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ANTIOXIDANT EFFECT OF FISH COLLAGEN HYDROLYSATE ADDITION TO MEATBALLS

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Abstract

In this study commercial fish collagen hydrolysate was used as an antioxidative ingredient for preparing meatball. Meatballs were prepared by adding the hydrolysates with tree different ratios (P1:1%, P2: 2%, P3: 3% (w/w) of meat), butylated hydroxytoluene (BHT) added group and control group (C). Meatballs have been stored at 4 °C during storage time (8 days). Color, thiobarbituric acid (TBA) values, peroxide values (POV), were done in 0., 2., 4., 6., 8., days and also sensorial analysis were done at the beginning of the storage. P1 group has the significantly ($p<0.05$) lowest TBA values. There was no significant difference found between the other peptide groups and BHT added group. POV of the BHT, P1, P2 groups also significantly ($p<0.05$) low from control and P3 groups. There was no significant difference were found the sensorial scores of meatballs between the control and treatment groups.

Keywords: Antioxidant, Collagen hydrolysate, Meatball, POV, TBA

KÖFTELERE BALIK KOLLAJEN HİDROLİZATI İLAVESİNİN ANTİOKSİDAN ETKİSİ

Özet

Bu çalışmada köfte hazırlanırken antioksidan etkili ingredient olarak ticari balık kollajen hidrolizatları kullanılmıştır. Köfteler farklı oranlarında hidrolizat ilavesi (etin %1'i (P1), % 2'si (P2), %3'ü (P3) oranında) bütillendirilmiş hidroksitoluen (BHT) ilaveli grup ve kontrol grubu (C) olarak hazırlanmıştır. Köfteler depolama süresi (8 gün) boyunca 4 °C'de muhafaza edilmişlerdir. Renk, tiyobarbütirik asit (TBA) değeri, peroksit (POV) değeri analizleri 0., 2., 4., 6., 8. günlerde yapılmıştır ve aynı zamanda duyusal analizler depolamanın başlangıcında gerçekleştirilmiştir. P1 grubunun istatistiki olarak önemli ölçüde ($p<0.05$) en düşük TBA değerine sahip olduğu belirlenmiştir. Diğer peptit ileveli gruplar ile BHT ilaveli grubun arasında farkın önemli olmadığı belirlenmiştir. BHT, P1 ve P2 gruplarının peroksit değerlerinin kontrol ve P3 grubundan istatistiki olarak önemli ($p<0.05$) ölçüde düşük olduğu belirlenmiştir. Kontrol grubu ile muamele gruplarının duyusal özellikleri arasında duyusal değerler arasında önemli bir farklılık olmadığı tespit edilmiştir.

Anahtar Kelimeler: Antioksidant, Kollajen Hidrolizatı, Köfte, POV, TBA

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1. Introduction

Fish skin collagen hydrolyzates show different bioactive properties so it can give functionality to food [1]. Due to the hesitation between consumers about bovine spongiform encephalopathy (BSE) the use of bovine and porcine collagen was restricted [2,3]. So researchers focus on alternative sources of collagen which are marine sources [3]. During the processing and filleting of fish, the skin constitutes a fairly large potential source of collagen [4]. Enzymatic hydrolysis of the proteins is a way of bioactive peptide production from marine products [5]. These peptides obtained as a result of hydrolysis have many bioactive properties as well as antioxidant activities. Some researches about the usage of different protein hydrolyzates from marine products focused on their potential antioxidant activities in food systems [6].

Some synthetic antioxidants like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) have long been used in the food industry for retarding or inhibiting the lipid oxidation [7]. The consumer wants to purchase products produced by natural additives and avoid from foods which have produced with synthetic additives [8].

The aim of the research was to determine the effect of collagen (obtained from fish) hydrolyzate addition on some quality characteristics of meatballs.

2. Materials and Methods

The beef and spices were bought from the local market in Afyonkarahisar. The fish collagen hydrolyzate was supplied from Rousseoult Angouléme S.A.S. (France). The analytical grade of chemicals and standards were used from Sigma or Merck (Darmstadt, Germany).

2.1. Meatball Preparation

Minced meats (PKM22/Arıcımakina) which purchased from the local market were quickly brought to the Nutrition and Dietetic laboratory in 10 minutes in thermoboxes and stored at refrigerator until meatball preparation. Mixtures of control, BHT added (200 ppm) group and three collagen hydrolyzate added group was prepared according to Table 1. And each group kneaded by hand for 30 minutes to obtain well-mixed dough. Meatballs prepared with a weight of 25 g and rounded. Each treatment group was sealed in plastic bags and stored at 4°C for 8 days. Analysis was done each two days (0, 2, 4, 6, 8). The experiment was designed with two replications.

Table 1. Ingredients of meatballs

Ingredients (g)	Control	BHT	P1	P2	P3
Meat	1000	1000	1000	1000	1000
Red pepper	7	7	7	7	7
Cumin	7	7	7	7	7
Black pepper	7	7	7	7	7
Onion	15	15	15	15	15
Salt	20	20	20	20	20
Collagen hydrolysate	-	-	10	20	30
BHT(ppm)	-	200	-	-	-

2.2. Analysis

Moisture and fat content were determined according to [9]. Mettler-Toledo model pH meter was used for pH readings. Water activity (aw) analyse was done by the method of [10]. The water activity (aw) values were determined by an aw-meter LabTouch-aw, (Novasina, Switzerland).

CIE color values (L*, a*, b*) of the meatballs were determined by X-Rite (Ci6X) colorimeter. The color parameters were measured at three different points of the samples.

Thiobarbituric acid (TBA) was calculated with the method of Tarladgis et al. [11]. TBA results given as malondialdehyde(MDA) per kg of sample.

POV was determination done by the description of Sallam et al., [12]. POV results given as milliequivalent peroxide per kg of sample.

The cooking yield and fat retention were determined by Murphy et al., [13] method.

Moisture retention and shrinkage were determined by El-Magoli et al., [14] method.

Sensory evaluation was conducted according to IFT [15] at the beginning of the storage. Meatball samples were grilled at 80 °C internal temperature and served in randomly. The serving temperature was approximately 60 °C. Water and bread were served between samples. Consumer panelists were 12 volunteers from the Nutrition and Dietetics department and nine-point hedonic scale was used.

Statistical Analysis

The obtained data were presented as mean±SD (means and standard deviation) and subjected to analysis of variance one-way ANOVA for proximate analyses, cooking and sensorial parameters and two way-ANOVA for color, TBA and POV values. Means were compared with Tukey test.

3. Results and Discussion

Table 2 showed the highest moisture content found in the control group and BHT added group, the lowest group is the P3 samples significantly different (P< 0.05) from the control group. Palamutoğlu and Sariçoban [16] suggested that the effect of collagen hydrolyzate addition to the sucuk samples lowers the moisture content.

Table 2. Proximate analysis results of meatballs

	Moisture (%)	Fat (%)	a_w	pH
C	57.83±0.57^{a}	15.25±0.01^{a}	0.94±0.00^{a}	5.81±0.03
BHT	57.60±0.31^{a}	12.10±0.01^{c}	0.93±0.00^{b}	5.76±0.06
P1	52.94±0.62^{ab}	14.43±0.01^{b}	0.93±0.00^{ab}	5.80±0.01
P2	53.40±1.98^{ab}	11.25±0.14^{e}	0.93±0.00^{ab}	5.83±0.02
P3	51.82±2.23^{b}	11.55±0.01^{d}	0.93±0.00^{ab}	5.83±0.04

$^{a–b}$Means in the same column with different superscripts are significantly different ($P < 0.05$).

There was no significant (P >0.05) difference found between water activity of control and hydrolyzate added groups and also there was no significant difference found between pH values of all samples. Ibrahim [17] showed that the addition of fish protein isolates to the beef meatballs significantly effects the protein, fat contents, and pH. Fish protein isolate addition lowers the fat content, increases the protein content and, pH also increases in the isolate added groups. In this research, there was no increase seen in the pH values of the meatball samples. Addition of other spices may decrease the pH values of the samples so difference not found.

Table 3 shows the color parameters of the meatball groups. According to Cheah and Abu Hasim [18] samples with galangal extract showed increasing a* values with increasing concentration. Addition of BHT or alfa-tocopherol didn't affect the L and b values. Tironi et al., [19] said that the red color decreased in both untreated and rosemary extract-treated samples. These results showed that the rosemary extract can preserve the red

Table 3. The color parameters of meatball surfaces

	Day	Control	BHT	P1	P2	P3
L*	0	45.15±3.19^{b}	44.57±0.03^{bD}	48.47±0.12^{a}	43.88±2.07^{bB}	46.44±1.81^{abC}
	2	46.98±3.48	46.27±1.49^{CD}	49.28±2.48	47.98±1.77^{A}	49.44±1.63^{AB}
	4	45.27±0.77^{c}	47.67±0.93^{abC}	46.73±0.75^{b}	48.10±1.18^{aA}	48.63±0.48^{aB}
	6	47.06±2.40	49.83±1.35^{B}	48.73±1.20	48.17±2.15^{A}	49.87±0.77^{AB}
	8	48.83±0.55^{cd}	51.89±1.89^{aA}	49.42±0.95^{bc}	47.62±1.15^{dA}	51.00±0.12^{abA}
a*	0	17.45±1.35^{aA}	17.32±0.23^{aA}	16.70±0.13^{aA}	15.42±0.50^{bA}	16.55±0.23^{aA}
	2	15.03±1.13^{B}	14.69±0.76^{B}	15.26±1.28^{B}	15.36±1.34^{A}	15.71±0.02^{B}
	4	12.73±1.12^{bC}	12.80±0.49^{bC}	13.12±0.35^{bC}	13.33±1.93^{bB}	16.52±0.48^{aA}
	6	12.66±0.46^{aC}	12.31±0.35^{aC}	10.03±0.01^{bD}	12.10±0.47^{aBC}	12.57±0.24^{aC}
	8	14.03±0.35^{aBC}	12.48±0.59^{bC}	10.27±0.44^{cD}	10.71±0.34^{cC}	11.96±0.28^{bD}
b*	0	19.78±0.41^{aAB}	19.69±0.88^{a}	20.45±0.27^{aA}	18.36±1.42^{b}	20.74±0.56^{a}
	2	19.44±1.36^{abABC}	19.32±0.87^{ab}	18.39±0.59^{bB}	18.54±0.41^{b}	20.50±1.15^{a}
	4	18.39±1.08^{cC}	19.62±0.52^{bc}	20.40±0.12^{abA}	19.56±1.40^{bc}	21.20±0.06^{a}
	6	18.96±0.32^{abBC}	19.17±1.21^{ab}	17.90±0.55^{bB}	17.79±0.94^{b}	20.26±0.98^{a}
	8	20.41±0.39^{bcA}	20.74±1.30^{ab}	19.92±0.66^{bcA}	19.36±0.69^{c}	21.78±0.02^{a}

$^{a-b}$Means in the same row with different superscripts are significantly different (P <0.05)
$^{A-D}$Means in the same column with different superscripts are significantly different (P <0.05)

color but rosemary extract shows only a partial preservation of red color, because there are other causes for color modification in addition to the oxidative processes. Conversion of oxy- and deoxyhemoglobin to met-hemoglobin causes a decrease at a* value, myofibrillar protein denaturation produces a change by interaction with hemoglobin, and surface dehydration.

The L* value of the control group did not show a statistically significant change depending on the days. The change in the L*values of P2, P3 and BHTt groups during storage was found to be statistically significant ($p<0.05$). BHT and P3 groups were found to have the highest L* value at the end of the storage (51.89±1.89, 51.00±0.12 respectively). The a* value decreased in all tretment groups with respect to time and the difference between the averages was found to be statistically significant ($p<0.05$). The lowest a values were determined in the P1 and P2 groups on the 8th day (10.27±0.44, 10.71±0.34 respectively). The difference between the b* values of the control and P1 groups was found to be not significant ($p>0.05$) depending on the day, but the difference between the mean values of BHT, P2 and P3 groups was significant ($p<0.05$).

Table 4 shows the TBA POV values of meatballs. Many spices contain phenolic substances which exhibit antioxidant activities. In this research, meatball formulas have some spices and they showed antioxidative activity during storage of the samples. TBA values of all groups showed some decrease at the beginning of storage. The reason for this decrease is due to the compounds in the spices added to the meatballs. When the addition of protein hydrolyzates to the meatballs, P1 group has the lowest TBA values but there was no significant difference found between other groups except P3 group.

Flaczyk et al., [20] found that the cracklings hydrolysate addition to meatballs limited the formation of lipid oxidation products and TBA reactive substances in samples during storage.

Sakanaka and Tachibana [21] determined the effect of egg yolk protein hydrolyzates in beef TBA reactive substances was dose-dependent. Peña-Ramos and Xiong [22] also showed the lipid oxidation can be retarded by 1% to 2% hydrolysate addition.

According to Ulu [23], soy protein isolate added meatballs had the lowest TBA values under all storage conditions. Kılıç et al. [24] also reported textured soy protein isolate addition was effective in reducing lipid oxidation in cooked meatballs during storage.

In research, whey protein isolate edible film treated group of meatballs had significantly lower TBA values compared to the control group during storage. Whey protein isolate based edible films lowers the TBA values during the storage [25].

When the POV values were examined, it was found that the differences between the averages of all application groups were statistically significant due to storage ($p<0.05$). At the end of storage, the difference between the mean POV values of control, P1 and P3 groups was not significant, but BHT and P2 groups were found to have a statistically lower value than the other groups.

POV was the lowest at the beginning of the storage and significantly increases during the time.

Table 5. Cooking parameters of meatballs

	Cooking Yield*	Fat Retention**	Moisture Retention**	Shrinkage
C	78.03±0.37^{a}	40.76±0.19^{d}	45.12±0.21^{a}	10.32±0.57
BHT	77.03±3.15^{ab}	63.16±2.58^{c}	44.35±1.81^{a}	7.75±0.22
P1	73.22±2.28^{b}	43.28±1.20^{d}	38.76±1.21^{b}	8.43±2.62

P2	75.35±0.07^{ab}	81.98±0.08^{a}	40.24±0.04^{b}	7.21±5.06
P3	76.44±0.030^{ab}	75.77±0.30^{b}	39.60±0.16^{b}	4.85±0.95

$^{a-b}$Means in the same column with different superscripts are significantly different (* p<0.05, **P<0.01)

Stadtman [26], concluded that the amino acids which they have active side chains (sulfhydryl, thioether, amino group, imidazole, and indole ring) are sensitive to the oxidation due to the oxidising lipids. Especially cysteine, methionine, lysine, arginine, histidine and tryptophan residues and also valine serine and proline are common targets of reactive oxygen species [27].

Table 4. TBA and POV of meatballs

	Day	Control	BHT	P1	P2	P3
TBA (mg MDA/ kg meat)	0	0.66±0.02^{aA}	0.48±0.01^{bcA}	0.37±0.02^{dA}	0.53±0.04^{bA}	0.41±0.07^{cdB}
	2	0.54±0.31^{AB}	0.30±0.01^{C}	0.25±0.00^{C}	0.29±0.04^{D}	0.20±0.09^{C}
	4	0.24±0.00^{dC}	0.33±0.06^{bcC}	0.39±0.03^{abA}	0.42±0.00^{aB}	0.30±0.01^{cdBC}
	6	0.39±0.00^{aBC}	0.24±0.04^{cD}	0.25±0.02^{bcC}	0.35±0.02^{abC}	0.34±0.07^{abcBC}
	8	0.37±0.02^{bBC}	0.42±0.01^{bB}	0.35±0.00^{bB}	0.40±0.03^{bB}	0.78±0.24^{aA}
POV (meq O_2/ kg meat)	0	4.90±1.70^{B}	5.13±1.02^{B}	3.22±0.06^{C}	5.16±2.06^{B}	5.12±0.36^{D}
	2	8.26±1.33^{aA}	4.63±0.50^{bB}	4.65±1.78^{bC}	8.06±1.18^{aA}	8.07±0.06^{aC}
	4	8.63±0.36^{aA}	5.75±1.82^{bAB}	7.54±0.50^{aB}	8.58±0.65^{aA}	7.66±1.72^{aC}
	6	8.56±0.47^{abA}	7.18±0.80^{cA}	8.14±1.23^{bcB}	7.88±1.01^{bcA}	9.53±0.68^{aB}
	8	9.60±0.96^{aA}	5.00±1.26^{bB}	10.10±0.42^{aA}	2.89±0.06^{cC}	11.41±0.46^{aA}

$^{a-b}$ Means in the same row with different superscripts are significantly different (P <0.05)
$^{A-D}$ Means in the same column with different superscripts are significantly different (P <0.05)

Table 6. Sensorial parameters of meatballs

	Appearance	Color	Flavor	Texture	Odor	Overall Acceptability
C	8.22±0.67	8.22±0,66	7.44±1.42	6.67±1.22	7.11±1.05	7.22±0.83
BHT	8.00±1.00	7.89±1.05	7.44±1.81	7.56±1.13	7.22±1.20	7.22±1.78
P1	7.67±0.70	7.56±1.01	6.33±1.73	6.33±1.22	6.67±1.87	6.33±1.12
P2	7.78±0.83	7.78±0.44	7.44±1.50	6.89±1.36	7.89±0.92	7.44±1.01
P3	7.78±0,97	7.67±0.70	7.22±1.48	7.11±1.27	7.00±0.70	7.44±1.13

Cooking yield of the P1 samples significantly differs from other groups and also haven't got any effect on the fat retention. P2 group has the significantly highest (81.98±0.08) fat retention values between the samples. Except for the control and BHT groups other groups have significantly lower moisture retention values. Table 6 shows that there was no significant difference found between the shrinkage of the samples.

Kılıç et al. [24] indicates there is a correlation between pH and cooking loss. Due to this phenomenon ph of raw meat significantly and positively correlated with moisture retention.
Ulu, [23] showed that the yield of meatball samples were not significantly different with wheat flour and whey protein concentrate or soy protein isolate added samples but all of the cooking yields were lower than the control group.

Ibrahim [17] showed that the fish protein isolates addition to meatballs effects the general acceptability, odor, texture, and flavor were significantly different. Fish protein isolate lowers the odor and general acceptability scores.

El-Magoli et al. [14] showed that whey protein concentrate addition is an effective means for retaining the added water and fat retention was significantly improved at %3 and %4 addition to low-fat meatballs. They also showed that the lower percentage of whey concentrate addition adversely affected the fat retention. Researchers concluded that the whey protein addition to the beef patties results in the emulsification of some of the fat.
The commercial protein hydrolyzate powder has a fishy odor so it can't be acceptable to consumers. But in this research, the odor of the hydrolyzate was masked with the addition of some spices and onion to the meatball recipe. So Table 6 indicate that there was no significance (P >0.05) difference found between the control and the treatment groups.

4. Conclusions

Addition of commercial fish collagen hydrolysates to the meatballs doesn't change the sensorial charactersitics and improves the inhibition of lipid oxidation. Consumption of the fish collagen hydrolysate is limited because of the fishy odor but in this research usage of hydrolysates with other spices and onion in the meatball

formula doesn't affect the sensorial parameters because spices have been masked the fishy odour. Peptide addition lowers the fat content of the samples and can be used for new products. Utilization of fish byproducts can be increase in the food industry because of their bioactive effects of peptides such as antioxidant activity.

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