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Determination of the Shelf-Life of Trout (*Oncorhynchus mykiss*) Raw Meatball That Packed under Modified Atmosphere

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Abstract: Raw meatball is a traditional food in Turkey which is produced by mixing and kneading of various ingredients. There isn't any thermal inactivation process during the production. Because of high initial microbial load and absence of a pasteurization process and because of its raw consumption, raw meatball has high risk of microbial infection. In this study, it is aimed to detect the shelf-life of trout (*Oncorhynchus mykiss*) raw meatball that was packed under 3 different conditions; Control Group, MAP1 (GroupA) and MAP2 (GroupB). Sensory, pH and microbiological analyses were done during the study. According to the results, it has been determined that the Control (%100 air) samples saved their freshness until 5th day, the MAP1 (5% O₂+ 35% CO₂+ 60% N₂) and the MAP2 (5% O₂+ 25% CO₂+ 70% N₂) groups saved their freshness until 9th. These two groups showed no difference between themselves about storage.

Key words: Trout, raw meatball, MAP, microbiological quality, shelf life

Introduction

Raw Meatball is mostly consumed in East and South East Anatolia which are economically and educationally less developed regions of Turkey. It also recently takes place in the menus of luxury hotels, restaurants and holiday villages as an appetizer which is appreciated. As there is no standard for the ingredients of raw meat ball, its contents may differ according to the region. The main contents are: small, boiled and pounded wheat and minced lean meat. Other ingredients are: onion, garlic, tomato sauce, parsley, red pepper, black pepper, thyme, cumin, allspice, ice or water. All these ingredients are mixed together in an order and are kneaded by hand (traditionally by naked hand). The quality of the raw meatball is directly depends on the quality of meat and spices, hygiene of the personnel and the process. It increases the risk of human health that raw meatball is consumed without any kind of cooking process. Generally, raw meatball is consumed as soon as it is prepared (Sagun *et al.*, 2003). Some studies, which have been done on raw meatball, have showed that the microbial load of this food is high and it may create an important risk for the public health (Göktaş and Tuncel, 1988; Küplülü *et al.*, 2003; Vural *et al.*, 2006). Göktaş and Tuncel (1988) have notified that raw meatball can be conserved in the fridge for 24 h. Foods may have some changes such as oxidation and microbiological effects during their storage. If food doesn't contact with O₂ from its process step to its arrival to the customer, it can save its freshness for a long time. This may occur by changing the atmosphere conditions in the package (Weber and Laux, 1992). Thus, it has been started to

use packing technology with modified atmosphere recently. When Modified Atmosphere Packaging (MAP) combined with refrigeration, it can extend the shelf-life of the seafood according to its hunting operation time and quality (Dhananjaya and Stroud, 1994). The main parameters which affect the shelf-life of the stocked products are the production form, the gas mixture inside the package, features of the package material, storage temperature, packing process and machines that used, etc. In this study, it is aimed to prepare the raw meatball with trout meat and then to detect the shelf life of this product by packaging at cold and by packaging with MAP at different gas mixtures.

Materials and Methods

In this study, totally 40kg cleaned rainbow trout (*Oncorhynchus mykiss*, W.,1792) which were obtained from LIMAN Alabalık Co. Ltd. as 200- 250g pieces was used. The frozen fish which was transported to the laboratory in foam boxes, were holded in hot water (85° C) for two minutes, then head, skin and fish bones were boned and meat was cleaned (As it is possible to be risky to consume much and deficient raw fish meat, it was thought to use cooked fish meat and a sensory panel test had been done about this. After evaluating this sensory test results which had been scored by 10 panellists group, it was decided to use cooked fish meat). These cleaned meat were kneaded by hand with small pounded wheat which had been kept waiting in drinking water for 60 min. The quantity of the small pounded wheat was half of the fish meat quantity. Different kinds of spices (red pepper, black pepper,

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cumin, hot pepper, sweet pepper sauce, garlic, onion, salt and garden rocket) were added. Kneading took 3 h and after this, the mixture was shaped as 40-50g flat meatballs. These meatballs were put in foam boxes as 10-12 pieces, 400-500g weight and were taken to the store at cold ($+4 \pm 1^{\circ}\text{C}$) packing with as follows:

The analysis groups

Control group: 100 % air, MAP₁ (Group A): 5 % O₂ + 35 % CO₂ + 60 % N₂, MAP₂ (Group B): 5 % O₂ + 25 % CO₂ + 70 % N₂.

The analyses were run on the first day and then the quality changes were determined by analysing once in two days. In our study, KOROZA PA/PE package (unit weight $37,24 \pm 10$ %), packing material with thickness 90 micron ± 10 %, packing machine (HENKOVAC E-173 model, The Netherlands) with mix gas mixture were used.

Sensory analyses: The sensorial quality of raw trout meatball was evaluated by 10 experienced panelist according to "the hedonic scale" by using 0-10 scores. According to this evaluation; a score of between 10-8 accepted as "very good", between 8-6 accepted as "good", between 6-5 accepted as "proper to consume" and a score of under 5 accepted as "spoil" for each sensorial characteristic. Fresh trout was evaluated according to its appearance, odour and colour. After frying in hot oil, it was evaluated according to its taste, odour, appearance and tissue by panellists (Amerina et al., 1965).

pH Measurements: These measurements were done with WTW 537 model micropressor pH meter. While measuring; 10g fish sample had been weighted, diluted 1:1 and the pH probe had been immersed into the solution. Four readings were made on each sample and the mean recorded (Manthey et al., 1988).

Microbiological analyses: 10 g samples, that had been taken from each group's each 3 packages under aseptic conditions, have been rarefied with 90 mL peptone water (Merck, Cat No: 107228) and homogenized in stomacher (IUL Instrument, Spain) for 1 min. The dilutions as rarefication solutions have been made from this homogenized sample with 1 mL rarefication and 9 mL peptone. From these dilutions, mesophilic aerobic bacteria, psychrophilic bacteria, *S. aureus* have been planted with spread plac method and total and fecal coliform have been planted with MPN (Most Possible Number) method. The counting results after the incubation were calculated as log colony forming units (cfu/g and MPN/g).

Total Mesophilic Aerobic Bacteria: To count Total Mesophilic Aerobic Bacteria, Plate Count Agar (PCA) medium (Oxoid CM 463) and spread plac method were

used. The results were evaluated by counting after 24 h incubation at 37°C (Anonymous, 1994).

Psychrophilic Bacteria: The results were evaluated by counting after 10 days incubation at 7°C by using Plate Count Agar (PCA) medium (Oxoid CM 463) and by spread plac method (Anonymous, 1994).

Staphylococcus aureus: The planting was done into medium which is poured into petri dish with Egg yok tellurit (Oxoid) addition into Baird Parker (BP) (Oxoid) medium by spread plac method. The results were evaluated by counting after 2 days incubation at 37°C (Anonymous, 1994).

Total coliform bacteria: To count total coliform bacteria, Lauryl Tryptose Broth (LST) (Oxoid CM 451), Brilliant Gren Bile 2 % Broth (Oxoid CM 31) medium were used and the planting was done by MPN (Most Possible Number) method. The tubes, which were planted into LST, were incubated for 24-48 h at 37°C. One full of transfer loop from each tube which showed positive reaction were planted into BGLB medium, and then were incubated for 24-48 h at 35-37°C. It was confirmed that coliform bacterias exist in the tubes which form gas (Baumgard, 1986).

Fecal coliform bacteria: It was planted from the tubes which were confirmed to have coliform by forming gas in the BGLB medium to EC Broth (Oxoid 10765) medium with transfer loop. The tubes were incubated at 45.5°C for 24 h. It was confirmed that fecal coliform bacterias exist in the tubes which formed gas and they were called as positive (Baumgard, 1986).

Salmonella spp.: 25g sample was taken under aseptic conditions and pre-enhanced in 225 mL phosphate buffer solution (Sigma, Cat No:P-4417) at 37°C for 16-20 h in autoclaved bottles. For selective enhancement, 1 mL pre-enhanced sample from each bottle were taken and planted into the tubes which have 10 mL Selenite Cystine Broth (Merck, Cat No: 1.07709) and Tetrathionate Broth (Merck, Cat No: 1.05285) inside and were waited for 24 h at 37°C. Passing to the selective solid medium was done by smearing method with Bismuth Sulfite Agar (Merck, Cat No: 1.05418) and Xylose Lysine Desoxycholate Agar (Merck, Cat No:1.05287) at 37°C for 24 h incubation. For Salmonella identification, planting was done with transfer loop into the medium that consisted TSI Flat Agar (Merck, Cat No: 1.03915) and LI Flat Agar (Merck, Cat No: 11640). The incubation time was 24 h at 37° C for TSI Agar and 48 h at 37°C for LI Agar. After incubations, Urea Tests were done for the suspicious colonies in the TSI Agar and LI Agar (The colour changes for the suspicious colonies were bottom yellow/top red for TSI Agar and purple for LI Agar). If there were urea negative isolates, biological and serological tests were done (Andrews, 1992).

Measurements of Packing Material Permeability: The O₂ measurements were done by Servomex Oxygen Analyser 574; the CO₂ measurements were done by Servomex Infra-Red Gas Analyzer PA (404 SVS; Servomex, Sussex, UK) Range: 0-100 % CO₂ vehicles in TUBITAK (The Scientific and Technical Research Council of Turkey).

Statistical analyses: Analyses results are reported as Mean \pm Standart Deviation (Sd). Differences in mean values were determined using the t-test method (significance was defined at P < 0.5) (Sokal and Rohlf, 1987).

Results and Discussion

The results of the pH and sensory microbial analyses are shown on Table 1 and Fig. 1-4. The values of the sensory and pH analyses of cooked and fresh fish that were used for this study are 6.29 \pm 0.01 and 6.33 \pm 0.01; 9.80 \pm 0.38 and 9.33 \pm 0.26. For fresh samples, the microbiological loads are; for mesophilic aerobic bacteria 1 log cfu/g and for psychrophil, coliform and fecal coliforms, the number was counted lower than the minimum level. For cooked trout, the microbiological loads were counted lower than the minimum level for mesophilic aerobic bacteria, psychrophil, coliform and fecal coliform microorganisms.

After the raw trout meatball had been prepared, the initial and the 5th day (the day on which the sensory spoilage was seen) pH values of Control group were determined as 6.18 \pm 0.01 and 6.03 \pm 0.01 (p<0.05); sensory analyses values were determined between 9.58 \pm 0.38 and 5.33 \pm 0.41 points (p>0.05). At the end of the 5th day, the microbial values of our Control Group samples were determined respectively; for total mesophilic aerobic bacteria count, as 4.42 and 6.88 log cfu/g (p<0.05); for psychrophil microorganism count, as 3.92 and 5.90 log cfu/g; for total coliform bacteria count, as 2.04 and 1.47 MPN/g (p<0.05) and fecal coliform bacteria count, as 0.35 and 1.48 log MPN/g (p<0.05).

After raw trout meatball had been prepared, the pH value of MAP₁ group, on the initial day (mixture) and on the 9th day which the sensory spoilage had been seen; were 6.18 \pm 0.01 and 6.04 \pm 0.01 (P<0.05) and sensory analyses values were detected between 9.58 \pm 0.38 and 5.42 \pm 0.58 (P>0.05). On the initial day and at the end of the 9th day, the microbial values of our MAP₁ group samples were determined relatively; for total mesophilic aerobic bacteria count, as 4.42 and 6.61 log cfu/g (P<0.05); for psychrophil microorganism count, as 3.92 and 6.77 log cfu/g (P<0.05); for coliform count, as 2.04 and 2.30 log MPN/g (P<0.05); for fecal coliform count, as 0.35 and 2.18 log MPN/g (P<0.05). After raw trout meatball had been prepared, the pH values of MAP₂ group were 6.18 \pm 0.01 and 6.02 \pm 0.02 (P<0.05) and sensory analyse values were detected between

9.58 \pm 0.38 and 5.75 \pm 0.32 (P>0.05), on the initial day (mixture) and on the 9th day which the sensory spoilage had been seen. The microbial values of our MAP₂ group samples on the initial day and on the end of the 9th day were determined relatively; for total aerob mesophilic bacteria count, as 4.42 and 5.0 log cfu/g (P<0.05); for psychrophil microorganism count, as 3.92 and 5.87 log cfu/g (P<0.05); for total coliform count, as 2.04 and 1.60 log MPN/g (P<0.05); for fecal coliform count as <0.35 and 1.60 log MPN/g (P<0.05). Also, we have never come across with *Salmonella* spp. and *S. aureus* during our study.

The gas permeability of the package material that was used in this study was determined as 31.5 cm³/m²/d bar for O₂ at +4°C; the water steam permeability was determined as 7.27 g/m²/bar.

Generally, one of the first chemical changes on the fish meat, is the pH changes. pH value of the fish is changeable according to the species. So, this is not an exact criteria for the detection of freshness and quality. This is used as a supporter for other quality control parameters. It is defined that for fresh fish meat, the pH value is 6-6.5 and this can exceed according to the storage time. The limit pH degree for consuming is 6.8-7.0 for fish (Varlik *et al.*, 1993). For the tilapia fillets which have been packed with normal air at 4°C, the pH value was analysed as 6.6 at the 9th day of the storage (Reddy *et al.*, 1994). For the herring vegetable salads which were packed under modified atmosphere conditions and stored, it was seen that the packaging rules and the changes of quality do not effect the product's pH significantly (Ahvenainen *et al.*, 1990). It is defined that; the pH values that analysed at the end of 24 h storage with different gas mixture packages, do not differ from the control group samples significantly. But, at the end of 21 days, it was seen that these samples differ from the control group samples very much (pH 8.03). In our study, the pH value decrease of the raw trout meatball has been caused by the acidity of the spices and boiled and pounded wheat.

If a kind of product is acceptable for its quality parameters but not acceptable for its sensory features, then it is not proper to consume this product (Varlik *et al.*, 1993). It has been determined that, after storing the Morina fillets at MA, the fillets were better than the control group at the 6th and 9th days of the storage. It has been notified that, the fillets in the control packages were spoilt at the 9th day (Woyewoda *et al.*, 1984). In one of the studies of Reddy *et al.* (1994) for the tilapia fillets, at the end of the 9th day of the storage at +4°C with normal air, fillets were spoilt according to the sensory evaluation. Brown *et al.* (1980) have been detected that, for rockfish and salmon fish, after packaging with MA, sensory control samples differ very much from the packed with gas samples at the end of the 7th day. Çetin and Bostan (2002) determined that control group samples of meatballs prepared from red meat spoil at the 4th day of

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Table 1: pH and Sensory analyses results of raw trout meatball packed under Modified Atmosphere

Analysis		Raw	Cooked	Mixture	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11
pH	Control	6.29±0.01	6.33±0.01	6.18±0.01	6.15±0.02	6.14±0.01	6.03±0.01	6.01±0.01	6.06±0.01	5.80±0.01
	MAP ₁			6.18±0.01	6.15±0.01	6.14±0.01	6.07±0.01	6.03±0.01	6.04±0.01	5.97±0.01
	MAP ₂			6.18±0.01	6.11±0.01	6.14±0.01	6.06±0.01	6.03±0.01	6.02±0.02	6.03±0.01
Sensory	Control	9.80±0.38	9.33±0.26	9.58±0.38	9.42±0.58	7.75±0.27	5.33±0.41	4.83±0.52		
	MAP ₁			9.58±0.38	9.25±0.52	7.67±0.27	5.91±0.58	6.50±0.32	5.42±0.58	4.17±0.41
	MAP ₂			9.58±0.38	9.50±0.32	7.83±0.26	7.25±0.27	7.17±0.26	5.75±0.32	4.58±0.49

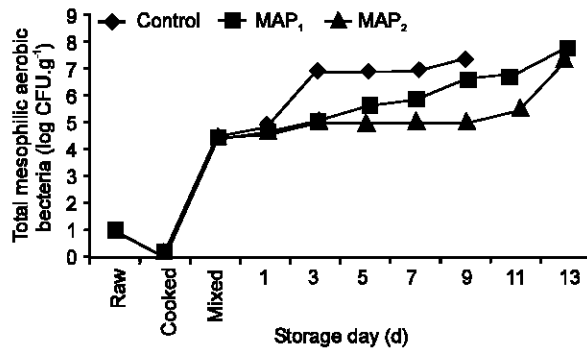


Fig. 1: Total mesophilic aerobic bacteria (log cfu/g) analyses results of raw trout meatball packed under Modified Atmosphere

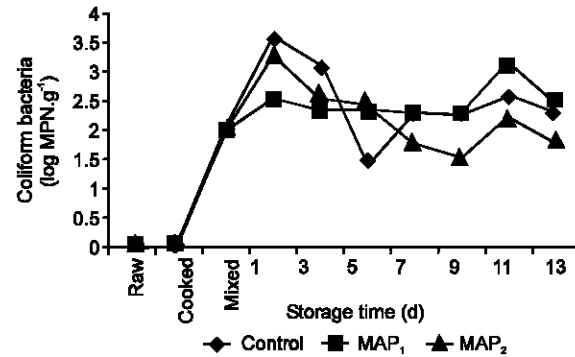


Fig. 3: Coliform bacteria (log MPN/g) analyses results of raw trout meatball packed under Modified Atmosphere

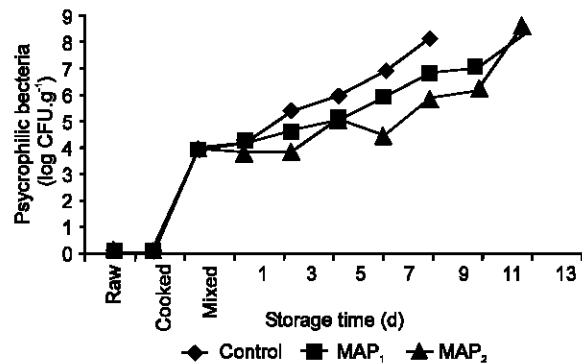


Fig. 2: Psychrophilic bacteria (log cfu/g) analyses results of raw trout meatball packed under Modified Atmosphere

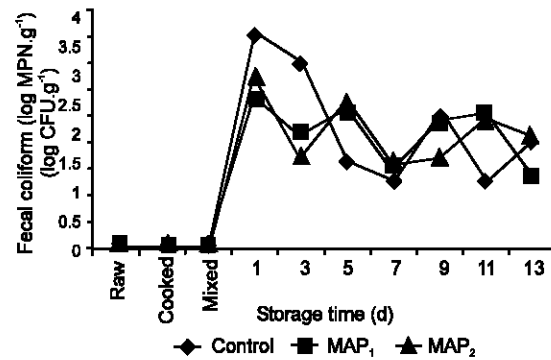


Fig. 4: Fecal coliform (log MPN/g) analyses results of raw trout meatball packed under Modified Atmosphere

the shelf-life. In our study, it was determined that control group samples are appropriate to consume till 5th day and both of the groups that operated by MAP are appropriate to consume till 9th day according to sensory. The limit value of the total aerob mesophilic bacteria is 7-8 log cfu/g. Thus, the standards and regulations suggest much lower values generally (Olafsdottir *et al.*, 1997). According to Anonymous (1992), the limit values for fresh or frozen seafood are 6-7 log cfu/g for total aerob mesophilic bacteria count, 160- 210g for coliforms and 9-12g for *E. coli*. Yildirim *et al.* (2005) defined that, in the study of determinations for the quality changes of the red meat Raw trout meatball; The total aerob mesophilic bacteria count is 4.74 log cfu/g, *S. aureus* number is

4.64 and coliform number is 3.04 log cfu/g in the control group samples. They also did not come across any *Salmonella* spp. Çetin and Bostan (2002), defined that, for the red meat raw trout meatball stored at cold, the initial and 10th day values are 6.15 and 9.59 log cfu/g for total aerob mesophilic bacteria, 4.41-7.91 log cfu/g for coliform bacteria, 3.98-6.91 lof cfu/g for *S. aureus*. In one of the studies related with the hamburger meatballs that packed and stored at 3°C under MA, because of the initial excess of microorganisms, at the 7th day of the storage total aerob mesophilic bacteria count was 6 log cfu/g, at the 13rd day the number was higher than 7 log cfu/g which was the limit value (Çiftçioglu and Gün, 1994). Dondero *et al.* (2004) did not come across with

Salmonella spp. and *S. aureus* in any step of the storage for their study which was about the cold storage of vacuum packages of smoked somon.

Küplülü *et al.* (2003) determined that; for different samples of red meat raw meat balls that were transported from different places of Ankara (Turkey), total aerob mesophilic bacteria count was 6 log cfu/g, coliform bacteria count was 3-5 log cfu/g, average number of *S. aureus* were 4 log cfu/g. They also did not come across any *Salmonella* spp. For the tilapia fillets which were packed with normal air at 4°C and stored for 13 days, the total mesophilic aerobic bacteria count was found as 9.6 log cfu/g and total anaerob bacteria load was found as 8.7 log cfu/g (Reddy *et al.*, 1995). As for the catfish which were packed with normal air at 0°C, as the initial total mesophilic aerobic bacteria count was 5.88 log cfu/g, this was determined as 8.22 log cfu/g at the 8th day. In another study for rainbow trout and herring fillets, after 6 days of storage with vacuum and wrap packaging, total aerob mesophilic bacteria count was 4 log cfu/g and more, for MA groups, it was about 3 log cfu/g. In the study of hamburger meatballs that stored at 3°C with MA, it was seen that the coliform amount increased logarithmically till the 13rd day and became lower than the initial degree of 5.64 log cfu/g for the rest days (Çiftçioglu *et al.*, 1994). Akkus *et al.* (2004), determined that the shelf-life of the anchovy meatballs is 9 days. In our study, there was no significant microbiological load for fresh and cooked fish. Especially, the microbiological content of the product has been increased after adding spicy mixture. Also, it has been thought that the reasons for the increase of coliform amount had been caused by packaging material, by washing water and by different people who kneaded. Elmali and Yaman, (2005) have been detected microbiological quality of raw meat balls produced in Turkey and they have concluded that consumption of raw meat ball poses a risk of food borne infections or toxication due to its raw meat for human health.

Conclusion: In our study, using cooked fish meat affected the shelf-life of the raw trout meatball by extending the duration. Although, it was determined that raw trout meatball had spoiled at the 7th day for microbiological control groups and at the 11th day of storage with MA, they lost their consuming features. Thus, our study is proper to the literature datas. It has been determined that normal vacuum packaged products save their freshness till 5 days and MAP₁ and MAP₂ samples save their freshness till 9 days.

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