Food Research 5 (2) : 62 - 66 (April 2021)
Journal homepage: http://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

Antioxidant effects of green tea extracts on lipid oxidation of half-dried eels

*Song, H.S.

Department of Food and Nutrition, Gwangju Health University, Gwangju, 62287, Korea

Article history:
Received: 30 October 2020
Received in revised form: 3 December 2020
Accepted: 5 February 2020
Available Online: 21 February 2021

Keywords:
Green tea,
Half-dried eel,
Peroxide,
Antioxidant,
TBA

DOI:
https://doi.org/10.26656/fr.2017.5(2).620

Abstract

In order to explore the change of lipid oxidation of half-dried eels treated with green tea extracts and stored frozen, acid values, peroxide values, carbonyl values, and TBA values were measured. The eels were placed into the plastic bag and soaked for 1 hr in the solution mixed with distilled water and green tea extracts, and also vacuumed and stored frozen at –18 ~ –20°C for 9 months after being dried with a hot-air blower for 15 hrs at 35°C (Aw 90-91). The control consisted of eels prepared in the same way without any pre-treatment. At the end of the 1st month, 3rd month, 6th month, and 9th month, eel oil obtained from the samples were tested for its lipid oxidation. The activity of green tea extracts 1 mL was very similar to Vitamin C 500 μM 0.8 mL. The acid values, peroxide values, carbonyl values, and TBA values of eels treated with green tea extracts were lower than those of the control during 9 months of frozen storage ($p<0.05$). While the acid values and peroxide values of the control and eels treated with green tea extracts highly increased after the first month of frozen storage, the TBA values increased greatly on the third month of frozen storage. Compared to the control, the pre-application of green tea extracts to half-dried eels meaningfully prevented the generation of TBA compound during the frozen storage ($p<0.01$). In conclusion, pre-application of green tea extracts was effective in delaying early-stage peracid inducement and preventing the generation of secondary oxidation compounds, such as carbonyl compound and TBA compound, in the course of the drying and frozen storage.

1. Introduction

The concerns on heart-related diseases and cancers sparked interest in this research and relates it to the consumption of a healthy diet, including w-3 fatty acid, found in fishes. However, unsaturated fat in food downgrades the food quality due to oxidation by oxygen, moisture, heat, etc. (Cho *et al*., 2011). It is also related to the difficulties of processing, storing, and distribution of fish with high unsaturated fat because of their easy rancidity. In order to resolve the problems of easy rancidity, research on peroxide prevention has been undertaken in various fish, such as half-dried eel (Song *et al*., 2018), salted mackerel (Nam *et al*., 2011), anchovy oil (Kang *et al*., 2007), saury (Cook, 1995), seasoned squid (Yang *et al*., 1999), white fish meat (Lee *et al*., 1997), and shellfish (Cho *et al*. 1998). Eel, one of Korea's most favourite health foods in summer, has high unsaturated fatty acid. Thanks to the increase of eel supplies based on recent developments in farming skills, not only has the annual consumption consequently increased, but HMR products containing eels have also appeared in the market. Therefore, it is essential to research how to prevent eel's peroxide activity to improve storage and distribution capabilities (Song *et al*., 2018). Green tea has been reported in many works of literature for its high antioxidant effects based on its polyphenol compound having the antioxidant capacity (Yang *et al*., 1999; Son *et al*., 2005; Kang *et al*., 2007; Nam *et al*., 2011; Ryu *et al*., 2017). This research aimed to explore the antioxidant effects and storage stability of green tea extracts in regard to half-dried eel's peroxide when they were pre-applied.

2. Materials and methods

2.1 Samples

Eels (*Anguilla bicolor pacifica*) used in the experiment were cultivated in the city of Naju, weighing around 250-300 g, eviscerated, and cleaned before use. The green tea used for extracting solutions to be pre-applied to eels for the purpose of preventing eel's

*Corresponding author.
Email: *songuta@ghu.ac.kr*

eISSN: 2550-2166

peroxide was dried green tea that was easily accessible in the market (Green tea, Damian, Korea). Approximately, 30 g of green tea was added to distilled water and then extracted for 6 hrs at 112°C using a pressured double boiler function in an electronic boiling pot (OC-2300R, OCOO, co., Ltd., Korea). Once the solution was obtained, it was filtered twice using gauze and filter paper (No.1, Whatman, UK), respectively.

2.2 Pre-treatment of eels and eel oil extraction

The eels were placed into the plastic bag and soaked for 1 hr in the solution mixed with distilled water and green tea extracts at a ratio of 3:2. Once treated, eels were cleaned with flowing water and were dried with paper towels. They were also dried with a hot-air blower for 15 hrs at 35°C to the extent that the water activity was maintained at 90~91 using HP23-AW water activity analyzer (Rotronic, Switzerland). Half-dried eels were cut into sizes that weighed about a 100 g and stored after being vacuumed in the freezer until the oil extracted. At the end of the 1st, 3rd, 6th, and 9th month, the antioxidant effects of eel oil obtained after 1 hr of defrosting were examined in terms of acid value and peroxide value.

Eel oil was extracted using the method specified in Folch *et al*. (1957). Chopped eel weighing 60 g were mixed with 300 mL of a blended solution of chloroform and methanol (2:1, v/v), then extracted using a homogenizer (SMG-G, Shinsang, Co., Ltd., Korea) and filtered using a filter paper (No.1). Another 250 mL of the blended solution of chloroform and methanol was added to the residue, then extracted using a homogenizer, which constitutes the second time of filtration. All obtained solutions were poured into a separate funnel, mixed with distilled water and left for 15 to 20 hrs. Next, the layer of chloroform was separated, dehydrated by Na_2SO_4 and filtered. Eel oil was obtained by concentrating all filtered solutions under reduced pressure using a rotary evaporator (Rotavapor R-215, Büchi, Germany) at 40°C (Song 2019).

2.3 DPPH radical scavenging effects

DPPH radical scavenging effects were measured using an extracted solution filtered via a filter paper (No.1) once. Sample diluted by 1 mL of distilled water was mixed with 2 mL of ethanol and 0.5 mL of 700 μM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution (Sigma Co. USA). DPPH radical scavenging effects were measured at 517 nm absorbance using a UV-spectrophotometer (UV-1650, Shimadzu, Japan) (Song *et al*., 2007).

2.4 Acid value

Eel oil collected in 1 to 2 g using a method specified in Korean food code was placed into a flask, and 100 mL mixed solution of methanol and ether (2:1) was then added. With 1-2 drops of phenolphthalein indicator injected, pink colour was chosen as the determination of endpoint through titration by 0.1 N KOH (Kim, Lee, Ma *et al*., 2015; Ministry of Food and Drug Safety, 2017).

2.5 Peroxide value

After 1 to 2 g of lipid was recovered using a method specified in Korean food code, 25 mL of a mixed solution of acetic acid and chloroform (3:2, v/v) was added. On top of this, 1 mL of KI saturated solution was also added. Then, it was left in a dark room for 10 mins after being shaken for 1 min. With 30 mL of distilled water and starch indicator solution injected, the endpoint through titration by 0.01 N $Na2S2O3$ was observed when the solution turned colourless. (Ministry of Food and Drug Safety, 2017; Song, 2019).

2.6 Carbonyl value

Eel oil of 0.05 g was placed into a 100 mL glass bottle with a cap, along with benzene 5 mL, 0.05% 2,4-DNPH (dinitrophenyl hydrazine) benzene 5 mL, and 4.3% trichloroacetic acid 3 mL that was injected into the bottle. Then, the mixture was warmed up in a double boiler of 60°C water bath for 30 mins. After cooling it off at room temperature and popping the colour with 10 mL of 4% KOH-ethanol, the absorbance was measured at 440 nm (Choi *et al.,* 2006; Song, 2018).

2.7 The measurement of TBA value

Mixing and sonicating 200 mg of TBA with 100 mL of 95% butanol at 60°C was done in a sonicator (Ultrasonic, JAC 4020, KODO, Korea) for 30 mins using a method specified in Korean food code. TBA (thiobarbituric acid) reagent was taken and mixed directly with glacial acetic acid at the ratio of 1:1. After the solution was cooled to room temperature the TBA value was obtained through the absorbance measurement at 530 nm, with a blend of 0.05 g eel oil, 10 mL benzene, and 10 mL of TBA reagent, which was kept for 2 hrs at 95°C in a water bath (Ministry of Food and Drug Safety, 2017; Song, 2018).

2.8 Statistical analysis

Comparative analysis among experimental groups was performed using IBM SPSS Statistics 20. After ANOVA was undertaken, Duncan's multiple range test was also performed with a 5% confidence level (α = 0.05).

3. Results and discussion

3.1 DPPH scavenging effect of green tea extracts

In order to measure the DPPH radical scavenging effects of green tea extract, a Vitamin C 10 mM solution was used as the positive control (Table 1). The correlation coefficient between concentration and absorbance was more than 0.9 ($r>0.9$). The effects of green–tea extracts in DPPH radical scavenging were found to be dose-dependent. The activity of 1mL green tea extract yielded similar activity to 0.8 mL of Vitamin C 500 μM. The activity of Vitamin C 500 μM, the control group, was found to be 10 times higher when in 1 mL than in 0.1 mL, but green tea extracts showed about 2% difference when compared between 0.1 mL and 1 mL. Therefore, green tea extracts were not only found to be effective in terms of antioxidant effects in undiluted solutions but also diluted to some degree. Song (2018) reported that the activity of green tea extracts under 0.1 mL was similar to that of Vitamin C.

3.2 Acid value of half-dried eel oil

The acid value is one of the measures taken to analyse lipid rancidity by measuring free fatty acid values (Cho *et al*., 2011). While the unfrozen and half-dried eel's acid value was 2.6 mg KOH/g, the green tea extract-treated eel's acid value was 2.4 mg KOH/g. (Table 2).

The difference in acid value is believed to be inconsequential and to come from a half-day drying process prior to frozen storage through the effective antioxidant effects of green tea extract treatment applied during the drying process. According to Song (2019) and Song and Kim (2018), there were antioxidant effects during the drying process if ethanol extracts from plants were applied to eels prior to the drying. Mackerel, which was widely known for ease of lipid rancidity due to high unsaturated fatty acid such as eels, was reported to have an acid value of 2.3 mg KOH/g when pre-treated with green tea extract in the pre-refrigeration stages (Nam *et al*., 2011). Overall, the acid value of half-dried eels pre-treated with green tea extracts was lower than half-dried eels in the control group throughout the nine months frozen in storage ($p<0.05$). The acid value rose steeply after the first month of storage, while the acid value of half-dried eels pre-treated with green tea extracts increased 2.5 times after the first month, and the control increased by 4 times during the same period ($p<0.01$). When eels pre-treated with ethanol extracts from plants, such as Hutgae, ginger, onion, green tea, etc., were refrigerated, their low acid values were reported to inhibit the increase of their free fatty acid values (Song and Kim, 2018; Song, 2019). Although the acid value was low in the case of eels pre-treated with green tea extracts ($p<0.05$), both testing groups showed 6 times an increase after six months of frozen in storage. However, the antioxidant effects from pre-treatment with green tea extracts were believed to be limited by the third month.

3.3 Peroxide value of half-dried eel oil

On the day zero of refrigeration, the peroxide values of eel of the control group were 13.35 meq/kg (Table 3), and those with green tea extracts were 10.03 meq/kg. Therefore, green tea extracts were effective in preventing the peroxide value of the eels during the drying process ($p<0.05$). During one month of frozen storage, the peroxide values of eels in the control and the eels treated with green tea extracts increased 2.4 times and 1.8 times more than the prior state. Since then, after six months of frozen storage, the peroxide values gradually increased. However, during the ninth month of frozen storage, the peroxide values decreased through re-decomposition of peroxide, which started after the sixth month of frozen storage. During nine months of frozen storage, the peroxide values of eels treated with green tea extracts were lower than those of eels in the control ($p<0.05$), and up to three months of frozen storage, pre-treatment of green tea extracts was comparatively effective in preventing peroxide activity ($p<0.01$). The researchers reported that green tea extracts effectively delayed the peracid inducement time of eel oil (Song and Kim, 2018). Additionally, the researchers found that water extract from green tea prevented the peroxide of anchovy oil (Kang *et al*., 2007), and green tea was used to

Table 1. Antioxidant effect of green tea extract on DPPH radical

	Antioxidant activity (%)			
	0.1 mL	0.2 mL	0.5 mL	1 mL
Green tea extract	76.22±6.70**	76.98±6.12**	77.55±6.10	78.54±5.46
Vitamin C 500 μM	9.4±1.73	19.05±2.34	60.34±3.18	94.48±2.04*

* and ** superscript are significantly different at $p<0.05$ and $p<0.01$, respectively within the same storage period by t-test.

Table 2. Acid value (mg KOH/g) of half-dried eel during storage at refrigerator

Extract	Storage				
	0 day	1 month	3 month	6 month	9 month
Control	2.62±0.46	13.19±0.76	13.72±2.00	18.43±0.19	20.49±0.95
Green tea extract	2.36±1.44	8.18±6.13**	10.79±5.54	16.41±6.67*	18.11±6.07*

* and ** superscript are significantly different at $p<0.05$ and $p<0.01$, respectively within the same storage period by t-test.

eISSN: 2550-2166

Table 3. Peroxide value (meq/kg) of half-dried eel during storage at refrigerator

Extract	Storage				
	0 day	1 month	3 month	6 month	9 month
Control	13.35±1.07	45.66±3.82	48.69±3.93	49.65±4.15	39.72±3.51
Green tea extract	10.03±0.90*	28.42±1.40**	32.69±0.43**	37.79±2.02*	29.61±1.64*

* and ** superscript are significantly different at $p<0.05$ and $p<0.01$, respectively within the same storage period by t-test.

improve the quality of sea-products in the process (Yang *et al*., 1999; Lee, 1999).

3.4 Carbonyl value of half-dried eel oil

Carbonyl compound, which can be generated by proteolysis and amino acid oxidation, can produce a toxic substance from the reaction to fatty acid compounds, such as malondialdehyde and 4-hydroxynonenal (Berlett and Stadtman, 1997). On day zero of storage, the carbonyl values of eels in the control and the eels treated with green tea extracts were 2.26 meq/kg and 2.19 meq/kg, respectively (Table 4). During nine months of frozen storage, the carbonyl values of eels treated with green tea extracts were lower than those of eels in the control. In the case of eels treated, the lower ratio of carbonyl compound was noted, which evidently showed that the green tea extracts not only helped prevent the oxidation of lipid in the early stages but also the second stage ($p<0.05$). During the three weeks of the refrigeration, green tea extracts were effective in preventing the generation of the carbonyl compound, a type of lipid oxidation (Song, 2018). The generation of carbonyl compound in meat increased significantly when proteolysis activities increased (Kim, Son, Kim *et al*., 2015). The carbonyl values of eels refrigerated for three weeks were 2.9 meq/kg (Song, 2018), while eels treated with green tea extracts were lower than eels in the control, and eel oil stored at 37℃ were 9 meq/kg after one week of storage (Choi, 2006). Considering other research done, the carbonyl compound generation speed of green tea extracts treated, vacuum-packed, and frozen eels were quite hindered.

3.5 TBA value of half-dried eel oil

TBA value is a degree of oxidative rancidity in fats, which measures the colour of a product, a reaction between thiobarbituric acid and malondialdehyde formed by oxidation of polyunsaturated fatty acids (Kim, Son, Kim *et al*., 2015). The TBA values of untreated eels were 4.24 on day zero, and the TBA values of green tea extracts-treated eels were 3.87 (Table 5). During nine months of frozen storage, the TBA values of eels treated with green tea extracts were meaningfully lower than those of eels in the control ($p<0.01$). While the TBA values of eels in the control increased 10.6 times more than the prior state, those treated with green tea extracts increased 8.8 times more, which indicated that the treatment with green tea extracts stopped secondary oxidation of lipid. Compared to the high rise of acid values and peroxide values during one month of frozen storage, the TBA values greatly increased after three months of frozen storage. There was a finding that the TBA values of eels treated with green tea extracts and refrigerated for three weeks were similarly low to those of eels treated with the Vitamin C solution (Song, 2018).

4. Conclusion

In conclusion, pre-application of green tea extracts was effective in delaying the early stage peracid inducement and preventing the generation of secondary oxidation compounds, such as carbonyl compound and TBA compound, in the course of the drying and frozen storage. Especially up to three months of frozen storage, pre-application of green tea extracts was found to be highly effective in preventing the lipid oxidation of eels.

Conflict of interest

The author declares no conflict of interest.

Table 4. Carbonyl value (meq/kg) of half-dried eel during storage at refrigerator

Extract	Storage				
	0 day	1 month	3 month	6 month	9 month
Control	13.35±1.07	45.66±3.82	48.69±3.93	49.65±4.15	39.72±3.51
Green tea extract	10.03±0.90*	28.42±1.40**	32.69±0.43**	37.79±2.02*	29.61±1.64*

* and ** superscript are significantly different at $p<0.05$ and $p<0.01$, respectively within the same storage period by t-test.

Table 5. TBA value of half-dried eel during storage at refrigerator

Extract	Storage				
	0 day	1 month	3 month	6 month	9 month
Control	4.24±0.80	17.97±1.23	35.16±5.68	41.53±4.13	49.31±4.29
Green tea extract	3.87±0.44	12.62±2.84**	21.92±2.00**	24.96±1.74**	38.04±3.65**

* and ** superscript are significantly different at $p<0.05$ and $p<0.01$, respectively within the same storage period by t-test.

Acknowledgment

The research was conducted with the Research Grant of Gwangju Health University with grant number 2019001.

References

Berlett, B.S. and Stadtman, E.R. (1997). Protein oxidation in aging, disease, and oxidative stress. *Journal of Biological Chemistry*, 272(33), 20313-20316. https://doi.org/10.1074/jbc.272.33.20313

Cho, H.S., Cho, T.Y., Lee, K.H. and Lee, N.G. (1998). Lipid oxidation in dried shellfish during the storage. *Journal of the Korean Society of Fisheries and Ocean Technology*, 31, 594-598

Cho, S.H., Cho, K.R., Kang, M.S., Song, M.R. and Joo, N.Y. (2011). Food Science. p. 101-106. Seoul, Korea: Kyomunsa

Choi, B.D., Kang, S.J., Ha, Y.L., Kim, S.Y. and Lee, J.J. (2006). Oxidative stability of lipids from eel (*Anguilla japonica*) fed conjugated linoleic acid. *Journal of the Korean Society of Food Science and Nutrition*, 35(1), 61-67. https://doi.org/10.3746/jkfn.2006.35.1.061

Cook, C.Y. (1995). Antioxidative effect of ethanol extract of ginger on mackerel pike (*Cololabis saira*) flesh. *Journal of The Korean Oil Chemists Society*, 12(1), 43-46.

Folch, J.M., Lees, M. and Stanley, G.H.S. (1957). A simple method for the isolation and purification of total lipids from animal tissue. *Journal of Biological Chemistry*, 226(1), 497-503. https://doi.org/10.1016/S0021-9258(18)64849-5

Kang, S.T., Yoo, U.H., Nam, K.H., Kang, J.Y. and Oh, K.S. (2007). Antioxidative effects of green tea extract on the oxidation of anchovy oil. *Journal of Agriculture and Life Science*, 41, 47-53.

Kim, H.J., Lee, K.J., Ma, K.H., Cho, Y.H., Lee, S., Lee, D.J. and Chung, J.W. (2015). Effect of tomato leaf extracts on anti-inflammatory and antioxidant activities. *Journal of Integrative Agriculture*, 27(4), 529-535. https://doi.org/10.12719/KSIA.2015.27.4.529

Kim, S.Y., Son, Y.J., Kim, S.H., Kim, A.N., Lee, G.Y. and Hwang, I.K. (2015). Studies on oxidative stability of *Tenebrio molitor* Larvae during cold storage. *Korean Journal of Food and Cookery Science*, 31(1), 62-71. https://doi.org/10.9724/kfcs.2015.31.1.062

Lee, K.H., Cho, H.S., Lee, J.H., Shim, K.H. and Ryu, H.S. (1997). Lipid oxidation in roasted fish meat: rancidity in roasted and/or reheated white muscled fish. *Journal of the Korean Society of Fisheries and Ocean Technology*, 30, 714-718.

Lee, S.K. (1999). Effect of extract from green tea and boxthorn on the storage stability of powdered anchovy. *Journal of Food Hygiene and Safety*, 14, 238-243.

Ministry of Food and Drug Safety. (2017). Korean Food standards codex. Retrieved July 29, 2020 from Food Safety Korea website: http://www.foodsafetykorea.go.kr/foodcode

Nam, K.H., Jang, M.S., Lee, D.S., Yoon, H.D. and Park, H.Y. (2011). Effect of green tea and lotus leaf boiled water extracts treatment on quality characteristics in salted mackerel during storage. *Korean Journal of Food Preservation*, 18(5), 643-650. https://doi.org/10.11002/kjfp.2011.18.5.643

Ryu, J.Y., Song, Y.W., Moon, J.Y., Jun, N.J. and Cho, S.K. (2017). Antioxidant activities of soymilk added with green tea and rosemary extract. *Korean Journal of Food Preservation*, 24(6), 871-878. https://doi.org/10.11002/kjfp.2017.24.6.871

Son, G.M., Bae, S.M., Chung, J.Y., Shin, D.J. and Sung, T.S. (2005). Antioxidative effect on the green tea and puer tea extracts. *The Korean Journal of Food and Nutrition*, 18(3), 219-224.

Song, H.S. (2018). Antioxidant effect of green tea extracts on peroxidation of eels. *Korean Journal of Food Preservation*, 25, 763-769. https://doi.org/10.11002/kjfp.2018.25.7.763

Song, H.S. (2019). Antioxidant effects of ethanol extracts from plants on semi-dried eels. *Korean Journal of Food Preservation*, 25(7), 763-769. https://doi.org/10.11002/kjfp.2018.25.7.763

Song, H.S., Kim, D.P., Jung, Y.H. and Lee, M.K. (2007). Antioxidant activities of red Hamcho (*Salicornia herbacea* L.) against lipid peroxidation and the formation of radicals. *The Korean Journal of Food and Nutrition*, 20(2), 150-157.

Song, H.S. and Kim, Y.M. (2018). Antioxidant effects of ethanol extracts from plants on peroxide content in semi-dried eels. *The Korean Journal of Food and Nutrition*, 31(5), 647-652.

Yang, S.Y., Kim, D.S., Oh, S.W. and Bang, H.A. (1999). Anti-browning activities of green tea water extracts on seasoned squid. *Korean Journal of Food Science and Technology*, 31(2), 361-367.

