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Microbiological, physicochemical, and sensorial quality of vacuum-packed sausage from thornback ray (*Raja clavata* L., 1758) at chilled storage

Eda Özer, Mustafa Ünlüsayin*, Ruhan Erdilal

Department of Fishing and Fish Processing Technology,
Fisheries Faculty, Akdeniz University, 07059, Antalya, Turkey

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Abstract

The aim of this study, sausage producing technology was used to increase the quantity of consumable thornback ray (*Raja clavata* L., 1758) owing to their low muscle quality and high cartilage tissue. The physicochemical and microbial qualities were investigated during 56 days under vacuum storage conditions at 4°C. In addition the analyses of chemical composition, color and sensorial quality were evaluated. Increasing in pH, Total volatile basic nitrogen (TVB-N), Thiobarbituric acid reactive substances (TBARs), Total mesophilic count (TMC), Enterococci, Lactic acid bacteria (LAB) were found statistically significant ($p<0.05$) in sausage throughout storage. The contents of water, protein, fat and ash were 66.34±0.10%, 22.35±0.47%, 2.4±0.56% and 3.73±0.21%, respectively. The Lightness (L), red-green tendency (a), yellow-blue tendency (b), whiteness (w) from the color measurements were established 49.19± 2.77, 10.77± 0.60 and 11.18± 2.02 and 46.85± 2.91, respectively. The sausage from thornback ray reached their maximum acceptable TMC on days 42 under vacuum storage conditions at 4°C. However, TVB-N, TMA-N and TBARs levels of sausage did not exceed the legal limit for 56 days.

Keywords: *Raja clavata*, fish frankfurter, seafood, shelf life, quality, vacuum packing

1. Introduction

Presently, there is a growing interest in developing meat analogues using alternative sources of protein; as a result, health-conscious consumers are promoting the research and development of different meat systems. Among these, restructured fish products have been used with the purpose of reaching young consumers. In a nutritional perspective, the seafood contains a well balanced protein source, high levels of minerals and trace elements, such as selenium and iodide and high levels of B-vitamins, high polyunsaturated fatty acids (PUFAs), omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [1-3].

Thus, fish can be tested as a meat analogue and several studies have addressed production of attractive fish sausages or surimi [4-13].

Sausages are one of the oldest forms of meat processing and modern sausage technology has its roots deeply embedded in history. Sausages are products in which fresh comminute meats are modified by various processing methods to yield desirable organoleptic and technological characteristics [14]. Thus, they can be specially suited for testing the potential of some fish species. In general, processors focus on making a less expensive product with lower quality raw materials [15]. For thornback ray is not preferred to consume, it has a low cost owing to their low muscle quality and high cartilage tissue.

* Corresponding author: *e-mail:* munlusayin@akdeniz.edu.tr

The total amount of *Raja sp.* caught in the World 170.209 tons in Turkey was 974 tons [16]. There are a few studies on the quality changing of thornback ray surimi products during chilling storage [17] and boiled ray during ice storage [18]. The processing of sausage technology and adding spices can improve the acceptability of thornback ray. Hence, a new product can be constituted to increase the diversity of seafood products. In addition, thornback ray that is generally assumed as a discard fish material could be gained to the economy.

The objective of this research was to study the microbiological and physicochemical changes in the sausage from thornback ray following vacuum packing treatment at chilling temperature.

2. Materials and methods

Materials. The fresh fillets of thornback ray, *Raja clavata* (L., 1758), were used as fish material for sausage production. Rays were caught in the Gulf of Antalya, the Mediterranean Sea by deep trawl nets. Rays were put in polystyrene boxes including ice just after being caught and then transported to our laboratory immediately. Totally, 62 rays were used for the treatment. Mean total body mass and length of individuals were 324.2± 402.3g and 35.66± 10.8cm, respectively.

Sausage preparation and handling. Thornback ray was gutted, removed skin and head and filleted. Thornback ray muscles were minced through a grinder with a plate having 3 mm-diameter perforations. Fish sausage formulations consist 1kg filleted ray, 24g corn starch, 20g vegetable oil, 50g ice water, 20g table salt, 1 piece egg yolk, 24.9g onion powder, 15g garlic powder, 15g cumin powder and 05g color additive (beetroot red, E 162). Sausage was then inserted into sausage polyethylene casings using a hand held sausage filling machine. Sausages were dried at 50-55^{o}C during 20-25 minutes. Then they were hot-smoked at 75^{o}C for 60 minutes. They were then chilled in ice water for 5 minutes, vacuum packed (Henkelman, Boxer42) (80g sausage for each pack) and stored at 4^{o}C for 56 days. Control group was sausage that made from ray mince without any additives and hot smoking process. Samples were taken every 7 days for analysis.

Color Analysis. The surface colour of samples was evaluated on the Hunter L, a, and b scale using a Chromameter (Konica Minolta, CR-400). Measurements were taken on the all body as 5 measurements per sample of smoked and control. The colorimeter was calibrated before each series of measurements using a white ceramic plate and the Hunter L, a, and b values were recorded. Mean L, a, and b values were used to determine the color difference between smoked and control groups. Value of W was calculated as described by [12].

Proximate analyses. The proximate contents of samples were determined according to the Official Methods of Analysis. Moisture content was determined according to the Official Method 950.46 [19]. Crude protein content (Nx6.25) was calculated using the Kjeldahl method 928.08 [20]. Lipid (fat) content was determined according to the Soxhlet method 960.39 [21]. Crude ash (inorganic matter) was determined according to the method 920.153 [22].

Analysis of microbiota. Ten grams of sample were weighed aseptically and homogenized in a Stomacher bag for 1 min with 90 mL of Maximum Recovery Diluent. Further decimal dilutions were made with the same diluent. Total mesophilic count (TMC) was determined on Plate count agar (PCA, Merck) by the spread plate method, and incubated at 30^{o}C for 48 h. Enterococci was counted using KF Agar (Merck) by spread plate and incubated at 30^{o}C for 48 h. Total coliform count (TCC) was made using Violet red bile agar (VRBA, Merck) by the spread plate method and incubated at 35^{o}C 24 h. Lactic acid bacteria (LAB) was counted using Man rogosa sharp agar (MRS, Merck) by pour plate method and incubated at 30^{o}C for 48 h. Yeast and mold were determined using Potato dextrose agar (PDA, Merck) by the spread plate method and incubated 25^{o}C for 7 days. Microbiological counts were all expressed as colony-forming units (cfu/g) of sample [23].

Biochemical Analysis. pH of homogenized sample measured using a Hanna Instruments digital pH meter, Model pH211 with a glass electrode. Total volatile basic nitrogen (TVB-N) was measured in accordance with the method of Antonacopoulos and Vyncke [24]. Trimethylamine nitrogen (TMA-N) content was measured according to the method of Schormüller [25].

Thiobarbituric acid reactive substances (TBARs) analysis was made according to the method of Ke *et al.* [26].

Sensory Analysis. Sensory analyses were carried out as described by Carbonell *et al.* [27]. Appropriate sensory descriptors were defined for the sensory experiment and questionnaires were designed including these descriptors. Sausage groups were assessed by a panel of 17 internally trained members of the Fisheries Faculty, Akdeniz University using a 10 cm line scale, 0 at the extreme left to 10 at the extreme right and rating subsequently scored in cm from the left. The parameters evaluated by the assessors on samples: Appearance (0 = extremely dislike to 10 = extremely like), tenderness (0 = extremely hard to 10 = extremely tender), intensity of the spices (0 = none to 10 = extremely intense), intensity of the oil (0 = oil-free to 10 = extremely oily), juiciness (0 = extremely dry to 10 = extremely juicy), oxidised flavour (0 = No oxidised flavour to 10 = extremely oxidised flavour), and overall level of acceptability (0 = extremely unacceptable to 10 =extremely acceptable). Panellists were also asked to identify a single 'most preferred' sample from those presented.

Statistical analysis. One-way analysis of variance of data was carried out using the SPSS 13 for Windows software package (SPSS Statistical Software, Inc., Chicago, IL, USA). The difference between pairs of means was resolved by means of confidence intervals using Tukey's tests; the level of significance was set at $p<0.05$.

3. Results and Discussion

The contents of proximate, biochemical and microbiota of control group and sausage samples were shown in Table 1. Moisture content (g/100g) of control group significantly decreased, but the contents of lipid and ash significantly increased with the sausage process ($p<0.05$). During drying and smoking, evaporation might result in a decrease of moisture content in the fish sausage. Major varieties of fish made into fish sausage are normally lean and fatty tissue of animal and / or shortening oil is generally mixed into product [10]. The increase of lipid content is probably due to both adding oil and high water losses. Similarly, the increase of ash content is probably due to adding spices.

The similar results for control group were reported by Turan and Sönmez [17] (77.47g of moisture /100g mince, 1.38g of ash/100g mince), Gögüş and Kolsarici [28] (18.2-24.2g of protein /100g mince, 0.1-1.6g of lipid/100g mince), Synnes *et al.* [29] (0.41g of lipid/100 g mince). Sausage process changed ($p<0.05$) control group colour towards a more yellow hue (higher b) and redder (higher a). The similar values for colour results were reported for mackerel (*Scomber scombrus)* sausages [12]. The pH value of fish sausage was lower than those of control group ($p<0.05$). This decrease is possibly related to the protein component and to the lipid material of the oil-in-water emulsion [30]. TBARs, TMA-N, TVB-N, TMC and LAB values did not change with sausage process. The similar results of quality analysis were reported by Mugica *et al.* [18] (pH 6.5-9), Velázquez-Barros *et al.* [31] (30.4 mg/100g of TVB-N for thornback ray mince). Enterococci and Coliforms, being facultative anaerobic bacterial species, were less or not detected in the sausage than control group which might be due to retardation of log phase as a result of reduced metabolic rate owing to sudden change in the physical environment (Table 1).

Table 1. The contents of proximate, biochemical and microbiota of samples

Contents	Control	Sausage
Moisture (%)	75.08 ± 0.05^{a}	66.34 ± 0.10^{b}
Crude Protein (%)	21.57 ± 0.13^{a}	22.35 ± 0.47^{a}
Crude Lipid (%)	0.43 ± 0.42^{a}	2.4 ± 0.56^{b}
Crude Ash (%)	1.47 ± 0.22^{a}	3.73 ± 0.21^{b}
L	55.75 ± 7.26^{a}	49.19 ± 2.77^{a}
a	4.93 ± 0.51^{a}	10.77 ± 0.60^{b}
b	0.24 ± 0.98^{a}	11.18 ± 2.02^{b}
W	55.44 ± 7.13^{a}	46.85 ± 2.91^{a}
pH	6.41 ± 0.01^{a}	6.18 ± 0.04^{b}
TBARs (mgMA/kg)	0.68 ± 0.03^{a}	0.45 ± 0.02^{a}
TMA-N (mg/100g)	0.64 ± 0.11^{a}	0.40 ± 0.11^{a}
TVB-N (mg/100g)	28.7 ± 0.09^{a}	31.5 ± 0.09^{a}
TMC (log cfu/g)	4.71 ± 0.05^{a}	4.09 ± 0.01^{a}
Enterococcus (log cfu/g)	4.37 ± 0.02^{a}	3.29 ± 0.03^{b}
LAB (log cfu/g)	4.24 ± 0.04^{a}	3.74 ± 0.14^{a}
TCC (log cfu/g)	4.32 ± 0.07	ND
Yeast and Mould (log cfu/g)	ND	ND

Values are shown as mean ± standard deviation of triplicate measurements for three independent and parallel experiments. Different superscript letters in the same row indicate significant differences between groups ($p<0.05$)

It could also be due to through cooking of sausage with hot-smoking which drastically injured and killed these bacterial species [32].

Yeast and mold can contaminate to fish by fisheries and processing devices [33, 34]. No yeast and mold were determined for control group and sausage samples. Hence, it was seen that there is no contamination after fishing and handling and processing.

pH varies during storage time (Figure 1), displaying an decreasing trend and such variation was statistically different ($p<0.05$). The reason of this, micro flora is dominated by LAB in vacuum packed products and count of them constantly increases during storage following reduction in pH of meat [35, 36].

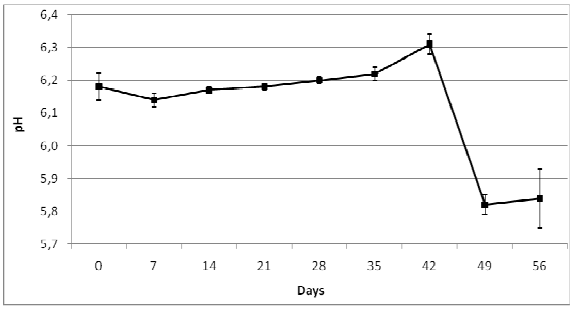

Figure 1. The changing of pH for the sausage during storage at 4°C

TBARs varies during storage time, displaying an increasing trend ($p<0.05$), like be descript by the previous studies [37-39], was statistically different (Figure 2). The limit of acceptability for chondrichthyes species is level of 7-8 mg malonaldehyde/kg flesh [40]. The data obtained in the present study suggest that TBARs values of sausage were within the very good quality limits.

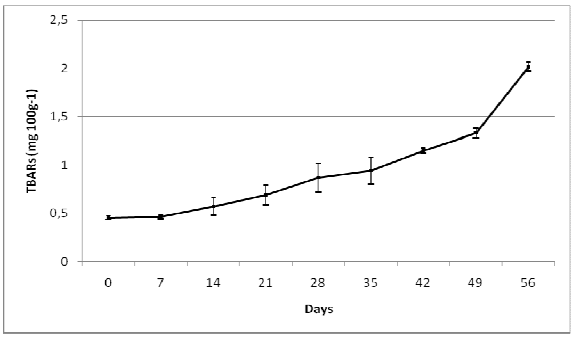

Figure 2. The changing of TBARs for the sausage during storage at 4°C

Trimethylamine (TMA) resulting from bacterial reduction of Trimethylamine oxide (TMA-O) is associated with the fishy odour of spoiling seafood [41]. TMA-N of sausage (Figure 3) did not exceed the legal limit of 8 mg/100g muscle [42] for during storage and did not show any significant variations ($p>0.05$).

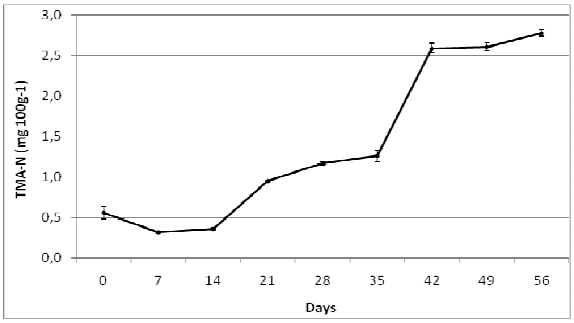

Figure 3. The changing of TMA-N for the sausage during storage at 4°C

TVB-N revealed a linear increase after 21st day of storage ($p<0.05$) (Figure 4). TVB-N did not exceed the legal limit of 50 mg/100g muscle [43] for sample even after 42 days of storage when microbiological analysis indicated an unacceptable quality. These results suggest that TVB-N, which is a widely used parameter to assess the quality evolution of marine fish species, is not useful as a quality indicator of ray sausage.

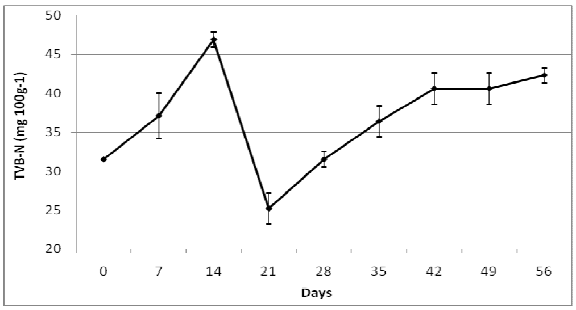

Figure 4. The changing of TVB-N for the sausage during storage at 4°C

The changing of microbiota for thornback ray sausage during chilling storage was shown in Table 2. TMC, Enterococci and LAB revealed a linear increase during storage ($p<0.05$). TMC of sausage on day 49 of storage exceeded the value of 6 log cfu/g, considered as the upper acceptable microbiological limit [33, 44].

The similar results for Enterococci count were reported for thornback ray [34]. The linear increase for LAB during storage is an expected situation in vacuum packed meat and meat product [44, 45]. No coliforms and yeast and mold were determined for sausage samples during storage.

Table 2. The changing of microbiota for the sausage during storage at 4°C (log cfu/g)

Days	TMC	TCC	Yeast and Mold	Enterecoccus	LAB
0	4.09 ± 0.01^{a}	ND	ND	3.29 ± 0.63^{a}	4.74 ± 0.14^{a}
7	4.18 ± 1.39^{a}	ND	ND	3.72 ± 0.03^{a}	5.06 ± 0.03^{b}
14	4.14 ± 0.03^{a}	ND	ND	3.79 ± 0.01^{a}	5.12 ± 0.06^{bc}
21	4.65 ± 0.64^{a}	ND	ND	3.88 ± 0.03^{a}	5.13 ± 0.01^{bc}
28	5.26 ± 0.01^{a}	ND	ND	3.90 ± 0.04^{a}	5.15 ± 0.01^{bc}
35	5.61 ± 0.01^{a}	ND	ND	3.97 ± 0.01^{a}	5.27 ± 0.08^{cd}
42	5.77 ± 0.01^{a}	ND	ND	4.05 ± 0.01^{a}	5.35 ± 0.01^{de}
49	7.32 ± 0.01^{b}	ND	ND	6.19 ± 0.02^{b}	5.41 ± 0.02^{e}
56	7.69 ± 0.12^{c}	ND	ND	6.21 ± 0.01^{b}	5.44 ± 0.01^{e}

Values are shown as mean ± standard deviation of triplicate measurements for three independent and parallel experiments. Different superscript letters in the same column indicate significant differences between groups ($p<0.05$)

The results of sensorial analysis for sausage samples were presented in Table 3. The intensity of the spices ($p<0.01$) and juiciness ($p<0.05$) sensory descriptors were positively correlated to the overall level of acceptability. There was not the external ammonia odour the limiting factor of acceptability in sausage. This was from the most remarkable advantage of adding spices ray sausage. Because the TBARs levels were 0.45 mg MDA/kg usually associated with rancid flavour/odour by sensory panellists and that the panellists found acceptable. Because ray mince was prepared as manual under the laboratory conditions, cartilaginous tissue could not removed completely, thus, panellists found the sausages too gristly. So, in the following studies, cartilaginous tissue must be reducing as possible as by special techniques.

Table 3. Results of sensory evaluation of sausages

Parameters	Means Scores
Appearance	5.69 ± 2.42
Tenderness	5.75 ± 1.65
Intensity of the spices	5.58 ± 1.70
Intensity of the oil	4.40 ± 2.17
Juiciness	4.58 ± 1.73
Oxidised flavour	3.33 ± 2.52
Overall level of acceptability	5.98 ± 2.17

Values are shown as mean ± standard deviation of triplicate measurements for seventeen independent panellists

4. Conclusion

In conclusion, thornback ray sausage technology owing to improved relatively the acceptability of fish, without hindering their nutritional and technological qualities, can be used by food processing industry to increase the diversity of seafood product. However, to keep a good overall sensory acceptance, future works should try to reduce thornback rays' cartilaginous tissue. Results of present study indicated that thornback ray flesh be efficiently used to process sausage with the storage stability. According to total mesophilic count thornback ray sausage can be consumed until 42nd day.

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