Suppressive Effects of Green Tea Polyphenols on Microbial Growth and Volatile Basic Nitrogen Content in Round Form Yellowtail (Seriola quinqueradiata) Meat during Ice Storage


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Suppressive effects of green tea polyphenols (GTP), which contain (+)-catechin, (-)-epicatechin, (-)-gallocatechin, (-)-epigallocatechin, (-)-galloatechin gallate, (-)-epicatechin gallate and (-)-epigallocatechin gallate, on microbial growth and volatile basic nitrogen (VBN) content in the cultured fish meat were investigated. The moist pellets supplemented with different concentrations of GTP (0, 0.02 and 0.2% (w/w)) were fed to young yellotails (Seriola quinqueradiata) of 0-year fish. These fish were killed on the 4th week after feeding. The numbers of microbial and psychrophilic bacteria in their meat were counted during ice storage of the round fish. The contents of VBN, which are known as their bacteria metabolites, content in the meat, were also determined. The increase of microbial counts and psychrophilic bacteria counts in their meat were suppressed by GTP feeding. The increase in VBN of their meat were also suppressed. The feeding of 0.2% GTP were more effective than that of 0.02% GTP. Thus, it was suggested that GTP were effective to control microbial growth and to maintain freshness of the meat during delivery of cultured fish.

(Received Jan. 12, 2001; Accepted Jul. 31, 2001)

Recently, it has been well investigated that green tea polyphenols (GTP), which are polyphenolic compounds in green tea (Camellia sinensis L.), have valuable chemical and biological properties1). There are especially numerous studies on antimicrobial effects of GTP.

We reported that GTP have antibacterial activities against various pathogenic bacteria in livestock industries2). Sakana et al.3,4) reported that GTP have antibacterial activities against cariogenic and periodontal bacteria. They also described that GTP exhibit antimicrobial effects against thermophilic spore-forming bacteria5). Furthermore, it was indicated that the inhibition on the extracellular release of Vero toxin from enterohemorrhagic Escherichia coli O157 : H7 also occurs6). Thus, GTP have been used as useful and effective additives in various foods and animal feed.
With the progress of aquacultural technology, the price of expensive fish has sufficiently been reduced so that they have become a part of our regular diet. Accordingly, there is currently great interest in the production of higher quality cultured fish since consumers always prefer natural, fresh and tasty fish\(^7\). For this reason, progress in the technology of maintaining freshness during delivery from the fish farm to the consumer has also recently gained much attention\(^8\). It is likely that GTP are suitable feed material for cultured fish. However, little is known about the biological effects of GTP on cultured fish.

The present study aims at investigating the suppressive effects on microbial growth during ice storage in the meat of round form cultured yellowtails. The suppressive effect of GTP on the increasing in content of volatile basic nitrogen (VBN) in fish meat, which are bacterial metabolites, was also examined at the same time.

**Materials and Methods**

1. **Fish**

Yellowtails (*Seriola quinqueradiata*) used in this experiment were juvenile fish (0-year fish) within one year after hatching, and were approximately each weighed 12 grams. They were obtained from a mariculture of Kowaura in Mie Prefecture on June 1998. They were cultured in net cages (5 m × 5 m × 5 m), which were located in the cove of Owase Bay at the Ozone Fish Culture Ground of Owase Branch, Fisheries Research Institute of Mie (Owase, Mie, Japan), and had been fed on commercial aquacultural extruded pellets (Hamachi Special EP, Marubeni Feed Co., Ltd., Chiyoda, Tokyo, Japan) until the starting our the experiment. The values of chemical analysis of aquacultural feed used was 48.1% crude protein, 10.2% crude fat, 2.9% crude fiber, 14.8% crude ash, 2.5% calcium, 1.5% phosphorus. The specimens of yellowtail used in this experiment were cultivated such that the young fish possessed an average fork length of 32.0 cm and average body weight of 596 g at the start of the experiment.

2. **Test material**

The GTP (a mixture of 6 kinds of polyphenolic compounds in green tea) tested in this study were commercially available as Teapecus® B (Taiyo Kagaku Co. Ltd., Yokkaichi, Mie, Japan). The specifications of GTP used are shown in Table 1. Teapecus® B containing 25.5% polyphenolic compounds was prepared as the feed mix. Chemical structures of polyphenolic compounds containing in Teapecus® B are shown in Fig.1.

<table>
<thead>
<tr>
<th>Item</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Color</td>
<td>Yellowish brown or brown</td>
</tr>
<tr>
<td>Appearance</td>
<td>Powder</td>
</tr>
<tr>
<td>Moisture</td>
<td>Less than 6.0%</td>
</tr>
<tr>
<td>Ash</td>
<td>Less than 16.0%</td>
</tr>
<tr>
<td>Polyphenols(^1)</td>
<td>25.0~26.0%</td>
</tr>
<tr>
<td>Free Reducing Sugar</td>
<td>13.0~14.0%</td>
</tr>
</tbody>
</table>

\(^1\) : Including \((-\) - epigallocatechin gallate, \(-\) - epigallocatechin, \(-\) - epicatechin gallate, \(-\) - epicatechin, \(+\) - gallocatechin and \(+\) - catechin.

![Chemical Structure of Green Tea Polyphenols](image_url)

**Fig. 1** Chemical Structure of Green Tea Polyphenols
3. Experimental design

The cultured young yellowtail used in this study were 495 specimens in total. They were divided equally into three experimental groups, the control group (A), the 0.02% GTP (B) and the 0.2% GTP (C). Each group was separately cultured in net cages as the above mentioned in the cove of Owase Bay. The moist pellets possessing the compositions shown in Table 2 were fed to the three groups during the test period. The commercial formula feed (Hamachi Ace 50) were purchased from Sakamoto Feed Co., Ltd., Katori, Chiba, Japan. The chemical analysis values of the formula feed were 51.0% crude protein, 4.2% crude fat, 3.9% crude fiber, 14.8% crude ash, 1.3% calcium, 1.9% phosphorus. Fresh feed used was thawed spotlined sardine (Sardinops melanostictus) meat. A vitamin premix (Hamachi Aid W–N) was purchased from Takeda Chemical Industries, Ltd., Chuo-Ku, Tokyo, Japan. The composition of the vitamin premix included 8.0 × 10^4 IU vitamin A, 1.6 × 10^4 IU vitamin D₃, 420mg dl-α-tocopherol acetate ester, 10mg vitamin K₃, 720mg vitamin B₁, 180mg vitamin B₆, 220mg vitamin B₂, 0.17mg vitamin B₁₂, 240mg calcium D-pantothenate, 600mg nicotinamide, 18mg folic acid, 6.0g choline chloride, 1.2mg D-biotin, 968mg calcium ascorbate, and 600mg inositol per kg. Fish oil (Feed Oil MS) was purchased from Riken Vitamin Co., Ltd., Chiyoda-Ku, Tokyo, Japan. Fish oil was produced from the liver oil of alaska pollack (Theragra chalcogramma) and contained 0.02% butylhydroxytoluene. The feed was supplemented with a ratio of 0.02% GTP and 0.2% GTP to Groups B and C, respectively.

The moist pellets were prepared every two weeks and stored at −20°C until feeding. These pellets were fed once every morning during the 4-week test period (from November 6 to December 3, 1998). The average water temperature at the depth of 2 m was nearly 20.6°C during the test period. The salinity and dissolved oxygen levels in the seawater ranged from 31.1 to 34.0 psu and 5.4 to 6.1mg/l, respectively.

For the analysis of the fish meat, 50 fish of each group were dispatched instantly on the 4th week after feeding. These fish were put into expanded polystyrene insulated boxes with crushed ice and carried to our laboratory.

These round form fish were stored in crushed ice in a ratio of 1:1 (w/w) inside expanded polystyrene insulated boxes provided with holes at the bottom to drain out melting water. Crushed ice were added to these boxes appropriately until the day of analysis.

4. Preparation of fish meat for analysis

Five fish were sampled from each group and were filleted, skinned and boned, and the red meat was separated from the fillets on the day of each storage period. These fillets were cut into dorsal, ventral and abdominal-cavity portions. The dorsal meat was used in the analysis of this study. The ventral, abdominal-cavity and red meat was used in other studies. The dorsal meat was chopped into small pieces and minced with a Waring blender while cooling with ice water, and then the sample meat was thoroughly mixed to compensate for specimen differences. The different three portions of mixed meat was sampled three times, and each sample was measured three times, respectively. The average figure obtained from 9 measurements was used as representative data. Deviation among 9 measurements fell within 5% in all experiments.

5. Estimation of freshness of the meat during their ice storage of round fishes

In this study, changes of total viable counts, psychrophilic bacteria counts and VBN contents in the meat during ice storage of round fish were investigated. Total viable bacteria and psychrophilic bacteria were counted on the day of dispatch, the 5th and 10th day after ice storage according to the method outlined by the Association of Official Analytical Chemists and Pharmaceutical Society of Japan.
VBN contents were determined on the day of dispatch, the 1st, 3rd, 5th and 10th day after ice storage by the Yamagata method. Measurements of microbial counts and determination of VBN contents for each sample were replicated three times.

Results and Discussion

The changes of total viable counts of bacteria in fish meat during ice storage are shown in Fig. 2. The total viable counts were approximately same levels \(0.17 \times 10^3 \text{CFU/g of meat in group A, } 0.10 \times 10^3 \text{CFU/g in group B and } 0.19 \times 10^3 \text{CFU/g in group C}\) among the three groups on the first day of dispatch. These counts in all groups increased with the passage of time during the storage period. In group A, the counts greatly increased to \(0.47 \times 10^3 \text{CFU/g of meat and } 1.62 \times 10^3 \text{CFU/g of meat, on the 5th and 10th day after their ice storage, respectively. In group B, the counts a little increased to } 0.16 \times 10^3 \text{CFU/g of meat and } 0.71 \times 10^3 \text{CFU/g of meat, on the 5th and 10th day after ice storage, respectively. In group C, the counts also a little increase to } 0.20 \times 10^3 \text{CFU/g of meat and } 0.36 \times 10^3 \text{CFU/g of meat, on the 5th and 10th day after their ice storage, respectively. The total viable counts in group A had a higher tendency relatively than those in groups B and C on the 5th and 10th day after their ice storage. In group A, the total viable counts of bacteria showed a remarkable increase tendency on the 10th day after their ice storage, while only a slightly increased on the 5th day. In groups B and C, a little increase in them were observed during ice storage. In group C, stronger microbial growth inhibition than group B was observed.

Fig. 3 shows the changes of psychrophilic bacterial counts in the meat during their ice storage of round fishes. Psychrophilic bacterial counts at the day of dispatch were approximately same level in the three groups, \(0.30 \times 10^3 \text{CFU/g of meat in group A, } 0.21 \times 10^3 \text{CFU/g in group B and } 0.43 \times 10^3 \text{CFU/g in group C}\). The counts among the three groups
increased during the storage period. In group A, the counts drastically increased to $1.50 \times 10^3$ CFU/g of meat and $8.13 \times 10^3$ CFU/g of meat, on the 5th and 10th day after their ice storage, respectively. In group B, the counts slightly increased to $0.56 \times 10^3$ CFU/g of meat and $2.00 \times 10^3$ CFU/g of meat, on the 5th and 10th day after ice storage, respectively. In group C, the counts also increased to $0.49 \times 10^3$ CFU/g of meat and $1.5 \times 10^3$ CFU/g of meat, on the 5th and 10th day after their ice storage, respectively. The psychrophilic bacterial counts in group A showed an abrupt increase on the 5th and 10th day after ice storage. These results showed nearly the same tendency to that obtained in the total viable counts of bacteria. In group A, counts of the bacteria on the 10th day were observed much higher than those on the 5th day after ice storage. On the other hand, the increase of psychrophilic bacterial count in groups B and C during ice storage had not a tendency to be so pronounced. The growth inhibition of the psychrophilic bacteria tended to be more pronounced than that of the total viable bacteria.

One of the most serious problems in maintaining freshness of fish and shellfish is the deterioration of their quality by bacteria growth with the passage of storage time. As their spoilage is mainly caused by microbiological decomposition, an increase in bacteria growth is correlated to the degree of their spoilage. What is known of the relationship between the microbial counts and the freshness of fish, is as follows: fresh fish meat keep their total viable counts to less than $5 \times 10^5$ CFU/g of meat, the counts in the fish and shellfish meat stored chilled range from $10^5$ to $10^6$ CFU/g of meat, and spoiled meat show their bacteria counts to be more than $1.5 \times 10^6$ CFU/g of meat. Therefore, bacteria count tests are performed to estimate the incidence of microbiological contamination, the self life of foods or the risk of food poisoning. In particular, counts of psychrophilic bacteria in fish and shellfish are very important indexes because they easily increase even at low temperature where they are usually stored. For these reasons, the analysis of psychrophilic bacteria counts during storage of fish and shellfish provides the most useful information. Thus, changes in total viable counts and psychrophilic bacteria counts were examined in this study.

Green tea polyphenols (GTP) used in this study are composed of (+) catechin, (-) epicatechin, (-) epigallocatechin, (-) gallocatechin gallate, (-) epicatechin gallate and (-) epigallocatechin gallate (Fig.1). Many studies have described that GTP, a mixture of the above chemicals, showed obvious antimicrobial effects against various kinds of bacteria, for example, were various domestic animal pathogens, cariogenic Streptococcus mutans, periodontal Porphyromonas spp., thermophilic spore-forming bacteria and intestinal Clostridium spp.

Ahn et al. reported that GTP inhibit the growth of intestinal Clostridium spp. in vitro. Okubo et al. demonstrated that GTP also decreased the number of human intestinal Clostridium spp. in human volunteer study, supporting the in vitro results reported by Ahn et al. We also described that intake of GTP prevented Holstein calves from digestive and respiratory organ diseases owing to improving intestinal microflora balance and inhibiting growth of pathogenic bacteria in the several field tests. Additionally, the growth inhibition of GTP against various fish pathogens, which were Vibrio spp., Streptococcus spp., Pasteurella piscida, Edwardiella tarda and Aeromonas spp., has already been studied.

So far, various physiological effects besides antimicrobial properties of GTP have been found in vivo studies. It was reported that an oral intake of GTP suppressed the production of 8-hydroxydeoxyguanosine (8 OH-dG), which are produced by oxidative stress in colon. The production of 8-OH-dG is induced by giving 1,2-dimethylhydrazine, resulting in colon cancer. Their study suggested that GTP could suppress physiological oxidation in vivo. Yokozawa et al. reported that urinary methylguanigine, which is known as a uremic toxin and is produced by the oxidation of creatine, was decreased by oral administration of GTP to rats with induced renal failure. They also found that the level of serum methylguanigine in renal failure patients who have
been hemodialyzing decreased by the administration of GTP\textsuperscript{19}. In their studies, it was suggested that GTP was able to act as a radical scavenger to the hydroxyl radicals \textit{in vivo}. It was also reported that the presence of GTP is determined in plasma and organs of both rats\textsuperscript{20} and human\textsuperscript{21,22} by oral administration of GTP. From these studies, it has been thought that administrated GTP might exist in organs. We have previously reported that the peroxide value (POV), thiobarbiturate value (TBA) and metmyoglobin formation ratio of meat during ice storage were suppressed in cultured yellowtail fed on GTP, and indicated that these effects were based on the possibility of suppressive effect of GTP on physiological oxidation in fish\textsuperscript{24}).

In this study, changes of total viable and psychrophilic bacterial counts in fish meat during ice storage were suppressed by feeding of GTP. These results indicate that GTP might be incorporated into the fish body in the same manner as in the cases of rats and human. Furthermore, in this study, it was suggested that GTP could show antimicrobial effects in fish, while counts of both total viable and psychrophilic bacteria in all groups (groups A, B and C) were kept to less than $5 \times 10^5$ CFU/g of meat, indicating that the raw fish meat was edible even on the 10th day after ice storage. Based on these results above mentioned, it may be suggested that GTP improved intestinal microflora balance, or inhibited fish pathogens in fish. For these reasons, it was thought inhibiting effect of GTP might lead those results of microbial counts in two groups (groups B and C). Thus, GTP showed a tendency to stronger effect on the growth of total viable bacteria and psychrophilic bacteria in group C than that in group B. However, further work is needed to support these results obtained in this study. We are presently attempting to confirm our results.

The changes of volatile basic nitrogen (VBN) contents in the meat during ice storage of round fish are shown in Fig. 4. In all groups, the VBN was increased during ice storage, although the increasing rates showed a suppressive tendency in groups B and C compared with it of group A. In particular, on the 10th day after ice storage, VBN contents in groups B and C were observed obviously lower than that in group A (14.0mg/100g of meat in group A, 12.3mg/100g of meat in group B and 11.8mg/100g of meat in group C, Fig. 4). The suppressing effect of GTP on the production of VBN was observed more strongly in group C than in group B.

In general, production of VBN is caused by the proliferation of various kinds of bacteria, and thus only a small volume of VBN is found at just after killing. VBN is comprised of ammonia, trimethylamine, dimethylamine, etc., and is produced by microbial enzymes which are generally known as reductase. The contents of VBN are used as an index for the freshness of fish meat. In this study, it was observed that the production of VBN in yellowtail meat during ice storage was suppressed by the feeding of GTP. The findings correspond to the results obtained in the bacteriological experiment (Fig. 1 and 2). It is suggested that the GTP fed to fish might express anti-bacterial effects, and subsequently the production of VBN was decreased.

Our experiment has suggested that the feeding of GTP to fish might be able to show a desirable influence on the freshness of fish meat during
storage. GTP may be available as an easy and preferable ingredient of feed to keep the freshness of cultured fish in the near future.

Acknowledgements

This work was partly supported by a Grant-in-Aid from the Regional Science Promoter Program from the Mie Prefectural Industrial Technology Advancement Center, and the Japan Science and Technology Corporation.

References

緑茶ポリフェノール給与飼育した
ブリ氷蔵中における細菌増殖および
揮発性塩基態窒素量抑制効果

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（+）-カテキン、（-）-エピカテキン、（-）-ガロ
カテキン、（-）-エピガロカテキン、（-）-ガロカテ
キンガレート、（-）-エピカテキンガレートおよび
（-）-エピガロカテキンガレートからなる緑茶ポリフェ
ノール（GTP）の飼育魚の細菌増殖および揮発性
塩基態窒素（VBN）含量の抑制効果を検討した。異
なる濃度のGTP（0, 0.02および0.2% (w/w)）を添
加したモイストペレットを当歳魚のハマチに給餌した。
給餌開始4週間後にハマチを活けじめした。ラウンド
氷蔵中の魚肉部の一般細菌および低温細菌の生菌数を
計数した。また、細菌の代謝産物として知られている
魚肉部のVBN含量も定量した。魚肉部の一般細菌お
よび低温細菌の増殖はGTP給餌により抑制された。
また、魚肉中のVBNの増加も抑制された。0.2%の
GTP添加は0.02%GTP添加より効果的であった。こ
れらのことから、GTPは養殖魚の配送段階における
細菌と鮮度保持に効果的であることが示唆された。

（平成13年1月12日受付、平成13年7月31日受理）