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CHEMICAL, MICROBIOLOGICAL AND SENSORIAL PROPERTIES OF ANCHOVY SAUSAGE (SUCUK)

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ABSTRACT

Today, consumers and food industry focused on nutrient rich products. Daily diet list must contain foods high in nutrients such as essential oils, minerals, and protein. Sausage type meat products are among the most consumed meat products, but the quality of the meat used in these products is not at the desired level. In Turkey anchovy is an important fish species and its processing on meat products will lead higher quality and nutritional content of meat products and there will be a good alternative product for person dislike to directly consume anchovy. In this study; the chemical, microbiological and sensorial properties of anchovy sausage were investigated. The anchovy sausage provided a high energy value (296 kcal/100g) due to its high contents of protein and fat. It comprised of polyunsaturated fatty acids (12%) as mainly eicosapentaenoic acid and docosahexaenoic acid. It was also rich in iron, zinc and selenium as essential minerals. The sensorial scores of the anchovy sausage were high. Study results show that this product can be consumed as a healthy meat product.

Key words: Anchovy, Sausage, Meat Products, Fatty Acids, Histamine, TBARS

HAMSİ SUCUĞUNUN KİMYASAL, MİKROBİYOLOJİK VE DUYUSAL ÖZELLİKLERİ

ÖZET

Günümüzde tüketiciler ve gıda endüstrisi besin içeriği zengin ürünlere odaklanmıştır. Günlük diyet listesi, uçucu yağlar, mineraller ve protein gibi besin değeri yüksek gıdalar içermelidir. Sucuk, salam, sosis türü et ürünleri en fazla tüketilen et ürünlerinin başında gelmektedir, ancak bu ürünlerde kullanılan et kalitesi istenilen seviyede değildir. Türkiye'de hamsi önemli bir balık türüdür ve et ürünlerinde işlenmesi, bu et ürünlerinin daha kaliteli ve daha zengin besin içeriğine sahip olmasını sağlayacaktır. Ayrıca hamsiyi doğrudan tüketmekten hoşlanmayan kişiler için iyi bir alternatif ürün olacaktır. Bu çalışmada; hamsi sucuğunun kimyasal, mikrobiyolojik ve duyusal özellikleri araştırılmıştır. Hamsi sucuğu, yüksek protein ve yağ içeriği nedeniyle yüksek enerji değerine (296 kcal.100 g^{-1}) sahiptir. Çoğunlukla eikosapentaenoik asit ve dokosaheksaenoik asit gibi çoklu doymamış yağ asitlerinden (%12) oluştuğu belirlenmiştir. Aynı zamanda temel mineraller olarak demir, çinko ve selenyum açısından da zengindir. Hamsi sucuğunun aldığı duyusal puanlar yüksektir ve çalışma sonuçları bu ürünün sağlıklı bir et ürünü olarak tüketilebileceğini göstermektedir.

Anahtar kelimeler: Hamsi, Sucuk, Et Ürünleri, Yağ Asitleri, Histamin, TBARS

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INTRODUCTION

The demand of red meat has showed decreasing trend, whereas the demand of white meat has exhibited increasing trend owing to health and economic reasons. Fish meat has a great importance in the white meat since it is cheap and has high nutritional value (Dinçoğlu, 2001). Fish meat includes proteins, fats, vitamins and minerals. Fish proteins have a high biological value because of a high proportion of essential amino acids. Fish oils are a good source of polyunsaturated fatty acids (PUFAs), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Fish has a high level of fat-soluble vitamins (A, D, and E) and B complex vitamins (B_3, B_6, and B_{12}). Fish is also excellent source of minerals such as iron, calcium, selenium and zinc minerals (Sidhu, 2003; Turan et al., 2007).

Nowadays, consumer preferences have been changed. They prefer foods having a healthier image. Meat products are rich in saturated fatty acids (SFAs). SFAs are associated to an increased risk for cardiovascular diseases. Therefore, the replacement of SFAs with unsaturated fatty acids is suggested to reduce the risk of cardiovascular diseases. Meat industries have developed new meat products to meet their consumers' demand. Meat products are enriched with omega-3 fatty acids to create a healthier meat product (Intarasirisawat et al., 2014; Santana et al., 2013) since omega-3 fatty acids are associated with various health benefits. Omega-3 fatty acids may show protective effects against heart diseases and cancer. Omega-3 fatty acids may reduce total cholesterol, LDL cholesterol and triglyceride levels, which are accepted as the risk factors for cardiovascular diseases. They may inhibit transactivation of transcription activator protein 1 (AP-1), which is responsible for gene expression causing cell proliferation and tumor formation in cancer. Omega-3 fatty acids are also required for brain and eye health (Gogus and Smith, 2010).

Sausage, one of the most consumed meat products in the world, is frequently made from red meat. Fish sausages have gained popularity in recent years due to the potential health benefits of omega-3 fatty acids (Maqsood et al., 2008).

Anchovy (*Engraulis encrasicolus* L.) is one of the most important fish species in the Black Sea and Mediterranean Sea. Anchovy is consumed as fresh. It is also processed into fishmeal (Köse and Erdem, 2004; Turan et al., 2004).

The aim of this study was production of an alternative and nutrient rich meat product for consumers, to investigate properties of a new sausage made from anchovy fillet. The proximate composition, physicochemical properties, fatty acid composition, quality characteristics, microbiological quality, and sensorial properties of the sausage were determined.

MATERIAL AND METHOD

Chemicals

All chemicals and solvents were obtained from Merck (Darmstad, Germany). FAME mix was supplied from Sigma-Aldrich (St. Louis, MO, USA).

Sausage production

Anchovy was purchased from local market. Anchovy fillet was obtained from fish after the cleaning and removing of the internal organs. Anchovy fillet was minced through a meat grinder (Arı Makine, PKM 1, Istanbul, Turkey). The minced fish fillet (70%) was mixed with tallow (23%), salt (2.2%), sugar (1%), various spices (3.5%), and garlic (0.3%) and the obtained sausage dough was filled to the artificial casing. Heat treatment applied at 80 °C for 20 min (Oven, Arı Machine, FPK 200, Istanbul, Turkey). After the heat treatment, samples were cooled and stored at 4 °C until analysis.

Proximate composition

Moisture, protein, fat, and ash analysis were carried out to the AOAC standards (AOAC, 2000) Crude protein (factor: 6.38) and crude fat were determined by the Kjeldahl method and Soxhlet method, respectively. Total carbohydrate was calculated by subtracting the moisture, protein, fat and ash contents from the total percentages.

Physicochemical properties

pH value was measured with a pH meter (HANNA 211, USA). Color values (*L*, a*, b**) were measured with a color meter (Konica Minolta-300, Japan).

Energy value: energy value of samples were calculated by adding protein, fat and carbohydrate energy amounts.

Energy (kcal/100g) = Protein*4 + Fat*9 + Carbohydrate*4

Fatty acid composition

The fatty acid composition of the samples was detected according to the analytical method ISO 12966-1:2014. Fatty acid methyl ester (FAME) was injected into a Shimadzu GC-2010 Plus gas chromatograph (Shimadzu Corporation, Japan) equipped with a flame ionization detector, a split/splitless injector and a long capillary column (TRACE TR-FAME GC column, 60 m × 0.25 mm × 0.20 µm, Fisher Scientific). The temperature conditions of the program were as follows: the initial temperature of the column was 90 ºC, and held for 5 min, followed by a 10 ºC/min ramp to 240 ºC, and held for 20 min. The carrier gas was nitrogen at a flow rate of 60 mL/min, and the split ratio was 25:1. The injection quantity was 1 µL. The identification of FAMEs was performed by using a standard FAME reference mixture.

Microbiological analyses

Detection of *Salmonella, Listeria monocytogenes, Vibrio cholera, Vibrio parahaemolyticus,* positive coagulase *Staphlycoccus,* coliform bacteria, aerobic bacteria and yeast and mold were performed in accordance with the ISO methods (ISO 6579, ISO 11290-1, ISO 21872-1, ISO 6888-1, ISO 4832, ISO 4833-1, ISO 21527- I-II). Cell count was expressed as colony forming units per gram ($cfu.g^{-1}$) of the sausage.

Mineral analysis

Mineral analysis was carried out according to NMKL method (NMKL 170 method, modification AFS). A 0.5 g sample was placed into a burning cup, and 1 mL of H_2O_2 and 5 mL of HNO_3 were added. The samples were burned in a microwave oven (Milestone, Start D, Italy) and analyzed with an inductively coupled plasma mass spectrometry (ICP-MS) (Agilent 7700 Series, USA). Na, Ca, K, Mg, Fe, Zn, and Se were determined.

TBARS value

Analysis was performed in accordance with the method developed by (Tarladgis et al., 1960). A 10 g sample mixed with 49 mL of water and 1 mL of sulfanomide solution was homogenized for 2 minutes with a blender (Waring, 8011 ES, USA). After washing with water, the solution was taken into a Kjedahl flask. A 2 mL of HCl was added to the flask. The Kjedahl flask was placed into the distillation unit. A 5 mL of distillate mixed with 5 mL of TBA solution (0.02 M) was kept at boiling water bath for 35 minutes. Absorbance at 538 nm was measured. The results are expressed as mg malonaldehyde.kg^{-1}.

Histamine analysis

Sausage sample was homogenized with a grinder (Waring, 8011 ES, USA). A 5 g of the homogenized sample was weighed into a tube, and 10 mL of perchloric acid was added to the tube. After homogenization for 4 minutes with Ultra-Trax homogenizer (IKA, T25 D, Germany), the tubes were centrifuged at 2440 g for 10 minutes. The solution was filtered via filter paper. Extraction was repeated, and extracts were combined. A 0.5 mL of extract was mixed with 0.1 mL of sodium hydroxide, 0.15 mL of sodium bicarbonate, and 1 mL of dansyl chloride. After the solution was incubated at 40 °C for 45 minutes, it was kept at room temperature for 10 minutes. A 50 µL of ammonium was added and mixed for 30 seconds. Ammonium acetate and acetonitrile were added, and filtered via filter (Millex-LH, PTFE, 0.45µm, 4 mm). The filtrate was analyzed via HPLC (Agilent 1260, USA) with a DAD detector, and a column (Nucleosil, 250x4.6 mm, 5µ). A gradient elution with 0.1 mol/L ammonium acetate (Mobil phase A), and acetonitrile (Mobil phase B) started with A 50% and finished with A 10% in 20 minutes. Column temperature was 40° C, and flow rate was 0.9 mL.min^{-1}.

TVB-N analysis

Exactly 10 g of sample was weighed into a flask and mixed with 90 mL of perchloric acid solution. After the sample was homogenized for two minutes with a blender, and it was filtered. Extract (50 mL) was put into a steam distillation apparatus. A few drops of phenolphthalein, silicone, and 6.5 mL of sodium hydroxide solution was added to the apparatus and started to steam distillation quickly. Approximately 100 mL of distillate was taken in 10 minutes. The distillation outflow tube was submerged in a receiver with 100 ml boric acid solution. The volatile bases in the receiver flask were determined by titration with standard hydrochloric acid solution (EC, 2005).

Sensorial properties

The surface color and appearance, greasiness, juiciness, aroma of the raw samples were determined by ten panelists. The sausage samples were fried at 180° C for 2 minutes in an oven (Arçelik MF 26 BK, Istanbul, Turkey). The texture, aroma, and overall acceptability of the cooked samples were evaluated. The samples were put in the cups coded randomly with three digit numbers. A 9-points scale was used for the evaluation of the samples.

Statistical analysis

Three independent samples were analyzed in triplicate. The mean and standard deviations values were calculated by using office program (Excel 2016).

RESULTS AND DISCUSSION

Proximate composition

The proximate composition of the anchovy sausage is shown in Table 1. The main macronutrient found in the anchovy sausage was fat (25.6%) followed by protein (12.5%). The anchovy sausage exhibited a higher fat content and a lower protein content compared to those reported for other fish sausages. Dallabona et al. (2013) reported 16.5% of protein and 14.4% of fat in the pasteurized sausage made from Nila tilapia. Pasteurized fish sausage from Bull's eye was found to have 18.9% of protein and 1.3% of lipid (Umesha Bhatta et al., 2015). Minced fish (Talang Queenfish) sausage was determined to have 19.4% of protein and 19.1% of fat (Yousefi and Moosavi-Nasab, 2014). Commercial fish sausages produced from fillets of crimson snapper had 19.7% of protein and 5.5% of lipid (Al-Bulushi et al., 2013). The anchovy sausage had a higher fat content and a lower content of protein than anchovy as well (Kaya and Turan, 2010). These findings may be explained by using beef tallow (20%) in the anchovy sausage formulation. Fat used in the fish sausages from Nila tilapia and Bull's eye was reported to be lower than 5% that might explain their lower fat content. The carbohydrate level (3.8%) of the sausage was lower compared to the fat and protein levels. This finding could be attributed to the absence of carbohydrate in anchovy, which was the main ingredient of the sausage. Sugar used in the sausage formulation were the source of carbohydrate.

Table 1. Proximate composition, energy value and physicochemical properties of the anchovy sausage

Moisture (g.100 g^{-1})	47.4±0.5
Protein (g.100 g^{-1})	12.5±0.2
Fat (g.100 g^{-1})	25.6±0.2
Carbohydrate (g.100 g^{-1})	3.8±0.2
Ash (g.100 g^{-1})	5.5±0.2
Energy value (kcal.100 g^{-1})	296±2
pH	5.47±0.09
L^* value	70.52±0.10
a^* value	3.75±0.02
b^* value	22.43±0.14

*Mean ± standard deviation

The sausage had a high energy value (296 kcal.100g^{-1}) owing to its high contents of fat and protein. The proximate composition can make the anchovy sausage a valuable food in human nutrition. Therefore, its consumption may be proposed to provide macronutrients and energy.

Physicochemical properties

The physicochemical properties of the anchovy sausage are shown in Table 1. The pH value of the anchovy sausage (5.47) was comparable with the pH value (< 5.60) of the heat treated sausages made from red meat and/or white meat (TFC, 2012). The L^*, a^*, and b^* values of the anchovy sausage were 70.52, 3.75, and 22.43, respectively. L^* and b^* values of the anchovy sausage exhibited higher values than the meat sausage while it had a* lower value than that sausage (Yıldız-Turp and Serdaroğlu, 2008). Similar results were reported for the fish sausage produced from saithe (Dincer et al., 2017). These findings could be attributed to differences in color values between red meat and fish meat. The anchovy sausage had higher L^*, a^*, and b^* values than the minced fish sausage (Yousefi and Moosavi-Nasab, 2014). Moreover, it showed higher L^* and b^* values than the commercial fish sausage (Al-Bulushi et al., 2013).

Fatty acid composition

The fatty acid composition of the anchovy sausage is shown in Table 2. The palmitic acid (26.3%) was the main saturated fatty acid found in the sausage followed by the stearic acid (19.2%) and myristic acid (5.5%). Oleic acid (27.3%) and palmitoleic acid (4.3%) were the most abundant monounsaturated fatty acids (MUFAs) detected in the sausage. The sausage comprised of EPA

(4.6%) and DHA (5.7%) as the main polyunsaturated fatty acids. Total SFAs, MUFAs and PUFAs of the anchovy sausage were 56%, 32% and 12% respectively. Both total SFAs and MUFAs of the sausage were higher than those of anchovy, whereas the total PUFAs was lower compared to anchovy (Turan et al., 2007). These results can be related to the use of tallow in the sausage production. Beef tallow comprises of SFAs (42-55%), MUFAs (39-47%) and PUFAs (1.5-5%).

Table 2. Fatty acid composition of the anchovy sausage

Fatty acid	(g.100 g^{-1})
C14:0 (myristic acid)	5.54±0.09
C14:1 (myristoleic acid)	0.49±0.02
C15:0 (pentadecanoic acid)	0.68±0.03
C16:0 (palmitic acid)	26.30±0.24
C16:1 (palmitoleic acid)	4.25±0.08
C17:0 (heptadecanoic acid)	1.78±0.03
C18:0 (stearic acid)	19.20±0.04
C18:1 (oleic acid)	27.32±0.07
C18:2 (linoleic acid)	0.78±0.02
C20:0 (arachidic acid)	0.37±0.01
C18:3 (γ-linolenic acid)	0.19±0.01
C20:3 (cis-8,11,14-eicosatrienoic acid)	0.27±0.02
C20:5 (cis-5,8,11,14,17-eicosapentadecaenoic acid)	4.59±0.02
C22:1 (erucic acid))	0.32±0.02
C22:6 (cis-4,7,10,13,16,19-docosahexaenoic acid)	5.73±0.05
C23:0 (tricosanoic acid)	1.96±0.03
C24:0 (lignoceric acid)	0.23±0.01
SFA	56.06±0.10
MUFA	32.38±0.05
PUFA	11.56±0.05

*Mean ± standard deviation

The anchovy sausage exhibited a higher PUFAs content (12%) than the red meat sausage (2.6%) (Yıldız-Turp and Serdaroğlu, 2008). This property may provide superiority over red meat sausage. PUFAs are known to show potential health benefits such as lowering cholesterol level, triglyceride level and blood pressure. Therefore, an increase in intake of PUFAs in human diet is recommended. The PUFAs of the anchovy sausage were comprised of mainly EPA, and DHA, which were previously associated with heart, brain and eye health (Gogus and Smith, 2010).

Mineral composition

The mineral composition of the anchovy sausage is shown in Table 3. The anchovy sausage included important minerals such as calcium, sodium, potassium, magnesium, iron, zinc, selenium. The macro minerals were calcium (55 mg.100 g^{-1}), sodium (740 mg.100 g^{-1}), potassium (240 mg.100g^{-1}), and magnesium (31 mg.100g^{-1}) detected in the sausage. Iron (2.1 mg.100g^{-1}), zinc (1.5 mg.100g^{-1}), and selenium (0.02 mg.100 g^{-1}) were the micro minerals found in the sausage. The anchovy sausage showed higher calcium and sodium contents than the Malaysian commercial fish sausages, which can be attributed to the sausage formulations (Huda et al., 2012).

Table 3. Mineral composition of the anchovy sausage

Mineral	**(mg.kg^{-1})**
Calcium	552±7
Sodium	7400±30
Potassium	2400±20
Magnesium	313±5
Iron	21±0.1
Zinc	15±0.1
Selenium	0.18±0.01

*Mean ± standard deviation

Calcium, sodium, potassium and magnesium have structural and functional roles in biological system. Calcium is a primary mineral found in bones and teeth and required for bone and teeth formation. It also regulates nerve and muscle functions. Sodium is the principal cation in extracellular fluids that modulates acid-base balance, osmotic pressure, and cell permeability. Potassium is the principal cation in intracellular fluids that regulates acid-base balance, osmotic pressure, and muscle contradiction. Magnesium has importance in bone health. It is also required for activation of several enzymes. Iron is responsible for transport of oxygen as hemoglobin. It is also a cofactor of enzymes involved in oxidation reactions. Zinc is a cofactor of enzymes taken place in macronutrient metabolism and cell replication. Selenium is an antioxidant mineral (Soetan et al., 2010).

A 100 g of the anchovy sausage meets approximately 15% of the recommended daily intake (RDI) value of iron, 20% of the RDI value of zinc, and 35% of the RDI value of selenium (NIH, 2016). It can be interpreted that the anchovy sausage can help to provide recommended daily intake of essential minerals.

Microbiological properties

The results of microbiological analysis are presented in Table 4. Pathogen microorganisms such as *Salmonella, L. monocytogenes, V. cholera,* and *V. parahaemolyticus* were not detected in the anchovy sausage. Presence of pathogens in fish and fish products leads to food safety problems. It was shown that *Salmonella,* and *L. monocytogenes* could be detected in fish and fish products even when strict hygienic practices were applied (Stollewerk et al., 2014). *V. cholera* and *V. parahaemolyticus* are two important pathogens widely distributed in aquatic environment. They are considered as the main contributor to outbreaks arisen from the consumption of raw and undercooked seafood (Tang et al., 2014).

Table 4. Microbiological results of the anchovy sausage

Total aerobic colonies	1.5 10^4 cfu. g^{-1}
Yeast and mould	<100 cfu. g^{-1}
Total coliform bacteria	< 10 cfu. g^{-1}
Coagulase-positive *Staphylococcus*	<100 cfu. g^{-1}
Salmonella	Not detected
Listeria monocytogenes	Not detected
Vibrio chlorea	Not detected
Vibrio parahaemolyticus	Not detected

When the count of specific spoilage organism exceeds 7 log cfu.g^{-1}, fish spoils very rapidly. The number of total aerobic colonies, yeast and mold, total coliform bacteria, and coagulase-positive *Staphyloccus* were found to be below the values set by Turkish Legislation (TFC, 2011). These findings indicated that the applied heat treatment was effective in controlling microorganisms, and the microbiological quality of the sausage could be considered as acceptable.

Quality characteristics

Quality properties of samples are given in Table 5. TBARS (Thiobarbituric acid reactive substances) value, total volatile basic-nitrogen (TVB-N), and histamine contents were determined to evaluate the quality characteristics of the sausage sample.

Table 5. Quality characteristics of the anchovy sausage

Characteristic	
TBA (mg MA.kg^{-1})	3.98±0.02
TVB-N (mg.100 g^{-1})	14.21±0.56
Histamine (mg.kg^{-1})	67.70±1.25

*Mean ± standard deviation

The TBARS value of the anchovy sausage was 4 mg.kg^{-1}. TBARS value is a criterion showing the degree of rancidity in meat. Lipid oxidation reduces sensorial quality and nutritional value of meat products. Undesirable color, odor, and taste changes are formed during oxidation reactions. Moreover, essential fatty acids and fat-soluble vitamins are degraded. Malonaldehydes, the secondary products of lipid oxidation reaction, are determined by TBARS value (Bozkurt and Erkmen, 2007). The TBARS value of the sausage was found to be lower than the acceptability limit value (8 mg.kg^{-1}) (Erdem et al., 2005).

The TVB-N content of the anchovy sausage was 14.2 mg.100 g^{-1}. TVB-N is used to evaluate the quality changes in seafood. In fish, TVB-N value of 15-20 mg.100 g^{-1} is accepted as good quality (Umesha Bhatta et al., 2015). According to TVB-N value, the quality of the sausage was considered as good.

Histamine level of the sausage was 68 mg.kg^{-1}. Histamine occurs in protein-rich foods. Histidine, an amino acid, is converted to histamine by means of bacterial decarboxylation. Histamine formation leads to toxicological risk. It can cause food poising if an exposure level is over a toxicity level (70-1000 mg). Histamine level in meat products is also related to product quality, especially microbiological quality. Some spoilage microorganisms (*Pseudomonas*, *Staphylococci*, *Micrococci*, and *Enterococci* etc) are involved in decarboxylation reactions. In the Turkish legislation, the maximum level set for histamine was 100 mg.kg^{-1} for fish and 200 mg.kg^{-1} for canned fish, respectively (Codex, 2011). The anchovy sausage had a lower histamine level than the maximum level, indicating that the sausage did not pose toxicological risk.

Quality characteristics of the anchovy sausage showed good quality.

Sensorial properties

Sensory properties of anchovy sausages are given in Table 6. Surface color and appearance, greasiness, juiciness, and aroma of the raw sausage and texture, aroma, and general acceptability of the cooked sausage were evaluated. The raw sausage exhibited values higher than 7, while the cooked sausage showed values higher than 8. These results indicated that sensorial acceptability of the sausage was high.

Table 6. Sensorial properties of the anchvoy sausage

Raw sausage		
	Surface color	7.2±1.2
	Greasiness	7.1±1.1
	Juiceness	7.2±1.6
	Aroma	8.0±1.3
Cooked sausage		
	Texture	8.6±0.9
	Aroma	8.6±0.6
	General acceptability	8.2±0.8

*Mean ± standard deviation

The sensorial scores of the anchovy sausages were higher compared to those reported for other fish sausages. The sensorial scores of Nile tilapia sausage (7.1) and fish sausage made from surimi and silver catfish (4.6-6.8) were found to be lower than 8 (Amiza and Ng, 2015; Dallabona, et al., 2013).

CONCLUSION

The proximate composition, fatty acid composition and mineral composition make the anchovy sausage a valuable food. It had a high energy value. It comprised of EPA and DHA as PUFAs, and iron, zinc, and selenium as essential minerals. Microbiological and chemical characteristics were acceptable for meat products. Sensorial scores of the sausage were high as well. Therefore, it may be proposed as a source of macronutrients and micronutrients for human diet. It can be interpreted that the anchovy sausage may be marketed as a healthier meat product.

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