



Research Article

**The Effects of Goji Berry (*Lycium barbarum* L.) Extract on Some Chemical, Microbiological and Sensory Characteristics of Liquid Smoked Common Carp (*Cyprinus carpio* L., 1758) Sausages**

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**Abstract:** In this study, the effects of goji berry extract on some quality characteristics of common carp (*Cyprinus carpio* L., 1758) sausages during 28 days of storage at 2±1°C were investigated. TBA (Thiobarbituric acid) values demonstrated that goji berry acted as a natural antioxidant and retarded lipid oxidation of common carp sausages during 28 days of storage. The lowest TVB-N (Total Volatile Basic Nitrogen) contents were noticed in S2 group (2% goji berry added common carp sausages) followed by S1 group (1% goji berry added common carp sausages). Total aerobic mesophilic bacteria (TAMB) of goji berry extract added samples were always lower than control samples. Goji berry extract was very effective to inhibit the growth of microorganisms. Addition of goji berry extract caused partially redness in sausage samples depending on concentration. Highest aroma and color score was found in S1 group. On the other hand, depending on increase in storage period, color and aroma scores also decreased significantly.

**Kurt Üzümü (*Lycium barbarum* L.) Ekstraktının Sazan (*Cyprinus carpio* L., 1758) Sosislerinin Bazı Kimyasal, Mikrobiyal ve Duyusal Özellikleri Üzerine Etkileri**

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**Anahtar kelimeler**

Sazan balığı sosisi,  
Kurt üzümü ekstraktı,  
Bazı kalite parametreleri.

**Öz:** Bu çalışmada, kurt üzümü ekstraktının 2±1°C'da 28 gün depolanan sazan (*Cyprinus carpio* L., 1758) sosislerinin bazı kalite özellikleri üzerine etkileri araştırılmıştır. TBA değerleri kurt üzümü ekstraktının doğal antioksidan gibi davrandığını ve 28 günlük depolama süresince sazan sosisinde lipid oksidasyonunu geciktirdiğini göstermiştir. En düşük TVB-N içerikleri S2 (%2 kurt üzümü ekstraktı eklenen sazan sosisleri) ve S1 (%1 kurt üzümü ekstraktı eklenen sazan sosisleri) gruplarında bulundu. Kurt üzümü ekstraktı eklenen sazan sosislerinin toplam aerobik mezofilik bakteri (TAMB) sayıları kontrol örneğine göre daha düşük çıktı. Kurt üzümü ekstraktı mikroorganizma gelişimini engellemede çok etkiliydi. Konsantrasyon artışına bağlı olarak kurt üzümü eklenmesi sosis örneklerinde kısmen kırmızılığa neden oldu. Aroma ve renk değerleri bakımından en iyi puanlama S1 grubunda tespit edildi. Diğer yandan, depolama süresindeki artışa bağlı olarak renk ve aroma değerleri de belirgin olarak azaldı.

## 1. Introduction

The trend in consumer preferences towards healthful, economical and confident foods lead sausage industry to look for new alternatives like fish. Fish added sausage formulations can be a solution to increase the nutritional quality of food and also to include fish in the daily diet of the people (El-Sherif et al., 2016). Due to its long chain n-3 fatty acids content, fish sausages have a beneficial effect on health Churi et al., 2016). Fish sausage is one of the most popular and value added products, prepared from minced fish meat (Raju et al., 2003). Fish sausage is obtained by mixing minced fish meat with salt, spices, seasonings, fat, etc. then stuffed into casings and cooked (Nithin et al., 2015).

Common carp (*Cyprinus carpio*, L., 1758) is the most dominant freshwater fish species in the world due to its abundance, high economic and nutritive values (Zhang et al., 2017). The global aquaculture production for common carp was approximately 4 557 000 tons in 2016 and it is the third highly produced freshwater fish species in the world (FAO, 2018). Taking into consideration the above mentioned, common carp can be used easily to increase the nutritional value of meat products like sausages, hot dog without changing sensorial properties and storage stability (Buchtova and Kaspar, 2016).

Smoking is one of the oldest technologies used to preserve of meat and fish (Hattula et al., 2001). Wood smokes or other fuel smokes contain many chemical contaminants including polycyclic aromatic hydrocarbons (PAHs) (Babic et al., 2018). PAHs are toxic contaminants and many of them have carcinogenic activity (Purcaro et al., 2013). Liquid smoke is ideal and safe for use in marinades, sauces and processed meat like sausage and ham to give a smoked flavor properties (Lingbeck et al., 2014). By the refining process, unwanted PAHs are removed (Varlet et al., 2010). Applicability in most foods, minimization of environmental pollution, different forms of practice (spraying, dipping, blending etc.), adding flexibility to the application techniques, using as a natural preservative in foods, effective control over the flavor and color are among the most important advantages of liquid smoking (Goncalves et al., 1999; Nithin et al., 2015).

Type of raw materials (fish, spices, other ingredients) and heat treatment affect chemical, microbiological and sensory deterioration of fish sausages (Sojic et al., 2017). Addition of natural antioxidants is one of the accepted methods to retard lipid oxidation and to prevent microbiological spoilage in processed meat products.

*Lycium barbarum*, L. belongs to family of Solanaceae and has beneficial effects on health because it owns a rich content. The antioxidant and antimicrobial effect of goji berry or wolfberry (*Lycium barbarum*, L.) comes from its polysaccharide, flavonoid, phenolic acids and carotenoid contents (Karakas et al., 2016). *Lycium barbarum* fruits, also known as Goji berries contains polysaccharide, vitamin C, B complex, E, free amino acids, polyphenols, organic acids, its derivatives and minerals like zinc, iron, copper, calcium, selenium (Jeszka-Skowron et al., 2017). Due to its high antioxidant content, goji berry is known as “super fruit” and has been used as herbal medicine and functional food in Asian countries (Amagase et al., 2011). There have been many studies on the antioxidant activity and phenolic compounds of goji fruits (Wang et al., 2010; Ioniaca et al., 2012; Donno et al., 2015; Jeszka-Skowron et al. 2017; Rukeya et al., 2017; Pires et al., 2018). Jeszka-Skowron et al., 2017 reported that antioxidant activities of goji fruits were higher than all tested dried fruits. Pires et al., 2018 found that goji berry fruits had highest phenolic contents and showed high antibacterial activity against gram-positive bacteria. Due to its bioactive compounds and functional properties, goji fruits can be used in food products (Pires et al., 2018).

There is no information in the literature about the preservative effects of goji berry extract on fish and seafood products and this is the first research to indicate the application of goji berry extract as a natural antioxidant in fish sausage. Therefore, the aim of this research is to determine the effects of goji berry extract on lipid stability, sensory attributes and microbiological quality of liquid smoked Common carp sausage.

## 2. Material and Methods

### 2.1. Materials

Goji berry fruit extract was purchased from Xi'an Xin Sheng Bio-Chem Company. Liquid smoke was acquired from A&D Chemical Company (Turkey) and it has Hallal and GRAS (by FDA) certificates. Cellulose casings were purchased from the Wienie-Pak (21mm×15m, Lommel, Belgium). Other sausage ingredients were obtained from local supermarket.

Fresh *Cyprinus carpio* obtained from Izmir (in Turkey) fish market were transported to the laboratory in styrofoam boxes containing ice and they were gutted, skinned and filleted. 16 kg of fish were used for sausage production.

### 2.2. Preparation of sausage

The sausage formula (Table 1) has been developed with minor modifications on formula of Dincer and Cakli, 2010. The batter was randomly assigned into three treatment lots: Lot 1) Control (C): without addition of goji berry extract, Lot 2) S1: 1% (w/v) goji berry extract added, Lot 3) S2: 2% (w/v) goji berry extract added.

For each treatment, carp fillets and beef fat were minced using meat mincer (Remta 12 No) (3 mm pore size) then salt, sugar, soy protein concentrate, sodium polyphosphate, sodium nitrite, modified potato starch, ice water, liquid smoke added to the minced meat and blended for 3 min. Afterwards, spices were added and mixed for 3 min. Goji berry extract was added to S1 and S2 batter and blended for 3 min. The temperature of blended common carp meat was kept below 10°C during preparation of mixture. Sausage emulsion was stuffed into cellulose casings (21-mm diameter×15-m long) by using a manual filler. The stuffed casings were linked in 10 cm length, and cooked at 80°C for 15 min in water bath until the internal temperature reached 75°C. The cooked sausages were immediately cooled in ice water. Sausages samples were put in plastic bags (low-density polyethylene (LDPE)) and stored at 2±1°C for 28 days. Lipid oxidation, sensory and microbiological analyzes were performed on 28 days of storage. Each treatment of packed sausage was randomly taken for analyses at day 0, 7, 14, 21 and 28. All analysis were performed with two samples from each batch in duplicate.

Table 1. Ingredients and additives (%) used in common carp sausage formulations

Ingredients	(C) Control	S1 (1% goji berry extract added)	S2 (2% goji berry extract added)
Minced Fish	68.2	68.2	68.2
Beef Fat	5	5	5
Sunflower oil	5	5	5
Soy protein concentrate	1.80	1.80	1.80
Salt	1.50	1.50	1.50
Sugar	0.30	0.30	0.30
Ascorbic acid	0.02	0.02	0.02
Hot paprika powder	0.15	0.15	0.15
Coriander	0.15	0.15	0.15
Caraway	0.15	0.15	0.15
Sodium nitrite	0.02	0.02	0.02
Modified potato starch	1.90	1.90	1.90
Sodium polyphosphate	0.20	0.20	0.20
Liquid smoke	0.50	0.50	0.50
Goji berry extract	-	1	2
Ice/water	15.2	14.2	13.2

### 2.3. Chemical analysis

TBA (Thiobarbituric acid) value used in the determination of lipid oxidation were analyzed according to method of Tarladgis et al., 1960 and expressed as mg of malondialdehyde (MDA)/kg of samples.

The amount of total volatile basic nitrogen (TVB-N) value was determined according to Varlik et al., 1993. The amount of TVB-N of samples were calculated as mg N/100 g common carp sausage.

## 2.4. Microbiological analysis

25 g of sample was taken aseptically from each group and homogenized with 225 mL of sterilized peptone water (BPW, Oxoid Ltd., Basingstoke, Hampshire, UK) for 2 min in stomacher. Other decimal dilutions (from  $10^{-1}$  to  $10^{-9}$ ) were prepared this dilution and plated in the appropriate medium. Plate Count Agar (Merck, incubation at 30°C for 3 days) was used for determination of total aerobic mesophilic bacteria (TAMB) (ICMSF, 1986).

Mold and yeast were determined using Yeast Extract Glucose Chloramphenicol Agar. Plates were incubated at 22°C for 5 days (Harrigan and McCance, 1976).

*Staphylococcus aureus* were determined using Baird–Parker Agar (Oxoid, incubation at 37°C for 1 day then catalase test was carried out). Coliform groups were determined using Violet Red Bile Glucose Agar (Oxoid, incubation at 37°C for 24 h) (Halkman, 2005). All microbiological results were expressed as log CFU/gram.

## 2.5. Sensory analysis

Sensory evaluation of the control and goji berry extract added sausages (1% and 2%) was performed by 6 untrained panelists in different ages. The cellulose casings of sausages were removed, were sliced into 4 mm thick pieces, were placed on a white plastic dishes and served at room temperature. Three pieces of each sample were served for evaluation of color and aroma attributes (ISO, 1993; ISO, 2008).

Assessment was made using five-point scale. On this scale: 5- very good; 4–good; 3–normal; 2–bad; 1–product unacceptable ((Kurtcan, 1987).

## 2.6. Statistical analysis

Statistical analysis of data was conducted using SAS software (SAS Institute Inc., Cary, NC, 1999). Analysis of variance (ANOVA) was performed and Duncan test was used to evaluate the significance of differences between mean values.

## 3. Results and Discussion

### 3.1. Thiobarbituric acid (TBA) content

Table 2. Thiobarbituric acid (TBA) value (mg MDA/kg) of common carp sausage treated with goji berry extract

Storage Time (days)	Treatments		
	Control	S1	S2
0	0.49 <sup>aA</sup> ±0.34	0.51 <sup>aA</sup> ±0.03	0.49 <sup>aA</sup> ±0.03
7	0.84 <sup>aB</sup> ±0.04	0.81 <sup>aB</sup> ±0.07	0.56 <sup>bB</sup> ±0.08
14	1.39 <sup>aC</sup> ±0.16	1.27 <sup>aC</sup> ±0.42	0.85 <sup>bC</sup> ±0.08
21	2.21 <sup>aD</sup> ±0.10	1.85 <sup>bD</sup> ±0.06	1.40 <sup>cD</sup> ±0.10
28	3.48 <sup>aE</sup> ±0.09	2.47 <sup>bE</sup> ±0.12	1.79 <sup>cE</sup> ±0.04

a-c: Means within each row with different letters are significantly different ( $p < 0.05$ )

A-E: Means within each column with different letters are significantly different ( $p < 0.05$ )

Sample denomination: C: control; S1: (1% goji berry extract added); S2: (2% goji berry extract added)

TBA value of common carp was found 0.42 mg MDA/kg. Table 2 shows the effect of goji berry extract addition on lipid oxidation of common carp sausages during storage period. The initial TBA values of control, S1 and S2 group were determined as 0.49, 0.51 and 0.49 mg MDA/kg of sample, respectively. At the beginning of the cold storage, there were no significant differences between goji berries extract added common carp sausages and control samples. TBA values of all groups increased during storage period ( $p < 0.05$ ). S2 group showed lowest TBA value than control and S1 group on each

of the sampling days ( $p < 0.05$ ). As the concentration level of extract increases, the TBA value decreased. During 14 days of storage, there was no significant difference between control and S1 group. By the 21 days of storage, significant differences were observed among all groups ( $p < 0.05$ ). At the last stage of storage, TBA values of control, S1 and S2 groups were determined as 3.48, 2.47 and 1.79 mg MDA/kg of sample, respectively. Addition of goji berry extract retarded lipid oxidation in common carp sausages during 28 days of storage. TBA values demonstrated that goji berry acted as a natural antioxidant in common carp sausage. Its antioxidant activity and its protective effect come from large content of polysaccharide, flavonoid, phenolic acid and carotenoid of goji berry. Flavonoids may act as hydrogen donors, radical scavengers and metal chelators (Pedro et al., 2018). Our results are in agreement with that of Kulkarni et al., 2011, Sojic et al., 2017 and Coban et al., 2018. Kulkarni et al., 2011 found that addition of grape seed extract reduced lipid oxidation in sausage samples. Sojic et al., 2017 reported that *Juniperus communis* L. essential oil added samples showed lower TBA values compared with control samples. El-Zainy et al., 2016 found that grape seed polyphenols extract added sausage samples showed lowest TBA values. Coban et al., (2018) found that lowest TBA values were measured in 1% propolis extract added smoked common carp (*Cyprinus carpio*) sausages during 9 weeks of storage.

### 3.2. Total volatile basic nitrogen (TVB-N) content

Table 3. TVB-N content (mg/100g) of common carp sausages treated with goji berry extract

Storage Time (days)	Treatments		
	Control	S1	S2
0	10.18 <sup>aA</sup> ±0.16	10.11 <sup>aA</sup> ±0.08	10.18 <sup>aA</sup> ±0.21
7	14.72 <sup>aB</sup> ±0.42	14.19 <sup>aB</sup> ±0.05	11.24 <sup>aA</sup> ±0.17
14	18.60 <sup>aC</sup> ±0.42	17.03 <sup>bC</sup> ±0.07	14.80 <sup>cB</sup> ±0.11
21	24.35 <sup>aD</sup> ±0.27	20.90 <sup>bD</sup> ±0.28	18.19 <sup>cC</sup> ±0.14
28	31.06 <sup>aE</sup> ±0.79	25.23 <sup>bE</sup> ±0.16	21.21 <sup>cD</sup> ±0.32

a–c: Means within each row with different letters are significantly different ( $p < 0.05$ )

A-E: Means within each column with different letters are significantly different ( $p < 0.05$ )

Sample denomination: C: control; S1: (1% goji berry extract added); S2: (2% goji berry extract added)

TVB-N content of carp sausages during 28 days of storage are shown in Table 3. At day 14, there was a significant difference among the samples ( $p < 0.05$ ). 2% goji berry added common carp sausages increased more slowly than control and 1% goji berry added common carp sausages through the storage period.

As shown in Table 3, TVB-N production increased in all groups throughout the storage period at  $+2^{\circ}\text{C} \pm 1$ . The increase in TVB-N values of all groups may be the result of removal of free amino acids and breakdown of nucleotides by autolytic enzymes and microbial activity (Ocano-Higuera et al., 2012).

The lowest TVB-N content were noticed in S2 group during 28 days of storage ( $p < 0.05$ ). At day 28, the highest value of TVB-N was recorded in control group that reached to 31.06 mg TVB-N/ 100g sample and then spoiled. Goji berry extract added sausage samples for both concentrations did not exceed the acceptable limit value of 30 mg muscle TVB-N/100 g Gokoglu et al., 1998 at day 28. Similarly, Coban et al., 2019 reported propolis extract added smoked carp sausages had lowest TVB-N values during 9 weeks of storage at  $2^{\circ}\text{C}$  and TVB-N values of all groups did not exceed the acceptable limit value.

### 3.3. Microbiological analysis

Table 4. Effect of goji berry extract addition on TAMB (log cfu/g) of smoked common carp sausages stored at +2°C±1

Storage Time (days)	Treatments		
	Control	S1	S2
0	2.55 <sup>aA</sup> ±0.17	2.26 <sup>aA</sup> ±0.09	2.26 <sup>aA</sup> ±0.09
7	3.44 <sup>aB</sup> ±0.07	3.44 <sup>aB</sup> ±0.10	2.89 <sup>bB</sup> ±0.11
14	4.81 <sup>aC</sup> ±0.25	4.03 <sup>bC</sup> ±0.16	3.26 <sup>cC</sup> ±0.09
21	5.78 <sup>aD</sup> ±0.14	5.67 <sup>aD</sup> ±0.14	3.63 <sup>bCD</sup> ±0.21
28	6.97 <sup>aE</sup> ±0.11	6.07 <sup>bE</sup> ±0.15	4.29 <sup>cD</sup> ±0.13

a-c: Means within each row with different letters are significantly different (p<0.05)

A-E: Means within each column with different letters are significantly different (p<0.05)

Sample denomination: C: control; S1: (1% goji berry extract added); S2: (2% goji berry extract added)

Total aerobic mesophilic bacteria (TAMB) of different common carp sausages during 28 days of storage at +2°C±1 are shown in Table 4. The TAMB of control and goji berry extract added common carp sausages increased significantly (p<0.05) during 28 days of storage at +2°C±1. The lowest increase was shown in 2% goji berry extract added samples.

The initial TAMB of control group was 2.55 log cfu/g common carp sausage and increased to 6.97 log cfu/g common carp sausage at the end of the storage period. TAMB of S1 and S2 group were 2.26 log cfu/g common carp sausage at the beginning of storage and increased to 6.07 and 4.29 cfu/g common carp sausage, respectively. TAMB of goji berry extract added common carp sausages were lower than control samples during storage period. Goji berry extract was very effective in inhibiting the growth of bacteria. Our results are similar with Coban et al., 2019. They found that propolis extract added carp sausages showed significantly lower total aerobic mesophilic bacteria (TAMB) count when compared to control samples during 9 weeks of storage.

Yeast and mold counts, coliform groups were not detected in all samples. In this work, we found *Staphylococcus aureus* count smaller than 1 log cfu/g. The value of *Staphylococcus aureus* was within the limit of 3 log cfu/g recommended by Purba et al., 2014 in good manufacturing practices.

### 3.4. Sensory analysis

Sensory analysis is a method of assessing the quality of a product by using the sensitivity of the human senses (Purba et al., 2014). The results of sensory (color and aroma) evaluation of goji berry extract added common carp sausages are seen in Figure 1.

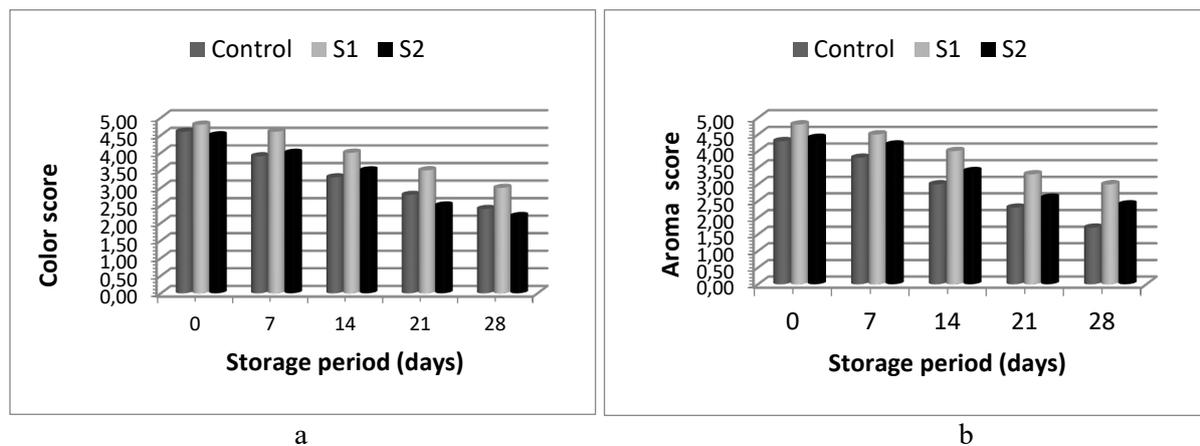


Figure 1. Changes in color (a) and aroma (b) scores of goji berry extract added common carp sausages Control: without extract, S1: 1% goji berry extract added, S2: 2% goji berry extract added.

Color and aroma scores of all groups significantly decreased during 28 days of storage (p<0.05). Significant differences were found in goji berry extract added fish sausages (p<0.05). Highest color

score was found in S1 group when compared to control and S2 groups ( $p < 0.05$ ). In fact, the acceptance of fish products could be related to cultural factors so S2 group can get the best acceptance in different countries and even in different regions of the country (Oliveira Filho et al., (2010).

Addition of goji berry extract caused partially redness in common carp sausage samples depending on concentration. Sojic et al., 2017 reported that batches produced with the *Juniperus communis*, L. essential oil addition were darker and redder compared to control. Maqsood et al., 2012 found that addition of kiam wood extract to the fish emulsion sausages caused an increase in redness during 20 days of refrigerated storage. As shown in Figure 1 (a, b), S1 showed the highest aroma scores at days 7, 14, 21 and 28. Decreasing of sensory scores with an increasing of storage time were also reported by Oliveira Filho et al., 2010; Choi et al., 2016 and Coban et al., 2018. Coban et al., 2018 found that aroma scores of sausages supplemented with propolis extract were decreased during storage period. At 28<sup>th</sup> day, the control group was unsuitable for human consumption due to its soft texture and its bad aroma. This was also demonstrated by the chemical and microbiological analysis measurements during storage time.

#### 4. Conclusion

According to our results, addition of goji berry extract to liquid smoked fish sausages retarded lipid oxidation as indicated by lower TBA values and lower TVB-N values. Goji berry extract prolonged the shelf life of liquid smoked fish sausages for 7 days and it can be used as a natural antioxidant and antibacterial agent to inhibit the quality loss of common carp sausages.

Goji berry extract could be added to common carp sausage but to keep good overall sensory acceptance, the suitable rate of goji berry extract in sausages should be 1%.

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