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Research note

천연 유래 복합(마늘+녹차) 주정추출물의 첨가에 따른 조미김용 옥수수유의 품질 특성

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Effect of a Natural Antibacterial·Antioxidant Blend (Garlic- and Green tea-Based Ethanol Extracts) on Quality Characteristics of a Corn Oil used for Seasoned Laver Products

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ABSTRACT - This study evaluated the antibacterial and antioxidant potentials of a mixture of ethanol-based extracts originating from garlic and green tea and its effects on the oxidative stability of a corn oil used for seasoned laver products. The extracts were screened I) for the antibacterial activity against food-borne pathogenic bacteria using an agar disc diffusion method and II) for the antioxidant activity by measuring 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging capabilities. The individual extracts exhibited a moderate degree of antibacterial and antioxidant potentials, and the mixture of garlic and green tea at a ratio of 5:5 effectively inhibited the growth of *Bacillus cereus* and *Staphylococcus aureus*, which was accompanied by a marked increase of the DPPH radical-scavenging capacity (155.16%). Particularly, the mixture significantly ($P<0.05$) delayed the lipid oxidation of a corn oil used for seasoned laver products at elevated storage temperatures, as compared with the untreated control group.

Key words: Antibacterial activity, Antioxidant activity, Garlic, Green tea, Seasoned laver products

Along with the very low content of lipids, edible seaweeds, such as laver, have been recognized as a promising source of essential nutrients, such as amino acids (i.e., alanine, aspartic acid, and glutamic acid), minerals (i.e., calcium, magnesium, and potassium), and vitamins (i.e., B_{12}), all of which contribute to their nutritional and physiological benefits to human health and wellbeing[1-3]. As well documented by Asanka Sanjeewa et al.[4], the consumption of laver has gained interest and popularity mainly due to its health-promoting benefits, including anticancer (e.g. phospholipid, polysaccharide, and sterol), antiinflammatory (e.g. glycoprotein and porphyran), and antioxidant (e.g. polyphenol and tocopherol) effects. Indeed, the global production of laver has experienced its significant growth, rising from approximately USD 1285.0 billion in 1997 to around USD 2319.7 billion by 2017[5].

Generally, the seasoned laver is made from dried seaweeds that are roasted and flavored with oils and salt, and its production processes involve "preparation of raw materials" → "the primary roasting (i.e. at 50-330°C for 2-10 s)" → "seasoning" → "the secondary roasting (i.e. at 260-400°C for 2-10 s)" → "packaging"[6]. Briefly, a sheet of dried raw materials coated with edible oils (e.g. perilla and/or sesame oils) are subjected to ultra high temperature (UHT)-

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based roasting procedures, particularly exceeding approximately 300°C. Considering the fact that the UHT treatments can induce the thermal oxidation of the vegetable oils during the manufacturing of seasoned laver products, a key determinant for maintaining the freshness and shelf life of seasoned laver products is the oxidative stability of the oils used[7]. Thus far, the mitigation and/or control of the oil's oxidation as a strategy for prolonging the shelf life of seasoned laver products can offer promising aspects for the Korean seaweed industry.

With the rising interest and popularity of laver-derived products, a plethora of studies have been conducted, primarily focusing on the control of food-borne pathogenic bacteria by chemical or physical intervention methods and the oxidation stability of edible oils in the relation to the shelf life extension. In a study conducted by Lee et al.[8], a 7-kGy electron beam irradiation resulted in approximately 2-log reductions in the population of total aerobic bacteria (TAB). The doses required to inactivate 90% of a population of *Salmonella enterica* serovar Typhimurium and *Staphylococcus aureus* by a gamma irradiation were 0.39±0.01 and 0.30±0.04 kGy, respectively, on dried laver products[9]. Heating dried laver products at 154-170°C for 10-18 s incorporated with an electron beam irradiation at a dose ranging from 1.8 to 3.0 kGy were effective for reducing or eliminating TAB by approximately 10^3 CFU/g, which was accomplished by the highest overall palatability score of 6.5 out of 7.0[10]. Additionally, several studies[8,11,12] have determined the positive effects of a natural antioxidant (i.e. a mixture of onion peel and rosemary ethanol-based extracts) or a blending ratio of vegetable oils (i.e. corn, perilla, and sesame oils) on the oxidative stability of edible oils used for manufacturing seasoned laver products. Oxidation of edible oils leads to the development of unpleasant odors, which can in turn deteriorate the quality of seasoned laver products, adversely affecting the consumer acceptance and satisfaction. However, the main oxidative stability of edible oils supplemented with natural antibacterial·antioxidant substances remains unclear, which can challenge the practical application of these additives during the long term storage of seasoned laver products. In the present study, a total of eight natural materials, such as blueberry, broccoli, *cheonggukjang*, garlic, green tea, onion, radish leaf, and *shiitake* mushroom, were screened for their potential antioxidative effects in a corn oil used for seasoned laver products. Based on the results, both garlic and green tea were selected as the most effective candidates. This study, therefore, aimed at investigating the effects of a natural antibacterial·antioxidant blend consisting of garlic and green tea on retarding the oxidation of a corn oil used for seasoned laver products in an attempt to determine the optimal blend concentration and ratio for improving the oxidative stability of the mixture-treated oils.

Materials and Methods

Preparation and storage

Powders of blueberry (Alldam food, Asan, Korea), broccoli (Sanmaeul Inc., Changnyeong, Korea), *cheonggukjang* (Hannong Village Agricultural Corporation Co., Ltd., Sangju, Korea), garlic (Jfood service Ltd., Anseong, Korea), green tea (Daehandaup Co., Ltd., Boseong, Korea), onion (Thevegan, Icheon, Korea), radish leaf (Malgeundeul Co., Ltd., Hongcheon, Korea) or s*hiitake* mushroom (Agricultural corporation Wellbeing Co., Ltd., Gimcheon, Korea) were purchased in a local grocery store located in Suncheon, Korea. Separately, each of the powders (weighing 5 g) was mixed with 50 mL of 25% ethanol (DAEJUNG Chemicals & Metals Co., Ltd., Siheung, Korea). After the vortexing procedure, the mixture was stirred in an oven (Daewon science Co., Bucheon, Korea) at a room temperature for 24 h to carry out its extraction. After the centrifugation at 1,500×g at 4°C for 15 min, only the supernatant was collected and subjected to a sterilization through a 0.22-μm pore size polycarbonate membrane filtration (Millipore® Ltd., Mississauga, Canada).

A garlic power and a green tea powder were mixed according to proportions specified in the supplementary Table S1. The mixtures (weighing a total of 5 g) were combined with 50 mL of 25% ethanol, vortexed, and stirred in an oven at a room temperature for 24 h to perform the extraction. After the centrifugation at 1,500×g at 4°C for 15 min, only the supernatant was collected and subjected to a sterilization through a 0.22-μm pore size polycarbonate membrane filtration.

Commonly, the resulting ethanol-based extracts were subjected to the vacuum concentration via the rotary evaporator (Sunileyela Co., Ltd., Seongnam, Korea) with a speed of 2,000×g and a temperature of 35°C for 5 h. Each of the ethanol-based extracts was combined with a corn oil (Umac Co., Ltd., Haenam, Korea), corresponding to its final concentration of 1 or 2%, and combined with 10 mL of DW and 1.5 g of lecithin (International Flavors & Fragrances Inc., New York, NY, USA), followed by maximally 32 d of storage at 25, 35, 50, and 70°C. Especially, the storage temperatures (i.e. 50 and 70°C) were applied as an accelerated oxidation-inducing model to stimulate a severe oxidative stress and to allow a rapid comparison of the oxidative stability of the oils.

Determination of antibacterial activities

The agar disc diffusion method described by a study

conducted by Hyun et al.[13] was applied in order to determine the diameter (mm) of clear zones imposed by an ethanol-based extract from blueberry, broccoli, *cheonggukjang*, garlic, green tea, onion, radish leaf or s*hiitake* mushroom against a total of 13 food-borne pathogenic bacteria. In this study, *Bacillus cereus* (ATCC 10876, ATCC 13061, and ATCC 14579), *Cronobacter sakazakii* (ATCC 12868, ATCC 29004, and ATCC 29544), *E. coli* O157:H7 (ATCC 35150, 43889, and 43890), *Listeria monocytogenes* (ATCC 19114 and ATCC 19117), *Salmonella enterica* serovar Enteritidis ATCC 13076, *Salmonella enterica* serovar Typhimurium (ATCC 19585, ATCC 43971, and DT104 Killercow), *Shigella flexneri* (KCTC 2517, KCTC 22192, and NCCP 10852), *Staphylococcus aureus* (ATCC 23235, ATCC 25923, and ATCC 40050), and *Yersinia enterocolitica* (ATCC 23715 and ATCC 55075) were obtained from the bacterial culture collections of the Sunchon National University (Sunchon National University, Suncheon, Korea). Individually, all the stock cultures were stored in a mixture of 1 mL of tryptic soy broth (TSB; Difco® Laboratories Inc., Detroit, MA, USA) and 0.5 mL of 50% glycerol (Junsei Chmical Co., Ltd., Kyoto, Japan) at -80°C and were activated in TSB, followed by 24 h of incubation at 37°C. Following the centrifugation at 8,000×g for 3 min at 4°C, each of the bacterial strains was washed with 0.1 M phosphate-buffered saline (PBS; BIONEER® Corp., Daejeon, Korea) and resuspended in 1 mL of 0.1 M PBS. TSB supplemented with 0.75% agar (Difco® Laboratories Inc.) was autoclaved at 121°C for 15 min, cooled in a water bath (JEIO TECH® Co., Ltd., Daejeon, Korea) at 45°C for 30 min, and inoculated with 100 μL of each of the bacterial solutions held in 0.1 M PBS. The fluids were poured into a sterile petri dish. After the solidification process, paper discs (diameter: 8 mm; SPL life sciences Co., Ltd., Pocheon, Korea) were placed on the surface of the agar plates. Then, 20 μL of each of the ethanol-based extracts (i.e. blueberry, broccoli, *cheonggukjang*, garlic, green tea, onion, radish leaf, s*hiitake* mushroom or a garlic and green tea blend at different formation ratios) was loaded onto the disc, followed by 24 h of incubation at 37°C.

Determination of antioxidant activities

The antioxidant activities of each of the ethanol-based extracts (i.e. blueberry, broccoli, *cheonggukjang*, garlic, green tea, onion, radish leaf, s*hiitake* mushroom or a garlic and green tea blend at different formation ratios) were evaluated according to a protocol described in a study conducted by Park et al.[14] and Yang et al.[15] for measuring 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich® Co., St Louis, MO, USA) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS; Sigma-Aldrich® Co.) radical-scavenging capabilities, respectively.

Measurement of acid values (AVS)

A mixed solution of ethanol and ether (DAEJUNG Chemicals & Metals Co., Ltd.) at a ratio of 1:2 (i.e. 100 mL) was transferred to a sterile glass bottle containing 5 g of the oil sample. Then, the AV was measured through a titration with an aqueous potassium hydroxide (KOH; DAEJUNG Chemicals & Metals Co., Ltd.) solution, using 1% phenolphthalein (DAEJUNG Chemicals & Metals Co., Ltd.) as an indicator.

$$\text{AV (mg/g)} = \frac{5.611 \times (a-b) \times f}{S} \times 10$$, where S is the weight (g) of samples, a denotes the input volume (mL) of 0.1 N ethanolic sodium hydroxide, b represents the blank input volume (mL) of 0.1 N ethanolic sodium hydroxide, and f is equivalent to the factor of 0.1 N ethanolic sodium hydroxide.

Measurement of peroxide values (POVs)

A mixed solution of acetic acid (DAEJUNG Chemicals & Metals Co., Ltd.) and chloroform (DAEJUNG Chemicals & Metals Co., Ltd.) at a ratio of 3:2 (i.e. 25 mL) was transferred to a sterile glass bottle containing 1 g of the oil sample. One mL of a saturated potassium iodide (KI; DAEJUNG Chemicals & Metals Co., Ltd.) solution (i.e. 70 g of KI was dissolved in 50 g of DW) was added to the mixture, followed by 10 min of incubation at an ambient temperature in the dark. Then, each of the samples was added with 30 mL of DW and 1 mL of a starch solution (1%; DAEJUNG Chemicals & Metals Co., Ltd.), and the POV was measured through a titration with an aqueous 0.01-N sodium thiosulfate (DAEJUNG Chemicals & Metals Co., Ltd.) solution.

$$\text{POV(meq/kg)} = \frac{(a-b) \times f}{S} \times 10$$, where S is the weight (g) of samples, a denotes the input volume (mL) of 0.01 N sodium thiosulfate, b represents the blank input volume (mL) of 0.01 N sodium thiosulfate, and f is equivalent to the factor of 0.01 N sodium thiosulfate.

Analysis

All the experiments were repeated three times with duplicate plates. The obtained results were expressed as the mean±standard deviation. Significant ($P<0.05$) differences among the treated and nontreated groups were determined using the analysis of variances (ANOVA) with the Duncan's multiple range test (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

It should be noted that an agar disc diffusion assay employed in this study is useful for comparing the antibacterial activities of the tested samples, but has a limitation as a quantitative method because the diameter of clear zones can vary, depending on the diffusibility of the tested materials on the surface of agar plates. Then, our results should be interpreted cautiously as a qualitative indicator of antibacterial potentials. Further studies using broth microdilution-based minimum inhibitory concentration (MIC) assays are warranted to more precisely evaluate the antibacterial efficacy of the selected samples. Table 1 summarizes the effects of individual ethanol-based extracts from blueberry, broccoli, *cheonggukjang*, garlic, green tea, onion, radish leaf, and s*hiitake* mushroom for inhibiting the growth of a broad range of food-borne pathogenic bacteria, followed by an agar disc diffusion assay. Overall, the majority of the natural ethanol-based extracts originating from blueberry, broccoli, *cheonggukjang*, onion, radish leaf, and s*hiitake* mushroom, exhibited no detectable antibacterial activities against most of the bacterial strains evaluated, as indicated by the absence of clear zones. Nevertheless, garlic was found to be the most effective for reducing or eliminating *B. cereus* and *St. aureus*, with the concomitant formation of clear zones being 12.26-12.83 and 9.96-10.62 mm, respectively, while having no or little effects against other food-borne pathogenic bacteria, including *C. sakazakii*, *E. coli* O157:H7, *L. monocytogenes*, *Salmonella*, *Sh. flexneri*, and *Y. enterocolitica*. Green tea was demonstrated for its ability to inhibit the growth of only *St. aureus* ATCC 23235 and *St. aureus* ATCC 25923, with the clear zones of 8.86±0.20 and 10.20±0.00 mm, respectively.

ABTS and DPPH activities of an ethanol-based extract from blueberry, broccoli, *cheonggukjang*, garlic, green tea, onion, radish leaf or s*hiitake* mushroom are shown in Fig.

Table 1. Effect of an ethanol-based extract from blueberry, broccoli, *cheonggukjang*, garlic, green tea, onion, radish leaf or *shiitake* mushroom for inhibiting the growth of food-borne pathogenic bacteria, following an agar disc diffusion assay (mm)

Bacterium	Ethanol-based extracts							
	Blueberry	Broccoli	*Cheonggukjang*	Garlic	Green tea	Onion	Radish leaf	*Shiitake* mushroom
B. cereus ATCC 10876	-	-	-	-	-	-	-	-
B. cereus ATCC 13061	-	-	-	12.82±0.67	-	-	-	-
B. cereus ATCC 14579	-	-	-	12.26±0.79	-	-	-	-
C. sakazakii ATCC 12868	-	-	-	-	-	-	-	-
C. sakazakii ATCC 29004	-	-	-	-	-	-	-	-
C. sakazakii ATCC 29544	-	-	-	-	-	-	-	-
E. coli O157:H7 ATCC 35150	-	-	-	-	-	-	-	-
E. coli O157:H7 ATCC 43889	-	-	-	-	-	-	-	-
E. coli O157:H7 ATCC 43890	-	-	-	-	-	-	-	-
L. monocytogenes ATCC 19114	-	-	-	-	-	-	-	-
L. monocytogenes ATCC 19117	-	-	-	-	-	-	-	-
S. Enteritidis ATCC 13076	-	-	-	-	-	-	-	-
S. Typhimurium ATCC 19585	-	-	-	-	-	-	-	-
S. Typhimurium ATCC 43971	-	-	-	-	-	-	-	-
S. Typhimurium DT104 Killercow	-	-	-	-	-	-	-	-
Sh. flexneri KCTC 2517	-	-	-	-	-	-	-	-
Sh. flexneri KCTC 22192	-	-	-	-	-	-	-	-
Sh. flexneri NCCP 10852	-	-	-	-	-	-	-	-
St. aureus ATCC 23235	-	-	-	9.96±0.33	8.86±0.20	-	-	-
St. aureus ATCC 25923	-	-	-	10.62±0.30	10.21±0.00	-	-	-
St. aureus ATCC 40050	-	-	-	-	-	-	-	-
Y. enterocolitica ATCC 23715	-	-	-	-	-	-	-	-
Y. enterocolitica ATCC 55075	-	-	-	-	-	-	-	-

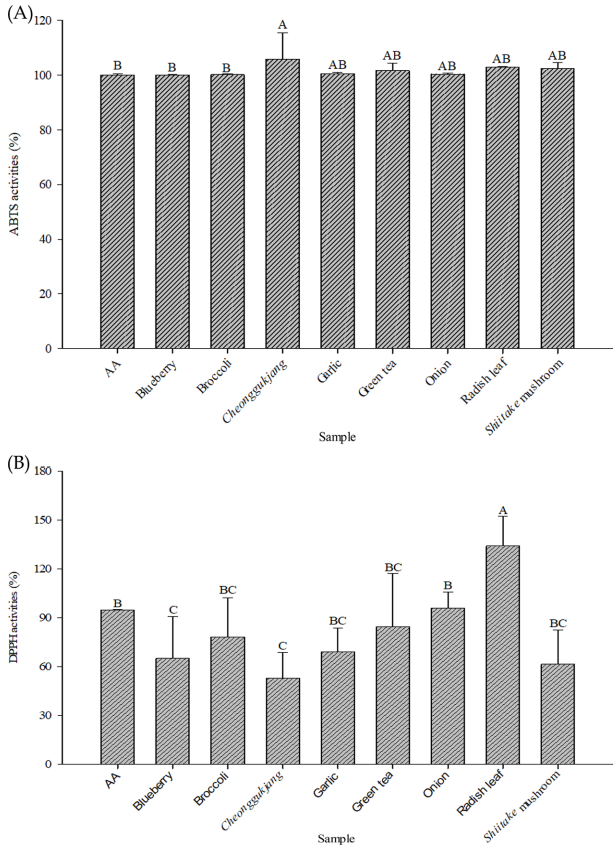

Fig. 1. (A) ABTS and (B) DPPH activities of an ethanol-based extract from blueberry, broccoli, c*heonggukjang*, garlic, green tea, onion, radish leaf or *shiitake* mushroom.

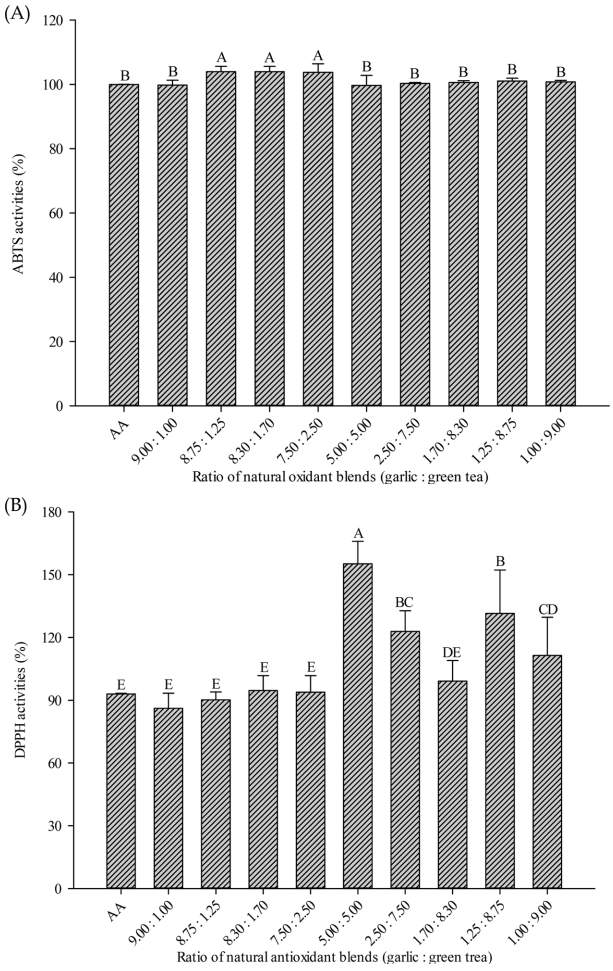

Fig. 2. (A) ABTS and (B) DPPH activities of mixtures containing ethanol-based extracts from garlic and green tea.

1. As a result, ABTS activities of blueberry, broccoli, *cheonggukjang*, garlic, green tea, onion, radish leaf or s*hiitake* mushroom were 100.00±0.13, 100.15±0.36, 105.81 ±9.68, 100.50±0.55, 101.68±2.72, 100.38±0.36, 102.98 ±0.20 or 102.39±2.13% in comparison with those of the control group (vitamin C; AA; 100.11±0.28%). Radish leaf was found to exhibit the strongest DPPH radical-scavenging activity (134.03±18.08%), followed by onion (95.86 ±9.91%), green tea (84.56±32.67%), broccoli (78.28 ±23.85%), garlic (69.06±14.83%), blueberry (65.04±25.80%), *shiitake* mushroom (61.50±21.02%), and *cheonggukjang* (53.06±15.70%).

ABTS and DPPH activities of mixtures consisting of ethanol-based extracts from garlic and green tea at various formation ratios are described in the supplementary Table 2 and Fig. 2. ABTS activities, ranging from 103.76% to 103.95%, of the mixtures (garlic+green tea) at a ratio of "8.75:1.25", "8.30:1.70", and "7.50:2.50" were significantly (P<0.05) higher than those at the other formation ratios of a mixture of garlic and green tea. Notably, the mixture of garlic and green tea at a ratio of 5:5 was found to exhibit the strongest DPPH radical-scavenging activity (155.16±10.78%), which was significantly (P<0.05) higher than that at the other formation ratios of a mixture of garlic +green tea (86.16-131.49%).

Table 3 presents the effects of an ethanol-based mixture of garlic and green tea at a ratio of 5:5 for inhibiting the growth of a broad range of food-borne pathogenic bacteria, as determined by carrying out an agar disc diffusion assay. As a result, the garlic+green tea mixture yielded selective antibacterial activities, with the formation of clear zones, ranging from 9.28±0.37 mm to 12.43±0.27 mm, achieved exclusively against certain gram-positive bacteria, such as *B. cereus* and *St. aureus*, but failed to inhibit the growth of other food-borne pathogenic bacteria.

Fig. 3 displays the change patterns of AV or POV of a corn oil containing 1-2% ethanol-based extracts from a mixture of garlic and green tea (formulated at a ratio of 5:00:5:00), following 32 d of storage at different storage temperatures. Commonly, all the AVs remained unchanged

Table 2. Formulation of mixtures containing ethanol-based extracts from garlic and green tea

Ratio (garlic:green tea)	Garlic (g)	Green tea (g)	Weight (g)
10.00:0.00	5.000	0.000	
9.00:1.00	4.500	0.500	
8.75:1.25	4.375	0.625	
8.30:1.70	4.150	0.850	
7.50:2.50	3.750	1.250	
5.00:5.00	2.500	2.500	5.000
2.50:7.50	1.250	3.750	
1.70:8.30	0.850	4.150	
1.25:8.75	0.625	4.375	
1.00:9.00	0.500	4.500	
0.00:10.00	0.000	5.000	

Table 3. Effect of ethanol-based extracts from garlic+green tea for inhibiting the growth of food-borne pathogenic bacteria, following an agar disc diffusion assay (mm)

Bacterium	Mixture (garlic+green tea)[1)]
B. cereus ATCC 10876	-
B. cereus ATCC 13061	9.34±0.19
B. cereus ATCC 14579	9.28±0.37
C. sakazakii ATCC 12868	-
C. sakazakii ATCC 29004	-
C. sakazakii ATCC 29544	-
E. coli O157:H7 ATCC 35150	-
E. coli O157:H7 ATCC 43889	-
E. coli O157:H7 ATCC 43890	-
L. monocytogenes ATCC 19114	-
L. monocytogenes ATCC 19117	-
S. Enteritidis ATCC 13076	-
S. Typhimurium ATCC 19585	-
S. Typhimurium ATCC 43971	-
S. Typhimurium DT104 Killercow	-
Sh. flexneri KCTC 2517	-
Sh. flexneri KCTC 22192	-
Sh. flexneri NCCP 10852	-
St. aureus ATCC 23235	10.31±0.32
St. aureus ATCC 25923	10.82±0.16
St. aureus ATCC 40050	12.43±0.27
Y. enterocolitica ATCC 23715	-
Y. enterocolitica ATCC 55075	-

[1)]Mixture containing ethanol-based extracts from garlic and green tea at a ratio of 5:5.

before and after 32 d of storage at 25, 35, 50, and 70 °C. A slight increase in the AVs of a corn oil supplemented with 1-2% mixtures of garlic and green tea was observed after 32 d of storage at 70°C, which was not significantly (P<0.05) different from those of a corn oil without any supplementation under the same storage conditions. Following 32 d of storage at 25°C, POVs of a corn oil without any supplementations were 12.37±2.06 meq/kg higher than those with 1-2 mixtures of garlic and green tea (3.68-10.36 meq/kg) albeit no significant (P≥0.05) difference between the former and the latter. POVs of a corn oil with or without supplementations increased to 38.11 or 20.06-22.07 meq/kg after 32 d of storage at 35°C. At 50°C, POVs of a corn oil with or without a 1% mixture of garlic and green tea rapidly rose, exceeding 105.32±40.55 or 156.47 ±40.73 meq/kg within 32 d, whereas those of a corn oil fortified with a 2% mixture still remained stable, ranging from 4.01±1.64 to 12.71±2.06 meq/kg during the entire storage periods. At 70°C, corn oils with no treatment, a 1% mixture, and a 2% mixture took 14, 29, and 29 d, respectively, to reach a POV of more than 150.00 meq/kg. On day 32, final POVs of a corn oil with no treatment, a 1% mixture, and a 2% mixture were 227.01±10.43, 215.31±32.37, and 209.96±22.28 meq/kg, respectively, at 70°C.

In this study, both garlic and green tea remained active in inhibiting the growth of certain gram-positive bacteria, such as *B. cereus* and *St. aureus*. It should be also noted that a mixture of garlic and green tea was found to effectively suppress *B. cereus* and *St. aureus*, with the formation of clear zones being 9.28-9.34 and 10.31-12.43 mm, respectively. There were no significant (P≥0.05) differences in the diameter of clear zones driven by individual and combined treatments with garlic and green tea against *B. cereus* and *St. aureus*. Earlier studies have determined that ethanol or water-based extracts from garlic have antibacterial[16)], antifungal[17)], and antiviral[18)] activities, which is closely associated with allicin which can interfere with RNA synthesis and, to a lesser extent, DNA/protein synthesis[19)]. Green tea has been demonstrated for its strong ability to reduce or eliminate total mesophilic bacteria and *Listeria innocua* inoculated on beet leaves by more than 3.0 and 2.0 log CFU/g, respectively[20)]. It is well known that the effectiveness of green tea extracts for inactivating food-borne pathogenic bacteria is positively correlated with the polyphenol content, which is dependent on the concentration of tea products[21)]. In parallel with the growing consumer demand for healthy and nutritious food products that do not contain synthetic chemical additives, our findings highlighted the potential applicability of garlic and green tea or their mixtures as a promising natural antibacterial and antioxidant alternative in food systems.

AV and POV serve as important quality criteria for evaluating the lipid deterioration in seasoned laver products. Especially, the Food Code published by the Korean Ministry of Food and Drug Safety[22], specifies maximum allowable values of 4.0 mg/g and 60.0 meq/kg for AV and PV, respectively. Regardless of the conditions under which the corn oils used for manufacturing seasoned laver products were formulated with or without ethanol-based extracts, higher storage temperatures led to a shorter time for the POV required to reach 60.0 meq/kg. Indeed, the POV of a corn oil containing a 2% mixture of garlic and green tea was in an acceptable range of less than 12.70±2.06 meq/kg after at least 32 d of storage at 50°C, whereas less than 27-29 d were needed for a corn oil with or without a 1% mixture of garlic and green tea to reach 60.0 meq/kg at the same storage temperature. In a study conducted by Choi et al.,[12] who investigated the effects of a mixture of onion and rosemary for retarding the lipid peroxidation of a corn oil stored at 70°C, POVs of a corn oil incorporated with a 2% mixture of onion and rosemary at a ratio 3:1 always were significant ($P<0.05$) lower than those without any supplementations. Previously, several studies[23,24] found that the addition of rosemary extracts into vegetable oils significantly improved the resistance of the oils to the oxidative deterioration, as characterized by increasing phenolic concentrations, along with the prolonged retention of unsaturated fatty acids and tocopherols. Considering that the peroxide formation represents an early stage of the oxidation of lipids, the peroxide-inhibitory effects of a mixture of garlic and green tea indicate that the incorporation of natural plant-derived extracts may effectively delay the initiation and progression of an oxidative deterioration in edible oils used for seasoned laver products.

The DPPH-scavenging activity values greater than 150% observed in this study warrant a careful interpretation. Under the conventional calculation, DPPH-scavenging activities

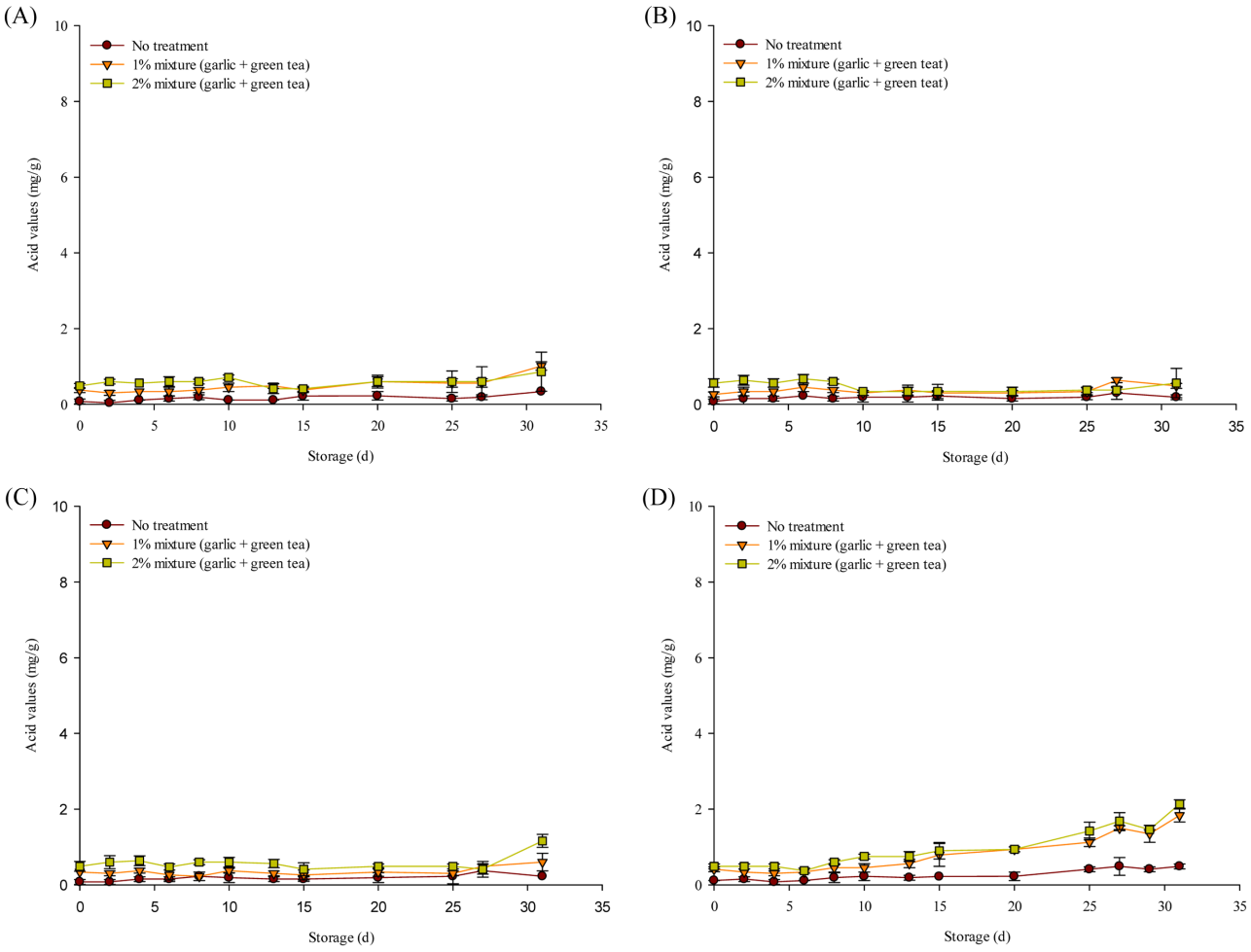

Fig. 3. Change on the (A-D) AV and (E-H) POV values of a corn oil supplemented with 1-2% ethanol-based extracts from a mixture of garlic and green tea (formulated at a ratio of 5:5), following 32 d of storage at (A and E) 25, (B and F) 35, (C and G) 50, and (D and H) 70°C.

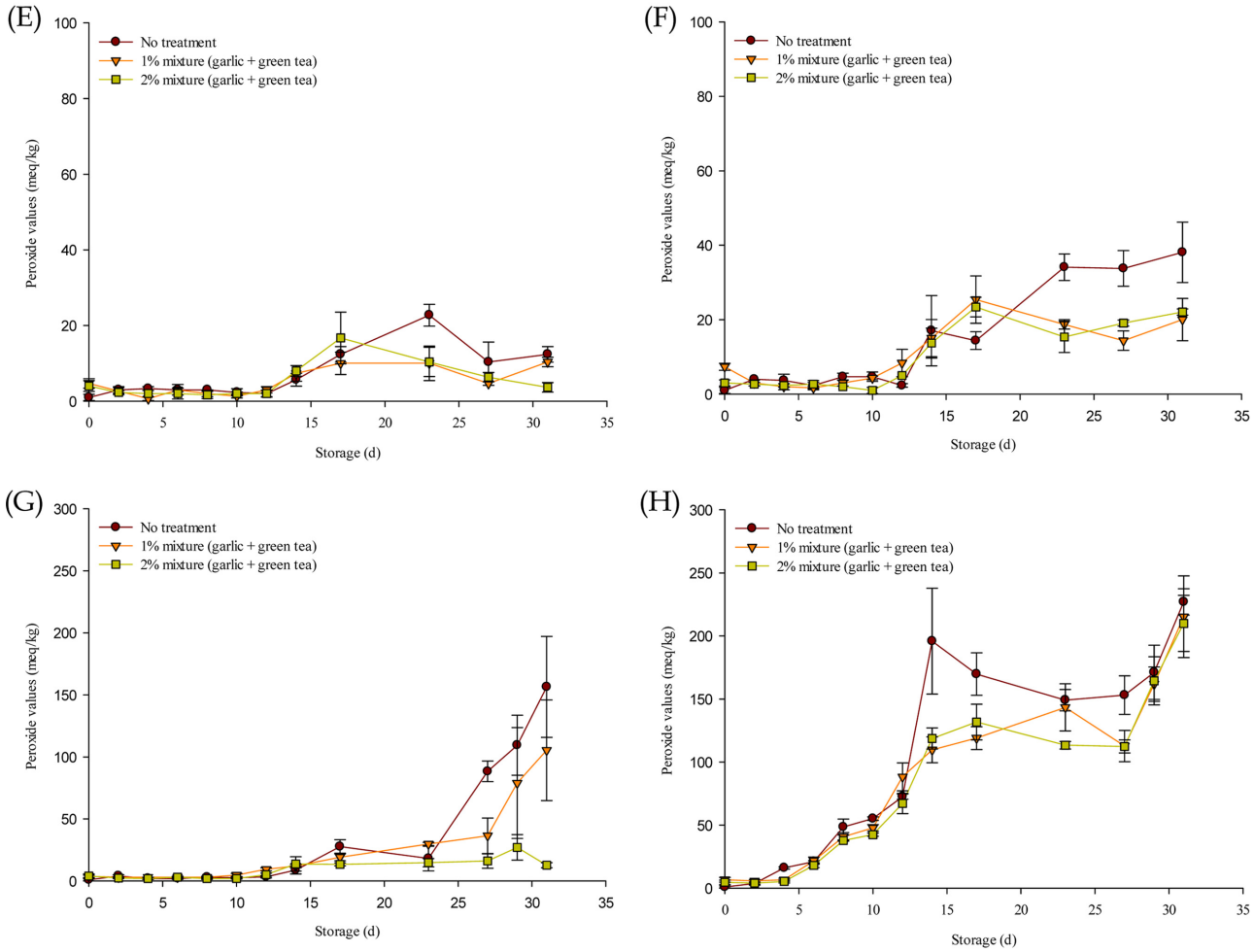

Fig. 3. (Continued) Change on the (A-D) AV and (E-H) POV values of a corn oil supplemented with 1-2% ethanol-based extracts from a mixture of garlic and green tea (formulated at a ratio of 5:5), following 32 d of storage at (A and E) 25, (B and F) 35, (C and G) 50, and (D and H) 70°C.

are generally expected to remain within the range of 0-100%. The values exceeding 100% indicate that the corrected absorbance of the samples was markedly lower than that of the control, which may result not only from the strong radical-quenching capacity, but also from analytical factors, including the sample's overestimation and/or turbidity during a blank correction. Since natural extracts are chemically complex and may contain a compound that interferes with spectrophotometric measurements, the high DDPH-scavenging activity values exceeding over 100% should be interpreted cautiously.

The profound variability in the standard deviations achieved for the 1% mixture-treated samples at 50°C suggests that the antioxidative effects of the 1% mixture of garlic and green tea remain inconsistent across three replicates under this condition. Considering the fact that POV reflects the accumulation of primary lipid oxidation-associated products, the largely variable results depicted in Fig. 3E-H may indicate that the onset and progression of lipid oxidation events substantially differed among individual samples. At 50°C, the oxidative deterioration was accelerated to promote the resultant formation of hydroperoxides, whereas the protective effects of the 1% mixture were still marginal and unstable. Under such a condition, small differences in the distribution of antioxidative compounds within the corn oil's matrix may cause marked differences in the oxidation rates among replicates, which can explain why some samples remained relatively less oxidized, whereas others underwent the extensive formation of peroxides during the same storage period.

In conclusion, this study evidenced that the natural ethanol-based blend of garlic and green tea not only provides strong antibacterial and antioxidant functions, but also effectively enhances the oxidative stability of a corn oil used for seasoned laver products. The findings may offer valuable insights for the development of natural

additives·preservatives that can be used for manufacturing seasoned laver products.

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국문요약

현대 식품산업에서 소비자의 건강과 식품 품질 유지, 그리고 인공첨가물에 대한 거부감을 고려하여 천연 유래의 기능성 소재 개발이 활발히 이루어지고 있다. 이러한 흐름 속에서 조미김은 대한민국을 비롯한 동아시아 국가에서 널리 소비되는 대표적인 가공 해조류 제품으로 제조 공정 중 식용 유지를 고온으로 가열하는 과정이 필수적이다. 하지만 300도 이상의 고온 가열 과정은 식용 유지의 산화를 유발하여 풍미 저하, 저장성 감소 및 궁극적으로 소비자 수용성 저하로 이어질 수 있다는 문제가 존재한다. 특히 조미김에서 사용되는 옥수수유, 참기름 및 들기름 등은 불포화지방산 함량 비율이 높아 산화에 더욱 취약하고 이로 인하여 그 품질 유지가 조미김 산업에서 중요한 과제로 부상하고 있다. 이에 따라 본 연구에서는 천연 식물성 원료인 마늘과 녹차로부터 이들의 주정 기반의 추출물을 활용하여 항산화 및 항균 효과를 검증하고 더 나아가 이 추출물들을 조미김용 유지(i.e. 옥수수유)에 첨가하여 산화 안전성에 미치는 영향을 종합적으로 평가하고자 하였다. 우선 국내산 마늘과 녹차 분말을 25% 에탄올에 침지시킨 후 24시간 동안 상온에서 교반하여 유효성분을 추출하였고 이를 원심분리 및 필터를 이용하여 여과 후 감압농축을 거쳐 최종 주정추출물을 확보하였다. 이렇게 마련된 개별 추출물들은 단독으로 사용되기도 하였으며, 무엇보다 마늘과 녹차를 여러 중량비로 혼합하여 복합 추출물로도 사용되었다. 항균활성 측정을 위하여 총 13종의 식품매개 병원성 세균(*Bacillus cereus, Escherichia coli* O157:H7, *Salmonella enterica serovar* Typhimurium, *Staphylococcus aureus* 등)을 대상으로 agar disc diffusion 방법을 적용하였다. 그 결과 마늘 추출물은 바실러스 세레우스와 황색포도상구균에 대하여 직경 약 12 mm 이상의 클리어존(clear zone)을 생성함으로써 강력한 생육(growth) 억제 효과를 나타내었으며, 녹차 추출물은 일부 황색포도상구균에 대하여 약 9-10 mm 크기의 클리어존 형성에 기여하였다. 특히 마늘과 녹차를 5:5 비율로 혼합한 복합 추출물의 경우에도 바실러스 세레우스와 황색포도상구균에 대하여 유의미한(P<0.05) 생육 억제능을 선보였다. 항산화 활성의 경우 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)와(과) 2,2-diphenyl-1-picrylhydrazyl (DPPH) 라디칼 소거 능력을 측정하였고 비타민 C를 대조군으로 설정하였다. 단일 추출물 중에서는 DPPH 기준으로 마늘과 녹차가 각 69.057±14.830% 및 84.556±32.670% 수준의 라디칼 소거능을 보여주었으며, 특히 이 두 성분을 5:5 비율로 혼합하였을 때 DPPH가 무려 155.158±10.783%까지 상승하여 단일 처리 대비 두 배 이상의 항산화 활성을 나타내었다. 이러한 항산화 및 항규 활성 결과를 바탕으로 마늘과 녹차 추출물을 5:5 비율로 혼합한 복합 추출물을 옥수수유에 1-2%의 농도로 첨가하여 저장온도에 따른 산화 안전성을 평가하였다. 이러한 실험은 25, 35, 50 및 70도의 네 가지 조건에서 32일간 진행되었고 일정 간격마다 산가(acid value, AV) 및 과산화물가(peroxide value, POV)를 측정하였다. 산가는 저장 중 큰 변동이 없었으나, POV에서는 뚜렷한 차이를 확인할 수 있었다. 특히 50도 조건에서는 대조군이 27일만에 POV를 60 meq/kg을 초과한 반면, 1% 혼합물 첨가군은 29일 및 2% 혼합물 첨가군은 32일이 지나도록 POV가 60 meq/kg을 초과하지 않아 보존기간을 유의적으로(P<0.05) 연장시키는 것으로 관찰되었다. 70도 조건에서도 유사한 경향이 나타났는데 대조군의 경우 14일만에 POV가 150 meq/kg을(를) 초과하였으나, 2% 혼합물 첨가군은 29일차에도 POV가 210 meq/kg 이하의 수준을 유지하여 산화 억제 효과가 지속됨을 확인할 수 있었다. 결론적으로 마늘과 녹차의 주정추출물 혼합물은 바실러스 세레우스 및 황색포도상구균과 같은 그람양성 식품매개 병원성 세균에 대하여 강력한 항균력을 가지며, 특히 DPPH 기준으로 그 항산화 활성은 단일 성분보다 복합 처리가 현저히 우수함을 확인하였다. 조미김 제조에 사용되는 유지의 산화 안전성 측면에서, 2% 혼합물 첨가는 고온에서도 과산화물 생성을 억제하여 보존성을 크게 높일 수 있었고, 이는 국내에서 규정된 조미김용 식용 유지 품질 기준을 만족하는 수준이었다. 이러한 결과는 천연 유래 복합(마늘+녹차) 주정추출물이 합성 산화방지제나 항균제 대용으로 사용할 수 있는 천연 소재로서의 활용가능성을 높이며, 궁극적으로 건강지향적이고 소비자 수용도가 높은 기능성 식품 원료로 발전할 수 있는 과학적 기반을 제공한다.

Conflict of interests

The authors declare no potential conflict of interest.

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Supplementary Information

The supplementary materials Table S1. Change on POV values of a corn oil supplemented with 1-2% ethanol-based extracts from a mixture of garlic and green tea (formulated at a ratio of 5:5), following 32 d of storage at 25, 35, 50, and 70°C.

Time (h)	Storage at 25°C			Storage at 35°C		
	No treatment	Mixture (1%)	Mixture (2%)	No treatment	Mixture (1%)	Mixture (2%)
0	1.00±1.00^{Aa}	4.68±1.53^{Babc}	4.01±1.74^{Bab}	1.00±1.00^{Aa}	7.36±1.16^{Bab}	3.01±1.00^{Aab}
4	3.34±0.58^{Cab}	0.67±0.58^{Aa}	2.01±0.00^{Ba}	3.68±2.09^{Aa}	2.01±1.00^{Aa}	2.34±0.58^{Aa}
8	3.01±0.00^{Aab}	2.01±1.00^{Aab}	1.62±1.16^{Aa}	4.68±1.16^{Ba}	3.01±1.00^{ABa}	2.01±0.00^{Aa}
12	2.01±1.00^{Aab}	3.01±1.00^{Aabc}	2.67±1.16^{Aa}	2.34±0.58^{Aa}	8.36±4.52^{Babc}	5.02±0.00^{ABabc}
14	5.68±2.09^{Aa-c}	7.36±2.52^{Acd}	8.02±1.74^{Aab}	17.05±11.57^{Aab}	15.05±6.10^{Abcde}	13.71±4.95^{Abcd}
17	12.30±2.52^{Ade}	10.03±3.62^{Ade}	16.72±8.35^{Ac}	14.38±2.90^{Aab}	25.41±7.79^{Befg}	23.40±3.22^{ABde}
20	4.68±0.58^{Aab}	6.02±2.01^{Abc}	5.35±3.52^{Aab}	11.37±1.16^{Aab}	10.70±5.05^{Aabcd}	24.41±11.97^{Ade}
23	22.73±3.52^{Bf}	10.03±5.58^{Ade}	10.36±4.74^{Ab}	37.10±4.37^{Bcd}	18.72±1.53^{Acdef}	15.38±5.15^{Acd}
27	10.36±6.45^{Acd}	4.68±0.58^{Aabc}	6.35±1.53^{Aab}	33.77±5.88^{Bcd}	14.38±3.22^{Abcd}	19.06±1.00^{Ad}
29	6.69±0.58^{Abc}	5.68±2.90^{Aabcd}	5.68±4.52^{Aab}	26.75±5.15^{Abc}	26.75±13.24^{Afg}	22.73±8.04^{Ade}
31	12.37±2.52^{Bde}	10.36±1.53^{Bde}	3.68±1.53^{Aab}	38.11±17.49^{Acde}	20.06±6.95^{Adefg}	22.07±1.00^{Ade}
35	17.05±4.60^{Be}	10.03±2.01^{Ade}	8.36±2.52^{Aab}	46.47±10.05^{Cdef}	30.09±8.57^{Bg}	13.04±3.62^{Aabcd}
39	15.71±4.18^{Ade}	14.71±4.63^{Ade}	10.03±2.65^{Ab}	61.52±9.10^{Bf}	29.09±1.74^{Afg}	32.76±11.54^{Ae}
42	12.04±3.47^{Ade}	10.36±2.52^{Ade}	10.03±2.65^{Ab}	52.49±19.92^{Bef}	15.05±3.62^{Abcdefg}	24.07±9.57^{Ade}

$^{A-C}$Within the same row, values with different uppercase letters among groups (i.e., no treatment, mixture (1%), and mixture (2%)) denote significant (P<0.05) differences.
$^{a-g}$Means in the same column with different lowercase letters denote significant (P<0.05) differences.

The supplementary materials Table S1. (*continuous from the previous page*) Change on POV values of a corn oil supplemented with 1-2% ethanol-based extracts from a mixture of garlic and green tea (formulated at a ratio of 5:5), following 32 d of storage at 25, 35, 50, and 70°C.

Time (h)	Storage at 50°C			Storage at 70°C		
	No treatment	Mixture (1%)	Mixture (2%)	No treatment	Mixture (1%)	Mixture (2%)
0	1.00±1.00^{Aa}	3.68±1.16^{Aa}	4.01±2.01^{Aab}	1.00±1.00^{Aa}	6.69±2.32^{Ba}	4.68±2.09^{ABa}
4	2.01±1.00^{Aa}	3.01±0.00^{Aa}	2.01±0.00^{Aa}	16.05±1.00^{Bab}	6.69±1.53^{Aa}	5.35±1.53^{Aa}
8	3.01±0.00^{Aa}	3.01±1.00^{Aa}	2.01±1.00^{Aa}	48.48±7.79^{Bbc}	40.79±4.05^{ABb}	37.78±0.58^{Abc}
12	3.34±1.16^{Aa}	9.36±1.53^{Ba}	4.68±1.53^{Aab}	72.55±2.52^{ABc}	88.26±13.57^{Bc}	67.20±9.88^{Ad}
14	8.69±3.80^{Aa}	12.04±1.74^{Aa}	13.71±7.12^{Abc}	195.92±51.44^{Bfg}	109.66±11.09^{Acd}	118.69±10.25^{Ae}
17	27.75±6.68^{Ba}	19.06±3.47^{ABa}	13.37±1.53^{Abc}	169.84±20.56^{Bef}	119.02±11.09^{Ade}	131.73±17.44^{Ae}
20	24.41±4.18^{Aa}	24.74±2.09^{Aa}	18.05±12.16^{Acd}	111.33±22.18^{Ad}	106.65±11.41^{Acd}	125.71±14.62^{Ae}
23	87.60±52.60^{Bb}	29.76±1.53^{ABab}	14.71±4.05^{Abc}	149.11±10.29^{Be}	143.43±22.94^{Bef}	113.34±3.62^{Ae}
27	88.26±10.18^{Bb}	36.44±17.53^{Aab}	16.05±7.02^{Ac}	153.12±18.64^{Be}	112.67±15.19^{Acd}	112.34±6.26^{Ae}
29	109.33±156.47^{Bb}	78.90±54.68^{ABbc}	27.08±12.57^{Ade}	171.18±26.39^{Aef}	161.82±16.61^{Af}	164.49±23.33^{Af}
31	156.47±49.88^{Bc}	105.32±49.67^{Bcd}	12.70±2.52^{Aabc}	227.01±12.78^{Ag}	215.31±39.64^{Ag}	209.96±27.29^{Ag}
35	179.20±34.11^{Ccd}	96.62±47.20^{Bc}	8.69±3.06^{Aabc}	ND[1)]	ND	ND
39	162.49±35.13^{Bc}	114.68±32.18^{Bcd}	12.70±3.06^{Aabc}	ND	ND	ND
42	213.30±15.60^{Bd}	154.80±52.02^{Bd}	29.42±3.06^{Ae}	ND	ND	ND

[1)]ND: Not determined.
$^{A-C}$Within the same row, values with different uppercase letters among groups (i.e., no treatment, mixture (1%), and mixture (2%)) denote significant (P<0.05) differences.
$^{a-g}$Means in the same column with different lowercase letters denote significant (P<0.05) differences.