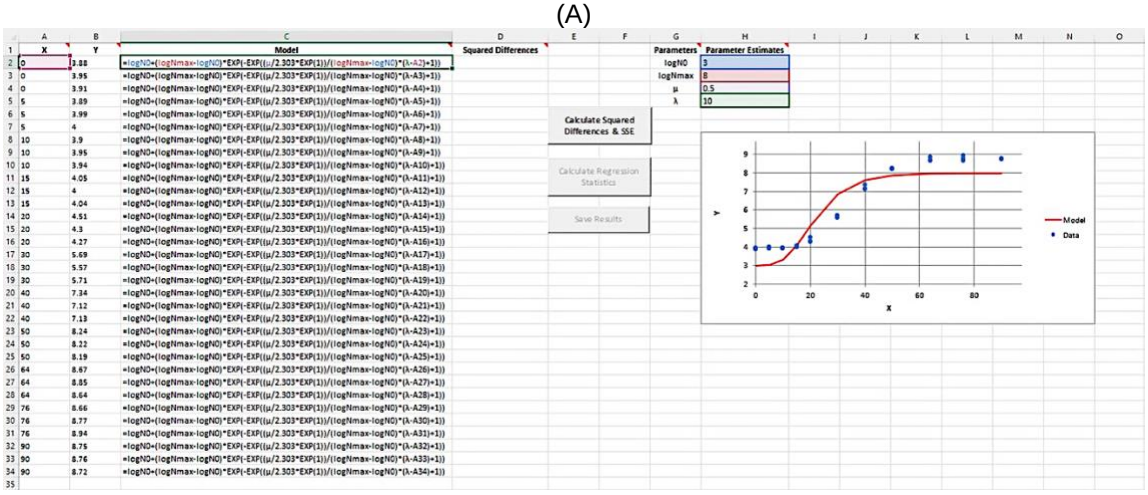
where $\log_{10}N(t)$ is the number of bacteria (dependent variable) and t is the time (independent variable). Model has four parameters: $\log_{10}N_{max}$ is the maximum bacterial load, $\log_{10}N_0$ is the initial number of bacteria, μ_{max} is the maximum growth rate and λ is the lag time.

Figure 7 shows the data, the model equation with the selected initial values of the parameters and the results. Model was written as: "=$\log N_0$+($\log N_{max}$-$\log N_0$)*EXP(-EXP((μ/2.303*EXP(1))/($\log N_{max}$-$\log N_0$)*(λ-A2)+1))". If the user enters "1" as the initial estimates of the parameters, the model [Equation (3)] will be undefined because $\log_{10}N_{max} - \log_{10}N_0$ will be zero. Furthermore, if $\log_{10}N_{max}$ is set to "2" leaving all parameters with "1" will not enough to obtain the best parameter estimates because Solver capacity is limited and number of iterations is not sufficient to have global minimum. This was also the case with van Deemter equation [Equation (2)] as discussed above, but not for Michealis-Menten equation [Equation (1)]. Users should be careful more and more about the initial estimates as the number of parameters in a model is high (≥ 3). Once again, the parameter estimates, standard errors of the parameters, confidence intervals and goodness-of-fit indices were all same with the other software programs (results not shown).

Table 3. Growth data of *L. monocytogenes* at 30 °C in 9% salt. Original data are from Lambert et al. [13]

Time (h)	$\log_{10}N(t)$ (CFU/mL)
0	3.88
0	3.95
0	3.91
5	3.89
5	3.99
5	4.00
10	3.90
10	3.95
10	3.94
15	4.05
15	4.00
15	4.04
20	4.51
20	4.30
20	4.27
30	5.69
30	5.57
30	5.71
40	7.34
40	7.12
40	7.13
50	8.24
50	8.22
50	8.19
64	8.67
64	8.85
64	8.64
76	8.66
76	8.77
76	8.94
90	8.75
90	8.76
90	8.72

(A)



(B)

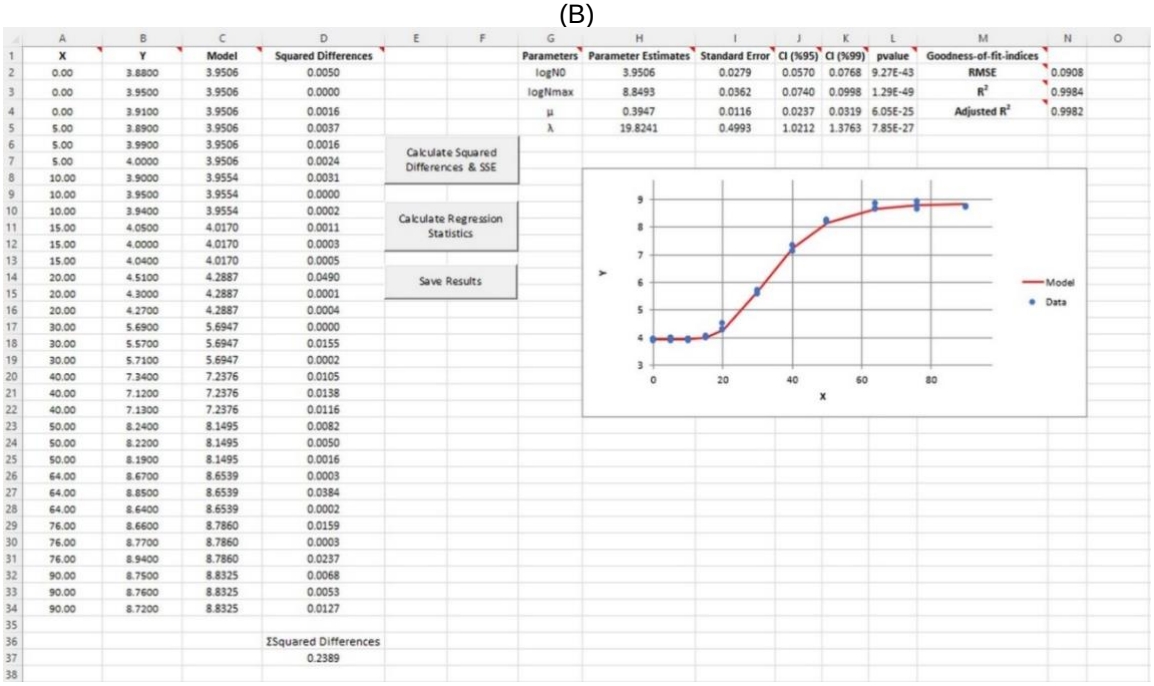


Figure 7. Entering the data given in Table 3, the model parameters (cells G2 to G5), initial estimates of the model parameters (cells H2 to H5) and the model equation [Equation (2)] (column C, starting from cell C2). (A). Results of the model fit [Equation (3)] which can be saved and extracted to another Excel® working page by clicking the "Save Results" button (B).

Workflow and Step-By-Step Guide to R-BioXL

R-BioXL is used to describe the experimental biological data in the form $y = f(x)$, where x is the independent variable and y is the dependent variable. R-BioXL focuses on models with one explanatory variable (X) and one response variable (Y) which is generally the case in biological sciences. Number of bacteria with respect to time, rate constant with respect to temperature and enzyme activity with respect to pH are the notable examples. Nevertheless, there may be cases where the response is non-linearly defined by using two explanatory variables such as lag time with respect to temperature and water activity. Users referred to other software packages which are capable to perform such fittings.

Before to use R-BioXL, experimental data in the form of X-Y and the mathematical equation to describe the data should be known by the user, so that he/she could enter the data and write down the model in Excel®. Moreover, regression assumptions are also valid for R-BioXL and these are listed below:

- Errors are normally distributed.
- All error (scatter) is in Y not in X viz., X is precisely known or there are no experimental errors in X.
- Data is homoscedastic i.e.; error or amount of scatter is the same for all Y values. If data is heteroscedastic, users need to transform the data before using R-BioXL or any other software for regression.
- The model used is the correct one and experimental errors are uncorrelated.

To use R-BioXL, the following steps should be followed:

1. Enter (or simply copy and paste) the raw data in two columns: X should be entered column A (starting from cell A2) and Y should be entered column B (starting from cell B2). After this step, data can be observed on the graph as the scatter plot – see Figs. 1 and 2a.
2. Write down the name of the parameters of the model to column G (starting from cell G2) – see Figure 2b.
3. Write down the initial values of the parameters to the adjacent cells i.e., column H (starting from cell H2). Initial values can be entered as "1" for the starting point – see Figure 2b.
4. Define the cells in column H (starting from cell H2): from Excel menu Formulas > Define name. Excel will define the cell as the name of the parameter in column G (starting from cell G2). Repeat this procedure for all parameters. Readers are referred to the works of Brown [4] and Kemmer and Keller [5] for naming the cells in Excel®.
5. Enter the model equation to column C (starting from cell C2). Since the cells in which the parameter values exist (column H) are defined in step 4, parameter names appeared in the model equation – also see Figure 2b. However, step 4 can be skipped and the model equation can be written without defining the cells. In this case, "$" should be used before and after the letter of the cell such as "$H$2" to fix the cell in the model equation – see Figure 5a.
6. The model will appear as the line plot (red line) – see Figure 2b.
7. Change the initial values of the parameters to make the red line (model equation) as close as possible to the blue data (experimental data) before using the Solver – see Figure 3a.
8. First button i.e., "Calculate the Squared Differences & SSE" is active after the step no. 5 – see Figs 2b and 3a.
9. Click on the button and R-BioXL will calculate the SSE – see Figure 3a.
10. User should use the Solver Routine of Excel® to obtain the best parameter estimates: from Excel® menu Data > Solver (Solver can be installed easily if it does not appear in Excel® menu through File > Options > Add-Ins > Excel® Add-Ins > Go > Solver), target should be selected as the cell of SSE and since this is a minimization problem i.e., our target is to minimize the SSE. Hence, minimum (Min) should be selected and this could be done by changing the parameters (By Changing Variable Cells). Several studies are present for the use of Solver in Excel® for minimizing SSE and obtaining parameter estimates [4–6]. Solver makes iteration to obtain the best parameter estimates and to be sure that the results are the global but not the local minimum, user can repeat this procedure with different initial estimates of the parameters. Nevertheless, it would be better to start the iteration with the closest possible initial values of the parameters to avoid (i) convergence failure, (ii) to obtain a local minimum – see step no. 7.
11. Click on "Calculate Regression Statistics" and R-BioXL will ask the number of parameters of the model entered to column C – see Figure 4b. Enter the number of the parameters and click "OK".
12. Asymptotic standard errors and confidence intervals (95 and 99%), p values, R^2, R^2_{adj} and RMSE values are calculated by R-BioXL – see Figure 4c.
13. Results can be saved into another worksheet by clicking on the "Save Results" button.

Features and Reliability of R-BioXL

R-BioXL is an Excel®-based application and Excel®'s Solver function need to be used before calculating parameters' precisions and goodness-of-fit statistics. Solver uses generalized reduced gradient (GRG) method as the iteration method although different algorithms such as Gauss-Newton, Marquardt-Levenberg and Neder-Mead methods are being used in non-linear regression [4] in different software programs. However, the outcome (parameter estimates and goodness-of-fit indices) would be the same for all methods.

The most popular statistic to compare the goodness-of-fit of different models is R^2; however, R^2 alone is not sufficient to judge the performance of a model's goodness-of-fit [15]. Therefore, R^2_{adj} and RMSE values are also given in R-BioXL. R^2 can be calculated as:

$$R^2 = 1 - \frac{\mathrm{SSE}}{\mathrm{SST}} \qquad (4)$$

where SSE is the sum of squared difference between experimental (observed) data (y_{exp}) and fitted (estimated) value by the model (y_{fitted}):

$$\mathrm{SSE} = \sum_{i=1}^{n}(y_{exp} - y_{fitted})^2 \qquad (5)$$

and SST (sum square total) is the sum of squared difference between experimental (observed) data (y_{exp}) and mean value of experimental data (y_{mean}):

$$\mathrm{SST} = \sum_{i=1}^{n}(y_{exp} - y_{mean})^2 \qquad (6)$$

R^2_{adj} is calculated as:

$$R^2_{\mathrm{adj}} = 1 - \frac{\mathrm{SSE}/n-p}{\mathrm{SST}/n-1} = 1 - \frac{\mathrm{MSE}}{\mathrm{MST}} \qquad (7)$$

where n is the number of data points, p is the number of parameters in the model, MSE is the mean square error and MST is the mean square total.

Another way to express R^2_{adj} is:

$$R^2_{\mathrm{adj}} = 1 - (1 - R^2) \times \frac{n-p}{n-1} \qquad (8)$$

Equation (8) reveals that R^2_{adj} is almost always lower than R^2. There are two occasions that R^2_{adj} equals to R^2: (i) when the model used has one parameter (p = 1), (ii) when the model has perfect fit i.e., R^2 = 1 and hence, R^2_{adj} = 1.

Last statistic is RMSE which is also known as standard error of the estimate or the model:

$$\mathrm{RMSE} = \sqrt{\frac{\mathrm{SSE}}{n-p}} \quad (9)$$

RMSE is known as the most informative indices for the microbiological [16] and biological data.

R-BioXL also calculates asymptotic standard errors and confidence intervals (95 and 99%) together with p values. Asymptotic standard errors are calculated by using SolverAid macro [9]. Reliability of this macro were also shown in other studies [17, 18]. Confidence intervals are calculated by using the standard error values and *t*-parameter for the confidence levels 95 or 99% and degrees of freedom ($n - p$), and p values are determined using a *t* distribution $n - p$ degrees of freedom.

Availability and user-friendliness are the most important characteristics of software packages used for non-linear regression and these are satisfied by R-BioXL. However, researchers generally neglect the numerical accuracy of software programs [19]. Excel® and its statistical features including non-linear regression in Solver has been improved throughout the years [20–24]. As mentioned above, the results of the examples shown in this study were all same (same parameter estimates with the same standard errors and same goodness-of-fit indices) for SigmaPlot 12.0, MATLAB R2017b and SPSS 22.0. Moreover, this comparison has done for different datasets including drying of foods, microbial inactivation, degradation/formation kinetics of several compounds etc. (results not shown) and R-BioXL produced the same exact results with SigmaPlot, MATLAB and SPSS showing the accuracy and reliability of the tool.

DISCUSSION

In this study, we showed that R-BioXL tool can be safely used to describe the biological data with suitable mathematical models. Users should use the Solver routine to obtain the parameter values and should enter the number of parameters in the model. The rest (standard errors, confidence intervals, p values and goodness-of-fit indices) are calculated automatically and instantly. The tool could be beneficial for the ones dealing with the biological data as well as chemical or physical data; however, users are expected to have some experience in Excel® since the model equation and initial parameter estimates should be entered by the users. Results also revealed that R-BioXL can be used not only for non-linear models, but also for the linear models; however, users are again expected to enter the initial estimates of the parameters which is not required for the linear regression.

Some Excel® based tools or Add-Ins are designed for unexperienced users on modeling. GInaFiT [25] for example, can be used to describe microbial inactivation and it contains 10 different models. The user can select a model or more than one model for the same dataset and can compare the results. Parameters, asymptotic standard errors and goodness-of-fit indices are also listed. DMFiT is another Excel® Add-In in which Baranyi model [26] can be used for microbial growth. The above examples are for the ones who are not expert in regression or mathematical modeling. Although some experience in Excel® is required for the use of R-BioXL, there is no restriction for entering the model i.e., user is free to input any model. However, there are two pitfalls in the usage of R-BioXL (i) Solver can find a local instead of a global minimum and (ii) standard errors may be underestimated. In fact, first issue is not specific to R-BioXL and can be easily solved because user can change the initial values of the parameters (as we did above), observe the model together with the data on the graph and select the suitable initial estimates to start the iteration. If the same parameter values are obtained with different initial values, a global minimum has likely been found [8]. Second problem has no direct solution because standard errors and confidence intervals are only approximate in R-BioXL. Therefore, confidence intervals calculated this way may be underestimated by a factor of 2-3 [8, 27]. Of course, this underestimation depends on the model structure or non-linearity of the model equation.

It may still be possible to calculate the asymmetrical confidence intervals in Excel® by using Monte Carlo (MC) simulation [13, 28] which can be considered as the best method to do that [8, 29, 30]. An Excel® based tool ÖK-BUZ GRoFiT [31] for microbial growth modeling can be given as an example. ÖK-BUZ GRoFiT has three growth models in it (Baranyi, modified Gompertz and three-phase linear models) and there are three versions of this tool. The third version of ÖK-BUZ GRoFiT is available both in Turkish and in English (https://drive.google.com/drive/folders/1X_sNdpdQ2dT3KKl6KIYGNW0dq_KzJV_Q) and uses linear approximation to calculate the confidence intervals just like R-BioXL. First version (available only in Turkish) on the other hand, calculates the confidence intervals by 100 MC simulations and depending on the computer speed, it may take about 10 to 30 seconds to finish the simulations. Normally, between 1000 and 10000 simulations are performed in MC analysis [7]; however, using higher number of simulations would not affect the results in microbial growth modeling [31, 32]. Moreover, increasing the number of simulations requires more time. Therefore, performing MC simulations in Excel® to calculate the asymmetrical confidence intervals for the non-linear models is an option, but this would affect the speed of the analysis. The protocol described by Kemmer and Keller [5] can also be used to determine confidence intervals of the parameters in Excel®; however, the protocol takes about an hour which makes it difficult to implement the analyses of the data.

CONCLUSION

We introduced R-BioXL (Regression tool for biological data in Excel®) which is an Excel®-based user-friendly tool for regression models to define *X*-*Y* data. Researchers who deal with mostly biological data can safely use it to describe their data with suitable (non-linear) mathematical models and obtain parameter estimates by using Excel® Solver. Moreover, parameters' precisions and goodness-of-fit of the models can be determined accurately in R-BioXL and

this was demonstrated with three different examples in this study.

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Araştırma Makalesi / Research Paper

Alabaş (*Brassica oleracea* var. *gongylodes*) Sebzesi ile Zenginleştirilmiş Erişte Üretimi

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ÖZ

Bu çalışmanın amacı, çiğ veya pişmiş olarak tüketilebilen lahanagiller familyasının bir üyesi olan yer lahanası olarak da bilinen alabaş sebzesini (*Brassica oleracea* var. *gongylodes*) farklı oranlarda kullanarak zenginleştirilmiş erişte üretmek ve böylece geleneksel erişteye (kontrol) göre besin değeri daha yüksek, alternatif bir lezzet geliştirmektir. Bu doğrultuda geliştirilen erişte reçetesine, pişmiş alabaş püresi (ağırlıkça, un esasına göre) %15 ve 30 oranlarında ilave edilmiştir. Alabaş püresi katkısının, eriştenin besin değerleri (nem, kül, protein, yağ, karbonhidrat ve toplam kalori), kalite özellikleri (ağırlık artışı, hacim artışı, pişme kaybı ve pişme süresi), renk değerleri (CIELAB) ve duyusal özellikleri (renk, koku, sertlik, yapışkanlık, elastik yapı, homojen yapı, lezzet ve genel beğeni) üzerine etkileri incelenmiştir. Yapılan analizler sonucunda alabaş püresi ilavesi erişteyi mineral madde (%1.08-1.29) ve protein içeriği (%12.81-15.22) açısından zenginleştirirken, kalite ve duyusal özellikleri bakımından kontrol ve katkılı erişte örnekleri arasında anlamlı bir farklılık olmadığı gözlemlenmiştir ($p>0.05$).

Anahtar Kelimeler: Erişte, Alabaş, Duyusal, Kalori, Kalite

Production of Turkish Noodles Enriched with Kohlrabi (*Brassica oleracea* var. *gongylodes*) Vegetable

ABSTRACT

The aim of this study is to produce noodles enriched with kohlrabi vegetable (*Brassica oleracea* var. *gongylodes*) known as a member of the cabbage family, which can be consumed raw or cooked and to develop an alternative flavor with high nutritional value compared to traditional noodles. Cooked kohlrabi puree was incorporated into the noodle recipe developed for this purpose at the rates of 15 and 30% (by weight, on a flour basis). The effects of the enrichment on the nutritional values (moisture, ash, protein, fat, carbohydrate and total calories), quality characteristics (weight gain, volume increase, cooking loss and cooking time), color values (CIELAB) and sensory parameters (color, odour, hardness, stickiness, elastic structure, homogeneous structure, flavor and general taste) of noodles were determined. Results indicated that the addition of kohlrabi puree enriched the noodles in terms of mineral content (1.08%-1.29%) and protein content (12.81%-15.22%), while there were insignificant differences in quality and sensory properties between the control and enriched noodle samples ($p>0.05$).

Keywords: Turkish noodle, Kohlrabi, Sensory, Calorie, Quality

GİRİŞ

Her bütçeye uygun, besin öğelerince zengin, kuru gıda çeşidi olduğu için uzun süre kalitesi bozulmadan muhafaza edilebilen, üretimi basit ve hazırlanması kısa süren bir ürün olması sebebiyle erişte gıda sektöründe hem üreticiler hem de tüketiciler için tercih sebebidir. Karbonhidrat içeriği yüksek olan eriştenin protein, vitamin ve mineral içeriği bakımından yetersiz olması besin içeriğinin arttırılması ve zenginleştirilmesi çalışmalarına yön vermiştir [1, 2]. İçerdiği kompleks karbonhidratlar nedeniyle iyi bir enerji kaynağı olan makarna ve türevleri hem çalışan kesimin hem de düşük gelirli ailelerin temel besin maddesi olarak kabul edilebilir. Çeşitli doğal katkılarla erişte ve makarna ürünlerinin zenginleştirilmesi günden güne önemi artan bir konu haline gelmiştir. Üretiminde buğday unu kullanılan ürünlerin yüksek lif ve protein içerikli çeşitli katkılarla zenginleştirilmesi besin değerlerinin ve fonksiyonelliklerinin arttırılması açısından önem arz etmektedir.

Turpgiller familyasının üyesi olarak da bilinen Brassica sebzeleri, dünyanın en çok yetiştirilen sebzelerinden olan lahana, brokoli ve karnabaharı içeren büyük bir otsu bitki grubundan oluşur. Brassica sebzeleri, gıda bileşenleri olarak ana kullanımlarının yanı sıra, farklı kanser türleri ve koroner kalp sorunlarının potansiyel riskini azaltmaya yardımcı olan antioksidanlarla doludur. C vitamini, folik asit gibi önemli vitaminler ve demir, potasyum ve selenyum gibi sayısız mineral kaynağıdır [3]. Literatürde başta erişte ve makarna olmak üzere pek çok çalışmada Brassica sebzelerinin kullanıldığı görülmektedir. Bu çalışmalara örnek olarak; %10, 20, 30 oranında brokoli tozu, karalahana tozu (%0, 5 ve 10), kırmızılahana suyu, püresi ve tozu (%1, 2, 10) ve karnabahar yaprağı püresi (%10, 15, 20 ve 25) kullanılarak erişte ve makarna üretimi yapılmış tekstürel, beslenme, kimyasal ve duyusal özellikler açısından incelenmiştir [4-7]. Alabaş bir diğer adıyla yer lahanası, daha çok kış aylarında yetiştirilen ve çiğ veya pişmiş olarak tüketilebilen lahanagiller familyasının bir üyesidir. Mutfaklarda kullanımı henüz yaygınlaşmamış olan, endüstriyel bazda konserve olarak da kullanılabilen çok keskin olmayan bir tat ve kokuya sahip alabaş, yaprakları da kullanılarak çorba, sarma, haşlama, salata gibi birçok farklı ürünün hazırlanmasında kullanılabilmektedir. Alabaş sebzesi 16. yüzyılda Avrupa'nın kuzeybatı kıyılarında yetiştirilmeye başlanan alabaş, bugün Avrupa'da, Kuzey Amerika'da, Kuzey Afrika kıyılarında ve Asya kıtasının bazı bölgelerinde yetiştirilmektedir [8, 9].

Gıda araştırmacıları sağlık açısından toksik potansiyele sahip yapay antioksidanların yerine kullanılabilecek doğal antioksidan arayışına girmiştir. Bu arayışın büyümesi üzerine bitkisel kaynaklı antioksidan elde etme ve bu antioksidan kaynaklarının gıda üretiminde kullanılması hedeflenmektedir. Özellikle çağımızın yaygın hastalıklarından olan ve tam olarak tedavisi bulunamayan kanser ve türevlerinde beslenmenin önemi düşünüldüğünde antioksidan kaynağı sebzelerin veya bunlardan elde edilebilecek bitkisel yağlar ve gıda maddelerinin tüketimi büyük önem arz etmektedir. Brassicacea ailesinin bir üyesi olan ancak ülkemizde çok fazla bilinmeyen ve tüketimi düşük olan alabaş; yüksek antioksidan içeriği ile sağlıklı ve dengeli beslenmede olması gereken sebzelerdendir [10].

Araştırmanın amacı, ülkemizde az bilinen bir yerel ürün olan alabaşı farklı oranlarda kullanarak zenginleştirilmiş erişte üretmektir. Günlük hayatımızda beslenmemizin temelinde yer alan ve karbonhidratça zengin olan eriştenin içeriğinin protein, vitamin ve minerallerce zengin olan alabaş ilavesiyle geliştirilmesi, besin değerinin arttırılması ve alternatif bir lezzet yaratılması bu çalışma kapsamında ele alınmıştır. Bu amaçla, üretilen eriştelerin besinsel öğeleri, renk değerleri ve kalite özellikleri belirlenmiş, ek olarak duyusal analiz ile tüketiciler açısından kabul edilebilirliği değerlendirilmiştir.

MATERYAL ve YÖNTEM

Materyal

Erişte üretiminde kullanılan buğday unu, yumurta, tuz ve alabaşlar Alanya'daki yerel bir marketten temin edilmiştir. Hamur yapma işleminde ve deneylerde distile su kullanılmıştır. Ayrıca bu çalışmada kullanılan tüm kimyasallar analitik saflıktadır.

Erişte Üretimi

Alabaş örnekleri sırasıyla soyulduktan ve eşit boyutlarda (2 cm^3) doğrandıktan sonra 95°C sıcaklıkta 7 dakika boyunca haşlanmış ve püre haline getirilmiştir. Alabaş örnekleri soyma, kesme, kaynatma ve püre haline getirme sırasına göre hazırlanmıştır. Bu çalışma kapsamında kontrol örneği olarak buğday unundan üretilen erişte kullanılmış olup, erişte örneklerinin hazırlanmasında Güllü ve Karagöz'ün [11] yöntemi modifiye edilerek kullanılmıştır (Tablo 1). Örnekler incelendiğinde %30'un üstüne çıkılan hamurun kıvamının aşırı yumuşak ve yapışkan olması sebebiyle erişte üretimine elverişli olmadığı, %15'in altına inildiğinde ise hamurun aşırı sert ve kuru olduğu gözlemlenmiştir. Bu sebeple %15 ve 30 oranında alabaş püresi kullanılmasına karar verilmiştir.

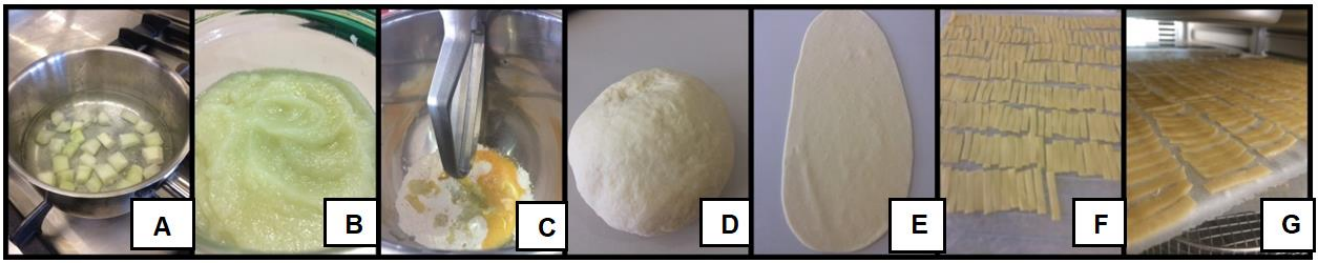
Un, yumurta, tuz ve alabaş püresi tartıldıktan sonra stant mikserine (Electrolux 602037 E-Mix Planetary Mixer, İsveç) alınmış, ele yapışmayacak, kulak memesi kıvamında bir hamur elde etmek için su kontrollü şekilde azar azar eklenerek 6ncı devirde 3 dakika karıştırılmış daha sonra yoğurma tezgâhına alınarak 3 dakika yoğurulmuştur. 30 dakika dinlendirme işleminden sonra hamur 4 eşit parçaya bölünerek makarna makinasında (Fackelmann, Almanya) açma işlemi yapılmış ardından geleneksel yöntemlerle şekil verme işlemi yapılmıştır. Daha sonra, sıcak hava fırınında (Memmert UF110, Almanya) 35°C'de 5 saat kurutma işlemi yapılmıştır. Üretim adımları Şekil 1 ve 2'de verilmiştir.

Tablo 1. Zenginleştirilmiş erişte formülasyonları
Table 1. Formulations of enriched Turkish noodles

% Alabaş İçeriği	Buğday Unu (g)	Yumurta (g)	Tuz (g)	Su (g)	Alabaş Püresi (g)
0	150	30	1.5	49.21	-
15	127.5	30	1.5	22	22.5
30	105	30	1.5	-	45

*Alabaş püresi miktarları buğday unu miktarına göre hesaplanmıştır.

Şekil 1. Erişte üretim basamakları
Figure 1. The production steps of Turkish noodle



Şekil 2. Alabaşın haşlanması (A), püre haline getirilmesi (B), malzemelerin karıştırılması (C), hamurun yoğurulması (D), hamurun açılması (E), hamurun kesilmesi (F), hamurun pişirilmesi (G)
Figure 2. Boiling the kohlrabi (A), pureeing it (B), mixing the ingredients (C), kneading the dough (D), rolling the dough (E), cutting the dough (F), baking the dough (G)

Besin Değerinin Belirlenmesi

Erişte örneklerinin nem, kül, protein ve yağ içeriği AOAC'nin [12] yöntemine göre belirlenmiştir. Kurutulmuş ve pişmemiş erişte örneklerinin kalori miktarları 100 gram erişte için karbonhidrat ve proteinin gramı 4 kcal ve yağın gramı 9 kcal ile çarpılmasıyla hesaplanmıştır. Karbonhidrat miktarı toplam besin değerlerinden yararlanılarak hesaplanmıştır [13].

Kalite Özellikleri

Erişte örneklerinin hacim artışı, pişme kaybı ve ağırlık artışı (%) değerleri, Yüksel ve diğ. [14] tarafından sunulan modifiye yönteme göre belirlenmiştir. Erişte örneklerinin pişme süreleri analizi ise manyetik karıştırıcıda 98°C (300°C/100 rpm, Heidolph, MR, Hei-Standard, Almanya) Köksel ve diğ. [15]'e göre yapılmıştır.

Renk Değerlerinin Saptanması

Pişmemiş erişte örneklerinin renk değerleri Konica Minolta Chroma Meter (CR-10, Tokyo, Japonya) renk cihazı ile AACC Metot No. 14-22 yöntemi kullanılarak belirlenmiştir. Ölçümlerde CIE *L** (parlaklık), *a** (kırmızılık-yeşillik) ve *b** (sarılık-mavilik) değerleri gün ışığı (D65/10°) ayarında okunmuştur. Tüm ölçümler 3 paralel şeklinde olup, ortalama değerleri alınmıştır [16, 17].

Duyusal Analiz

Duyusal analizde renk, koku, sertlik, yapışkanlık, elastikiyet, homojenlik, tat ve genel kabul edilebilirlik hedonik skala kullanılarak puanlanmıştır (1 aşırı derecede beğenmedim, 5 çok beğendim). Duyusal değerlendirmede Onoğur ve Elmacı'nın [18] yöntemlerinden faydalanılmıştır. Ayrıca panelistlerden erişte örnekleri ile ilgili görüş ve önerilerini yazmaları istenmiştir. Analiz öncesi panelistlere kısa bilgiler verilmiştir. Daha sonra optimum pişirme süresine göre panelist görüşlerini etkilememesi adına sos, tuz, baharat ve yağ kullanılmaksızın haşlanarak pişirilen erişte örnekleri şeffaf bir kapta servis edilmiştir. Erişte örneklerinin yüzeyinin nişastalı olup olmadığına parmakla, iki cam levha arasında ezerek sertliğine, içerisinde damar halinde pişmemiş kısım kalıp kalmadığına bakılmıştır. Alanya HEP Üniversitesi Gastronomi ve Mutfak Sanatları Bölümü'nde çalışan/okuyan 25 panelist (13 kadın ve 12 erkek) ile duyusal değerlendirme yapılmıştır.

İstatistiksel Analiz

Araştırmanın deneysel sonuçları ortalama ± standart sapma olarak hesaplanmıştır. Sonuçlar, SPSS 21.0 istatistik paket programı (IBM, USA) ve %95 güven aralığında Tek Yönlü Varyans Analizi (ANOVA) ile test edilmiştir. Örnekler arasındaki farklılık ise Duncan çoklu karşılaştırma testi ile belirlenmiştir. Ayrıca analizler 3 tekrarlı, örnek üretimleri ise 2 tekrarlı olarak yapılmıştır.

BULGULAR ve TARTIŞMA

Besin Değerine İlişkin Bulgular

Bu çalışmada, farklı yüzdelerde (%0, 15 ve 30) alabaş püresi eklenerek üretilen erişte örneklerinin besin değerine (nem, kül, protein, yağ, toplam karbonhidrat, toplam kalori) ilişkin yapılan analiz sonuçları Tablo 2'de verilmiştir. Alabaşın nem içeriğine bakıldığında; çiğ alabaşın ortalama %91.46, haşlanmış alabaşın ise ortalama %93.97 civarında nem içerdiği sonucuna ulaşılmıştır. Analiz sonuçlarına göre erişte örneklerinin içerdiği alabaş oranı arttıkça nem içeriğinin düştüğü görülmüştür. Alabaş püresi katkısı arttıkça eklenen su miktarı %15 alabaş katkılı eriştede yarı yarıya azaltılmış, %30 alabaş katkılı eriştede ise su ilavesi yapılmamıştır, bu sebeple nem içeriğinin düşmüş olabileceği düşünülmektedir. Ancak alabaş oranı değişiminin nem içeriği üzerine istatistiksel olarak anlamlı bir değişime sebep olmadığı ($p>0.05$) gözlemlenmiştir. Kontrol ve alabaş katkılı eriştelerin nem içeriği değerlerinin Türk Gıda Kodeksi Makarna Tebliği'nde belirtilen sınır değer olan en çok %13 değerinin altında ve uygun olduğu görülmektedir [19]. Ayrıca Fu [20] makarnaların nem içeriğinin %14'ün altında olmasının raf ömrü ve depolama için önem arz ettiğini belirtmiştir. Bu bağlamda, alabaş katkılı eriştelerin nem içeriği bakımından uzun süre depolamaya uygun olduğu sonucuna varılabilir. Alabaş katkılı erişte örneklerinin kül içeriği %1.08 ile 1.29 arasında değişmektedir. Eriştelerin mineral madde içeriği arasındaki fark istatistiksel olarak anlamlı bulunmuştur ($p<0.05$). Literatüre bakıldığında çiğ ve pişmiş gölevez unundan (%0, 20, 40 ve 60) glutensiz erişte üretimi [21], şeker pancarı lifi ve balkabağı lifi katkılı (%0, 2, 4, 6, 8 ve 10) erişte üretimi [22] ve kavuksuz arpa, tritikale ve yulafın tüm tane veya normal unları ile üretilen erişte [23] çalışmalarının sonuçlarıyla benzerlik göstermektedir. Literatürde alabaşın protein içeriği 100 g/1,9 g olarak belirtilmiştir. Bu nedenle alabaş oranı arttıkça eriştelerin protein içeriğinin de arttığı düşünülmektedir [8]. %0 alabaş katkılı erişte örneği (%12.81) en düşük protein içeriğine sahipken %30 alabaş örneğinin (%15.22) en yüksek protein içeriğine sahip olduğu gözlenmiştir. Benzer olarak, kavun çekirdeği tozu ile yapılan çalışmada katkı oranı arttıkça eriştelerin protein içeriğinin arttığı gözlemlenmiştir [24]. %0, 2, 4, 6, 8 ve 10 oranında şeker pancarı lifi ve balkabağı lifi kullanarak ve farklı ikame oranlarında (%0, 10, 15 ve 20) karnabahar yaprağı tozu ilave edilerek erişte üretimi yapılan diğer çalışmalarda da katkı miktarı arttıkça protein içeriğinin de arttığı görülmüştür [22,25].

Erişte örneklerindeki alabaş oranı arttıkça yağ içeriğinin de arttığı görülmüştür ($p<0.05$). İstatiksel analiz sonuçlarına göre kontrol örneği ile %30 alabaş ilaveli erişte arasında anlamlı bir fark olduğu tespit edilmiştir. Literatüre bakıldığında alabaşın yağ oranının eriştelik una göre daha düşük olduğu görülmüştür [8, 26]. Erişte örneklerindeki yağ içeriği artışının yumurtaların yağ oranlarının farklılık göstermesinden kaynaklandığı düşünülmektedir. Mete [26] farklı oranlarda (%5, 10, 20, 30 ve 40) kestane unu kullanarak erişte zenginleştirme çalışmasında alabaş katkılı erişte örneklerine benzer olarak, kestane unu ilavesinin eriştelerin yağ içeriğini arttırdığı ve istatiksel açından anlamlı bulunduğu belirtmiştir ($p<0.05$). Bunun sebebi olarak ise kullanılan kestane unu (%3.70) katkısının eriştelik una (%1.38) göre yağ oranının daha yüksek olmasından kaynaklandığı belirtilmiştir. Eriştelerin karbonhidrat içeriği 71.25 ile 74.64 arasında değiştiği görülmektedir. Bu durumda alabaş katkısının eriştelerin karbonhidrat içeriğini istatiksel olarak anlamlı düzeyde düşürdüğü söylenebilir ($p<0.05$). Literatürde bahsedildiği üzere

standart bir yumurtalı eriştenin karbonhidrat içeriği %79 makarnaların ise %72 bulunmuştur [27]. Alabaş katkılı eriştelerin karbonhidrat içeriğinin literatürle uyuştuğu söylenebilir. Birleşmiş Milletler istatistik verilerine göre; 100 gram yumurtalı eriştenin kalorisi 380 kcal, 100 gram makarnanın kalorisi ise 370 kcal olarak belirtilmiştir [28]. Alabaş katkılı erişte örneklerinin kalori içeriği 378.53 ile 374.28 arasındadır. Bu farklılığın alabaşın eriştelerin yağ ve protein oranının yükseltmesinden kaynaklanmış olduğu söylenebilir.

Tablo 2. Erişte örneklerinin temel kompozisyonu (100 g için)
Table 2. Basic composition of noodle samples (for 100 g)

	Besin Değeri					
Alabaş Oranı (%)	Nem (%)	Kül (%)	Protein (%)	Yağ (%)	Toplam Karbonhidrat	Toplam Kalori (kcal)
0	8.76±0.10^{a}*	1.08±0.01^{a}	12.81±0.41^{a}	2.72±0.11^{a}	74.64±0.37^{c}	374.28±0.76^{a}
15	8.66±0.10^{a}	1.25±0.01^{b}	13.31±0.14^{b}	2.80±0.13^{a}	73.98±0.25^{b}	374.34±0.88^{a}
30	8.62±0.25^{a}	1.29±0.01^{c}	15.22±0.38^{c}	3.63±0.10^{b}	71.25±0.39^{a}	378.53±1.30^{b}

*Her sütunda farklı üst simgeler örnekler arasında anlamlı bir farklılık olduğunu gösterir ($p<0.05$).

Erişte Örneklerinin Kalite Parametreleri

Erişte ile ilgili önemli kalite parametrelerinden biri pişirme kalitesidir. Pişirme kalitesi, pişmiş eriştenin yüzey özellikleri ve pişme sonrası sertliği sağlayan viskoelastik yapı ile belirlenmektedir. Genel olarak iyi kalitede pişirilen erişte, yüzeyde parçalanma ve yapışmaya dayanıklı ve yapıyı koruyan erişte olarak kabul edilmektedir. Erişte kalitesini, elastiklik, ağırlık artışı, pişme kaybı miktarı, hacim artışı, protein miktarı, gluten miktarı vb. özelikler de etkilemektedir [28]. Bu çalışmada, farklı yüzdelerde (%0, 15 ve 30) alabaş püresi eklenerek üretilen erişte örneklerinin kalite parametrelerine (L*, a*, b*, hacim artışı, ağırlık artışı, suya geçen madde miktarı, pişme süresi) ilişkin yapılan analiz sonuçları Tablo 3'te verilmiştir. Erişte için önemli kalite kriterlerinden olan hacim ve ağırlık artışı sonuçlarına bakıldığında alabaş miktarındaki değişimin eriştelerin hacim ve ağırlık artışında istatiksel olarak anlamlı bir etkisinin olmadığı görülmüştür ($p>0.05$). Benzer şekilde Dilek [21]'in çiğ ve pişmiş gölevez ununu farklı oranlarda (%0,20,40 ve 60) pirinç unu:mısır nişastası paçalı ile yer değiştirerek glutensiz erişte üretimi yaptığı çalışmada hacim ve ağırlık artışı alabaş katkılı örneklerle benzer bulunmuştur. Bu çalışmada ortaya çıkan sonuç, bizim çalışmamızın sonuçlarını destekler niteliktedir.

Erişte örneklerinin pişme kaybının %5.99-6.54 arasında değiştiği gözlenmiştir. Bu durumda kontrol örneği ve %15 alabaş katkılı erişte örneği arasında istatiksel olarak benzerlik ($p>0.05$) varken %30 alabaş katkılı erişte örneğiyle farklılık gösterdiği söylenebilir. Ancak alabaş miktarı değişiminin eriştelerin pişme kaybına anlamlı etkisi görülmemiştir ($p>0.05$). Gölevez unu (%0, 20, 40 ve 60) ile nohut unu (%0, 10, 20, 30, 40 ve 50) kullanılarak farklı oranda erişte üretimi yapılan çalışmalarda pişme kaybı oranlarının alabaş katkılı erişte örneklerinin pişme kaybı oranıyla benzerlik gösterdiği görülmektedir [21, 29]. Bunun yansıra, optimum pişme süreleri bakımından alabaş miktarı değişiminin anlamlı bir etkisinin olmadığı gözlemlenmiştir ($p>0.05$). Benzer çalışmalara bakıldığında Çalışkan Koç ve Pandiselvam [30] yulaf ve kinoa unu kullanarak (%50, 100) glutensiz erişte üretimi yapmış olduğu çalışmada erişte örneklerinde pişme süresinin 10.00-14.75 dakika arasında olduğu sonucuna ulaşmıştır. Bir diğer çalışmada ise %0, 3 ve 6 oranlarında pazı ve hindiba kullanılarak zenginleştirilmiş erişte üretimi yapılmış pişme süresi ortalama 12.00 dakika olarak hesaplanmıştır [31]. Bu durumda alabaş katkılı erişte örneklerinin pişme süresi kriterine göre literatürle benzer olduğu söylenebilir.

Tablo 3. Erişte örneklerinin kalite parametreleri
Table 3. Quality parameters of noodle samples

	Kalite Parametreleri						
Alabaş Oranı (%)	*L** (Parlaklık)	*a** (kırmızılık-yeşillik)	*b** (sarılık-mavilik)	Hacim Artışı (%)	Ağırlık Artışı (%)	Pişme Kaybı (%)	Optimum Pişme Süresi (dakika)
0	39.36±0.73^{b}*	-4.87±0.05^{a}	6.07±0.37^{a}	203.70±5.74^{a}	190.62±0.55^{a}	5.99±0.27^{a}	15.00±0.00^{a}
15	40.96±0.91^{c}	-2.70±0.03^{c}	7.28±0.34^{c}	203.70±5.74^{a}	190.37±0.36^{a}	6.43±0.62^{ab}	15.00±0.00^{a}
30	37.80±0.39^{a}	-3.52±0.02^{b}	6.77±0.19^{b}	203.70±5.74^{a}	190.38±0.41^{a}	6.54±0.17^{b}	15.00±0.00^{a}

*Her sütunda farklı üst simgeler örnekler arasında anlamlı bir farklılık olduğunu gösterir ($p<0.05$).

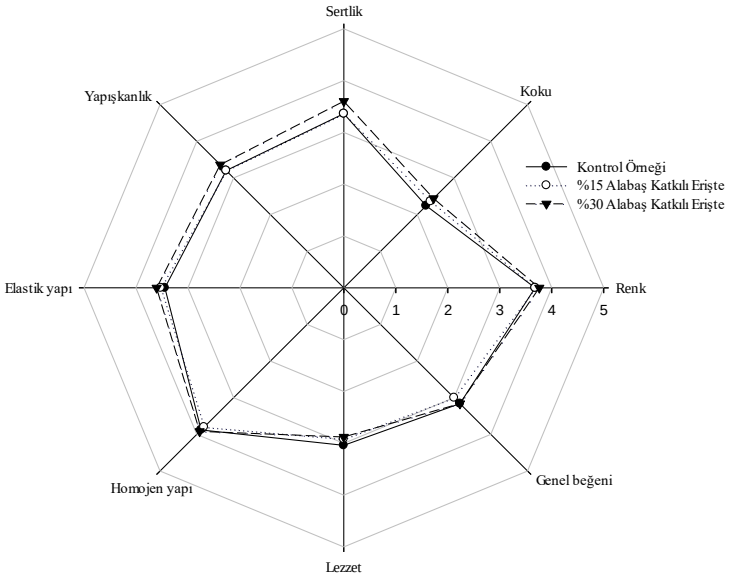
Renk, makarna ürünleri için önemli bir kalite parametresidir. Bir makarnanın *b** değeri sarılığı gösterir ve makarna ürünlerinde parlak sarı renk istenmektedir. Alabaş miktarı değişiminin eriştelerin *L** (parlaklık), *a** (kırmızılık-yeşillik) ve *b** (sarılık-mavilik) değerleri üzerinde anlamlı bir farka sebep olduğu görülmüştür ($p<0.05$). Renk tayini sonuçlarına göre en yüksek *L** değeri 40.96 ile %15 alabaş katkılı eriştede gözlenirken en düşük değer ise 37.80 ile %30 alabaş katkılı eriştede gözlemlenmiştir. *a** değeri incelendiğinde en yüksek değer -2.70 ile %15 alabaş katkılı erişte örneğinde iken en düşük değer ise -4.87 ile kontrol örneğinde gözlenmiştir. *b** değerleri incelendiğinde 7.28 ile %15 alabaş katkılı erişte örneği en yüksek değere sahipken, 6.07 ile en düşük değer kontrol örneğindedir. Kılıcı [15] şeker pancarı ve balkabağı lifi ilavesinin eriştenin renk değerlerinde anlamlı bir etkisi olduğu sonucuna ulaşmıştır. Eyidemir [1] çalışmada kayısı çekirdeği unu

kullanılan eriştelerin *a** ve *b** değerlerinde anlamlı bir fark gözlenmediği ($p>0.05$) ancak *L** değerinde anlamlı bir etki olduğu sonucuna ulaşılmıştır ($p<0.05$). Başka bir çalışmada ise %20 oranında pirinç kepeği ve mısır kepeği ilavesinin eriştelerin renk değerlerinde anlamlı bir değişikliğe ($p<0.05$) yol açtığı sonucuna ulaşılmıştır [32].

Duyusal Analiz Bulguları

Duyusal analiz sonuçlarına göre renk, koku, sertlik, yapışkanlık, elastik yapı, homojen yapı, lezzet ve genel beğeni gibi duyusal özellikler bakımından örnekler arasında istatiksel olarak anlamlı bir fark olmadığı saptanmıştır ($P>0.05$) (Şekil 3). Farklı oran kombinasyonlarda (%10, 20 ve 30) yalancı tahıl unlarından; amarant, karabuğday ve kinoanın eriştenin özelliklerine etkisini araştırmak için yapılan benzer bir çalışmada, duyusal özellikleri bakımında istatiksel olarak anlamlı bir fark olmadığı saptanmıştır [33]. Alabaş miktarındaki artışın eriştelerin besin içeriğini arttırırken duyusal özelliklerini etkilememesi istenilen bir durumdur.

Renk, koku, sertlik, yapışkanlık, homojen yapı, elastik yapı kriterleri incelendiğinde %30 alabaş katkılı erişte daha çok tercih edilirken %0 alabaş katkılı erişte ve %15 alabaş katkılı erişte benzer sonuçlar göstermektedir. Lezzet kriteri incelendiğinde, en çok beğenilen örneğin %0 kontrol ürün olduğu ve bunu sırası ile %30 ve %15 alabaş katkılı erişte örneklerinin takip ettiği gözlemlenmiştir. Panelistlerden tatmaları için sunulan erişte örneklerini genel beğenilerine göre puanlamaları istenmiştir. Alabaş kullanılarak üretilen erişte örneklerinde, en az beğenilen %15 alabaş katkılı erişte örneği olurken %0 ve 30 alabaş katkılı erişte örneği eşit derecede beğenilmiştir. Benzer bir çalışmada, kurutulmuş domates, yeşil fasulye ve bamya tohumu tozu (%4, 8, 12) kullanılarak erişte zenginleştirme çalışması yapılmıştır. Duyusal değerlendirmede panelistlerden görünüm, koku, renk, tat-lezzet ve genel beğenilerini 1-9 puan arasında değerlendirmeleri istenmiştir. Analiz sonuçları domates ve yeşil fasulye katkılı erişte örneklerinden miktarların arttıkça puanlarında arttığını göstermiştir. Bamya tohumu tozunda ise diğer örneklere göre daha düşük puanlar verilmiş ancak istatiksel olarak anlamlı bir farka sebep olmadığı belirtilmiştir [34]. Ek olarak, panelistlere sorulan soruların dışında farklı bir görüş yorum veya önerisi olup olmadığını sormak için duyusal analiz testinin sonuna bir bölüm daha eklenmiştir. Panelist yorumları incelendiğinde genel olarak tükettikleri standart eriştelerden pek bir farkının olmadığı, ayırt etmekte zorlandıkları yorumunda bulunmuşlardır.



Şekil 3. Alabaş katkılı erişte örneklerinin duyusal analiz sonuçları
Figure 3. Sensory scores of kohlrabi enriched noodles

SONUÇLAR

Bu çalışmada, buğday ununa yer değiştirme esasıyla alabaş (%0, 15 ve 30) eklenerek zenginleştirilmiş erişte üretimi amaçlanmıştır. Bu amaca uygun olarak, erişte hamurunun yapısını ve kalitesini bozmayacak, hamurun kırılma ve kopma olmaksızın kesilmesini ve kurutulmasını mümkün kılacak reçeteler geliştirilmiştir. Erişte üretimi yapıldıktan sonra, alabaş katkısının eriştenin besin değerleri (nem, kül, protein, yağ, karbonhidrat, kalori), kalite özellikleri (ağırlık artışı, hacim artışı, suya geçen kuru madde miktarı, pişme süresi), renk değerleri (*L**, *a** ve *b**) ve duyusal özelikleri (renk, koku, sertlik, yapışkanlık, elastik yapı, homojen yapı, lezzet ve genel beğeni) üzerine etkileri incelenmiştir. Yapılan analizler sonucu alabaş katkılı erişte örneklerinin nem ve kül içeriğinin kabul edilebilir seviyede ve literatürle uyum içinde olduğu gözlemlenmiştir. Alabaş katkısının eriştelerin protein ve yağ içeriğinde istatiksel anlamda farka yol açtığı gözlenirken kalite özelliklerini bozmadığı görülmüştür. Alabaş katkısındaki artış eriştelerin renk değerlerinde istatiksel olarak anlamlı bir farka yol açmıştır ($p<0.05$). Duyusal özellikleri bakımında eriştelerin kabul edilebilir oldukları ve eriştelerin duyusal özelliklerinin istatiksel açıdan anlamlı bir farklılığa sebep olmadığı görülmüştür ($p>0.05$). Bu kapsamda alabaş katkısının eriştenin besin içeriğini zenginleştirdiği, kalite ve duyusal özelliklerini

olumsuz anlamda etkilemediği dolayısıyla erişte üretiminde kullanılmaya elverişli olduğu sonucuna varılmıştır.

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Etik Beyanı

Dergi yayın politikası, makalelerin değerlendirilmesi ve etik hususlar ile ilgili detaylı bilgilere Etik Beyanı kısmından ulaşılabilir.

Çalışmaların Hazırlanması

1. Çalışmalar A4 boyutunda hazırlanmalı, üstten 2.45 cm, alttan 2.45 cm, sağ ve soldan 1.75 cm boşluk bırakılmalı ve tek kolon olarak hazırlanmalıdır. Metin çift satır aralıklı yazılmalı, paragraflar arasında tek satır boşluk bırakılmalıdır. Metinde bütün satırlar (sürekli) numaralandırılmalıdır.

2. Çalışma başlığı 14 punto Arial, koyu, küçük harflerle ve ortalanmış olarak yazılmalıdır. Başlıktan sonra bir satır boşluk bırakılmalı (11 punto); yazar isimleri (yalnızca ilk harfler büyük) 10 punto Arial ve ortalanmış olarak verilmelidir. Yazarların adresleri, telefon ve faks bilgileri ile yazışmalardan sorumlu yazarın e-posta adresi hemen alt satırda 9 punto Arial, ilk harfler büyük olacak şekilde ve ortalanmış olarak yazılmalıdır. Yazarların çalıştıkları kuruluşlar (ve/veya adresler) farklı ise her bir yazar isminin sonuna rakamlarla üst indis konulmalıdır.

3. Metin içindeki kısımların başlıkları (ÖZ, ABSTRACT, GİRİŞ vb.) 10 punto Arial ve koyu olarak büyük harflerle yazılmalı, başlıktan sonra bir satır boşluk bırakılarak metine geçilmelidir. Alt başlıklarda ilk harfler büyük, 10 punto Arial ve koyu yazı karakteri kullanılmalıdır. ÖZ'ün altına bir satır boşluk bırakıldıktan sonra en fazla 5 adet Anahtar Kelime konmalıdır. Anahtar Kelimelerden sonra bir satır boşluk bırakılarak İngilizce başlık ve altına ABSTRACT ve Keywords yazılmalıdır. Bir satır boşluk bırakılarak ana metine geçilmelidir.

4. Ana metin 9.5 punto Arial olarak hazırlanmalıdır.

5. Çalışma başlıca şu kısımlardan oluşmalıdır: Başlık, Yazar İsimleri, Adresleri, İletişim Bilgileri, Yazışmalardan Sorumlu Yazarın E-posta adresi, Öz, Abstract, Ana Metin (Giriş, Materyal ve Metot, Bulgular ve Tartışma, Sonuç), Teşekkür (gerekiyorsa), Kısaltmalar (gerekiyorsa), Kaynaklar.

6. Öz ve Abstract 250 kelimeyi geçmemeli, çalışmanın amacını, metodunu ve önemli sonuçlarını içermelidir. Öz tek paragraf olarak yazılmalı ve öz içinde kaynaklara atıf yapılmamalıdır.

7. Çalışma içerisinde geçen mikroorganizma isimleri ile Latince ifade ve isimler italik olarak yazılmalı ve kısaltmalarda uluslararası yazım kuralları göz önünde bulundurulmalıdır.

8. Tablo başlıkları tablonun üstüne, şekil başlıkları ise şeklin altına yazılmalı ve numaralandırılmalıdır. Kullanılan tablo ve şekillere metin içinde mutlaka atıf yapılmalıdır. Metin içinde geçen veriler tablo ve şekillerin tekrarı olmamalıdır. Tablo ve şekillerin başlıkları içerikleriyle uyumlu ve anlaşılabilir olmalıdır. Şekiller ve resimlerin yüksek çözünürlükte olmasına dikkat edilmelidir. Resimler (ve gerekiyorsa Şekiller) *.jpg formatında metin içerisinde yer almalıdır.

9. Metin içerisinde atıflar köşeli parantez içerisinde rakamlarla yapılmalı [1] ve Kaynaklar bölümünde bu numara sırasıyla detayları yazılmalıdır. Kaynakların numaralandırılması MS Word Numaralandırma Kitaplığı kullanılarak yapılmalıdır.

10. Kullanılan matematiksel denklemler numaralandırılmalı ve metin içerisinde bu denklemlere atıf yapılmalıdır.

11. Kaynaklar kısmı APA yazım stili kullanılarak hazırlanmalıdır. Kaynakların yazımında aşağıdaki örnek yazım biçimleri kullanılmalı ve makalelerin yayınlandığı dergi isimleri kısaltma kullanılmadan ve italik olarak yazılmalıdır. Web adreslerine atıf yapılacağında (mümkün olduğunca Resmi web sayfalarına atıf yapılmalıdır) mutlaka ilgili web adresine erişim tarihi verilmelidir.

Makale

[1] Bozkurt, H., İçier, F. (2009). İnegöl köfte üretiminde ohmik pişirmenin uygulanabilirliğinin incelenmesi. *Akademik Gıda,* 9(1), 6-12.

Kitap

[2] Kılıç, S. (2001). Süt Endüstrisinde Laktik Asit Bakterileri. Ege Üniversitesi Ziraat Fakültesi Yayınları, Ege Üniversitesi Matbaası, Bornova, İzmir.

Kitap Bölümü

[3] Gibson, G.R., Saavedra, J.M., MacFarlane, S., MacFarlane, G.T. (1997). Probiotics and Intestinal Infections. In Probiotics 2: Applications and Practical Aspects, Edited by R. Fuller, Chapman & Hall, 2-6 Boundary Row, London SE1 8HN, England, 212p.

Kongre-Sempozyum Bildirisi

[4] Gürsoy, O., Akdemir, O., Hepbaşlı, A., Kınık, Ö. (2004). Recent situation of energy consumption in Turkey dairy industry. *International Dairy Symposium: Recent Developments in Dairy Science and Technology*, May 24-28, 2004, Isparta, Turkey, Book of Proceedings, 10-16p.

12. Hakem görüşleri doğrultusunda düzeltilmek üzere yazar(lar)a gönderilen çalışmaların gerekli düzeltmeleri yapılarak yayın ofisine ulaştırılması gereklidir. Editörler tarafından belirtilen süre zarfında gönderilmeyen çalışmalar "ilk defa gönderilmiş çalışma" olarak değerlendirilecektir.

13. Yukarıdaki kurallara uygun olarak hazırlanmamış çalışmalar değerlendirmeye alınmaz.

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- Authors must guarantee that the submitted manuscript is not published anywhere previously and will not be submitted to anywhere before the editorial board makes a final decision on the manuscript.
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Manuscripts pass through initial screening in the editorial office followed by internal review by Editors. After the first evaluation, manuscripts are double-blind-reviewed by a peer review system involving at least two independent reviewers to ensure high quality of manuscripts accepted for publication. The Editors have the right to decline formal review of a manuscript if it is (i) on a topic outside the scope of the Journal, (ii) lacking technical merit, (iii) fragmentary and providing marginally incremental results or (iv) poorly written.

Publication fee

There is a 1000 TL submission fee for Turkish manuscripts submitted after February 1, 2024. This fee may not be waived. 750 TL fee is charged for articles submitted to the journal in English.

Ethics Statement

Detailed information about journal publication policy, evaluation of manuscripts and ethical issues can be found in the Ethics Statement section.

Preparation of a manuscript

1. Manuscripts should be prepared in A4 size, and the text must be prepared in a single column format. The text must be double-spaced, and a single space should be left between paragraphs. All lines and pages must be continuously numbered.

2. The title must be 14pt Arial, bold, small letters and centered. A blank line should be left after the title, and the names of authors should be given in 10pt Arial and centered. In addition to each author's contact address, the phone and fax numbers and e-mail address of the corresponding author should be provided. If the institutions of the authors are different, superscript numbers should be used to indicate their addresses.

3. The headings (e.g. Abstract, Introduction, Materials and Methods etc.) must be 10pt Arial, and should be typed in bold capital letters. Each heading should appear on its own separate line. A blank line should be left after each heading. A list of keywords, a maximum of 5, should be provided below the abstract section of the manuscript.

4. The main text should be prepared in 9.5pt Arial.

5. Typical articles mainly consist of the following divisions: Title, Author Names, Addresses, Contact Information, Corresponding author's e-mail address, Abstract, Main text (Introduction, Materials and Methods, Results and Discussion, Conclusions), Acknowledgements (if necessary), Abbreviations (if necessary) and References.

6. The abstract should not exceed 250 words, and the main purpose and method and the most significant result and conclusion should be presented in the abstract. The abstract should be prepared as a single paragraph, and should not include any citation.

7. Latin names in the text should be in italics, and names and abbreviations should follow international rules. If abbreviations that are not standard are unavoidable, they must be defined at their first mention in the text. Consistency of abbreviations throughout the article must be ensured. Internationally accepted rules and conventions must be followed, and the international

system of units (SI) must be used. If other units are mentioned, their equivalents in SI must be provided.

8. Table headings should be on the top of each table and figure captions below each figure. Each table or figure must be numbered consecutively in accordance with their appearance in the text. All figures and tables should be cited in the text. The data presented in the tables and figures should not be repeated in the text. Table headings and figure captions should be self-explanatory. Figures and pictures must be provided in high resolution, and pictures (and, if necessary figures) should be included in the text as *. jpg format.

9. References in the text should be cited in numbers in square brackets [1] and details of the citations must be provided in the Literature or References section with their respective numbers.

10. Mathematical equations should be numbered and cited in the text.

11. References should be given according to the APA manual of style. The following formats should be used for the details of cited references, and the journal names must be typed in italics. References to the Web addresses (if necessary, the official web pages should be preferred) must include full web address and the date of access.

Article

[1] Güzeler, N., Kaçar, A., Say, D. (2011). Effect of milk powder, maltodextrin and polydextrose use on physical and sensory properties of low calorie ice cream during storage. *Akademik Gıda,* 9(2), 6-12.

Book

[2] Kilic, S. (2001). Lactic Acid Bacteria in Dairy Industry. Ege University Faculty of Agriculture Publications, Ege University Press, Bornova, Izmir, Turkey.

Book Chapter

[3] Gibson, G.R., Saavedra, J.M., MacFarlane, S., MacFarlane, G.T. (1997). Probiotics and Intestinal Infections. In Probiotics 2: Applications and Practical Aspects, Edited by R. Fuller, Chapman & Hall, 2-6 Boundary Row, London, England, 212p.

Proceedings of the Congress-Symposium

[4] Gursoy, O., Akdemir, O., Hepbasli, A., Kinik, O. (2004). Recent situation of energy consumption in dairy industry in Turkey. *International Dairy Symposium: Recent Developments in Dairy Science and Technology*, May 24-28, 2004, Isparta, Turkey, Book of Proceedings, 10-16p.

12. A list of the corrections requested by the referees must be provided by the authors, and it must be sent to the editorial office.

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Akademik Gıda dergisine gönderilen tüm makaleler Dergi Editörleri için Davranış Kuralları ve En İyi Uygulama Kılavuzları ve Dergi Yayıncıları için Davranış Kurallarında (Code of Conduct and Best Practice Guidelines for Journal Editors and Code of Conduct for Journal Publishers) belirtilen Genel Kılavuzlara uygun olarak değerlendirilmektedir. Bilimsel yazılar dergiye gönderilmeden önce derginin Yazım Kurallarının okunmasını önemle tavsiye ederiz. Yazarlar aynı zamanda Avrupa Bilim Editörleri Birliği'nin (EASE) (European Association of Science Editors) İngilizce olarak basılacak makaleler için "Bilimsel Makalelerin Yazarları ve Çevirmenleri İçin Rehber"e uymalıdır. Yazarlar, insan veya hayvan verilerini içeren araştırmaları için Uluslararası Tıp Dergisi Editörleri Komitesinin (International Committee of Medical Journal Editors) önerilerini takip etmelidir.

Makalelerin Değerlendirilmesi

Dergiye gönderilen tüm makaleler, bilimsel içeriklerinin özgünlüğü ve kalitesi ölçütlerine göre değerlendirilir.

- Dergiye gönderilen tüm yazılar, ilk olarak yayın ofisindeki (teknik ve genel kalite değerlendirilmesi açısından) eleme işleminden geçer ve ardından teknik ve bilimsel editörler tarafından değerlendirilir.
- İlk değerlendirmeden sonra, editörler (i) dergi kapsamı dışında kalan bir konu hakkında hazırlanmış makaleleri (ii) teknik olarak eksik/yetersiz makaleleri, (iii) kısmi ve marjinal artan sonuçları içeren makaleleri veya (iv) kötü yazılmış makaleleri reddetme hakkına sahiptir.
- İlk inceleme sonucunda makalenin ileri değerlendirme için uygun olduğuna karar verilirse, dergide yayımlanmak üzere kaliteli makalelerin seçimini yapmak amacıyla, makaleler çift-körlü (hakemin ve yazar/yazarların birbirlerini görmedikleri) değerlendirme sistemi ile en az iki bağımsız hakemden oluşan bir değerlendirme sürecinde bilimsel incelemeye alınır.
- Hakemler tarafından talep edilirse, makalenin hakem görüşleri doğrultusunda yazarlar tarafından revize edilmiş versiyonu orijinal hakemler tarafından tekrar değerlendirilir. Değerlendirmelerin ardından editörler hakem önerileri doğrultusunda makale hakkındaki nihai kararlarını verirler. Gerekirse editörler, hakemlerin istedikleri tüm şartların yerine getirilmesi için yazarlardan ilave revizyon isteyebilir.
- Kabul edilen makalelerin son versiyonu, yayın öncesi taslağın (galley proof) hazırlanması için teknik editörlere gönderilir. Yazarlardan, makalelerinin dizgisi hazırlanmış taslaklarını son kontrol için yayın öncesinde incelemeleri istenir.
- Tüm makaleler, nihai formlarında DOI numarası almış ve çevrimiçi olarak pdf dosyaları halinde yayımlanır. İlgili veritabanlarında bu şekilde indekslenir.

Yayın Ücreti

Akademik Gıda dergisinde makalelerin yayınlanması için herhangi bir yayın ücreti talep edilmemektedir.

Gizlilik

Editörler, Akademik Gıda'ya gönderilen tüm makaleleri tam bir gizlilikle ele alır. Editörler, hakemler haricinde, COPE tavsiyelerine uyulmadığı takdirde, üçüncü şahıslara makale ile ilgili hiçbir bilgi vermezler. Yayınlanmak üzere dergiye gönderilen makaleler hakemler için de gizlidir ve bilimsel değerlendirme için aldıkları makalelerin herhangi bir bölümünü üçüncü şahıslarla paylaşmalarına veya dağıtmalarına izin verilmez. Suiistimal şüphesi olduğunda, hakemlerin derhal gizli bir şekilde yayın ofisine başvurmaları önerilir. Hakemler ayrıca, Dergi Editörleri İçin Davranış Kuralları ve En İyi Uygulama Kuralları ile Dergi Yayıncıları için Davranış Kuralları'nı (Code of Conduct and Best Practice Guidelines for Journal Editors and Code of Conduct for Journal Publishers) takip ederek editöre gizli yorumlarında belirli bir eylem önerebilirler.

Akademik Gıda, çift-kör bir hakem inceleme süreci yürütür, yani çalışmanın eleştirel değerlendirmesini sağlamak için hakemlerin isimleri gizlidir. Hakemlerden, raporlarında adlarını veya irtibat bilgilerini açıklamamaları istenir. Hakem raporları yazarlara gönderilemeden önce bu açıdan kontrol edilir.

Yazarlık

Bir yazar, bir araştırmanın fikrine veya tasarımına, verilerin elde edilmesine, verilerin analizine veya yorumlanmasına büyük ölçüde katkıda bulunan, makalenin hazırlanmasında, yazılmasında veya gözden geçirilmesinde entelektüel içeriğe eleştirel katkı yapan bireydir. Katkıda bulunanlar diğer kişiler makalenin Teşekkür bölümünde belirtilmelidir ve çalışmanın yazarı olarak kabul edilemez. Tüm yazarların doğru ve tam isimleri ile ORCID kimlikleri dergiye gönderilen

makalenin başlık sayfasında yer almalıdır. Yazarların isimlerinin yanında çalıştıkları kurumlar ve yazışmalardan sorumlu yazarın geçerli bir adresi verilmelidir. Yazışmalardan sorumlu yazarın telefon ve faks numaraları ile e-posta adresi makalenin ilk sayfasında belirtilmelidir. Tüm yazarlar, gönderilen makalenin daha önce herhangi bir yerde yayınlanmadığını ve makale hakkında Akademik Gıda dergisi nihai bir karar vermeden önce makaleyi başka bir dergiye göndermeyeceklerini garanti etmelidir.

Destekleyen/Finans Sağlayan Kuruluşlar

Araştırmanın tüm finans kaynaklarına ilişkin detaylar, Teşekkür bölümünde belirtilmelidir. Yazarlar, resmi finansman kurum/larının tam isimlerini ve proje/hibe numaralarını belirtmelidir.

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Yayınlanan verilerin doğruluğundan tüm yazarlar sorumlu olmalıdır. Verilerin düzeltilmesi için, yazışmalardan sorumlu yazardan yayın öncesi taslağı (galley proof) incelemesi ve makalenin yayınlanmasından 4 gün önce dikkatlice düzeltmesi istenir.

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Bir makalenin geri çekilmesi, gönderim veya yayın hatalarını düzeltmek için kullanılır. Yazarlar makaleyi geri çekebilir ve bu durumda Yayın Etiği Komitesi (COPE) Geri Çekme Kurallarına [(COPE) retraction guidelines] uymalıdır. Tekrarlanan veya benzerlik oranı yüksek bir yayın, verilerin hileli kullanımı, intihal veya etik dışı araştırma yapılması durumunda, makale editör tarafından geri çekilecek ve geri çekilen makale linklerine bağlantı korunacak ancak elektronik veri tabanına (makale sayfasına) bir geri çekme bildirimi eklenecektir.

Etik Hususlar

Çıkar çatışması:

- Yazar/lar başvuru sırasında herhangi bir çıkar çatışması varsa beyan etmelidir. Yazar/ların başvuru sırasında bilimsel değerlendirme için en az üç potansiyel hakem önermeleri istenir. Önerilen hakemler çalışma arkadaşları, ortak çalıştıkları kişiler veya çalıştıkları kurumların üyeleri olamazlar.
- Hakemler makaleyi değerlendirmelerini önleyen herhangi bir çıkar çatışması olması durumunda Editörleri bilgilendirmesi ve bu konuda COPE kurallarına uyması tavsiye edilmektedir.
- Editörler Kurulu üyeleri veya kurul üyelerinin ortak çalıştıkları kişiler tarafından dergiye gönderilen makaleler için, değerlendirme sırasındaki önyargıları en aza indirgemek amacıyla, değerlendirme süreci ilgili kurul üyelerini dışarıda tutacak şekilde değiştirilerek uygulanır.
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İnsan denekleri, hayvan veya bitki içeren araştırmalar

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- İnsan denekleri içeren çalışmalarda, deneklerin çalışmaya katılmak için imzaladıkları onamlar yazarlar tarafından sağlanmalıdır. 18 yaşın altındaki deneklerin çalışmaya katılmaları için ebeveyn veya velileri tarafından izin verilmelidir.
- Test edilen tüm denekler için, makalenin, ilgili kurallara ve/veya uygun izinlere veya lisanslara uyumunu gösteren belgelerin sunulması gerekir.
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- Bitki materyallerinin toplanması dahil, bitkiler üzerinde yapılan deneysel araştırmalar, kurumsal, ulusal veya uluslararası kurallara uygun olmalıdır.
- Saha çalışmaları yerel mevzuata uygun olarak yapılmalı ve uygun izinleri ve/veya lisansları belirten bir açıklama makalede yer almalıdır.

Yayın suistimali

- Akademik Gıda dergisi, Dergi Editörleri İçin Davranış Kuralları ile En İyi Uygulama Kılavuzları ve Dergi Yayıncıları İçin Davranış Kurallarını (Code of Conduct and Best Practice Guidelines for Journal Editors and Code of Conduct for Journal Publishers) takip eder.
- Makalenin aynı anda birden fazla dergiye gönderilmesi, intihal, yayınlanmış makalenin yeniden yayınlanması, etik kuralların ihlali vb. şüpheli bir suiistimal durumunda, araştırmacılar, hakemler veya okuyucular Yayın Ofisi (ogursoy@yahoo.com) ile iletişime geçmeye teşvik edilir.
- Makaledeki benzerlik oranı tek bir kaynaktan %10'dan fazla olmamak üzere en fazla %25 ile sınırlandırılmıştır. Bu koşula uymayan makaleler reddedilir. Bu şartların ihlal edilmesi durumunda, COPE (COPE recommendations) tavsiyeleri izlenecek ve ilgili tüm taraflara bildirilecektir.

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Peer Review

All contributions are evaluated according to the criteria of originality and quality of their scientific content.

- All manuscripts pass through an initial screening process (technical and overall quality evaluation) in the editorial office followed by an internal review by the technical and scientific editors.
- After the first evaluation, editors have the right to decline formal review of a manuscript if it is (i) on a topic outside the scope of the Journal, (ii) lacking technical merit, (iii) fragmentary and providing marginally incremental results or (iv) poorly written.
- If the manuscript is considered suitable for further evaluation, manuscripts are double-blind-reviewed by a peer review system involving at least two independent reviewers to ensure high quality of manuscripts accepted for publication.
- If requested, the revised version is evaluated by the reviewers, and editors make a decision about final acceptance based on their suggestions. If necessary, further revision can be asked for to fulfil all the requirements of the reviewers.
- The final version is then sent to the technical editor in order to produce a galley proof, and the authors receive this proof for final check before publishing.
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Funding Sources

Details for all funding sources of the research should be stated in the Acknowledgements. Authors should provide

the full official funding agency name(s) and grant number(s).

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