Preservation of fish using instant extract of green and black tea

Pengawetan ikan menggunakan ekstrak instan teh hijau dan teh hitam

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Abstract

The use of illegal and hazardous preservative agents such as formalin by traditional fishermen and trader has become a problem in Indonesia. In this research, instant extract powder of green tea and black tea were used as natural preservatives for fish. Both substances are known for their anti-bacterial and anti-oxidative activities due to the presence of various types of polyphenols, particularly the catechin groups. The tea extract powder was produced by drying using a spray drying device. The two different tea extract powders were tested separately, and in combination. The tests were done by dipping pieces of fresh indian mackerel fish (Rastrelliger kanagurta), *also known locally as kembung banjar, in the solution containing the tea extract. The microbial tests were done using total plate count (TPC) method. The result showed that instant green and black tea extracts was capable in suppressing bacterial growth in fish. When the two substances were mixed together, the preservation activities were found to be stronger than if they were used separately. In addition, histamine and total volatile base nitrogen (TVB-N) tests which are regularly used to test the freshness of fish also showed substantial improvement in the quality of the fish sample. The sensory tests also showed that the extracts were capable of improving the freshness of the fish sample.*

Keywords: *tea extract, green tea, black tea, fish preservation, fishermen*

Abstrak

Penggunaan bahan-bahan pengawet yang ilegal dan berbahaya, seperti formalin, oleh para nelayan dan pedagang ikan tradisional Indonesia telah menjadi permasalahan di Indonesia. Dalam penelitian ini, serbuk ekstrak instan dari teh hijau dan teh hitam digunakan sebagai pengawet ikan. Kedua jenis teh ini diketahui memiliki sifat antibakteri dan juga antioksidan karena mengandung berbagai senyawa polifenol, terutama katekin. Serbuk ekstrak teh dihasilkan dengan pengeringan menggunakan alat *spray-dryer*. Kedua jenis ekstrak teh dicoba secara terpisah dan dalam kombinasi. Pengujian dilakukan dengan merendam potongan ikan kembung banjar (*Rastrelliger kanagurta*) segar di dalam larutan ekstrak teh. Uji mikrobiologis dilakukan menggunakan metode *total plate counts* (TPC). Hasil penelitian menunjukkan bahwa ekstrak instan teh hijau dan teh hitam mampu menekan pertumbuhan bakteri pada ikan. Aktivitas campuran ekstrak instan teh

hijau dan teh hitam dalam pengawetan ikan bersifat sinergis, lebih kuat dibandingkan jika digunakan sendiri-sendiri. Sebagai tambahan, uji histamin dan *total volatile base nitrogen* (TVB-N) yang biasa digukan untuk menguji kesegaran ikan menunjukkan perbaikan kualitas ikan. Uji inderawi menunjukkan juga bahwa kedua ekstrak instan teh tersebut dapat memperbaiki kesegaran ikan.

Kata kunci: ekstrak teh, teh hijau, teh hitam, pengawetan ikan, nelayan

INTRODUCTION

Indonesia has a massive sea area of about 7.9 million km^2 and is one of the largest fish producers in the world. Data from Directorate General of fisheries (2007) showed that the total production of fish being caught in Indonesia in 2006 reached 4.5 kilo-ton. Unfortunately until now, the large quantity of this natural resource still cannot be used optimally because of many factors; such as facilities provided by the government, and lack of knowledge of preservation particularly for traditional fishermen. Lack of facilities, such as roads for transportation, and nearby fish market equipped with cold storage facilities still become serious problems. Furthermore, the best way to preserve fresh fish until now is using ice using a comparison of 1:1 (Wibowo and Yunizal 1998). However, another reason is that not all fishermen could purchase the ice because of economic problem or availability. Lack of knowledge and economic problem have led them to the use of dangerous and illegal chemical to be used as food preservatives such as formalin.

In this research, instant green tea and black tea extracts acquired from the leaves of *Camellia Sinensis* var. *assamica* were used as natural preservatives agent to preserve the freshly captured fish. Both green and black teas are known to contain substances that possess strong antibacterial activities which corresponds to the presence of polyphenol compounds (Sakanaka *et al.*, 2000; Ooshima *et al*., 1993; Jain *et al.*, 2006; Okubo *et al.*,1991; Neyestani *et al.*, 2007). In green tea, major antibacterial polyphenol compounds are the catechin groups (Ooshima *et al*., 1993; Bhagwat *et al.*,2003). However, black tea contains also catechin groups at lower percentage but higher in tannins which can also act as important antibacterial polyphenolic compounds (Okubo *et al.*, 1991; Bhat *et al.*, 1998). In this investigation, the instant green and black tea extracts were tested for their efficacy when they were used separately, as well as simultaneously in a combined mixture.

Under normal circumstances, the fishermen could utilize ice to preserve the newly caught fish. However, in many areas, ice is simply unavailable. Thus an alternative to the use of ice is needed for these fishermen particularly those who live in remote coastal regions. Tea, whether in the forms of dried leaves or its extracts, present a good alternative to the use of ice. The outcome of this research is hoped to give positive contribution on the quality of fish being caught by traditional fishermen. It is noted, however, that the finding in this research has a wide potential to be applied

in various industries particularly food industries to preserve various food products. Further studies in this matter should be undertaken to explore the potential of green and black tea extracts to be used as natural preservative agents.

MATERIALS AND METHODS

Fish sample preparation

The fish samples used were the Indian Mackerel (*Rastrelliger kanagurta*) which were also known locally as the Kembung Banjar. The fish were bought from PT. Arta Mina Tama, Muara Baru, North Jakarta. The size of the selected fish was medium, of which weights were approximately 150g. The fishes were already frozen upon purchase, and brought to the laboratory inside a cooling box, and later frozen again inside a Sanyo chest freezer at -18^{0}C. Before being used for the study, all the fish was washed. Prior to use, the fish was thawed at a temperature of 0^{0}C (±1^{0}C) for 12 hours inside a refrigerator. Small pieces of fish (5g) were then cut aseptically inside a sterile biosafety cabinet. The samples were then ready to be used.

Green and black tea extracts preparation

The extraction process of the dried tea leaves involved grinding of the samples until they passed 0.5 mm sieve. Following this, 50g of the powder was then extracted by using aquadest at 70^{0}C for 15 minutes with continuous stirring. The mixture was then pre-filtered first using nylon cloth followed by sieving using 20 µm filtration, and then filtered by membrane filtration process at 3 different stages of pores sizes, starting from 5 µm, 1 µm and finally 0.5 µm. The filtration would not only provide solutions that were ready for spray drying, but also provided sterilization stage of the final extract.

The filtered-tea extract was then dried using a spray dryer with inlet temperature of 270^{0}C and outlet temperature between 80-85^{0}C, to become instant tea extracts (GEA Niro A/S 3049).

The strength of antibacterial activities of both green tea and black tea extracts was evaluated based on the concentration of polyphenols that are contained within the reconstituted extracts. No attempt in this study to differentiate or to compare the different types of polyphenols that existed in the two different tea extracts. In this work, the tea polyphenols from the two types of tea extracts were tested using Folin-ciocalteu method with gallic acid as the standard.

Method of preservation

For the fish sample dipping experiment, three different concentrations of tea polyphenols were prepared i.e. 1000, 3000 and 6000 ppm of polyphenol.

The pieces of fish samples were dipped into preservatives solution (instant tea extract) for 15 minutes. One sample also put in an empty sterile container as blank. Following the dipping process, the fish samples were then allowed to stand inside room temperature for 0 hour, 6 hour and 12 hour. This delay would simulate the storage period of the fish following capture in the sea before processing. The 0 hour (i.e. no delay) samples were included as a point of comparison for the growth of bacteria in samples with longer delay periods.

Microbial evaluation

When the delay time was over, the fish samples were then put in a blender and added with 45 ml of 2% Buffered Peptone Water (BPW, Tecra, Canada). The ratio of sample to BPW solution was 1:9. The mixture was blended for 3 minutes until the solution was homogenized. The solution was then ready for the total plate count analysis.

The activity of microorganisms is the main factor limiting the shelf life of raw fish. The aerobic plate count (Total Plate Count or TPC) would indicate the level of microorganisms in a product. The aim of using this method was to observe bacteria's growth among the unpreserved fish and the fish treated with preservatives at different concentrations. In the process, 23.5 g of Standard Method Agar (SMA) powder was weighed and put into an Erlenmeyer flask containing 1 liter of aquadest. The flask was brought to a boil with frequent stirring to dissolve. The media was also sterilized by autoclaving at 121^{o}C for 15 minutes. The media was cooled to 44-46^{o}C for not more than 3 hours prior to use. Following this, 1 ml of the desired dilution, for example 10^{-5}, was pipetted and put into a sterile Petri dish. The standard method agar was poured into the Petri dish about 12-15 ml (cooled to 45 ± 1^{o}C). The sample solution inside the Petri dish was mixed immediately by making movement in the form of "8" on the table. After the agar solidified, the Petri dish was incubated in the upside down position for 24 ± 2 hours at 37 ± 1^{o}C. These steps were repeated with the next serial dilutions. At the end of the incubation period, the numbers of colony forming units were then counted and reported.

Total volatile based-nitrogen (TVB-N) analysis

The TVB-N analysis was carried out to evaluate the quality of the preserved fish in comparison to the unpreserved ones in terms of the production of volatile based nitrogen compounds caused by bacterial activities. The TVB-N could provide measures of the degree of fish decomposition due to bacterial activities which typically occurred after prolonged storage of more than one day. The TVB-N level contributes to the sensory characteristic usually noted as the fishy odor that occurred in spoiled fish.

For the TVB-N analysis, 25g of minced homogenized sample was weighed and blended with 75 ml TCA (Trichloroacetic Acid) 7.5% for 2 minutes. The mixture was filtered through filter paper into an Erlenmeyer, or centrifuged until clear filtrate was obtained. 1 ml boric acid 1% was put into the inner chamber of the same Conway dish. Meanwhile, using another pipette, 1 ml filtrate was put into the outer chamber of a Conway dish and 1 ml of potassium carbonate was added into the opposite side of the outer chamber. In this time, the Conway dish should be put at an angle to avoid both solutions in the outer chamber to get mixed up. A few drops of potassium carbonate were given in the edge of the Conway dish to have an air tight covering. Both solutions in the outer chamber of Conway dish were mixed carefully for a minute. Along with the sample, blank was made by replacing filtrate with TCA 5% solution. All Conway dishes which had been prepared above were incubated at 35^{o}C for 2 hours or at room temperature overnight. After being incubated, boric acid in the inner chamber of each Conway dish

of blank was titrated with HCl 1/70N until the color of boric acid turned pink. Afterward, boric acid in the Conway dishes of filtrate was titrated until similar pink color to Blank was acquired. Total Volatile Bases was calculated based on this titration data.

Histamine analysis

Histamine analysis is another parameter that can evaluate the freshness of fish. The toxic histamine is formed by the breakdown of histidine, which is found at fairly high levels in the muscles of fish belonging to the Scrombroidae family. Histamine formation is induced by high temperatures after harvest, thus the analysis is relevant for fish freshly caught in the tropical regions where high temperature exists. Poisoning due to the presence of histamine is commonly caused by consumption of spoiled fish.

The histamine was extracted from it's the fish sample by using methanol. 10 gram of fish sample was extracted in 50 ml 100% MeOH solution. The mixture was blended for 2 minutes. The sample solution was heated to 60ºC in a water bath. Then it was cooled down to 25 ºC. The sample solution was diluted to 100 ml with aqua DM. Then the solution was filtered through a Whatmann filter paper. The sample was ready for purification. The purification used ion exchange chromatography method, where the histamine from the sample extract would pass through the column but the histidine and other free amino acids were retained. Purification process began by transferring resin to a Dower column with 8 cm in height. One ml sample extract was added to 8 cm prepared Dower column. Immediately initiate flow by adding 5 ml aqua DM.

A large volume of water was added into a flask containing 5 ml of HCl 1N until 35 ml has been eluted. The solution in flask was then diluted to 50 ml. The 50 ml sample was ready for assay. 5 ml of eluted sample was added with 10 ml 0.1 N HCl and 3 ml 1N NaOH. One ml 0.1% OPA solution was added and then it was incubated for 4 minutes. 3 ml of 3.57 N Phosphoric Acid was added into the mixture. The histamine content in the column effluent was detected by fluorescent detection. λ ex = 350 nm and λ em = 444 nm. The quantitative histamine concentrations were determined by comparing sample fluorescence values to a standard curve.

Sensory analysis

Sensory analysis was done to measure, analyze and interpret reactions to characterized the food as perceived through the senses of sight, smell, taste, touch and hearing. Descriptive test is one of many sensory analysis tests. This type of testing is concerned with obtaining *subjective* data, or how well products are described and likely to be accepted. For this test session, the preserved fish fillet sample has to be steamed and then being evaluated for odor and flavor. This test also could provide complete sensory descriptions of products, determine how the preservatives concentration, as tea polyphenol affects product characteristics, and identify key sensory attributes that promote product acceptance. In this research, the untrained panelists were asked to give the range value of certain aspect quality of fish.

RESULTS AND DISCUSSION

Microbial analysis

Microbial condition of live fresh fish is normally considered to be sterile. However, microorganisms could be found on the skin, in the gills and also, naturally in the guts. Normally the number of microorganisms on the skin is in range of 10^2-10^7 cfu (colony forming units)/cm^2 and in the gills and intestine between 10^3-10^9cfu/g (Liston 1980). Other than that, the microorganism may also be introduced from the surrounding area following the capture of the fish.

Results from the dipping of the fish sample in instant green tea extract showed reduction of bacterial growth on the plate count agar which showed that some preservation action have already taken place. It showed that the higher the concentration of green tea polyphenol, which was measured by folin-ciocalteau method, the fewer the bacteria that grew on plate count agar (Fig.1). The antibacterial compounds in green tea are from catechins group which could inhibit the growth of bacteria in fish. The galloyl moiety which presents in the Epigalocatechingallate (EGCg) and Epicatechingallate (ECg) but absents from the Epigalocatechin (EGC) and Epicatechin (EC) is believed to have antibacterial activity.

Similar suggestion from two different research concluded that the antibacterial of catechins could originated from the ability to complex with extracellular proteins and soluble proteins and to complex with bacterial cell walls (Haslam 1996 and Stern *et al.*, 1996). However, this interaction mechanism between catechins and bacterial membrane was still being investigated (Yong-su, 2002).

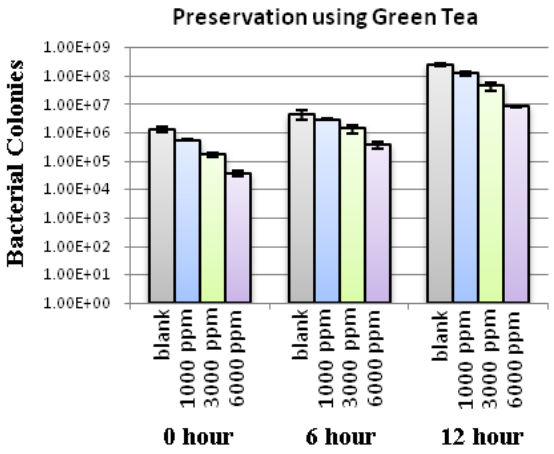

FIGURE 1
Bacterial growth in fish preserved with instant green tea extract

The instant black tea polyphenol treatment also produces similar result, that there was a decrease in bacterial growth after dipping. The higher the concentrations of black tea polyphenol were, the fewer were the bacterial growth on the plate count agar (Fig. 2). The antibacterial compounds from black tea are from the theaflavin, thearubigin and tannin groups which are polymer from catechins. These compounds still have the galloyl moiety, which are believed as the antibacterial compound. Study of tannins as antibacterial agent were already done (Scalbert, A. 1991; Tirang *et al.*, 2007; Yu W. and Chi-Tang H., 2010).

However, the mechanisms of inhibition towards the growth of bacteria by black tea polyphenols until now were still studied. The action of tannin as antimicrobial activities in many literatures are believed to be due to digestibility that leads to the inactivation of peptidoglycon protein compound (bacterial cell wall), cell membrane disruption, and iron metal ion chelation, which is an essential micronutrient required for bacterial growth (Zoetendal *et al.* 2005; Stern *et al.* 1996; and Scalbert 1991).

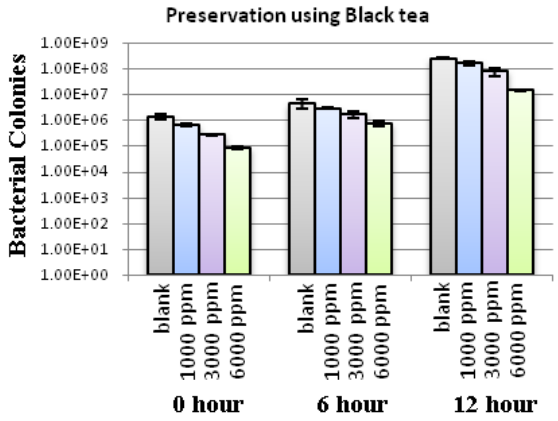

FIGURE 2
Bacterial growth in fish preserved with instant black tea extract

The reduction of total number bacteria colonies by using instant green tea was stronger compared to the instant black tea extract. The used of mixture of both instant tea extract were also tested. The results are shown in Figure 3. The data showed that the combination of both types of tea yielded a stronger reduction of forming bacterial colonies. However, further study is still needed to confirm if there were any synergisms between both instant tea extracts. It might also be argued that, upon comparison of Figure 1 and Figure 3, the action of instant green tea extract polyphenol seemed to be more dominant in the preservation action.

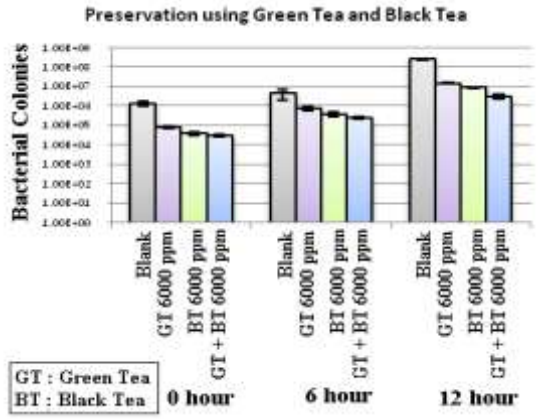

FIGURE 3
Bacterial growth in fish preserved with instant green tea and black tea extracts

TVB-N analysis results

One of the most widely used measurements for seafood quality is the Total Volatile Basic Nitrogen (TVB-N). Comparison of the chemical compounds developing in naturally spoiling fish and sterile fish has shown that most of the volatile compounds are produced by bacteria. Enzymes from spoilage microorganisms, particularly proteolytic enzyme, can metabolize the amino acids of the fish muscle producing a wide variety of volatile compounds resulting off-flavors and odors. The total chemical compounds of; trimethylamine or TMA (produced by spoilage bacteria), dimethylamine or DMA (produced by autolytic enzymes during frozen storage), ammonia (produced by the deamination of amino-acids and nucleotide catabolites), and other volatile basic nitrogenous compounds associated with seafood spoilage are measured in TVB-N test (Huss, 1995). The TVB-N value between the ranges of 30–35 mg/100 g for flesh are generally regarded as the limit of acceptability for ice stored cold-water fish. (Castro *et al*, 2004; Huss, 1988).

For the TVB-N analysis, only fish samples dipped in a combination of instant green tea extract (6000 ppm polyphenol) and instant black tea extract (6000 ppm polyphenol) were studied due to the time constrain of the studies. This mixture was believed to be the combination that would yield the best outcome. The results of the analysis are shown below (Fig. 4).

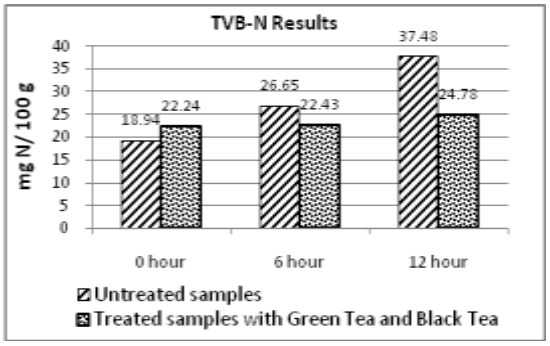

FIGURE 4
Results of TVB-N analysis for fish treated with combination of instant green and black tea extract

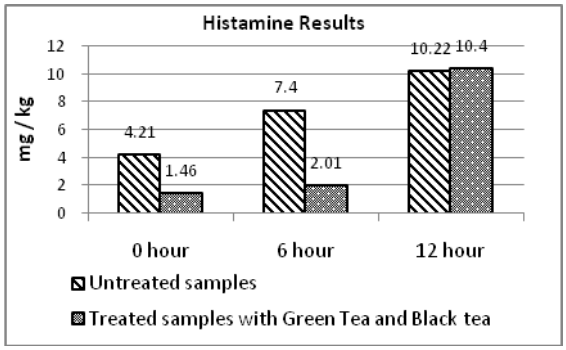

FIGURE 5
Results of histamine analysis for fish treated with combination of instant green tea and black tea extract

The result showed that fish without treatment had higher TVB – N level compared to dipped sample in the preservative. At 0 hour, the TVB-N value already developed and possibly the tea polyphenols were not yet working on the bacteria which were responsible to the presence of TVB-N value.

Histamine analysis results

Histamine fish poisoning or Scombroid fish poisoning is a foodborne chemical intoxication caused by the consumption of spoiled, or bacterially contaminated fish. The bacteria responsible for forming histamine may occur naturally in the living fish or may be added during handling. Moreover, these bacteria could rapidly grow when the storage temperature near 32.2^0C. The free histidine in fish was the substrate for microbial decarboxylation to produce histamine. Thus, histamine is more commonly the result of high temperature spoilage than of long term and relatively low temperature spoilage (Bell, 2003). It is for this reason that the histamine analysis was also carried out in conjunction with the antibacterial studies.

The fish dipped in preservative agent solutions at 0 and 6 hour exposure time showed significantly decreasing level of histamine. However, the histamine level at 12 hours storage time showed no decreasing value. In contrast, the treated fish had slightly higher level compare to the untreated fish sample level. Research from Ndaw *et al*. (2008) showed that the freshness of fish is also influenced by the variety of microflora in every fish. The characters of the microflora could be influenced by the fish's feeding habits, geographical location, the season, ocean temperature, etc. In tropical waters, where Indian mackerel mostly exist, the indigenous microflora may be more important histamine-producing organisms, and it could be worse if the fish had died before landing. Under these conditions, it is possible for histamine to be formed before the fish is brought to land and chilled.

Sensory analysis results

Sensory analysis was run in order to see the organoleptic impact from the antibacterial action. If the preservatives agent was able to inhibit the growth of bacteria at certain concentration, the acceptance of that sample would have to be examined. The

variations among the panelists in the response of the same level of certain test could contribute to a non-conclusive answer. The untrained panelists can differ widely in their response to their sensitivity to chemical response. However, the diversity of the value could be lowered if the trained panelists are involved and also the required trained panelist could be smaller. Since, there was no trained panelists, this research was using 30 untrained panelists. There were 4 kinds of attributes to be examined by the panelists; foreign taste, fishy odor, freshness, and overall acceptance. Each attributes measured the level of liking of the panelist of the samples. With this technique, statistics could be used to measure variability and to compare products. The fish samples were (i) the fish without treatment, (ii) fish dipped in green tea solution, (iii) fish dipped in black tea solution, and (iv) fish soaked in a mixture of green tea and black tea solution.

The results of this test showed that tea polyphenols could suppress the presence of fishy odor of fish samples (Fig. 6). The foreign taste however showed some significance possibly due to the astringent taste of tea in treated fish sample compared to the untreated ones. This taste is due to the presence of polyphenolic substances, and depends very much on the chemical structures. The more polymerized is the polyphenol chemical structure, the stronger is the astringent taste (Valentova *et al.*, 2001). On the other hand, in terms of freshness evaluation, panelists reported that treated fish samples had better freshness compared to the untreated ones. Furthermore, dipping of the fish samples in green tea extract solution was preferred compared to other fish samples.

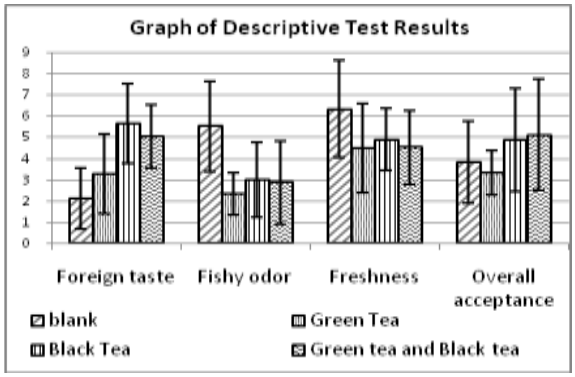

FIGURE 6
Results of the descriptive test
Note: The higher number on the graph indicates the stronger the characteristic of each test parameters

CONCLUSIONS

The instant green tea and black tea extracts have been shown to be capable of suppressing the bacterial growth in the fish sample. The antibacterial activities were shown to correlate with the polyphenol concentrations within the tea extracts. Hence, they are potential to be used as natural preservatives agent for fish particularly under circumstances relevant to traditional fishermen. In addition, the use of combination of both instant tea extract have been shown to be able to suppress the level of toxic histamine and TVB-N compounds in every parameters. However, the results showed that instant green tea extract might be more advantageous when used as preservatives. It does not produce strong flavor and foreign taste that might affect the sensory characteristics of preserved fish. The proposed method that can be used for traditional fishermen could be through dipping of the whole fish inside the instant tea extract solution, since most of the spoilage bacteria in fish originate from the skin, gills that had been in contact with the outside environment. Hence, tea polyphenols could prolong the fish freshness.

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