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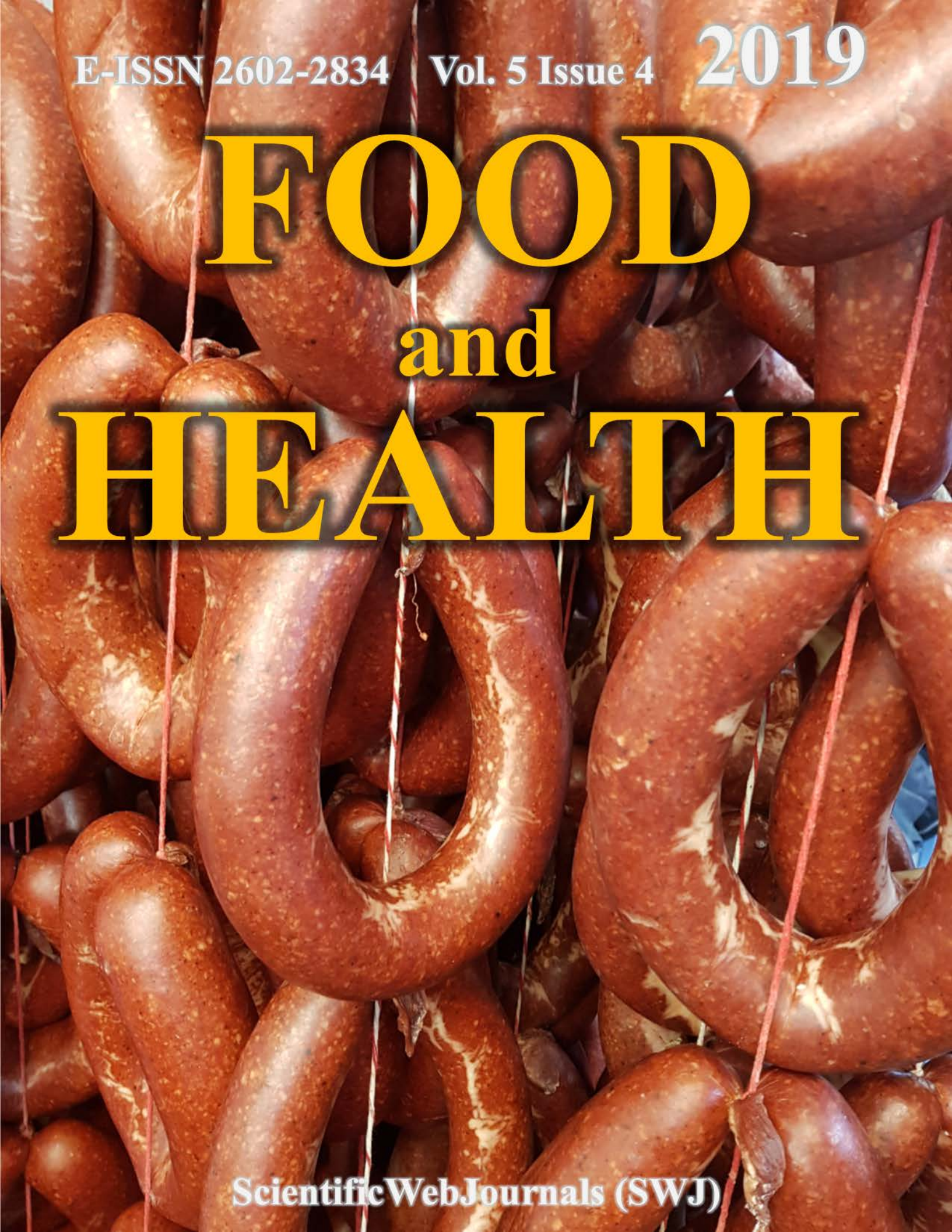
FOOD and HEALTH



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FOOD and HEALTH

Aims and Scope

FOOD and HEALTH

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QUALITY ATTRIBUTES OF CITRUS FIBER ADDED GROUND BEEF AND CONSUMER ACCEPTANCE OF CITRUS FIBER ADDED TURKISH MEATBALLS

Ayça Gedikoğlu[1], Andrew Douglas Clarke[2]

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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. Over all 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 (Scheneeman, 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 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 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.

$$\text{WHC} = \left[\frac{\text{Area of free water}}{\text{Area of meat}}\right] \quad (1)$$

Cooking Yield

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

$$\text{Cooking Yield \%} = \left[\frac{\text{Cooked weight of the product}}{\text{Uncooked weight of the product}}\right]^{*} \quad (2)$$

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

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

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 capac-ity 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 treat-ments are shown in Table 2. The moisture content of the con-trol 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 mois-ture 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 com-ing from citrus fiber, increase in the protein content was nor-mal 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 Faneshil (1986) also reported that phospholipids can react with bicinchoninic 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 the control and CF 1%. 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

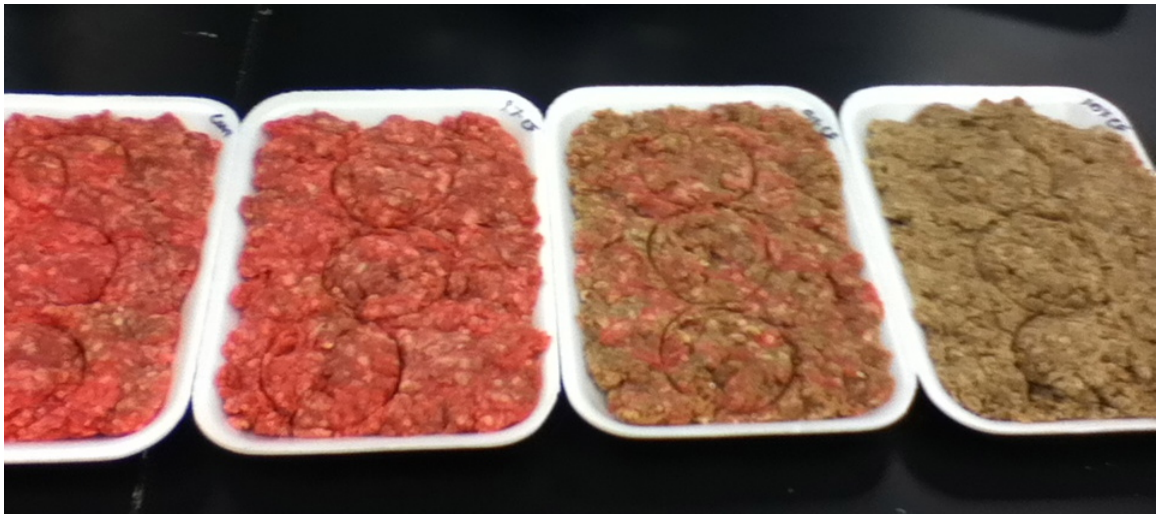

Table 2. Addition of different levels of citrus fiber on physico-chemical properties of ground beef meatballs

	Citrus Fiber Treatment Levels			
	0%	1%	5%	10%
pH raw	5.54 ±0.139^{ab}	5.62 ±0.133^{a}	5.59 ±0.096^{a}	5.47 ±0.104^{b}
pH cooked	5.65 ±0.100^{a}	5.74 ±0.136^{a}	5.66 ±0.104^{a}	5.49 ±0.121^{b}
WHC	0.68 ±0.13^{a}	0.49 ±0.05^{b}	0.44 ±0.09^{bc}	0.36 ±0.07^{c}
Cooking Yield (%)	71.43 ±4.54^{c}	78.91 ±4.64^{b}	86.62 ±4.54^{a}	92.21 ±4.79^{a}
Moisture Content (%)	60.75 ±2.51^{a}	60.51 ±2.14^{a}	58.49 ±1.86^{ab}	56.35 ±3.88^{b}
Fat Content (%)	21.30 ±3.01^{a}	20.59 ±2.76^{ab}	19.81 ±2.90^{b}	19.68 ±3.26^{b}
Protein Content (%)	14.46 ±0.69^{d}	16.49 ±0.49^{c}	19.28 ±0.81^{b}	21.16 ±0.64^{a}

Each value in the Table is represented as mean ± standard deviation (n=6).

$^{a, b, c, d}$ 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™

Total Pectin	9390 mg / 100g
Protein	6.37 %
Total Sugars	1.7%
Total Dietary Fiber	82.7%
Soluble Fiber	23.4%
Insoluble Fiber	59.3%
Potassium	453 mg/100g
Sodium	210 mg/100g
Calcium	78 mg /100g
Vitamin A (Beta Carotene)	117 IU/100g
Vitamin C	0.91 mg/100g

Source: Natural Citrus Products

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

Citrus Fiber Levels	Textural Properties					
	Hardness	Springi-ness	Cohesive-ness	Gumminess	Chewiness	Resilience
CF 0%	1356.13 ±500.65^{a}	0.746 ±0.051^{a}	0.553 ±0.036^{a}	743.90 ±252.42^{a}	563.01 ±221.8^{a}	0.228 ±0.021^{a}
CF 1%	1088.89 ±396.29^{ab}	0.714 ±0.051^{a}	0.480 ±0.03^{b}	521.59 ±179.94^{b}	378.63 ±150.78^{b}	0.194 ±0.019^{b}
CF 5%	887.17 ±243.74^{b}	0.656 ±0.046^{b}	0.346 ±0.052^{c}	304.06 ±78.86^{c}	201.22 ±59.97^{c}	0.145 ±0.019^{c}
CF 10%	819.69 ±246.72^{b}	0.611 ±0.05^{c}	0.244 ±0.074^{d}	198.62 ±76.31^{c}	120.83 ±46.79^{c}	0.121 ±0.023^{d}

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

Citrus Fiber Levels	Hunter Color		
	L Value	*a* Value	*b* Value
CF 0%	48.24 ±0.742^{a}	23.36 ±1.01^{a}	9.98 ±0.240^{a}
CF 1%	44.42 ±0.117^{bc}	18.64 ±0.96^{b}	9.35 ±0.177^{b}
CF 5%	42.92 ±0.801^{c}	11.89 ±2.10^{c}	9.39 ±0.164^{b}
CF 10%	45.61 ±0.848^{b}	8.23 ±2.28^{c}	10.29 ±0.268^{a}

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

Citrus Fiber Levels	Flavor	Texture	Overall Likeness
CF 0 %	6.52 ± 1.4^{a}	6.69 ± 1.52^{a}	6.69 ± 1.37^{a}
CF 1 %	6.61 ± 1.44^{a}	6.27 ± 1.75^{b}	6.55 ± 1.51^{a}
CF 3 %	5.94 ± 1.76^{b}	5.9 ± 1.67^{c}	5.9 ± 1.66^{b}
CF 5 %	5.49 ± 1.73^{c}	5.46 ± 1.89^{d}	5.47 ± 1.68^{c}

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.

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

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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, Biogen amine, Starter culture, Heat treated, Sucuk

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 (Ravyts 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 carnosus*, *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; Gençcelep 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, and Chaudhari, 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 Zantopoulos, 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 microorganism. 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 Karabulut and Candogan, 2017). 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 3.2×10^{11} CFU/mL

and 8.4×10^{11} 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 *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 $CaCl_2$ (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 centrifuge 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 Figure1. In heat treated sucuk production, heat treatment was carried out 24 h after stuffing in a cabin 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 4ºC 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 sucuks 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 and bacteria counts from previous study (Bilenler, Karabulut, and Candogan, 2017) 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).

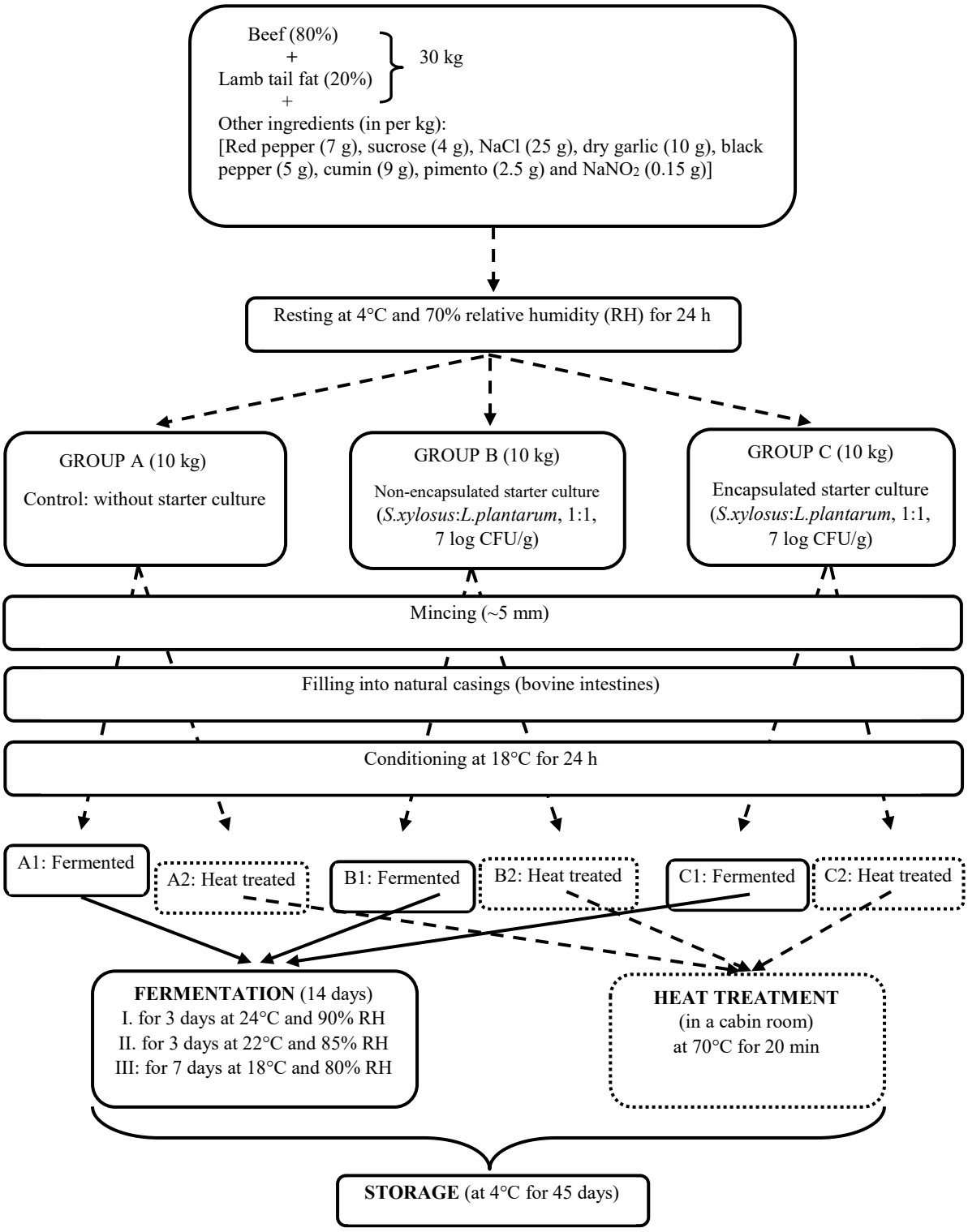

Figure 1. Flow chart for production process of the fermented and heat treated sucuks

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 at 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 sucuks 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.

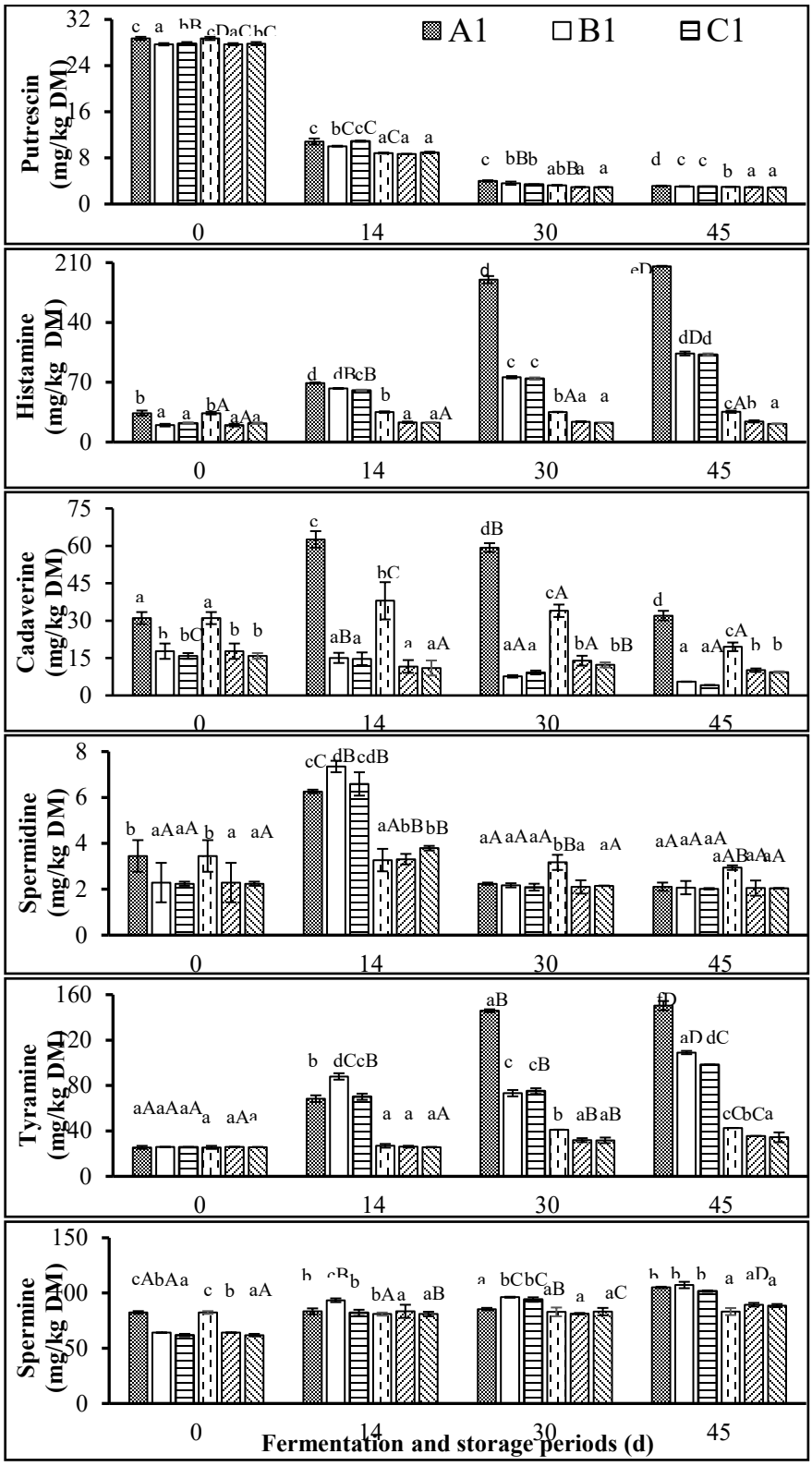

Figure 2. Changes of biogenic amines amounts during fermentation and storage periods. Different lowercase letters (a-d) in the same production day for the different sucuk samples indicate significant difference ($p < .05$). Different uppercase (A-D) between the storage days for the same sample indicate significant difference ($p < .05$)

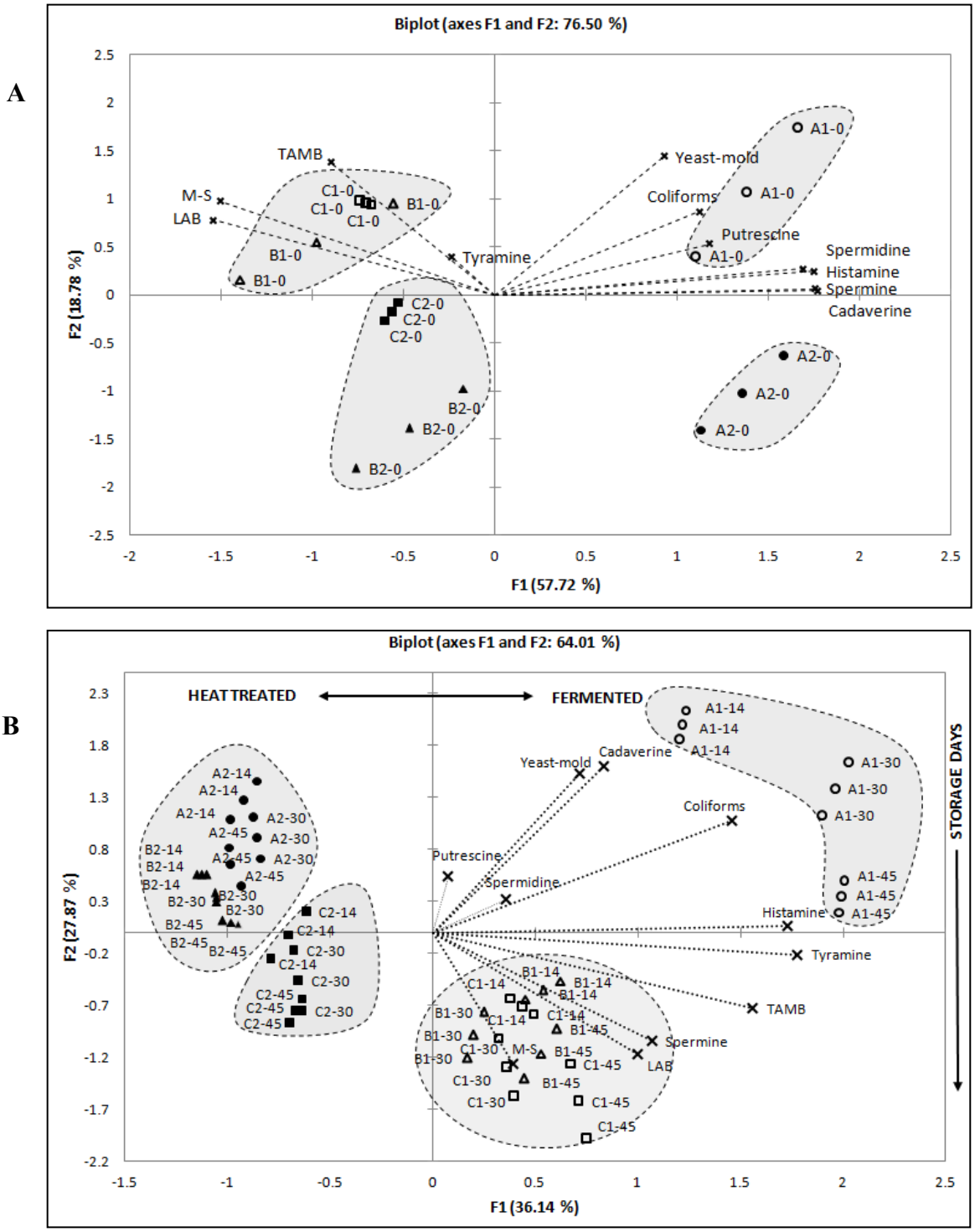

Figure 3. Biplot of the principal component analysis (PCA) carried on data of biogenic amines and microbial counts of sucuk samples just after production (A) and storage periods (B). The variables (Loadings) used for the analysis were indicated in red letters and sucuk samples (Scores) were indicated 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 3 B 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 sucuk 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 sucuk 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.

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

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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-Krajnik *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*., 2017; Omac *et al*., 2018). It can grow at a temperature range of 1-45 °C, low pH and high salinity (10% NaCl) (Drevets and Bronze, 2008; Gahan and Hill, 2014; Omori *et al*., 2017; Santos *et al*., 2018). The low pH in acidic foods plays an important role in terms of microbial stability and food safety. However, recent studies have shown that *L. monocytogenes* have an increased survival level in foods with low pH due to its acid tolerance mechanism (Gahan *et al*., 1996; Koutsoumanis *et al*., 2003; Chung *et al*., 2018).

The acid tolerance response (ATR) mainly forms as a result of the exposure of certain pathogenic bacteria to moderately acidic (pH 5.0-6.0) environments for a certain period of time, resulting in resistance to high acidic environments by synthesizing acid shock proteins controlled by specific genes (Lou and Yousef, 1997; Giaouris *et al*., 2014; Park *et al*., 2016; Ijabadeniyi and Mnyandu, 2017; Kapetanakou et al., 2017; Santos et al., 2018). It is known that the resistance of *L. monocytogenes* to acidic pH depends on the composition of the growing environment, bacterial strain, the phase of bacterial growth and the acid type used (Phan-Thanh *et al*., 2000; Cataldo *et al*., 2007). Acid-tolerant pathogenic bacteria are of importance in terms of both food industry and human health (Leyer *et al*., 1995; Gahan *et al*., 1996; Cheng *et al*., 2003; Öztürk and Halkman, 2015; Omori *et al*., 2017; Guariglia-Oropeza *et al*., 2018).

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 (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).

Acid Tolerance of L. monocytogenes

To determine the resistance of *L. monocytogenes* cells to strongly acidic conditions, 0.5 mL of acid-adapted and non-treated 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 % $NaNO_3$ and 0.01 % $NaNO_2$ 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* (10^5 CFU/g) was added to the sucuk batter of the first experimental group, while non-acid-tolerated *L. monocytogenes* (10^5 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. monocytogene*s 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). Koutsoumanis 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 HCI (pH 4.5) adapted and nontreated *L. monocytogenes* in pH 2.5 (log CFU/mL)

Incubation (h)	Acid-adapted period (h)				Nontreated
	1	2	3	4	
0	$7.02A^{a}\pm0.06$	$7.00A^{a}\pm0.04$	$7.02A^{a}\pm0.14$	$7.05A^{a}\pm0.22$	$7.06A^{a}\pm0.21$
1	$6.21B^{a}\pm0.06$	$6.23B^{a}\pm0.05$	$6.27B^{a}\pm0.07$	$6.10B^{a}\pm0.02$	$5.60B^{a}\pm0.06$
2	$5.78B^{a}\pm0.38$	$5.98B^{a}\pm0.28$	$5.91B^{a}\pm0.17$	$5.95B^{a}\pm0.10$	$4.48C^{b}\pm0.33$
3	$4.03C^{b}\pm0.26$	$4.65C^{ab}\pm0.00$	$5.30C^{a}\pm0.44$	$4.71C^{ab}\pm0.78$	$2.33D^{c}\pm0.10$
4	$3.42D^{bc}\pm0.24$	$3.00D^{c}\pm0.00$	$5.09C^{a}\pm0.44$	$4.08D^{b}\pm0.25$	$<1.00E^{d}$
5	$2.08E^{b}\pm0.25$	$1.95E^{b}\pm0.00$	$3.42D^{a}\pm0.43$	$<1.00E^{c}$	$<1.00E^{c}$

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 HCI (pH 4.5) adapted and nontreated *L. monocytogenes* in pH 3.0 (log CFU/mL)

Incubation (h)	Acid-adapted period (h)				Nontreated
	1	2	3	4	
0	$7.41A^{a}\pm0.05$	$7.23A^{ab}\pm0.19$	$7.06A^{ab}\pm0.10$	$6.93A^{b}\pm0.35$	$7.28A^{ab}\pm0.08$
1	$6.47B^{a}\pm0.13$	$6.32B^{ab}\pm0.02$	$6.28B^{ab}\pm0.00$	$6.06B^{b}\pm0.10$	$6.25B^{ab}\pm0.21$
2	$6.25B^{a}\pm0.02$	$6.23BC^{a}\pm0.05$	$6.22B^{a}\pm0.04$	$6.02B^{ab}\pm0.07$	$5.67C^{b}\pm0.06$
3	$6.19BC^{a}\pm0.01$	$6.15BC^{a}\pm0.04$	$6.16B^{a}\pm0.05$	$5.82B^{a}\pm0.11$	$5.18D^{b}\pm0.03$
4	$6.10BC^{a}\pm0.02$	$6.06BC^{a}\pm0.02$	$6.12B^{a}\pm0.04$	$5.76BC^{a}\pm0.14$	$4.56E^{b}\pm0.15$
5	$5.87C^{a}\pm0.12$	$5.92C^{a}\pm0.04$	$6.02B^{a}\pm0.02$	$5.45C^{a}\pm0.15$	$4.17F^{c}\pm0.38$

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 HCI (pH 4.5) adapted and nontreated *L. monocytogenes* in pH 3.5 (log CFU/mL)

Incubation (h)	Acid-adapted period (h)				Nontreated
	1 saat	2 saat	3 saat	4 saat	
0	7.40	7.21	7.16	6.64	7.31
1	7.25	6.82	6.75	6.35	7.17
2	7.02	6.55	6.27	6.29	7.01
3	6.71	6.40	6.10	6.00	6.74
4	6.25	6.32	6.06	5.85	6.32
5	5.83	6.16	5.43	5.67	6.06
Ort.	6.74A±0.18	6.57A±0.11	6.29A±0.18	6.13A±0.10	6.77A±0.14

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, beefs 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. Tchuenchieu (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* spp. as starter culture were added to the sucuk dough of the experimental and control group, respectively. The difference between the *Staphylococcus* spp. 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. monocytoges* during ripening and storage periods at 4 °C of the sucuk samples (log CFU/mL)

Days	Acid-adapted	Nontreated
H0*	5.89	5.69
Ripening		
0	5.87	5.14
1	5.78	5.13
2	5.57	4.96
3	5.51	4.94
4	5.43	4.69
6	4.76	4.13
Storage		
8	4.44	3.66
15	3.53	2.97
30	3.37	2.70
40	2.89	<2.00

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)

	Acid-adapted		Nontreated	
Days	LAB	*Staph.* spp.	LAB	*Staph.*spp.
HO*	6.48	6.43	6.45	6.33
Ripening				
0	6.65	6.39	6.52	6.29
1	9.25	6.69	9.14	6.17
2	9.42	6.09	9.31	6.28
3	9.35	6.16	9.36	6.12
4	9.19	6.09	9.20	5.98
6	9.21	6.69	9.32	6.08
Storage				
8	9.22	6.90	9.12	7.15
15	8.75	6.21	8.79	7.24
30	8.83	5.75	8.57	6.15
40	8.47	5.91	8.33	6.19

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*

	Acid-adapted		Nontreated	
Days	pH	% Moisture	pH	% Moisture
HO*	5.78	59.19	5.75	59.22
Ripening				
0	5.80	57.15	5.75	58.62
1	4.65	56.11	4.62	58.51
2	4.42	54.35	4.39	54.00
3	4.56	50.21	4.59	50.47
4	4.54	48.20	4.52	49.19
6	4.48	43.01	4.44	38.49
Storage				
8	4.61	39.82	4.59	35.88
15	4.74	35.37	4.60	31.04
30	4.76	34.39	4.72	30.06
40	4.74	33.78	4.80	29.42

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.

Compliance with Ethical Standard

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

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DETERMINATION OF THE BEST FUNCTIONAL CHICKPEA CULTIVARS BY TOPSIS TECHNIQUE

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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* arientum) 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; Özer 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; Özer et al., 2010; Ozkilinc et al., 2011; Atalay and Babaoglu, 2012; Siddique et al., 2012; Neugschwandtner et al., 2015; Sadras and Dreccer, 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 (Coşkuner and Karababa, 2004; Bibi et al., 2007; Özer et al., 2010; Çelik 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 Yemenicioglu (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 Yemenicioglu, 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 (Özcan 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 phenolic 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, Aydın, Azkan, Çakır, Diyar, Gülümser, Hasanbey, Ilgaz, İzmir, İnci, 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, $K_2O_8S_2$, NaH_2PO_4, Na_2HPO_4, NaCl, Na_2CO_3, (±)-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 $FeCl_2$ which were purchased from Merck KGaA (Germany), and ethylene diamine tetraacetic acid (EDTA), $CuSO_4$, Na-K tartrate, NaOH, gallic acid, sodium caseinate, 2, 2′-Azino-bis (3-ethyl benzothiazoline-6-sulfonic acid) diammonium 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) Na_2CO_3, 2.5 mL of 1% (w/v) $CuSO_4.5H_2O$ 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 as average of three different sample measurements 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 Yemenicioglu, 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) Na_2CO_3 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 $K_2O_8S_2$ 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 6^{th} minutes. The % inhibition of ABTS radical cation was determined by calculating the differences between absorbance read at 6^{th} 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 $FeCl_2.4H_2O$ 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

$x\_ij = a\_ij/\sqrt{(\sum\_(k = 1)\^m a\_kj\^2)}$ (1)

k = 1,2,3 …, i, …, k, i=1,2,…

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

$$v_{ij} = x_{ij} \times w_{ij} \quad (2)$$

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

$S^* = \{v_{1*}, v_{2*}, v_{3*}, \dots, v_{n*}\}$ (maximum values)

$S^- = \{v_{1-}, v_{2-}, v_{3-}, \dots, v_{n-}\}$ (minimum values)

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

$$d_i^* = \sqrt{\left(v_{ij} - v_j^*\right)^2} \quad (3)$$

$$d_i^- = \sqrt{\left(v_{ij} - v_j^-\right)^2} \quad (4)$$

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

$C = (d\_i\^-)/(d\_i\^ * + d\_i\^-)$ (5)

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 by 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 should be completely removed from food extracts by evaporating, drying, etc. due to toxicity 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-Ciocalteu 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. Dıblan 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 (Dıblan 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 (IC_{50}), and 22.85 ±0.25 (% inhibition) which were the lower than our findings (Sreerama et al., 2012; Jogihalli 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; Jogihalli 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 were 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 Çakır 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

Alternatives	TPC	ICA	FRSA	WSPC	WBC	OBC
			All samples			
Aksu	0.2828	0.2409	0.2767	0.2727	0.2850	0.2300
Arda	0.2884	0.2788	0.3037	0.2619	0.2914	0.3202
Aydın	0.3201	0.4254	0.3051	0.3302	0.2663	0.2732
Azkan	0.2804	0.3591	0.3041	0.2595	0.2789	0.2421
Çakır	0.2811	0.3168	0.2655	0.2840	0.2943	0.3567
Diyar	0.3146	0.2795	0.3048	0.2922	0.2653	0.2811
Gülümser	0.3083	0.2133	0.2976	0.2901	0.2725	0.3046
Hasanbey	0.2674	0.2333	0.2723	0.2834	0.3262	0.2698
Ilgaz	0.2895	0.2609	0.2862	0.2967	0.3114	0.2564
İnci	0.2771	0.2233	0.3001	0.2955	0.2932	0.3283
İzmir	0.2533	0.2993	0.2570	0.2721	0.3040	0.2965
Seçkin	0.2942	0.2616	0.2855	0.3175	0.2685	0.2795
			Adana in 2014 and 2015			
Aksu	0.2685	0.2583	0.2689	0.2648	0.3165	0.2401
Arda	0.2952	0.3156	0.3026	0.2639	0.3016	0.3028
Aydın	0.3112	0.4387	0.3064	0.3289	0.2593	0.2790
Azkan	0.2796	0.4225	0.3110	0.2656	0.2792	0.2653
Çakır	0.2817	0.3344	0.2496	0.2700	0.2758	0.3476
Diyar	0.3310	0.2731	0.3041	0.3027	0.2675	0.2815
Gülümser	0.3031	0.1387	0.2967	0.2778	0.2764	0.3290
Hasanbey	0.2741	0.1881	0.2910	0.2685	0.3265	0.2659
Ilgaz	0.2821	0.1975	0.2615	0.3034	0.3210	0.2392
İnci	0.2793	0.2441	0.3061	0.2803	0.2896	0.3310
İzmir	0.2694	0.2464	0.2879	0.2929	0.2924	0.2904
Seçkin	0.2823	0.2496	0.2702	0.3336	0.2462	0.2688
			Adana and Erzurum in 2015			
Aksu	0.2692	0.1640	0.2575	0.2781	0.2915	0.2320
Arda	0.2952	0.2445	0.3183	0.2431	0.2928	0.2995
Aydın	0.3058	0.4121	0.2877	0.3271	0.2583	0.2705
Azkan	0.2609	0.3492	0.2881	0.2462	0.2770	0.2172
Çakır	0.3047	0.3325	0.2822	0.3025	0.3007	0.3632
Diyar	0.2939	0.2128	0.2829	0.2878	0.2562	0.3017
Gülümser	0.3188	0.2038	0.2894	0.2877	0.2785	0.2808
Hasanbey	0.2795	0.2825	0.2729	0.2790	0.2918	0.2768
Ilgaz	0.2893	0.3092	0.3104	0.3013	0.3095	0.2725
İnci	0.2882	0.1317	0.3045	0.3066	0.2996	0.3175
İzmir	0.2374	0.3451	0.2552	0.2683	0.3110	0.2813
Seçkin	0.3111	0.3352	0.3075	0.3228	0.2912	0.3218

Table 2. Weighted normalized decision matrix

Alternatives	TPC	ICA	FRSA	WSPC	WBC	OBC
			All samples			
Aksu	0.0471	0.0401	0.0461	0.0454	0.0475	0.0383
Arda	0.0481	0.0465	0.0506	0.0436	0.0486	0.0534
Aydın	0.0533	0.0709	0.0509	0.0550	0.0444	0.0455
Azkan	0.0467	0.0599	0.0507	0.0432	0.0465	0.0404
Çakır	0.0469	0.0528	0.0443	0.0473	0.0490	0.0595
Diyar	0.0524	0.0466	0.0508	0.0487	0.0442	0.0469
Gülümser	0.0514	0.0356	0.0496	0.0484	0.0454	0.0508
Hasanbey	0.0446	0.0389	0.0454	0.0472	0.0544	0.0450
Ilgaz	0.0483	0.0435	0.0477	0.0495	0.0519	0.0427
İnci	0.0462	0.0372	0.0500	0.0493	0.0489	0.0547
İzmir	0.0422	0.0499	0.0428	0.0453	0.0507	0.0494
Seçkin	0.0490	0.0436	0.0476	0.0529	0.0448	0.0466
			Adana in 2014 and 2015			
Aksu	0.0448	0.0430	0.0448	0.0441	0.0528	0.0400
Arda	0.0492	0.0526	0.0504	0.0440	0.0503	0.0505
Aydın	0.0519	0.0731	0.0511	0.0548	0.0432	0.0465
Azkan	0.0466	0.0704	0.0518	0.0443	0.0465	0.0442
Çakır	0.0469	0.0557	0.0416	0.0450	0.0460	0.0579
Diyar	0.0552	0.0455	0.0507	0.0505	0.0446	0.0469
Gülümser	0.0505	0.0231	0.0494	0.0463	0.0461	0.0548
Hasanbey	0.0457	0.0314	0.0485	0.0447	0.0544	0.0443
Ilgaz	0.0470	0.0329	0.0436	0.0506	0.0535	0.0399
İnci	0.0466	0.0407	0.0510	0.0467	0.0483	0.0552
İzmir	0.0449	0.0411	0.0480	0.0488	0.0487	0.0484
Seçkin	0.0470	0.0416	0.0450	0.0556	0.0410	0.0448
			Adana and Erzurum in 2015			
Aksu	0.0449	0.0273	0.0429	0.0464	0.0486	0.0387
Arda	0.0492	0.0408	0.0530	0.0405	0.0488	0.0499
Aydın	0.0510	0.0687	0.0479	0.0545	0.0430	0.0451
Azkan	0.0435	0.0582	0.0480	0.0410	0.0462	0.0362
Çakır	0.0508	0.0554	0.0470	0.0504	0.0501	0.0605
Diyar	0.0490	0.0355	0.0471	0.0480	0.0427	0.0503
Gülümser	0.0531	0.0340	0.0482	0.0479	0.0464	0.0468
Hasanbey	0.0466	0.0471	0.0455	0.0465	0.0486	0.0461
Ilgaz	0.0482	0.0515	0.0517	0.0502	0.0516	0.0454
İnci	0.0480	0.0219	0.0507	0.0511	0.0499	0.0529
İzmir	0.0396	0.0575	0.0425	0.0447	0.0518	0.0469
Seçkin	0.0519	0.0559	0.0513	0.0538	0.0485	0.0536

Tablo 3. Positive (S^*) and negative (S^-) ideal solutions for the criteria

Criteria	All samples		Adana 2014-2015		Adana-Erzurum 2015	
	S^*	S^-	S^*	S^-	S^*	S^-
TPC	0.053	0.042	0.055	0.045	0.053	0.040
ICA	0.071	0.036	0.073	0.023	0.069	0.022
FRSA	0.051	0.043	0.052	0.042	0.053	0.042
WSPC	0.055	0.043	0.056	0.044	0.055	0.041
WBC	0.054	0.044	0.054	0.041	0.052	0.043
OBC	0.059	0.038	0.058	0.040	0.061	0.036

Tablo 4. TOPSIS evaluation of chickpea samples

Alternatives	All samples			Adana 2014- 2015			Adana-Erzurum 2015		
	d_i^{*a}	d_i^{-b}	C^c	d_i^*	d_i^-	C	d_i^*	d_i^-	C
Aksu	0.0399	0.0085	1.75E-01	0.0390	0.0233	3.75E-01	0.0494	0.0115	1.89E-01
Arda	0.0287	0.0214	4.27E-01	0.0258	0.0341	5.69E-01	0.0334	0.0280	4.56E-01
Aydın	0.0171	0.0403	7.02E-01	0.0164	0.0530	7.64E-01	0.0186	0.0512	7.33E-01
Azkan	0.0271	0.0261	4.91E-01	0.0214	0.0489	6.95E-01	0.0322	0.0371	5.35E-01
Çakır	0.0224	0.0284	5.59E-01	0.0257	0.0377	5.95E-01	0.0154	0.0449	7.44E-01
Diyar	0.0299	0.0198	3.98E-01	0.0317	0.0282	4.71E-01	0.0372	0.0234	3.86E-01
Gülümser	0.0381	0.0177	3.17E-01	0.0519	0.0187	2.65E-01	0.0386	0.0233	3.76E-01
Hasanbey	0.0374	0.0137	2.67E-01	0.0464	0.0178	2.77E-01	0.0291	0.0293	5.02E-01
Ilgaz	0.0332	0.0155	3.18E-01	0.0459	0.0174	2.75E-01	0.0238	0.0360	6.02E-01
İnci	0.0357	0.0199	3.58E-01	0.0354	0.0263	4.27E-01	0.0478	0.0241	3.35E-01
İzmir	0.0290	0.0193	4.00E-01	0.0363	0.0228	3.85E-01	0.0265	0.0385	5.92E-01
Seçkin	0.0322	0.0172	3.48E-01	0.0382	0.0228	3.74E-01	0.0151	0.0435	7.42E-01

[a] d_i^*, [b] d_i^-, and [c] C are positive ideal solution of Euclidean distance, negative ideal solution of Euclidean distance, and the closeness coefficient of each alternative, respectively.

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 Yemenicioglu, 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, or ICA either significant or not. This indicated that the seeds with high antioxidant activity may have poor functional properties. 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.

Table 5. Correlations between different parameters determined for chickpea cultivars

			All samples		
	TPC[a]	**ICA**	**FRSA**	**WSPC**	**WAC**
ICA[b]	0.195*				
FRSA[c]	0.577*	0.245*			
WSPC[d]	0.255*	0.200*	0.152		
WAC[e]	-0.217*	-0.100	-0.285*	-0.093	
OAC[f]	-0.028	0.068	0.074	0.109	-0.154
			Adana 2014-2015		
	TPC	**ICA**	**FRSA**	**WSPC**	**WAC**
ICA	0.233*				
FRSA	0.539*	0.410*			
WSPC	0.188	0.239*	0.104		
WAC	-0.192	-0.179	-0.220**	-0.113	
OAC	0.064	0.113	0.046	0.150	-0.301
			Adana-Erzurum in 2015		
	TPC	**ICA**	**FRSA**	**WSPC**	**WAC**
ICA	-0.054				
FRSA	0.492*	0.025			
WSPC	0.215**	0.192	0.182		
WAC	-0.312*	-0.183	-0.287*	-0.229**	
OAC	0.086	-0.005	0.055	0.205**	-0.029

* P <0.05, ** P<0.1

[a]TPC: Total phenolic content (mg GAE/g), [b]ICA: Iron chelating activity (µmolEDTA/g),[c] FRSA: Free radical scavenging activity (µmol trolox/g), [d]WSPC: Water soluble protein content (mg caseinate /g), [e]WAC: Water absorption capacity (g/g), [f]OAC: 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.

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CELIAC DISEASE AND NEW ATTEMPTS TO DEVELOP GLUTEN-FREE MEAT PRODUCT FORMULATIONS

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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 prolamins and the polymeric glutenins. When reacted with alcohol, the toxic fractions of prolamins, 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 maldigestion and malabsorption of nutrients, vitamins, and minerals (Niewinski, 2008, Scherf et al., 2016). CD commonly appears in early childhood, with sore 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 (Niewinski, 2008, Scherf et al., 2016, Cui et al., 2017). Moreover, CD also places patients at greater risk for certain cancers (Cui et al., 2017), mycotoxins and major depressive disorder (Gobbetti et al., 2018).

CD is one of the most common genetically based disease, about 97% of individuals with CD have genetic markers on chromosome 6p21, called class-II human leukocyte antigen (HLA), specifically HLA-DQ2 and HLA-DQ8 (Niewinski, 2008). Although genetic factors play an important role in the development of CD, environmental factors are also effective. Since the disease would not be seen as far as wheat and other gluten-containing cereals are included in the diet, the incidence of CD has increased in societies where wheat has an important place in nutrition, or in some ethnic groups that have changed their dietary habits (Demirçeken, 2011). According to Lionetti et al. (2015), the widespread diffusion of CD is not surprising given that its causal factors show a worldwide distribution. In addition, the common use of refined grains and leavening by chemical and baker's yeast agents are some of the examples of the most recent trend (Gobbetti et al., 2018).

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.

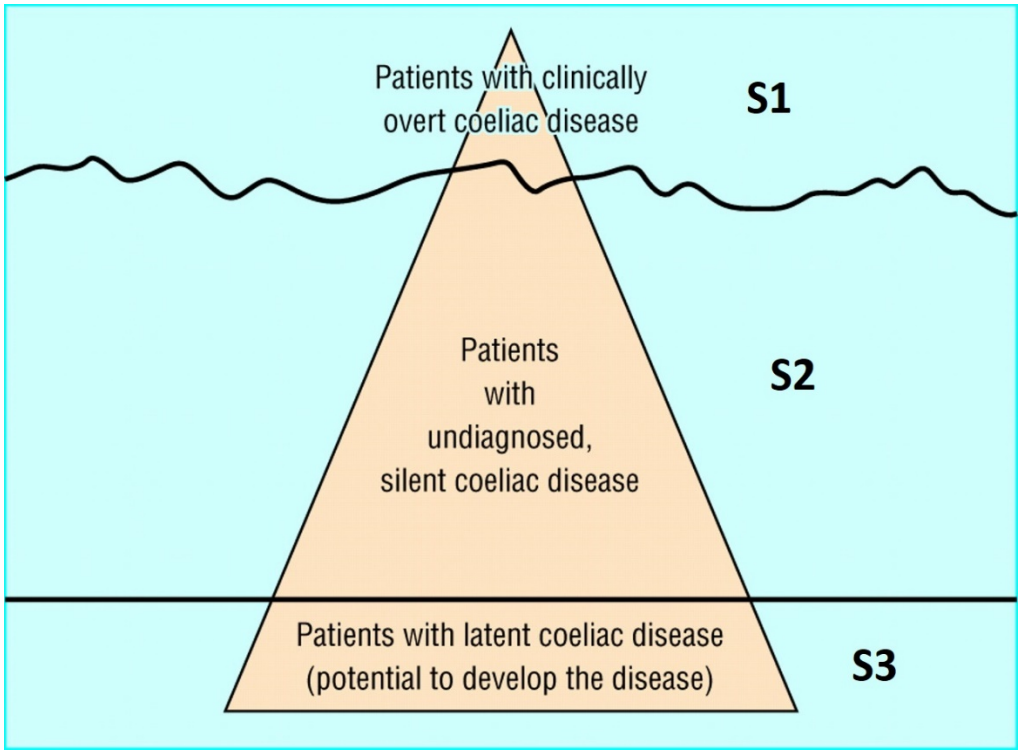

Figure 1. The iceberg model of CD (Feighery, 1999).

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). Gökçe 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, fitosteroids, 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

Product	Gluten-free ingredient(s)	Highlights	Reference
Chicken nugget	Rice flour	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.	Jackson et al. (2006)
Chicken nugget	Sorghum flour	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.	Devatkal et al. (2011)
Kibbeh	Pearl millet flour	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.	Brasil et al. (2015)
Chicken nugget	Pecans	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.	Jackson (2016)
Chicken nugget	Gluten-free wheat flour in combination with cellulose, egg powder, whey powder or pectin	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.	Taşbaş et al. (2016)
Chicken nugget	Amaranth flakes with soy oil, eggs, oregano or basil	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.	de Carvalho et al. (2018)
Chicken nugget	Rice flour (RF), chickpea flour (CF), oat fiber (OF) or Jerusalem artichoke powder (JAP)	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.	Öztürk et al. (2018)
Fish patty	Rice, corn, amaranth or quinoa flours	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.	Romero et al. (2018)

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.

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PROBİYOTİK- İNSAN BAĞIŞIKLIK SİSTEMİ ETKİLEŞİMLERİ

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ÖZ

Probiyotikler yeterli miktarda tüketildiklerinde sağlık üzerine yararlı etkiler gösteren canlı mikroorganizmalardır. Bu mikroorganizmalar, patojenlerle mücadele, epitel hücre kararlılığını sağlama ve bağışıklık sistemini düzenleyebilmeleri ile sağlık üzerine olumlu etkiler göstermektedirler. Bağışıklık düzenleyici, anti-inflamatuar, anti-mikrobiyal, anti-oksidan etkilerini, açığa çıkardıkları metabolitleriyle, ürettikleri moleküllerle ve hücre yapı bileşenleriyle sağlamaktadırlar. Peptidoglikan, teikoik asit, lipoteikoik asit, hücre yüzey polisakkaritleri, salgılanan proteinler ve yüzey proteinleri gibi probiyotik etken molekülleri doğal bağışıklıkta görevli reseptörler tarafından tanınarak bağışıklık sistemini harekete geçirmektedir. Yapılan çalışmalarla bu etken moleküllerinden bazılarının etki mekanizmaları aydınlatılmıştır.Bu derlemede probiyotiklerin, probiyotik-konak etkileşimleri için kullandıkları bilinen etken molekülleri hakkında ve etkileri üzerine bilgi verilmesi amaçlanmıştır.

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

Dost mikroorganizmalar olarak adlandırılan probiyotikler, FAO/WHO tarafından yeterli miktarda tüketildiklerinde insan sağlığına yararlı etkileri olan canlı mikroorganizmalar olarak tanımlamaktadır (FAO/WHO, 2002). Bulgarlar' ın sağlıklı ve uzun yaşam sürmelerinin sırrını merak eden Elie Metchnikoff 1900' lü yılların başlarında probiyotiklerin sağlık üzerine yararlı etkilerinin olduğunu gösteren ilk araştırmayı yapmış ve yapılacak diğer çalışmaların da yolunu açmıştır. Yapılan araştırmalarla, fermente süt ürünleri tüketimi ile sağlıklı yaşam ilişkilendirilmiş ve bu etkinin kaynağının fermente süt ürünlerinde bulunan bu dost bakteriler olduğu ispatlanmıştır (Villena vd., 2013; Nagpal vd., 2014). Tablo 1' de bir bakterinin probiyotik olabilmesi için sahip olması gereken özellikler verilmiştir.

Probiyotiklerin insan sağlığı üzerine etkileri farklılık gösterebilmektedir. Tüketilen probiyotik bakteri suşu ve sayısı, probiyotik ürünün formulasyonu, tüketen bireyin bağırsak mikroflorası ve metabolik durumuna göre etkiler değişiklik göstermektedir. Bu nedenle, tüketilen probiyotik ürünün herkeste aynı etkiyi göstermesi beklenmemektedir (Corcoran vd., 2008).

Probiyotiklerin yararlı etkilerinin ortaya çıkabilmesi için bağırsaklara yeterli sayıda ulaşmaları gerekmektedir. Probiyotikler, çoğunlukla *Lactobacillus* ve *Bifidobacterium* cinslerinden oluşmakta ve ticari olarak kullanılmaktadır. İlk probiyotik ticari ürün olarak, 1930' larda *Lactobacillus casei* Shirota suşunu içeren probiyotik süt ürünü 'Yakult' piyasaya sürülmüş ve sonrasında diğer birçok probiyotik ürün ticarileştirilmeye başlamıştır (Villena vd., 2013; Nagpal vd., 2014). Ticari probiyotik ürünlerin artmasıyla, Türk Gıda Kodeksinde 29/12/2011 tarihli 28157 sayılı yönetmeliğe göre ürünün probiyotik ürün olabilmesi için içerisinde raf ömrü sonuna kadar yeterli miktarda canlı probiyotik mikroorganizma (en az 1.0x 10^6 kob/g) bulundurması ve bu canlılığı muhafaza etmesi gerektiği bildirilmiştir (Gıda, Tarım ve Hayvancılık Bakanlığı, 2011).

Günümüzde tüketicilerin bilinçli ve sağlıklı beslenmeye yönelmesiyle probiyotiklerin önemi giderek artmaktadır. Sağlık üzerine etkileri sayesinde probiyotik ürünlere ticari olarak büyük yatırımlar yapılmış ve bu ürünler tüketici tercihine yönelik çeşitlendirilmiştir (Agrawal, 2005; Rodgers, 2008; Villena vd., 2013). Probiyotiklerin sağlık üzerine etkilerinin araştırılmasına yönelik çalışmalar yeni fonksiyonel probiyotik ürünlerin geliştirilmesine de ortam hazırlamıştır.

Bu derlemede genel olarak probiyotikler, probiyotiklerin sağlık üzerine etkileri ile ilintili moleküler mekanizmalar ve probiyotiklerin hem insan bağırsak epitel hücreleri hem de bağışıklık hücreleriyle etkileşimlerini sağlamak için kullandıkları etken moleküller ile ilgili yapılan çalışmalar derlenmiştir.

Tablo1. Probiyotik Mikroorganizmaların Özellikleri

Probiyotik Mikroorganizmaların Özellikleri	Referanslar
Anti-alerjik, anti-toksik, anti-mutajenik ve anti-kanserojen	Ouwehand vd., 2002, Sağdıç vd., 2004, Guarner vd., 2005, Singh vd., 2011, Sarkar, 2013, Nagpal vd., 2014
Bağırsaklarda mukus ya da insan epitel hücrelerine yapışarak kolonizasyon	
Bağırsakta patojen yapışmasını azaltma	
B-galaktosidaz aktivitesi	
Genetik stabilite	
Kolay ve tekrar üretilebilme	
Üretim ve depolama süresinde dayanıklılık	
Patojenlere karşı antimikrobiyal aktivite	
Patojen özellik taşımama	
Sağlık üzerine yararlı etkiler	
Sindirim enzimlerine, mide asidine ve safra tuzlarına karşı direnç	

Probiyotiklerin Etki Mekanizmalari ve Sağlik Üzerine Etkileri

Probiyotiklerin Etki Mekanizmaları

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

Probiyotikler, bağırsak epitel hücrelerine tutunarak patojenlerin tutunmasını ve besin rekabetine girerek patojenlerin gelişimini engellerler (Sarkar, 2013). Probiyotikler, ayrıca ürettikleri anti-mikrobiyal maddelerle (defensin, bakteriyosin ve/veya hidrojen peroksit) ve ürettikleri organik asitlerin ortam pH' sını düşürmesiyle patojenlerin gelişimini baskılarlar (Nagpal vd., 2014). Örneğin; kronik gastrite neden olan patojen *Helicobacter pylori'* nin tedavisindeki antibiyotik uygulamalarıyla beraber probiyotik kullanımının, *H. pylori*' nin mide epitelinde kolonizasyonunu önleyerek inflamasyonu azalttığı bilinmektedir (Gotteland vd., 2006).

Probiyotikler, ürettikleri moleküllerle ya da hücre yapı bileşenlerini kullanarak bağırsaklarla doğrudan etkileşime girerek bağışıklık sistemini düzenleyebilmektedirler (Boirivant ve Strober, 2007; Villena vd., 2013). Peptidoglikan, lipopolisakkarit, teikoik asit, lipoteikoik asit, bakteriyal DNA, ekzopolisakkarit ve flagella (kamçı) gibi Mikrop İlişkili Moleküler Yapılar (MAMP) bağışıklık sisteminde görevli bu yapıları tanıyan reseptörlerle (desen tanıma reseptörü, PRR) etkileşime girerek doğal ve kazanılmış bağışıklık sistemini harekete geçirirler (Lee vd., 2013). Bu alanda kayda değer sayıda çalışma yapılmasına rağmen hala çözülememiş moleküler mekanizmalar mevcuttur. Ayrıca, her probiyotik suşunun bağışıklık etkisi farklıdır. Bu nedenle, her suşun spesifik olarak incelenerek etkileşim mekanizmalarının açığa çıkarılması gereklidir (Lebeer vd., 2008; Sarkar ve Mandal, 2016).

Son olarak, patojenlerin ürettikleri toksinlerle ve bağışıklık sistemi tarafından patojenlere karşı üretilen pro-inflamatuar sitokinlerle epitel bariyer zarar görmekte ve epitel hücrelerin geçirgenliği artmaktadır. Böylece, patojenler ve istenmeyen metabolitler epitel bariyeri geçerek kan akışına karışmaktadır (Boirivant ve Strober, 2007; Sarkar ve Mandal, 2016; Gleeson, 2017). Probiyotikler çeşitli sinyal yolaklarını harekete geçirerek programlı hücre ölümlerinin önlenmesi, defensin üretimi, epitel hücreler arasındaki bağların güçlendirilmesi, mukus salınımının arttırılması gibi mekanizmaları tetikleyerek epitel hücrelerin kararlılığının sağlanmasına yardımcı olurlar (Lebeer vd., 2008; Lee vd., 2013; Krishna Rao ve Samak, 2013).

Probiyotiklerin Sağlık Üzerine Etkileri

Probiyotik tüketiminin sağlık üzerine birçok yararı olduğu bilinmektedir, hatta probiyotikler bazı hastalıklarda önleyici ve/veya tedavi edici olarak kullanılmaktadır. Probiyotiklerin sağlık üzerine etkilerini belirlemek için birçok klinik çalışma yapılmıştır. Ancak bu araştırmalar sonucunda bazı rahatsızlık ve hastalıklarda kesin olumlu etkileri belirlenebilmişken, bazıları üzerinde ise bu etkiler belirlenememiştir (Kleerebezem vd., 2019).

Probiyotiklerin önleyici ve/veya tedavi edici olarak kullanıldığı hastalıkların başında inflamatuar bağırsak hastalıkları (IBD) gelmektedir. Kronik, tekrarlayabilen ve çeşitli faktörlere bağlı olan IBD, sindirim sistemi boyunca bağışıklık sisteminin düzensiz ve aşırı yanıt vermesiyle inflamasyon oluşması nedeniyle görülen bir hastalıktır. Genetik faktörler, çevresel faktörler, bağışıklık sistemi bozuklukları, patojenler ve oksidatif stres IBD' nın oluşmasına neden olabilecek faktörlerdir (Boirivant ve Strober, 2007; Pandey vd., 2015). IBD' ler içerisinde yer alan ülserit kolit (UC) , Crohn (CD) ve poşit hastalıklarının tedavisinde ve hastalık semptomlarının hafifletilmesinde probiyotik tüketiminin etkili olduğu yapılan klinik araştırmalarla gösterilmiştir (Sheil vd., 2007; Kelesidis ve Pothoulakis, 2012; Veerappan vd., 2012).

En yaygın fonksiyonel sindirim sistemi rahatsızlığı olan inflamatuar bağırsak sendromu (IBS), daha çok psikolojik, sosyal ve biyolojik faktörlerin etkili olduğu, yaşam kalitesini düşüren kronik bir rahatsızlıktır (Tanaka vd., 2011). Probiyotik tüketiminin IBS semptomlarını azaltıcı etkiler gösterdiği çalışmalarla belirlenmiş ancak bu etkilere sebep olan mekanizmalar tamamen aydınlatılamamıştır (Hoveyda vd., 2009; Tanaka vd., 2011; Pandey vd., 2015).

Probiyotiklerin tedavi yardımcısı olarak kullanıldığı başka bir sağlık problemi ishaldir. 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 olumlu sonuçlar verdiği bilinmektedir (Macfarlane ve Cummings, 2002; Agrawal, 2005; McFarland, 2006; Sudha ve Bhonagiri, 2012; Pandey vd., 2015) .

Probiyotik tüketiminin, kolon kanseri riskini ve tümörlerini azaltıcı etki gösterdiği çalışmalarla gösterilmiştir (Rafter, 2002; Fotiadis vd., 2008; Pandey vd., 2015). Probiyotiklerin ürettikleri anti-kanserojenik ve anti-mutajenik maddelerle tümör oluşumu ve kanser riskini azalttığı düşünülmektedir. Probiyotiklerin, bağırsaklarda oluşan kanserojen bileşikleri etkisiz hale getirmesi, bağırsak mikroflorasını değiştirerek kanserojen bileşik oluşumuna sebep olan mikroorganizmaların azalmasını sağlaması ve hücre döngüsüne yardımcı olan

ve tümör hücrelerinin gelişimini engelleyen bileşenler üretmesi kanseri 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 yanıtının düzenlenmesi üzerine etkilerinin olduğu bilinmektedir. Probiyotiklerin bu mekanizmaları kullanarak alerji semptomlarının hafifletilmesi üzerine olumlu etkiler gösterdiği klinik çalışmalarla belirlenmiştir (Kalliomäki ve Isolauri, 2004; Parvez vd., 2006; Rupa ve Mine, 2012).

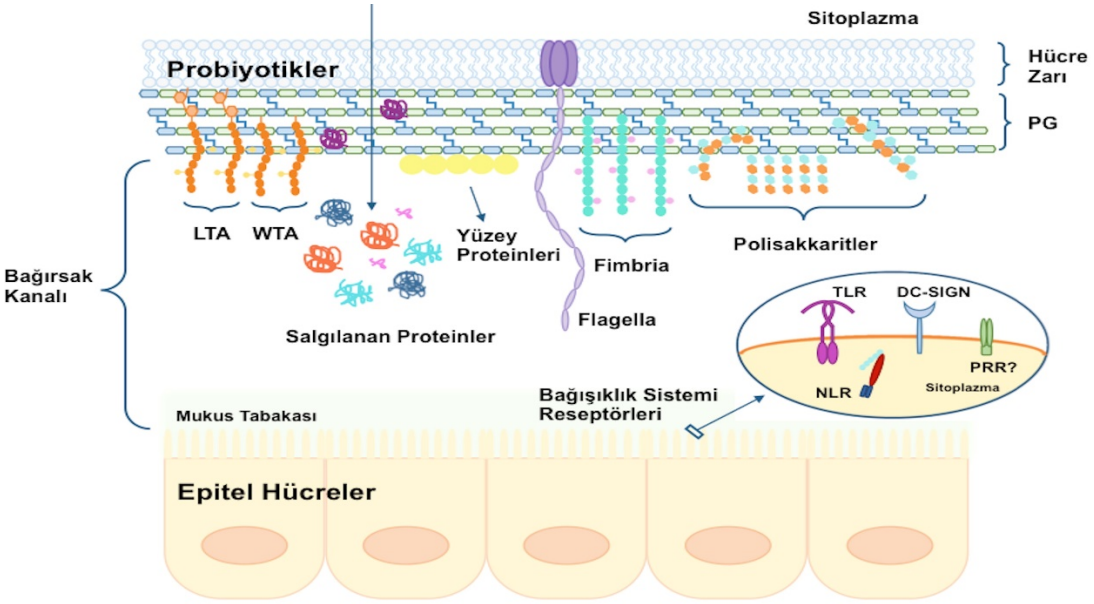
Probiyotikler, ayrıca, laktoz intoleransı, gastrit, hipertansiyon, ağız sağlığı, obezite, diyabet, yüksek kolestrol, karaciğer hastalıkları ve çeşitli enfeksiyon hastalıkları üzerine olumlu etkiler göstermektedir. (Parvez vd., 2006; Singh vd., 2011; Rupa ve Mine, 2012; Nagpal vd., 2014; Pandey vd., 2015).

Probiyotiklerin sağlık üzerine olumlu etkilerinin moleküler mekanizmalarının belirlenmesi için yapılan araştırmalar son yıllarda hız kazanmıştır. Özellikle probiyotiklerin etken moleküllerinin belirlenmesi ve bu moleküllerin doğrudan hastalıkları önleyici ya da tedavi amacı ile kullanılması konusunda çalışmalar yapılmaktadır.

Probiyotiklerin Etken Molekülleri

Probiyotikler sağlık üzerine etkilerini hücre yapı bileşenleriyle, ürettikleri moleküllerle ve fermantasyon sonucu oluşan metabolitleriyle sağlamaktadır. Bu moleküller kısa zincirli yağ asitleri, peptitler, enzimler ve proteinler, teikoik asit, lipoteikoik asit, peptidoglikan, endo- ve ekzo- polisakkaritler, hücre yüzey proteinleri, vitaminler, organik asitler, flagella, fimbria, hidrojen peroksit ve karbondioksit olarak sıralanabilir (Rupa ve Mine, 2012; Matsuki ve Pe, 2013; Tsilingiri ve Rescigno, 2013; Tabasco vd., 2014; Kang ve Im, 2015; Sarkar ve Mandal, 2016; Aguilar-Toalá vd., 2018). Şekil 1' de probiyotiklerin etken molekülleri şematik olarak gösterilmiştir.

Probiyotiklerin bağışıklık düzenleyici etkileri, probiyotik-konak etkileşimleriyle başlar. Bağışıklık sisteminin düzenlenmesi ve sürdürülmesi için; epitel hücrelerde ve bağışıklık hücrelerinde bulunan (makrofajlar, dendritik hücreler vb.) doğal bağışıklıkta görevli PRR' ler çeşitli bakterilerde bulunan MAMP' ları tanır ve bağışıklık sisteminin sinyal iletimini başlatır (Akira vd., 2006; Kumar vd., 2009; Kawai ve Akira, 2011; Kumar vd., 2011). Probiyotiklerin peptidoglikan, teikoik asit, lipoteikoik asit, hücre yüzey polisakkaritleri, salgılanan proteinler ve yüzey proteinleri gibi moleküllerinin PRR tarafından tanınarak doğal bağışıklık sinyal iletimini başlattığı son yıllarda yapılan çalışmalarla ortaya konmuştur (Lebeer vd., 2010; Bron vd., 2012b; Lee vd., 2013; Ruiz vd., 2014; Hevia vd., 2015).



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

Peptidoglikan (PG)

Gram (+) bakterilerde kalın bir tabaka olarak görülen peptidoglikan tabakası, glikan zincirlerine β-1,4 N-asetilglukozamin (GlcNAc) ve N-asetilmuramik asit (MurNAc) alt birimlerinin bağlanmasıyla oluşan ve hücreye şeklini verip hücre bütünlüğünü koruyan temel hücre duvarı bileşenidir (Delcour vd., 1999). PG üzerinde kovalent bağlı teikoik asit, ekzopolisakkaritler ve proteinler yer almaktadır. PG yapısındaki farklı bağ oluşumlarına dayalı çeşitlilik bakteri suşlarına spesifik özellikler sağlar (Kleerebezem vd., 2010). PG' nin otolizi ve enzimatik parçalanmasıyla oluşan parçaları sindirim sisteminde yayılarak PRR' ler tarafından tanınırlar. PG parçalarının tanınması TLR2 ve Nod-Benzeri Reseptörler (NLR 'ler) aracılığıyla gerçekleşir (Lebeer vd., 2010). Probiyotik *Lactobacillus plantarum* ATCC 14917 ve *Lactobacillus johnsonii* JCM 2012 ile yapılan bir çalışmada, bu bakterilere ait peptidoglikan parçalarının pro-inflamatuar sitokin IL-12 üretimini TLR2 yoluyla baskılayıcı etki gösterilmiştir (Shida vd., 2009).

L. casei Shirota, *Lactobacillus rhamnosus* ATCC 53103, *Lactobacillus plantrarum* ATCC 14917 ve *L. Johnsonii* Y 50092 ile yapılan başka bir çalışmada, izole edilen polisakkarit-PG komplekslerinin bağışıklık düzenleyici etkileri incelenmiştir. *L. casei* Shirota dışındaki suşlar ile sonuç alınamazken, *L. casei* Shirota' dan izole edilen polisakkarit-PG kompleksinin pro-inflamatuar sitokin IL-6 üretimini düşürdüğü ve anti-inflamatuar NF-κB yolağını başlattığı gösterilmiştir. Böylece *L. casei* Shirota' nın IBD tedavilerinde yararlı bir probiyotik olabileceği ileri sürülmüştür (Matsumoto vd., 2005) .

NOD2 reseptörlerinin de PG türevi muramil peptitleri tanıdığı ve Crohn hastalığında NOD2 reseptörlerinin rol oynadığı bilinmektedir (Ogura vd., 2001; Girardin vd., 2003). Probiyotik *Lactobacillus salivarius* Ls33 ve *Lactobacillus acidophilus* NCFM bakterilerinden saflaştırılan PG' nin muramil peptit parçalarıyla kolit fareler üzerinde çalışma yapılmıştır. Yapılan çalışmada Ls33 PG' nin NOD2 sinyal iletimine bağlı olarak anti-inflamatuar sitokin IL-10 salınımını başlattığı ve bu yolla da koruyucu etki gösterdiği belirlenmiştir. NCFM' nin ise kolit fareler üzerinde herhangi bir koruyucu etki göstermediği görülmüştür. Bu sonuçlar, PG fragmanlarının NLR sinyal iletimi üzerine etkisini gösterirken, bu etkinin suşa spesifik olarak ortaya çıktığını göstermiş ve PG yapısındaki çeşitliliğin öneminin anlaşılmasına olanak sağlamıştır (Fernandez vd., 2011).

Teikoik Asit (TA)

Gram (+) bakterilerin hücre duvarlarının ikinci temel bileşeni teikoik asittir (TA). Anyonik bir bileşen olan TA, PG' ye tekrarlanan poligliserol fosfat ve poliribitol fosfat alt birimlerinin kovalent bağ ile bağlanmasıyla oluşmaktadır. TA hücre duvarına bağlı (WTA) ya da membrana bağlı (LTA) olarak bulunabilmektedir (Neuhaus ve Baddiley, 2003). LTA hemen hemen tüm Gram (+) bakterilerde bulunduğu için bağırsak epitel hücreleri LTA' ya karşı tolerans mekanizmaları geliştirmiştir (Melmed vd., 2003; Lebeer vd., 2010). TA bileşenlerinin mekanizmalarını belirlemeye yönelik çalışmalarda doğrudan canlı hücreyle ve izole edilen LTA' lar ile *in vivo* ve *in vitro* deneyler yapılmıştır. LTA' nın yapısındaki farklılıkların bağışıklık düzenleyici etkisi üzerine katkıları henüz netleştirilememiştir (Lee vd., 2013).

Gram (-) bakterilerin lipopolisakakritleriyle epitel hücrelerde pro-inflamatuar IL-8 sitokininin salınımına neden olduğu, *L. johnsonii* La1 ve *L. acidophilus* La10 probiyotik bakterilerinden izole edilen LTA' ların ise IL-8 salınımını baskıladığı gösterilmiştir. Bu çalışmayla, *Lactobacillus* suşlarının Gram (-) bakteri ve bileşenleri kaynaklı hastalıkların tedavisinde ve bağırsak homeostazının sağlanmasında rol oynayabileceği önerilmiştir (Vidal vd., 2002). Benzer şekilde, *Staphylococcus aureus* LTA' sı ile indüklenen pro-inflamatuar TNF-α salınımına, *L. plantarum* 'dan izole edilen LTA' nın antagonistik etki gösterdiği ve TLR2 yoluyla TNF-α salınımını engellediği gösterilmiştir (Kim vd., 2008).

LTA' nın yapısal farklılıklarının mekanizmaları üzerine etkisini belirlemek için de çalışmalar yapılmıştır. *L. plantarum* L-137 ve *L. plantarum* JCM1149 suşlarının LTA' ları ile çalışılmış ve daha iyi bir IL-12 indüktörü olan L-137 'nin LTA' sında daha fazla alanin olduğu bulunmuştur (Hirose vd., 2010). Altı farklı *Lactobacillus* suşuna ait LTA' ların fare bağışıklık hücreleri üzerine etkileri araştırılarak, *L. casei* ve *Lactobacillus fermentum'* dan izole edilen LTA' ların TLR2 yoluyla makrofajlarda pro-inflamatuar sitokin TNF-α salınımını indüklediği, ancak doza ve suşa bağlı farklılıklar olduğu gösterilmiştir (Matsuguchi vd., 2003). *L. acidophilus* NCFM' nin LTA sentezleyen fosfogliserol transferaz geni çıkarılarak elde edilen mutant LTA ile pro-inflamatuar IL-12 ve TNF-α salınımını azalırken, anti-inflamatuar IL-10 salınımının arttığı görülmüştür. Böylece, LTA' nın IL-12 salınımındaki temel faktör olduğu doğrulanmıştır (Saber vd., 2011; Mohamadzadeh vd., 2011). Ayrıca NCFM 'nin LTA' sı ile kolon kanseri olan farelerde yapılan çalışmalar polipleri hafifletici etkisi olduğunu göstermiştir (Khazaie vd., 2012). Kronik kolit olan farelerle yapılan çalışmada, *L. rhamnosus* GG' nin LTA' sının D-alanizasyonundan sorumlu dlt geninin inaktivasyonuyla mutantlar elde edilmiştir. Bu mutantların TLR

ekpresyonunu ve pro-inflamatuar sitokin üretimini azaltıcı etki gösterdiği belirlenmiştir (Claes vd., 2010; Claes vd., 2012).

Bağışıklık 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 salınımını indüklerken, zayıf olarak IL-12 indüksiyonu da yapmıştır. *L. plantarum'* un tersine, *L. casei* IL-12 salınımını indüklerken, zayıf olarak IL-10 indüksiyonu yapmıştır. İki suşun beraber kullanımıyla yapılan çalışmada, makrofajların aktivasyonunu sağlayan WTA ve LTA' nın TLR2 yoluyla IL-10 üretimini sağlayarak sinerjik etki oluşturdukları görülmüştür (Kaji vd., 2010).

Yapılan araştırmaların çoğu LTA' nın bağışıklık düzenleyici etkisini belirlemeye yöneliktir. Diğer bir hücre duvarı bileşeni olan WTA' nın etki mekanizmasını anlamaya yönelik yapılan bir çalışmada, WTA üretmeyen *L. plantarum* WCFS1 mutantı, *L. plantarum* WCFS1 WTA' sı ve D-Alanin eksikliği bulunan *L. plantarum* WCFS1 WTA mutantı üzerine çalışılmıştır. Mutant ve doğal suşa ait WTA' nın TLR2/6 sinyal yolunu doğrudan etkilemediği görülmüştür. Saflaştırılan WTA' lar insan dentrik hücrelerinde herhangi bir sitokin üretimine neden olmazken, pro-inflamatuar sitokin IL-12 ve TNF-α salınımınlarını önemli seviyede azalttığı belirlenmiştir (Bron vd., 2012a). Bu çalışmada da görüldüğü gibi, WTA 'nın mekanizması üzerine kesin bir sonuca varılamadığı için, bağışıklık düzenleyici etkisini belirlemeye yönelik araştırmalar yapılmaya devam edilmelidir.

Hücre Duvarı Polisakkaritleri

Hücre duvarı polisakkaritleri, hücre yüzeyine gevşekçe bağlı bulunan ve çevreye salınabilen formda (ekzopolisakkaritler-EPS), daimi olarak hücreye bağlı ve kalınca etrafını örtecek formda (kapsüler polisakkaritler- CPS) ve hücre duvarını örtmeyecek şekilde kovalent bağlı olarak tutunmuş ya da tutunmamış formda (hücre duvarı polisakkaritleri- WPS) olarak sınıflandırılırlar. Polisakkaritlerin konumu ve miktarı bakterinin büyüme koşullarına ve bakteri türüne/suşuna bağlı olarak değişmekte ve dolayısıyla WPS, CPS ve EPS' de fazlasıyla rastgele farklılaşmalara olanak sağlamaktadır. Genellikle heteropolisakkarit kompleksi yapısında olan polisakkaritlerdeki farklılaşmayı belirleyen faktörler; şeker monomeri çeşidi, bağlanma ve dallanma şekilleri ve modifikasyonlar olarak sıralanabilir (Kleerebezem vd., 2010; Chapot-Chartier ve Kulakauskas, 2014). Son yapılan çalışmalarda, probiyotik polisakkaritlerinin bağırsak kolonizasyonunda önemli rolü olduğu ve sindirim sistemi koşullarına karşı koruyucu etki gösterdiği bulunmuştur (Mozzi vd., 2009).

Polisakkaritlerin doğrudan bağışıklık düzenleyici etkisi olduğunu gösteren çalışmalar mevcuttur. *L. casei* Shirota hücre duvarı polisakkaritlerinin makrofajların ürettiği pro-inflamatuar IL-12 sitokin üretimini durdurarak pro-inflamatuar yanıtı baskıladığı gösterilmiştir (Yasuda vd., 2008). *Escherichia coli* Nissle 1917' in CPS 'sinin fonksiyonunu belirlemeye yönelik yapılan çalışmada, *E. coli* Nissle 1917 ve kapsülsüz *E. coli* Nissle 1917 mutanlarıyla çalışılmıştır. İki suşta ile de pro-inflamatuar sitokin indüksiyonu görülmesine rağmen, kapsül içermeyen mutantın pro-inflamatuar sitokin ekpresyonunda ciddi şekilde azalma olduğu ve *E. coli* Nissle 1917' den saflaştırılan CPS' nin sitokin üretimini indüklemediği belirlenmiştir. Bu sonuçlar doğrultusunda, CPS' nin bağırsak epitel hücreleriyle bakteri hücresinin etkileşimine aracılık ettiği gösterilmiş ancak mekanizması henüz belirlenememiştir (Hafez vd., 2009). *L. rhamnosus* GG EPS' sinin bakteriyi sindirim sisteminde önemli ölçüde koruyucu etki gösterdiği ve bağışıklık sistemi tepkisine karşı EPS üretiminin arttığı gösterilmiştir (Lebeer vd., 2011).

Probiyotik EPS' nin bağışıklık sistemini etkilemesinin patojenlerle mücadelede dolaylı olarak önemli bir faktör olduğunu gösteren çalışmalar yapılmıştır. *Bifidobacterium breve* UCC2003 EPS' sinin fare modellerinde pro-inflamatuar sitokin üretimini baskıladığı ve böylece, *Citrobacter rodentium* kaynaklı enfeksiyonu azalttığı belirlenmiştir (Fanning vd., 2012). Benzer şekilde, *L. plantarum* N14' den saflaştırılan EPS' lerin domuz bağırsak epitellerinde enteratoksik *E. coli* 'nin neden olduğu inflamasyona karşı pro-inflamatuar sitokin IL-6, IL-8 ve MCP-1 üretimini azalttığı görülmüştür. Sonuçlar doğrultusunda, domuzlarda patojen kaynaklı bağırsak enfeksiyonlarına karşı koruyucu etkisi olduğu belirlenmiştir (Murofushi vd., 2015). *Bifidobacterium longum* BCRC 14634 'ten izolen edilen EPS fraksiyonunun bağışıklık düzenleme etkisine yönelik çalışmada, IL-10 anti-inflamatuar sitokin üretimini arttırdığı ve *in vitro* makrofaj gelişimini teşvik ettiği gösterilmiştir (Wu vd., 2010). *L. rhamnosus* KL37 EPS' lerinin ise diğerlerinden farklı olarak fare makrofajlarından hem pro-inflamatuar sitokin TNF-α, IL-6 ve IL-12 üretimini hem de anti-inflamatuar sitokin IL-10 üretimini uyardığı görülmüştür. TNF-α/IL-10 arasındaki dengenin incelenmesiyle potansiyel pro-inflamatuar etki gösterdiği belirlenmiş ve patojen enfeksiyonlarında klinik olarak bağışıklık düzenleyici ajan olarak kullanılabileceği önerilmiştir (Ciszek-Lenda vd., 2011). Başka bir çalışmada da, *Bifidobacterium animalis* ssp. lactis A1 ve IPLA-R1, *B. longum* NB667 ve *L. rhamnosus* GG' nin ürettikleri EPS' lerin bakteriyel patojenlerin toksik etkisini yok ettiği belirlenmiştir (Ruas-Madiedo vd., 2010).

Hücre Yüzeyinde Bulunan ya da Hücre Dışına Sentezlenen Proteinler

Hücre yüzeyine tutunmuş halde olan ve hücre dışına salgılanan proteinler/peptitler mikroorganizma-konak hücre etkileşimlerinde önemli rol oynar (Lebeer vd., 2010). Bu peptitlerden biri olan Serin-Treonince zengin peptit (STp) *L. plantarum* BMCM12 tarafından üretilmektedir. STp' nin mekanizmasının aydınlatılması için bağırsakta bağışıklık cevabı/toleransı arasındaki mekanizmayı kontrol eden dentrik hücreler ile çalışılmış ve STp' nin anti-inflamatuar IL-10 salınımını artırdığı görülmüştür (Bernardo vd., 2012). Ayrıca STp' nin kolit hastalarında bağısak homeostazının sağlanmasına yardımcı olduğu bildirilmiştir (Al-Hassi vd., 2014).

Hücre dışına sentezlenen bir serin proteaz inhibitörü olan serpin genom taramalarıyla *B. Longum* sekansında bulunmuş (Schell vd., 2002) ve saflaştırılan serpinin, serin pankreatik elastaz ve nötrofil elastazını inhibe ederek inflamasyonu ortadan kaldırdığı görülmüştür. Böylece bağırsak homeostazına katkı sağladığı gösterilmiştir (Ivanov vd., 2006).

Hücre dışına sentezlenen bir diğer protein ise *Lactobacillus paracasei* tarafından üretilen proteaz lactocepindir. Lactocepin, pro-inflamatuar IFN-ɣ' yı indükleyerek epitel hücrelerce salınan IBD' de de görülen temel pro-inflamatuar kemokin olan IP-10 proteinini parçalamakta ve böylece anti-inflamatuar etki göstermektedir. Lactocepin üreten *L. paracasei* ve lactocepin üretemeyen *L.paracasei* mutantıyla yapılan çalışmada, mutantın IP-10' u daha az parçaladığı belirlenmiş ve böylece lactocepinin bağışıklık düzenleyici etkisi ortaya konmuştur (Von Schillde vd., 2012).

Probiyotikler bağırsakta hücre dışına anti-mikrobiyal proteinler ya da bakteriyosinlerini salgılarlar. Patojen enfeksiyonlarının önlenmesinde yer alan bakteriyosinlerin etkilerini belirlemek için, patojen *Listeria monocytogenes* enfeksiyonuna karşı *L. salivarius* UCC118' un ürettiği bakteriyosin Abp18 ile çalışılmıştır. Abp18 üretmeyen mutantın aksine doğal suşun farelerde konakla doğrudan etkileşime geçerek enfeksiyona karşı koruyucu etki gösterdiği görülmüştür (Corr vd., 2007). Ayrıca, bazı *L. plantarum* bakteriyosinlerinin doğrudan anti-inflamatuar sitokin üretimine etkisi olduğu gösterilmiştir (Meijerink vd., 2010; Van Hemert vd., 2010).

Probiyotiklerin ürettikleri proteinlerle bağışıklık düzenleyici etki mekanizmalarının araştırılmasına öncü olan çalışmalardan birinde; VSL#3 probiyotik karışımındaki probiyotiklerin ve ürettikleri proteinlerin, epitel bariyer fonksiyonuna katkı sağladığı belirlenmiştir (Otte ve Podolsky, 2004; Lee vd., 2013). Bu katkının *L. rhamnosus* GG' nin ürettiği 2 adet proteinden, (p40 ve p75) kaynaklı olduğu sonraki çalışmalarla gösterilmiştir. p40 ve p75 proteinlerinin, insan ve fare bağırsak epitel hücrelerinde spesifik sinyal iletimi yoluyla programlı hücre ölümlerini (apoptosis) önlediği, epitel hücrelerin çoğalmasını teşvik ettiği ve epitel zararları azalttığı görülmüştür (Yan vd., 2007; Yan vd., 2013, Seth vd., 2008). Böylece, probiyotik proteinlerin doğrudan bağırsak epitel hücre homeostazına katkı sağladıkları ortaya konmuştur. Yapılan genomik araştırmalarla, *L. casei*, *L. paracasei* ve *L. rhamnosus* suşlarının genomlarında p40 ve p75 genlerini kodlayan diziler bulunmuş ve benzer rollere sahip oldukları gösterilmiştir. Böylece probiyotiklerin ürettiği hücre dışı proteinlerin de probiyotik etkilerin oluşmasında rol oynadığı anlaşılmıştır (Bäuerl vd., 2010).

Probiyotikler ürettikleri yüzey proteinleri ile de bağışıklık sistemiyle etkileşime girmektedir. *L. acidophilus* NCFM tarafından üretilen yüzey proteini SlpA dendritik hücreye bağlanarak anti-inflamatuar sitokin IL-10 üretimini arttırırken pro-inflamatuar sitokin IL-12 üretimini düşürdüğü belirlenmiştir. Mutasyon çalışmalarıyla SlpA üretmeyen suşların dentrik hücre lektin reseptörlerine (DC-SIGN) bağlanmasında azalma olduğu görülmüştür. Yapılan çalışmada, saflaştırılan SlpA' nın doğrudan DC-SIGN' a bağlanmasıyla etkileşimleri doğrulanmış ve DC-SIGN' a bağlanan ilk probiyotik bakteri ligandı olarak tanımlanmıştır (Konstantinov vd., 2008). Daha sonra yapılan çalışmalar ile *L. acidophilus* NCK2187 ürettiği SlpA proteinin de benzer fonksiyonel özellikler gösterdiği ortaya konmuştur (Lightfoot vd., 2015).

Probiyotik *Lactobacillus helveticus* R0052' den ekstrakte edilen SlpA ile yapılan çalışmadaysa, patojenlerin bağırsak epitel hücrelerine yapışmasında azaltıcı etkisi olduğu gösterilmiştir (Johnson-Henry vd., 2007). *L. helveticus* fb213, *L. acidophilus* fb116 ve *L. acidophilus* fb214 suşları ile yapılan çalışmalarda SlpA üretmeyen mutantların epitel hücre yapışma kapasitelerinin azaldığı gösterilmiştir (Meng vd., 2014). Genomik yaklaşımlar kullanılarak yapılan taramalarda da, genelde yapışmada rollü olan çeşitli olası Slp proteinlerinin *L. acidophilus*, *L. helveticus*, *L. brevis*, *L. gasseri, L. johnsonii, L. crispatus, L. kefir, L. parakefir, L. amylovorus*, *L. sobrius,* ve *L. mucosae*' nin genomlarında bulunduğu tespit edilmiştir (Kleerebezem vd., 2010; Hynönen ve Palva, 2013).

Lactobasillerin ve VSL#3 probiyotik kokteylinin pro-inflamatuar sinyal yolağını indükleyerek, insanda doğal bağışıklığın cevabı olarak üretilen ve antimikrobiyal bir peptit olan β-defensin-2 geninin indüksiyonuna yol açtığı gösterilmiştir. Ancak bu etkiyi sağlayan molekülün ne olduğu belirlenememiştir (Schlee vd., 2008). *E. coli* Nissle 1917 flagellalıdır ve β-defensin-2 salınımını indüklediği bilinmektedir. Flagella

çeşitli bakterilerde hücre duvarına bağlı olarak bulunur. Konağın bağırsak epitel hücre reseptörleriyle ilk temasa geçtiği hareketli yüzey proteinidir (Ruiz vd., 2014). Yapılan çalışmada, flagellası olmayan *E. coli* Nissle 1917 mutantlarında bağışıklık düzenleme kapasitesi bozulurken, flagellanın tekrar aktifleştirilmesiyle bağışıklık düzenleme kapasitelerinin eskiye döndüğü belirlenmiştir. Başka bir *E. coli* suşunun *E. coli* Nissle 1917' nin tersine β-defensin-2 salınımını indüklemediği görülmüş, dolayısıyla, *E. coli* Nissle 1917' nin flagellasının temel uyarıcı faktör olduğu doğrulanmıştır (Schlee vd., 2007).

Probiyotiklerde hücre duvarına bağlı olarak bulunan bir diğer protein ise fimbria (pili)' dır. Biyosentez ve yapılarının farklı olmasına rağmen hem Gram (+) hem de Gram (-) bakterilerde fimbria görülmektedir. Genom sekanslarından yola çıkarak bifidobakter ve lactobasillerde fimbrianın kodlandığı açığa çıkarılmıştır (Lebeer vd., 2010). Fimbrianın bağırsak epitel hücrelerine yapışabilme özelliği sağlamasının yanı sıra bağışıklığı uyarıcı özelliğinin de belirlendiği çalışmalar mevcuttur. Sağlık üzerine etkileri ve bağırsak epitellerine yapışabilme özelliği bilinen *L. rhamnosus* GG' nin hücre yüzeyinde bulunan fimbriaları ile bunu sağladığı bilinmektedir (Reunanen vd., 2012). Model olarak kullanılan *L. rhamnosus* GG ve fimbriası olmayan mutantlarıyla çalışılmış, fimbrianın epitel hücrelere bağlanmada önemli olduğu ve pro-inflamatuar sitokin IL-8' in ekspresyonunun dengelenmesinde fonksiyonel rolü olduğu belirlenmiştir (Lebeer vd., 2012). Benzer şekilde, *L. rhamnosus* GG' nin fimbriasının makrofajlar üzerindeki etkisi fimbriası olmayan *L. rhamnosus* GG mutantı ve doğal suşlarıyla çalışılarak fare modellerinde incelenmiştir. Fimbrianın makrofajlara bağlanmada önemli olduğu ve anti-inflamatuar sitokin IL-10 salınımını arttırarak ve pro-inflamatuar sitokin IL-6 salınımını azaltarak anti-inflamatuar etki gösterdiği görülmüştür (Vargas García vd., 2015). Aynı zamanda *L. rhamnosus* GG' nin patojen enfeksiyonları üzerine etkisine bakılmış, *Salmonella enterica serovar Typhimurium* SL1344' nin neden olduğu inflamasyona karşı, fimbriasıyla bağırsak epitellerine bağlanıp doğal bağışıklık sitokin salınımını doğrudan uyardığı belirlenmiştir (Ganguli vd., 2015). *L. rhamnosus* GG' nin fimbrialarının patojen bakterilerin bağlanmasını önlediği belirtilerek, enfeksiyonlara karşı *L. rhamnosus* GG' nin fimbriasının tedavi amaçlı kullanılabileceği önerilmiştir (Tytgat vd., 2016). *Bifidobacterium bifidum* PRL2010 'nin fimbriası ile de çalışılmış, benzer şekilde fimbrianın yapışmada kritik önemi olduğu ve böylece bağırsak epitel hücre reseptörleriyle etkileşime girerek bağışıklık düzenleyici etki gösterdikleri belirtilmiştir (Turroni vd., 2013).

Sonuç

Son yıllarda yapılan çalışmalarla, probiyotiklerin bağışıklık sistemi üzerine birçok etkilerini ürettikleri moleküllerin insan hücreleri ile etkileşimleri sonucu sağladıkları ortaya çıkmıştır. Probiyotik hücre yapı bileşenleri ve ürettikleri moleküllerin bağışıklık sistemini yönlendirme mekanizmalarının belirlenmesi için birçok çalışma yapılmıştır. Sonuçlar probiyotiklerin insan bağışıklık sistemi üzerine aynı anda birçok etkiyi gerçekleştirebildiklerini göstermektedir. Probiyotiklerin tüketimiyle kesin sonuçların ön görülememesi nedeniyle, hastalıkların önleme ve tedavisinde probiyotiklerin değil ürettikleri etken moleküllerinin tüketiminin daha kontrollü bir yaklaşım olduğu anlaşılmaktadır. Bu da sentetik probiyotik etken moleküllerinin ilaç ya da gıda takviyesi olarak yakın zamanda hayatımıza gireceğini işaret etmektedir. Moleküler bilimlerle desteklenen, protein yapı çözümlemesi ve genomik gibi yaklaşımlar probiyotiklerin etken moleküllerinin ve etki mekanizmalarının belirlenmesine olanak sağlayacaktır.

Etik Standart ile Uyumluluk

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

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LAKTOZ İNTOLERANSIN PREVALANSI, TEŞHİSİ VE LAKTOZSUZ BESLENME TAVSİYELERİ

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ÖZ

Laktoz, sadece sütte bulunan bir disakkarit olup, emmeyi bıraktıktan sonra çoğu kişide laktozu parçalayan laktaz enziminin sentezi durmakta veya azalmaktadır. Laktoz intolerans olarak bilinen bu durum sütün sindirilememesiyle sonuçlanmakta ve dünyada çok sık görülmektedir. Laktaz geni üzerinde meydana gelen tek nükleotit polimorfizmleri nedeniyle bazı kişilerde laktaz enzimi yaşam boyu sentezlenebilmektedir. Laktaz kalıcılığı olarak adlandırılan bu durumun prevalansı etnik gruplara göre değişmektedir. Laktoz intoleransın teşhisinde çeşitli yöntemler uygulanmasına rağmen, çoğu zaman semptomların gözlenmesi hastalık teşhisi için yeterli olmaktadır. Laktoz intolerans olanlar ömür boyu sürecek laktozsuz bir diyet uygulamalıdır. Süt kalsiyum gibi bazı önemli besin öğeleri açısından zengin bir kaynak olduğu için, laktoz intolerans hastalarının kalsiyum gibi ihtiyaçlarını peynir ve yoğurt gibi laktoz içeriği düşük olan gıdalardan karşılamaları önemlidir. Bu çalışmada, laktoz intolerans hakkında bilgi verilerek, laktoz intoleransın prevalansı ve teşhis yöntemleri ile laktozsuz beslenme üzerinde durulmuştur. Bu çalışma ile laktoz intolerans farkındalığının hem hastalar tarafından hem de gıda endüstrisinin paydaşları tarafından arttırılması hedeflenmektedir.

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 immünoglobülin gibi insan yaşamı için önemi bulunan pek çok besin öğesi açısından zengin bir kaynaktır. Süt ve süt ürünleri tüketicilerin beslenme gereksinimlerini karşılamalarının yanında obezite, kemik erimesi, diş çürüğü, zayıf gastrointestinal sağlık, kardiyovasküler hastalıklar, hipertansiyon ve kolorektal kanser gibi çeşitli rahatsızlıkların önlenmesi ve semptomların azaltılmasındaki rolleri için de tüketilebilmektedir (Demirgül ve Sağdıç, 2018).

Laktoz çiftlik hayvanları da dâhil olmak üzere neredeyse tüm memelilerin (denizaslanı ve denizaygırı (mors) hariç) sütünde bulunan temel karbonhidrattır. İnsan sütünde yaklaşık 7.2 g/100 mL laktoz bulunurken; insan beslenmesinde önemli bir yer tutan inek sütünün yaklaşık 4.7 g/100 mL'si laktozdan oluşmaktadır (Lomer vd., 2007). Laktoz, yaşamın ilk yılında, bebeklerin ihtiyaç duyduğu toplam enerjinin neredeyse yarısını karşılayan en önemli enerji kaynağıdır (Silanikove vd., 2015). Laktoz ayrıca, kalsiyum, fosfat, manganez ve magnezyum gibi önemli minerallerin emilimini kolaylaştırmaktadır. Laktoz bunlara ek olarak bağırsak mikrobiyotası tarafından fermente edilerek *Bifidobacterium* türleri gibi probiyotik Gram pozitif bağırsak bakterilerinin gelişimine katkıda bulunur. Böylece konakta patojen mikroorganizmaların gelişmesi önlenebilmektedir (Ugidos-Rodriguez vd., 2018).

Laktoz, meme bezlerinin epitel hücrelerinin Golgi aygıtı içinde, glukoz ve galaktozun, laktoz sentaz enzimi tarafından katalizlenen reaksiyonlar sonucu oluşan bir disakkarittir (Kuhn ve White, 2009; Silanikove vd., 2015). Laktozun vücutta metabolize edilebilmesi için ince bağırsaktaki epitel hücrelerden salgılanan laktaz (β-D-galaktozidaz) enzimi tarafından monosakkarit bileşenlerine (β-D-glukoz ve β-D-galaktoza) hidrolize edilmesi gerekmektedir. Laktaz, fetüste sadece doğumdan birkaç gün önce ortaya çıkar ve tam da bebeğin anne sütünden laktoz alması için doğru zamanda zirveye ulaşır. Bebeğin sütten kesilmesinden 6-12 ay sonra ise laktaz azalmaya başlar (Campbell vd., 2005). Laktaz aktivitesinin yaşla birlikte azalması, normal fizyolojik bir durum olarak kabul edilmektedir (Gaskin ve Ilich, 2009). Çoğu insanda laktozu sindirme yeteneği çocuklukta kaybolurken, bazı popülasyonlarda laktaz aktivitesi yaşam boyunca devam etmektedir. Bu durum laktaz kalıcılığı (laktoz toleransı) olarak bilinir (Laland vd., 2010).

Laktaz enziminin yokluğunda süt tüketimi, gaz sancısı, şişkinlik, gurultu, mide krampları, ishal ve bulantı gibi rahatsız edici semptomlara neden olmaktadır (Demirgül ve Sağdıç, 2018). Bu semptomlar süt tüketiminin ardından genellikle 30 dakika ile 2 saat veya daha uzun sürelerde açığa çıkmaktadır (Gaskin ve Ilich, 2009).

Bu çalışma ile dünya genelinde yaygın bir şekilde görülen laktoz intoleransın biyolojisi, farklı etnik gruplardaki prevalansı ve teşhis yöntemleri hakkında bilgi verilmesi amaçlanmıştır. Ayrıca laktoz intolerans olan kişilere yönelik laktozsuz beslenme tavsiyelerinde bulunmak da çalışmanın diğer bir amacıdır.

Laktoz İntoleransın Biyolojisi

Laktoz intolerans, süt ve süt ürünlerinin tüketilmesinden sonra kolondaki sindirilmemiş laktoz nedeniyle meydana gelen sindirim bozukluğunu tanımlamak için kullanılan bir terimdir (Gaskin ve Ilich, 2009).

Bağırsaklarda çok az O_2 bulunur ve bağırsak bakterilerinin glikoliz ile ATP üretebilmeleri için, aerobik koşullarda olduğu gibi H_2O'ya çevrilemeyen karbonhidratlardaki hidrojenin, hidrojen gazı şeklinde veya bir dizi metabolit aracılığıyla (alkoller, dioller, aldehitler, ketonlar ve asitler) uzaklaştırılması gerekmektedir. Bağırsak florasında bulunan Archaebacteria ise hidrojen gazını metan gazına dönüştürebilir (Campbell vd., 2010). Campbell vd. (2010) tarafından öne sürülen bakteriyel-toksin hipotezine göre, laktoz intolerans semptomları, ince bağırsakta emilemeyen laktoz gibi karbonhidratların ve diğer gıdaların anaerobik parçalanmasının bir sonucu olarak, bağırsak bakterileri tarafından alkoller, dioller (bütan 2,3 diol), ketonlar, asitler ve aldehitler (metilglioksal) gibi çeşitli olası 'toksik metabolitlerin' üretilmesiyle açıklanabilir. Bu toksinler bakterilerde kalsiyum sinyallerini indükleyerek, bakterilerin gen ekspresyonlarını ve gelişmelerini etkilerler ve böylece bağırsaktaki mikroflora dengesinde değişikliğe neden olurlar. Bu bakteriyel toksinlerin aynı zamanda, konakçı ökaryot hücrelerdeki nöronlar, kas ve bağışıklık sistemi hücreleri ile bağırsak ve sistemik semptomlara neden olan diğer hücreler üzerindeki sinyal mekanizmalarını da etkileyerek, gıda intoleransı olan kişilerde çeşitli semptomlara yol açtığı bildirilmiştir (Campbell vd., 2010).

Çoğu insan normalde sütten kesilmesinin ardından laktaz üretemez ve sonuçta laktoz intolerans ortaya çıkar. Dünya üzerindeki yetişkin insanların yaklaşık %70'inin laktoz intolerans olduğu düşünülmektedir (Lomer vd., 2007). Laktaz enzimindeki düşük aktivite, tüketilen laktozun gastrointestinal kanalın sindirme kabiliyetini aşması halinde, laktoz maldigesyonuna (sindirim bozukluğu) ve gastrointestinal stres semptomlarına neden olabilmektedir (Brown-Esters vd., 2012).

Laktoz intolerans ve laktoz maldigesyonu terimleri sıklıkla birbirlerinin yerine kullanılmaktadır (Gaskin ve Ilich, 2009). Maldigesyonla sonuçlanan laktoz intoleransı, tüketilen laktoz miktarı, öğün zamanları, yoğurtta olduğu gibi laktozla

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

Birçok Güney Avrupalının süt içtikten sonra rahatsızlandıkları ilk kez Hipokrat tarafından gözlemlenmiştir. Ancak bunun sebebinin sütteki şekere karşı biyokimyasal intolerans olduğu 20. yy. başlarına kadar bilinmemekteydi. Laktozun diyareye neden olabileceği ilk kez 100 yıldan fazla bir süre önce bildirildi (Jacobi, 1901; Campbell vd., 2005).

Çinliler ve Japonlarda laktaz miktarı ve aktivitesi 2-3 yaşından sonra hızla azalır, 5-10 yaşlarında ise laktaz kalıcılığı nadiren görülür. Buna karşın, yetişkinlikte laktaz kaybı beyaz Kuzey Avrupalılarda nadiren gerçekleşmekte ve bu kayıp daha yavaş seyirli olmaktadır (Campbell vd., 2005). Bazı etnik gruplarda yaşamları boyunca laktaz aktivitesinin gözlenmesi genellikle laktaz enzimini kodlayan gende meydana gelen tek nükleotit polimorfizmlerinden kaynaklanmaktadır (Brown-Esters vd., 2012).

Laktoz maldigesyonuna neden olan laktaz eksikliğinin farklı sebepleri bulunmaktadır. 'Konjenital laktaz eksikliği', nadir görülen, doğuştan gelen bir metabolizma hatası olup, laktaz enzimi doğumda bulunmadığında veya aktivitesi ciddi oranda azaldığında ortaya çıkar ve yaşam boyunca bu şekilde devam eder (Gaskin ve Ilich, 2009). Konjenital laktaz eksikliğine laktaz geninin (*LCT* geni) kendisinde gerçekleşen mutasyonların neden olduğu belirtilmiştir. Konjenital laktaz eksikliği özellikle Finlandiya'da nispeten çok görülmektedir (Campbell vd., 2005).

Zaman içinde gelişen 'primer laktaz eksikliği' en sık gözlenen laktaz eksikliği tipidir. 2 yaşından önce laktaz aktivitesi, bebeklerin/küçük çocukların tükettiği süt ve süt ürünlerinin sindirimi için genellikle yeterince yüksektir. Ayrıca, anne sütü laktoz içerdiği 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).

"Sekonder laktaz eksikliği", laktaz eksikliğinden ve bunun yol açtığı laktoz maldigesyonundan sorumlu, altta yatan bir patofizyolojik durumdan kaynaklanmaktadır (Gaskin ve Ilich, 2009). Bu durum genellikle bağırsak mukozasının bakteriyel, viral veya protozoan enfeksiyonlar ile zarar görmesi sonucu ortaya çıkmaktadır (Campbell vd., 2005). Örneğin, rotavirüsün neden olduğu akut enfeksiyon, ince bağırsakta hasara yol açabilir ve ekspresyonu epitel hücrelerin yüzeylerinde gerçekleşen laktaz enziminin kaybına neden olabilir. Ayrıca, *Giardia* gibi parazitlerden kaynaklanan enfeksiyonlar da doğrudan proksimal ince bağırsağı etkileyerek laktoz maldigesyonuna neden olabilmektedir (Gaskin ve Ilich, 2009).

Farklı Etnik Gruplardaki Laktoz İntolerans Prevalansı

Dünya genelinde insanların önemli bir kısmı doğumdaki laktaz seviyelerinin büyük bir kısmını erken çocuklukta kaybetmeye başlar ve bu kayıp yaşam boyunca devam eder. Ancak hipolaktazya (laktaz yetersizliği) prevalansı etnik kökene bağlı olarak değişiklik göstermektedir (Tablo 1) (Swagerty vd., 2002).

Çoğu beyaz Kuzey Avrupalı 1-2 bardak sütü (250-500 mL) hiçbir yan etki görülmeksizin içebilirken, bazı etnik gruplar laktoza karşı o kadar hassastırlar ki, bir fincan kahvedeki sadece 10-20 ml süt bile onları 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 düşüktür. Bu durum sütten kesildikten sonraki birkaç yıl içinde laktaz enziminde gerçekleşen %75-90 oranındaki azalmadan kaynaklanmaktadır (Matthews vd., 2005).

Mądry vd. (2010), yetişkinlerdeki hipolaktazyanın Avrupa'nın güneyine ve doğusuna gidildikçe arttığını, Güney İtalya ve Türkiye'deki yetişkinlerde hipolaktazya görülme prevalansının %70'lerde olduğunu bildirmişlerdir. Ancak Türkiye'deki laktoz intolerans prevalansının ve bunun altında yatan sebeplerin daha net ortaya konabilmesi için; güncel teşhis yöntemlerinin kullanıldığı geniş kapsamlı çalışmalara ihtiyaç bulunmaktadır. Bu çalışmalarla toplumdaki laktoz intolerans farkındalığı arttırılabileceği gibi, gıda endüstrisinin de laktoz intolerans olanlara yönelik alternatif gıda üretme motivasyonu arttırılabilecektir.

Laktaz kalıcılığı, laktaz geninin regülasyon bölgesindeki en az 5 bağımsız tek nükleotit varyantıyla ilişkilidir. *-13907**G, *-13910**T, *-13915**G, *-14009**G ve *-14010**C alelleri laktaz kalıcılığıyla en çok ilişkilendirilen alellerdir (Liebert vd., 2017). Belirli bir gen varyantının frekansı etnik kökene özgüdür. Başka bir ifadeyle, Kuzey Avrupalılarda süt tüketimine izin veren gen varyantı, Batı Afrikalılarda süt tüketimine izin veren gen varyantı ile aynı değildir (Cook, 2014).

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)

Etnik Grup (belirtilmediği sürece yetişkinler)	***Düşük laktaz (potansiyel laktoz intolerans) prevalansı (%)***
Beyaz Avustralyalılar	10
Beyaz Kuzey Avrupalılar	10
Barselonalılar (İspanya özerk bölgesi, Kuzeydoğu İspanya)	13-15
Finler	20
Amerikalılar (tüm yetişkinler)	30
Galiçyalılar (İspanya özerk bölgesi, Kuzeybatı İspanya)	32.5
İspanyollar	> 40
İtalyanlar	> 40
Yunanlar	> 40
Orta Avrupalılar (örneğin Macar ve Çingeneler)	> 40
Güney Amerikalılar (tüm yetişkinler)	> 50
Meksikalılar	> 50
Afrika kökenli Amerikalılar	60-70
Eskimolar	> 70
Amerikan Kızılderelileri	> 70
Siyah Afrikalılar	> 75
Avustralya Aborjinleri	> 80
Hintliler ve diğer Asyalı gruplar	> 80
Çinliler	> 90
Japonlar	> 90
Yetişkinler (tüm etnik gruplar)	70
Huzursuz bağırsak sendromu hastaları (tüm etnik gruplar)	> 50
2 ile 10 yaş arasındaki çocuklar (tüm etnik gruplar)	0-40
2 yaşın altındaki çocuklar (tüm etnik gruplar)	0-20

Avrupalılarda laktaz kalıcılığının, *MCM6* genindeki (*-13910**T) tek bir C'den (sitozinden) T'ye (timine) geçiş ile güçlü bir şekilde ilişkili olduğu tespit edilmiştir (Itan, vd., 2009). Ayrıca, in vitro çalışmalarda *-13910**T alelinin *LCT* geninin promotör aktivitesini doğrudan etkileyebileceği gösterilmiştir (Lewinsky vd., 2005; Itan, vd., 2009). *-13910**T aleli frekansı, Doğu ve Güney Avrupa'da %6-36, Orta ve Batı Avrupa'da %56-67, Britanya Adaları ve İskandinavya'da %73-95 arasında değişirken (Bersaglieri vd., 2004; Mulcare, 2006), laktaz kalıcılığı, Doğu ve Güney Avrupa'da %15-54, Orta ve Batı Avrupa'da %62-86, Britanya Adaları ve İskandinavya'da %89-96 arasında değişmektedir (Itan vd., 2009). Bu durum, *-13910**T alelini Avrupa'daki laktaz kalıcılığını tahmin etmek için iyi bir araç haline getirmiştir. Bununla birlikte, genotip/fenotip frekans karşılaştırmaları, *-13910**T alelinin Afrikalı (Mulcare vd., 2004) ve Orta Doğulu popülasyonların (Enattah vd., 2008) çoğunda laktaz kalıcılığı frekanslarını etkilemediğini göstermiştir (Itan, vd., 2009). Bunun yerine Orta Doğu ve Afrika'daki laktaz kalıcılığı ile en fazla ilişkilendirilen aleller, *-13907**G, *-13915**G, *-14009**G ve *-14010**C alelleridir (Tishkoff vd., 2007; Ingram vd., 2007; Enattah vd. 2008; Ingram vd., 2009; Liebert vd., 2017).

Etnik gruplar arasındaki laktaz kalıcılığı frekansı ile süt tüketimi ve süt çiftçiliğiyle uğraşma durumları arasında güçlü bir ilişki bulunmaktadır (Laland vd., 2010). Bersaglieri vd., (2004) yaşam boyu laktaz üretebilme yeteneğinin, sığır gibi süt hayvanlarının evcilleştirilmesine paralel olarak 5000 ile 10000 yıl önce ortaya çıktığını tahmin ettiklerini bildirmişlerdir. Kültürel-tarihsel hipotez olarak adlandırılan hipoteze göre, laktozun metabolize edilmesini sağlayan mutasyon yaklaşık 10000 yıl önce, Kuzey Avrupa'da yaşayanlarda ortaya çıkmıştır. Bu dönem, kıtanın bu bölümünde Neolitik dönemin başlangıcı ve Kuzey Avrupalıların hayvancılık ve süt üretimine başladıkları tarihlerle örtüşmektedir. Diyetlerindeki kalori (enerji) ve kalsiyum ile D vitamini miktarını arttıracak

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

Laktaz kalıcılığının ortaya çıkma nedenine dair öne sürülen bir başka hipotez ise kalsiyum asimilasyon hipotezidir (Flatz ve Rotthauwe, 1973). Bu hipoteze göre güneş ışınlarının deriye yeterince ulaşamadığı ve dolayısıyla yeterli D vitamini sentezlenemeyen bölgelerde yaşayanlarda laktaz kalıcılığı alellerinin ortaya çıkacağı iddia edilmiştir. D vitamini kalsiyumun emilimi için önemli olup, süt hem D vitamini hem de kalsiyum açısından iyi bir kaynaktır. Bu gibi faktörlerin, kalorili ve protein bakımından zengin bir besin kaynağını tüketme kabiliyetini arttırması muhtemel görünmektedir. Bu faktörlerin sağladığı göreceli avantajların Avrupa ve Afrika'da farklı olması da olasıdır (Itan, vd., 2009).

Laktoz İntoleransın Teşhisi

Laktoz intolerans teşhisinde çeşitli yöntemler bulunmakla birlikte, teşhiste izlenen ilk yol genellikle hastanın diyetinden laktoz içeren ürünlerin çıkarılması ve semptomların bu süre zarfında izlenmesi olmaktadır. Bununla birlikte, süt tüketimi ile bazı hastalarda laktoz intolerans semptomlarının gözlenmemesi, teşhisi zorlaştırmaktadır. Ayrıca semptomlar gözlense bile bunun nedeninin huzursuz bağırsak sendromu gibi altta yatan başka bir intestinal rahatsızlığın göstergesi olma ihtimali de bulunmaktadır (Gaskin ve Ilich, 2009).

Laktoz intolerans varlığının belirlenmesinde kullanılan yöntemlerden birisi kan şekeri (glukoz) miktarının ölçülmesidir. Laktaz enzimini üreten kişiler laktoz içeren bir ürün tükettikleri zaman laktozun parçalanmasına bağlı olarak kandaki glukoz miktarlarında artış olmaktadır (Itan vd., 2010; Cook, 2014). Laktoz tolerans testi, kg vücut ağırlığı başına yaklaşık 1-1.5 g oral laktoz dozunun uygulanması ve kan glukoz seviyelerinin ölçülmesi için seri kan numunelerinin elde edilmesiyle yapılır. Bağırsak semptomları meydana gelirse ve kan şekeri seviyesi, açlık seviyesinin üstünde 20 mg/dL'den daha az artarsa test pozitiftir. Ancak değişken gastrik boşalma ve glukoz metabolizmasının etkisinden dolayı testlerin yaklaşık %20'sinde yanlış (false) pozitif ve yanlış negatif sonuçlar ortaya çıkabilmektedir (Swagerty vd., 2002).

Laktoz intoleransın belirlenmesinde yararlanılan diğer bir yöntem ise hidrojen solunum testidir. Laktoz, kendisini oluşturan glukoz ve galaktoza sindirilmediğinde, disakkarit sindirilmeden kolona geçer (Gaskin ve Ilich, 2009) ve burada bazı bağırsak bakterileri tarafından (bifidobakteriler, laktobasiller ve *Escherichia coli* gibi bazı mikroorganizmalar laktozu metabolize edebilmektedir) fermente edilerek; laktik asit ve yağ asitleri ile hidrojen, karbondioksit ve metan gazı gibi çeşitli gazlar açığa çıkar. Hidrojen kandan akciğerlere geçer ve bir hidrojen analizörü kullanılarak nefeste algılanabilir (Corgneau vd., 2015). Laktoz alımından sonra bazal hidrojenin 20 ppm'den daha yüksek bir konsantrasyona yükselmesi, hipolaktazyayı gösterir. Laktoz malabsorbsuyonu olan hastaların yaklaşık %90'ında hidrojen solunum testi pozitiftir. 25-50 g laktoz alımından sonra nefesteki hidrojen miktarının ölçümü, kan yoluyla yapılan laktoz tolerans testinden daha hassas ve spesifiktir. Ancak hidrojen solunum testi kolondaki mikrobiyal floradan, oral antibiyotik kullanımından ve kolonik lavman kullanımından dolayı yanlış negatif sonuçlar verebilmektedir. Ayrıca uyku, egzersiz, aspirin kullanımı ve sigara kullanımı laktoz ile ilgisi olmayan nefesteki hidrojen miktarını arttırabilmektedir (Swagerty vd., 2002).

Hem kandaki glukoz miktarının belirlenmesi yönteminde hem de hidrojen solunum testinde doğru sonuçların alınabilmesi için, bireylere gece boyu aç bırakıldıktan sonra laktoz verilmelidir (Cook, 2014).

Laktoz maldigesyonunun tanısı için kullanılan en güvenilir yöntemlerin başında, laktaz aktivitesinin doğrudan belirlenmesini sağlayan intestinal biyopsi yöntemi yer almaktadır. Bu yöntemin esası, laktozdan serbest hale geçen glukozun, glukoz oksidaz reaktifiyle belirlenmesine dayanmaktadır. Ancak yöntemin sağlıklı kişilerde kullanılması çeşitli sorunlara yol açtığı için, yöntem laktoz intolerans şüphesinin belirgin olduğu durumlarda kullanılmalıdır (Mattar vd., 2012).

Laktoz intoleransın teşhisinde, laktaz enzimini kodlayan gende meydana gelen polimorfizmlerin belirlenmesi esasına dayanan genetik testler de kullanılabilmektedir (Misselwitz vd., 2013). Bu amaçla en çok, Avrupalı popülasyonlarda laktaz kalıcılığıyla en fazla ilişkilendirilen *MCM6* genindeki -*13910**T polimorfizminin belirlenmesine yönelik testler uygulanmaktadır (Ugidos-Rodriguez vd., 2018).

Genetik testlerin, klinik bulguları olan ve hidrojen solunum testi negatif olan 8 yaşın üzerindeki bireylerde yararlı olabileceği belirtilmektedir. Genetik testler, tükürükten izole edilen DNA'nın spesifik amplifikasyonu kullanılarak yapılabilir. Yöntemin esası, real-time PCR (Polimeraz Zincir Reaksiyonu) kullanılarak ilgili polimorfizmlerin floresan problarla bulunarak işaretlenmesine dayanmaktadır. PCR ile analizin spesifikliği yaklaşık %100; duyarlılığı ise yaklaşık %93'tür. Bu test, hızlı, kesin ve non-invaziv bir tanı konulmasına imkân sağlamaktadır (Ugidos-Rodriguez vd., 2018).

Hidrojen solunum testine kıyasla genetik testler, semptomlara neden olmadan laktoz intolerans teşhisine imkân veren, daha basit ve non-invaziv yöntemlerdir. Ayrıca genetik testler hipolaktazya veya laktaz kalıcılığı hakkında daha doğrudan bir sonuç verirken; hidrojen solunum testinin yorumlanması üzerine, verilen laktoz dozu, test süresi ve bireyin yaşı gibi fazla sayıdaki değişken etkilidir (Mattar vd., 2012). Ancak laktaz kalıcılığına neden olan polimorfizmlerin bölgelere

ve etnik gruplara göre farklılık göstermesi genetik testleri sınırlandırmaktadır. Bu konuda yapılacak daha ileri çalışmalarla gelecekte genetik testlerin etkinliği arttırılabilir. Bununla birlikte genetik testlerin sekonder laktaz eksikliğini teşhis etmek amacıyla kullanılamayacağı açıktır (Misselwitz vd., 2013).

Laktozsuz Beslenme Tavsiyeleri

Laktoz sadece memelilerin sütünde bulunan bir disakkarit olmasına rağmen, süt ve süt ürünleri pek çok gıdanın üretiminde kullanılmaktadır. Ayrıca sütten saflaştırılan laktoz, teknolojik özelliklerinden dolayı endüstriyel gıdalarda katkı maddesi olarak, ilaçlarda ise dolgu maddesi olarak sıklıkla kullanılmaktadır. Laktozun renk ve su bağlama özelliği bulunmaktadır. Ayrıca tekstür üzerine de olumlu etkileri vardır. Laktoz yaklaşık olarak glukozun yarısı, sakkarozun ise üçte biri oranında tatlılık vermektedir. Laktoz bu özelliklerinden dolayı tatlılarda, şekerlemelerde, ekmeklerde, sosis gibi işlenmiş et ürünlerinde çok kullanılmaktadır (Vesa vd., 2000). Bunların yanında enteral beslenmede kullanılan birçok sıvı da laktoz içermektedir (Campbell vd., 2005). Laktozun, Batı diyetlerinde tüketilen karbonhidratların yaklaşık %6'sını oluşturduğu tahmin edilmektedir (Ugidos-Rodriguez vd., 2018).

Bazı Asya restoranları geleneksel Asya mutfaklarında kullanılan laktoz içermeyen Hindistan cevizi sütü gibi malzemelerin yerine son yıllarda süt kreması veya yoğunlaştırılmış süt kullanmaktadır. Oysa birçok laktoz intolerans hastası, süt tozu ve yoğunlaştırılmış sütün eşdeğer miktardaki sütten daha fazla laktoz içerdiğini bilmemektedir. Benzer şekilde pek çok insan, peynir altı suyu gibi ürünlerin sütteki laktozun neredeyse tamamını içerdiğinin farkında değildir (Campbell vd., 2005). Laktoz intolerans olan kişilere uygun bilgilendirmelerin yapılması oldukça önemlidir. Laktoza toleransı çok düşük olan kişiler, yukarıda zikredilen ve Tablo 2'de verilen gıdalar da dâhil olmak üzere, değişik miktarlarda laktoz içerebilen birçok gıda ürünü olduğunu bilmeli ve gıda ambalajlarındaki uyarıları dikkate almalıdır.

Tablo 2. Bazı gıdaların içerdiği yaklaşık laktoz miktarları (Topçu ve Saldamli, 2006; Chandan ve Shah, 2006; Misselwitz vd., 2013; Corgneau vd., 2015; Hertzler vd., 2017)

Table 2. Approximate amount of lactose contained in some foods (Topçu and Saldamli, 2006; Chandan and Shah, 2006; Misselwitz et al., 2013; Corgneau et al., 2015; Hertzler et al., 2017)

Gıda	*Laktoz miktarı (g/100g)*	*Referans*
İnek sütü	4.9	Chandan ve Shah, 2006
Yoğurt	4.5	Misselwitz vd., 2013
Taze beyaz peynir (1 gün depolanan)	2.05	Topçu ve Saldamli, 2006
Olgunlaştırılmış beyaz peynir (90 gün depolanan)	0.74	Topçu ve Saldamli, 2006
Peynir altı suyu (beyaz peynir)	4.57	Topçu ve Saldamli, 2006
Cheddar	0.18	Corgneau vd., 2015
Mozarella	0.07	Corgneau vd., 2015
Ekşi 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
Vanilyalı puding	1.80	Corgneau vd., 2015
Balık kroket	0.12	Corgneau vd., 2015
Çikolata bar	8.21	Corgneau vd., 2015

Laktoz malabsorpsiyon derecesi, hastalar arasında geniş ölçüde değişmekle birlikte, çoğu hasta, tamamen laktoz içermeyen veya ciddi ölçüde kısıtlanmış bir diyete gerek duymaz. Bu hastaların diyetinden kalsiyum, fosfor, riboflavin, A ve D vitaminleri gibi temel besin öğelerini sağladıkları için, süt ürünleri tamamen çıkarılmamalıdır. ABD'de diyetten alınan kalsiyumun yaklaşık %75'i süt ürünleri ile karşılanmaktadır (Swagerty vd., 2002). Kalsiyum tüketimindeki azalmanın osteoporoz, hipertansiyon ve bazı kanser türleri ile ilişkili olduğuna dair veriler bulunmaktadır (Yıldırım ve Özen, 2017). Laktoz intolerans olan yetişkinler, semptomlarının gözlenmediği miktara kadar süt ürünleri de dâhil olmak üzere günlük 1200-1500 mg kalsiyum alımını sürdürmelidirler (Swagerty vd., 2002). Bazı süt ürünleri üretim teknolojileri gereği düşük laktoz içermektedir. Çoğu peynir çeşidinde laktozun büyük bir kısmı peynir altı suyuna geçmektedir (Corgneau vd., 2015). Yoğurt üretiminde ise sütteki laktozun bir kısmı parçalanmaktadır. Ancak yine de azımsanmayacak miktarda laktoz içeren yoğurdun laktoz intoleranslılar tarafından daha rahat tüketilebiliyor olması, yoğurt bakterilerinin laktaz enzimi üretme yeteneğine ve laktaz enziminin mide asitliğini geçebilmesine bağlanmaktadır (Adolfsson vd., 2004).

Bunların yanı sıra laktozun sütten uzaklaştırılmasıyla üretilen laktozsuz sütler de, laktoz intolerans olanlar için iyi bir alternatif oluşturmaktadır. Laktozu azaltılmış ve laktozsuz süt üretiminde genellikle iki yöntemden yararlanılmaktadır. Bunlardan en sık uygulanan yöntem, laktozun enzimatik olarak hidroliz edilmesidir. Süt, *Kluyveromyces lactis*, *Aspergillus oryzae* ve *A. niger* gibi mikroorganizmalardan elde edilen mikrobiyal laktaz enzimiyle uygun şartlarda inkübe edilerek laktozun glukoz ve galaktoza parçalanması sağlanır. Glukoz ve galaktozun toplam tatlılık derecesinin laktozdan fazla olması sebebiyle, bu sütler normal sütlere göre daha tatlıdır (Ugidos-Rodriguez vd., 2018). Bu, bazı tüketiciler tarafından hoş karşılanmamaktadır. Ancak, çikolatalı süt gibi bazı ürünlerin laktozsuz süt kullanılarak üretilmesi durumunda, ürünlerin hem laktozsuz özellik kazanması, hem de bu ürünlere daha az eklenti şeker ilave edilmesi sağlanabilir (Corgneau vd., 2015). Enzim hidroliz yöntemi peynir altı suyundaki laktozun uzaklaştırılması için de kullanılabilmektedir. (Ugidos-Rodriguez vd., 2018). Laktozsuz süt üretiminde kullanılan diğer yöntem ise ilk yöntemin bir varyasyonu olan; süte uygulanan ultrafiltrasyon işleminin ardından, laktaz enziminin süte katılmasıdır. Ultrafiltrasyon membranları ile sütteki laktoz miktarı azaltılır, daha sonra laktaz enziminin eklenmesi ile de kalan laktozun hidrolizi sağlanır (Corgneau vd., 2015; Ugidos-Rodriguez vd., 2018).

Hastaların laktaz enzim takviyeleri kullanmalarının da yararlı olduğu düşünülmektedir (Swagerty vd., 2002). Laktaz enzimi bitkiler, hayvansal organlar, küf, maya ve bakteri gibi pek çok kaynaktan elde edilebilir, ancak yaygın olarak çözünen laktazlar genellikle mikrobiyal kaynaklıdır (Corgneau vd., 2015). Enzim takviyesi olarak daha çok *A. oryzae* ve *K. lactis* gibi mikroorganizmalardan elde edilen ekzojen laktaz enzimi kullanılmaktadır (Di Rienzo vd., 2013). Bu amaçla, laktaz enzimi içeren kapsüller, çiğnenebilir tabletler ve sıvı preparatlar kullanılabilir. Enzim takviyesi kullanımındaki seçeneklerden biri, sütün ticari laktaz enzimi ile birkaç saat (tüketimden 10 saat önce yapılan işlemin hastalar tarafından daha iyi tolere edilebileceği belirtilmektedir) inkübe edilmesidir (Ugidos-Rodriguez vd., 2018). Ekzojen laktaz enziminin laktoz malabsorbsiyonu olanların yemeğine eklenmesi ile yan etki gözlenmeksizin laktoz emiliminin artabileceği bildirilmektedir. Ayrıca laktaz aktivitesi bulunan probiyotik mikroorganizmaları içeren gıdaların tüketilmesinin de faydalı olduğu düşünülmektedir. Bu mikroorganizmalar, süt ve süt ürünlerine eklenebileceği gibi gıda takviyesi şeklinde de tüketilebilir (Di Rienzo vd., 2013). Bununla birlikte, semptomlara neden olmayacak laktaz dozunu tam olarak ayarlamanın zor olduğu ve bu nedenle tam bir iyileşme sağlanamayacağı hastalara bildirilmelidir (Swagerty vd., 2002).

Halen tartışmalı bir konu olmasına rağmen, literatürde bazı hastaların düzenli laktoz tüketimi ile laktoza toleranslarını arttırabildiklerine ve dolayısıyla laktoz intolerans semptomlarının ve nefes hidrojen miktarlarının azaldığına dair veriler bulunmaktadır. Szilagyi (2015), şu anki verilerin (Johnson vd., 1993; Hertzler ve Savaiano, 1996) uzun süre (en az 3-4 hafta) laktoz içeren süt ürünleri tüketilmesinin, laktoz intolerans semptomlarını azalttığını gösterdiğini bildirmiştir. Semptomlardaki iyileşmenin sebebi tam olarak aydınlatılamamış olsa da bunu açıklayabilmek için farklı hipotezler ileri sürülmüştür. Kolonik mikrobiyota ve kolonik fonksiyonlarda (motilite, transit veya pH) meydana gelen adaptasyonlar; daha az bakteriyel hidrojen üretimi; sürekli laktoz tüketimi ile bireylerin semptom algısının azalması ve plasebo etkiler, bu gözlemleri açıklayabilmek için öne sürülen hipotezlerden bazılarıdır (de Vrese vd., 2001).

Sonuç

Pek çok beslenme otoritesi, içerdiği önemli besin elementleri nedeniyle süt ve süt ürünlerinin, diyetimizin bir parçası olması gerektiğini belirtir. Bununla birlikte, sütte bulunan temel karbonhidrat olan laktoza karşı dünya nüfusunun önemli bir kısmında, rahatsız edici çeşitli semptomlara neden olan aşırı hassasiyet bulunmaktadır. Laktoz intolerans olarak adlandırılan bu durumun sebebi, anneyi emmeyi bıraktıktan sonra laktozu parçalayan laktaz enziminin sentezinin durması veya azalmasıdır. Laktoz intolerans prevalansı etnik gruplara

göre değişmektedir. Türkiye'de laktoz intolerans prevalansını belirlemeye yönelik az sayıda çalışma bulunmaktadır. Bu çalışmalardan birinde (Mądry vd., 2010) Türkiye'deki yetişkinlerde hipolaktazya prevalansının %70 olduğu bildirilmektedir. Ancak güncel teşhis yöntemlerinin uygulandığı geniş kapsamlı çalışmalarla, bölgelere göre Türkiye laktoz intolerans haritasının ortaya konması gerekmektedir. Bu durum gıda endüstrisinin laktozsuz ürün geliştirme motivasyonunu arttırabileceği gibi bölgesel olarak yapılan üretimlerde farklılaşmaya gidilmesine de imkân tanıyacaktır.

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 toplumda sık rastlanılan hassasiyet veya alerjenlere karşı bulunan uyarılar daha belirgin olmalıdır. Ayrıca belirli gıdalara karşı hassasiyeti veya alerjisi olanların, gıda seçiminde daha bilinçli hareket etmeleri için yetkili otoriteler tarafından doğru yönlendirilmeleri gerekmektedir.

Etik Standart ile Uyumluluk

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

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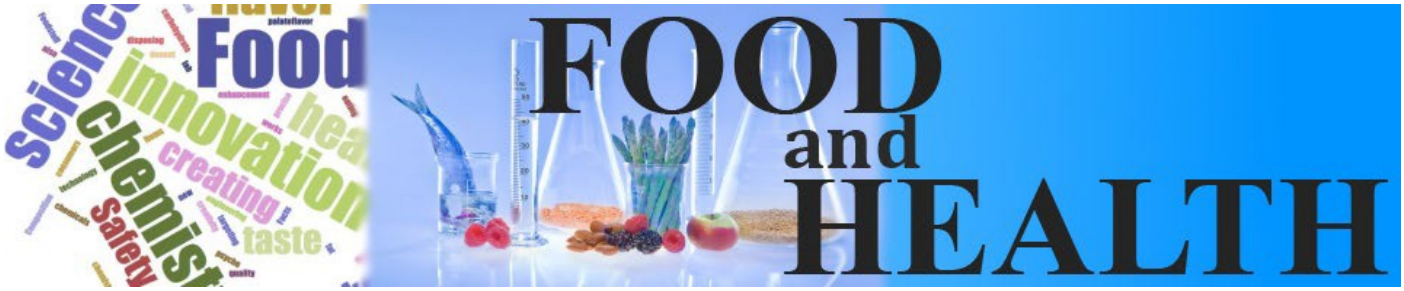

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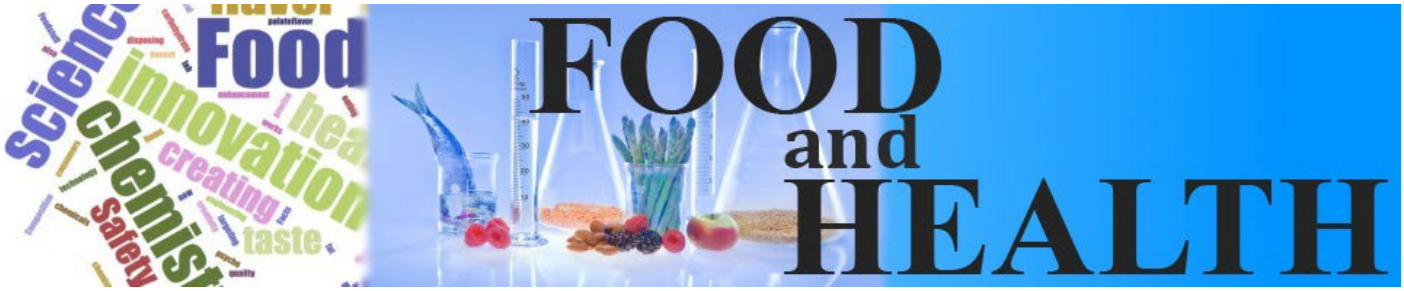

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Full Name(s) and Surname (s) of author(s)

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

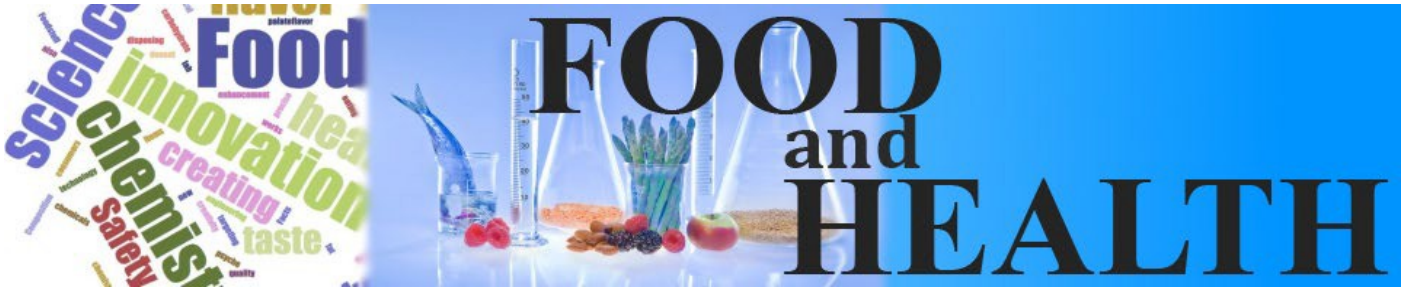
Material and Methods

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

Type of manuscript	Page	Abstract word limit	Reference limit
Original Article	≤25	180	40
Review Article	no limits	180	60
Short Communication	≤5	150	20

Tables

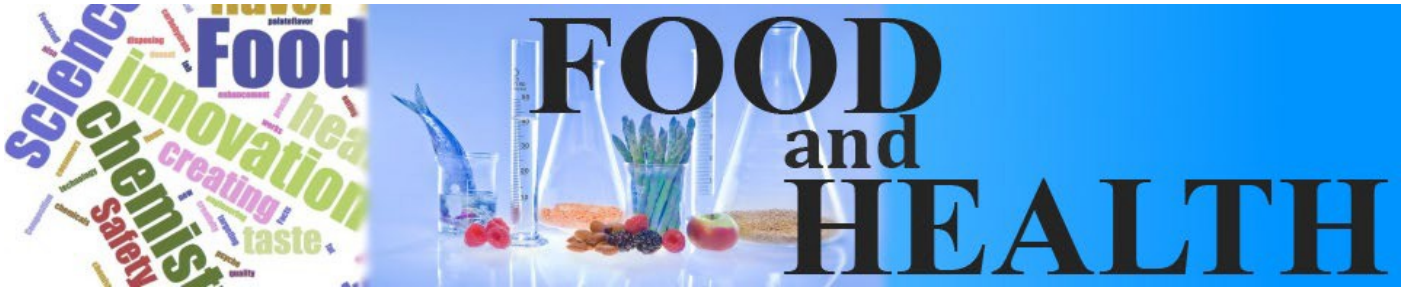
Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables 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 2.

Material Type	Reference List/Bibliography
A book in print	Baxter, C. (1997). *Race equality in health care and education.* Philadelphia: Ballière Tindall, p. 110-115, ISBN 4546465465
A book chapter, print version	Haybron, D.M. (2008). Philosophy and the science of subjective well-being. In M. Eid & R. J. Larsen (Eds.), *The science of subjective well-being* (p. 17-43). New York, NY: Guilford Press. ISBN 4546469999
An eBook	Millbower, L. (2003). *Show biz training: Fun and effective business training techniques from the worlds of stage, screen, and song*. p. 92-90. Retrieved from http://www.amacombooks.org/ (accessed 10.10.2015).
An article in a print journal	Carter, S., Dunbar-Odom, D. (2009). The converging literacies center: An integrated model for writing programs. *Kairos: A Journal of Rhetoric, Technology, and Pedagogy,* 14(1), 38-48.
Preview article in a journal with DOI	Gaudio, J.L. & Snowdon, C.T. (2008). Spatial cues more salient than color cues in cotton-top tamarins (*Saguinus oedipus*) reversal learning. *Journal of Comparative Psychology,* https://doi.org/10.1037/0735-7036.122.4.441
Websites - professional or personal sites	*The World Famous Hot Dog Site.* (1999, July 7). Retrieved January 5, 2008, from http://www.xroads.com/~tcs/hotdog/hotdog.html (accessed 10.10.2015).
Websites - online government publications	U.S. Department of Justice. (2006, September 10). Trends in violent victimization by age, 1973-2005. Retrieved from http://www.ojp.usdoj.gov/bjs/glance/vage.htm (accessed 10.10.2015).
Photograph (from book, magazine or webpage)	Close, C. (2002). *Ronald.* [photograph]. Museum of Modern Art, New York, NY. Retrieved from http://www.moma.org/collection/object.php?object_id=108890 (accessed 10.10.2015).
Artwork - from library database	Clark, L. (c.a. 1960's). *Man with Baby.* [photograph]. George Eastman House, Rochester, NY. Retrieved from ARTstor.
Artwork - from website	Close, C. (2002). *Ronald.* [photograph]. Museum of Modern Art, New York. Retrieved from http://www.moma.org/collection/browse_results.php?object_id=108890 (accessed 10.10.2015).

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.