Aims and Scope

FOOD and HEALTH

Abbreviation: FOOD HEALTH

e-ISSN: 2602-2834

Journal published in one volume of four issues per year by ScientificWebJournals (www.ScientificWebJournals.com)

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

Food/Seafood/Food Technology/Food Chemistry/Food Microbiology/Food Quality/Food Safety/Food Contaminant/Food Allergen/Food Packaging/Modified Food/Functional Food/Dietary Supplements/Nutrition and their health effect is the general topics of journal.

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

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

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

“Food and Health” journal is indexed in TUBITAK ULAKBIM TR Index, FAO/AGRIS, ERIH PLUS, SciLit and Bielefeld Academic Search Engine (BASE).

Processing and publication are free of charge with the journal. No fees are requested from the authors at any point throughout the evaluation and publication process. All manuscripts must be submitted via the online submission system, which is available at http://dergipark.gov.tr/journal/1646/submission/start.

The journal guidelines, technical information, and the required forms are available on the journal’s web page.

Statements or opinions expressed in the manuscripts published in the journal reflect the views of the author(s) and not the opinions of the ScientificWebJournals, editors, editorial board, and/or publisher; the editors, editorial board, and publisher disclaim any responsibility or liability for such materials.

All published content is available online, free of charge at http://jfhs.scientificwebjournals.com.

ScientificWebJournals (http://scientificwebjournals.com) holds the international copyright of all the content published in the journal.

Editor in Chief: Prof. Nuray ERKAN
Address: Istanbul University, Faculty of Aquatic Sciences, Department of Seafood Processing Technology, Ordu Cad. No: 8, 34134 Fatih/Istanbul, Turkey
E-mail: nurerkan@istanbul.edu.tr

Tablo of Contents/İçerik

RESEARCH ARTICLES/ARAŞTIRMA MAKALESİ

**QUALITY ATTRIBUTES OF CITRUS FIBER ADDED GROUND BEEF AND CONSUMER ACCEPTANCE OF CITRUS FIBER ADDED TURKISH MEATBALLS** / 205-214

Ayça Gedikoğlu, Andrew Douglas Clarke

**EFFECT OF ENCAPSULATED STARTER CULTURE INCLUSION AND HEAT TREATMENT ON BIOGENIC AMINES CONTENT OF SUCUK** / 215-226

Tuğça Bilenler, İhsan Karabulu

**CAN ACID ADAPTATION OF Listeria monocytogenes INCREASE SURVIVAL IN SUCUK (A TURKISH DRY-FERMENTED SAUSAGE)?** / 227-238

Fatma Öztürk, Abdulkadir Halkman

**DETERMINATION OF THE BEST FUNCTIONAL CHICKPEA CULTIVARS BY TOPSIS TECHNIQUE** / 239-252

Levent Yurdaer Aydemir, Fatma Gizem Akçakaya

REVIEW/DERLEME

**CELIAC DISEASE AND NEW ATTEMPTS TO DEVELOP GLUTEN-FREE MEAT PRODUCT FORMULATIONS** / 253-264

Burcu Öztürk Kerimoğlu, Meltem Serdaroğlu

**PROBİYOTİK- İNSAN BAĞIŞIKLIK SİSTEMİ ETKİLEŞİMLERİ** / 265-280

Dicle Dilara Akpınar, Burcu Kaplan Türköz

**LAKTOZ İNTOLERANSIN PREVALANSI, TEŞHİSİ VE LAKTOZSUZ BESLENME TAVSİYELERİ** / 281-290

Furkan Demirgül, Recep Demirgül
QUALITY ATTRIBUTES OF CITRUS FIBER ADDED GROUND BEEF AND CONSUMER ACCEPTANCE OF CITRUS FIBER ADDED TURKISH MEATBALLS

Ayça Gedikoğlu¹, Andrew Douglas Clarke²

Cite this article as:

ABSTRACT

The objectives of this study were (I) to determine the addition of different citrus fiber (CF) levels (0%, 1%, 5%, and 10%) on the quality attributes of ground beef meatballs, (II) to determine consumer preferences for ground beef meatballs made with different CF levels (0%, 1%, 3% and 5%). Both water holding capacity and cooking yield of samples significantly (p<0.05) increased with addition of citrus fiber. There is no significant (p>0.05) difference found between the control CF 0% and the CF 1% for hardness and springiness values. Hunter color L, a, b values were significantly (p<0.05) impacted by the addition of citrus fiber. Results of the consumer panel showed that CF 1% got the highest flavor score with 6.61 followed by CF 0% with 6.52 (p>0.05). CF 5% had the lowest texture scores with 5.46. Overall likeness was highest for control with 6.69 followed by CF 1% with 6.56, CF 3% with 5.9, and CF 5% with 5.47. In conclusion, citrus fiber can be used in comminuted meat products at 1% level.

Keywords: Citrus Fiber, Meatballs, Water Holding Capacity, Flavor, Texture, Color
Introduction

In recent years, consumers’ food choices have shifted towards healthy foods due to increased incidence of coronary heart disease (CHD), diabetes, obesity and cancer (Rosamond et al., 2008). Food products associated with high fat content and high cholesterol have been linked to incidences of CHD (Micha, Wallace, & Mozaffarian, 2010), diabetes mellitus (Lajous et al., 2011), and risk of stroke (Larsson, Virtamo, & Wolk, 2011). Processed meat products have been closely linked to these diseases due to their high cholesterol content and saturated fat (Cross, Leitzmann, & Gail, 2007; Micha et al., 2010). New food products have been developed to have high protein content, low fat content as well as high fiber content to provide healthier food alternatives to consumers. Plant based proteins such as legumes (Serdaroglu, Yildiz-Turp, & Abrodimov, 2005) and soy protein (Singh, Kumar, Sabapathy, & Bawa, 2008) have been studied as extenders to increase protein content and mimic or replace fats to reduce the use of saturated fat in meat products. Additionally, fiber has been studied for both health and functional benefits. It has been reported that consumption of fiber helps with decreased cholesterol levels, with the absorption of glucose (Schneeman, 1987), and decreased incidence of hemorrhoids and colon cancer (Kritchersky, 1990). Also, dietary fiber such as psyllium and β-glucan have been approved by the Food and Drug Administration (FDA) for health claims for protection against coronary heart disease (USDHHS, 1997, 1998). It has been reported that insoluble fiber such as cellulose has been successfully used as a fat replacement in many food products such as frozen desserts, cheese spreads, salad dressing and processed meat products (Akoh, 1998). Functional properties of processed meat products made with different fiber sources have been studied. Use of peach fiber in low fat frankfurters (Grigelmo-Miguel, Motilva-Casado, & Martin-Belloso, 1997), β-glucan rich fiber in breakfast sausage (Aleson-Carbonell, Fernandez-Lopez, Perez-Alvarez, & Kuri, 2005), rice bran fiber in reduced fat frankfurters (Choi et al., 2010), orange fiber in fermented sausage called Sucuk (Yalinkilic, Kaban, & Kaya, 2012), yellow passion fruit fiber in pork burgers (Lopez-Vargas, Fernandez-Lopez, Perez-Alvarez, & Viuda-Martos, 2014) and carrot and lemon fiber in low-fat beef hamburgers (Soncu et al., 2015) have been helpful for improving functional properties of meat products. According to Gorinstein et al. (2001), citrus peel (albedo and flavedo) is rich in soluble fiber and can be used in meat products as a functional ingredient. Also, it has been reported that due to citrus fiber high vitamin C content and presence of bioactive compounds such as phenolic acids and flavonoids, it may provide further benefits as an antioxidant (Aleson-Carbonell et al., 2005; Fernandez-Lopez et al., 2004). Citrus fiber, by product of juice industry, provides great opportunity to be used as a fiber source and functional ingredient in comminuted meat products.

Based on this information, the objectives of our study were (I) to determine the impact of adding citrus fiber on the quality attributes of beef meatballs. The quality attributes investigated were the pH of both the raw and cooked meatballs, water holding capacity (WHC), cooking yield (%), textural properties, Hunter color L, a, and b values, and proximate composition. (II) to determine consumers’ acceptance for flavor, texture and overall liking of ground beef meatballs made with citrus fiber.

Materials and Methods

Sample Preparation

Beef cattle were slaughtered and their carcasses placed in a cooler for 48 hours. Later, two bottom rounds were collected from the carcass and weighed. After cutting the beef bottom rounds into smaller pieces, they were two-step (course and fine) ground using a LEM™ Products .35 P stainless steel electric meat grinder (West Chester, OH). Once they were ground, they were separated into four treatment groups and weighed. The treatment group with 0% citrus fiber, in other words control (CF 0%) was made into ground beef meatballs using a 50-mm diameter ice cream scoop; the meatballs were then placed onto four Styrofoam® trays for day 0, day 3, day 6, and day 9, and were covered with stretch film and labeled for replication, treatment group, and experimental days. Packages were then placed into a refrigerator. Treatments of 1%, 5%, and 10% citrus fiber were weighed based on the ground beef weight, and the fiber was mixed into the ground beef using a KitchenAid® blender. After each mixing, the blender was cleaned before mixing the next treatment group. Later, meat from each group was also made into meatballs using a 50-mm diameter ice cream scoop. The meatballs were then placed onto Styrofoam® trays covered with stretch film, and labeled for replication, treatment group, and experimental days. Packages were placed into the refrigerator until their use in the experiment. This procedure was replicated two more times on different slaughtering days to provide three total replications.
**pH**

A 5 g sample was homogenized with 45 mL distilled water by using a blender. Then, the pH of the slurry was determined by using a Fisher Accumet® model 230A pH/ion meter (Fisher Scientific Inc., Salt Lake City, UT). The pH measurements of both the raw and cooked samples of the three replicates were determined in duplicates.

**Water Holding Capacity**

The water holding capacity of the samples was determined according to methods reported by (Wierbicki, 1958). The formula used to calculate the water holding capacity (WHC) is shown below (Price and Schweigert, 1987); WHC was determined in triplicate for each treatment. Lower values indicate better water holding capacity.

\[
\text{WHC} = \frac{\text{Area of free water}}{\text{Area of meat}}
\]

(1)

**Cooking Yield**

The cooking yield of the ground beef meatballs was calculated by using the formula shown below (Bishop et al., 1993).

\[
\text{Cooking Yield} \% = \left( \frac{\text{Cooked weight of the product}}{\text{Uncooked weight of the product}} \right) \times 100
\]

(2)

**Determination of Moisture, Fat and Protein Content**

The moisture and fat content of the meat samples was determined based on the CEM SMART Trac system. This two-step system uses microwave for determining the moisture content of a meat sample. Next, it uses nuclear magnetic resonance (NMR) analysis for determining a fat content of the microwaved sample (Keeton et al., 2003). The protein content was determined using bicinchoninic acid (BCA) colorimetric detection and quantitation of the total protein method, according to Smith et al. (1985).

**Texture Profile Analysis**

After ground beef meatballs were cooked and their weight was recorded for the cooking yield procedure, they were cooled to room temperature before texture profile analysis (TPA). Each meatball was compressed to 50 percent of its original height in two consecutive cycles at a crosshead speed of 50 mm/min by using a TA-TX2 texture analyzer (Stable Micro Systems, Surrey, UK) with a 38-mm diameter probe for the evaluation of the texture profile analysis, as described by Bourne (1978). Triplicates of each treatment were evaluated for hardness, springiness, cohesiveness, gumminess, chewiness, and resilience.

**Hunter Color Values**

Hunter color L (lightness), a (redness) and b (yellowness) values were evaluated using a Minolta colorimeter (Konica Minolta Chroma Meter CR-410, Minolta Ltd., Milton Keynes, UK). The raw ground beef treatments were placed onto Styrofoam® trays individually, and treatments were spread flat on the tray to provide an even surface for color measurement. The Minolta colorimeter was placed directly on the surface of the ground beef samples. Color values were measured in triplicate for each treatment.

**Consumer Survey**

**Meatball Manufacture**

Ground beef (with 90% meat and 10% fat) and other ingredients were bought fresh from a store the day before the consumer panel. A Turkish köfte recipe was used for the formulation of the meatballs, and this recipe produced approximately 35-40 small meatballs. Table 1 shows the formulation of control (CF 0 %) treatment of ground beef meatballs. The rest of the treatments were made the same way with the exception of the addition of citrus fiber in 1%, 3% and 5% levels. After establishing the four ground beef foundations, onion and garlic were peeled and parsley leaves were picked; they were washed, diced and chopped. Ground beef and other ingredients were all mixed together. The meatballs were made using a 36-mm diameter ice cream scoop to make sure that all the meatballs were the same size. Meatballs were placed on a tray with a rack and each rack had a label with the treatment name on it. Once all the meatballs of a treatment were placed on a rack, the tray was placed in an oven, which was preheated to 190°C. A probe was placed into one of the meatballs and the temperature was set up for 72°C. Once the meatballs were properly cooked, the tray was taken out from the oven to cool down. The same procedure was followed for all the treatments. Meatballs were placed into labeled glass containers with lids for each treatment. Because the consumer panel room had only five available seats, the containers were kept in a refrigerator to insure safe handling practices between sets of panels. In order to serve warm meatballs to
the panelists, the meatball treatments were placed in individual Crock-Pot slow cookers with tomato sauce. The temperature of the sauce was kept above 60°C to provide safe and warm meatballs to panelists, and verified by calibrated temperature probes. The recipe of the tomato sauce is shown in Table 1. Meatballs were removed from the refrigerator to the Crock-Pots as needed.

*Sensory Evaluation*

Untrained panelists (164) of students, faculty and staff of the University of Missouri volunteered to participate in the consumer taste panel. Each panelist evaluated four warm meatball samples. One whole meatball for each treatment was placed into a labeled plastic cup. Each treatment was coded with randomly selected 3-digit numbers, and the four treatments were served to panelists in a randomized order. Panelists were also provided with a glass of water and were instructed to cleanse their pallets before trying the next sample. The rating test employed the hedonic scale of dislike extremely (1) to like extremely (9) (IFT, 1981). Panelists were instructed to evaluate the samples based on their degree of likeness for flavor, texture and overall likeness. Hedonic scale results were converted to numerical scores for statistical analysis.

**Statistical Analysis**

Three replications of ground beef meatballs were evaluated for cooking yields, WHC, pH, TPA, Hunter color values, and proximate analysis. Both data for quality attributes and consumer panel was analyzed by the analysis of variance (ANOVA), using the general linear model (GLM) procedure of the (SAS, 2011). Quality attributes data was randomized complete block design in which the block was a carcass. The treatments were arranged as a 4×4 factorial (4 levels of citrus fiber, 4 days). Means were separated by the Tukey test when significant ($p<0.05$) treatment effects were found.

### Table 1. List of ingredients for the Turkish meatball and the tomato sauce

<table>
<thead>
<tr>
<th>List of Ingredients for Meatball</th>
<th>Weight (g) or Quantity</th>
<th>List of Ingredients for Tomato Sauce</th>
<th>Weight (g or ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground beef (90% Lean)</td>
<td>454 g</td>
<td>Water</td>
<td>1000 ml</td>
</tr>
<tr>
<td>Onion</td>
<td>240 g (1 medium size)</td>
<td>Butter</td>
<td>227 g</td>
</tr>
<tr>
<td>Parsley</td>
<td>12 g</td>
<td>Tomato paste</td>
<td>120 g</td>
</tr>
<tr>
<td>Garlic</td>
<td>3 g (1 and half garlic)</td>
<td>Dry mint flakes</td>
<td>1 g</td>
</tr>
<tr>
<td>Egg</td>
<td>46 g (1 shelled egg)</td>
<td>Black pepper</td>
<td>0.8 g</td>
</tr>
<tr>
<td>Olive oil</td>
<td>15 g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pepper paste</td>
<td>14 g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salt</td>
<td>2.3 g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cumin</td>
<td>2.2 g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black pepper</td>
<td>1.2 g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sweet paprika</td>
<td>1 g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nutmeg</td>
<td>0.8 g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cinnamon</td>
<td>0.2 g</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Results and Discussion

pH
Table 2 shows the effect on pH of adding citrus fiber to both raw and cooked ground beef samples. The pH range of the raw samples ranged between 5.47 and 5.62 for treatments. Cooking caused a rise in the pH of all treatments except the CF 10% treatment. Similar results were also observed by Bilek and Turhan (2009). The pH range of the cooked samples ranged between 5.49 and 5.74. Adding 10% citrus fiber caused a significant (p<0.05) change in the pH of the cooked samples. However, the change in the pH of treatments with 1% and 5% citrus fiber was not significant (p>0.05) in comparison to change in the pH of the control.

Water Holding Capacity (WHC) and Cooking Yield (CY%)
The addition of citrus fiber boosted both the WHC and cooking yield. Table 2 illustrates the impact of adding citrus fiber on the water holding capacity and cooking yield of ground beef meatball treatments. Besbes et al. (2008) reported that an increase in the addition of wheat fiber caused a rise in the water holding capacity of beef burgers in comparison to the control burger samples. Furthermore, the cooking yield of CF 10% was highest at 92.21, and all the citrus treatments had significantly (p<0.05) higher cooking yields than the control (CF 0%). Serdaroglu et al. (2005) found similar results with the use of lentil flours on improving the water holding capacity and cooking yield of low fat meatballs. Cengiz and Gokoglu (2007) also reported that the addition of citrus fiber reduced the cooking loss for frankfurter-type sausages. Since the citrus fiber is high in pectin, it can allow binding with free water from meat samples. Thus, it can help with improving water holding capacity and cooking yield.

Determination of Moisture, Fat and Protein Content
The moisture, fat and protein content of the ground beef treatments are shown in Table 2. The moisture content of the control was highest, and an increase in the addition of the dry ingredient—citrus fiber—caused a decrease in the moisture content of all treatments. While the gradual decrease in moisture content was expected due to addition of dry powder in different levels, the major increase in the protein content was not expected. Even with the addition of 6.37% protein coming from citrus fiber, increase in the protein content was normal than higher. This could be due to BCA colorimetric methodology. Smith et al. (1985) reported that presence of glucose caused artificially high protein content values. Kessler and Fanesihil (1986) also reported that phospholipids can react with bichinchoninic acid (BCA) that can cause artificially high protein content. Since, citrus fiber has sugars, such as glucose that may interfere with our results and therefore it may cause artificially high protein content. Table 3 displays the nutritional facts associated with CitraFiber™ citrus fiber. Huang et al. (2011) reported similar results: The addition of wheat fiber into Chinese-style sausages caused a decrease in the moisture content and an increase in the protein content.

Textural Properties
The textural properties of ground beef meatballs made with or without citrus fiber are shown in Table 4. Our results showed that the addition of citrus fiber caused a decrease in hardness. The control had the highest hardness values, and there were no significant (p>0.05) differences between all treatments. However, there were significant (p<0.05) differences between treatments in terms of all of the textural properties. Yang et al. (2007) reported similar results: Adding hydrated oatmeal and tofu caused a decrease in the hardness of low-fat pork sausages. There were also reports of the hardening of meat products with the addition of fiber. Cofrades et al. (2000) stated that the addition of soy fiber caused an increase in the hardness of bologna-type sausage. Huang et al. (2011) also found hardening in Chinese-type sausages made with wheat or oat fiber. Most of the studies observed increase in hardness with addition of fiber were emulsified meat products. Springiness slightly decreased with the addition of citrus fiber, the significant difference (p<0.05) was observed between the control and CF 5 and 10%. The cohesiveness of ground beef meatballs made with 0% and 1% citrus fiber was significantly higher (p<0.05) than the meatballs made with 5% and 10% citrus fiber. Samples made with 10% citrus fiber had less cohesiveness and resilience than those of other treatments.

Hunter Color L, a, b Values
Results of the Hunter color L, a, b values are summarized in Table 5. The addition of citrus fiber caused significant (p<0.05) decrease in lightness, redness and yellowness values for raw ground beef treatments. Only exception, there was no significant (p>0.05) difference found between yellowness values for the control and the CF 10%. The changes in color of treatments were visually apparent and can be seen by the Picture 1. Bilek and Turhan (2009) observed similar results, where the addition of flax seed flour caused a decrease in the
lightness values of the beef patties made with 20% fat content. The control treatment redness values were significantly higher (p<0.05) than all of the other treatments. The addition of citrus fiber caused a decrease in the redness values for raw ground beef samples. Fernandez-Gines et al. (2003) reported an increase in the redness values when citrus fiber was first added to bolognas but a decrease in the redness values during storage time. The addition of citrus fiber to raw ground beef significantly (p<0.05) increased the b values of all treatments. While the addition of citrus fiber at 10% level had the highest yellowness values, it was not significantly (p>0.05) different than the control. Cofrades et al. (2000), and Cengiz and Gokoglu (2007) reported similar results: Increasing the addition of fiber caused a rise in b values. The difference between our findings and those of prior studies could result from our product being raw and mixed ground beef whereas other studies were conducted with cooked emulsified products.

Picture 1. Hunter color measurement of raw ground beef treatments

![Hunter color measurement of raw ground beef treatments](image)

Table 2. Addition of different levels of citrus fiber on physico-chemical properties of ground beef meatballs

<table>
<thead>
<tr>
<th></th>
<th>Citrus Fiber Treatment Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>pH raw</td>
<td>5.54 ±0.139&lt;sup&gt;a,b&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH cooked</td>
<td>5.65 ±0.100&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>WHC</td>
<td>0.68 ±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cooking Yield (%)</td>
<td>71.43 ±4.54&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Moisture Content (%)</td>
<td>60.75 ±2.51&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat Content (%)</td>
<td>21.30 ±3.01&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Protein Content (%)</td>
<td>14.46 ±0.69&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Each value in the Table is represented as mean ± standard deviation (n=6).
<sup>a,b,c,d</sup> Different superscripts in the same row indicate significant difference by the Tukey’s test (p<0.05).
### Table 3. Nutritional facts about citrus fiber CitraFiber™

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Pectin</td>
<td>9390 mg / 100g</td>
</tr>
<tr>
<td>Protein</td>
<td>6.37%</td>
</tr>
<tr>
<td>Total Sugars</td>
<td>1.7%</td>
</tr>
<tr>
<td>Total Dietary Fiber</td>
<td>82.7%</td>
</tr>
<tr>
<td>Soluble Fiber</td>
<td>23.4%</td>
</tr>
<tr>
<td>Insoluble Fiber</td>
<td>59.3%</td>
</tr>
<tr>
<td>Potassium</td>
<td>453 mg/100g</td>
</tr>
<tr>
<td>Sodium</td>
<td>210 mg/100g</td>
</tr>
<tr>
<td>Calcium</td>
<td>78 mg /100g</td>
</tr>
<tr>
<td>Vitamin A (Beta Carotene)</td>
<td>117 IU/100g</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>0.91 mg/100g</td>
</tr>
</tbody>
</table>

Source: Natural Citrus Products

### Table 4. Addition of different levels of citrus fiber on textural properties of ground beef meatballs

<table>
<thead>
<tr>
<th>Citrus Fiber Levels</th>
<th>Hardness</th>
<th>Springiness</th>
<th>Cohesiveness</th>
<th>Gumminess</th>
<th>Chewiness</th>
<th>Resilience</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF 0%</td>
<td>1356.13</td>
<td>0.746</td>
<td>0.553</td>
<td>743.90</td>
<td>563.01</td>
<td>0.228</td>
</tr>
<tr>
<td>±500.65</td>
<td>±0.051</td>
<td>±0.036</td>
<td>±252.42</td>
<td>±221.8</td>
<td>±0.021</td>
<td></td>
</tr>
</tbody>
</table>

| CF 1%               | 1088.89   | 0.714       | 0.480        | 521.59    | 378.63    | 0.194      |
| ±396.29             | ±0.051    | ±0.03      | ±179.94      | ±150.78   | ±0.019    |

| CF 5%               | 887.17    | 0.656       | 0.346        | 304.06    | 201.22    | 0.145      |
| ±243.74             | ±0.046    | ±0.052     | ±78.86       | ±59.97    | ±0.019    |

| CF 10%              | 819.69    | 0.611       | 0.244        | 198.62    | 120.83    | 0.121      |
| ±246.72             | ±0.05     | ±0.074     | ±63.14       | ±46.79    | ±0.023    |

Each value in the Table is represented as mean ± standard deviation (n = 9).
a, b, c, d Different letters in the same column indicate a significant difference by the Tukey’s test (p<0.05).

### Table 5. Effect of citrus fiber on hunter color L, a, b values of raw ground beef treatments

<table>
<thead>
<tr>
<th>Citrus Fiber Levels</th>
<th>L Value</th>
<th>Hunter Color</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>a Value</td>
<td>b Value</td>
</tr>
<tr>
<td>CF 0%</td>
<td>48.24 ±0.742</td>
<td>23.36 ±1.01</td>
</tr>
<tr>
<td>CF 1%</td>
<td>44.42 ±0.117</td>
<td>18.64 ±0.96</td>
</tr>
<tr>
<td>CF 5%</td>
<td>42.92 ±0.801</td>
<td>11.89 ±2.10</td>
</tr>
<tr>
<td>CF 10%</td>
<td>45.61 ±0.848</td>
<td>8.23 ±2.28</td>
</tr>
</tbody>
</table>

Each value in the Table is represented as mean ± standard deviation (n = 9).
a, b, c, d Different letters in the same column indicate a significant difference by the Tukey’s test (p<0.05).
Sensory Evaluation of Meatballs

Consumers’ acceptance of ground beef meatballs made with different levels of citrus fiber is shown in Table 6. Results showed that meatballs made with 1% citrus fiber (CF 1%) had the highest flavor score with 6.61, followed by the control treatment with 6.52. There was no significant difference (p>0.05) in flavor scores between CF 1% and the control treatment, however, both treatments had significantly (p<0.05) higher flavor scores than CF 3% and CF 5%. Besbes, Attia, Deroanne, Makni, and Blecker (2008) reported similar results. Beef burgers made with pea and wheat fiber received the highest flavor scores. In another study, Yildiz-Turp and Serdaroglu (2010) reported that low fat beef patties made with 10% plum puree received higher flavor scores than the control. On the other hand, Bilek and Turhan (2009) reported that the addition of flaxseed flour to beef patties caused a decrease in flavor scores.

Results showed that texture attribute of ground beef meatballs were significantly (p<0.05) impacted by the addition of citrus fiber. The control meatball treatments received the highest scores of 6.69, followed by the CF 1% treatment with 6.27. Treatments with the highest citrus fiber, the CF 5%, received the lowest score in texture with 5.46, which is like slightly. Besbes et al. (2008); Bilek and Turhan (2009) reported similar results: an increase in the fiber levels caused a decrease in texture sensory scores for beef patties. There were also reports of improvements in sensory texture scores for sausage products. Huang, Tsai, and Chen (2011) reported that Chinese style sausages made with oat fiber received higher scores than the control. Yalinkilic et al. (2012) reported that a fermented sausage product called Sucuk made with citrus fiber received slightly higher sensory texture results than the control.

Results of overall likeness for the four treatment groups are shown in Table 6. The control has the highest overall likeness scores with 6.69 followed by the CF 1% with 6.56, the CF 3% with 5.9 and the CF 5% with 5.47. There was no significant (p>0.05) difference in overall likeness scores between the control and the CF 1%. However, there were significant (p<0.05) differences between the control with the CF 3% and the CF 5%. Fernadez-Gines, Fernandez -Lopez, Sayas- Barbera, Sendra, and Perez-Alvarez (2003) reported similar findings. They found that, at the highest concentration, the addition of citrus fiber to bolognas caused a decrease in overall quality scores. Serdaroglu et al. (2005) reported that meatballs made with legume flour extenders received high scores (6.8 and above) in overall acceptability. Additionally, in another study low fat pork sausage made with oatmeal or tofu received higher overall acceptability scores than control pork sausages (Yang, Choi, Jeon, Park, & Joo, 2007). In a recent study, Tomaschunas et al. (2013) reported that low fat Lyon style sausages made with inulin and citrus fiber had similar sensory characteristics to full fat reference.

Table 6. Consumers’ acceptance of Turkish meatballs made with different levels of citrus fiber

<table>
<thead>
<tr>
<th>Citrus Fiber Levels</th>
<th>Flavor</th>
<th>Texture</th>
<th>Overall Likeness</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF 0 %</td>
<td>6.52 ±1.4a</td>
<td>6.69 ±1.52a</td>
<td>6.69 ±1.37a</td>
</tr>
<tr>
<td>CF 1 %</td>
<td>6.61 ±1.44a</td>
<td>6.27 ±1.75b</td>
<td>6.55 ±1.51a</td>
</tr>
<tr>
<td>CF 3 %</td>
<td>5.94 ±1.76b</td>
<td>5.9 ±1.67c</td>
<td>5.9 ±1.66b</td>
</tr>
<tr>
<td>CF 5 %</td>
<td>5.49 ±1.73c</td>
<td>5.46 ±1.89d</td>
<td>5.47 ±1.68c</td>
</tr>
</tbody>
</table>

Each value in the Table is represented as mean ± standard deviation.

a, b, c, d Different letters in the same column indicates significant difference (p<0.05) analyzed by the Tukey’s test.
Conclusion

Results of this study indicate that citrus fiber at 1% level can be used in comminuted meat products to increase the cooking yield and water holding capacity, and it can have high acceptability by the consumer. Both industry and consumers can benefit from using citrus fiber in meat products.

Compliance with Ethical Standard

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

References


EFFECT OF ENCAPSULATED STARTER CULTURE INCLUSION AND HEAT TREATMENT ON BIOGENIC AMINES CONTENT OF SUCUK

Tuğça Bilenler, Ihsan Karabulut


1 Inonu University, Faculty of Engineering, Department of Food Engineering, 44280 Malatya, Turkey

ORCID IDs of the authors:
T.B. 0000-0001-7831-6337
I.K. 0000-0002-9014-8863

Submitted: 30.01.2019
Revision requested: 09.04.2019
Last revision received: 09.04.2019
Accepted: 12.04.2019
Published online: 12.07.2019

ABSTRACT

In this study, the effect of encapsulated starter culture (Lactobacillus plantarum plus Staphylococcus xylosus) inclusion on biogenic amines (BAs) content of sucuk was investigated comparatively in heat treated (at ~70 °C for 20 min) and fermented sucuks. The highest contents of histamine, which is known as the most toxic BA, were observed in the fermented samples including non-encapsulated (103.6 mg/kg) and encapsulated (102.3 mg/kg) starter cultures, while the lowest values were detected in the heat treated sucuks (p < .05), including non-encapsulated (24.2 mg/kg) and encapsulated (21.4 mg/kg) starter cultures, at the end of 45 days of storage. Based on the principal component analysis of the microbiological count and BAs content of the samples, the heat treated and encapsulated starter culture included sucuks discriminated themselves from control groups and non-encapsulated encapsulated starter culture included samples with lower histamine and tyramine contents as well as their microbiological loads.

Keywords: Encapsulation, Biogenic amine, Starter culture, Heat treated, Sucuk

Correspondence:
Tuğça BİLENLER
E-mail: tugca.bilenler@inonu.edu.tr

©Copyright 2019 by ScientificWebJournals
Available online at
http://jfhs.scientificwebjournals.com
Introduction

Fermentation is one of the oldest food preservation methods. Meat fermentation results in lactic acid production by certain species of lactic acid bacteria (LAB) that are naturally occurring microflora on meat or are subsequently added at known levels as starter culture (Ravys Vuyst and Leroy, 2012). Sucuk is one of the most popular traditional dry fermented meat products in Turkey (Soyer, Ertaş and Üzümcüoğlu, 2005). Starter cultures are frequently used in fermented sucuk in order to reduce fermentation time, enhance sensory quality, and improve product safety and lower cost of fermented products. LAB are preferably used as starter culture in sucuk production together with micrococci because of their acidification ability as well as proteolytic and lipolytic activities (Leroy Verluyten and De Vuyst, 2006). Among the starter cultures Pediococcus acidilactici, Pediococcus pentosaceus, Lactobacillus plantarum, Staphylococcus xylosus and Pediococcus pentosaceus, generally used as alone or their binary mix in traditional sucuk production (Bozkurt and Erkmen, 2002; Dalmış and Soyer, 2008; Genccelep Kaban and Kaya, 2007; Kaban and Kaya, 2009; Kurt and Zorba, 2010).

Biogenic amines (BAs) are organic bases with low molecular weight that widely occur at low pH in fermented foods by decarboxylation of amino acids via microbial action (Jairath Singh Dabur Rani, 2015). The role of microorganisms and other external factors on BAs formation was extensively discussed in many of reviews (Bover Cid Miguélez-Arrizado Becker Holzapfel and Vidal-Carou, 2008; Galgano Favati Bonadio Lorusso and Romano, 2009; Kaniou Samouris Mouratidou Eleftheriadou, and Zantopoulou, 2001; Claudia Ruiz-Capillas and Jiménez-Colmenero, 2004). The presence of BAs in foods is important for several reasons such as the level of BAs is used as an indicator of quality and/or acceptability in some foods (Hernández-Jover Izquierdo-Pulido Veciana-Nogués Mariné-Font and Vidal-Carou, 1997), and high level BAs intake could cause toxic effects (Bardócz, 1995). Formation of free amino acids with proteolytic events during fermentation provides precursors for BAs. The pH level of fermented meat products has critical importance on the level of BAs. Decarboxylase activity increases with decreased pH levels thereby the production of BA increases (Bover Cid et al., 2008). One way to prevent excessive BA accumulation is inclusion of amine-negative starter culture to carry out a controlled fermentation (Bover-Cid Izquierdo-Pulido and Vidal-Carou, 2000). The most extensively studied BAs in meat and fermented meat products are tyramine, cadaverine, putrescine and histamine (Claudia Ruiz-Capillas and Jiménez-Colmenero, 2004).

In recent years, because of the great consumer awareness and developments regarding new technologies, attempts to improve the food quality with innovative techniques have become very popular. As an innovative application, encapsulation can be used to protect the sensitive biomaterials from environmental influences and provide controlled release through the semi permeable shell structure. (Corbo et al., 2016; De Prisco and Mauriello, 2016; Kailasapathy, 2002). Viability of LAB in fermented foods has been successfully improved by encapsulation (Martín Lara-Villoslada Ruiz and Morales, 2015).

Heat treated sucuk is defined as “sucuk like product” in Turkey. Manufacturers have been included a heat treatment step (at 68-70°C for 15-30 min) to sucuk production process just after filling of sucuk dough into casings. The advantages of this step include; extending shelf life, reducing of production period and production cost (Ercoşkun Tağı and Ertaş, 2010). The main aim of the heat treatment is to destroy unwanted microbiorganism. Meanwhile, beneficial bacteria such as LAB and Micrococcus-Staphylococcus (M-S) are also destroyed. However, development of the quality properties of fermented foods is largely dependent on maintaining the desired bacteria in an active state. To overcome such deficiencies, we used microencapsulated starter cultures including Lactobacillus plantarum and Staphylococcus xylosus in heat treated and traditional sucuks. Higher survival rate for starter cultures was achieved by encapsulation. Encapsulation procedures, morphological properties, survival rate and release behavior of starter cultures and the effect of non-encapsulated and encapsulated starter cultures inoculation on physicochemical and microbiological characteristics of heat treated and fermented sucuks were reported in our previous study (Bilenler Üzümcüoğlu, 2010). However, it was not possible to give the results of BA contents within the same article due to limited scope.

Therefore, the purpose of the present paper was to report the effect of encapsulated starter culture inclusion on BAs formation in heat treated sucuks during fermentation and storage periods, and also to compare with the formation of BAs in traditional fermented sucuk.

Materials and Methods

Starter Culture Preparation

Lactobacillus plantarum (ATCC No: 2331) and Staphylococcus xylosus (ATCC No: 29971) were activated and refreshed in MRS (Merck, Darmstadt, Germany) and BHI broth (Merck), respectively, at 37°C for 48h. Starter cultures in late-log phase (with the cell numbers of $3.2 \times 10^{11}$ CFU/mL...
and 8.4 × 10^{11} \text{ CFU/mL}, respectively) were harvested by centrifugation at 3000 rpm for 10 min, washed in sterile saline solution (0.8% NaCl) (Bilenler Karabulut and Candogan, 2017).

**Microencapsulation**

Starter cultures were encapsulated according to the emulsion technique as commonly applied Sultana et al. (2000). The detailed microencapsulation process was described in the preceding paper (Bilenler Karabulut and Candogan, 2017). Sodium alginate/starch blends (Sigma-Aldrich, Steinheim, Germany) were used as wall material to encapsulate starter cultures. The highest survival rates for both encapsulated bacteria were obtained with alginate-starch blend with percentage ratios of 2:2 and 0.5:0.5 for *S. xylosus* and *L. plantarum*, respectively. The experimental materials were properly sterilized before use. Shell mixture solution was sterilized at 121°C for 15 min. After cooling to room temperature, the mixture containing 40 mL of alginate-starch and 10 mL of active cell suspension (with 11.5 log CFU/mL and 11.92 log CFU/mL for *S. xylosus*, *L. plantarum* and *S. xylosus*, respectively) were prepared. The suspension was added dropwise into 250 mL of sunflower oil containing 0.5% of Tween 80 and stirred for 20 min. The emulsion was broken by quickly adding 0.1 M calcium chloride (100 mL) into the mixture while stirring. The mixture was allowed to stand for 20 min to settle alginate beads and they were washed with a solution containing 0.9% salt and 5% glycerol. Microcapsules were harvested by low speed centrifugation at 15000 rpm for 5 min. Then the microcapsules were frozen at −18°C for 24 h and freeze-dried overnight using a freeze-dryer (Armfield, Ringwood, England). Dry microcapsules were stored at 4°C until use (Bilenler Karabulut and Candogan, 2017).

**Sucuk Manufacture**

Beef, lamb tail fat and additives were mixed to prepare sucuk dough according to the recipe described by Kaban and Kaya, (2009) using industrial scale equipment at a local meat factory (Malatya Meat and Meat Products) as described recently (Bilenler Karabulut and Candogan, 2017). Detail of production steps and sucuk groups with and without starter cultures in non-encapsulated or encapsulated forms are described in a flow chart (Figure 1). Non-encapsulated or encapsulated starter cultures [*S. xylosus* + *L. plantarum*] (1:1) at a level of 7 log CFU/g were used. The batter stuffed into natural casings and conditioned. Fermented sucuk groups were subjected to stepwise 14-day fermentation under the conditions given in Figure 1. In heat treated sucuk production, heat treatment was carried out 24 h after stuffing in a cabinet room heated conventionally at 70°C for 20 min until the internal temperature of the sucuk coils reached 70°C. Then the both sucuk groups were stored at 4°C for 45 days. The sucuk manufacturing was replicated two times under the same production conditions. Triplicate analyses were carried out at Days 0 (after stuffing), 14 (after production), 30 and 45 during refrigerated storage.

**Biogenic Amine Analysis**

The extraction and derivatization of BAs were done as described by Eerol et al. (1993). Briefly, 4 g of sucuk sample was weighed to a test tube and homogenized with 10 mL of perchloric acid (70%), followed by centrifugation at 3000 rpm for 10 min. The extraction was repeated twice. Both supernatants were combined, and the final volume was adjusted to 25 mL with perchloric acid. In order to derivatization of the amines in samples, each extract was mixed with 200 µL of 2 M sodium hydroxide and 300 µL of saturated sodium hydrogen carbonate (8.5 g/100 mL pure water), then reacted with 1 mL dansyl chloride solution (10 mg/mL acetone) at 40°C for 45 min. After that, the reactant was mixed with 100 µL of 25% ammonium hydroxide and incubated at room temperature for 30 min to remove the residual dansyl chloride. The mixture was adjusted to 5 mL with 0.1 M ammonium acetate/acetonitrile (1:1) and filtered through a 0.45 µm syringe filter (Lubitech, Songjiang, China) for HPLC analysis.

Twenty microliters of the filtrate were injected into HPLC system (Shimadzu, Kyoto, Japan) equipped with a Spherisorb ODS-2 column (5 µm, 125 x 4 mm; Waters, Milford, MA, USA). The column temperature was set 25°C. The mobile phase consisted of solvent A (100% acetonitrile) and solvent B (0.01 M ammonium acetate). The flow rate was 1 mL/min. The separation was carried out by gradient elution procedure started at 50% B, and then solvent B was raised to 90% within 25 min. Individual BAs were detected at 254 nm and quantified by calibration curve prepared with seven point concentrations of authentic standards (putrescine, histamine, cadaverine, spermidine, tyramine and spermine) purchased from Sigma-Aldrich (Steinheim, Germany). BA analysis was carried out in triplicate and results of two independent batches of sukuks were expressed as mg/kg of dry matter (DM).

**Statistical Analysis**

The effects of starter culture inclusion in non-encapsulated or encapsulated forms and heat treatment on the BA contents of sucuk were analyzed by analysis of variance (ANOVA). Duncan’s multiple-comparison test was used as a tool for comparisons of means at a level of p < 0.05 using the SPSS package programme version 16.0 (SPSS Inc., Chicago, IL, USA). For the principal component analysis (PCA) 24 observations (sucuk samples) and 11 variables including 6 BAs (putrescine, histamine, cadaverine, spermidine, tyramine and spermine) were used in total. Correlation coefficients between the variables determined by the coefficient of Pearson and PCA were made by using XLStat software, version 2010.2.02 (Addinsoft, Paris, France).
**Figure 1.** Flow chart for production process of the fermented and heat treated sucuks
Results and Discussion

Sucuk samples produced by fermentation or heat treatment and with/without starter culture in encapsulated or non-encapsulated forms were analyzed for their BA contents and the results are shown in Figure 2. The investigated BAs (putrescine, histamine, cadaverine, tyramine, spermidine and spermine) were detected in all sucuk samples. Histamine, tyramine and spermine were predominant amines in all sucuk samples. In general, starter culture inclusion in non-encapsulated or encapsulated forms slowed down the rate of BAs formation during fermentation and storage periods. One of the most important methods to prevent BAs formation is addition of the amine negative starter cultures to fermented meat products as reported by several reports (Bover-Cid Izquierdo-Pulido and Vidal-Carou, 1999; Gençcelep Kaban and Kaya, 2007; Papavergou, 2011; Suzzi and Gardini, 2003).

Initial amounts of BAs in control samples (A1: fermented and A2: heat treated) were higher (p < .05) than those of other samples at Day 0. Our previous paper (Bilenler Karabulut and Candogan, 2017) reported that control samples had significant microbial load before fermentation and heat treatment. The microbial load in these samples produced high amount of BAs during resting within 24 h (Figure 1), before fermentation process or heat treatment. This microbial flora including mainly Enterobacteriaceae was thought to be responsible for the high levels of BAs in control samples (A1 and A2). There is a strong relationship between the BA contents and some groups of microorganisms that are able to decarboxylate amino acids. For example, mainly putrescine and cadaverine production is a consequence of microbial activity of Enterobacteriaceae and tyramine production is due to the activity of Enterococci (Jairath Singh Dabur Rani and Chaudhari, 2015). In addition, the pH value of the sucuk is another factor that influences the formation of biogenic amines. Biogenic amine formation is promoted by decarboxylase activity at lower pH (Gençcelep et al., 2008; Santos 1998).

Starter culture inclusion (non-encapsulated and encapsulated forms) in both production methods [fermented (B1: non-encapsulated and C1: encapsulated) and heat treated (B2: non-encapsulated and C2: encapsulated)] affected putrescine contents at Day 0 (p < .05). The highest amount of putrescine contents were measured at Day 0 due to the activity of Enterobacteriaceae. Significant decreases were observed in putrescine contents of all samples at 14 days of storage (p < .05). This can be explained mainly by the competitive relationship between added starter culture and undesired microbial flora during storage days. As reported in our preceding report (Bilenler Karabulut and Candogan, 2017), starter cultures become dominant in the sucuk samples during storage. Another reason for decreasing of putrescine may be due to its conversion to spermidine. This decreasing pattern of the concentration of spermidine was reported by Rabie et al. (2014). Putrescine levels remained unchanged in the heat treated samples within 14-45 days of storage, while significant reductions were observed in the fermented group samples during this period due to higher starter culture activity. The same performance was observed with both starter culture forms (non-encapsulated or encapsulated) in reducing of putrescine and cadaverine contents. Similar reduction was observed in Spanish dry-cured "chorizo" sausage which was attributed to the lower counts of lactic acid bacteria during long storage periods (Ruiz-Capillas Jiménez Colmenero Carrascosa and Muñoz, 2007). Effect of starter cultures on reducing of putrescine and cadaverine was slightly higher in the heat treated samples (B2 and C2) than fermented ones at Day 14. On the contrary, the amount of cadaverine in fermented group was reduced faster during storage periods. Putrescine contents in all the samples decreased to about 3 mg/kg level in B1, C1, B2 and C2 samples at the end of storage period, while cadaverine reduced to 4-5 mg/kg in fermented samples and 9-10 mg/kg in heat treated samples. These amounts of putrescine and cadaverine were lower than the values reported in the literature for Turkish type sucuk (Gençcelep Kaban and Kaya, 2007), Sichuan-style sausage (Sun et al., 2016) and Italian dry fermented sausages (Tabanelli et al., 2012). Principally, putrescine and cadaverine are considered as non-toxic BAs and there are no any suggestions on their acute doses.

Among the BAs in fermented foods, histamine and tyramine are considered as the most toxic by EFSA Panel on Biological Hazards (BIOHAZ) (2011). The highest histamine level was observed in control samples (A1 and A2) at Day 0. The histamine content increased in both groups (non- and encapsulated starter culture used sucuks) after Day 14, while the increase in fermented group being higher. Histamine accumulation in fermented samples (A1, B1 and C1) continued as the storage time elapsed. Among the starter culture included sucuk samples, the highest histamine content was observed in fermented samples (103.6 and 102.3 mg/kg for B1 and C1, respectively) at Day 45, whereas the lowest (p < .05) was detected in heat treated samples (24.2 and 21.4 mg/kg for B2 and C2, respectively). Histamine levels in heat treated sucsuks are in tolerable upper intake level recommended by EFSA who stated that intake up to 50 mg of histamine for healthy individuals can be considered safe for healthy individuals (EFSA, 2011). Histamine accumulation in heat treated samples, including non-encapsulated (B2) and encapsulated (C2) starter cultures, were not significant (p > .05) during fermentation and storage periods with the exception of 45 days of
storage. Histamine content in non-encapsulated starter culture used sample (B2) was higher than that of encapsulated starter culture used sample at Day 45 ($p < .05$). This can be explained by the higher number of starter cultures in C2 sample (Bilenler Karabulut and Candogan, 2017). At the end of storage period, histamine contents of encapsulated starter culture included and heat treated sucuk were considerably lower than those of reported values for different types of sausages (Bozkurt and Erkmen, 2002; Sun et al., 2016; Tabanelli et al., 2012).

Tyramine content of the samples at Day 0 was around 25 mg/kg ($p > .05$) and increased drastically to 150.20, 109.03 and 98.25 mg/kg ($p < .05$) after 45 days of storage for control, non-encapsulated starter culture used sample and encapsulated starter culture used sample in fermented group, respectively. Those levels in heat treated group were considerably low (42.61, 35.40 and 34.46 mg/kg, respectively) which may be due to destruction of the non-starter microbial flora. This effect was also observed in heat treated samples during storage period; tyramine formation within Day 30 and 45 was almost controlled ($p > .05$) in encapsulated starter culture included sample (C2). Effect of starter culture inclusion on reducing of tyramine was also confirmed in previous studies (Bover-Cid Izquierdo-Pulido and Vidal-Carou, 2000; Latorre-Moratalla et al., 2010). EFSA (2011) stated that intake up to 600 mg of tyramine can be considered safe for healthy individuals not taking monoamine oxidase inhibitor drugs. In this respect, the tyramine content of the samples may be considered within the safety ranges and considerably lower than the levels reported for Turkish dry fermented sausages (316.3 mg/kg) (Bozkurt and Erkmen, 2004), Felino-type sausages (254.38 mg/kg) (Tabanelli et al., 2012), and Italy and South Belgium fermented sausage (187 and 176 mg/kg, respectively) (Ansorena et al., 2002).

Initial amounts of spermidine for control, non-encapsulated and encapsulated starter culture included samples were 3.45, 2.29 and 2.24 mg/kg, respectively. These values increased in fermented group at Day 14 ($p < .05$) and then decreased to about 2 mg/kg during storage, while the changes in heat treated samples were not significant ($p > .05$) during fermentation and storage periods. Similar changes with spermidine were observed for spermine contents. Higher increases in fermented group after Day 14 and during storage periods indicating that heat treatment and encapsulated starter culture inclusion effectively prevented formation of these amines. As stated before, at certain levels, spermidine and spermine are not considered to be indicators of spoilage because they can be naturally exist in meats (Hernández-Jover Izquierdo-Pulido Veciana-Nogués Mariné-Font and Vidal-Carou, 1997). Kurt and Zorba (2009) reported higher level of spermidine (5.27 mg/kg) and lower level of spermine (36.35 mg/kg) in heat treated Turkish dry fermented sausage. It was reported that the concentrations of spermidine were always lower than those of spermidine due to conversion of spermidine to spermine (Hernández-Jover et al., 1997; Rabie et al., 2014).

The data were subjected to PCA on two different dataset composed of i) initial values (Day 0) and ii) fermentation and storage periods, in order to better describe the relations between factors affected by starter culture forms (non-encapsulated and encapsulated) and production methods (fermentation and heat treatment). The biplot projection of the loading and score values of the PCA for initial and fermentation-storage periods are shown separately in Figure 3 A and B, respectively. As it can be seen from Figure 3 A, 76.50 % of the total variance can be explained by the first two principal components. Control samples, starter culture forms and production methods were clearly separated on the PC1 which accounts for 57.72 % of the total variance. By looking at the orientations of the variables (loadings) and the samples (scores), it is clearly seen that the control samples discriminate themselves other sucuk samples with their higher yeast-molds and coliform counts (Bilenler Karabulut and Candogan, 2017), and higher BA contents with the exception of tyramine. As stated before, natural microbial flora probably formed a considerable amount of BAs in the sample Day 0. Among the BAs, the only putrescine correlated positively with LAB ($r = 0.204$) and M-S counts ($r = 0.149$) indicating that these microorganisms were able to generate putrescine in fermented and heat treated samples at Day 0. Negative correlation between the starter cultures and BA contents at initial stage of the production showed that there were no favorable conditions for BA formation yet. There were positive correlations between the coliforms and histamine, cadaverine, spermidine and spermine ($p < .05$) at Day 0. However, fermented and heat treated groups discriminated themselves from control samples with high counts of LAB and M-S including *L. plantarum* and *S. xylosus*, respectively.
Figure 2. Changes of biogenic amines amounts during fermentation and storage periods. Different lowercase letters (a-d) in the same production day for the different sucuk samples indicate significant difference ($p < .05$). Different uppercase (A-D) between the storage days for the same sample indicate significant difference ($p < .05$).
Figure 3. Biplot of the principal component analysis (PCA) carried on data of biogenic amines and microbial counts of sucuk samples just after production (A) and storage periods (B). The variables (Loadings) used for the analysis were indicated in red letters and sucuk samples (Scores) were indicated with different colors and symbols. Percentages in brackets correspond to the explained variances of the corresponding components. See Figure 1 for abbreviated sample codes.
The fermented and heat treated samples included starter culture in non-encapsulated or encapsulated forms exhibited distinct properties which was also dependent on storage periods. Figure 3B showed the positioning of the production methods (heat treated or fermented sucuks) in the first principal plane as deducted from PCA. About 66.82% of the total variance was explained by the first principal component. Heat treated and fermented sucuk samples clearly separated on PC1 axis. High counts of M-S and LAB, and spermine discriminated non-encapsulated and encapsulated starter culture included fermented samples. Control and non-encapsulated starter culture included samples in the heat treated group could be separated from encapsulated starter culture included sucek in the graph due to mainly similar microbiological properties and BA contents. As given in previous study, heat treatment nearly destroyed the beneficial microbial flora as well as coliforms but not influenced survival of LAB and M-S. Correlations between the variables and observations were found to be different from that of initial stage due to biochemical changes occurred during storage period. In accordance with the literature findings (Jairath et al., 2015), coliforms were found to be primarily responsible for the formation of two most toxic BAs as well as cadaverine, since higher correlations ($p < .05$) were exist between coliforms and tyramine ($r=0.637$), histamine ($r=0.730$) and cadaverine ($r=0.813$). Total aerobic mesophilic bacteria (TAMB) also contributed significantly ($p < 0.05$) to the formation of the BAs with the exception of putrescine and cadaverine, while effect of M-S on BAs formation, except spermidine ($r=0.308$), was found to be not significant ($p > 0.05$). It should be noted that there was a high correlation between fungi (yeast-mold) and cadaverine ($r=0.818$), but their role is debated and, for many aspects, controversial (Gardini Özogul Suzzi Tabanelli and Özogul, 2016).

**Conclusion**

BAs formation in heat treated and fermented sucuks was comparatively investigated by incorporation of amine negative starter culture in non-encapsulated and encapsulated forms. Heat treatment reduced formation of BAs by destruction of amine producing microorganisms. Fermentation with encapsulated (amine negative) starter cultures reduced the presence of BAs in the heat treated product respect to the traditional fermentation. In this respect, heat treatment and encapsulated starter culture inclusion could be especially proposed for reducing histamine content of sucek to the safer level, although any reduction would always depend on other factors influencing BAs formation.

**Compliance with Ethical Standard**

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

**Financial disclosure:** This study was supported by Inonu University, Directorate for Scientific Research (Project No. 2015/36).

**References**


PMid:19200122

PMid:18206769


CAN ACID ADAPTATION OF *Listeria monocytogenes* INCREASE SURVIVAL IN SUCUK (A TURKISH DRY-FERMENTED SAUSAGE)?

Fatma Öztürk¹, Abdulkadir Halkman²

Cite this article as:
Öztürk, F., Halkman, A. (2019). Can acid adaptation of *Listeria monocytogenes* increase survival in sucuk (a turkish dry-fermented sausage)?. *Food and Health*, 5(4), 227-238. [https://doi.org/10.3153/FH19024](https://doi.org/10.3153/FH19024)

¹ İzmir Katip Celebi University, Faculty of Fisheries, Department of Fishing and Processing Technology, Çiğli, İzmir, Turkey
² Ankara University, Faculty of Engineering, Department of Food Engineering, Gölbaşı, Ankara, Turkey

ORCID IDs of the authors:
F.O. 0000-0003-4763-3801
A.H. 0000-0001-9987-0732

Submitted: 04.04.2019
Revision requested: 09.05.2019
Last revision received: 15.05.2019
Accepted: 04.06.2019
Published online: 10.08.2019

ABSTRACT

In this research, acid resistance levels of *Listeria monocytogenes* have been examined under the conditions of acid adaptations. In addition, the effect of acid adaptation on the survival of *L. monocytogenes* in sucuk have also been determined. *L. monocytogenes* were adapted to pH 4.5 for the periods of 1, 2, 3 and 4 hours. The survival of *L. monocytogenes* that were adapted to acid have been ascertained at pH 2.5, 3.0 and 3.5 respectively. It has been found that HCl acid adaptations at pH 3.5 have resulted in no increase in the survival of *L. monocytogenes*. A three-hour adaptation process has led to an increase in survival level at pH 2.5 while 1, 2, 3 or 4-hour adaptation processes lead to an increase in survival level at pH 3.0. However, it was found that the survival level of *L. monocytogenes* in sucuk did not increase as a result of acid adaptation procedure. Acid adapted pathogens have many risks for food safety and human health. These pathogens maintain their viability in acidic foods and cause foodborne diseases. Therefore, understanding the mechanisms of acid adaptation of pathogens will help to create more effective food safety systems and will play a role in the prevention of foodborne diseases.

Keywords: Acid adaptation, Inorganic acids, HCl, *Listeria monocytogenes*, Sucuk
Introduction

*Listeria monocytogenes* is a Gram-positive, facultative anaerobe, non-spore, rod-like bacterial species that causes sporadic or epidemic infections in humans and animals (Ferrari *et al*., 2017; Mikš-Krajník *et al*., 2017; Suo *et al*., 2018). Contamination occurs by the consumption of infected foodstuffs (Bergholz *et al*., 2018). Although it is rarely seen in healthy individuals, it is an important pathogen for individuals with weak immune system, newborns, elderly and pregnant women. It causes gastroenteritis, septicemia, meningitis, meningoencephalitis, also miscarriages in pregnant women and stillbirths (Drevets and Bronze, 2008; Giaouris *et al*., 2014; Calvo *et al*., 2016; No *et al*., 2016; Bergholz *et al*., 2018).

*L. monocytogenes* is an important pathogen in terms of public health, which can spread widely in the environment, develop at refrigerator temperature, maintain its viability even under adverse conditions such as refrigeration, freezing, heating and drying processes (Cacace *et al*., 2010; Hingston *et al*., 2010; Omari *et al*., 2016; Hettich EBA 12; Germany) for 10 minutes. Cell pellets suspended using physiological saline water (PSW) were washed two times more by centrifugation. The pH values of the obtained cell pellets were adjusted to 4.5 using 6 N HCl (37%; Merck) solution and left to incubate for 1, 2, 3 or 4 hours in 10 mL TSB. As a result, acid-adapted cells were obtained. For the preparation of the nontreated cells, the cell pellet was suspended in 10 mL TSB medium at pH 7.0 (Cheng *et al*., 2003).

The aim of this study was to obtain the acid-tolerated *L. monocytogenes* cells which cause food poisoning and to determine the tolerance of these cells to strong acidic conditions. It was also aimed to investigate the effect of this acid adaptation on the survival level of *L. monocytogenes* in sucuk (A Turkish Dry-Fermented Sausage).

Materials and Methods

Bacterial Cultures

The *L. monocytogenes* ATCC 7644 strain used in the trial was obtained from Ankara University Food Engineering Department’s culture collection.

Preparation of Acid-Adapted *Listeria monocytogenes* Cells

The cells of 15-hour stationary phase of *L. monocytogenes* cells were used in the trials. Accordingly, 5 µL of active *L. monocytogenes* was transferred to 50 mL TSB and incubated for 15 hours at 37 °C. At the end of this period, 9 mL cultures were placed in centrifuge cells and centrifuged at 5000 rpm for 15 hours at 37 °C (Cheng *et al*., 2003). At the 0th, 1st, 2nd, 3rd, 4th, and 5th hours of incubation, samples were inoculated in Tryptic Soy Agar (TSA, Merck) by spread plate method, incubated at 37 °C and bacterial count was determined as CFU/mL.

Acid Tolerance of *L. monocytogenes*

To determine the resistance of *L. monocytogenes* cells to strongly acidic conditions, 0.5 mL of acid-adapted and nontreated cells were inoculated in 50 mL TSB with 2.5, 3.0 or 3.5 using HCl and incubated at 37 °C (Cheng *et al*., 2003). At the 0th, 1st, 2nd, 3rd, 4th, and 5th hours of incubation, samples were inoculated in Tryptic Soy Agar (TSA, Merck) by spread plate method, incubated at 37 °C and bacterial count was determined as CFU/mL.

Production of Sucuk

Beef and tail fat (25 %) were chopped and minced through a 3-mm-diameter plate. The minced meat was irradiated at 25 kGy at Turkish Atomic Energy Authority Sarayköy Nuclear Research and Training Center and kept at -20 ± 2 °C until use. The minced meat was thawed the night before the production
of sucuk, and 1.6 % NaCl, 1.2 % garlic, 0.5 % sucrose, 0.5 % bitter red pepper, 0.6 % sweet pepper, 0.6 % black pepper, 0.8 % cumin, 0.04 % NaNO₃ and 0.01 % NaNO₂ were added (Soyer et al., 2005). Commercial starter culture (Staphylococcus carnosus, Staphylococcus xylosus ve Lactobacillus curvatus) was homogeneously added to the mix using a mixer. Following this stage, two experimental groups were formed. Acid-tolerated L. monocytogenes (10⁵ CFU/g) was added to the sucuk batter of the first experimental group, while non-acid-tolerated L. monocytogenes (10⁵ CFU/g) was added to the sucuk batter of the second experimental group (control group). The prepared sucuk batter was kept in the refrigerator (at 4 °C) overnight and the additives were allowed to diffuse to the meat. Sucuk batter was filled in artificial casings in 50-60 g portions using a manual meat mincer. The sucuk samples were ripened in a conditioner room where temperature and moisture can be adjusted automatically at 85-90 % for 3 days at 22 °C, at 80-85 % for 3 days at 22 °C and at 65-70 % for 3 days at 20 °C (Soyer et al., 2005) sequentially. Following the ripening process, the sucuk samples were stored at 4 °C.

Bacteriological Analyses of Sucuk

For the bacteriological analyses of sucuk samples, 10 g sample was transferred into stomacher bags containing 90 mL Maximum Recovery Diluent (MRD, Merck) and homogenized in the stomacher (Seward Stomacher®400 Circulator; England) at 235 rpm for 1 minute. Inoculations from appropriate dilutions prepared using 9 mL MRD were carried out using selective media by spread plate method. PALCAM Agar (Merck) was used for the L. monocytogenes, and incubated at 37 °C for 48 hours. Baird Parker Agar (Merck) was used for the Staphylococcus spp. and incubated at 37 °C for 48 h. For the lactic acid bacteria (LAB), De Man Rogosa Sharp Agar (Merck) was used and incubated at 30 °C for 72 h (Harrigan 1998).

Physical and Chemical Analysis of Sucuk

For pH determination, 100 mL pure water was added to 10 g sucuk sample and homogenized. The pH of the mixture was measured using an Inolab (level 2) pH meter (AOAC 2000). In order to determine the dry matter, approximately 5 g sucuk sample was weighed and dried at 105 °C until a constant weight was obtained (AOAC 2000).

Statistical Analysis

In terms of the studied characteristics (different pH and different adaptation times), the findings were analyzed by repeated measurement ANOVA in factorial order. DUNCAN test was used to determine the different groups. In the variance analysis, CMSTAT package program was used while SPSS 15 package program was used for the DUNCAN tests.

Results and Discussion

Acid Adaptation

The survival rate of L. monocytogenes ATCC 7644 strain with acquired tolerance to at pH 4.5 using HCl to pH 2.5 varied depending on the adaptation time. Bacterial counts in the acid-adapted group exhibited a faster decrease trend compared to those in the other nontreated group. It was found that, at the 4th and 5th hour of the incubation, the group with the highest level of tolerance to acid was the experimental group which was adapted to acid for 3 hours. Acid adaptation performed at pH 4.5 for 1, 2, 3 or 4 hours using HCl increased the resistance of L. monocytogenes to pH 3.0 (P<0.05). Different adaptation times did not have any different effects on the increase in resistance (Table 1, 2 and 3). Acid adaptation performed at pH 4.5 for 1, 2, 3 or 4 hours using HCl caused no increase in the resistance of L. monocytogenes to pH 3.5.

The studies conducted in recent years focused on food pathogens such as L. monocytogenes have revealed that these bacteria have mechanisms that enable them to adapt to acidic environments (Leyer et al., 1995; O’driscoll et al., 1996). Koutoumanis and Sofos (2004), in their study on the survival levels of E. coli O157:H7, L. monocytogenes and S. typhimurium subjected to pH values ranging between 4.0 and 6.0, it was found that, pathogens were protected from lethal acidic conditions with acid adaptation procedure and the acid tolerance varied depending on the species and pH. At pH values between 5.0 and 6.0, acid resistance of L. monocytogenes increased, and the highest resistance was determined at pH 5.5. Phan-Thanh et al. (2000) adapted the L. monocytogenes LO28 strain to HCl for a couple of hours at pH 5.5 before the acid stress. The researchers found that the highest tolerance to pH 3.7 in groups where adaptation was carried out for 2 and 3 hours. The researchers have also reported that acid tolerance decreased when adaptation period was extended to 24 hours. Giaouris et al. (2014) have reported an increase was determined in the resistance to lethal acidic (pH 2) conditions of the L. monocytogenes Scott A strain, which was acid-
adapted in TSB containing glucose. Koutsoumanis et al. (2003) have reported that the acid tolerance of *L. monocytogenes* which was acid-adapted in TSB containing glucose at pH values 5.0, 5.5 and 6.0 for 90 minutes increased, however acid adaptation procedures carried out at pH values 4.0, 4.5 and 7.0 did not cause any increase in acid tolerance. In the present study, acid adaptation performed at pH 4.5 for 1, 2, 3 or 4 hours caused increase in the resistance of *L. monocytogenes* to pH 2.5 and pH 3.0. The highest increase in the survival level at pH 2.5 was determined in the experimental group adapted to acid for 3 hours.

### Table 1. The survival level of HCl (pH 4.5) adapted and nontreated *L. monocytogenes* in pH 2.5 (log CFU/mL)

<table>
<thead>
<tr>
<th>Incubation (h)</th>
<th>Acid-adapted period (h)</th>
<th>Nontreated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>0</td>
<td>7.02A ±0.06</td>
<td>7.00A ±0.04</td>
</tr>
<tr>
<td>1</td>
<td>6.21B ±0.06</td>
<td>6.23B ±0.05</td>
</tr>
<tr>
<td>2</td>
<td>5.78B ±0.38</td>
<td>5.98B ±0.28</td>
</tr>
<tr>
<td>3</td>
<td>4.03C ±0.26</td>
<td>4.65C ±0.00</td>
</tr>
<tr>
<td>4</td>
<td>3.42D ±0.24</td>
<td>3.00D ±0.00</td>
</tr>
<tr>
<td>5</td>
<td>2.08E ±0.25</td>
<td>1.95E ±0.00</td>
</tr>
</tbody>
</table>

A, B, C (↓); a, b, c (→): The difference between averages having the same letters is not statistically significant (P>0.05).

### Table 2. The survival level of HCl (pH 4.5) adapted and nontreated *L. monocytogenes* in pH 3.0 (log CFU/mL)

<table>
<thead>
<tr>
<th>Incubation (h)</th>
<th>Acid-adapted period (h)</th>
<th>Nontreated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>0</td>
<td>7.41A ±0.05</td>
<td>7.23A ±0.19</td>
</tr>
<tr>
<td>1</td>
<td>6.47B ±0.13</td>
<td>6.32B ±0.02</td>
</tr>
<tr>
<td>2</td>
<td>6.25B ±0.02</td>
<td>6.23BC ±0.05</td>
</tr>
<tr>
<td>3</td>
<td>6.19BC ±0.01</td>
<td>6.15BC ±0.04</td>
</tr>
<tr>
<td>4</td>
<td>6.10BC ±0.02</td>
<td>6.06BC ±0.02</td>
</tr>
<tr>
<td>5</td>
<td>5.87C ±0.12</td>
<td>5.92C ±0.04</td>
</tr>
</tbody>
</table>

A, B, C (↓); a, b, c (→): The difference between averages having the same letters is not statistically significant (P>0.05).

### Table 3. The survival level of HCl (pH 4.5) adapted and nontreated *L. monocytogenes* in pH 3.5 (log CFU/mL)

<table>
<thead>
<tr>
<th>Incubation (h)</th>
<th>Acid-adapted period (h)</th>
<th>Nontreated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 saat</td>
<td>2 saat</td>
</tr>
<tr>
<td>0</td>
<td>7.40</td>
<td>7.21</td>
</tr>
<tr>
<td>1</td>
<td>7.25</td>
<td>6.82</td>
</tr>
<tr>
<td>2</td>
<td>7.02</td>
<td>6.55</td>
</tr>
<tr>
<td>3</td>
<td>6.71</td>
<td>6.40</td>
</tr>
<tr>
<td>4</td>
<td>6.25</td>
<td>6.32</td>
</tr>
<tr>
<td>5</td>
<td>5.83</td>
<td>6.16</td>
</tr>
</tbody>
</table>

A, B, C (↓); a, b, c (→): The difference between averages having the same letters is not statistically significant (P>0.05).
It was stated that the resistance of *L. monocytogenes* to low pH varied depending on the bacterial strain (Phan-Thanh et al., 2000). Vialette et al. (2003) have reported that the adaptation ability of clinical isolates of *L. monocytogenes* to adverse conditions such as acid and osmotic stress was higher than those isolated from foods. Berk et al. (2005) have reported that *S. typhimurium* strains isolated from humans had a higher acid tolerance compared to those of isolated from foods. In addition, numerous studies have reported that the development phase was also effective on acid resistance. In the present study, in which stationary phase cells were used, acid adaptation procedure carried out using HCl at pH 4.5 for 1, 2, 3 or 4 hours lead to an increase in the acid tolerance of *L. monocytogenes* to pH 2.5 and 3.0 whereas it did not cause any increase in survival levels at pH 3.5. Lee et al. (1994), on their study on *S. typhimurium*, have reported that the tolerance of stationary phase cells to pH 3.0 were 1000 times higher than those of the logarithmic phase cells. O’Driscoll et al. (1996) have reached similar results, reporting that the stationary phase cells of *L. monocytogenes* were naturally resistant to pH changes (pH 3.5 however, for logarithmic phase cells to survive at pH 3.5, they should be acid-adapted to pH 5.5 environment and acid tolerance response should be induced. Similarly, Lou and Yousef (1997) have reported that, as a result of one-hour adaptation at pH 4.5 and 5.0, acid tolerance of logarithmic phase cells of *L. monocytogenes* Scott A strain to pH 3.5 increased.

### *L. monocytogenes* Count in Sucuk

After 3 hours of incubation in a TSB medium adjusted to pH 4.5 using HCl, the acid-adapted *L. monocytogenes* were added to the sucuk batter. *L. monocytogenes* counts in sucuk samples during the ripening and storage period are given in Table 4.

Acid-adapted and nontreated *L. monocytogenes* counts added to sucuk batter decreased during the ripening and storage periods and determined to be 2.89 log CFU/g in the experimental group and <2.00 log CFU/g in the control group at the 40th day of the storage period. However, no significant difference was found between the experimental groups (P>0.05). It was determined that the survival rate of *L. monocytogenes* did not increase with acid adaptation in sucuk samples. Similar results were determined by Calicioglu et al. (2002). In their study, beers inoculated with acid-adapted *L. monocytogenes* strains (LM101, LM103, N7143, N7144, TB2000) were marinated with different solutions and dried at 60 °C for 10 hours. As a result of the study, it has been reported that acid adaptation did not cause an increase in the survival level in *L. monocytogenes*. Gahan et al. (1996) determined that the survival levels of lactic acid-adapted *L. monocytogenes* increased in yoghurt and cottage cheese containing lactic acid, orange juice containing citric acid and salad sauce containing acetic acid. However, in foods with higher pH, such as mozzarella cheese and low-fat cheddar cheese, acid adaptation did not increase the survival level. Unlike the results reported in the studies mentioned above, Francis and O’Beirne (2001) have reported that survival level of *L. monocytogenes* (ATCC 19114) which was acid-adapted for 1 hour at TSB medium at pH 5.5 using lactic acid increased in vegetables packaged under modified atmospheric conditions. In another study, it has been stated that the survival level of *L. monocytogenes* Scott A strains acid adapted using TSYB increased in non-neutralized berry juices (pH 3.70-4.89) (Karabiyikli et al., 2017). In the present study, it was determined that the acid tolerance of the *L. monocytogenes* ATCC 7644 increased with acid adaptation in the experiments performed under *in vitro* conditions. However, in the trials performed in sucuk samples, no increase was observed in the survival level values in acid-adapted cells. It was thought that this might be due to the combined effect of protective factors including starter culture, low water activity, low pH, nitrite and sodium chloride in fermented sausages (Erol et al. 1999; Lindqvist and Lindblad 2009; Kaya and Gökalp, 2004; Kara and Akkaya 2010). In the studies conducted in different food systems, it was determined that the acid tolerance varied depending on the composition of the media. In the study conducted by Gahan et al. (1996), acid-adapted *L. monocytogenes* and acid-resistant mutant strains showed higher survival rates in commercial yoghurt and home cheese produced under laboratory conditions. The acid-resistant mutant showed higher resistance during the ripening of hard cheeses such as cheddar cheese, and a high number of cells were recovered after 70 days of ripening. Chung et al. (2018) have reported that, with the acid adaptation, the survival rate of *L. monocytogenes* (ATCC 19111, 19115 and 9117) increased in commercial fruit juices, however it was eliminated by adding carvacrol and thymol to the composition of the media. Tchuenchiew (2016) has reported that the acid types used in acidification of fruit juices was effective in the inactivation of *L. monocytogenes* 56 LY cells adapted to citric acid. Malic and hydrochloric acid added fruit juices
were found to have higher inactivation rates compared to citric acid.

**LAB and Staphylococcus count in Sucuk**

LAB and *Staphylococcus* spp. counts determined in sucuk samples during the ripening and storage are given in Table 5. *L. curvatus* was added to sucuk batter as the starter culture at 6.48-6.45 log CFU/g. LAB counts increased with the onset of fermentation and reached 9.25 log CFU/g in the experimental group and 9.14 log CFU/g in the control group on the 1st day of fermentation (P>0.05). After the 8th day, which was the onset of the storage period, LAB counts started to decrease, and determined to be 8.75 and 8.79 log CFU/g in the experimental and the control groups, respectively. It was determined that pH decreased as the LAB count increased, and this interaction was found to be statistically significant (P<0.05).

The use of starter culture on *L. monocytogenes* in fermented sucuk is known to be effective. Kaya and Gökalp (2004) showed that the use of starter culture inhibited the development of *L. monocytogenes*. The number of *L. monocytogenes* in sucuk produced without using starter culture increased by 10^3 log CFU/g on the 3rd day of ripening period. In sucuk produced using starter culture, it was stated that in the first three days, LAB number reached 10^9 log CFU/g, pH value decreased below 5.0 and *L. monocytogenes* could not develop. Porto-Fett et al. (2008) reported that, fermentation and drying stage of fermented semi-dry sucuk, when pH 5.3 and 4.8, the number of *L. monocytogenes* was decrease 0.07-0.74 log CFU/g. In this study, the number of *L. monocytogenes* continued to decrease from the beginning of ripening period. Erol et al. (1999), with the addition of starter cultures producing bacteriocin, the number of *L. monocytogenes* at 10^5 CFU/g decreased to 0.03 EMS/g at the end of the ripening period (14 days), this value decreased 2.4 EMS/g in sucuk samples containing *L. curvatus* strain which not produce bacteriocin. In this study, using the same starter culture, the number of *L. monocytogenes*, which was 5.89-5.69 log CFU/g at the start of fermentation, reached 3.53-2.97 log CFU/g on the 15th day.

*Staphylococcus* spp. counts determined in sucuk samples during the ripening and storage are given in Table 5. 6.43 log CFU/g and 6.33 log CFU/g of *Staphylococcus* as starter culture were added to the sucuk dough of the experimental and control group, respectively. The difference between the *Staphylococcus* numbers determined in the experimental and control groups was not statistically significant (P>0.05).

Ensoy (2004) reported that the most commonly used species in fermented meat products in the family of *Micrococcaceae* were *S. carnosus* and *S. xylosus*. It is stated that these starter cultures are used to improve the flavor and color characteristics of the product.

### Table 4. The survival level of *L. monocytogenes* during ripening and storage periods at 4 °C of the sucuk samples (log CFU/mL)

<table>
<thead>
<tr>
<th>Days</th>
<th>Acid-adapted</th>
<th>Nontreated</th>
</tr>
</thead>
<tbody>
<tr>
<td>H0*</td>
<td>5.89</td>
<td>5.69</td>
</tr>
<tr>
<td>Ripening</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>5.87</td>
<td>5.14</td>
</tr>
<tr>
<td>1</td>
<td>5.78</td>
<td>5.13</td>
</tr>
<tr>
<td>2</td>
<td>5.57</td>
<td>4.96</td>
</tr>
<tr>
<td>3</td>
<td>5.51</td>
<td>4.94</td>
</tr>
<tr>
<td>4</td>
<td>5.43</td>
<td>4.69</td>
</tr>
<tr>
<td>6</td>
<td>4.76</td>
<td>4.13</td>
</tr>
<tr>
<td>Storage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>4.44</td>
<td>3.66</td>
</tr>
<tr>
<td>15</td>
<td>3.53</td>
<td>2.97</td>
</tr>
<tr>
<td>30</td>
<td>3.37</td>
<td>2.70</td>
</tr>
<tr>
<td>40</td>
<td>2.89</td>
<td>&lt;2.00</td>
</tr>
</tbody>
</table>

H0*: Keeping the sucuk batter at 4 °C overnight. Values are the average of 2 replicates. No significant difference was found between the experimental groups (P>0.05).
Table 5. The number of LAB and *Staphylococcus* spp. in the sucuk samples inoculated with *L. monocytogenes* cells (log CFU/mL)

<table>
<thead>
<tr>
<th>Days</th>
<th>Acid-adapted</th>
<th>Nontreated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LAB</td>
<td><em>Staph.</em> spp.</td>
</tr>
<tr>
<td>HO*</td>
<td>6.48</td>
<td>6.43</td>
</tr>
<tr>
<td>Ripening</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>6.65</td>
<td>6.39</td>
</tr>
<tr>
<td>1</td>
<td>9.25</td>
<td>6.69</td>
</tr>
<tr>
<td>2</td>
<td>9.42</td>
<td>6.09</td>
</tr>
<tr>
<td>3</td>
<td>9.35</td>
<td>6.16</td>
</tr>
<tr>
<td>4</td>
<td>9.19</td>
<td>6.09</td>
</tr>
<tr>
<td>6</td>
<td>9.21</td>
<td>6.69</td>
</tr>
<tr>
<td>Storage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>9.22</td>
<td>6.90</td>
</tr>
<tr>
<td>15</td>
<td>8.75</td>
<td>6.21</td>
</tr>
<tr>
<td>30</td>
<td>8.83</td>
<td>5.75</td>
</tr>
<tr>
<td>40</td>
<td>8.47</td>
<td>5.91</td>
</tr>
</tbody>
</table>

H0*: Keeping the sucuk batter at 4 °C overnight. Values are the average of 2 replicates. No significant difference was found between the experimental groups (P>0.05).

**The Changes in pH and Moisture Level in Sucuk**

In the experimental and control groups, moisture values, which were initially 59.19% and 59.22%, showed a rapid decrease especially during the drying period. (Table 6). On the 8th day, moisture value was determined to be 39.82% in the experimental group and 35.88% in the control group. On the 40th day, moisture value was determined to be 33.78% in the experimental group and 29.42% in the control group. The difference between the moisture levels of the experimental and control groups was statistically not significant (P>0.05). It was determined that there was a positive interaction between pH value and moisture level, the moisture decreased with the decrease in pH (P<0.05). It was seen that the survival level of *L. monocytogenes* decreased as the moisture level decreased (P<0.05). Kaya and Gökalp (2004) reported that while the moisture content of the sucuk produced by using starter culture was between 59.38% and 60.11% at the beginning of ripening period, this value was between 36.72- 37.34% on the 12th day of ripening period. Dalmış ve Soyer (2008) stated that, the moisture content of starter cultured sucuk was 60.12% at the beginning of ripening period this value was decreased to 39.5% on the 9th day of ripening period. In this study, while moisture value was measured 59.19-59.22% in beginning of ripening period; this value was measured 39.82-35.88% on the 8th day of ripening period.

It was observed that the pH of the experimental group decreased to 5.78 in the sucuk batter and while it decreased to 5.75 in the sucuk batter in the control group with the onset of fermentation. Changes were determined in pH values during the ripening and storage periods, however no significant difference was found between the experimental groups (Table 6). Similar to this study, the decrease in pH due to lactic acid bacteria which increased during the fermentation process was also determined by many researchers. Hampikyan and Uğur (2007) stated that the initial pH value in fermented sucuk was 5.87-5.90 and the pH reached 4.72-4.82 on the 30th day of the ripening. According to Yıldız-Turp and Serdaroğlu (2008), the pH value of fermented sucuk with an initial pH of 5.49-5.59 reached 4.60-4.82 on the 12th day of ripening period. In the study conducted by Erkmen (2009), it was stated that in the sucuk produced using starter culture, during the fermentation, pH decreased rapidly and reached the lowest level on day 3 (pH 4.82-4.92).
Table 6. pH values and moisture levels (%) of the sucuk samples inoculated with *L. monocytogenes*

<table>
<thead>
<tr>
<th>Days</th>
<th>Acid-adapted</th>
<th>Nontreated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
<td>% Moisture</td>
</tr>
<tr>
<td>HO*</td>
<td>5.78</td>
<td>59.19</td>
</tr>
<tr>
<td>Ripening</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>5.80</td>
<td>57.15</td>
</tr>
<tr>
<td>1</td>
<td>4.65</td>
<td>56.11</td>
</tr>
<tr>
<td>2</td>
<td>4.42</td>
<td>54.35</td>
</tr>
<tr>
<td>3</td>
<td>4.56</td>
<td>50.21</td>
</tr>
<tr>
<td>4</td>
<td>4.54</td>
<td>48.20</td>
</tr>
<tr>
<td>6</td>
<td>4.48</td>
<td>43.01</td>
</tr>
<tr>
<td>Storage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>4.61</td>
<td>39.82</td>
</tr>
<tr>
<td>15</td>
<td>4.74</td>
<td>35.37</td>
</tr>
<tr>
<td>30</td>
<td>4.76</td>
<td>34.39</td>
</tr>
<tr>
<td>40</td>
<td>4.74</td>
<td>33.78</td>
</tr>
</tbody>
</table>

H0*: Keeping the sucuk batter at 4 °C overnight. Values are the average of 2 replicates. No significant difference was found between the experimental groups (P>0.05).

Conclusion

In this study, it was determined that *L. monocytogenes* ATCC 6644 was adapted to acid by exposure to moderately acidic (pH 4.5) conditions and can survive at certain levels in highly acidic environments which are lethal for the bacteria without acid adaptation. However, acid adaptation did not cause an increase in the *L. monocytogenes* counts in sucuk. Acid adapted pathogens, such as *L. monocytogenes*, pose a risk to food safety and human health. These pathogens taken through the food have resistance to gastric acidity. The virulence of these pathogens increases and the infective doses decrease. They also gain resistance to other environmental stresses such as high temperature, salinity, cold storage and freezing-thawing. Therefore, it is necessary to reconsider the preservation methods used in the food industry.

References


Compliance with Ethical Standard

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

Acknowledgement: This research includes a part of the PhD Thesis entitled “The determination of Acid and Salt Adapted *Escherichia coli* O157: H7 and *Listeria monocytogenes* in Turkish Sucuk”. The part of this study related to *E. coli* O157:H7 published as an article.


https://doi.org/10.1016/j.ijfoodmicro.2013.11.013
PMid:24296256

Guariglia-Oropeza, V., Orsi, R.H., Guldimann, C., Wiedmann, M., Boor, K.J. (2018). The *Listeria monocytogenes* bile stimulon under acidic conditions is characterized by strain-specific patterns and the upregulation of motility, cell wall modification functions, and the prfa regulon. *Frontiers in Microbiology*, 9(120), 1-18. 
https://doi.org/10.3389/fmicb.2018.00120
PMid:29467736 PMCid:PMC5808219

https://doi.org/10.1016/j.meatsci.2006.11.014
PMid:22064303


https://doi.org/10.1371/journal.pone.0180123
PMid:28662112 PMCid:PMC5491136

https://doi.org/10.4081/ijfs.2017.5916
PMid:28713785 PMCid:PMC5505079

https://doi.org/10.1016/j.ijfoodmicro.2017.01.015
PMid:28189900


https://doi.org/10.1111/jfpp.12840


PMid:14660405 PMCid:PMC309912

https://doi.org/10.1111/j.1472-765X.2004.01491.x
PMid:15214733

https://doi.org/10.1128/jb.176.5.1422-1426.1994
PMid:8113183 PMCid:PMC205208


https://doi.org/10.9734/BMRJ/2016/27272

https://doi.org/10.1016/S0168-1605(02)00249-0

https://doi.org/10.1016/j.meatsci.2007.07.013
PMid:22062464
DETERMINATION OF THE BEST FUNCTIONAL CHICKPEA CULTIVARS BY TOPSIS TECHNIQUE

Levent Yurdaer Aydemir , Fatma Gizem Akçakaya


ABSTRACT

Technique for order preference by similarity to ideal solution (TOPSIS) analysis was firstly applied to rank the most suitable registered chickpea cultivars among (12×3=36 samples) alternatives based on their functional properties. Chickpeas were grown in controlled trial fields of state research institutes in Adana (in 2014-2015) and Erzurum (2015) regions which had mild-hot and cold climate conditions, respectively. Total phenolic (TPC) and water-soluble protein (WSPC) contents, free radical scavenging (FRSA) and iron chelating (ICA) activities, and water binding (WBC) and oil binding (OBC) capacities of extracts were determined. Equal weights were assigned for the parameters in TOPSIS application and the distances of each alternative from ideal positive and negative solution points and closeness coefficients were determined. Considerable variations were observed for TPC, FRSA and ICA. The average values of determined parameters in each group (location, year, location and year) were close to each other. Significant low positive correlations were not determined between TPC, FRSA and ICA while any significant correlations were determined between the WSPC, OBC, and WBC (P˂0.05). Aydın cultivar had the highest score for its antioxidant and technical functions (closeness coefficient was 7.02E-01) and followed by Çakır (5.59E-01) and Azkan (4.91E-01). This study showed the suitability of TOPSIS analysis in agriculture and food science area when the sample number was high and many different properties of samples were considered.

Keywords: Chickpea, Antioxidant activity, Water absorption, Oil absorption, TOPSIS
Introduction

Chickpea (Cicer arietum) is one of the most important pulse crops with high carbohydrate and dietary fiber content, considerable protein content and of various minerals (Bibi et al., 2007; Özter et al., 2010; Mafakheri et al., 2011; Torutaeva et al., 2014; Çelik et al., 2016). Due to its high nitrogen utilization efficiency and high protein yield under drought conditions, chickpea is mostly grown in arid or semiarid Mediterranean environment of West Asia and North Africa and adopted in North America, western Canada, Australia, New Zealand, and Central Europe (Oweis et al., 2004; Özter et al., 2010; Ozkilinc et al., 2011; Atalay and Babaoglu, 2012; Siddique et al., 2012; Neugschwandtner et al., 2015; Sadras and Drecce, 2015). However, there are some challenges to develop new chickpea varieties due to its restricted genetic variations, many registered cultivars have been planting around the world (Mafakheri et al., 2011; Atalay and Babaoglu, 2012; Siddique et al., 2012). Due to its suitable climatic conditions, Turkey is the fifth biggest producer of chickpea after India, Australia, Myanmar, and Ethiopia (FAO, 2012). In the market high yield registered chickpea cultivars resistant or tolerant to biotic and abiotic stress factors are being grown and consumed as flour, canned, roasted, boiled, fermented, fried steamed, or snack food (Coskuner and Karababa, 2004; Bibi et al., 2007; Ozter et al., 2010; Celik et al., 2016). The studies also showed that chickpea seeds had good functional properties which allowed them to be used as additive in processed foods, cosmetics and pharmaceuticals. Aydemir and Yemencigiolu (2013) compared the functional properties of chickpea globulins with commercially produced soy protein isolate and concentrate, whey protein isolate, fish gelatine, bovine gelatine, and egg white protein and they reported that chickpea globulins had the potential to be used as functional protein source alternative to those commercial proteins due to their higher water and oil absorption capacities, better gelation properties, and more stable emulsion and foam formation abilities (Aydemir and Yemencigiolu, 2013). Chickpea extracts had also showed considerable antioxidant activity based on free radical scavenging and metal chelating properties which were associated with better food quality protection and health benefits (Zhao et al., 2014; Kou et al., 2015; Torres-Fuentes et al., 2015).

In this study functional properties of 12 registered chickpea cultivars were grown in different locations in different growing seasons were determined. Although the climate conditions and seasonal variances were highly effective on physical and chemical properties on the same cultivars, it was aimed to determine the best cultivars with high functional properties. 6 different criteria were determined and measured associated with the functional properties of chickpeas but to evaluate the results was difficult because one sample might be preferred regarding one functional property (such as antioxidant activity), the other sample might be preferred considering the other functional property (such as water absorption capacity) (Ozturk et al., 2014). In order to overcome this difficulty, multi criteria decision methods could be applied to evaluate the results and to determine the best cultivars which had different functional properties. Multi criteria decision methods are used for the evaluation of alternatives based on determined criteria by using a number of qualitative and/or quantitative criteria (Ozcan et al., 2011). Different types of multi criteria decision methods have been applied in different studies and among them TOPSIS (technique for order preference by similarity to ideal solution) technique is one of methods which is widely used to obtain decision hierarchy by making pairwise comparison between criteria. In TOPSIS method, positive and negative ideal solutions are calculated, and the best alternative is determined which is nearest to the positive ideal solution and farthest from the negative ideal solution (Lin et al., 2008; Balli and Korukoglu, 2009). Although TOPSIS technique have been extensively used in many different areas (management, computer, electrical sciences, etc.), only a few numbers of studies using this technique are found in food science literature. Mostly researchers used TOPSIS technique for optimization of new food formulations such as cheese nuggets, vegetable juice, prebiotic pudding, hot chocolate beverage, and milk based herbal tea. (Gurmeric et al., 2013; Ozturk et al., 2014; Ansarifar et al., 2015; Dogan et al., 2016, 2018; Gul and Dervisoglu, 2017). Kou et al., (2015) and Sun et al., (2011) were also applied TOPSIS technique to determine the best alternatives among different jujube cultivars based on their bioactive properties such as phe nolic content or antioxidant activity (Sun et al., 2011; Kou et al., 2013).

In this study TOPSIS technique was applied to determine the best registered chickpea cultivars among 36 samples with high functional properties such as free radical scavenging and iron chelating activity, water and oil binding capacity, soluble protein content and total phenolic content which were grown in two different locations (Adana and Erzurum) or two different years (2014 and 2015).

Materials and Methods

Materials

12 registered chickpea seeds were kindly provided from Dr. Dürdane Mart from Eastern Mediterranean Agricultural Research Institute, Adana, TURKEY. Registered chickpea cultivars were abbreviated as follow: Aksu, Arda, Aydin, Azkan, Çakır, Diyar, Gülümser, Hasanbey, Ilgaz, İzmir, Inci, Seçkin...
as AK, AR, AY, AZ, CA, DI, GU, HA, IL, IZ, IN, SE, respectively. Location of Adana and Erzurum were abbreviated as A and E while grown year of 2014 and 2015 were abbreviated as 14 and 15 as suffix for cultivar name, respectively. Example: AKA14 was an abbreviation AKSU-ADANA-2014 that meant Aksu cultivar grown in Adana location in 2014. The chemicals used in the study were listed as Folin Ciocalteu’s reagent, K₂O₅S₂, NaH₂PO₄, Na₂HPO₄, NaCl, Na₂CO₃, (±)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (trolox), 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p'-disulfonic acid monosodium salt hydrate (FerroZine) and FeCl₂ which were purchased from Merck KGaA (Germany), and ethylene diamine tetraacetic acid (EDTA), CuSO₄, Na-K tartrate, NaOH, gallic acid, sodium caseinate, 2, 2'-Azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) di-ammonium salt (ABTS) which were purchased from Sigma-Aldrich (Germany).

**Determination of Water and Oil Binding Capacity of Chickpea Flours**

The water (WBC) and oil binding capacities (OBC) of chickpea flour were determined by interacting 50 mg of chickpea flour and 1.5 mL of liquid (distilled water or commercial sunflower oil) for 30 minutes at room temperature after mixing in a test tube for 20 seconds. After incubation, free liquid phase was separated by centrifugation (15000 × g, 25 °C, 20 min) (Aydemir et al., 2014). The absorbed liquid content was calculated as average of three measurement and WBC and OBC of flour samples were expressed as g liquid/g dry weight flour. Total moisture content of chickpea flours was measured with moisture analyser (Ohaus MB 45, Switzerland).

**Preparation of Water Soluble Chickpea Extracts**

500 mg of chickpea flour were stirred in 5 mL deionized water in orbital shaker overnight about 18-20 hours at 25 °C to maximize the extraction of water soluble components in chickpea flour (pH of the solution was 6.5 ±0.2). Then the suspensions were centrifuged, and clear supernatants were separated and named as chickpea soluble extract (15000×rcf, 25°C, 30 min) (Aydemir et al., 2014).

**Determination of Water Soluble Protein Content of Chickpea Extracts**

The water-soluble protein content (WSPC) of chickpea extracts was spectrophotometrically determined by using Lowry method (Lowry et al., 1951). 0.2 mL of chickpea extract were reacted with 2.1 mL of Lowry reactive for 10 min. Lowry reactive was prepared with 245 mL of 2% (w/v) Na₂CO₃, 2.5 mL of 1% (w/v) CuSO₄.5H₂O and 2.5 mL 1% (w/v) Na-K tartrate dissolving in 0.1 mol/L NaOH solution. Then 0.2 mL of 10-fold diluted Folin Ciocalteu’s reagent was added into the mixture and further incubated for 1 hour at ambient temperature in dark conditions. The absorbances of the test samples were determined at 750 nm and WSPC results were expressed as sodium caseinate equivalents (mg of caseinate / g dry seed).

**Determination of Total Phenolic Content of Chickpea Extracts**

Total phenolic content (TPC) of chickpea extracts were determined spectrophotometrically by using Folin Ciocalteu’s reagent as described by Aydemir et al. (2013) (Aydemir and Yemencioğlu, 2013). Firstly, 400 μL of chickpea extract were reacted with 1000 μL of 10-fold diluted Folin Ciocalteu’s reagent (in distilled water) for 3 minutes and then 800 μL of 7.5% (w/v) Na₂CO₃ were added into the mixture and further incubated for 2 hours at room temperature in dark conditions. The absorbances of the test samples were determined at 765 nm and TPC results as average of three different sample measurements were expressed as gallic acid equivalents (μg of GA/g dry seed).

**Determination of Free Radical Scavenging Activity of Chickpea Extracts**

The free radical scavenging activity (FRSA) of chickpea extract was spectrophotometrically determined by measuring the inhibition of ABTS radical cations by antioxidants in chickpea extract for 6 minutes (Re et al., 1999). Firstly, 7 mmol/L ABTS radical solution was prepared by dissolving ABTS in 2.45 mmol/L K₂O₅S₂ and left for incubation about 16-18 h at room temperature in dark conditions. Before the tests, absorbance of the solution was set 0.700 ±0.020 at 734 nm diluting with 75 mmol/L phosphate buffer saline containing 150 mmol/L NaCl, pH 7.4. Then, 0.1 mL chickpea extract was reacted with 1.9 mL ABTS radical solution and the absorbance of the mixture was read at 6th minutes. The % inhibition of ABTS radical cation was determined by calculating the differences between absorbance read at 6th min and absorbance set for the ABTS solution. The FRSA results of the test samples were average of three different sample measurements and were expressed as trolox equivalents (μmol Trolox/g dry seed).

**Determination of Iron Chelating Activity of Chickpea Extracts**

The iron chelating activity (ICA) of chickpea extract was spectrophotometrically determined according to the method described in Aydemir et al. (2014) (Aydemir et al., 2014). Firstly, 2 mL of chickpea extract was reacted with 0.1 mL of 1 mmol/L FeCl₂.4H₂O solution and for 30 minutes at room temperature in dark conditions. Then, 0.1 mL of 0.5 mmol/L 

---

**Determination of Water Soluble Protein Content of Chickpea Extracts**

0.2 mL of chickpea extract was spectrophotometrically determined by using Lowry method (Lowry et al., 1951). 0.2 mL of chickpea extract were reacted with 2.1 mL of Lowry reactive for 10 min. Lowry reactive was prepared with 245 mL of 2% (w/v) Na₂CO₃, 2.5 mL of 1% (w/v) CuSO₄.5H₂O and 2.5 mL 1% (w/v) Na-K tartrate dissolving in 0.1 mol/L NaOH solution. Then 0.2 mL of 10-fold diluted Folin Ciocalteu’s reagent was added into the mixture and further incubated for 1 hour at ambient temperature in dark conditions. The absorbances of the test samples were determined at 750 nm and WSPC results were expressed as sodium caseinate equivalents (mg of caseinate / g dry seed).

**Determination of Total Phenolic Content of Chickpea Extracts**

Total phenolic content (TPC) of chickpea extracts were determined spectrophotometrically by using Folin Ciocalteu’s reagent as described by Aydemir et al. (2013) (Aydemir and Yemencioğlu, 2013). Firstly, 400 μL of chickpea extract were reacted with 1000 μL of 10-fold diluted Folin Ciocalteu’s reagent (in distilled water) for 3 minutes and then 800 μL of 7.5% (w/v) Na₂CO₃ were added into the mixture and further incubated for 2 hours at room temperature in dark conditions. The absorbances of the test samples were determined at 765 nm and TPC results as average of three different sample measurements were expressed as gallic acid equivalents (μg of GA/g dry seed).

**Determination of Free Radical Scavenging Activity of Chickpea Extracts**

The free radical scavenging activity (FRSA) of chickpea extract was spectrophotometrically determined by measuring the inhibition of ABTS radical cations by antioxidants in chickpea extract for 6 minutes (Re et al., 1999). Firstly, 7 mmol/L ABTS radical solution was prepared by dissolving ABTS in 2.45 mmol/L K₂O₅S₂ and left for incubation about 16-18 h at room temperature in dark conditions. Before the tests, absorbance of the solution was set 0.700 ±0.020 at 734 nm diluting with 75 mmol/L phosphate buffer saline containing 150 mmol/L NaCl, pH 7.4. Then, 0.1 mL chickpea extract was reacted with 1.9 mL ABTS radical solution and the absorbance of the mixture was read at 6th minutes. The % inhibition of ABTS radical cation was determined by calculating the differences between absorbance read at 6th min and absorbance set for the ABTS solution. The FRSA results of the test samples were average of three different sample measurements and were expressed as trolox equivalents (μmol Trolox/g dry seed).

**Determination of Iron Chelating Activity of Chickpea Extracts**

The iron chelating activity (ICA) of chickpea extract was spectrophotometrically determined according to the method described in Aydemir et al. (2014) (Aydemir et al., 2014). Firstly, 2 mL of chickpea extract was reacted with 0.1 mL of 1 mmol/L FeCl₂.4H₂O solution and for 30 minutes at room temperature in dark conditions. Then, 0.1 mL of 0.5 mmol/L
ferrozine was added into the solution and further incubated for 10 minutes. The absorbance of test samples was determined at 562 nm and ICA results of average of three different sample measurements were expressed as EDTA equivalents (μmol of EDTA/g dry seed).

**TOPSIS Comprehensive Evaluation Method**

TOPSIS method was applied to determine the best chickpea samples based on grown location, grown year, and all samples (Ozturk et al., 2014). The steps of TOPSIS method were as follow: In step 1, the normalized decision matrix was established by the following equation

\[ x_{ij} = a_{ij}/\left(\sum_{(k = 1)^{m}a_{kj}}^{}\right)^{2} \]  

where \( x_{ij} \) is the normalized value and \( a_{ij} \) is the real value of the criteria. In step 2, the weighted normalized decision matrix was calculated using the following equation

\[ v_{ij} = x_{ij} \times w_{ij} \]  

where \( v_{ij} \) is the weighted normalized value and \( w_{ij} \) is the weight of the criteria. In this study equal weight was assigned for each criteria. In step 3, the positive and negative ideal solutions are determined

\[ S^{+} = \{v_{1+}, v_{2+}, v_{3+}, \ldots, v_{n+}\} \]  

\[ S^{-} = \{v_{1-}, v_{2-}, v_{3-}, \ldots, v_{n-}\} \]  

where \( S^{+} \) is the maximum values and \( S^{-} \) is the minimum values.

In step 4, the distance of each alternative from the positive and negative ideal solution is calculated according to the following equations

\[ d_{i}^{+} = \left(\sum_{i}^{n}(v_{ij} - v_{ij})\right)^{2} \]  

\[ d_{i}^{-} = \left(\sum_{i}^{n}(v_{ij} - v_{ij})\right)^{2} \]  

where \( d_{i}^{+} \) and \( d_{i}^{-} \) is the distance of alternative from positive and negative ideal solution, respectively. In step 5, the closeness coefficient of each alternative \( C \) is obtained using following equation

\[ C = (d_{i}^{-})/(d_{i}^{+} + d_{i}^{-}) \]  

In step 6, the ranking of alternatives is determined based on their \( C \) values.

**Statistical Analysis**

Analysis of variances (ANOVA) and correlations were done using Minitab 17 software (Minitab Ltd., United Kingdom).

**Results and Discussion**

**Functional Properties of Chickpea Cultivars**

Registered chickpea cultivars were grown in Adana and Erzurum regions which had mild and cold climate conditions, respectively. Annual average temperature and total rainfall were 18.9 °C and 646.6 mm in Adana and (1927-2016); 5.7 °C and 432.8 mm for Erzurum (1929-2016). In Adana region, the chickpeas were grown in 2014 and 2015 while for Erzurum region the harvest year was only 2015. The growth of chickpeas in Adana region in successive years provided the chance of better comparison of some functional properties of chickpea cultivars by minimizing the effects of harvest year variations on functional properties while the growth of chickpeas in Adana and Erzurum regions at the same year provided the chance of better comparison of those properties by minimizing the effects of harvest location and climate variations. On the other hand, these conditional differences also gave the opportunity to determine the effects of different harvest locations and years on considered properties of chickpea cultivars.

The chickpea extracts used in the study were obtained by using water as a solvent. Generally organic solvents such as methanol, ethanol, acetone, or their aqueous solutions are used for sample extractions to determine phenolic content and antioxidant activity because organic solvents provide better phenolic extractions from food samples and mostly those phenolic compounds are the main contributors to the antioxidant activity of that food sample. However, organic solvents provide better phenolic extraction they require additional steps and increase cost in food processing since organic solvents are toxic and risk for human health and being not acceptable for food industry (Durante et al., 2014; Hou et al., 2016). Therefore, deionised water was used as sole solvent in this study. The production of water soluble chickpea extracts was also easy, cheap, and completely safe. In addition, to interpret data obtained from analysis were closer to the potential real food process applications. The previous study conducted our group has also reported that water extraction of chickpea samples yielded more total phenolic content than the samples extracted by ethanol, acetone, and acidified acetone (Dıblan et al., 2018). In that study, it was seen that Folic-Ciocaliceu method measured more phenolic contents in water extracts of legumes than organic extracts since the water soluble proteins made contribution to the results due to their amino acid residues containing aromatic ring. The bands belonging soluble proteins were only determined in water extracts in legumes according the FT-IR characteriza-
tion. Moreover, some phenolic compounds can be found either free or complexed form with proteins. When the water extraction was applied to the legumes, protein-phenolic complexes might become soluble in water extracts which were not be soluble in organic extracts.

Considerable variations in each parameter were determined between the cultivars in Adana 2014, Adana 2015, and Erzurum 2015 (P<0.05). The differences between chickpea extracts were broader in their TPC, ICA and FRSA values which were more associated with antioxidant activity. Antioxidant in legumes had the potential to be used as additive in food formulas to prevent lipid oxidation and food supplement (Escarpa and Gonzalez, 2001). On the other hand, less variations between chickpea extracts were determined in their WSPC, WBC, and OBC values which were more associated with their technological properties during food processing because these properties are related to their foaming, emulsifying and gelling properties (Aydemir and Yemenicioglu, 2013). The average values of TPC, ICA, FRSA, WSPC, WBC, and OBC of chickpea seeds grown in Adana 2014 were 1955 ±260 μg GA/g, 13.0 ±4.7 μmol EDTA/g, 20.4 ±3.8 μmol Trolox/g, 72 ±5 mg caseinate/g, 2.88 ±0.38 g/g, and 0.95 ±0.19 g/g; those of grown in Adana 2015 were 1875 ±220 μg GA/g, 9.5 ±5.4 μmol EDTA/g, 19.9 ±2.2 μmol Trolox/g, 61±9 mg caseinate/g, 2.85 ±0.34 g/g, and 0.88±0.12 g/g; those of grown in Erzurum 2015 were 1930 ±214 μg GA/g, 11.5 ±3.2 μmol EDTA/g, 21.5 ±2.7 μmol Trolox/g, 67 ±8 mg caseinate/g, 2.47 ±0.31 g/g, and 0.96 ±0.17 g/g, respectively. AYA14 cultivar was one of the prominent chickpea samples with its high TPC, ICA, FRSA and WSPC values (P<0.05). According to ANOVA results, chickpea samples grown in Adana 2014 had better functional properties than those of cultivars grown in Adana and Erzurum 2015. On the other hand, the lowest functional properties were mostly owned by the cultivars grown in Adana 2015. Any statistical differences were not observed between the average values of each criterion had by chickpea extracts when classified as Adana 2014, Adana 2015 and Erzurum 2015 (P<0.05). When the functional properties of chickpea extracts were evaluated for their harvest location and harvest year, the variations between the cultivars in each criterion were decreased even any statistical differences were not observed in WBC of chickpea cultivars grown in 2015 (P<0.05). This situation made more difficult to decide the best cultivars with good functional properties. Because the functional property values of chickpea extracts were similar to each other and between these values significant differences mostly did not observed. Diblan et al., (2018) investigated the effects of different solvents on TPC of chickpea extracts and reported that water extraction provided the highest phenolic content (1829 ±12 μg GAE/g that was similar to our findings) than ethanol (1478 ±79 μg GAE/g), acetone (875 ±21 μg GAE/g) and acidified acetone (729 ±24 μg GAE/g) extraction methods (Diblan et al., 2018). Arab, Helmy, and Bareh (2010) measured the WBC and OBC of chickpea flours to be used in functional pasta production and determined the similar OBC values but lower WBC values than our findings (Arab et al., 2010). It is common to see some differences in functional properties of chickpea flours due to cultivar variations. In the literature mostly, aqueous organic solvents such as methanol, ethanol, acetone, hexane, etc. were mostly used for chickpea extraction. The reported TPC values were between 0.45 and 10.84 mg GAE/g flour which were similar to our findings and FRSA were 1.26 ±0.09 μmol TE/g, 31.4 ±1.4 μg/mL (IC50), and 22.85 ±0.25 (% inhibition) which were the lower than our findings (Srirama et al., 2012; Jogi halli et al., 2017; Rocchetti et al., 2017; Xu et al., 2017). OBC of registered cultivar flours were similar to the results reported in the literature whereas WBC were found mostly higher than those of literature. OBC was varied from 0.85 to 1.25 g/g and WBC was between 0.89 and 2.30 g/g (Kaur and Singh, 2005; Joshi et al., 2007; Xu et al., 2014, 2017; Jogi halli et al., 2017). Unfortunately, metal chelating activity of chickpea flour extract could not be obtained from the reachable literature. Some studies were also investigated the functional properties of chickpea proteins where the water was used for protein extraction (Arcan and Yemenicioglu, 2007, 2010; Yust et al., 2010; Aydemir and Yemenicioglu, 2013; Mokni Ghribi et al., 2015; Torres-Fuentes et al., 2015; Jogihalli et al., 2017). For chickpea protein extraction, alkali conditions were generally created by using chemicals such as NaOH, KOH, etc. and additional centrifugation steps and drying processing (lyophilization) were employed. Aydemir and Yemenicioglu (2013) determined the TPC, WSPC, WBC, and OBC of four different chickpea globulin proteins (Aydemir and Yemenicioglu, 2013). They found that chickpea proteins had higher TPC, WBC and OBC by 4, 2, and 14 times. Arcan and Yemenicioglu (2007) applied heat treatment to chickpeas to determine the effect of heat to the antioxidant properties of chickpeas and measured FRSA and ICA values of protein extracts. The measured values were considerably higher than our values because antioxidant proteins were concentrated on chickpea proteins due to bound phenolics and electron transferring groups on amino acids to free radicals (Arcan and Yemenicioglu, 2007).

In order to determine the best chickpea cultivars with good functional properties, 36 alternatives were ranked based on each functional criterion. The rankings were completely different from each other. The first three rankings for TPC was DIA14, AYA14, AYE15; for ICA was AYA14, AZA15, DIA14; for FRSA was ILE15, DIA14, AYA14; for WSPC was AYA14, AYE15, SEA15; for WBC was HAA14,
AKA15, ILA15; for OBC was CAE15, ARA14, ARE15. This ranking which had 36 alternatives in each criterion made the decision more difficult because in practical application the main objective of this breeding program was to test the chickpea cultivars in different growing conditions such as location and year. Among tested cultivars, high quality and productive samples would be chosen and announced as the primary cultivars to be grown. For this reason, it looked more economical to choose the cultivars that can be grown in different conditions with high quality. The functional properties analysed in this study were the tools that might attach higher importance to the cultivars for value added product production such as natural additive, food supplement or etc.

For this reason, a new ranking of 12 registered cultivars were done by using the average values of the same chickpea cultivar for each criterion (for example: average value of Aksu cultivar in TPC criterion was calculated by averaging TPC of Aksu extract in Adana 2014, Adana 2015, and Erzurum 2015). However, Aydın cultivar had the first rank in TPC, ICA, FRSA, and WSPC, it was still difficult to decide the best cultivars because the rankings were again completely different in each criterion. It was Aydın, Diyar, Gülümser for TPC; Aydın, Azkan, Çakır for ICA; Aydın, Diyar, Azkan for FRSA; Aydın, Seçkin, Ilgaz for WSPC; Hasanbey, Ilgaz, İzmir for WBC; and Çakır, İnci, Arda for OBC. All of these challenges were considered, the best way was to apply one of the multi criteria decision techniques to decide the best cultivars with good functional properties.

**TOPSIS Comprehensive Evaluation for Ranking Cultivars**

In order to determine the best chickpea cultivars with good functional properties, TOPSIS, multi criteria decision technique, was applied for 12 alternatives considering 6 criteria. Alternatives were the cultivars: Aksu, Arda, Aydın, Azkan, Çakır, Diyar, Gülümser, Hasanbey, Ilgaz, İnci, İzmir, and Seçkin. Criteria were TPC, ICA, FRSA, WSPC, WBC, and OBC. The TOPSIS evaluation was used for three purposes: to determine the best cultivars (alternatives) grown in i) only Adana region, ii) in 2015, iii) all location and harvest years. The average values of the same chickpea cultivars grown in different location and years were calculated. After decision matrix was constructed, the normalized decision matrix was constructed (Table 1). This technique gives the researcher the advantage of being involved in the analysis process by assigning “weight” to the criteria considering the importance of the criteria. In this study equal weights were assigned to each criterion as 0.17 (total weight should be 1.00 for 6 criteria). Because it was aimed to determine the best chickpea cultivars which were good at in all functional properties. However, different weights could be assigned according to the purposes.

For example, if someone aimed to determine the cultivars good at more antioxidant properties, the weights would be assigned higher for FRSA, ICA, and TPC than WSPC, WBC, OBC. On the other hand, if the aim was to determine the cultivars good at more technological properties such as WSPC, WBC, and OBC, the higher weights would be assigned for these criteria than TPC, ICA, FRSA. The weighted normalized decision matrix was given in Table 2. According to the weighted normalized decision matrix, positive (S⁺) and negative (S⁻) ideal solutions for each criterion were determined in Table 3. These ideal solutions were important for TOPSIS technique because the distances of alternatives (chickpea cultivars) from these points are used in the analysis to rank the alternatives. The being closest to the positive ideal solution and farthest to the negative ideal solution were associated with the closeness coefficient of alternatives (Table 4). According to the closeness coefficient of alternatives, the first three rank was Aydın, Azkan, and Çakir cultivars among those grown in only Adana region (closeness coefficients varied from 1.75E-01 to 7.02E-01); Çakır, Seçkin, Azkan cultivars among those grown in 2015 (closeness coefficients varied from 1.89E-01 to 7.33E-01); Aydın, Çakır, and Azkan cultivars among those all grown in all locations and harvest years (closeness coefficients varied from 1.75E-01 to 7.02E-01). According to three TOPSIS analysis, İnci and Gülümser cultivars were the worst samples with the lowest closeness coefficients.

However, many decision problems including multi criteria have been encountered in food science area, it is not very common to use multi criteria decision techniques to solve the problems. In food science, the researchers were benefited from TOPSIS technique in food for either optimization of new food formulations or to determine the best alternatives among the samples (Gurmeric et al., 2013; Ozturk et al., 2014; Ansarifar et al., 2015; Dogan et al., 2016, 2018; Gul and Dervisoglu, 2017). Similar to our study, Kou et al. (2015) applied TOPSIS technique to evaluate the nutrition of 15 different jujube cultivars (alternatives) based on their total flavonoids, proanthocyanidins, ascorbic acid, total triterpene, total polyphenol, total polysaccharide, cAMP (7 criteria) values and reported that TOPSIS technique was an efficient ranking method (Kou et al., 2015). Sun et al. (2011) ranked the 10 batches of sour jujube fruits based on their polyphenols, flavonoids, anthocyanins, saponins, alkaloids, polysaccharides, carotenoids, vitamin C and selenium contents and concluded that TOPSIS method can be efficiently utilised in the assessment of total natural antioxidant content and quality of sour jujube fruits (Sun et al., 2011).
Table 1. Normalized decision matrix

<table>
<thead>
<tr>
<th>Alternatives</th>
<th>TPC</th>
<th>ICA</th>
<th>FRSA</th>
<th>WSPC</th>
<th>WBC</th>
<th>OBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>All samples</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aksu</td>
<td>0.2828</td>
<td>0.2409</td>
<td>0.2767</td>
<td>0.2727</td>
<td>0.2850</td>
<td>0.2300</td>
</tr>
<tr>
<td>Arda</td>
<td>0.2884</td>
<td>0.2788</td>
<td>0.3037</td>
<td>0.2619</td>
<td>0.2914</td>
<td>0.3202</td>
</tr>
<tr>
<td>Aydın</td>
<td>0.3201</td>
<td>0.4254</td>
<td>0.3051</td>
<td>0.3302</td>
<td>0.2663</td>
<td>0.2732</td>
</tr>
<tr>
<td>Aşkın</td>
<td>0.2804</td>
<td>0.3591</td>
<td>0.3041</td>
<td>0.2595</td>
<td>0.2789</td>
<td>0.2421</td>
</tr>
<tr>
<td>Çakır</td>
<td>0.2811</td>
<td>0.3168</td>
<td>0.2655</td>
<td>0.2840</td>
<td>0.2943</td>
<td>0.3567</td>
</tr>
<tr>
<td>Diyar</td>
<td>0.3146</td>
<td>0.2795</td>
<td>0.3048</td>
<td>0.2922</td>
<td>0.2653</td>
<td>0.2811</td>
</tr>
<tr>
<td>Gülümser</td>
<td>0.3083</td>
<td>0.2133</td>
<td>0.2976</td>
<td>0.2901</td>
<td>0.2725</td>
<td>0.3046</td>
</tr>
<tr>
<td>Hasanbey</td>
<td>0.2674</td>
<td>0.2333</td>
<td>0.2723</td>
<td>0.2834</td>
<td>0.3262</td>
<td>0.2698</td>
</tr>
<tr>
<td>Iğdır</td>
<td>0.2895</td>
<td>0.2609</td>
<td>0.2862</td>
<td>0.2967</td>
<td>0.3114</td>
<td>0.2564</td>
</tr>
<tr>
<td>İnci</td>
<td>0.2771</td>
<td>0.2233</td>
<td>0.3001</td>
<td>0.2955</td>
<td>0.2932</td>
<td>0.3283</td>
</tr>
<tr>
<td>İzmir</td>
<td>0.2533</td>
<td>0.2993</td>
<td>0.2570</td>
<td>0.2721</td>
<td>0.3040</td>
<td>0.2965</td>
</tr>
<tr>
<td>Seçkin</td>
<td>0.2942</td>
<td>0.2616</td>
<td>0.2855</td>
<td>0.3175</td>
<td>0.2685</td>
<td>0.2795</td>
</tr>
</tbody>
</table>

**Adana in 2014 and 2015**

<table>
<thead>
<tr>
<th>Alternatives</th>
<th>TPC</th>
<th>ICA</th>
<th>FRSA</th>
<th>WSPC</th>
<th>WBC</th>
<th>OBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aksu</td>
<td>0.2685</td>
<td>0.2583</td>
<td>0.2689</td>
<td>0.2648</td>
<td>0.3165</td>
<td>0.2401</td>
</tr>
<tr>
<td>Arda</td>
<td>0.2952</td>
<td>0.3156</td>
<td>0.3026</td>
<td>0.2639</td>
<td>0.3016</td>
<td>0.3028</td>
</tr>
<tr>
<td>Aydın</td>
<td>0.3112</td>
<td>0.4387</td>
<td>0.3064</td>
<td>0.3289</td>
<td>0.2593</td>
<td>0.2790</td>
</tr>
<tr>
<td>Aşkın</td>
<td>0.2796</td>
<td>0.4225</td>
<td>0.3110</td>
<td>0.2656</td>
<td>0.2792</td>
<td>0.2653</td>
</tr>
<tr>
<td>Çakır</td>
<td>0.2817</td>
<td>0.3344</td>
<td>0.2496</td>
<td>0.2700</td>
<td>0.2758</td>
<td>0.3476</td>
</tr>
<tr>
<td>Diyar</td>
<td>0.3310</td>
<td>0.2731</td>
<td>0.3041</td>
<td>0.3027</td>
<td>0.2675</td>
<td>0.2815</td>
</tr>
<tr>
<td>Gülümser</td>
<td>0.3031</td>
<td>0.1387</td>
<td>0.2967</td>
<td>0.2778</td>
<td>0.2764</td>
<td>0.3290</td>
</tr>
<tr>
<td>Hasanbey</td>
<td>0.2741</td>
<td>0.1881</td>
<td>0.2910</td>
<td>0.2685</td>
<td>0.3265</td>
<td>0.2659</td>
</tr>
<tr>
<td>Iğdır</td>
<td>0.2821</td>
<td>0.1975</td>
<td>0.2615</td>
<td>0.3034</td>
<td>0.3210</td>
<td>0.2392</td>
</tr>
<tr>
<td>İnci</td>
<td>0.2793</td>
<td>0.2441</td>
<td>0.3061</td>
<td>0.2803</td>
<td>0.2896</td>
<td>0.3310</td>
</tr>
<tr>
<td>İzmir</td>
<td>0.2694</td>
<td>0.2464</td>
<td>0.2879</td>
<td>0.2929</td>
<td>0.2924</td>
<td>0.2904</td>
</tr>
<tr>
<td>Seçkin</td>
<td>0.2823</td>
<td>0.2496</td>
<td>0.2702</td>
<td>0.3336</td>
<td>0.2462</td>
<td>0.2688</td>
</tr>
</tbody>
</table>

**Adana and Erzurum in 2015**

<table>
<thead>
<tr>
<th>Alternatives</th>
<th>TPC</th>
<th>ICA</th>
<th>FRSA</th>
<th>WSPC</th>
<th>WBC</th>
<th>OBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aksu</td>
<td>0.2692</td>
<td>0.1640</td>
<td>0.2575</td>
<td>0.2781</td>
<td>0.2915</td>
<td>0.2320</td>
</tr>
<tr>
<td>Arda</td>
<td>0.2952</td>
<td>0.2445</td>
<td>0.3183</td>
<td>0.2431</td>
<td>0.2928</td>
<td>0.2995</td>
</tr>
<tr>
<td>Aydın</td>
<td>0.3058</td>
<td>0.4121</td>
<td>0.2877</td>
<td>0.3271</td>
<td>0.2583</td>
<td>0.2705</td>
</tr>
<tr>
<td>Aşkın</td>
<td>0.2609</td>
<td>0.3492</td>
<td>0.2881</td>
<td>0.2462</td>
<td>0.2770</td>
<td>0.2172</td>
</tr>
<tr>
<td>Çakır</td>
<td>0.3047</td>
<td>0.3325</td>
<td>0.2822</td>
<td>0.3025</td>
<td>0.3007</td>
<td>0.3632</td>
</tr>
<tr>
<td>Diyar</td>
<td>0.2939</td>
<td>0.2128</td>
<td>0.2829</td>
<td>0.2878</td>
<td>0.2562</td>
<td>0.3017</td>
</tr>
<tr>
<td>Gülümser</td>
<td>0.3188</td>
<td>0.2038</td>
<td>0.2894</td>
<td>0.2877</td>
<td>0.2785</td>
<td>0.2808</td>
</tr>
<tr>
<td>Hasanbey</td>
<td>0.2795</td>
<td>0.2825</td>
<td>0.2729</td>
<td>0.2790</td>
<td>0.2918</td>
<td>0.2768</td>
</tr>
<tr>
<td>Iğdır</td>
<td>0.2893</td>
<td>0.3092</td>
<td>0.3104</td>
<td>0.3013</td>
<td>0.3095</td>
<td>0.2725</td>
</tr>
<tr>
<td>İnci</td>
<td>0.2882</td>
<td>0.1317</td>
<td>0.3045</td>
<td>0.3066</td>
<td>0.2996</td>
<td>0.3175</td>
</tr>
<tr>
<td>İzmir</td>
<td>0.2374</td>
<td>0.3451</td>
<td>0.2552</td>
<td>0.2683</td>
<td>0.3110</td>
<td>0.2813</td>
</tr>
<tr>
<td>Seçkin</td>
<td>0.3111</td>
<td>0.3352</td>
<td>0.3075</td>
<td>0.3228</td>
<td>0.2912</td>
<td>0.3218</td>
</tr>
</tbody>
</table>
Table 2. Weighted normalized decision matrix

<table>
<thead>
<tr>
<th>Alternatives</th>
<th>TPC</th>
<th>ICA</th>
<th>FRSA</th>
<th>WSPC</th>
<th>WBC</th>
<th>OBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aksu</td>
<td>0.0471</td>
<td>0.0401</td>
<td>0.0461</td>
<td>0.0454</td>
<td>0.0475</td>
<td>0.0383</td>
</tr>
<tr>
<td>Arda</td>
<td>0.0481</td>
<td>0.0465</td>
<td>0.0506</td>
<td>0.0436</td>
<td>0.0486</td>
<td>0.0534</td>
</tr>
<tr>
<td>Aydin</td>
<td>0.0533</td>
<td>0.0709</td>
<td>0.0509</td>
<td>0.0550</td>
<td>0.0444</td>
<td>0.0455</td>
</tr>
<tr>
<td>Azkan</td>
<td>0.0467</td>
<td>0.0599</td>
<td>0.0507</td>
<td>0.0432</td>
<td>0.0465</td>
<td>0.0404</td>
</tr>
<tr>
<td>Çakır</td>
<td>0.0469</td>
<td>0.0528</td>
<td>0.0443</td>
<td>0.0473</td>
<td>0.0490</td>
<td>0.0595</td>
</tr>
<tr>
<td>Diyar</td>
<td>0.0524</td>
<td>0.0466</td>
<td>0.0508</td>
<td>0.0487</td>
<td>0.0442</td>
<td>0.0469</td>
</tr>
<tr>
<td>Gülümser</td>
<td>0.0514</td>
<td>0.0356</td>
<td>0.0496</td>
<td>0.0484</td>
<td>0.0454</td>
<td>0.0508</td>
</tr>
<tr>
<td>Hasanbey</td>
<td>0.0446</td>
<td>0.0389</td>
<td>0.0454</td>
<td>0.0472</td>
<td>0.0544</td>
<td>0.0450</td>
</tr>
<tr>
<td>İlgaz</td>
<td>0.0483</td>
<td>0.0435</td>
<td>0.0477</td>
<td>0.0495</td>
<td>0.0519</td>
<td>0.0427</td>
</tr>
<tr>
<td>İnci</td>
<td>0.0462</td>
<td>0.0372</td>
<td>0.0500</td>
<td>0.0493</td>
<td>0.0489</td>
<td>0.0547</td>
</tr>
<tr>
<td>İzmir</td>
<td>0.0422</td>
<td>0.0499</td>
<td>0.0428</td>
<td>0.0453</td>
<td>0.0507</td>
<td>0.0494</td>
</tr>
<tr>
<td>Seçkin</td>
<td>0.0490</td>
<td>0.0436</td>
<td>0.0476</td>
<td>0.0529</td>
<td>0.0448</td>
<td>0.0466</td>
</tr>
</tbody>
</table>

**Adana in 2014 and 2015**

<table>
<thead>
<tr>
<th>Alternatives</th>
<th>TPC</th>
<th>ICA</th>
<th>FRSA</th>
<th>WSPC</th>
<th>WBC</th>
<th>OBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aksu</td>
<td>0.0448</td>
<td>0.0430</td>
<td>0.0448</td>
<td>0.0441</td>
<td>0.0528</td>
<td>0.0400</td>
</tr>
<tr>
<td>Arda</td>
<td>0.0492</td>
<td>0.0526</td>
<td>0.0504</td>
<td>0.0440</td>
<td>0.0503</td>
<td>0.0505</td>
</tr>
<tr>
<td>Aydin</td>
<td>0.0519</td>
<td>0.0731</td>
<td>0.0511</td>
<td>0.0548</td>
<td>0.0432</td>
<td>0.0465</td>
</tr>
<tr>
<td>Azkan</td>
<td>0.0466</td>
<td>0.0704</td>
<td>0.0518</td>
<td>0.0443</td>
<td>0.0465</td>
<td>0.0442</td>
</tr>
<tr>
<td>Çakır</td>
<td>0.0469</td>
<td>0.0557</td>
<td>0.0416</td>
<td>0.0450</td>
<td>0.0460</td>
<td>0.0579</td>
</tr>
<tr>
<td>Diyar</td>
<td>0.0552</td>
<td>0.0455</td>
<td>0.0507</td>
<td>0.0505</td>
<td>0.0446</td>
<td>0.0469</td>
</tr>
<tr>
<td>Gülümser</td>
<td>0.0505</td>
<td>0.0231</td>
<td>0.0494</td>
<td>0.0463</td>
<td>0.0461</td>
<td>0.0548</td>
</tr>
<tr>
<td>Hasanbey</td>
<td>0.0457</td>
<td>0.0314</td>
<td>0.0485</td>
<td>0.0447</td>
<td>0.0544</td>
<td>0.0443</td>
</tr>
<tr>
<td>İlgaz</td>
<td>0.0470</td>
<td>0.0329</td>
<td>0.0436</td>
<td>0.0506</td>
<td>0.0535</td>
<td>0.0399</td>
</tr>
<tr>
<td>İnci</td>
<td>0.0466</td>
<td>0.0407</td>
<td>0.0510</td>
<td>0.0467</td>
<td>0.0483</td>
<td>0.0552</td>
</tr>
<tr>
<td>İzmir</td>
<td>0.0449</td>
<td>0.0411</td>
<td>0.0480</td>
<td>0.0488</td>
<td>0.0487</td>
<td>0.0484</td>
</tr>
<tr>
<td>Seçkin</td>
<td>0.0470</td>
<td>0.0416</td>
<td>0.0450</td>
<td>0.0556</td>
<td>0.0410</td>
<td>0.0448</td>
</tr>
</tbody>
</table>

**Adana and Erzurum in 2015**

<table>
<thead>
<tr>
<th>Alternatives</th>
<th>TPC</th>
<th>ICA</th>
<th>FRSA</th>
<th>WSPC</th>
<th>WBC</th>
<th>OBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aksu</td>
<td>0.0449</td>
<td>0.0273</td>
<td>0.0429</td>
<td>0.0464</td>
<td>0.0486</td>
<td>0.0387</td>
</tr>
<tr>
<td>Arda</td>
<td>0.0492</td>
<td>0.0408</td>
<td>0.0530</td>
<td>0.0405</td>
<td>0.0488</td>
<td>0.0499</td>
</tr>
<tr>
<td>Aydin</td>
<td>0.0510</td>
<td>0.0687</td>
<td>0.0479</td>
<td>0.0545</td>
<td>0.0430</td>
<td>0.0451</td>
</tr>
<tr>
<td>Azkan</td>
<td>0.0435</td>
<td>0.0582</td>
<td>0.0480</td>
<td>0.0410</td>
<td>0.0462</td>
<td>0.0362</td>
</tr>
<tr>
<td>Çakır</td>
<td>0.0508</td>
<td>0.0554</td>
<td>0.0470</td>
<td>0.0504</td>
<td>0.0501</td>
<td>0.0605</td>
</tr>
<tr>
<td>Diyar</td>
<td>0.0490</td>
<td>0.0355</td>
<td>0.0471</td>
<td>0.0480</td>
<td>0.0427</td>
<td>0.0503</td>
</tr>
<tr>
<td>Gülümser</td>
<td>0.0531</td>
<td>0.0340</td>
<td>0.0482</td>
<td>0.0479</td>
<td>0.0464</td>
<td>0.0468</td>
</tr>
<tr>
<td>Hasanbey</td>
<td>0.0466</td>
<td>0.0471</td>
<td>0.0455</td>
<td>0.0465</td>
<td>0.0486</td>
<td>0.0461</td>
</tr>
<tr>
<td>İlgaz</td>
<td>0.0482</td>
<td>0.0515</td>
<td>0.0517</td>
<td>0.0502</td>
<td>0.0516</td>
<td>0.0454</td>
</tr>
<tr>
<td>İnci</td>
<td>0.0480</td>
<td>0.0219</td>
<td>0.0507</td>
<td>0.0511</td>
<td>0.0499</td>
<td>0.0529</td>
</tr>
<tr>
<td>İzmir</td>
<td>0.0396</td>
<td>0.0575</td>
<td>0.0425</td>
<td>0.0447</td>
<td>0.0518</td>
<td>0.0469</td>
</tr>
<tr>
<td>Seçkin</td>
<td>0.0519</td>
<td>0.0559</td>
<td>0.0513</td>
<td>0.0538</td>
<td>0.0485</td>
<td>0.0536</td>
</tr>
</tbody>
</table>
Properties were not very associated to each other. These activities could have iron chelating activities, but these two showed that the compounds with free radical scavenging activities as 0.577 (for all extracts) or 0.539 (harvested in Adana region) were low as 0.195 (for all samples) or 0.233 (harvested in Adana 2014) (P<0.05). The significant correlations between TPC and ICA were positive correlations between TPC, FRSA, and ICA but no significant correlations were between WBC and OBC. Functional properties are mostly related to the carbohydrate and protein content which had the ability to bound water and oil and most of these contents were mostly eliminated during water soluble extraction process. Therefore, there could not be found any correlation between the bioactive and functional properties of the extracts.

Correlations Between Determined Parameters

Correlation analyses between determined parameters of chickpea cultivars were done in three groups; cultivars grown in i) only Adana region, ii) in 2015, iii) all location and harvest years (Table 5). In each group, there were significant positive correlations between TPC, FRSA, and ICA but no significant correlations were between WBC and OBC (P<0.05). The significant correlations between TPC and ICA were low as 0.195 (for all samples) or 0.233 (harvested in Adana region) and between TPC and FRSA were moderate as 0.577 (for all extracts) or 0.539 (harvested in Adana region) or 0.492 (harvested in 2015). Correlation analysis showed that the compounds with free radical scavenging activities could have iron chelating activities, but these two properties were not very associated to each other. These activities are mostly generated by soluble proteins in the extracts because it is known that soluble chickpea proteins have both free radical scavenging and iron chelating activities (Arcan and Yemencioglu, 2007). Moreover, soluble free phenolics in the extracts are greatly contributed to the free radical scavenging activities. In all groups, WBC and OBC were negatively or almost zero correlated with TPC, FRSA, and ICA.

### Table 3. Positive ($S^+$) and negative ($S^-$) ideal solutions for the criteria

<table>
<thead>
<tr>
<th>Criteria</th>
<th>All samples</th>
<th>Adana 2014-2015</th>
<th>Adana-Erzurum 2015</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$S^+$</td>
<td>$S^-$</td>
<td>$S^+$</td>
</tr>
<tr>
<td>TPC</td>
<td>0.053</td>
<td>0.042</td>
<td>0.055</td>
</tr>
<tr>
<td>ICA</td>
<td>0.071</td>
<td>0.036</td>
<td>0.073</td>
</tr>
<tr>
<td>FRSA</td>
<td>0.051</td>
<td>0.043</td>
<td>0.052</td>
</tr>
<tr>
<td>WSPC</td>
<td>0.055</td>
<td>0.043</td>
<td>0.056</td>
</tr>
<tr>
<td>WBC</td>
<td>0.054</td>
<td>0.044</td>
<td>0.054</td>
</tr>
<tr>
<td>OBC</td>
<td>0.059</td>
<td>0.038</td>
<td>0.058</td>
</tr>
</tbody>
</table>

### Table 4. TOPSIS evaluation of chickpea samples

<table>
<thead>
<tr>
<th>Alternatives</th>
<th>All samples</th>
<th>Adana 2014-2015</th>
<th>Adana-Erzurum 2015</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$d_i^*$</td>
<td>$d_i^-$</td>
<td>$C^*$</td>
</tr>
<tr>
<td>Aksu</td>
<td>0.0399</td>
<td>0.0085</td>
<td>1.75E-01</td>
</tr>
<tr>
<td>Arda</td>
<td>0.0287</td>
<td>0.0214</td>
<td>4.27E-01</td>
</tr>
<tr>
<td>Aydın</td>
<td>0.0171</td>
<td>0.0403</td>
<td>7.02E-01</td>
</tr>
<tr>
<td>Azkan</td>
<td>0.0271</td>
<td>0.0261</td>
<td>4.91E-01</td>
</tr>
<tr>
<td>Çakır</td>
<td>0.0224</td>
<td>0.0284</td>
<td>5.59E-01</td>
</tr>
<tr>
<td>Diyar</td>
<td>0.0299</td>
<td>0.0198</td>
<td>3.98E-01</td>
</tr>
<tr>
<td>Gülümser</td>
<td>0.0381</td>
<td>0.0177</td>
<td>3.17E-01</td>
</tr>
<tr>
<td>Hasanbey</td>
<td>0.0374</td>
<td>0.0137</td>
<td>2.67E-01</td>
</tr>
<tr>
<td>Ilgaz</td>
<td>0.0332</td>
<td>0.0155</td>
<td>3.18E-01</td>
</tr>
<tr>
<td>İnci</td>
<td>0.0357</td>
<td>0.0199</td>
<td>3.58E-01</td>
</tr>
<tr>
<td>İzmir</td>
<td>0.0290</td>
<td>0.0193</td>
<td>4.00E-01</td>
</tr>
<tr>
<td>Şeklin</td>
<td>0.0322</td>
<td>0.0172</td>
<td>3.48E-01</td>
</tr>
</tbody>
</table>

$d_i^*$, $d_i^-$, and $C^*$ are positive ideal solution of Euclidean distance, negative ideal solution of Euclidean distance, and the closeness coefficient of each alternative, respectively.
Table 5. Correlations between different parameters determined for chickpea cultivars

<table>
<thead>
<tr>
<th></th>
<th>All samples</th>
<th>Adana 2014-2015</th>
<th>Adana-Erzurum in 2015</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TPC*</td>
<td>ICA</td>
<td>FRSA</td>
</tr>
<tr>
<td>ICA</td>
<td>0.195*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FRSA</td>
<td>0.577*</td>
<td>0.245*</td>
<td>0.410*</td>
</tr>
<tr>
<td>WSPC</td>
<td>0.255*</td>
<td>0.200*</td>
<td>0.152</td>
</tr>
<tr>
<td>WAC</td>
<td>-0.217*</td>
<td>-0.100</td>
<td>-0.285*</td>
</tr>
<tr>
<td>OAC</td>
<td>-0.028</td>
<td>0.068</td>
<td>0.074</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TPC</td>
<td>ICA</td>
</tr>
<tr>
<td>ICA</td>
<td>0.233*</td>
<td>0.492*</td>
</tr>
<tr>
<td>FRSA</td>
<td>0.539*</td>
<td>0.215**</td>
</tr>
<tr>
<td>WSPC</td>
<td>0.188</td>
<td>0.025</td>
</tr>
<tr>
<td>WAC</td>
<td>-0.192</td>
<td>-0.005</td>
</tr>
<tr>
<td>OAC</td>
<td>0.064</td>
<td>0.113</td>
</tr>
</tbody>
</table>

* P <0.05, ** P<0.1

aTPC: Total phenolic content (mg GAE/g), bICA: Iron chelating activity (μmolEDTA/g), cFRSA: Free radical scavenging activity (μmol trolox/g), dWSPC: Water soluble protein content (mg caseinate/g), eWAC: Water absorption capacity (g/g), fOAC: Oil absorption capacity (g/g)

Conclusions

This study revealed that for ranking of the alternatives, TOPSIS is suitable technique to be used in multi criteria decision making process when the sample size is big, and the determined parameters related to the same property are existed. 12 registered cultivars grown in different location and year studied for their functional properties and their potential to be processed as value added bioactive or functional product was highlighted for the first time. However, the individual cultivars had varying results by different harvest locations and years, they had similar average values when they grouped as the same location or year. This situation showed that the chickpeas could have those potentials independent from their harvest location and year. For this reason, the chickpeas studied in this study are suitable legumes which can be used for functional food additives due to their good techno-functional and bioactive properties. They also have potential to be used as functional plant protein sources for different purposes in food, pharmaceutical and cosmetic industries which extensively benefited from plant sourced natural products. After more detailed phenolic, protein and mineral characterization of chickpea cultivars grown in different location and year in Turkey, the effects of growing conditions on functional and bioactive properties of cultivars will also be determined.

Compliance with Ethical Standard

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

Financial disclosure: This study was supported by Adana Science and Technology University Scientific Research Coordination Unit. Project Number MÜHDBF.GIDA.2015-14.

Ethics committee approval: No ethics committee approval is needed.
References


https://doi.org/10.3390/mca14020119


Ozturk, G., Dogan, M., and Said Toker, O. (2014). Physicochemical, functional and sensory properties of mellorine enriched with different vegetable juices and TOPSIS approach to determine optimum juice concentration. *Food Bioscience*, 7, 45-55. [https://doi.org/10.1016/j.fbio.2014.05.001](https://doi.org/10.1016/j.fbio.2014.05.001)


PMid:25766818


ABSTRACT

Celiac disease (CD) is one of the most common diseases related to nutrition affecting consumers all over the world. There has been a steady increment in the production of ready-to-eat meat products with rapid changes in dietary habits, urbanization, and globalization. Despite this increase in manufacturing of ready-to-eat meat products, there is still a market demand present for gluten-free meat products. Since the only treatment for CD is a lifelong gluten-free diet, an undeniable need is present for meeting the nutritive demands of CD sufferers by improving the range of gluten-free products with both high nutritive and technological quality. The present paper overall covers the CD and its impacts in connection with the development strategies for gluten-free meat product formulations.

Keywords: Gluten, Gluten-free diet, Gluten-free meat products, Celiac disease
Introduction
In the last decades, there has been a rising demand for ready-to-eat food products depending on the progress of production technologies, globalization, and the modernization of everyday life. Coated meat products are one of the ready-to-eat foods that are very popular and widely consumed in mass consumption areas as well as at home. These kinds of products, commonly named as “nuggets”, present advantages in terms of short preparation time, high sensory quality and low costs (Dogan et al., 2005, de Carvalho et al., 2018). The industrial production of nuggets mainly consists of portioning the dough that contains meat, fat, and salt; pre-dusting the surface of each portion with a dry flour; battering with a semi-liquid mixture of flours, starches, eggs, water, spices and other ingredients; coating the outer surface with flours and/or breadcrumbs and finally deep-fat frying to stabilize the coating material (Akgün, 2006, Gökçe et al., 2016, de Carvalho et al., 2018). Battering and coating materials provide many desired characteristics to the product such as appealing appearance, attractive color, crispness, adhesion and flavor (Jackson, 2016).

One of the most common battering and/or coating ingredients in nugget formulations is wheat flour which contains approximately 60% gluten (Jackson et al., 2006). Gluten is the main structural protein in wheat flour, which’s main protein fractions, glutenin and gliadin, are highly responsible for the technological and sensory characteristics of many baked products (Gallagher et al., 2004, Jnawali et al., 2016). Regarding coated meat products, gluten is mentioned to play a key role in holding the gases that are formed by the impact of leavening agents and thereby creating a porous structure that enhance the texture. This underlines the fact that increased concentrations of gluten in the coating material could improve the crispness and the color of the deep-fat fried product (Akgün, 2006). Due to its high fat and water binding capacity, gluten also has a considerable application potential in the formulation of different meat, poultry, and fish products, besides it has a strong ability to bind meat pieces in restructured meat products leading enhancement in sliceability and decrement in cook losses (Taşbaş et al., 2016).

Despite all these highlighted benefits, consumption of gluten-containing food products might cause serious health issues for some consumers. Celiac disease (CD) is one of the most remarkable gluten-related disorders that affects approximately 1% of the world population (Cui et al., 2017, Gobbetti et al., 2018). Since the only treatment for CD is a lifelong gluten-free diet (Gobbetti et al., 2018), it is a notable issue to enhance the options for gluten-free food products that could meet the demands such as sensory and nutritional quality, as well as product costs.

Although a plenty of gluten-free food products, such as bread, pasta, cookies, and cakes are today available in the market, there is still a need for gluten-free meat products for consumption of CD sufferers. Within this review, it was objected to emphasize the general impacts of CD and its relation to the consumption of coated meat products in terms of new strategies to design novel gluten-free formulations.

A Brief Look at Celiac Disease and Its Impacts
Although CD is the most common gluten-related disorder, in fact, there are many different diseases caused by gluten consumption including non-celiac gluten sensitivity, dermatitis herpetiformis (Duhring disease), wheat allergy, gluten ataxia, and irritable bowel syndrome (Sapone et al., 2012, Cui et al., 2017, Gobbetti et al., 2018). However, within this review, we will mostly focus on the influences of CD and its connection with the diet, in particular with coated meat products. CD (gluten-sensitive enteropathy or coeliac sprue) is a permanent food intolerance induced by gluten present in some cereals like wheat, barley and rye (Gallagher et al., 2004, Demir and Kılınç, 2016, Foschia et al., 2016, Cui et al., 2017). Accounts of CD date back to the first century A.D. in the medical books of Aretaeus from Cappadocia (Farrell and Kelly, 2002). After the Neolithic revolution that began in the area surrounding modern Turkey, Iraq, and Iran called “Fertile Crescent”, agricultural practices, the major living and thereby dietary changes led to the appearance of “new” diseases such as CD (Rostami et al., 2004). Today it is one of the most common lifelong disorders based on nutrition affecting mankind all over the world (Lionetti et al., 2015, Demir and Kılınç, 2016). Gluten proteins are storage proteins that occur exclusively in the starchy endosperm of the grains and make up approximately 70-80% of total grain proteins (Scherf
et al., 2016). Gluten proteins have two major groups: the ethanol-soluble fraction termed prolaminids and the polymeric glutenins. When reacted with alcohol, the toxic fractions of prolaminids, which are called “gliadins” from wheat, “secalins” from rye and “hordeins” from barley are formed (Ciclitira et al., 2005, Niewinski, 2008). These residues are toxic to the small intestinal mucosa of patients with CD and cannot be completely digested by peptidases from the stomach, pancreas, and intestinal brush borders (Ciclitira et al., 2005, Cui et al., 2017). CD predominantly affects the mucosa of the upper small intestine and is characterized by “villous atrophy” that leads a broad flat villi which is associated with malabsorption of nutrients, vitamins, and minerals (Niewinski, 2008, Scherf et al., 2016). CD commonly appears in early childhood, with some symptoms including chronic diarrhea and failure to thrive, or the symptoms can also develop later in life (Foschia et al., 2016). The disease can occur at any age with females being more commonly affected than males with a suggested ratio of around 2:1 (Scherf et al., 2016). The symptoms mainly include abdominal pain, chronic or intermittent diarrhea, vomiting, chronic constipation, abdominal distention, weight loss, weakness, severe malnutrition and many other extra-intestinal associations that could cause further complications (Cui et al., 2017). Moreover, CD also places patients at greater risk for certain cancers (Niewinski, 2008). The prevalence of CD is commonly explained by the “iceberg model” (Gallagher et al., 2004), which is presented in Figure 1. According to this model, cases which have been properly diagnosed make up the visible section (S1). The CD sufferers who follow a gluten-free diet and show a normal mucosa represent the lower part of this section. Under the waterline (finish line of S1), there begins the silent section (S2), which is referred to undiagnosed patients that have no symptoms or have some symptoms not related to CD. The bottom section of the iceberg (S3) indicates a small group of person with a latent CD that shows a normal mucosa at present but carry the potential to develop CD in the future (Feighery, 1999, Gallagher et al., 2004). The model clearly indicates that the actual prevalence of CD could be much higher than is reported due to the big invisible areas. Kerpes et al. (2017) also reported that the validity of the prevalence value of CD is unclear because of silent forms and low clinical rates of detection.

In some recent studies, the worldwide prevalence of CD has been reported as between 0.3-2% (Lionetti et al., 2015, Cui et al., 2017), briefly, the overall prevalence has been stated as 1% on average (Lionetti et al., 2015, Jackson, 2016). Although CD was previously considered as a typical disease of European origin, later it was theorized that the pattern of agriculture spreading could explain the higher CD incidence in some western countries like Ireland (Rostami et al., 2004). Nevertheless, the highest reported prevalence is in western Europe and in places where Europeans emigrated like North America and Australia. However, it is also found in northwest India and may be underdiagnosed in South America, North Africa and Asia (Farrell and Kelly, 2002, Lionetti et al., 2015). These regional differences may arise from variation in HLA or other genetic factors, dietary habits, infant feeding practices, gastrointestinal infections, socioeconomic status, hygiene, or other unknown effects (Unalp-Arida et al., 2017). Also, it should be noted that in Western countries the overall incidence of the disease is on the rise, probably due to the environmental components (Lionetti et al., 2015) and as reported by Cui et al. (2017), the increasing rate doubles every 20 years.

The prevalence of CD is commonly explained by the “iceberg model” (Gallagher et al., 2004), which is presented in Figure 1. According to this model, cases which have been properly diagnosed make up the visible section (S1). The CD sufferers who follow a gluten-free diet and show a normal mucosa represent the lower part of this section. Under the waterline (finish line of S1), there begins the silent section (S2), which is referred to undiagnosed patients that have no symptoms or have some symptoms not related to CD. The bottom section of the iceberg (S3) indicates a small group of person with a latent CD that shows a normal mucosa at present but carry the potential to develop CD in the future (Feighery, 1999, Gallagher et al., 2004). The model clearly indicates that the actual prevalence of CD could be much higher than is reported due to the big invisible areas. Kerpes et al. (2017) also reported that the validity of the prevalence value of CD is unclear because of silent forms and low clinical rates of detection.
Gluten-Free Diet: Challenges and Needs

The only treatment of CD is to follow a strict and permanent lifelong gluten-free diet, which results in complete remission (Feighery, 1999, Jnawali et al., 2016). The treatment of CD with a gluten-free diet has positive effects on the mucosal histology as it will normalize it and the clinical symptoms will ease (Foschia et al., 2016). Following this diet may seem really easy at the first look, however treating the disease in a developing country can be extremely difficult due to the challenges in its institution and maintenance of adherence (Lionetti et al., 2015). Jnawali et al. (2016) notified that a gluten-free diet not only involves eliminating gluten-containing cereals and their products but also requires constant vigilance and a complete change in lifestyle. Even though most patients self-report strict dietary gluten adherence, a significant number have persistent mucosal damage 2 years after they started a gluten-free diet (Rubio-Tapia et al., 2010). Silvester et al. (2016) assessed the relationship between self-reported adherence to this diet and found out that individuals who believe they are following a gluten-free diet are not able to correctly identify foods that are gluten-free, which suggests ongoing gluten consumption may be occurring. Gobbetti et al. (2018) reported that gluten is safe avoidance of its main sources like wheat and barley, but especially when present as minor ingredients like sausages, instant soups, and confectioneries, it becomes very difficult. Thus novel approaches for definition and categorization of gluten-free food sources and products are needed. As mentioned by Foschia et al. (2016), in order to avoid any type of contamination, gluten-containing ingredients have to be located and manipulated in areas strictly separated from the gluten-free ones.

Lionetti et al. (2015) emphasized that today there is neither an organized sector nor industry for gluten-free food products in developing countries and these products are not readily available. Therefore, it is a notable point to increase the awareness of the disease in both society and governments by improving legislation strategies for gluten labeling, educational applications, and periodical free screen tests. The U.S. Food and Drug Administration (FDA) issued a final rule in 2013 defining the term “gluten-free” for voluntary use in the labeling
of foods for the benefit both people with CD and the food industry. This rule defines gluten-free as meaning that the food is either inherently gluten-free or does not contain an ingredient that is: (1) a gluten-containing grain (e.g. spelt wheat), (2) derived from a gluten-containing grain that has not been processed to remove gluten (e.g. wheat flour), or (3) derived from a gluten-containing grain that has been processed to remove gluten (e.g. wheat starch), if the use of that ingredient results in the presence of 20 ppm or more gluten in food. Also, any unavoidable presence of gluten in the food must be less than 20 ppm (U.S. Food and Drug Administration, 2018).

Today the consumers are highly aware of nutrition-related diseases and would like to improve physical and mental well-being besides just satisfying hunger (Jnawali et al., 2016). Although CD sufferers have to follow a strict gluten-free diet, also some consumers without CD prefer to take a gluten-free diet due to some health issues, weight management and/or minimizing future risk of gastrointestinal diseases (Gobbetti et al., 2018), also due to the cultural, ecological, civic, historical or ethnical interest of quality (Foschia et al., 2016). In a population-based regional study, it was reported that 0.7% of participants had CD, while 1.1% of them avoid gluten without CD (Unalp-Arida et al., 2017). From 2013 to 2015, the gluten-free industry enjoyed a growth of 136%, leaving behind the awareness of the disease (Reilly, 2016). For this reason, the number of consumers seeking for gluten-free product options is remarkable since both of the groups are in need of that kind of diet type. Therefore, it becomes more of an issue to increase the high-quality gluten-free product range and to maximize the options. In a recent review of Jnawali et al. (2016), some specific considerations in the development of gluten-free products have been well-described that includes avoidance of gluten-containing sources (as the challenges are underlined above), ensuring sensory characteristics and nutritional value of the products, meeting the recommended dietary allowances and product costs.

**Novel Approaches for Designing Gluten-Free Meat Product Formulations**

Consumers with CD have the opportunity to eat certain types of gluten-free products that are categorized as naturally occurring gluten-free foods (fruits, vegetables, eggs, unprocessed meat, poultry, and fish) and gluten-free substitute foods (bread, pasta, cereals, crackers and snack foods) in which wheat flour is replaced by a gluten-free ingredient (Taşbaş et al., 2016, Cui et al., 2017). In literature, there exists a plenty of studies regarding gluten-free production and related quality parameters of bakery food products like bread (Lazaridou et al., 2007, de la Barca et al., 2010, Hager and Arendt, 2013, Martínez and Gómez, 2017, Rinaldi et al., 2017), pasta (Giuberti et al., 2015, Sanguinetti et al., 2015, Larrosa et al., 2016), cake (Levent and Bilgiçli, 2011, Preichardt et al., 2011, Talens et al., 2017), cookies (Rai et al., 2014, Brito et al., 2015, Molinari et al., 2018), crackers (Radočaj et al., 2014), and also some other foods like tarhana (Yalçin et al., 2008, Bilgiçli, 2009), snacks (Kahlon et al., 2016) and beer (Kerpes et al., 2017). On the other hand, only a limited research data is available on developing gluten-free meat product formulations. Taşbaş et al. (2016) reported that although unprocessed meats are free of gluten, people with CD need to be aware of meats and poultry with added ingredients that make them into ready-to-cook or processed meat products which may contain gluten. Considering the rising trend in production of all kinds of ready-to-eat meat products; since wheat flour and its derivatives are the most common ingredients used in the formulation of these products, there is an irrefutable demand for formulating that kind of products in gluten-free form.

Wheat flour as a traditional batter ingredient of nuggets contains a considerable amount of proteins that are necessary to form elasto-plastic batter (Taşbaş et al., 2016) and thus improve technological quality. Jnawali et al. (2016) stated that since eliminating wheat from the diet would mean the exclusion of a good protein source and sticking to a high carbohydrate diet, the protein content of the alternate source has to be considered. Since most of the gluten-free products are starch-based and thereby have low nutritional value, it is very important to enrich such products for consumers who are obliged to follow a gluten-free diet (Demir and Kılınç, 2016). Also, gluten-free products are mostly known as lacking mineral or to have less fortification with micronutrients and fibers compared to wheat-containing products (Gobbetti et al., 2018). Vici et al. (2016) reported that a gluten-free diet may lead to possible nutrient deficiencies or nutrient excess (e.g. saturated fats). For these reasons, the
alternative ingredients for gluten replacement should present the potential to overcome the nutritive deficiencies and simultaneously to increase product yield and quality.

According to Adedeji and Ngadi (2011), incorporation of some alternative flours or hydrocolloids in batter formulations of meat products could improve functionality and quality as well as cost-effectiveness, which points out that it could be possible to develop high-quality meat products by using proper gluten-free ingredients. So far, some functional ingredients in formulation of coated meat products have been used with combination of wheat flour to improve overall quality: Dogan et al. (2005) reported that soy flour was found to be an effective ingredient in improving quality of deep-fat fried chicken nuggets in terms of crispness and color, while both soy and rice flours provided reduced oil absorption. Kilincceker and Hepsag (2011) suggested the utilization of yellow lentil flour and chickpea flour as the batter materials of fish balls that improve the yield and sensory properties. In another study, it was reported that the addition of oat flour in breading mixes of chicken meatballs positively affected sensory properties and yield (Kilincceker 2013). Gökcə et al. (2016) investigated some quality characteristics of deep-fat fried chicken nuggets formulated with wheat, corn, rye and soy flours. They reported that the highest cooking yield was found in corn and wheat flour samples, the use of rye flour significantly increased the penetrometer values, and incorporation of corn flour showed the highest yellowness among samples. Kwaw et al. (2017) evaluated different single and composite flours from wheat, millet, sorghum, and soybean as breading agents in the deep frying of chicken breast. They recorded an increase in fat absorption in the single cereal coated samples compared to the composite flours and found that the samples coated with an equal ratio of soybean and sorghum composite flour had the highest overall acceptability. As is seen, in these studies the incorporation of the ingredients did not directly target to replace wheat or to formulate gluten-free formulations, yet this does not mean that these alternates have not a potential to be used in gluten-free meat products. In addition, so far various sources as gluten replacers have been suggested for general use in the formulation of different food products. Demirçeken (2011) stated that rice and corn are the main sources which do not contain toxic prolamine and can safely be consumed in the diet of celiac patients. Some minor cereals such as teff, millets, and Jungle rice, and some legumes such as chickpea, lentil, and soybeans have been mentioned by Jnawali et al. (2016) and Gobbetti et al. (2018) as gluten alternatives carrying potential nutritive benefits. Besides, in the near past, a comprehensive list was also presented by Niewinski (2008), who stressed some other gluten-free ingredients like buckwheat, oats (uncontaminated), sago and sorghum flour. Quinoa, a type of pseudo-cereal, has been suggested as a good alternative for CD patients since it contains high biological valued proteins, low-glycemic indexed carbohydrates, fitosteroiids, w-3 and w-6 fatty acids, micro-nutritional and bioactive compounds (Demir and Kılınç, 2016). Oat, another functional source that contains proteins, essential amino acids, and various antioxidants, is tolerated by almost all CD sufferers, although oat intolerance has been described (Ciclitira et al., 2005, Smulders et al., 2018). Smulders et al. (2018) reported that the prolamine storage protein called “avenin” in oat does not contain any of the known CD epitopes from gluten of wheat, barley, and rye, and long-term food studies confirm the safety of oats for CD patients. Some other gluten-free sources that can be used for developing food products for CD sufferers include nuts (e.g. almonds, hazelnuts, walnut), seeds (e.g. flax seeds, chia seeds, pumpkin seeds) and tubers (e.g. arrowroot, tapioca, potato) (Jnawali et al., 2016). As a result, all these ingredients could be counted as potential alternatives for wheat flour and alike materials to be further incorporated into gluten-free meat product formulations. In addition to the replacement of wheat flour and other gluten-containing ingredients by these types of alternatives, Gobbetti et al. (2018) also mentioned some recent approaches in gluten-free diet such as pre-digestion of dietary gluten, treatments of prebiotics that are capable of hydrolyzing gluten, degradation of wheat flour by use of sourdough and production of genetically modified wheat.
Table 1. The studies on the formulation of gluten-free meat products

<table>
<thead>
<tr>
<th>Product</th>
<th>Gluten-free ingredient(s)</th>
<th>Highlights</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken nugget</td>
<td>Rice flour</td>
<td>Value was added to the products by utilizing rice flour in the production of gluten-free products without diminishing sensory quality as well as lipid reduction through baking.</td>
<td>Jackson et al. (2006)</td>
</tr>
<tr>
<td>Chicken nugget</td>
<td>Sorghum flour</td>
<td>Use of sorghum flour significantly increased the product yield, texture and dietary fiber content. It was concluded that 5% sorghum flour is optimum to prepare gluten-free nuggets in terms of sensory quality.</td>
<td>Devatkal et al. (2011)</td>
</tr>
<tr>
<td>Kibbeh</td>
<td>Pearl millet flour</td>
<td>Kibbeh prepared with millet flour presented good oxidation stability. Baked kibbehs with millet flour presented good acceptability and did not differ from the samples with wheat flour in terms of appearance, texture, and flavor.</td>
<td>Brasil et al. (2015)</td>
</tr>
<tr>
<td>Chicken nugget</td>
<td>Pecans</td>
<td>The pecans did not have a significant effect on moisture content, batter adhesion, or consumer acceptability while nuggets made with pecans had a higher lipid content.</td>
<td>Jackson (2016)</td>
</tr>
<tr>
<td>Chicken nugget</td>
<td>Gluten-free wheat flour in combination with cellulose, egg powder, whey powder or pectin</td>
<td>The addition of whey powder in the formulation was resulted in decreased oil uptake, increased pick up and water holding capacity. Lipid oxidation was decreased in the samples manufactured with wheat flour or whey powder. The results showed that 2% whey powder can be used in gluten-free nugget manufacture without posing any quality problems.</td>
<td>Taşbaş et al. (2016)</td>
</tr>
<tr>
<td>Chicken nugget</td>
<td>Amaranth flakes with soy oil, eggs, oregano or basil</td>
<td>All of the formulations had similar yields. The samples with soy oil showed the highest lipid, carbohydrate, and mineral content and had a better acceptance for all evaluated sensory attributes.</td>
<td>de Carvalho et al. (2018)</td>
</tr>
<tr>
<td>Chicken nugget</td>
<td>Rice flour (RF), chickpea flour (CF), oat fiber (OF) or Jerusalem artichoke powder (JAP)</td>
<td>Utilization of OF and JAP decreased moisture, RF and OF increased protein, JAP decreased fat and OF decreased carbohydrate content of the samples. Samples with OF and JAP was effective to provide an equivalent cooking yield to wheat flour samples. JAP samples had the lowest oil absorption among treatments.</td>
<td>Öztürk et al. (2018)</td>
</tr>
<tr>
<td>Fish patty</td>
<td>Rice, corn, amaranth or quinoa flours</td>
<td>Flours addition affected proximate composition increasing carbohydrates, total fat and mineral content compared to control. No differences were found in the aroma of products. Addition of rice flour increased juiciness and tenderness whereas taste, overall acceptance and buying intention were higher in control patty, followed by patties made with corn flour.</td>
<td>Romero et al. (2018)</td>
</tr>
</tbody>
</table>
Getting back to the main idea, some key highlights of recent studies on gluten-free meat products are summarized in Table 1. As well as the general potential of the alternative ingredients has been emphasized above, it could be seen that some of these ingredients have been already used in the formulation of different kinds of meat products. In the studies, gluten-free meat products have been formulated with some ingredients such as gluten-free wheat flour itself, rice flour, sorghum flour, pecans, amaranth flakes, chickpea flour, oat fiber, and Jerusalem artichoke powder, as wheat flour replacers. It was recorded that most of these ingredients were able to increase product yield in terms of cook loss and adhesion ratios without negatively affecting sensory quality, to improve nutritional value, and to retard lipid oxidation, meaning that these compounds could present multifunctional benefits on overall quality. Jackson et al. (2006) reported that rice-based products can be used to reduce the fat content of deep-fried, battered chicken because rice flour batter absorbs less oil than wheat flour batter due to chemical differences between proteins and thus prevents products against lipid oxidation. Sorghum flour was mentioned to be a good source of dietary fiber as a non-glutinous flour that could be used in the scope of making gluten-free meat products (Devatkal et al., 2011). Jackson (2016) stressed that pecans can be used as an ingredient in breaded chicken nuggets with its high dietary fiber content, nutritive features, and health benefits. In another study, it was suggested that the combination of gluten-free wheat flour and whey protein can be a suitable alternative to produce good-quality gluten-free coated chicken products (Taşbaş et al. 2016). de Carvalho et al. (2018) stated that amaranth (Amaranthus) grains have emerged as an attractive raw material to replace wheat in the development of products for celiac individuals, with a high content of high biological quality protein, minerals, and vitamins. Öztürk et al. (2018) recommended that especially dietary fiber sources could supply to formulate gluten-free poultry products that have equivalent cooking characteristics to standard gluten-containing products, meanwhile improving health profile. Except for coated meat products, some non-glutinous flours have been also mentioned to improve nutritive, sensory and technological quality of different gluten-free meat products like fish patties (Romero et al., 2018) and kibbeh (Brasil et al., 2015).

Consequently, overall data indicated that the utilization of alternative gluten-free natural sources in the formulation of meat products presents the opportunity to produce high-quality and nutritive products, as well as to decrease product costs.

Conclusion

CD is effective on approximately 1% of the world population and showing a steadily increasing trend. Also, the real prevalence is thought to be much higher due to the silent forms in the iceberg. Today the only available and accepted solution for CD is a strict and permanent gluten-free diet. Besides, it is a fact that not only celiac patients but also those who would like to consume gluten-free products prefer this diet. However, currently, most of the gluten-free products in the market are mentioned to have low nutritional value and overall quality. At the same time, the labeling and classification practices are insufficient as well as some consumers could barely obey the rules of the gluten-free diet. Therefore, it is a fact that there is still a gap between gluten-free products and quality. Secondly, although some bakery foods are already available in the market, there are only a few options present for the gluten-free meat products. Since meat is one of the main sources of proteins and essential nutrients in the diet, the lack of meat and meat products may cause serious deficiencies. Accordingly, the development of novel strategies in meat products for consumers with gluten sensitivity is important to increase the consumption of such products in the diet of these individuals. In particular, increasing the variety of gluten-free ready-to-eat meat products like nuggets, sausages, and meatballs is necessary for especially adolescents and children who mostly prefer to consume that kind of products and whom self-adherence is much lower than adults. In addition, future studies are needed to formulate new gluten-free meat products by using dietary fiber sourced ingredients.

Compliance with Ethical Standard

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

Financial disclosure: This study was supported by The Republic of Turkey, Ministry of Science, Industry, and Technology (Project No: 0764.STZ.2014, SANT-TEZ Program).
References

[https://doi.org/10.1080/10942910903131423](https://doi.org/10.1080/10942910903131423)


[https://doi.org/10.1080/09637480802112546](https://doi.org/10.1080/09637480802112546)


[https://doi.org/10.1016/j.bpg.2005.01.003](https://doi.org/10.1016/j.bpg.2005.01.003)

[https://doi.org/10.1016/j.anai.2017.01.008](https://doi.org/10.1016/j.anai.2017.01.008)

[https://doi.org/10.1080/15428052.2017.1310071](https://doi.org/10.1080/15428052.2017.1310071)

[https://doi.org/10.1007/s11130-010-0187-z](https://doi.org/10.1007/s11130-010-0187-z)

[https://doi.org/10.3153/JFHS16011](https://doi.org/10.3153/JFHS16011)


[https://doi.org/10.1111/j.1745-4557.2010.00367.x](https://doi.org/10.1111/j.1745-4557.2010.00367.x)


[https://doi.org/10.1056/NEJMra010852](https://doi.org/10.1056/NEJMra010852)

[https://doi.org/10.1136/bmj.319.7204.236](https://doi.org/10.1136/bmj.319.7204.236)

[https://doi.org/10.1016/j.ijfoodmicro.2016.06.014](https://doi.org/10.1016/j.ijfoodmicro.2016.06.014)


PROBİYOTİK- İNSAN BAĞİŞKLİK SİSTEMİ ETKİLEŞİMLERİ

Dicle Dilara Akpınar1, Burcu Kaplan Türköz2

Cite this article as:

1 Ege Üniversitesi, Fen Bilimleri Enstitüsü Gıda Mühendisliği Bölümü, Bornova, 35040, İzmir, Türkiye
2 Ege Üniversitesi, Mühendislik Fakültesi Gıda Mühendisliği Bölümü, Bornova, 35040, İzmir, Türkiye

ORCID IDs of the authors:
D.D.A. 0000-0002-8318-169X
B.K.T. 0000-0003-3040-3321

Submitted: 06.02.2019
Revision requested: 05.05.2019
Last revision received: 09.05.2019
Accepted: 10.05.2019
Published online: 04.09.2019

Correspondence:
Buruń KAPLAN TÜRKÖZ
E-mail: burcu.kaplan.turkoz@ege.edu.tr

ÖZ


Anahtar Kelimeler: Probiyotikler, Sağlık etkileri, Probiyotik-konak etkileşimleri, Bağışıklık düzenleme, Etken moleküller

ABSTRACT

PROBIOTIC-HUMAN IMMUNE SYSTEM INTERACTIONS

Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host. These microorganisms are known to provide beneficial health effects by competing with pathogens, providing epithelial cell stability and showing regulatory effects on the immune system. They provide immunomodulatory, anti-inflammatory, anti-microbial, antioxidant effects with their released metabolites, produced molecules and cell structure components. Effector molecules of probiotics such as peptidoglycan, teichoic acid, lipoteichoic acid, cell surface polysaccharides, secreted proteins and surface proteins are recognized by innate immunity receptors and activate the immune system. The mechanisms of some effector molecules of probiotics have been elucidated. The aim of this review is to give information on the effects of probiotics and their active molecules known to be used for probiotic-host interactions.

Keywords: Probiotics, Health Effects, Probiotic-host interactions, Immunomodulation, Effector molecules
Giriş


Tablo 1. Probiyotik Mikroorganizmaların Özellikleri

<table>
<thead>
<tr>
<th>Probiyotik Mikroorganizmaların Özellikleri</th>
<th>Referanslar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bağışıklarda mukus ya da insan epitel hücrelerine yapışarak kolonizasyon</td>
<td></td>
</tr>
<tr>
<td>Bağışakta patojen yapışmasını azaltma</td>
<td></td>
</tr>
<tr>
<td>B-galaktosidaz aktivitesi</td>
<td></td>
</tr>
<tr>
<td>Genetik stabilite</td>
<td></td>
</tr>
<tr>
<td>Kolay ve tekra üretilebilme</td>
<td></td>
</tr>
<tr>
<td>Üretim ve depolama süresinde dayanıklılık</td>
<td></td>
</tr>
<tr>
<td>Patojenlere karşı antimikrobiyal aktivite</td>
<td></td>
</tr>
<tr>
<td>Patojenler tarafından antimikrobiyal aktivite</td>
<td></td>
</tr>
<tr>
<td>Sağlık üzerine yararlı etkiler</td>
<td></td>
</tr>
<tr>
<td>Sindirim enzimlerine, mide asidine ve safra tuzlarına karşı direnç</td>
<td></td>
</tr>
</tbody>
</table>
Probiyotiklerin Etki Mekanizmaları ve Sağlık Üzerine Etkileri

Probiyotikler insan sağlığını doğrudan ya da dolaylı olarak etkilemektedir. Probiyotiklerin en temel etkileri arasında patojenlerle mücadele, bağırsak sistemini yönlendirme ve bağırsak epitel barieri koruma ve iyileştirme sayılabilir (Lebeer vd., 2010).


Probiyotiklerin Sağlık Üzerine Etkileri

Probiyotik tüketiminin sağlık üzerine birçok yararı olduğu bilinmektedir, hatta probiyotik bazi hastalıklarla ilgili olmayan ve/veya tedavi edici olarak kullanılmaktadır. Probiyotiklerin sağlık üzerine etkilerini belirlemek için birçok klinik çalışma yapmıştır. Ancak bu araştırmalar sonucunda bazı rahatsızlıklar ve hastalıklarla kesin olarak etkisi belirlenebilmişken, bazıları üzerinde ise bu etkiler belirlenememiştir (Kleerebezem vd., 2019).

Probiyotiklerin önleyici ve/veya tedavi edici olarak kullanıldığı hastalıklardan başında inflamatur bağırsak hastalıkları (IBD) gelmektedir. Kronik, tekrarlayabildi ve çeşitli faktör lere bağlı olan IBD, sindirim sistemi boyunca bağırsık sisteminin düzünsüz ve aşırı yanıt vermesiyle inflamasyon oluşması nedeniyle görülen bir hastalıdır. Genetik faktörler, çevresel faktörler, bağırsık sistem bozuklukları, patojenler ve oksidatif stres IBD’ nin oluşmasına neden olabilecek faktörlerdir (Boirivant ve Strober, 2007; Pandey vd., 2015). IBD’ ler içerisinde yer alan ülserit kolit (UC), Crohn (CD) ve postibid hastalıklarının tedavisinde ve hastalık semptomlarının hafifletiminde probiyotik tüketiminin etkili olduğu yapılan klinik araştırmalarla gösterilmiştir (Sheil vd., 2007; Kelesidis ve Pothoulakis, 2012; Veerappan vd., 2012).


Probiyotiklerin tedavi yardımı olarak kullanıldığı başka bir sağlık problemi de vardır. Antibiyotik kullanımı, patojen bakteriler ve virüsler bağırsak mikroflorasının değişmesine sebep olarak ishal olunmasına neden olabilir. Akut, antibiyotik kaynaklı ve seyahat ishali çeşitlerinin hepsinde probiyotik tüketiminin etkili olduğu yapılan klinik araştırmalarla gösterilmiştir (Macfar-
ve tümör hücrelerinin gelişimini engelleyen bileşenler üretmesi kanser önleyici mekanizmaları olarak öne sürülmektedir. Ayrıca olası mekanizmalardan biri de bağışıklık cevabının probiyotikler tarafından düzenlenmesi olarak gösterilmektedir (Rafter, 2002; Gürsoy ve Kinik, 2005; Parvez vd., 2006; Fotiadis vd., 2008; Kumar vd., 2010).

Probiyotiklerin bağırsak bariyer fonksiyonunu koruduğu ve bağışıklık yanının düzenlenmesi üzerine etkilerini olduğu bilinmektedir. Probiyotiklerin bu mekanizmaları kullanarak alerji semptomlarının hafifletilmesi üzerine olumlu etkiler gösterdiği klinik çalışmalarla belirlenmiştir (Kalliömäki ve Isolauri, 2004; Parvez vd., 2006; Rupa ve Mine, 2012).

Probiyotikler, ayrıca, laktoz intoleransı, gastrit, hipertansiyon, ağız sağlığı, obezite, diyabet, yüksek kolestrol, karaciğer hastalıkları ve çeşitli enfeksiyon hastalıkları üzerine olumlu etkiler göstermektedir. (Parvez vd., 2006; Singh vd., 2011; Rupa ve Mine, 2012; Nagpal vd., 2014; Pandey vd., 2015).

Probiyotiklerin sağlığı üzerine olumlu etkilerin molekül mekanizmalarının belirlenmesi için yapılan araştırmalar son yıllarda hız kazanmıştır. Özellikle probiyotiklerin etken molekülünün belirlenmesi ve bu molekülerin doğrudan hastalıkları önleyici ya da tedavi amacı ile kullanılarak konusunda çalışmalar yapılmaktadır.

Probiyotiklerin Etken Molekülleri


**Şekil 1.** Probiyotiklerin etken molekülerinin şematik gösterimi. Soldan sağa, teikoik asit (LTA ve WTA), probiyotiklerin hücre dışına salgılantığı protein ve hücre yüzey proteinleri, fimbria, flagella, polisakkaritler (EPS, WPS ve CPS) ve peptidoglikan ile bağırsak epitellerinde bulunan bağımsız sisteminde görevli PRR’ler (TLR, NLR ve DC-SIGN gibi reseptörler) etkileşime girerek bağışıklık yanıtını oluştururlar.
**Peptidoglikan (PG)**


NOD2 reseptörlerinin de PG türevi muramil peptitleri tanıdıği ve Crohn hastalığında NOD2 reseptörlerinin rol oynadığı lightklediği (Ogura vd., 2001; Girardin vd., 2003). Probiyotik Lactobacillus salivarius L33 ve Lactobacillus acidophilus NFCM bakterilerinden saflaştırılan PG’nin muramil peptit parçalarıyla kolit fareler üzerinde çalışma yapılmıştır. Yapılan çalışmada L33 PG’nin NOD2 sinyal estimine bağlı olarak anti-inflamatuar sitokin IL-10 salınıma başlattığı ve bu yolla da koruyucu etki gösterdiği belirlenmiştir. NFCM’nin ise kolit fareler üzerinde herhangi bir koruyucu etki göstermediği görülmüştür. Bu sonuçlar, PG fragmanlarının NLR sinyal estimine üzerine etkisini gösterirken, bu etkinin suşa spesifik olarak ortaya çıktığını göstermiş ve PG yapısındaki çeşitliliğin önemini anlaşımasına olanak sağlamıştır (Fernandez vd., 2011).

**Teikoik Asit (TA)**


Gram (+) bakterilerin lipopolisakkitrilerleyepit etik hücrelerde pro-inflamatuar IL-8 sitokininin salınımında neden olduğu, L. johnsonii La1 ve L. acidophilus La10 probiyotik bakterilerinden izole edilen LTA’lardan ise IL-8 salınımını baskılı göstermiştir. Bu çalışmayla, Lactobacillus salivarius LTA’da ise ilâklediilen pro-inflamatuar TNF-α salınımını azalırken, antiinflamatuar sitokin IL-12 üretimini düşürmüştü. LTA’nın yapısındaki farklılıkların bağışıklık düzenleyici etkilerine katkıları henüz Tôi görülmüştür. Böylece, LTA’nın LTA’ nin antagonsistik etki gösterdiği ve TLR2 yoluyla makrofajlarda pro-inflamatuar etki göstermiştir (Kim vd., 2008).

LTA’nın yapısaldaki farklılıkların mekanizmalarını üzerine etkisini belirlemek için de çalışmalar yapımıştır. L. plantarum L.137 ve L. plantarum JCM1149 suşlarının LTA’ları ile HCT-116 ve HL-60 hücrelerinde TNF-α ile pro-inflamatuar etkileri araştırılmış ve daha iyi TNF-α salınımını azaltan bir etkisi göstermişlerdir (Kim vd., 2008). LTA’nın yapısaldaki farklılıkların mekanizmalarını üzerine etkisini belirlemek için de çalışmalar yapımıştır. L. plantarum L.137 ve L. plantarum JCM1149 suşlarının LTA’ları ile HCT-116 ve HL-60 hücrelerinde TNF-α ile pro-inflamatuar etkileri araştırılmış ve daha iyi TNF-α salınımını azaltan bir etkisi göstermişlerdir (Kim vd., 2008).
Bağırlık cevabı oluşturulurken, IL-10 (anti-inflamatuar)/IL-12 (pro-inflamatuar) sitokin salınımı arasındaki denge kritik bir öneme sahiptir. Farklı Lactobacillus suşları IL-10 veya IL-12 salınımını farklı seviyelerde indüklemektedirler. L. plantarum IL-10 salınımı induklerken, yayılan olarak IL-12 indükşiyonu da yapmıştır. L. plantarum’un tersine, L. casei IL-12 salınımını induklerken, yayılan olarak IL-12 indükşiyonu yapmıştır. İki suşun beraber kullanımla yapılan çalışmada, makrofajların aktivasyonunu sağlayan WTA ve LTA’nın TLR2 yoluya IL-10 üretimini sağlayarak sinirli etki oluşturduklarını belirtmiştir (Kaji vd., 2010).

Yapılan araştırmaların çoğu LTA’nın bağırlık düzenleyici etkisini belirlemeye yönelik araştırmaları devam edilmelidir. Diğer bir hücre duvarı bileşeni olan WTA’nın etki mekanizmasını anlamaya yönelik yapılan bir çalışmadı, WTA üretmeyen L. plantarum WCFS1 mutlantı, L. plantarum WCFS1 WTA’si ve D-Alanin eksikliği bulunan L. plantarum WCFS1 WTA mutlantı üzerine çalışılmıştır. Mutant ve doğal suş ait WTA’nın TLR2/6 sinyali yoluya doğrudan etkilemediği görülmüştür. Saflaştırılan WTA’lar insan dentrik hücrelerinde herhangi bir sitokin üretimine neden olmamak, pro-inflamatuar sitokin IL-12 ve TNF-α, LTA’nın salınımını önemli seviyede azalttığı belirlemiş (Brdn vd., 2012a). Bu çalışmada da görüldüğü gibi, WTA’nın mekanizması üzerine kesin bir sonuca varılamadığını, bağırlık düzenleyici etkisini belirlemeye yönelik araştırmalar yapılmaya devam edilmelidir.

Hücre Duvarı Polisakkaritleri

Hücre Yüzeyinde Bulunan ya da Hücre Dışında Sentezlenen Proteinler


Hücre dışına senezte olan bir serpin genom taramalarıyla B. Longum seksandasında bulunmuş (Schell vd., 2002) ve saflaştırılan serpinin, serin pankreatik elastaz ve nötrofil elastazını inhibe etme yeteneğini de göstermiş (Ivanov vd., 2006).

Hücre dışına senezte olan bir diğer protein ise Lactobacillus paracasei tarafından üretilen proteaz lactopeptindir. Lactopeptin, pro-inflamatuar IFN-γ’yi indükleyen peptid hücresel salınının en güçlü temel pro-inflamatuar kemokin olan IP-10 proteinini parçalamaktadır ve böylece anti-inflamatuar etkiler göstermektedir. Lactopeptin üretken L. paracasei ve lactopeptin üretmen L. paracasei mutan tonları ile yapılan çalışmadan, mutantın IP-10’u daha az parçaladığı belirlenmiş ve böylece lactopeptinin bağışıklık düzenleyici etkisi ortaya konmuştur (Von Schillde vd., 2012).


Probiyotikler ürettiği yüzey proteinerleri ile de bağışıklık sistemine etki etmektedir. L. acidophilus NCFM tarafından üretilen yüzey proteini SlaP dendritik hücreye bağlanarak anti-inflamatuar sitokin IL-10 üretimini artırmak için pro-inflamatuar sitokin IL-12 üretimini düştürdüğü belirlenmiştir. Mutasyon çalışmalarıyla SlaP üretilen suşların dendritik hücre letkin reseptörlerine (DC-SIGN) bağlanmasını azalma üstünlüğü ortaya konmuştur. Yanılan çalışmadan, saflaştırılan SlaP’ın doğrudan DC-SIGN’a bağlanmaya etkisini doğrulayarak anti-PMN’ye karşı koruyucu etki göster arastırılmıştır (Konstantinov vd., 2008). Ayrıca, SlaP’ın ve NCF2187 üretilen yüzey proteininin bağışıklık düzenleyici etkilerini ortaya koyan SlpA proteininin de benzer fonksiyonel özellikler gösterdiği ortaya konmuştur (Lightfoot vd., 2015).


Lactobasillerin ve VSL#3 probiyotik kolyeylemin pro-inflamatuar sitokin yolo yoluyla etkilemektedir, insanda doğal bağışıklığı gevşetirerek epitel zararlara ve antimikrobiyal bir peptit olan β-defensin-2 geninin inaktüsyonuna yol açtığı gösterilmiştir. Ancak bu etkiye sahip molekülün ne olduğu belirlenemediştir (Schlee vd., 2008). E. coli Nissle 1917 flagellalıve ve β-defensin-2 salınınını indüksiyonu birlikte etkilenmiştir. Flagella
çeşitli bakterilerde hücre duvara bağlı olarak bulunur. Ko-
nağın bağırsak epitel hücre reseptörleriley ilik teması geçtiği hareketli yüzey proteinidir (Ruiu vd., 2014). Yaptılağın
mada, flagellasi olmayan *E. coli* Nissle 1917 mutantlarında
bağışıklık düzenleme kapasitesi bozulurken, flagellasi tür-
harakтиleştirilmeleriyle bağışıklık düzenleme kapasitelerinin
eski öndüğü belirlenmiştir. Başka bir *E. coli* suçunun *E.
coli* Nissle 1917’nin tersine β-defensin-2 salınımını indükle-
mediği görülmuştur, dolayısıyla, *E. coli* Nissle 1917’nin flagel-
lasının temel uyarıcı faktör olduğu doğrulanmıştır (Schlee
d., 2007).

Probiyotiklerde hücre duvara bağlı olarak bulunan bir diğer
protein ise fimbria (pili)’dir. Biyosept ve yazıların farklı
olmasa rağmen hem Gram (+) hem de Gram (-) bakterilerde
fimbria görülmemektedir. Genom sekanslarından yola çıkarak
bifidobakter ve lactobasillerde fimbriyanın kodlandığı açığa
çarşınmıştır (Lebeer vd., 2010). Fimbriyanın bağırsak epitel
hücrelerine yapışabilme özelliğini sağlamak için sira başğı-
şıklığı uyari özellikinin de belirlendiği hücreler mevcut-
tur. Sağlıklı üzerine etkileri ve bağırsak epitellerine yapışa-
bilme özelliği bilinen *L. rhamnosus* GG’ nin hücre yüzeyinde
bulunan fimbrialar ile bunu sağladıgı bilinmektedir (Reuna-
en vd., 2012). Model olarak kullanılan *L. rhamnosus* GG ve
fimbriasy olmayan mutlantlaryla çalışılmış, fimbriyanın epitel
hücrelerine bağlanmadada önemli olduğu ve pro-inflamatur sit-
okin IL-8’in ekspresyonunun düşürülmesinde rolü olduğu belirlenmiştir (Lebeer vd., 2012). Benzer şekilde, *L. rhamnosus* GG’ nin fimbrialanın makrofajlar üzerindeki
etkisi fimbriasy olmayan *L. rhamnosus* GG mutanti ve doğal
şüşlaryla çalışılarken fare modellerinde incelenmiştir. Fimb-
riyanın makrofajlar ile bağlanmadada önemli olduğu ve anti-infla-
matur sitokin il-10 salınımlarını artıramak ve pro-inflamatur sitokin IL-6 salınımlarını azaltarak anti-inflamatur etki göster-
diği görülmüştür (Vargas Garcia vd., 2015). Aynı zamanda*
L. rhamnosus* GG’ nin patojen enfeksiyonları üzerine etkisine
bakılmış, *Salmonella enterica serovar Typhimurium SL1344’nin
neden olduğu inflamasyona karşı, fimbriasya bağırsak epitellerine bağlanan doğal bağısıklık sitokin salınımını
doğrudan uyardığı belirlenmiştir. (Ganguli vd., 2015). *L.
rhamnosus* GG’ nin fimbriyaların patojen bakterilerin bağ-
lammasını önlediği belirtilerek, enfeksiyonla karşı *L. rham-
osus* GG’ nin fimbriyanın tedavi amaçlı kullanılıabileceği
önerilmiştir (Tytgat vd., 2016). *Bifidobacterium bifidum*
PRL2010’ın fimbriasi ile de çalışılmış, benzer şekilde fimb-
riyanın yapışmada kritik öneşi olduğu ve böylece bağırsak
epitel hücre reseptörleriyle etkileşime girerek bağısıklık dü-
zeleyici etki gösterdikleri belirtilmiştir (Turroni vd., 2013).

Sonuç
Son yıllarda yapılan çalışmalarla, probiyotiklerin bağışıklık
sistemi üzerine birçok etkilerini üretikleri moleküllerin insan
hücreleri ile etkileşimleri sonucu sağladıkları ortaya çıkmış-
tır. Probiyotik hücre yapısı ile etkilerleri ve üretikleri moleküle-
rın bağışıklık sisteminin yönendirme mekanizmalarının belir-
lenmesi için birçok çalışma yapılmıştır. Sonuçlar probiyotik-
lerin insan bağışıklık sistemi üzerine aynı anda birçok etkiye
gerekleştirilmişdirleriğini göstermektedir. Probiyotiklerin tü-
ektimiyile kesin sonuçları ön görülemememi nedeniyle, hast-
alıkların önleme ve tedavisinde probiyotiklerin diğer üretik-
lerin moleküllerinin tüketicinin daha kontrollü bir yak-
laşım olduğu anlaşılmaktadır. Bu da sentetik probiyotik etken
moleküllerin ilacıya olma zorunluluğunu sağlar ve bu yakını
zamanında hayatımıza gireceğini işaret etmektedir. Moleküller bilimlerle
desteğen, protein yapısı çözümlenecek ve genom gibi yak-
laşmalar probiyotiklerin etken moleküllerin ve etki meka-
nizmalarının belirlenmesine olanak sağlayacaktır.

Etki Standart ile Uyumluluk
Çıkar çatışması: Yazarlar bu yazı için gerçek, potansiyel veya al-
gılanaan çıkar çatışması olmadığını beyan ettirmişler.

Teşekkür: Bu derlemedeki bilgi birikiminin oluşmasına katkı sağ-
ilen TÜBİTAK ve Ege Üniversitesi Bilim ve Araştırma Projeleri (Proje No: 116Z299) ve Ege Üniversitesi Bilimsel Araştırma Projeleri (Proje No: 16-Müh-083) desteklerine
teşekkür ederiz.

Kaynaklar
ment with health benefits. *Food Biotechnology*, 19(3), 227-
246. https://doi.org/10.1080/08905430500316474

Aguilar-Toalá, J.E., García-Varela, R., García, H.S., Mata-
Haró, V., González-Córdova, A.F., Vallejo-Cor-
evolving term within the functional foods field. *Trends in
Food Science & Technology*, 75, 105-114. https://doi.org/10.1016/j.tifs.2018.03.009


https://doi.org/10.1073/pnas.1115621109


https://doi.org/10.1136/gut.2010.232918


https://doi.org/10.1038/pr.2015.5

https://doi.org/10.1074/jbc.C200651200


https://doi.org/10.1111/nbu.12260

https://doi.org/10.1111/j.1365-2036.2006.02868.x

https://doi.org/10.1079/BJN20051428


https://doi.org/10.1128/IAI.00040-09

https://doi.org/10.3389/fmicb.2015.01285

https://doi.org/10.1111/j.1348-0421.2009.00189.x

https://doi.org/10.1186/1471-230X-9-15


Sudha, R.M., Bhonagiri, S. (2012). Efficacy of Bacillus Coagulans Strain Unique Is-2 in the Treatment of Patients With...


LAKTOZ İNTOLERANSIN PREVALANSI, TEŞHİSİ VE LAKTOZSUZ BESLENME TAVSİYELERİ

Furkan Demirgül¹, Recep Demirgül²

Cite this article as:

¹ Doğuş Üniversitesi, Sanat ve Tasarım Fakültesi, Gastronomi ve Mutfak Sanalari Bölümü, İstanbul, Türkiye
² Necmettin Erbakan Üniversitesi, Meram Tıp Fakültesi, Genel Cerrahi Ana Bilim Dalı, Yoğun Bakım Bilim Dalı, Konya, Türkiye

ORCID IDs of the authors:
F.D. 0000-0002-0141-0058
R.D. 0000-0001-8809-1522

Submitted: 01.03.2019
Revision requested: 26.04.2019
Last revision received: 06.05.2019
Accepted: 14.05.2019
Published online: 16.09.2019

Correspondence:
Furkan DEMİRGÜL
E-mail: furkandemirgul@gmail.com

ÖZ


Anahtar Kelimeler: Süt, Laktoz intolerans, Laktaz kalıcılığı, Laktozsuz beslenme

ABSTRACT

THE PREVALENCE AND DIAGNOSTIC METHODS OF LACTOSE INTOLERANCE AND LACTOSE-FREE DIET RECOMMENDATIONS

Lactose is a disaccharide found only in milk, and after weaning, the synthesis of lactase, which breaks down lactose, stops or decreases in most individuals. This condition, known as lactose intolerance, results in the inability of the digestion of milk and is very common in the world. Because of the single nucleotide polymorphisms occurring on the lactase gene, the lactase enzyme can be synthesized for life in some individuals. The prevalence of this condition called lactase persistence varies according to ethnic groups. Although there are various methods for the diagnosis of lactose intolerance, observation of symptoms is often sufficient for the diagnosis of the disease. Those who have lactose intolerance should apply a life-long lactose-free diet. Since milk is a rich source of some important nutrients such as calcium, it is important for lactose intolerance patients to meet their needs like calcium from foods with low lactose content such as cheese and yogurt. In this study, information about lactose intolerance was given and lactose intolerance prevalence, diagnosis methods, and lactose-free diet were examined. This study aims to increase the lactose intolerance awareness of both patients and food industry stakeholders.

Keywords: Milk, Lactose intolerance, Lactase persistence, Lactose-free diet
Giriş

Süt; protein, yağ, laktoz, vitamin, mineral, enzim, hormon ve immünoglobulin gibi insan yaşamını için önemli bulunan pek çok besin öğesi açısından zengin bir kaynaktır. Süttüne olan gıpta ve tüketimden gelen karbonhidratların aksı olarak akciğer dokusunda, kemik erimesi, diş çürüğü, alerji, astım, resim, hipertansiyon, kolorektal kanser veduğ gibi önemli rahatsızlıkların nedeni olarak kabul edilmektedir (Demirgül ve Sağdıç, 2018).

Laktaz ciftlik hayvanları da dahi olmak üzere neredeyse tüm memelilerin (denizesi ve deniz aygırı (mors) hariç) süttünde bulunan temel karbonhidratdır. İnsan sütündeki yaklaşık 7.2 g/100 mL laktoz bulunurken; insan beslenmesinde önemli bir yer tutan ine sütünün yaklaşık 4.7 g/100 mL’i laktozdan oluşmaktadır (Lomer vd., 2007). Laktoz, yaşamın ilk yılında, beşerlerin ihtiyaç duydukları toplam enerjinin neredeyse yarısını karşılamak için kullanılan bir tekrarlanabilen enerji kaynağıdır (Silanikove vd., 2010). Laktoz, herhangi bir yetişkin hayvanın, özellikle süt tüketen memelilerin, meme bezlerinin epitel hücrelerinin Golgi ağısty ortam içinde, glukoz ve galaktozun, laktoz sentaz enzimi tarafından ferment edilerek Bifidobacterium türleri gibi probiyotik Gram pozitif bağırsak bakterilerinin gelişimine katkıda bulunan önemli bir monosakkarit bileşendir (Kuhn ve White, 2009; Silanikove vd., 2010).


Bu çalışma ile dünya genelinde yaygın bir şekilde görülen laktoz intoleransının biyolojisi, farklı etnik gruplardaki prevalansı ve tıbbi öğretimlerdeki bilgi verilmesi amaçlanmıştır. Ayrıca laktoz intolerans olan kişiler için akciğer dejeneratif hastalıklarını veOTHER DOMESTIC SECTOR, ANNUAL REPORT 2018


birlikte ekzojen β-galaktozidaz tüketimi ve kolon bakterilerine uygun süt ürünleri tüketimi gibi pek çok faktör etkilemektedir (Brown-Esterson vd., 2012).


Zaman içinde gelişen ‘primer laktaz eksikliği’ en sık gözlenen laktaz eksikliğidir. 2 yaşından önce laktaz aktivitesi, bebeklerin/küçük çocukların tükettiği süt ve süt ürünleri nedeniyle nadir görülür. Ancak, anne sütü laktaz içerdigi için, primer laktaz eksikliği, herhangi bir etnik popülasyonun emzirilen bebeklerinde çok nadir görülür. Bununla birlikte, 2 yaşından sonra laktaz üretimi yavaş yavaş azalır ve bu durum hemen veya daha sonra çeşitli semptomlara neden olabilir (Gaskin ve Ilich, 2009).


Farklı Etnik Gruplara Laktaz Yetersizliğinin prevalansı

Dünya genelinde insanların önemli bir kısmı doğumda laktaz seviyelerinin büyük bir kısmını erken çocukluğa kaybetmiş ve bu kayıp yaşam boyunca devam eder. Ancak hipolaktazaya (laktaz yetersizliği) prevalans etnik kökene bağlı olarak değişiklik göstermektedir (Tablo 1) (Swagerty vd., 2002).

Çoğu beyaz Kuzey Avrupa 1-2 bardak sütü (250-500 mL) hiçbir yan etki görülmemesini içermektedir, bazı etnik gruplar laiska karşı o kadar hassastırlar ki, bazı kanserlerdeki hastaların %10-20 ml süt bile onlara hasta etmeye yetebilir (Campbell vd., 2005). Beyaz Kuzey Avrupalılar ve diğer bazı etnik gruplar (Bedeviler ve Afrika’da yaşayan bazı topluluklar gibi) dışındaki tüm memelilerin laktaz aktiviteleri düsükür. Bu durum, laktaz kalıcılığına neden olabilmektedir (Matthews vd., 2005).

Madyr vd. (2010), yetişkinlerdeki hipolaktazyanın Avrupa’nın güneyine ve doğusuna gidildiğinde arttığını, Güney İtalya ve Türkiye’deki yetişkinlerde hipolaktazaya görülme prevalansının %70’lerde olduğunu bildirmişlerdir. Türkiye’deki laktaz intolerans prevalansının ve bunun altında yatan beş yan etkisi nedeniyle etnik kökenle en çok ilişkilendirilen alellere ait (Liebert vd., 2005). 

Tablo 1. Farklı etnik gruplarda gözlenen laktaz eksikliği prevalansı (Campbell vd., 2005; Lomer vd., 2007; Ugidos-Rodriguez vd., 2018)

Table 1. Prevalence of lactase deficiency observed in different ethnic groups (Campbell et al., 2005; Lomer et al., 2007; Ugidos-Rodriguez et al., 2018)

<table>
<thead>
<tr>
<th>Etnik Grup (belirtilmediği sürece yetişkinler)</th>
<th>Düşük laktaz (potansiyel laktoz intolerans) prevalansı (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beyaz Avustralyalılar</td>
<td>10</td>
</tr>
<tr>
<td>Beyaz Kuzey Avrupalar</td>
<td>10</td>
</tr>
<tr>
<td>Barselonalılar (İspanya özerk bölgesi, Kuzeydoğu İspanya)</td>
<td>13-15</td>
</tr>
<tr>
<td>Finler</td>
<td>20</td>
</tr>
<tr>
<td>Amerikalılar (tüm yetişkinler)</td>
<td>30</td>
</tr>
<tr>
<td>Galiçyalılar (İspanya özerk bölgesi, Kuzeybatı İspanya)</td>
<td>32.5</td>
</tr>
<tr>
<td>İspanyollar</td>
<td>&gt; 40</td>
</tr>
<tr>
<td>İtalyanlar</td>
<td>&gt; 40</td>
</tr>
<tr>
<td>Yunanlar</td>
<td>&gt; 40</td>
</tr>
<tr>
<td>Orta Avrupalar (örn. Macar ve Çingeneler)</td>
<td>&gt; 40</td>
</tr>
<tr>
<td>Güney Amerikalılar (tüm yetişkinler)</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>Meksikalılar</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>Afrika kökenli Amerikalılar</td>
<td>60-70</td>
</tr>
<tr>
<td>Eskimolar</td>
<td>&gt; 70</td>
</tr>
<tr>
<td>Amerikan Kızılderelemeleri</td>
<td>&gt; 70</td>
</tr>
<tr>
<td>Siyah Afrikalılar</td>
<td>&gt; 75</td>
</tr>
<tr>
<td>Avustralya Aborjinleri</td>
<td>&gt; 80</td>
</tr>
<tr>
<td>Hintliler ve diğer Asyalı gruplar</td>
<td>&gt; 80</td>
</tr>
<tr>
<td>Çinliler</td>
<td>&gt; 90</td>
</tr>
<tr>
<td>Japonlar</td>
<td>&gt; 90</td>
</tr>
<tr>
<td>Yetişkinler (tüm etnik gruplar)</td>
<td>70</td>
</tr>
<tr>
<td>Huzursuz bağırsak sendromu hastaları (tüm etnik gruplar)</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>2 ile 10 yaş arasındaki çocuklar (tüm etnik gruplar)</td>
<td>0-40</td>
</tr>
<tr>
<td>2 yaşın altında çocuklar (tüm etnik gruplar)</td>
<td>0-20</td>
</tr>
</tbody>
</table>

olan süt tüketiminin avantajlarının, laktaz kalıcılığı teşvik ettiği düşünülmektedir (Ugidos-Rodriguez vd., 2018).


**Laktoz İntoleransının Teşhisi**

Laktoz intolerans teşhisinde çeşitli yöntemler bulunmaktadır. İlk olarak laktaz enzimini kodlayan genetik testler, tükürükten izole edilmiş DNA’nın spesifik amplifikasyonu kullanılarak yapılabılır. Genetik testler, klinik bulguları olan ve hidrojen solunum testine negatif olan 8 yaş altı çocuklar üzerinde bileşikleri yararlı olarak değerlendirilmektedir. Genetik testler, tükürükten izole edilen DNA’nın spesifik amplifikasyonu kullanılarak yapılabilir. Genetik testler, real-time PCR (Polimeraz Zincir Reaksiyonu) kullanılarak, laktaz enzimini kodlayan genin spesifik amplifikasyonu testi olarak değerlendirilir. PCR ile analizin spesifikliği yaklaşık %100; dayaranlığı ise yaklaşık %93’tür. Bu test, hızlı, kesin ve non-invaziv bir tanı konulmasına imkân sağlamakta (Ugidos-Rodriguez vd., 2018).

Hidrojen solunum testine kıyaslal genetik testler, semptomlara neden olan ve laktaz intolerans teşhisinde imkan veren, daha basit ve non-invaziv yöntemlerdir. Ayrıca genetik testler, hidrojen solunum testine kıyaslal genetik testler, real-time PCR (Polimeraz Zincir Reaksiyonu) kullanılarak, laktaz enzimini kodlayan genin spesifik amplifikasyonu testi olarak değerlendirilir. PCR ile analizin spesifikliği yaklaşık %100; dayaranlığı ise yaklaşık %93’tür. Bu test, hızlı, kesin ve non-invaziv bir tanı konulmasına imkân sağlamakta (Ugidos-Rodriguez vd., 2018).


Genetik testlerin, klinik bulguları olan ve hidrojen solunum testine negatif olan 8 yaş altı çocuklar üzerinde bileşikleri yararlı olarak değerlendirilmektedir. Genetik testler, tükürükten izole edilen DNA’nın spesifik amplifikasyonu kullanılarak yapılabilir. Genetik testler, real-time PCR (Polimeraz Zincir Reaksiyonu) kullanılarak, laktaz enzimini kodlayan genin spesifik amplifikasyonu testi olarak değerlendirilir. PCR ile analizin spesifikliği yaklaşık %100; dayaranlığı ise yaklaşık %93’tür. Bu test, hızlı, kesin ve non-invaziv bir tanı konulmasına imkân sağlamakta (Ugidos-Rodriguez vd., 2018).

Hidrojen solunum testine kıyaslal genetik testler, semptomlara neden olan ve laktaz intolerans teşhisinde imkan veren, daha basit ve non-invaziv yöntemlerdir. Ayrıca genetik testler, laktaz kalıcılığı veya laktaz kalıcılığı hakkında daha doğru olduğunu pierden bir sonuc verirken; hidrojen solunum testinin yorumlanması üzerine, verilen laktaz dozu, test süresi ve bireyin yaşı gibi fazla saydaki değişken etkildir (Mattar vd., 2012). Ancak laktaz kalıcılığı neden olan polimorfizm genlerinin
ve etnik gruplara göre farklılık göstermesi genetik testler sı-
nırlırdıktadır. Bu konuda yapılacak daha ileri çalışma-
larla gelecekte genetik testlerin etkinliği artırılabilir. Bu-
nunla birlikte genetik testlerin sekonder laktaz eksikliğini teş-
his etmek amacıyla kullanılamayacağı açıktır (Misselwitz
vd., 2013).

**Laktozuz Beslenme Tavsiyeleri**

Laktaz sadece memelilerin sütünde bulunan bir disakkarit ol-
masına rağmen, süt ve süt ürünleri pek çok gıdanın üreti-
minde kullanılmaktadır. Ayrıca sütten saflaştırılan laktoz,
technolojik özelliklerinden dolayı endüstriyel gıdalarda katı
maddesi olarak, ilaclarda ise dolgu maddesi olarak sıkılkla
kullanılmaktadır. Laktozun renk ve su bağlama özelliğini bul-
unmaktadır. Ayrıca tekstür üzerine de olumlu etkileri vardır.
Laktoz yüksek alan ürünlerde glukozun yarısını, sakkarozun ise ölçücü
biri oranında tatlılak simultaneously vermektedir. Laktuz bu özelliklerinden
dolaylı tatlılarda, şekerlemelerde, ekmeklerde, sosis gibi iş-
lenmiş et ürünlerinde çok kullanılmaktadır (Vesa vd., 2000).

**Tablo 2. Bazı gıdaların içerdiği yaklaşık laktoz miktarları**

<table>
<thead>
<tr>
<th>Gıda</th>
<th>Laktoz miktarı (g/100g)</th>
<th>Referans</th>
</tr>
</thead>
<tbody>
<tr>
<td>İnek sütü</td>
<td>4.9</td>
<td>Chandan ve Shah, 2006</td>
</tr>
<tr>
<td>Yoğurt</td>
<td>4.5</td>
<td>Misselwitz vd., 2013</td>
</tr>
<tr>
<td>Taze beyaz peynir (1 gün depolanan)</td>
<td>2.05</td>
<td>Topçu ve Saldamli, 2006</td>
</tr>
</tbody>
</table>
| Olgunlaştırılmış beyaz peynir (90 gün de-
   polanan)                    | 0.74                    | Topçu ve Saldamli, 2006|
| Peynir altı suyu (beyaz peynir) | 4.57                   | Topçu ve Saldamli, 2006|
| Cheddar                       | 0.18                    | Corgneau vd., 2015     |
| Mozarela                      | 0.07                    | Corgneau vd., 2015     |
| Eksi krema                    | 2.91                    | Corgneau vd., 2015     |
| Tereyağı                      | 1                       | Hertzler vd., 2017     |
| Dondurma                      | 6                       | Misselwitz vd., 2013   |
| Çok tahıllı ekmek             | 0.56                    | Corgneau vd., 2015     |
| Aromalı enerji içeceği        | 0.20                    | Corgneau vd., 2015     |
| Salata sosu                   | 1.40                    | Corgneau vd., 2015     |
| Vanilyali puding              | 1.80                    | Corgneau vd., 2015     |
| Balık kroket                  | 0.12                    | Corgneau vd., 2015     |
| Çikolata bar                  | 8.21                    | Corgneau vd., 2015     |

Bazı Asya restoranları geleneksel Asya mutfağlarında kulla-
nılan laktoz içermeyen Hindistan cevizi sütü gibi malzemele-
rin yerine son yıllarda süt kremasını veya yoğunlaştırılmış süt
kullanmaktadır. Oysa birçok laktaz intolerans hastası, süt
zu ve yoğunlaştırılmış sütün eşdeğer miktarındaki süt daha
fazla laktaz içerdigini bilmemektedir. Benzer şekilde pek çok
insan, peynir altı suyu gibi ürünlerin sütten nere
deyse tamamını içerdigini farkında değildir (Campbell vd.,
2005). Laktaz intolerans olan kişilere uygun bilgilendirmeye-
rin yapılması oldukça önemlidir. Laktoz toleransı çok düşük
olan kişiler, yukarıda zikredilen ve Tablo 2’de verilen gıdalar
da dâhil olmak üzere, değerli miktarlarda laktoz içeren birço
gıda ürünü olduğunu bilmedi ve gıda ambalajlarındaki
uyarları dikkate almalıdır.

Sonuç

Pek çok beslenme otoritesi, içerdiği önemli besin elementleri nedeniyle süt ve süt ürünlerinin, diyetizmin bir parçası olması gerektiğini belirtir. Bununla birlikte, süt bulununun temel kARBONhidrat olan laktoza karşı dünyada nüfusunun önemli bir kısmını, rahatsız edici çeşitli semptomlara neden olan aşırı hassasiyet bulunmaktadır. Laktoz intoleranslar adı verilen bu durumda, anneyi emmevi, zehirli bir etkileşim olabileceği için, semptomları azaltmak için laktaz enzim kompresyonu gerektiğine dair raporlar bulunmaktadır (Swagerty vd., 2002).

Bununla birlikte, bazı hastaların düzenli laktoz tüketimi ile laktozu toleranslarını düzeltmeleri gerektiğini belirtirler. Bu hastaların laktaz enzim takviyeleri kullanmalarının da yararlı olduğunu, ancak bu yöntemlerin her zaman etkili olmayacağı belirtmektedir (Swagerty vd., 2002).

Laktoz sahip olduğu teknolojik özellikler nedeniyle pek çok gıdada katkı maddesi olarak da kullanılmaktadır. Laktoz içeren gıdaların ambalajlarında laktoz içerdiğine dair bir uyarı yer almasına rağmen, bu uyarı bazen belirgin olmayabilmektedir. Ambalajlı gıdalarda topluma sık rastlanılan hassasiyet veya alerjenler karşı kayıpların, gıda seçiminde daha bilişçili hareket etmeleri için yetkilili otoriteler tarafından doğru yönlendirmeleri gerektirmektedir.

Etik Standart ile Uyumluluk
Çıkar çatışması: Yazalar bu yazı için gerçek, potansiyel veya algılanan çıkar çatışması olmadığını beyan etmişlerdir.

Kaynaklar


Enattah N.S., Jensen, T.G., Nielsen, M., Lewinski, R., Kuokkanen, M., Rasinpera, H., El-Shanti, H., Seo, J.K.,...


Instructions to Authors

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

Originality, high scientific quality, and citation potential are the most important criteria for a manuscript to be accepted for publication. Manuscripts submitted for evaluation should not have been previously presented or already published in an electronic or printed medium. The journal should be informed of manuscripts that have been submitted to another journal for evaluation and rejected for publication. The submission of previous reviewer reports will expedite the evaluation process. Manuscripts that have been presented in a meeting should be submitted with detailed information on the organization, including the name, date, and location of the organization.

Manuscripts submitted to “Food and Health” will go through a double-blind peer-review process. Each submission will be reviewed by at least two external, independent peer reviewers who are experts in their fields in order to ensure an unbiased evaluation process. The editorial board will invite an external and independent editor to manage the evaluation processes of manuscripts submitted by editors or by the editorial board members of the journal. The Editor in Chief is the final authority in the decision-making process for all submissions.

An approval of research protocols by the Ethics Committee in accordance with international agreements (World Medical Association Declaration of Helsinki “Ethical Principles for Medical Research Involving Human Subjects,” amended in October 2013, www.wma.net) is required for experimental, clinical, and drug studies. If required, ethics committee reports or an equivalent official document will be requested from the authors.

For manuscripts concerning experimental research on humans, a statement should be included that shows the written informed consent of patients and volunteers was obtained following a detailed explanation of the procedures that they may undergo. Information on patient consent, the name of the ethics committee, and the ethics committee approval number should also be stated in the Materials and Methods section of the manuscript. It is the authors’ responsibility to carefully protect the patients’ anonymity. For photographs that may reveal the identity of the patients, signed releases of the patient or of their legal representative should be enclosed.

“Food and Health” journal requires experimental research studies on vertebrates or any regulated invertebrates to comply with relevant institutional, national and/or international guidelines. The journal supports the principles of Basel Declaration (https://www.basel-declaration.org/) and the guidelines published by International Council for Laboratory Animal Science (ICLAS) (http://iclasm.org/). Authors are advised to clearly state their compliance with relevant guidelines.

“Food and Health” journal advises authors to comply with IUCN Policy Statement on Research Involving Species at Risk of Extinction and the Convention on the Trade in Endangered Species of Wild Fauna and Flora for research involving plants.

All submissions are screened by a similarity detection software (https://intiha.net).

In the event of alleged or suspected research misconduct, e.g., plagiarism, citation manipulation, and data falsification/fabrication, the Editorial Board will follow and act in accordance with COPE guidelines.

Each individual listed as an author should fulfill the authorship criteria recommended by the ICMJE. The ICMJE recommends that authorship be based on the following 4 criteria:

1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
2. Drafting the work or revising it critically for important intellectual content; AND
3. Final approval of the version to be published; AND
4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

In addition to being accountable for the parts of the work he/she has done, an author should be able to identify which co-authors are responsible for specific other parts of the work. In addition, authors should have confidence in the integrity of the contributions of their co-authors.

All those designated as authors should meet all four criteria for authorship, and all who meet the four criteria should be identified as authors. Those who do not meet all four criteria should be acknowledged in the title page of the manuscript.

“Food and Health” journal requires corresponding authors to submit a signed and scanned version of the authorship contribution form (available for download through http://scientificwebjournals.com/JFHS/FHCopyrightandAuthorContributionForm2019.pdf) during the initial submission process in order to act appropriately on authorship rights and to prevent ghost or honorary authorship. If the editorial board suspects a case of “gift authorship,” the submission will be rejected without further review. As part of the submission of the manuscript, the corresponding author should also send a short statement declaring that he/she accepts to undertake all the responsibility.
for authorship during the submission and review stages of the manuscript.

“Food and Health” journal requires and encourages the authors and the individuals involved in the evaluation process of submitted manuscripts to disclose any existing or potential conflicts of interests, including financial, consultant, and institutional, that might lead to potential bias or a conflict of interest. Any financial grants or other support received for a submitted study from individuals or institutions should be disclosed to the Editorial Board. To disclose a potential conflict of interest, the ICMJE Potential Conflict of Interest Disclosure Form should be filled in and submitted by all contributing authors. Cases of a potential conflict of interest of the editors, authors, or reviewers are resolved by the journal’s Editorial Board within the scope of COPE and ICMJE guidelines.

The Editorial Board of the journal handles all appeal and complaint cases within the scope of COPE guidelines. In such cases, authors should get in direct contact with the editorial office regarding their appeals and complaints. When needed, an ombudsperson may be assigned to resolve cases that cannot be resolved internally. The Editor in Chief is the final authority in the decision-making process for all appeals and complaints.

When submitting a manuscript to “Food and Health” journal, authors accept to assign the copyright of their manuscript to ScientificWebJournals. If rejected for publication, the copyright of the manuscript will be assigned back to the authors. “Food and Health” journal requires each submission to be accompanied by a Copyright Transfer Form (available for download at http://scientificwebjournals.com/JFHS/FHCopyrightandAuthorContributionForm2019.pdf). When using previously published content, including figures, tables, or any other material in both print and electronic formats, authors must obtain permission from the copyright holder. Legal, financial and criminal liabilities in this regard belong to the author(s).

Statements or opinions expressed in the manuscripts published in “Food and Health” journal reflect the views of the author(s) and not the opinions of the editors, the editorial board, or the publisher; the editors, the editorial board, and the publisher disclaim any responsibility or liability for such materials. The final responsibility in regard to the published content rests with the authors.

MANUSCRIPT PREPARATION

The manuscripts should be prepared in accordance with ICMJE-Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (updated in December 2017 - http://www.icmje.org/icmje-recommendations.pdf). Authors are required to prepare manuscripts in accordance with the CONSORT guidelines for randomized research studies, STROBE guidelines for observational studies, STARD guidelines for studies on diagnostic accuracy, PRISMA guidelines for systematic reviews and meta-analysis, ARRIVE guidelines for experimental animal studies, TREND guidelines for non-randomized studies, and COREQ guidelines for qualitative studies.

Manuscripts can only be submitted through the journal’s online manuscript submission and evaluation system, available at http://dergipark.gov.tr/journal/1646/submission/start.

Manuscripts submitted to the journal will first go through a technical evaluation process where the editorial office staff will ensure that the manuscript has been prepared and submitted in accordance with the journal’s guidelines. Submissions that do not conform to the journal’s guidelines will be returned to the submitting author with technical correction requests.

Authors are required to submit the following forms during the initial submission.

- Copyright Transfer Form,
- Author Contributions Form (one form for copyright and contributions available in http://scientificwebjournals.com/JFHS/FHCopyrightandAuthorContributionForm2019.pdf)
- ICMJE Potential Conflict of Interest Disclosure Form (should be filled in by all contributing authors) Download this form from http://www.icmje.org/conflicts-of-interest/ fill and save. Send this to the journal with your other files.

Preparation of the Manuscript

Title (should be clear, descriptive and not too long)
Full Name(s) and Surname (s) of author(s)
ORCID ID for all author(s) (http://orcid.org/)
Address (es) of affiliations and e-mail (s)
Complete correspondence address and e-mail
Abstract
Key words (indexing terms), normally 3-6 items
Introduction
Material and Methods
Results and Discussion
Conclusion
Compliance with Ethical Standard

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

Financial disclosure: If there is any, the institutions that support the research and the agreements with them should be given here.

Acknowledgment: Acknowledgments allow you to thank people and institutions who assist in conducting the research.

References

Tables

Figures

Manuscript Types

Original Articles: This is the most important type of article since it provides new information based on original research. The main text should contain Introduction, “Materials and Methods”, “Result and Discussion” and Conclusion sections.

Statistical analysis to support conclusions is usually necessary. Statistical analyses must be conducted in accordance with international statistical reporting standards. Information on statistical analyses should be provided with a separate subheading under the Materials and Methods section and the statistical software that was used during the process must be specified.

Units should be prepared in accordance with the International System of Units (SI).

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

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

Table 1. Limitations for each manuscript type

<table>
<thead>
<tr>
<th>Type of manuscript</th>
<th>Page limit</th>
<th>Abstract word limit</th>
<th>Reference limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original Article</td>
<td>≤25</td>
<td>180</td>
<td>40</td>
</tr>
<tr>
<td>Review Article</td>
<td>no limits</td>
<td>180</td>
<td>60</td>
</tr>
<tr>
<td>Short Communication</td>
<td>≤5</td>
<td>150</td>
<td>20</td>
</tr>
</tbody>
</table>

Tables

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

Figures and Figure Legends

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

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

When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: “Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)”
All references, tables, and figures should be referred to within the main text, and they should be numbered consecutively in the order they are referred to within the main text.

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

References
Reference System is APA 6th Edition

In-text Citation with APA

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

...(Crockatt, 1995).

Direct quote from the text

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

Major Citations for a Reference List in Table 2.

<table>
<thead>
<tr>
<th>Material Type</th>
<th>Reference List/Bibliography</th>
</tr>
</thead>
</table>

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

REVISIONS

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

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