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Determination of Microbiological Quality of Fish Burgers Enriched with Orange Peel Extract

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Abstract

In this study, the effect of orange peel extracts (OPE) on the microbiological quality and shelf life of fish burgers was determined. To complete this objective, orange peels were extracted by using 70% ethanol. Fish burgers were divided into three groups. First group (G1) were treated with 1% concentration of OPE, second group (G2) were treated with 2% concentration of OPE and third group were prepared without OPE known as control group. All fish burgers were stored at 4±1^{o}C for 15 days. Total mesophilic bacteria (TMB) and total psychrophilic bacteria (TPB) count were determined. Conferring to the results of analysis, the TMB count was 6.28 log CFU/g and 5.44 log CFU/g in G1 and G2 groups, respectively by the end of the storage period, whereas in control it reached to 7.42 log CFU/g. Total psychrophilic bacteria count was 6.8 log CFU/g and 5.96 log CFU/g in G1 and G2 groups, respectively by the end of the storage period while in control it was determined as 7.19 log CFU/g. So it was concluded that the TMB and TPB counts in fish burgers treated with 1% and 2% OPE were significantly ($P<0.05$) lower than the control group throughout storage. Results of this study showed that the usage of OPE is an effective approach to prevent microbial growth in the fish burgers during the refrigeration storage.

Key words: Orange peel extract, psychrophilic bacteria, mesophilic bacteria, shelf life, fish burger

Research article
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INTRODUCTION

Fish has high quality nutrients like well-balanced proteins, great amount of essential n-3 polyunsaturated fatty acids (PUFA), vitamins and minerals (P, Se, Mg, and I) (Atitallah et al., 2019). Fish fatty acids are much important to humans for the inhibition of cardiovascular diseases that's why fish is a good substitute of red meat protein which can cause heart diseases (Conner, 2000; Mozaffarian et al., 2005; Vicente and Torres, 2007). Seafood is the most perishable food so it's more susceptible to microbiological and enzymatic spoilage due to oxidative reactions (Metin et al., 2002).

Fish and readymade fishery products like fish burgers, fish fingers and fish meat balls are the much important amongst the globally dealt food item however also they are most fragile food products because of great amount of polyunsaturated fatty acids. To stop the decline in fish product quality because of microbial growth and lipid oxidation, artificial food preservers like Butylated hydroxyl toluene, Butylated hydroxyanisole, and Tert-Butylhydro quinine are used (Frankel, 1993). These artificial antioxidants can be dangerous for human health as they could be carcinogenic (Ito et al., 1986). Therefore, it is essential to find out natural antioxidants, specifically plant derived compounds to increase the shelf life of fish and readymade fishery products through preventing and deferring lipids oxidation (Sanchez-Alonso et al., 2008). Many plants, herbs and spices which have been used in several food formations have antioxidant activities (Liu et al., 1992). The bioactive compounds naturally existing in plants have been proven to have antimicrobial, antioxidant and anti-carcinogenic properties (Ahn et al., 2007; Kanatt et al., 2007).

Citrus fruits are the globally prevalent fruit type by annual yield of more than 100 million tons (Shehata et al., 2021), and their industrial usage produce a great content of fruit wastes include pulp, seed and peel, which has come to be an ecological threat if not used and predisposed well. The peels of these citrus fruits are not eatable, they have enough bioactivities mostly antioxidant, antimicrobial, and anti-carcinogenic (Kumar et al., 2020). Citrus fruit waste have great importance because it consists high content of several flavonoids, polyphenols, carotenoids, sugars, dietary fiber, ascorbic acid, essential oils, and few other minor elements (Sharma et al., 2017).

Total bioactive compounds are considerably higher in peels of oranges, lemons, and grapefruits as compared to their fruits (Ramful et al., 2011). Therefore Recycling these fruit byproducts in a proper manner might give substantial economic advantage to food manufactures because of their valued industrial and nutritious specificities which provide possible health security for consumers, similarly, minimalize the worst ecological effect and enhance worth of this remains. It was also reported that many of these fruit byproducts might be utilized as functional elements during processing and manufacturing of healthy food products because of its high amount of bioactive compounds worked as natural stabilizers, antimicrobials, antioxidants, flavorings, pigments, and emulsifying mediators (Marın et al., 2002; Ayala-Zavala et al., 2011).

Approximately 25 to 30% fruits productivity are non-eatable products like peels and seeds (Ajila et al., 2010). Generally, fruit peels comprise about 50 to 65% of the whole fruit mass and known as the major byproduct of fruit industry. If they are not administered properly, they converted to waste and caused bad odor, insect's production leads to severe ecological pollution (Mandalari et al., 2006). Orange (*Citrus sinensis*) is a citrus fruit links to the family "Rutaceae", and orange peel waste by-product contained many nutrients, high content of fiber, vitamin C, vitamin B6, calcium, folate and other essential nutrients and minerals, which works as antimicrobial and antioxidants (Okwu, 2008). In this study it was aimed to determine the antimicrobial effects of orange peel extract in fish burgers.

MATERIALS and METHODS

Orange peel extraction

Oranges *(Citrus sinensis)* were bought from a local market and they peeled prudently by using a sharp knife. Then the peels were washed through distilled water and dried for 48 hours at 45°C. After that, orange peels were converted into powder by grinding. Subsequently 10 gram powder of orange peels was added in 100 mL of 70% ethanol for extraction and put into ultrasonic water bath for 30 minutes. When powder was mixed after 30 minutes it was filtered. Ethanol was vaporized by means of a rotary evaporator at 50°C under vacuum and then orange peel extract was obtained (Ucak and Khalily, 2022).

Preparation of fish burgers

Fish (anchovy) bought from local market, Nigde, Turkiye and quickly brought to laboratory in styrofoam boxes filled with ice. In the laboratory, fish were cleaned with distilled water, then cut the head, clean the fish gut, cut into fillets and minced them by using a meat mixer. After that minced fish meat was distributed into three groups known as Control (C), Group 1 (G1) and Group 2 (G2). Subsequently mixture for fish burgers formation was prepared by using the process proposed by Tokur et al. (2004) by means of slight modification. All spice ingredients were mixed with minced fish meat separately in each group, and two groups were supplemented with 1% and 2% concentration of orange peel extract in Group 1 and Group 2 respectively and mixed completely by using domestic mixer. Orange peel extract was not added to the control group. After mixing fish burgers were shaped from each group by using shaper. Each group has sixty burgers and each burger have a weigh of 50 g, comprising of 87.8% minced fish meat, 4% wheat flour, 6% corn flour, 0.2% onion powder, 0.2% garlic powder, 1.2% salt and 0.6% sugar. Then, burgers were kept at 4°C in refrigeration up to 15 days for periodic investigation of microbial growth (Ucak et al., 2011).

Microbiological analyzes

Fish burgers samples from each group were taken for the evaluation of microbiological analyses including total mesophilic and psychrophilic bacteria counts. 10 gr of fish burger from each group was taken and mixed with 90 mL of Ringer solution for 3 minutes to make it homogenized. More dilutions were prepared up to 10^{-8} concentration and then 0.1 mL from each dilution was spread on plate count agar plates (ICMSF, 1982). Then these plates were incubated at 8^{o}C for 7 days for total psychrophilic bacteria counts and at 37^{o}C for 24-48 h for total mesophilic bacteria counts, respectively.

Statistical analysis

Statistical analyzes were done with SPSS software (Statistical Analysis System, Cary, NC, USA) and Duncan multiple comparison test (One-way Anova at $P<0.05$ significance level) were applied for data comparison.

RESULTS and DISCUSSION

Total mesophilic bacteria count

According to the recommended limited value (7 log CFU/g) for fresh fish (ICMSF, 1986), the results of this study specify that fish burgers which were preserved by using OPE of 1% and 2% concentration were in good quality throughout the storage period as compared to control group. The influence of OPE on the growth of mesophilic bacterial growth on fish burgers is given in Figure 1. Total mesophilic bacterial count was 2.17 log CFU/g at the beginning of storage which increased to 6.28 log CFU/g and 5.44 log CFU/g in G1 and G2 groups, respectively at the end of the storage period, whereas in C it reacheD to 7.42 log CFU/g. It was perceived that the total mesophilic bacteria counts in fish burgers prepared with 1% and 2% concentration of OPE were significantly ($P<0.05$) lower than the C group through the storage period.

It was noticed in another study in which peppermint essential oil was used with chitosan coating to preserve mackerel fish meat balls and it was concluded that Total mesophilic bacteria count in C and CF groups were 6.24 and 5.93 log CFU/g respectively on the 18th day of storage, the lowermost Total mesophilic bacteria count 5.18 log CFU/g was found in fish meatballs samples coated with chitosan added with 1% peppermint oil emulsion (Ucak and Afreen, 2022). There was a study in which scientists reported that rosemary and laurel essential oils decelerated the growth of bacteria however in that study total bacterial count of the trout fish meatballs supplemented with rosemary and essential oils was found to be significantly higher (5.24 log CFU/g) than the present study (Keser and İzci, 2020).

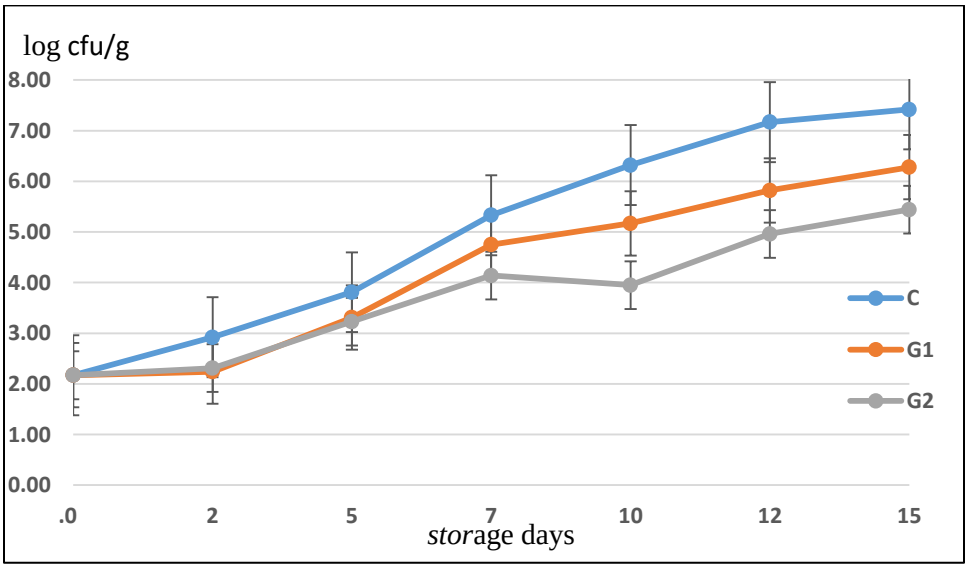

Figure 1. Effect of OPE on the growth of total mesophilic bacterial growth in fish burgers

Total psychrophilic bacteria count

The main reason of fish and fishery products spoilage is bacterial growth. The influence of OPE on the growth of total psychrophilic bacterial in fish burgers is given in Figure 2. Total psychrophilic bacteria count was determined as 2.04 log CFU/g at the beginning of storage which increased to 6.8 log CFU/g and 5.96 log CFU/g in G1 and G2 groups, respectively at the end of the storage period. In control group this value reached to 7.19 log CFU/g. Total psychrophilic bacteria count value in fish burgers treated with 1% and 2% OPE was significantly ($P<0.05$) lower than the control group during the storage period.

It was noticed in another study in which peppermint essential oil was used with chitosan coating to preserve mackerel fish meat balls and it was concluded that Total psychrophilic bacteria count in C and CF groups were 7.59 and 7.15 log CFU/g on the 18th day of storage, while 6.14 log CFU/g in the PMO group.

The lowest Total psychrophilic bacteria count was found in fish meatballs samples coated with chitosan added with 1% peppermint oil emulsion (Ucak and Afreen, 2022). It was observed in another study, in which rosemary extract was added with concentrations of 0.4% and 0.8% in the formation of mackerel fish burgers, and the total bacterial growth during storage was lesser in rosemary concentrated groups as compared to the control group (Ucak et al., 2011). It was found in one study that initial psychrophilic bacterial count was 4.22 log CFU/g in trout meat (Keser and Izci, 2020). Different antioxidants including thyme, laurel, sage and green tea were used to preserve fish burgers and initial count of psychrophilic bacteria was found to be 4.90 log CFU/g, and it was detected at the end of the study that total psychrophilic bacteria in those groups which were supplemented with antioxidant during the frozen storage was lesser as compared to the control group (Ozogul and Ucar, 2013).

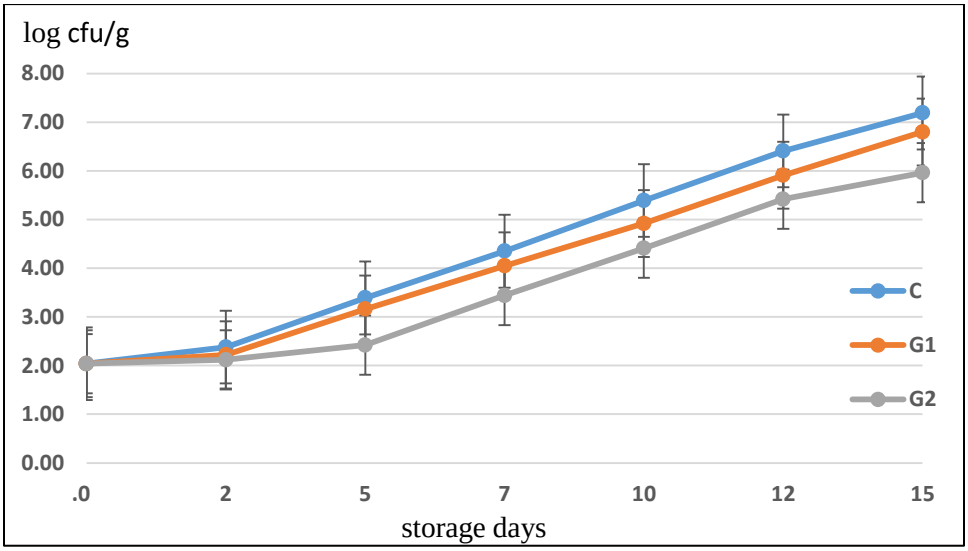

Figure 2. Effect of OPE on the growth of total psychrophilic bacterial growth in fish burgers

Many previous studies shows that microbiological growth was considerably ((P<0.05) influenced by adding pomegranate peel extract and edible coating. It was noticed that those fish fillets which treated with pomegranate peel extract have longer shelf life throughout refrigerated storage and have less growth of total viable count as compared to control samples (Prashanth et al., 2000; Shahidi and Naczk, 2004; Tomas-Barberan et al., 2001). Rosemary extract was also establish efficient in controlling the microbial growth of in fishery products (Sacchetti et al., 2005).

CONCLUSION

It was concluded from the present study that supplementation of fish burgers with orange peel extract suppressed the bacterial growth in the fish burgers. Throughout the 15 days of storage period, fish burgers incorporated with 1 and 2% orange peel extract presented the lowest total mesophilic bacteria and total psychrophilic bacteria counts as compared to the control group. Moreover, these bacterial count did not exceed the limit values of microbiological quality of fish.

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