British Journal of Applied Science & Technology
4(19): 2684-2698, 2014

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Quality Evaluation of Gilthead Sea Bream (*Sparus aurata*) Patties Formulated with Corn Flour

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Authors' contributions

Author MM designed the study, performed the laboratory tests, the statistical analysis, wrote the protocol and the manuscript and managed literature searches. Author XD participated in certain laboratory tests. The authors read and approved the final manuscript.

Original Research Article

Received 1st April 2014
Accepted 28th April 2014
Published 15th May 2014

ABSTRACT

Aims: To evaluate the effect of different concentrations of corn flour on the quality of gilthead sea bream patties, and their refrigerated storage stability under aerobic conditions.
Study Design: Completely randomised block design.
Place and Duration of Study: Department of Fisheries and Aquaculture Technology, Technological Educational Institute of Western Greece, Messolonghi, Greece, between April 2012 and July 2013.
Methodology: Gilthead sea bream (*Sparus aurata*) patties were extended with corn flour at levels 2.5%, 5%, 7.5% and 10% of the weight of fish in the patties and their quality was evaluated with tests related to chemical composition, cooking properties, instrumental color and texture and sensory assessments. The stability of patties formulated with 5% corn flour was evaluated daily and up to seven days of storage at $4^{0}C$ using microbiological (total viable count), chemical (total volatile basic nitrogen (TVB) and thiobarbituric reactive substances (TBARS) and instrumental texture (gel strength) measurements.
Results: The proximate composition and color of patties were significantly (*P=0.05*) affected by the addition of corn flour. Cooking yield increased with increasing levels of corn flour in patties. Using corn flour increased hardness, gumminess and chewiness of

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the cooked products. The highest gel strength value was obtained at a corn flour concentration of 5% (*P=0.05*). Sensory assessment showed that patties with corn flour up to 5% were the most acceptable. Corn flour at 5% substitution was considered optimum as an extender of gilthead sea bream patties. The total viable counts of patties increased throughout storage and exceeded the critical limit of 6.0 log cfu/g on the sixth day of storage. However, TVB and TBARS levels of patties remained under the limit for rejection until the end of the storage period.

Conclusion: Corn flour at 5% substitution was recommended as an extender in production of patties made from gilthead bream minced muscle. Based on microbiological evaluation, the shelf life of patties with 5% corn flour was determined to be 5 days during refrigerated storage at 4ºC.

Keywords: Gilthead sea bream; patties, corn flour; proximate composition; texture; color; sensory assessment; shelf-life.

1. INTRODUCTION

Gilthead sea bream (*Sparus aurata*) is one of the most important fish species farmed in the Mediterranean region. Nevertheless, the increased supply of gilthead sea bream caused prices to decline by more than 30% between 2002 and 2010 [1]. In certain, also, periods of the year, i.e. during autumn, there is plentiful supply of fresh fish in market, which causes a further decline of the prices [2]. There is, therefore, a need to look for the development of value-added products for commercial or industrial use, which could fulfill consumers' demands and make gilthead sea bream farming industries more profitable.

Recent studies suggested that farmed gilthead sea bream minced muscle can be used successfully for the manufacture of heat-induced gel products [3]. In addition, the use of corn flour in the formulation of gilthead sea bream burgers resulted in more tender and acceptable products than those prepared with potato or wheat flour [4]. Moreover, several studies have suggested that the concentration of flours in the formulations of meat burgers affects the physicochemical properties and acceptability of the products [5,6,7,8].

The aim of the present study was to evaluate the effect of different concentrations of corn flour on the quality of gilthead sea bream patties, and their refrigerated storage stability under aerobic conditions.

2. MATERIALS AND METHODS

2.1 Raw Fish

Gilthead sea breams (*Sparus aurata*; average weight and length 568±38 g and 32.1±0.89 cm (average±S.D.), respectively) were purchased from a commercial cage culture unit located in Western Greece. Fish were fasted for two days prior to harvesting and were slaughtered by immersion in ice-cold water (hypothermia). They were packed into an insulated polystyrene container with flaked ice and delivered to the Department of Fisheries and Aquaculture Technology in Messolonghi on the same day of their harvesting. At the laboratory, the fish were deboned, eviscerated, filleted and skinned by hand. The fillets were washed by water immersion and minced using a mincer with a whole diameter of 3mm. The minced gilthead sea bream muscle was divided into three equal portions and packed individually in polyethylene bags. The packages of minced fish were stored frozen at -80°C until they were used for the preparation of patties, which was completed within the first month of storage. In the present study, the temperature of -80ºC was used to preserve the minced fish since it has been shown that protein and lipid contents of frozen fish were unaffected within the first month of storage at -80ºC [9].

2.2 Preparation of Gilthead Sea Bream Patties

The portions of frozen gilthead sea bream minced muscle were thawed in a laboratory refrigerator at 4ºC overnight (12 hours). Then, a part of the gilthead sea bream minced muscle was used for the determination of the proximate composition and the rest for the preparation of the gilthead sea bream patties. The composition of the gilthead sea bream patties is shown in Table 1. The minced gilthead sea bream muscle, flour and the rest ingredients were mixed thoroughly in a bowl mixer with a spiral dough hook. Once the dough became smooth, a portion of the dough was used for the determination of proximate composition and the rest was shaped into patties using Petri dishes (mean weight of patties was 30 g approximately). The gilthead sea bream patties were used immediately for the analyses reported in the following sections.

Table 1. Gilthead sea bream formulations

Ingredients (g)	0%CF §	2.5%CF	5%CF	7.5%CF	10%CF
Fish minced meat	100	97.50	95.00	92.50	90.00
Corn flour	0	2.5	5	7.5	10
Salt	1.1	1.1	1.1	1.1	1.1
Spices	1.15	1.15	1.15	1.15	1.15
Sugar	0.9	0.9	0.9	0.9	0.9
Polyphosphate	0.2	0.2	0.2	0.2	0.2
Water	6	6	6	6	6
Olive oil	0.9	0.9	0.9	0.9	0.9
	110.25	110.25	110.25	110.25	110.25

§ CF=Corn flour (% of wet weight of fish minced meat)

2.3 Handling of Gilthead Sea Bream Patties

To evaluate the effects of different concentrations of corn flour on the quality of gilthead sea bream patties, three independent experiments were performed using the mince from ten gilthead sea breams in each experiment. Six gilthead sea bream patties per formulation were prepared in each experiment. The gilthead sea bream patties were weighed and baked in a preheated laboratory oven at 180±1ºC. During baking, the temperature of the thermal centre of each gilthead sea bream patty was monitored using a thermocouple and a recording thermometer. Once the centre temperature of the gilthead sea bream patties reached 70ºC (10 minutes), they were transferred in a thermostatically controlled oven and allowed to cool for one hour at 25ºC. Then, the gilthead sea bream patties were re-weighed for cooking yield determinations, the color measurements were taken and a cylindrical portion was excised from the central part of each sea bream patty for the instrumental textural determinations. The remaining portions of the gilthead sea bream patties with the same formulation were pooled first and then minced in a domestic mincer. Fractions of the minced portions of the gilthead sea bream patties were used for determination of water and ash contents and the rest were lyophilized for the crude protein and fat determinations.

Minced muscle from twenty gilthead sea breams was used in order to evaluate the stability at 4ºC of gilthead sea bream patties with the optimum corn flour level in terms of acceptable sensory characteristics (5% corn flour). Fifty-six gilthead sea bream patties were prepared and placed on plastic trays covered with cling film. The trays were placed in a laboratory refrigerator at 40ºC and the quality of gilthead sea bream patties was evaluated after 1,2,3,4,5,6 and 7 days of storage using microbiological (total viable count), chemical (total volatile basic nitrogen and thiobarbituric reactive substances) and instrumental texture (gel strength) measurements. Each day of sampling, three gilthead sea bream patties were used for the microbiological and chemical analyses and five patties were cooked and used for the instrumental texture measurements.

2.4 Chemical and Microbiological Analyses

Water content was measured following the method of [10]. The ash content was obtained by heating the residue from the moisture determination in a furnace at 550°C for 24 hours. Crude protein of the sea bream burgers were analyzed by the Kjeldahl method [10]. Total fat content (%) was determined from 2 g sample using petroleum ether and a Soxtherm S-360D extraction unit (Gerhardt, Germany). Thiobarbituric reactive substances (TBARS) were determined by the method of [11]. Total volatile basic nitrogen (TVB-N) was estimated using the direct distillation method with MgO according to the method of [12]. For total viable counts (TVC) measurements, 10 g of minced patties were homogenized with 90 ml sterile 0.1% peptone using a Waring blender. Appropriate dilutions of samples were prepared in sterile 0.1% peptone water. Samples (1 ml) of the dilutions were spread on the growth media using the pour plate method and then incubated at 30°C for 48 h [13].

2.5 Cooking Properties

Cooking yield was determined by measuring the weight of gilthead sea bream patties before and after cooking and was calculated according to [14]:

Cooking yield (%) = (Weight of cooked burger/ Weight of raw burger) x100

Moisture retention was determined according to [15] following the equation:

Moisture retention = Cooking yield x (% moisture in cooked patties/% moisture in raw patties)

Fat retention was determined according to [14] following the equation:

Fat retention (%) = Cooking yield x (% fat in cooked patties/% fat in raw patties)

2.6 Measurement of Color

Color measurements were carried out using a Handerlab Miniscan EZ Meter (Hunter Associates Laboratory, Inc., USA). The instrument was standardized against a white and black tile before each measurement. Results were expressed in L* (lightness), a* (redness) and b* (yellowness) Hunder scale parameters. Instrumental color determinations were made on three measurements in different areas of the surface of the patties.

2.7 Instrumental Texture Measurements

Instrumental texture measurements were performed using a universal testing machine (Stable Micro System, Model TA-XT plus, Texture Exponent, Surrey, UK).

Tests were performed using cylindrical portions (20 mm diameter and 10 mm height) taken from the central part of gilthead sea bream patties.

Texture Profile Analysis (TPA) was used to determine hardness, cohesiveness, chewiness, elasticity and gumminess of the gilthead sea bream patties [16]. This test was carried out by using compression platen with 24.5 mm diameter. TA-XT plus settings for TPA test were: load cell 5 kg; pre-test speed 2.0 mm/s; test speed 2.0 mm/s; post-test speed 5.0 mm/s; distance 50% and trigger type Auto-30 g.

A shear test was used to determine the force required to cut through the cylindrical portions. A V shaped blade was used. Settings for this test were: load cell 5 kg; pre-test speed 2.0 mm/s; test speed 2.0 mm/s; post-test speed 5.0 mm/s; distance 40 mm and trigger type Auto-30 g.

Penetration test was used to determine the braking force (g) and penetration distance (mm) of the portions of the gilthead sea bream patties. TA-XT plus settings for this test were load cell 5 kg; pre-test speed 2.0 mm/s; test speed 2.0 mm/s; post-test speed 5.0 mm/s; distance 75% and trigger type Auto-10 g. A 2 mm diameter penetration probe was used.

2.8 Sensory Analyses

A ranking test to assess the effect of corn flour content on overall acceptability of gilthead sea bream patties was performed according to [17] and [18]. For this test, 26 untrained assessors coming from the Department of Aquaculture and Fisheries Technology were used. Portions of cooked gilthead sea bream patties were wrapped with aluminum foil, coded with three digit random numbers and presented to panelist using random permutations. All evaluations were performed at room temperature.

2.9 Statistical Analyses

Statistical analyses of data were performed with Minitab14 for Windows (Minitab Inc., 2002). The results of proximate composition of raw and cooked gilthead sea bream patties were examined for significant differences by generalized linear model (GLM). The results of cooking characteristics, color and instrumental texture of gilthead sea bream patties formulated with different levels of corn flour and the results of the storage experiment were analyzed using one-way analysis of variance (ANOVA). Tests showing significant differences were followed by a Tukey HSD. The Friedman test was applied to sensory data for overall comparison and to establish the significance of differences between pairs of samples. Pearson's correlation coefficients between the means of the instrumental textural attributes and the content of corn flour in the patties were also calculated. Significance was accepted when $P=0.05$ [19].

3. RESULTS AND DISCUSSION

3.1 Proximate Composition of Gilthead Sea Bream Minced Muscle

The mean values of water, protein, lipid and ash of minced sea bream muscle were 73.52±0.86, 19.98±1.05, 3.93±0.83 and 1.37±0.07g/100g of minced muscle (means ± S.D.), respectively. According to [20], mean values of water, protein, lipid and ash of fresh skinless gilthead sea bream fillets, coming from diverse Greek fish farm units, from 70.3 to 75.3, 21.9 to 23.3, 32.6 to 7.38 and 1.30 to 1.48 g/100g of tissue, respectively. Therefore, the results of the water content, protein, lipid and ash content of gilthead sea bream mince of the present study are similar to those reported by [20].

3.2 Effect of Corn Flour Incorporation on Proximate Composition of Gilthead Sea Bream Patties

A significant decrease of moisture in gilthead sea bream patties was observed due to the cooking process (*P=0.05*; Table 2). This moisture loss resulted in an increase of the other nutrients in cooked gilthead sea bream patties. No differences were observed in fat content among the different formulations of the gilthead sea bream patties (P>0.05). However, the gilthead sea bream patties formulated with 7.5% and 10% corn flour had significantly lower water content than controls (*P=0.05*). Gilthead sea bream patties formulated with 10% corn flour had significantly lower ash content than controls (0% corn flour) and patties formulated with 2.5% corn flour (*P=0.05*). The protein content of gilthead sea bream patties formulated with 10% corn flour was significantly lower than all the other formulations (*P=0.05*). These changes can be attributed to the substitution of a portion of gilthead sea bream muscle with an ingredient (i.e. corn flour) that contained less water, ash and protein than the gilthead sea bream muscle. No significant cooking x formulation interaction was observed in the proximate composition (P>0.05). The remaining percentages of the total chemical composition analyses are likely due to carbohydrate. Significant differences in carbohydrate content were observed among the formulations. Gilthead sea bream patties formulated with 7.5% and 10% corn flour had similar carbohydrate contents that were significantly higher (*P=0.05*) than those of all the other formulations. The formulations with 2.5% and 5% corn flour had similar carbohydrate content and significantly higher than that of controls (*P=0.05*). In general, fish are known to have low concentrations of carbohydrate in their muscle [21]. Therefore, the amount of carbohydrate found in the gilthead sea bream patties can be attributed to the ingredients used in the preparation of the patties mainly corn flour and sugar. Chemical composition of fish fingers made from mince of unwashed mirror carp were 68.5%, 15.5%, 6%, 2.2% and 7.8% for moisture, protein, ash, lipid, ash, and carbohydrate content, respectively [21]. Burgers prepared from mince of silver catfish had moisture, protein, ash, lipid, ash and carbohydrate content equal to 68.3%, 18.9%, 6.1%, 2.6, and 4.2%, respectively [22]. Therefore, the chemical composition results of the present study are similar to those found by these other studies. Overall, the proximate composition of the gilthead sea bream patties was significantly affected by the addition of corn flour.

Table 2. Proximate composition of uncooked and cooked gilthead sea bream patties using different concentrations of corn flour

Formulation	Moisture	Ash	Fat	Protein	Carbohydrate
Effect of formulation					
Control(0%)	71.76±1.99a §	2.18±0.24a	5.78±0.68	16.38±1.28a	3.92±1.56c
2.5 %	70.65±1.67a	2.17±0.25a	5.11±0.42	16.03±1.15a	6.07±1.17b
5.0 %	69.16±1.51ab	2.03±0.20ab	5.57±0.51	16.06±1.04a	7.12±0.58b
7.5%	66.54±0.50b	2.08±0.15ab	5.40±0.19	15.87±0.52a	10.14±0.33a
10%	67.50±1.56b	1.90±0.15b	5.50±0.81	14.34±1.22b	10.75±0.52a
Effect of cooking					
Uncooked	70.28±2.36a	1.92±0.15b	5.06±0.34b	14.95±1.01b	7.78±2.79a
Cooked	67.96±1.91b	2.22±0.16a	5.88±0.38a	16.52±0.83a	7.41±2.84a

§Value is the mean ± standard deviation (n=3). GLM modelling revealed no significant formulation x cooking interaction; therefore only means of main effects are presented and compared. Numbers within the same column followed by a different letter (a, b, c) are significantly different (P=0.05).

3.3 Effect of Corn Flour Incorporation on Cooking Characteristics of Gilthead Sea Bream Patties

Cooking yield of meat products is an important parameter for the meat industry in predicting the behavior of products during cooking due to non-meat ingredients or other factors [23]. The cooking characteristics of gilthead sea bream patties are shown in Table 3. ANOVA showed significant differences in cooking yield values obtained from controls and gilthead sea bream patties formulated with corn flour (*P=0.05*). Differences in the cooking yield values between the gilthead sea bream patties formulated with different concentrations of corn flour were not found (P>0.05). However, an increasing trend in cooking yield values with increasing concentrations of corn flour in gilthead sea bream patties was observed. Similarly, moisture retention increased with increasing corn flour content in gilthead sea bream patties. Thus, gilthead sea bream patties with 10% corn flour had significantly higher water retention value compared to the other formulations (*P=0.05*). There were no marked differences in the moisture retention between the gilthead sea bream patties formulated with 2.5%, 5% and 7.5% corn flour (P>0.05). Water retention values of controls were significantly lower than those of gilthead sea bream patties formulated with 7.5% and 10% corn flour (P>0.05). Differences in fat retention between the formulations of gilthead sea bream patties were not found (P>0.05). Thus, incorporation of corn flour in formulations favored cooking yield and moisture retention of cooked gilthead sea bream patties. Chicken nuggets extended with cow-pea and/or peanut flour exhibited increased water retention and cooking yield [24]. Oat flour improved the cooking characteristics of beef patties [7]. Beef patties extended with bambara groundnut flour (BGF) exhibited increased cooking yield, moisture and fat retention compared to controls (0% BGF) [5]. In meat products, cooking yield is determined by the ability of the protein matrix to retain water and bind fat [25,26]. However, the results of the present study suggest that the improvement in cooking yield due to corn flour addition in gilthead sea bream patties is mainly associated with the water retention rather than with the lipid retention. During heating of flour, gelatinization of starch and swelling of the flour fiber occur. The swelled starch and fiber may interact with the protein of fish matrix preventing the migration of moisture from the product during cooking [27].

Table 3. Cooking characteristics of gilthead sea bream patties

Concentration of flour (%)	Cooking Yield (%)	Water Retention (%)	Lipid Retention
0	91.97±1.18b§	88.44±0.66c	99.08±1.35a
2.5	92.86±1.34a	88.94±0.74bc	97.64±3.20a
5	93.42±2.59a	89.44±0.02bc	95.41±7.66a
7.5	93.60±1.28a	90.59±0.00b	96.95±0.64a
10	94.29±2.48a	92.24±0.34a	94.04±0.67a

§Value is the mean ± standard deviation (n=18). Numbers within the same column followed by a different letter (a,b) are significantly different (P=0.05)

3.4 Effect of Corn Flour Incorporation on Color of Gilthead Sea Bream Patties

The color of comminuted fish products (e.g. fish gels) is a quality attribute as important as texture and flavor [28]. Table 4 shows the color values of the cooked gilthead sea bream patties with and without added corn flour. Gilthead sea bream patties with 5 to 10 % corn flour were significantly lighter (higher L* values; *P=0.05*) than the gilthead sea bream patties prepared with 0% and 2.5% corn flour. Gilthead sea bream patties containing corn flour at levels of 7.5 and 10% were more yellow (higher b* values; *P=0.05*) than the gilthead sea bream patties containing 0% to 5% corn flour. These results are similar to those reported for fish burgers prepared from sea mearger [29] and cat fish [22]. In addition, beef sausages extended with common bean flour at levels from 5% to 10% of the weight of meat were lighter and more yellow than controls [8]. The study of [30] showed that when surimi-corn flour gels were heated at 70°C the granules of corn flour were not fully swollen and consequently the gels were lighter and more yellow as the corn flour concentration was increasing. Since the gilthead sea bream patties were cooked at a core temperature of 70ºC, the findings of the present study can be explained by the suggestions reported in the study of [30].

Table 4. Hunter color L*, a*, b* values of gilthead sea bream patties

Concentration of corn flour (%)	L*	a*	b*
0	66.60±2.27 b§	0.26±0.47 b	18.55±1.63 b
2.5	67.04±3.42 b	0.46±0.34 b	18.55±2.12 b
5	68.38±1.70 a	0.52±0.63 b	18.87±1.71 b
7.5	68.45±1.66 a	0.66±0.33 a	19.99±1.53 a
10	69.20±1.39 a	0.83±0.41 a	19.63±1.54 a

§ Value is the mean ± standard deviation (n=18). Numbers within the same column followed by a different letter (a, b) are significantly different (P=0.05).

Gilthead sea bream patties prepared with 7.5 and 10% corn flour were more red (higher a* values, *P=0.05*) than patties extended with 0% to 5% corn flour (*P=0.05*). As was mentioned earlier in this paper, patties prepared with 7.5 and 10% corn flour contained less water than patties prepared with 0% to 5% corn flour (Table 2). Consequently, incorporation of corn flour at levels 7.5 and 10% could have caused less dilution of myoglobin of gilthead sea bream meat. As a result, gilthead sea bream patties prepared with 7.5 and 10% corn flour had higher a* values (more red) compared to those prepared with 0%, to 5% corn flour.

3.5 Effect of Corn Flour Incorporation on Texture of Gilthead Sea Bream Patties

The effects of corn flour content on the texture profile attributes and shear forces of cooked gilthead sea bream patties are shown in Table 5. With the exception of springiness, the remaining texture profile attributes showed significant changes with rising levels of corn flour in the formulations of gilthead sea bream patties (*P=0.05*).Furthermore, increasing corn flour content resulted in high regression coefficients for hardness (R2=0.951) gumminess (R2=0.910) and chewiness (R2=0.871) indicating that these attributes are sensitive to the functionality of the corn flour. Similar results are reported for sausages of geelbeck croaker formulated with different concentrations of corn flour [6]. Regardless of the level of corn flour, gilthead sea bream patties required significantly higher forces to shear compared to control patties (*P=0.05*). Shear forces obtained from gilthead sea bream patties exhibited no significant correlation as a function of starch content (P>0.05).

Penetration breaking force (g*) indicates the gel strength, while penetration distance (mm) denotes the gel brittleness. Gel strength values of gilthead sea bream patties with 5% corn flour were significantly higher than those of controls and the other formulations (*P=0.05*; Table 6). Differences in the penetration distance between the formulations were not found (P>0.05). Penetration braking force and penetration distance, exhibited no significant correlation as a function of starch content (P>0.05).

When surimi-starch gels are heated, starch granules absorb water from the surroundings and expand. The expanded starch granules exert pressure to the gel matrix contributing to the overall gel texture [31]. In the present study, cooking of gilthead sea bream patties at a core temperature of 700C would cause swelling of starch granules (even partial; [30]) and that would cause the aforementioned effects of corn flour on the textural attributes of the gilthead sea bream patties.

The decreased strength values of gilthead sea bream patties with 7.5 and 10% corn flour compared to those of gilthead sea bream patties with 5% corn flour can be attributed to the dilution of myofibrillar proteins, which are gelling agents much stronger than starch, as the corn flour concentration increased. Moreover, the water content of gilthead sea bream patties formulated with 7.5% and 10% corn flour, could be insufficient for both starch granules and myofibrillar proteins to reach peak gelatination and gelation points, respectively. Thus, a weaker minced muscle – starch gel was obtained at higher starch concentrations (7.5% and 10%) compared to 5% corn flour. Similar observations and explanations were reported for surimi-starch gels formulated with different concentrations of corn starch [30].

3.6 Effect of Corn Flour Incorporation on Overall Acceptance of Gilthead Sea Bream Patties

Sensory data of cooked gilthead seabream patties revealed that substitution of fish meat with corn flour up to 5% did not have a significnt effect on overall acceptability (Table 7; P>0.05). However, the scores of overall acceptance from patties with 7.5% and 10% corn flour were significantly different compared to to those of gilthead sea bream patties formulated with 5% corn flour. Therefore, substitution of gilthead sea bream minced mucle with corn flour at 5% is considered optimum for use as extender/ binder in gilthead sea bream patties. This level of corn flour was chosen for storage stability evaluation of gilthead sea bream patties.

Table 5. Texture profile analysis of gilthead sea bream patties

Concentration of flour (%)	Hardness (g)	Springiness	Cohesiveness	Gumminess	Chewiness	Shear Force N
0	6470±918 c§	0.89±0.05a	0.63±0.04ab	4122.8±333.6c	3678.9±214.1c	6.87±0.65b
2.5	7141±1031c	0.88±0.04a	0.60±0.03b	4188.0±395.9c	3681.3±286.2c	8.57±0.66a
5	7441±445bc	0.87±0.02a	0.61±0.02b	4539.1±228.8c	3928.9±203.0bc	7.93±0.73ab
7.5	7987±810b	0.86±0.01a	0.62±0.01b	4941.0±487.1bc	4260.8±432.3b	8.96±0.78a
10	9593±780a	0.90±0.02a	0.65±0.01a	6261.4±498.6a	5603.9±412.3a	8.08±0.32a
Pearson's correlation coefficient (r)	0.952	-0.183	0.585	0.910	0.871	0.410
Significance of correlation	S	NS	NS	S	S	NS

§Value is the mean ± standard deviation (n= 6). Numbers within the same column followed by a different letter (a, b, c) are significantly different (P=0.05).S: significant (P=0.05), NS: Not significant (P=0.05)

Table 6. Penetration test of gilthead sea bream patties

Concentration of flour (%)	Penetration Breaking force (g)	Penetration Distance to rupture (cm)
0	309.35±44.52 b§	0.47±0.08 a
2.5	353.95±58.10 b	0.49±0.14 a
5	642.22±156.65 a	0.40±0.04 a
7.5	434.09±77.03 b	0.40±0.07 a
10	419.19±39.05 b	0.39±0.09 a
Pearson's correlation coefficient (r)	0.706	-0.852
Significance of correlation	NS	NS

§Value is the mean ± standard deviation (n=6). Numbers within the same column followed by a different letter (a, b) are significantly different (P=0.05). S: significant (P=0.05), NS: Not significant (P=0.05)

Table 7. Sensory evaluation of sea bream burgers

Concentration of flour (%)	Overall acceptance
0	79.5ab§
2.5	82.5ab
5	95.0a
7.5	72.5b
10	60.5b
P (adjusted for ties)	0.041

§Numbers are the sum ranks from the Friedman test applied to sensory data (n=26).

3.7 Effect of the Length of Time at 4^0C on Chemical, Textural and Microbiological Characteristics of Gilthead Sea Bream Patties Formulated with 5% Corn Flour

Total viable count is an important quality index because of the effect of bacteria in spoilage. TVC of fresh minced fish was found as 3.54± 0.06 log CFU/g. After one day of storage, the TVC value of gilthead sea bream patties was 4.02 ± 0.09 log CFU/g. This result indicates that the non-fish ingredients introduced bacteria to patties since the ingredients were not sterilized before addition (Table 8) [32]. The total viable counts of gilthead sea bream patties increased throughout storage and exceeded the critical limit of 6.0 log cfu/g (Kilinc et al. 2008) on the sixth day of storage. This result suggests that the microbiological shelf-life of the patties stored at 40C was 5 days. Similar results are reported for sardine patties stored at 4°C for 7 days [33].

Thiobarbituric acid values are indicatives of the degree of rancidity in the products. TBA values more than 3-4 mg malonaldehyde per kg imply quality loss in fish products [34]. TBA values ranged from 0.323±0.002 to 0.924±0.01 malondialdehyde per kg of gilthead sea bream patties (Table 8). Thus, the TBARS levels of patties remained under the limit for rejection until the end of the storage period. This can be attributed to the presence of anti oxidants supplied by some of the ingredients, including onion and garlic [29].

Total volatile basic nitrogen (TVB-N) is an index of spoilage due to bacterial and endogenous enzymes action in fish [12]. TVB-N values of gilthead sea bream patties increased significantly ($P=0.05$) during storage. The initial TVB-N of gilthead sea bream patties was determined as 14.06±0.83 mg N/100g (Table 8). This value increased to 17.94±0.29 mg N/100g at the end of the storage period of 7 days and it did not exceed the acceptability limit (35 mg/100g). These results are similar to those reported for Atlantic mackerel fish burgers stored at 40C [35].

There was no sign of a tendency for strength values of gilthead sea bream patties to change in up to 6 days of storage at 4ºC. However, the mean strength value of cooked patties stored for 7 days was significantly lower than that of gilthead sea bream patties stored for one day (Table 8;$P=0.05$). This can be attributed to microbial reactions, as revealed by the increases in TVC values mentioned in this paper [36]. Changes in the brittleness values of gilthead sea bream patties were not observed throughout the storage period of the 7 days (data not shown).

Table 8. Effect of the length of time at 4ºC on chemical and microbiological characteristics of gilthead sea bream patties formulated with 5% corn flour

Analysis	Storage Time (days)						
	1	**2**	**3**	**4**	**5**	**6**	**7**
TVC (log_{10}cfu/g,n=3)	4.02±0.09d§	4.25±0.06dc	4.25±0.09dc	4.57±0.40c	5.74±0.10b	6.52±0.11a	6.86±0.21a
TBARS **(mg malondialdehyde/kg, n=3)**	0.323±0.002e	0.518±0.007d	0.554±0.004d	0.768±0.006c	0.753±0.006c	0.843±0.006b	0.924±0.01a
TVB-N (mg N/100g, n=3)	14.06±0.83d	14.63±0.09cd	16.79±0.75ab	15.91±0.84bc	16.91± 0.24ab	17.75±0.48 a	17.94±0.29a
Penetration Breaking Force (g, n=5)	716.23±119.19a	609.14±62.25ab	676.23±116.48ab	705.97±90.00a	688.97±65.75ab	536.69±95.41ab	487.84±68.51b

§Value is the mean ± standard deviation. TVC= Total Viable Count, TBARS=Thiobarbituric reactive substances, TVB-N=Total volatile basic nitrogen. Numbers within the same raw followed by a different letter (a, b, c, d) are significantly different (P=0.05)

4. CONCLUSION

The following conclusions are drawn from the present study:

- Incorporation of different levels of corn flour affected the proximate composition of gilthead sea bream patties. Formulations with corn flour up to 5% had similar water, ash, fat and protein content.
- Incorporation of corn flour improved cooking yield and water retention of gilthead sea bream patties.
- Formulations with 5% to 10% corn flour were the lightest, whereas formulations with corn flour up to 5% were the most red and yellow.
- Formulations with 10% corn flour were the hardest, the gummiest and chewiest, whereas the highest gel strength values were obtained at a corn flour concentration of 5%.
- Sea bream patties formulated with corn flour up to 5% were the most acceptable to the panel of the assessors.

Thus, corn flour at 5% substitution was recommended as an extender in production of patties made from gilthead bream minced muscle. Based on microbiological evaluation, the shelf life of gilthead sea bream patties with 5% corn flour was determined to be 5 days during refrigerated storage at 4^0C. However, the shelf life of gilthead sea bream patties can be extended by using different methods, including heat treatments, antimicrobials and effective packaging.

COMPETING INTERESTS

Authors have declared that there are no competing interests.

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